

THE EFFECTS OF ABRUPT DIETARY CHANGES ON THE HINDGUT ENVIRONMENT
OF THE HORSE

by

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Abstract

Abrupt dietary changes increase a horse's risk for developing gastrointestinal diseases, such as colic or laminitis. Understanding the impact of various feeds and feeding practices on feeding behavior and gastrointestinal function creates a whole-animal perspective that allows for a more holistic interpretation of the effects of abrupt dietary changes on the hindgut environment. Unfortunately, few reports exist that have examined the effects of abrupt dietary changes in the horse. This study was designed to determine the effects of various abrupt dietary changes on the hindgut environment. In 4 sequential experiments, horses were exposed to an abrupt change from a baseline ration to a complete pelleted diet, an abrupt change from a baseline ration to a 100% grass hay diet, an abrupt change from a prairie hay ration to an alfalfa hay ration, and an abrupt change from a baseline ration to a large concentrate meal. These dietary challenges were chosen to mimic real-world scenarios that horse owners are likely to encounter. These experiments were arranged into a longitudinal trial in which the effects of the abrupt dietary change on cecal and fecal pH, total lactate and volatile fatty acid (VFA) concentrations, cecal lactate-utilizing bacterial populations, and fecal dry matter (DM) were compared to values obtained while horses were consuming the baseline diet. In the first experiment, decreased cecal ($P < 0.0001$) and fecal ($P < 0.0001$) pH values combined with increased cecal total lactate ($P < 0.001$) and fecal VFA concentrations ($P < 0.0001$) indicate that the abrupt change to a complete pelleted diet disrupted the stability of the hindgut environment. Because cecal pH values were below 6.0, this dietary challenge may be significant enough to elicit subclinical fermentative acidosis and, thereby, increase colic risk. The dietary change to grass hay had little impact on the hindgut environment, as pH, total lactate, and VFA concentrations remained stable ($P \geq 0.05$). In general, horses may well tolerate an abrupt increase in the fibrous component of the diet and the

elimination of concentrate, a dietary shift that presents a more natural diet to the horse. The abrupt change to alfalfa hay elicited alterations in cecal pH ($P < 0.01$), total lactate ($P < 0.0001$) and VFA concentrations ($P < 0.05$), and lactate-utilizing bacterial populations; however, fecal parameters varied little in response to the dietary change ($P \geq 0.05$), indicating that the distal hindgut may be more tolerant to abrupt changes in forage sources than the cecal environment. Here, the potentially adverse shifts in cecal parameters indicate that an abrupt change in hay type and quality alters the fermentative environment of the proximal hindgut and may increase a horse's risk for gastrointestinal disease. Similarly, the abrupt introduction of a large concentrate meal elicited a decrease in cecal pH ($P < 0.005$) along with increases in total lactate ($P < 0.001$) and VFA concentrations ($P < 0.05$) in the cecum that were consistent with previously reported experiments in which horses were presented with large increases in dietary concentrates. Notable shifts in lactate-utilizing bacterial growth curves were also observed. Overall, these results provide evidence of environmental alterations in the equine hindgut that support epidemiological reports that associate abrupt changes in the amount and type of concentrate, hay type and quality, and forage:concentrate ratio with increased risk for gastrointestinal disease in horses.

Key words: cecum, dietary change, equine, hindgut, lactate-utilizing bacteria

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Dedication

This thesis is dedicated to all equine enthusiasts who have devoted their lives to these remarkable animals through education, research, and the promotion of the equine industry.

Chapter 1 - Literature Review: The Impact of Feedstuffs and Feeding Management on Ingestive Behaviors, Digestion, and Gastrointestinal Health in Equines

Abstract

Dietary composition and feeding practices affect feeding behaviors, digestive parameters, and gastrointestinal health in equines. Large incidence estimates for abnormal behaviors and stereotypies and gastrointestinal diseases in equine populations have prompted researchers to investigate the relationships between management practices and health factors. Dietary composition impacts ingestive behaviors, intake preferences, and may influence the development of abnormal and stereotypical behaviors that may indicate digestive distress. Dietary components, as well as the degree of feed processing, alter mastication and prececal digestion. Dry matter content, seasonality of feedstuffs, and palatability of feedstuffs affect intake preferences and may be related to gastrointestinal and metabolic disorders. Abnormal behaviors and stereotypies often develop in environments associated with intensive management practices and low-fiber and high-concentrate diets. Together, these prandial factors impact carbohydrate digestion and absorption throughout the gastrointestinal tract. Additionally, the extent of mastication affects the degree of prececal carbohydrate digestion in the small intestine, and the physical form of the feed and composition of the diet alter mean retention times and rates of passage through the gut. Impaired prececal digestibility disrupts the microbial ecosystem of the hindgut and increases an animal's risk for developing intestinal illnesses and metabolic disorders, such as colic and laminitis, when digestive contents spill over into the hindgut. These

conditions are of particular concern when horses encounter abrupt dietary changes.

Understanding the impact of various feedstuffs and feeding practices on feeding behavior and gastrointestinal function creates a whole-animal perspective that allows for a more holistic interpretation of the effects of abrupt dietary changes on the hindgut environment and may lead to improved equine management practices.

Introduction

Although equipped with a monogastric foregut, horses have a set of unique hindgut adaptations that enable them to thrive on forages. An augmented and specialized large intestine, in addition to several modified accessory organs, allows them to overcome the limitations of the foregut, slow the rate of passage through the hindgut, use microbial fermentation to break down complex carbohydrates, and obtain up to 80% of their energy requirements from the fermentation by-products of forage-based diets (Vermorel et al., 1997a). While this system functions well in a natural environment, it predisposes horses to digestive disturbances under modern management schemes. Furthermore, intensely managed feeding regimens, along with other environmental factors, can prompt the development of stereotypical behaviors and vices. Although the majority of horses tolerate these by-products of domestication, annual economic losses from affected horses have been estimated to be \$115.3 million for colic alone (Traub-Dargatz et al., 2001). Costs associated with abnormal behaviors and vices associated with dental injury and property damage have not been estimated.

Prevalence of Abnormal Behaviors and Gastrointestinal Disease

Animal welfare concerns have prompted investigation into the causal factors associated with stereotypical behaviors and vices in the horse. Current theories suggest that these behavioral responses compensate for environmental inadequacies or are expressions of frustration

(Hothersall and Nicol, 2009). For example, coprophagia, geophagia, wood-chewing, and bedding consumption are perceived to be compensatory responses to dietary deficiencies (Hothersall and Nicol, 2009). On the other hand, cribbing and windsucking are thought to be attempts at increasing salivation, although these behaviors could also originate from frustration or pain (Hothersall and Nicol, 2009). Other vices, such as weaving, pawing, headshaking, and self-mutilation, are more severe behaviors that may be rooted in environmental frustrations (Cooper and Mason, 1998). These behaviors are not only dangerous to the animals and their handlers, but also are energetically expensive and can be accompanied by muscle damage and fatigue (McGreevy et al., 1995). Wood-chewing is prevalent in 30.3% of young horses, and 74.0% of young horses that crib have previously demonstrated wood-chewing behaviors (Waters et al., 2002). In the general horse population, incidence estimates for these behaviors vary, but as much as 6.8 to 11.8% of horses chew wood while 4.1 to 4.4% of horses crib (Nicol, 1998; Albright et al., 2009; Malamed et al., 2010). Interestingly, these behaviors are not reported in feral horse populations, but they have been noted in Przewalski horses maintained in captivity (Boyd, 1986). Therefore, domestication and modern management practices may be to blame for the prevalence of these abnormal behaviors and stereotypes.

Gastrointestinal disorders, such as gastric ulcers, fermentative acidosis, laminitis, and colic, are more prevalent in intensively managed animals and are often attributed to dietary composition and feeding management practices (Durham, 2009). Andrews and Nadeau (1999) estimated that 25 to 50% of young, growing horses are afflicted with gastric ulcers, partly due to weaning stress and the addition of concentrate to the diet. Other researchers investigating adult horse populations have reported gastric ulcers in 70.9% of Thoroughbred broodmares (le June et al., 2009), 58% of performance horses (McClure et al., 1999), and 66 to 93% of racehorses

(Andrews and Nadeau, 1999). In the hindgut, large quantities of readily fermentable carbohydrates (CHO-F_R) promote fermentative acidosis, which could result in laminitis and lead to debilitating lameness (Hoffman et al., 2001). Microbial populations in the hindgut ferment CHO-F_R to produce lactic acid. The sudden accumulation of lactate acidifies the lumen of the intestine and prompts a physiological cascade that may result in the loss of useful life or the death of the animal. Fermentative acidosis and laminitis are common threats to equines under modern management practices, particularly obese and metabolically compromised horses (Trieber et al., 2006; Carter et al., 2009). Recently, researchers in Great Britain estimated the annual disease incidence of laminitis to be 0.5% (Wylie et al., 2013), whereas previous investigators reported the disease incidence to be between 2.4 to 23.8% for the general horse population (Dorn et al., 1975; Buckley et al., 2007). In a recent study, pasture-associated laminitis was reported to affect 23.5% of an equine population in England (Menzies-Gow et al., 2010). Here, researchers observed horses at a rescue farm and associated the disease incidence with the increased age of the horses. Differences in study design and disease definitions may partially explain the disparity between these reports (Wylie et al., 2013). Colic is a gastrointestinal disorder marked by severe abdominal pain. Risk assessments have implicated recent dietary changes, housing environments, deworming protocols, cribbing, previous colic episodes, and age in the etiology of this disease (Tinker et al., 1997; Cohen et al., 1999; Traub-Dargatz et al., 2001; Hudson et al., 2001; Little and Blikslager, 2002; Hillyer et al., 2002). Additionally, epidemiological studies have reported 5 to 10 colic cases per 100 horses, making colic one of the most prevalent diseases within equine populations (Tinker et al., 1997, Traub-Dargatz et al., 2001).

Due to overwhelming evidence implicating management practices in the development of unwanted behaviors and gastrointestinal disease, researchers are charged with identifying specific causal factors, elucidating the mechanisms through which they occur, and recommending preventative measures to horse owners and keepers. Understanding how specific dietary ingredients and feeding practices act in concert with other environmental circumstances to disrupt the functionality of the equine gastrointestinal tract (GIT) allows for improved dietary formulations and animal management strategies that promote disease prevention and animal welfare.

Feeding Behaviors Influenced by Diet

Ingestive Behaviors

Horses are nonruminant herbivores that have evolved over 50 million years into selective, continuous grazers (Houpt, 1990). When grazing, horses ingest several bites of grass and move to a new feeding area while chewing and swallowing their food. Additionally, horses will use this period of time to survey their surroundings for potential threats and predators (Houpt, 1990). Free ranging, feral horses devote 70 to 75 % of their day to grazing or foraging activities (Salter and Hudson, 1979; Mayes and Duncan, 1986). Horses maintained on pasture or provided with *ad libitum* hay allocate similar amounts of time to eating (Houpt, 1990; Fleurance et al., 2010). Willard et al. (1977) observed that horses fed only concentrate devoted only 3% of their time to eating and exhibited more wood-chewing and searching behaviors. Similarly, horses fed a complete pelleted diet spent only 10% of their time consuming feed and also displayed more searching behaviors compared to horses offered orchardgrass hay *ad libitum*. The nutritional compositions of these diets were similar, although the orchardgrass hay provided more dietary fiber (Elia et al., 2010). Other researchers have noted increases in bedding consumption,

particularly wood shavings, when horses were presented with pelleted and low-fiber diets (Houpt et al., 1988; Boswinkel et al., 2007). Therefore, it appears that horses will modify their feeding behaviors in response to dietary fiber deficiencies and changes in the physical form of the diet, perhaps to satisfy an inherent motivation for foraging activities.

Dietary composition also affects mastication parameters, which subsequently affect digestion and could pose a threat to gastrointestinal health. In horses, chewing stimulates salivary secretions and reduces the particle size of the feed in order to optimize later digestion (Alexander, 1966). Dietary particle size influences the range of mandibular motion and chewing rate, thereby affecting dental wear and saliva production (Ellis and Hill, 2005; Bonin et al., 2007, Elia et al., 2010). Horses fed a pelleted diet exhibit less range in mandibular movement, shorter chewing cycles, and a greater chewing frequency when compared to hay-fed horses (Bonin et al., 2007, Elia et al., 2010). Elia et al. (2010) observed that hay-fed horses had a 4-fold increase in total chews per day over horses fed pellets. Faster consumption and reduced mastication of concentrate and pelleted meals results in decreased saliva secretion, thus reducing salivary buffering in the stomach (Reese and Andrews, 2009). Accordingly, decreased saliva production has been implicated as a causal factor for gastric ulcers in horses maintained on high-concentrate diets (Reese and Andrews, 2009). Additionally, altered mastication patterns have been implicated in abnormal dental wear patterns commonly seen in horses fed high-concentrate diets (Bonin et al., 2007; Elia et al., 2010); accordingly, these wear patterns are rarely observed in feral horses, zebras, and Przewalski horses that rely on roughage-based diets (Becker, 1962). Abnormal tooth wear and a lack of routine dental care have been correlated with increased risk for simple colonic obstruction and distention (SCOD) colic (Hillyer et al., 2002). Although the mechanisms have not been extensively studied, inadequate mastication that reduces feedstuff

digestibility and subsequently alters rate of passage may be, at least in part, to blame for the increased risk of colic (Archer and Proudman, 2006). Therefore, providing adequate amounts of long-stem forage in the diet and routine dental care will promote proper mastication, salivation, and dental wear, and may reduce the risk for gastrointestinal disease.

Intake Preferences

One theory regarding equine feeding behaviors suggests that horses regulate their feed intake in order to maintain energy balance (Laut et al., 1985). Although there have been contradictory results in research trials, this may be true for horses maintained on forage-only diets or for horses fed poor quality feedstuffs (Laut et al., 1985; Berger et al. 1999). Voluntary dry matter intake (VDMI) for mature horses grazing on fresh forages is approximately 2.0% of body weight (BW), ranging from 1.5 to 3.1% BW (NRC, 2007). Reported VDMI are 2.0 to 2.5% BW and 1.5 to 2.2 % BW for hays and ensiled forages, or haylages, respectively (NRC, 2007). In horses, plant quality and maturity, in addition to forage type and specie, affect dry matter intake (DMI; Goodwin et al., 2005a; NRC, 2007; Edouard et al., 2008; Fleurance et al., 2010). While improved digestibility and palatability of better quality forages are generally associated with greater VDMI (NRC, 2007), contrary reports suggest that some horses maintained on high-quality pastures may in fact reduce forage intake to maintain energy balance (Hoskin and Gee, 2004). Accordingly, horses have been shown to increase DMI as forage digestibility and quality decreased (Edouard et al., 2008). Individual changes in intake compensated for decreases in digestible energy (DE) and crude protein (CP) of the forages, allowing horses to fulfill their maintenance requirements (Edouard et al., 2008).

Seasonal variations in intake have also been noted, with horses devoting more time to grazing activities in spring and fall and less time during summer and winter (Kaseda, 1983;

Fleurance et al., 2010). These seasonal intake patterns have been attributed to changes in available plant species, differences in forage quality and DE content and environmental factors (NRC, 2007). Plant components are divided into structural (SC) and nonstructural carbohydrate (NSC) fractions. Structural carbohydrates include components of the plant cell wall, such as cellulose, hemicellulose, and lignin and determine the neutral detergent fiber (NDF) content of feedstuffs. Nonstructural carbohydrates include water-soluble carbohydrates (WSC) and starch. Simple sugars, such as glucose, fructose, and sucrose, as well as fructans comprise the WSC component (Dey and Harborne, 1997; Hoffman et al., 2001; NRC, 2007). Cool-season (C₃) grasses, such as orchardgrass (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), Kentucky bluegrass (*Poa pratensis*), perennial ryegrass (*Lolium perenne*), timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*), smooth brome grass (*Bromus inermis*), and Matua prairiegrass (*Bromus willdenowii*), store and metabolize fructans and simple sugars at different rates throughout their growing season (NRC, 2007; Kagan et al., 2011a). Seasonal and diurnal variations in WSC content have been well documented in C₃ grasses (Waite and Boyd, 1953a; Lechtenburg et al., 1972; Pollock and Jones, 1979; Fisher et al., 1999; Shewmaker et al., 2006). More recently, Kagan and colleagues (2011a) observed greater fructan and simple sugar concentrations in fresh and dried orchardgrass sampled in early spring compared to samples collected during the summer. Samples collected in the afternoon also had greater WSC concentrations compared to forage samples collected in the morning (Kagan et al., 2011a). Interestingly, dried forage samples contained 33 to 50% more fructan on a dry matter basis than fresh forage samples collected in early spring, presumably due to continued accumulation of the sugar post-harvest (Kagan et al., 2011a). As a result of increased WSC content, C₃ grasses are highly palatable and have greater DE values during the spring and fall; therefore increases in the

VDMI of these forages should be expected during these growth seasons (NRC, 2007). However, overconsumption of NSC, primarily fructans, is the primary causal factor in the etiology of pasture-associated laminitis (PAL; Watts, 2010). Intake of C₃ grasses should be regulated in the spring and fall, as well as in the late afternoon, when fructan concentrations are greatest. Additionally, fructan content should be monitored in conserved C₃ forages (Watts, 2010).

Large quantities of dietary starch and simple sugars have been implicated in the development of insulin resistance, fermentative acidosis, colic (Durham, 2009), and laminitis (Hoffman, 2003; Pollit and Visser, 2010). Warm-season (C₄) grasses, such as bermudagrass (*Cynodon dactylon*), bahiagrass (*Paspalum notatum*), dallisgrass (*Paspalum dilatatum*), big bluestem (*Andropogon gerardii*), Caucasian bluestem (*Bothriochloa ischaemum*), pearl millet (*Pennisetum glaucum*), and crabgrass (*Digitaria* spp.) do not accumulate fructans, but rather store carbohydrates in the form of simple sugars and starch (NRC, 2007; Kagan et al., 2011b). Similar to C₃ grasses, seasonal variations in WSC and starch concentrations have been noted in C₄ species (McKell et al., 1969; Wilson and Ford, 1973; Chatterton et al., 1989). A recent examination of seasonal and diurnal variations of bermudagrass showed an inverse relationship between WSC and starch concentrations as the C₄ growing season progressed. Sugar content was greater in immature plants, whereas starch preferentially accumulated later in the growing season (Kagan et al., 2011b). Additionally, starch concentrations were increased in fresh forages sampled in the afternoon compared to those collected in the morning, whereas sucrose concentrations were greatest in dried forages (Kagan et al., 2011b). Kagan et al. (2011b) demonstrated that C₄ grasses accumulate less NSC than C₃ grasses and, therefore, are a safer forage source as they are less likely to cause digestive disturbances. However, warm-season hays originating from late cuttings may be problematic for horses supplemented with grains or

concentrates due to increased starch accumulation at this time (Kagan et al., 2011b).

Consequently, horse owners should monitor the NSC content of their pastures and hays, soak their forages, and adjust equine rations early in the growing season to avoid digestive disturbances associated with excessive NSC in the diet.

Feed intake preferences of horses also are associated with feedstuff palatability.

Palatability, determined through orosensory inputs such as smell, taste, and texture, is influenced by plant species, maturity, and feed processing methods (NRC, 2007). Researchers have established a preference among horses for sweet flavors (Hawkes et al., 1985; Goodwin et al., 2005a). This partially explains increased forage intakes when forage NSC contents are greatest and may be a mechanism through which grazing horses maintain their energy balance. On the other hand, domesticated horses sometimes require supplementation of concentrates to meet their energy demands for optimal growth, body condition, reproductive performance, and work (NRC, 2007). When offered palatable, energy-dense concentrates, however, horses tend to eat in excess of their energy requirements (Southwood et al., 1993; Argo et al., 2002; Cairns et al., 2002). While this may simply be an effect of reduced gut fill, relying on dietary DE for feed intake regulation is not advisable, especially when horses are fed palatable diets with elevated NSC content.

Abnormal Behaviors, Stereotypies, and Vices

Horses require a minimum of 1% of their body weight (BW) in roughage per day to maintain gut health and mental well-being (Zeyner et al., 2004; NRC, 2007). These fibrous sources may come from grazing fresh pasture, preserved forages, such as hay and haylage, or forage alternatives, including hay cubes and grain by-products (Coverdale et al., 2004; Hill, 2007). High-quality forages and fiber alternatives often are sufficient to support horses at

maintenance without supplementation of concentrates. However, grain sources are routinely supplemented to optimize growth, reproduction, and performance. As grains or other concentrates are introduced into the diet, the amount of forage a horse consumes decreases proportionately, as horses are only able to consume 2.0 to 2.5 % BW in DM d⁻¹ on average (NRC, 2007). As a result, diets fed to production and performance horses often contain minimal roughage and greater amounts of soluble CHO-F_R. Horses fed low-fiber diets are at an increased risk for developing abnormal behaviors, stereotypies, and gastrointestinal disease (Hothersall and Casey, 2012).

Abnormal behaviors, such as coprophagia, geophagia, wood-chewing, and bedding consumption, are thought to be physiological responses to suboptimal environments and management practices (NRC, 2007; Hothersall and Casey, 2012). These behaviors are most often noted in animals consuming diets deficient in fiber, minerals, or protein (Hothersall and Casey, 2012). Coprophagia, or fecal consumption, is common in foals and is thought to aid in inoculating the gut with appropriate microbial populations (Ike et al., 1984; Crowell-Davies and Houpt, 1985; Egan et al. 2010). In adult animals, on the other hand, the incidence of coprophagia increases when hay rations are limited (Zeyner et al., 2004; Vervuert et al., 2013) or when low-fiber, high-concentrate diets are fed (Zeyner et al., 2004). However, when horses are fed 1.0 kg hay/100 kg BW d⁻¹, no abnormal behaviors are noted (Zeyner et al., 2004). Coprophagia also has been noted in feral horses in seasons of reduced forage availability (NRC, 2007). Coprophagia is a concern due to the potentiation of potentially harmful parasitic populations in the feces. Horses that ingest large parasitic loads due to coprophagia, therefore, may be at an increased risk for colic, as heavy parasitic loads are a known risk factor for colic (Hillyer et al., 2002).

Geophagia, or eating soil, is considered to be an attempt at rectifying mineral deficiencies in the diet, particularly iron and copper deficiencies, or is thought to be a sign of “boredom” (Ralston, 1986; McGreevy et al., 2001; NRC, 2007). Aytekin et al. (2011) found reduced serum concentrations of iron and copper in horses displaying geophagia and other abnormal behaviors. Additionally, the authors noted numerically reduced serum concentrations of phosphorus in these horses, which has been implicated as a factor in the etiology of similar behaviors in other species (Jain and Chopra, 1994; Ghergariu et al., 1994; Sahin et al., 2001). McGreevy et al. (2001) tested soil samples from areas sought by geophagic horses and found increased iron and copper concentrations compared to control soil samples. No differences in phosphorous concentrations were found, therefore the authors suggested that iron and copper deficiencies may be a driving force for geophagic behavior (McGreevy et al., 2001). Geophagia is often concerning to owners due to the increased risk of gastrointestinal disorders associated with soil consumption, such as sand colic (McGreevy et al., 2001). Iron and copper supplementation may benefit geophagic horses by reducing the incidence of geophagia and thereby reducing their risk for sand colic; however, this theory has not been tested.

Wood-chewing and bedding consumption are most commonly observed in horses fed low-fiber or high-concentrate diets. Wood-chewing behavior destroys property, leads to abnormal wear of the incisors, and could develop into cribbing, a stereotypic behavior (Hothersall and Casey, 2012). Willard et al. (1977) noted a greater incidence of wood-chewing and coprophagia in horses fed only a pelleted concentrate diet compared to those consuming an all-forage ration. Wood-chewing was positively correlated with increases in cecal propionate and lactate, as well as decreases in cecal acetate, when horses were fed the concentrate diet, potentially indicating a causal relationship (Willard et al., 1977). Similar shifts in cecal acetate,

propionate, and lactate are typical for concentrate-based diets (Hintz et al., 1971) and such shifts in the VFA profile have been noted with greater magnitude in horses with induced laminitis (Garner et al., 1977b). Adding sodium bicarbonate to the cecum reduces the incidence of wood-chewing and coprophagia, indicating that hindgut acidity and adverse shifts in fermentation parameters may prompt the expression of these behaviors (Willard et al., 1977; Johnson et al., 1998). In light of these findings, Johnson et al. (1998) tested the effects of non-therapeutic doses of virginiamycin, an oral antibiotic also known as Founderguard, on the behaviors of intensively managed horses fed a high-concentrate diet. The authors found an increase in wood-chewing, bedding consumption, and grasping behaviors when concentrate represented 50 to 75% of the total ration without the inclusion of virginiamycin (Johnson et al., 1998). Furthermore, there was a negative correlation between fecal pH and the incidence of the abnormal behaviors, supporting the theoretical relationship between hindgut acidity and behavior (Johnson et al., 1998). Virginiamycin supplementation ameliorated the effects of the high-concentrate diets by moderating fecal pH and was associated with fewer incidences of behavioral abnormalities (Johnson et al., 1998). However, recent concerns for antibiotic resistance have limited the use of direct-fed antimicrobials such as virginiamycin, particularly in the European Union (Menzies-Gow and Young, 2011).

Stereotypies, sometimes referred to as vices, are repetitive oral or locomotive behaviors that serve no apparent function (Johnson et al., 1998; NRC, 2007; Hothersall and Nicol, 2009; Hothersall and Casey, 2012). These behaviors, including cribbing, weaving, box-walking, pawing, and headshaking, have not been observed in feral horse populations. Some have interpreted this to mean that these behaviors are likely coping mechanisms or expressions of frustration due to unnatural environments and intensive management (Hothersall and Nicol,

2009; Hothersall and Casey, 2012; Fureix et al., 2013). However, research attempts to link stereotypical behavior to physiological indicators of stress (i.e. glucocorticoids) have been contradictory (Clegg et al. 2008; Fureix et al., 2013).

Cribbing, or crib-biting, is the most thoroughly studied stereotypical behavior in horses. When cribbing, horses grasp a fixed surface with their incisors, contract their neck muscles, draw air into their esophagus, and emit a characteristic grunt (McGreevy et al., 1995). In a longitudinal study, Waters et al. (2002) found that 74% of young horses that developed cribbing had previously demonstrated wood-chewing. If wood-chewing is indeed a manifestation of hindgut acidity due to inadequate fiber, then high-concentrate diets ultimately may lead to cribbing behaviors. Epidemiological studies have identified low-fiber, high-starch diets as risk factors for cribbing and noted that horses bedded in straw, potentially an alternative fiber source, were less likely to crib than those bedded on other materials (McGreevy et al., 1995; Redbo et al., 1998; Waters et al., 2002; Bachman et al., 2003; Christie et al., 2006). However, other researchers have demonstrated that cribbing horses maintained on pasture or provided *ad libitum* forage will still perform the behavior, indicating that a lack of dietary fiber is not the only motivation for cribbing (Garcia, 2004; O'Reilly, 2006). Alternatively, concentrates themselves and meal feeding regimens may contribute to an increase in the behavior, as cribbing incidents increase when grains are provided in the diet and are more noticeable around feeding time (Gillham et al., 1994; Brown et al., 2007). The practice of feeding meals, as opposed to *ad libitum* feeding, regardless of the composition of those meals, often results in abnormal dental wear and mastication parameters, reduced salivary secretions, increased gastrointestinal acidity, and altered feed digestibilities and passage rates (Bonin et al., 2007; Clegg et al., 2008; Elia et al., 2010; Vervuert et al., 2013). These shifts in the digestive paradigm subsequently affect microbial

fermentation in the large intestine and could increase an animal's risk for developing gastrointestinal disease (Malamed et al., 2010). Consequently, researchers suggest that cribbing may develop over time as either an attempt to increase salivation to prevent gastric ulceration, or it may be an expression of frustration or pain associated with abdominal discomfort (Nicol et al., 2002; Clegg et al., 2008; Moeller et al., 2008; McCall et al., 2012). In support of this theory, Nicol et al. (2002) found more inflammation and ulceration in the gastric mucosa of cribbing foals than in their normal counterparts. Furthermore, cribbing behaviors and gastric ulceration were reduced when horses were administered antacids (Nicol et al., 2002; Mills and Macleod, 2002). Upon further investigation, Moeller and colleagues (2008) determined that cribbing does indeed lead to increased salivary production. Saliva samples were collected from cribbing and non-cribbing horses onto sponges from the exit of the submaxillary gland. Cribbing horses initially produced less saliva than non-cribbers before the cribbing bout; however, total salivary volume was not different between groups. When cribbing was prevented, cribbers produced less saliva. Therefore, the authors concluded that cribbing might stimulate oral stretch receptors and allow horses to produce more saliva, potentially alleviating some of the discomfort resulting from gastric ulcers (Moeller et al., 2008). McCall et al. (2012) noted that cribbing horses fed concentrates *ad libitum* cribbed less often than control horses maintained on a restricted feeding regimen. The authors supposed that chewing more food led to greater saliva production for the cribbing horses, therefore reducing their need to crib. However, Houpt (2012) reported contradictory findings when she examined the effects of cribbing on parotid salivary secretions. In her study, cribbing did not result in increased salivary secretions and there was no correlation between the number of crib bites and the amount of saliva produced. Contrary to the conclusions of Moeller et al. (2008), Houpt (2012) proposed that cribbing may, instead, elicit the

development of gastric ulcers by stimulating the parasympathetic nervous system, which increases gastric acid secretion and slows gastrointestinal motility. Albanese et al. (2013) found that intra-abdominal pressure was greater during and after cribbing incidents when compared to both control (non-cribbing) horses and baseline levels in cribbing horses. Increased intra-abdominal pressure, coupled with greater gastric acidity in cribbing horses, could expose the non-glandular gastric mucosa to ulcer-inducing conditions; however, this theory needs further investigation.

Another explanation for the development of stereotypical behaviors, such as cribbing, relates to stress-induced central nervous system (CNS) dysfunction. Similar to what has been described in other species, equine stereotypies may arise from a genetic predisposition for upregulated, or over-sensitized, dopamine receptors in the basal ganglia of the brain (McBride and Hemmings, 2009). McBride et al. (2005) discovered that cribbing horses have greater D1 and D2 receptor distributions consistent with individuals in other species that show stress-induced stereotypies. Considering that the basal ganglia and this dopaminergic pathway are responsible for reward-motivated behavior, stimulation of this sensitized region due to chronic stress or endorphin release may activate and potentiate cribbing behaviors (McBride and Hemmings, 2009). Stress may be induced by meal feeding practices or suboptimal environmental conditions that prevent “reward” attainment; whereas reward attainment, such as ingesting palatable feedstuffs and exercise, results in the release of endorphins (McBride and Hemmings, 2009). Similarly, cribbing behaviors stimulate the release of endorphins and, as a result, may be potentiated over time in chronic stress situations (McBride and Hemmings, 2009). In a sense, horses may become “addicted” to the behavior in the same way a person may become addicted to exercise or psychostimulants (McBride and Hemmings, 2009). In research settings, these

behaviors are attenuated pharmacologically by administering dopamine antagonists and through the use of acupuncture. From a management perspective, eliminating restrictive feeding protocols and extending meal consumption times may have similar effects (McBride and Hemmings, 2009). Indeed, McCall and others (2012) noted reductions in the number of crib bites and the duration and number of cribbing bouts when horses were fed concentrates *ad libitum*. Due to the impracticality of such a feeding regimen, the authors suggested that further research is warranted to investigate the effects of *ad libitum* feeding of reduced-starch concentrates and palatable, high-quality forages on cribbing behaviors (McCall et al., 2012).

The Effects of Diet and Dietary Changes on Carbohydrate Digestion and Absorption in the Equine

Carbohydrates (CHO) in forages and grains are fundamental components of equine diets and serve as the primary source of dietary energy. In terms of plant physiology, carbohydrates are categorized as either SC, part of the cell wall, or NSC, cellular contents (Hoffman et al., 2001). In regards to equine nutrition, however, categorizing carbohydrates as hydrolyzable (CHO-H), readily fermentable (CHO-F_R), or slowly fermentable (CHO-F_S) provides a clearer illustration of how differing dietary carbohydrates are processed in the digestive tract (Hoffman et al., 2001). Unfortunately, accurate laboratory analyses that distinguish between these carbohydrate fractions are not yet commercially available (Geor, 2007). Still, researchers can use traditional methods, such as proximate analysis and gas chromatography, in conjunction with proposed regression equations, to estimate CHO-H, CHO-F_R, and CHO-F_S (Hoffman et al., 2001; Geor, 2007). Understanding the interplay of these dietary components with gastrointestinal physiology should allow for improved dietary formulation, minimize adverse alterations in feeding behaviors, and reduce the prevalence of gastrointestinal diseases.

Carbohydrate Digestion in the Equine Foregut

The Stomach

After swallowing, feed particles move through the esophagus and enter the stomach via the cardiac sphincter. The equine stomach, like that of other monogastric species, is a simple, C-shaped organ. It is divided into 2 distinct regions, the nonglandular mucosa and the glandular mucosa, by the margo plicatus. The stomach can be further subdivided into the cardiac, or esophageal, area and the fundus (together known as the saccus caecus) in the nonglandular region and the corpus and pylorus, or antrum, in the glandular region, although exact definitions and terminology may vary with source (Al Jassim and Andrews, 2009). After mixing with gastric secretions, chyme exits the stomach through the pyloric sphincter and enters the small intestine.

The nonglandular mucosa comprises the proximal third of the stomach, while the glandular mucosa accounts for the remaining two-thirds. The nonglandular portion is comprised of stratified squamous epithelium that lacks the ability to secrete mucus, leaving these cells unprotected from acidic assault and prone to ulceration. In fact, 80% of equine gastric ulcers occur in the nonglandular region of the stomach (Al Jassim and Andrews, 2009). Therefore, high-roughage diets that promote saliva production are essential for maintaining the integrity of the nonglandular mucosa, as saliva provides the only cellular protection against acidic insult in this region of the stomach (Al Jassim and Andrews, 2009). The glandular mucosa, on the other hand, is lined with secretory columnar epithelial cells that differentiate into surface mucous cells, chief cells, parietal cells, or various endocrine cells (Merritt, 1999). Surface mucous cells line the exposed gastric epithelium and extend into gastric pits where they are termed mucous neck cells. These cells secrete mucins and bicarbonate, creating a protective pH gradient in the outer glycocalyx layer that promotes normal cellular function. Therefore, only 20% of equine gastric

ulcers are found in the glandular mucosa despite the increased acidity of the region (Al Jassim and Andrews, 2009). Chief cells and parietal cells line the gastric glands within the gastric pits and produce pepsinogen, a zymogen protease, and hydrochloric acid (HCl). The equine stomach continuously secretes HCl, an evolutionary adaptation to continuous grazing behaviors (Nadeau et al., 2000). Intensive management scenarios that employ restricted feeding protocols create unnatural feeding and fasting states. These feeding protocols limit the buffering effects of ingested feed and decrease salivary production, which create large swings in gastric pH that can have deleterious effects on the integrity of the gastric mucosa (Husted et al., 2009). Instead, *ad libitum* feeding of roughage is recommended to protect gastric health as it promotes a more stable gastric pH and leads to nearly continuous salivary production (Husted et al., 2009).

The stomach functions primarily as a temporary storage site for ingested feedstuffs, and it regulates the flow of chyme into the small intestine. Digesta generally moves through the stomach within 2 to 6 h following ingestion. However, under natural feeding situations, the equine stomach is rarely empty due to continuous grazing behavior (Medina et al., 2002; Van Weyenberg et al., 2006). Gastric motility, largely controlled by the parasympathetic nervous system, is dictated by migrating myoelectric complexes (MMC; Merritt, 1999). Gastric motility is often referred to as gastroduodenal motility, as the stomach and duodenum work in concert with each other to progress digesta through the GIT (Merritt, 1999). In the fasted state, the stomach is partially contracted (Merritt, 1999). Swallowing, esophageal distention, and gastric distention provide the neural stimulus for relaxing the fundus and corpus to accommodate gastric fill. These stimuli also initiate postprandial contractions in the antrum that propel chyme into the small intestine and mix ingesta with gastric secretions. These antral contractions occur at a rate

of 3 to 5 per min and are regulated by feedback mechanisms in the small and large intestines (Merrit, 1999).

Dietary bulk (meal size), the extent of feed processing, and nutritional composition impact the time required for digesta to flow through the GIT, a process commonly referred to as rate of passage (ROP; Van Weyenberg et al., 2006). Mean retention time (MRT), measured through the use of solid and liquid phase indigestible rare earth markers, is used to estimate the ROP of digesta through each compartment of the GIT (Drogoul et al. 2000). Reports of dietary effects on gastric ROP are limited, with total prececal and hindgut parameters being more fully defined. Early reports of gastric emptying that utilized radiographic scintigraphy reported the amount of time required for half of the digesta to leave the stomach (T_{50}) was 29 min and 68 min, respectively, for liquid and solid phase digesta (Sojka and Cantwell, 1988). More recently, preferential retention of liquid and solid phase digesta was demonstrated to be more prominent in the hindgut (Drogoul et al., 2000). However, due to increased starch solubility resulting from grain processing, faster transit of digesta via the liquid phase through the stomach and small intestine could challenge hindgut function in horses fed large concentrate rations (Julliand et al., 2006). Metayer et al. (2004) determined that meal size and starch content both affect gastric emptying rates in horses. Gastric emptying was faster in horses fed a small meal of low-starch concentrate (93 ± 7.6 min) compared to those fed a small meal of high-starch concentrate (143 ± 29.2 min; Metayer et al., 2004). The authors concluded that fiber content and caloric content may affect T_{50} , although further research is needed to isolate the main effects of each. Additionally, gastric emptying was faster in horses fed small meals of high-starch concentrate when compared to those fed large meals of high-starch concentrate (265 ± 36.3 min; Metayer et al., 2004). However, when comparing the rates at which feed (g min^{-1}) and DE (Kcal min^{-1}) passed through

the stomach, gastric emptying was greater for horses fed the large meal of high-starch concentrate than it was for horses fed the small meal (Metayer et al., 2004). Although the larger meal emptied at a slower rate in terms of the percentage of the original meal, the quantity of starch-rich ingesta flowing to the small intestine was much greater than with smaller meals (Metayer et al., 2004). Increased flow of starch could exceed small intestinal capacity for digestion and absorption, increase the quantity of starch reaching the hindgut and, thus, compromise hindgut function by predisposing horses to fermentative acidosis. Therefore, horses that require high-concentrate diets for optimal performance and production should receive their rations in several small meals throughout the day to minimize digestive disturbances.

Due to its relatively short MRT, there is limited gastric mixing and very little CHO digestion in the stomach. This, coupled with continuous secretion of HCl, creates a pH gradient throughout the lumen. First described in the horse by Murray and Grodinsky (1989), gastric pH ranges from approximately 5.46 to 4.00 in the nonglandular mucosa and gradually becomes more acidic (3.09 to 1.85) in the pyloric region of the glandular mucosa. More recent reports have noted similar values and large variations in gastric pH (ranging from 7.0 to 1.0), due, in part, to feeding state, dietary composition, and collection methods used (Nadeau et al., 2000; de Fombelle et al., 2003; Al Jassim, 2006; Varloud et al., 2007; Husted et al., 2009; McCall et al., 2012). The acidic environment of the distal stomach limits microbial colonization and, therefore, provides antimicrobial protection against potentially pathogenic bacteria that may be present in chyme. The low pH in this region also allows for some acid hydrolysis of CHO, although the extent is largely unknown (Hoffman, 2003). Methods of feed processing and MRT are probably determinants of the degree of acid hydrolysis of dietary CHO that occurs in the equine stomach. Blood glucose responses to various diets may indirectly implicate the impact of gastric CHO acid

hydrolysis on small intestinal digestion; however, these relationships have not been examined. Gastric microbial fermentation of feedstuffs may be more biologically important and, therefore, has received more recent investigation (Al Jassim and Andrews, 2009).

Due to its relatively moderate pH, the nonglandular mucosa supports microbial colonization in the saccus caecus (Al Jassim and Andrews, 2009). Recently, the contribution of gastric microbial fermentation to feedstuff digestibility and its role in gastric ulcer development has been examined. Researchers have begun to identify specific microbial populations that are important to gastric fermentation, characterize their end products, and elucidate the effects of various diets on gastric microbial metabolism. The extent of gastric CHO fermentation by resident microbial populations could be significant as it relates to prececal digestion, although it is currently considered to be nominal when compared to the amount of CHO fermentation that occurs in the hindgut (de Fombelle et al., 2003; Hoffman, 2003; Varloud et al., 2004). Even so, VFA concentrations in the nonglandular mucosa associated with microbial fermentation of readily available CHO-H and CHO-F_R in high-concentrate rations has been implicated in the etiology of gastric ulcers (Reese and Andrews, 2009). The role of microbial fermentation in ulcer development may be exacerbated by other factors, such as concurrent reductions in salivary secretions that often accompany high-concentrate and low-roughage diets and the presence of bile acids and pepsin (Andrews et al., 2005). Nevertheless, increases in VFA and lactate concentrations resulting from starch fermentation, coupled with continuous HCl secretion, further acidify the gastric environment and compromise the integrity of the nonglandular mucosa (Nadeau et al., 2003ab).

Recent studies utilizing culture methods have isolated substantial microbial populations from equine gastric contents (de Fombelle et al., 2003; Varloud et al., 2007). De Fombelle et al.

(2003) reported total anaerobic, cellulolytic, lactobacilli, streptococci, and lactate-utilizing gastric bacterial concentrations to be 9.1, 1.4, 7.8, 7.3, and 6.8 log₁₀ colony forming units (c.f.u.) ml⁻¹, respectively, for horses fed a high-fiber pelleted diet. In the same study, gastric contents collected from horses fed a high-starch pelleted diet yielded microbial cultures of 9.0, 1.0, 8.4, 7.5, and 7.6 log₁₀ c.f.u. ml⁻¹ for the same populations, respectively. The authors concluded that increased concentrations of lactobacilli, streptococci, and lactate-utilizing bacteria in horses fed the high-starch diet indicated a greater propensity for starch degradation. Additionally, total VFA concentrations were nearly 2-fold greater in horses fed the high-starch diet, presumably resulting from the activity of these bacterial populations. Conversely, the modest concentrations of cellulolytic bacteria noted in both groups of horses imply a limited capacity for cellulose degradation in the stomach (de Fombelle et al., 2003). In horses fed the high-starch diet, the greater concentration of lactate-utilizing bacteria, coupled with decreased lactate and increased VFA concentrations, indicate that lactate likely was further metabolized into propionate (de Fombelle et al., 2003). Others have reported that bacterial concentrations cultured from the gastric contents of horses fed a pelleted concentrate and meadow hay are lesser than those reported by de Fombelle et al. (2003), but are still indicative of significant gastric microbial fermentation (Varloud et al., 2007). Biochemical parameters, such as total lactate and VFA concentrations, were more consistent with those reported by de Fombelle et al. (2003) as similar high-fiber diets were used in both studies (de Fombelle et al., 2003; Varloud et al., 2007).

Culture-independent techniques have been utilized to identify specific microbial strains in the stomach associated with starch fermentation. Al Jassim et al. (2005) identified several lactate-producing bacterial strains using polymerase chain reaction (PCR) and 16S rDNA sequencing techniques, including *Lactobacillus mucosae*, *L. delbrueckii*, and *L. salivarius*, from

horses fed an *ad libitum* forage diet. Perkins et al. (2012) employed 16S rDNA bacterial tag-encoded pyrosequencing (bTEFAP) and fluorescence *in situ* hybridization (FISH) technologies to characterize microbial profiles of the nonglandular, glandular, and antral gastric regions of the equine stomach, as well as ulcerated mucosa. Although the phylogenetic analysis provided no statistical correlation between microbial strain and gastric region, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* were most abundant in the gastric mucosa. *Lactobacilli* and *Streptococci* were observed from all gastric regions using FISH, with *Streptococci* being more prevalent in the glandular mucosa. Furthermore, the authors reported significant individual variation and strong environmental influences on gastric microbial populations (Perkins et al., 2012). These methods are useful in providing more specific characterization of gastric microbial populations, as many newly identified strains are uncultivable. At this time, little is known about the impact of these species on digestive function in the horse or their implications for gastric health.

Acid hydrolysis and microbial fermentation both contribute to starch digestion in the stomach. However, due to limited absorptive mechanisms, the energetic value of the products of gastric digestion are largely unknown and, therefore, not accounted for in nutrient requirement or digestibility equations (Argenzio et al., 1974). Furthermore, the fate of gastric fermentation products has yet to be elucidated. There is some evidence from *in vitro* experiments that supports the idea that there is limited VFA absorption in the glandular mucosa (Argenzio et al., 1974); however, similar measurements have not been quantified *in vivo*.

The Small Intestine

As with monogastric species, the small intestine (SI) is the primary site of enzymatic digestion and nutrient absorption in the horse. Beginning at the pyloric sphincter and terminating at the ileocecal junction, the small intestine is divided into 3 distinct regions: the duodenum,

jejunum, and ileum. In total, the small intestine is approximately 22 m (72 ft) in length (Al Jassim and Andrews, 2009). Crypts, villi, and microvilli increase the epithelial surface area of the small intestine and maximize its secretory and absorptive capacity (Gray, 1992).

Gastrointestinal accessory organs in the horse epitomize evolutionary adaptations that accommodate feeding behaviors. Presumably because horses evolved as continuous grazers, they have a relatively large basal flow of pancreatic secretions, producing approximately 30 to 40 L of pancreatic fluid per day (Merrit, 1999), which results in diluted enzymatic concentrations. As a result, “meal feeding” practices may, in fact, overwhelm the small intestine’s capacity for carbohydrate digestion.

Digestive secretions are primarily released in the duodenum, the first segment of the small intestine. Acidic chyme flowing from the stomach stimulates a moderate increase in pancreatic and biliary secretions that enter the duodenum through various ducts. Secretory crypts are also more concentrated in the duodenum. Undifferentiated crypt epithelial cells secrete water and ions into the duodenal lumen presumably to dilute the chyme, promote mixing of chyme with digestive secretions, and facilitate nutrient absorption. As a result, duodenal pH typically ranges from 5.6 to 6.6 (de Fombelle et al., 2003; Al Jassim, 2006). As digesta moves through the small intestine, pH becomes more basic as a result of additional pancreatic and intestinal bicarbonate secretions. Therefore, pH ranges from 6.5 to 7.2 in the jejunum and 7.2 to 7.4 in the ileum (de Fombelle et al., 2003; Al Jassim, 2006).

Villi, small fingerlike projections on the intestinal mucosa, are covered with absorptive epithelial cells known as enterocytes. Microvilli, small projections on the surface of enterocytes, constitute the brush-border membrane, which is the focal point for nutrient absorption. Villi and microvilli, although present throughout the small intestine, are longest and most concentrated in

the distal duodenum and proximal jejunum. These structures adapt to nutrient availability by altering length and density to optimize nutrient absorption (Shirazi-Beechey, 2008). As digesta moves through the duodenum and jejunum and into the ileum, digestive secretions are reduced and there is a shift toward net water, ion, and nutrient absorption. If digestion and absorption are optimized, then digesta entering the large intestine should primarily consist of CHO-F_S. However, if small intestinal function is impaired or overwhelmed, significant quantities of CHO-H and CHO-F_R may escape enzymatic digestion and enter the hindgut.

Most sugar and starch digestion normally occurs in the small intestine, as there is little salivary amylase activity in horses and gastric contributions to carbohydrate digestion are limited (Julliard et al., 2006). Only monosaccharides are absorbed across the intestinal lumen, so starch and other hydrolyzable polysaccharides must be reduced to glucose, fructose, and galactose for absorption to occur. Starch digestion in the small intestine is initiated by pancreatic α -amylase, the saccharidase present in pancreatic secretions (Gray, 1992). Pancreatic α -amylase has a pH optimum of 6.9 and functions primarily in the duodenum. This enzyme acts on starch residues (amylose and amylopectin) that are 5 or more glucose molecules in length. By cleaving the α -1,4 linkages between glucose molecules, pancreatic α -amylase reduces starch into maltose (2 glucose molecules), maltotriose (3 glucose molecules), and branched α -limit dextrins (4 to 6 glucose molecules containing an α -1,6 linkage; Gray, 1992). These oligosaccharides are further hydrolyzed into monosaccharides by the brush-border glycanases maltase-glucoamylase and sucrase- α -dextrinase. These enzymes, produced by enterocytes, embed in the glycocalyx layer of the microvilli where they interact with luminal contents (Gray, 1992). Monosaccharides are then transported across the apical membrane of the enterocyte via Na⁺/glucose co-transporter isoform 1 (SGLT1) and glucose transporter 5 (GLUT5; Gray, 1992; Dyer et al., 2002). Monosaccharides

are also transported outside of the enterocyte via Na⁺-independent facilitated diffusion (GLUT2; Shirazi-Beechey, 2008).

In spite of this efficient digestive process, equine evolutionary adaptations limit the rate of starch degradation in the small intestine, predisposing horses that consume high-starch rations to gastrointestinal dysfunction and disease (Richards et al., 2004). Due to large pancreatic fluid output and the resulting dilution of digestive enzymes, the horse has limited activity of α -amylase in the small intestine (Richards et al., 2004). Indeed, starch digestion, as measured by glycemic responses, improves when exogenous α -amylase is added to equine diets alone and in combination with amyloglucosidase (maltase-glucoamylase; Richards et al., 2004). Thus, it appears that limited quantities of endogenous α -amylase may indeed limit starch digestion in the equine small intestine (Richards et al., 2004). Furthermore, an adaptive effect on glycemic response has been noted, suggesting that exogenous α -amylase supplementation may be ineffective long-term (Richards et al., 2004). On the other hand, Dyer et al. (2002) found significant disaccharidase activity within the brush-border membrane of horses maintained on pasture, with sucrase activity greatest in the proximal duodenum and jejunum. Maltase activity was noted in all regions of the small intestine and was much greater in horses compared to values reported for other species (Roberts, 1974; Kienzle and Radicke, 1993; Dyer et al., 2002). More recent work by Dyer et al. (2009) characterized the adaptive capacity of disaccharidases and SGLT1 to concentrate-supplemented diets. Disaccharidase activity in horses supplemented (long-term) with concentrate was similar to that of horses maintained on pasture; however, glucose uptake was enhanced in the jejunum and ileum of concentrate-fed horses (Dyer et al., 2009). The authors hypothesized that this response resulted from an increase in the number of SGLT1 transporters and indeed, upon further analysis, a 2-fold and 5-fold increase in SGLT1

protein abundance was observed in jejunal and ileal tissue samples, respectively, obtained from horses fed concentrates (Dyer et al., 2009). To assess the adaptive capacity of SGLT1, intestinal tissue samples were collected from horses acclimated to an all-hay ration, switched to a 60:40 hay:concentrate ration, and then again switched to a 40:60 hay:concentrate ration. After a 1-wk adaptation period to the 60:40 ration, ileal SGLT1 expression increased 2-fold; a similar response was seen in duodenal expression after 1 mo of adaptation to the diet (Dyer et al., 2009). Changing the diet to the 40:60 ration elicited an increase only in ileal expression of SGLT1 (Dyer et al., 2009). Cumulatively, the results from this study demonstrated the adaptive ability of the small intestine to increase glucose uptake by increasing SGLT1 expression, particularly in the distal regions of the SI, in response to increasing concentrates in the diet (Dyer et al., 2009). A recent report from the same laboratory indicated that SGLT1 upregulation is facilitated by the sweet taste receptor, T1T2-T1T3 (Daly et al., 2012). From an evolutionary perspective, horses may retain ample disaccharidase activity and the ability to increase glucose uptake in order to adapt to seasonal variations in soluble sugar concentrations noted in many grass species (Kienzle and Radicke, 1993; Dyer et al., 2002; Richards et al., 2004; Kagan et al., 2011ab). Conversely, as starch is a minor component of natural equine diets, moderate pancreatic α -amylase activity may have matched dietary demands. Feed processing methods may be the most practical combatant to overcome this physiological limitation by enhancing starch availability. The effects of processing on starch digestion have been well documented, although some reports are contradictory (McLean et al., 1999abcd; de Fombelle et al., 2001; Varloud et al., 2004; Al Jassim, 2006; Julliand et al., 2006). The caveat to improving starch digestion and sugar absorption in the small intestine is that it may lead to increased circulating concentrations of glucose, which, over time, could lead to insulin resistance (Harbour et al., 2003).

Modern feed processing technologies commonly utilized in generating equine diets include grinding, micronizing, flaking, and pelleting, although grains may also undergo other mechanical and thermal processing methods (Julliand et al., 2006; Hill, 2007). In addition to enhancing starch availability, feed processing also alters small intestinal MRT, which in turn affects the extent of enzymatic digestion and nutrient absorption that occurs. Gastrointestinal motility is dictated by the MMC, meaning it undergoes 4 distinct contractile patterns in an effort to mix luminal contents and propel digesta through the gut (Merritt, 1999). The MMC is characterized by initial inactivity (Phase 1) followed by a period of irregular segmentary and peristaltic contractions (Phase 2) and then an intense activity front characterized by stronger and more frequent peristaltic contractions (Phase 3). Finally, rapid contractions subside and the cycle starts over (Phase 4; Merritt, 1999). As a result, small intestinal transit time is roughly 1 to 2 h in horses fasted or fed *ad libitum* forage, with digesta moving at 30 cm min⁻¹ in the duodenum and decreasing in speed in the distal regions of the foregut (Merritt, 1999). Total prececal ROP may range from 1.6 to 9.9 h (mean 6.8 ± 1.2 h), depending on dietary and feed management factors (Julliand et al., 2006; Van Weyenberg et al., 2006). Recent reviews have highlighted these effects as they relate to feed processing, prececal MRT, and subsequent digestibilities of various feedstuffs (Van Weyenberg et al., 2006; Julliand et al., 2006; Hill, 2007). In general, particle size reduction (grinding), decreasing the hay:concentrate ratio of a diet, and restricted feeding practices increase prececal MRT and slow ROP, enhancing dietary digestibility (Medina et al., 2002; Julliand et al., 2006; Van Weyenberg et al., 2006). On the other hand, increasing the amount of fiber in the ration, reducing fiber length, and incorporating alfalfa into the diet decreases prececal MRT (Drogoul et al., 2000; Moore-Colyer et al., 2003; Julliand et al., 2006; Van Weyenberg et al., 2006). Furthermore, liquid phase digesta passes through the small

intestine faster than solid phase digesta (Julliand et al., 2006). As a result of this phenomenon, large amounts of soluble CHO present in the diet may be rapidly transported to the large intestine, where they may alter the microbial profile, reduce CHO-F_S digestibility, and impair hindgut function (Varloud et al., 2004; Julliand et al., 2006). Al Jassim (2006) examined total tract apparent digestibilities of processed sorghum and whole oat grains and assessed the effects of dry rolled or steam flaked sorghum on the characteristics of digesta. Overall, processing led to improved DM digestibility, and starch digestibility was increased with the diets containing dry-rolled and steam-flaked sorghum compared to the unprocessed oats. No differences were observed in pH or VFA and lactate concentrations of digesta between horses fed the dry-rolled and steam-flaked sorghum, indicating both processing methods had similar effects on prececal sorghum grain digestibility (Al Jassim, 2006). Van Weyenberg et al. (2006) reported no effect of feeding frequency of a complete pelleted meal on dietary digestibility or blood glucose parameters, as had been previously suggested (Houpt et al., 1988). Generally speaking, reports regarding feed processing and the effects of other dietary components on prececal MRT are conflicting and not well defined (Julliand et al., 2006; Van Weyenberg et al., 2006). Because of the rapid passage rate of digesta through the equine small intestine, coupled with limited α -amylase activity, significant quantities of starch may flow into the hindgut when high-starch diets are fed.

Carbohydrate Digestion in the Equine Hindgut

Collectively referred to as the hindgut, the cecum and large colon are the primary sites of microbial fermentation in the equine GIT. Resident microbial populations in these segments of the large intestine degrade dietary starch (CHO-H); oligosaccharides, fructans, and pectins

(CHO-F_R); and cellulose and hemicellulose (CHO-F_S) into monosaccharides that are used for cellular metabolism and proliferation (Hoffman et al., 2001). Within microbial cells, these monosaccharides undergo glycolysis, known as the Embden-Meyerhof-Parnas pathway, where they are converted to pyruvate to produce adenosine triphosphate (ATP; Hobson and Stewart, 1997; Shirazi-Beechey, 2008). During this process, nicotinamide adenine dinucleotide (NAD⁺), an oxidizing agent, is reduced to NADH. Fermentation pathways re-oxidize NADH and convert pyruvate into VFA and lactate, releasing carbon dioxide (CO₂), hydrogen (H₂), and methane (CH₄) as by-products (Hobson and Stewart, 1997; Shirazi-Beechey, 2008). Volatile fatty acids and lactate are absorbed across the apical membrane of colonocytes, epithelial cells lining the large intestine, through several mechanisms. First, many VFA are protonated in the intestinal lumen, rendering them highly lipid soluble. Thus, diffusion is a significant absorptive mechanism. There also are 2 carrier-mediated transporters that link VFA absorption to NaCl and H₂O absorption and bicarbonate (HCO₃⁻) secretion, as has been reported in other species (Shirazi-Beechey, 2008). The primary transporter along the basolateral membrane of colonocytes is monocarboxylate transporter-1 (MCT-1). This transporter couples the removal of one ionized VFA molecule and one hydrogen atom (H⁺) from the cell, thereby preventing cellular acidification (Shirazi-Beechey, 2008). Relative absorption and transportation rates for individual VFA vary with luminal concentrations and pH; however, acetate and propionate are preferentially transported to the bloodstream, whereas butyrate is largely retained by colonocytes for intracellular metabolism (Argenzio et al., 1974). In the body, peripheral tissues utilize acetate for energy production, the liver takes up propionate for gluconeogenesis, and butyrate is converted into ketone bodies, precursors for fatty acid synthesis (NRC, 2007).

The VFA concentrations in the lumen are reflective of the net production (gross production minus absorption) of VFA by resident microbial populations. Thus, measurements of luminal VFA concentrations provide insight into dietary effects on the microbial environment of the hindgut. Specifically, the relative proportions of acetate, propionate, and butyrate, the predominant VFA produced, currently provide the most understanding (Hobson and Stewart, 1997). Additionally, culture-dependent and culture-independent techniques have been used to further characterize the microbial diversity within the differing regions of the hindgut. Various bacterial, protozoal, and fungal strains have been identified; however, it is believed that bacteria are predominantly responsible for carbohydrate fermentation in equines (Moore and Dehority, 1993). Moore and Dehority (1993) found that defaunation, or removal of protozoa from the hindgut, does not appear to impact cellulose digestion and only nominally reduces DM digestibility in horses fed a high-forage ration. In horses fed a 60:40 hay:grain ration, DM digestibility was slightly improved by defaunation (Moore and Dehority, 1993). Thus, it appears that protozoa play only a minor role in total carbohydrate digestion in the equine, although they may be more important in starch digestion and in regulating bacterial populations (Moore and Dehority, 1993; Hobson and Stewart, 1997). Generally speaking, carbohydrate-digesting bacteria can be classified as cellulolytic, amylolytic, or lactate-utilizing (Hobson and Stewart, 1997). The primary bacterial phyla represented in the equine hindgut include *Firmicutes*, *Proteobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Actinobacteria*, and *Fibrobacter* (Daly et al., 2001; Daly et al., 2012; Shepard et al., 2012).

Within *Firmicutes*, the most abundant bacterial phyla identified in the hindgut, several classes and species are of particular concern when discussing carbohydrate digestion in the horse, specifically cellulolytic, lactate-producing, and lactate-utilizing bacterial populations. The

predominant cellulolytic species belong to the *Clostridia* class and include several from the Ruminococcaceae family and some from the Clostridiaceae and Eubacteriaceae families. More specifically, *Ruminococcus flavefaciens* has been identified as the primary cellulolytic bacterial species in the cecum using FISH technology (Julliand et al., 1999). To a lesser extent, *R. albus* and *Fibrobacter succinogenes* are also present (Julliand et al., 1999). Other known cellulolytic digesters present in the equine hindgut include *Bacteroides* sp., *Bacillus cellulolase dissolvens*, *Clostridium* sp., *Eubacterium* sp., and *Butyrivibrio fibrisolvens* (Julliand et al., 1999).

Additionally, the actions of cellulolytic fungi may play an important role in cellulose digestion; however, research is limited in horses (Julliand et al., 1998). A separate class of *Firmicutes*, known as *Bacilli*, includes bacteria from *Lactobacillus* sp. and *Streptococcus* sp. These bacteria rapidly ferment starch and other dietary sugars to lactate (Hobson and Stewart, 1997). Bailey et al. (2003) determined that certain species, including *S. bovis*, *L. mucosae*, *L. reuteri*, *L. salivarius*, *L. delbrueckii*, and *L. fermentum*, are capable of converting amino acids into amines, potentially contributing to the biological cascade of laminitis. Thus, these populations are often monitored when evaluating the effects of various diets on the hindgut ecosystem (de Fombelle et al., 2003; Al Jassim et al., 2005; Daly et al., 2012). In contrast, several Gram-negative bacteria from the Veillonellaceae family, including *Megasphaera* sp. and *Selenomonas* sp., convert lactate to butyrate and other intermediate VFA (Hobson and Stewart, 1997). Often collectively termed “lactate-utilizing” bacteria, the growth of these populations often follows rapid proliferation of the aforementioned lactate-producing bacteria. Monitoring lactate-utilizing bacterial populations provides an indicator for an animal’s tolerance for abrupt increases in lactate production within the hindgut.

The Cecum

As digesta leaves the small intestine, it flows through the ileocecal junction into the cecum, an enlarged, specialized compartment of the large intestine that precedes the large colon. The cecum is a blind fermentation reservoir that is approximately 1 m long and has a capacity of roughly 25 to 35 L (Al Jassim and Andrews, 2009). Often compared to the bovine rumen, the cecum is a primary site of microbial fermentation in the equine GIT. Volatile fatty acids absorbed from the cecum alone can provide up to 30% of the energy requirements for horses maintained on all-forage rations (Glinsky et al., 1976).

Secretion of bicarbonate, coupled with VFA absorption and the addition of basic digesta from the small intestine, buffers the cecum and promotes the proliferation of a diverse microbial community. Cecal pH generally ranges from 6.5 to 7.2, with an approximate mean of 6.8; however, cecal pH can decrease to 6.1 with large inclusions of grain in the diet. When horses are maintained on high-fiber diets, total cultivable anaerobic bacteria range from 7.6 to 7.9 log₁₀ c.f.u ml⁻¹ (Medina et al., 2002; de Fombelle et al., 2003). Respective population concentrations for cellulolytic bacteria, *Lactobacillus* sp., *Streptococcus* sp., and lactate-utilizing bacteria are 6.0 to 6.1, 6.2 to 6.4, 6.2 to 6.6, and 6.1 to 7.0 log₁₀ c.f.u ml⁻¹ (Medina et al., 2002; de Fombelle et al., 2003). In horses acclimated to high-starch diets, total anaerobic bacterial concentrations increased to range from 7.7 to 8.6 log₁₀ c.f.u ml⁻¹ (Medina et al., 2002; de Fombelle et al., 2003). This response mainly reflects an increase in *Lactobacillus* sp. and *Streptococcus* sp. populations, as cellulolytic bacteria decrease to 5.0 to 5.2 log₁₀ c.f.u ml⁻¹ (Medina et al., 2002; de Fombelle et al., 2003). The population shifts reported in lactate-utilizing bacteria are varied, depending on sampling protocols (Medina et al., 2002; de Fombelle et al., 2003). Other changes in the forage:concentrate ratio have produced pH and microbial population shifts similar to those reported by Medina et al. (2002) and de Fombelle et al. (2003; Kern et al., 1973; Kern et al.,

1974; Julliand et al., 2001). Changing forage type and physical form, as well as the inclusion of high-fiber alternatives, seems to elicit little variation in cecal parameters (Drogoul et al., 2000; McLean et al., 2000; Moore-Colyer et al., 2000; Coverdale et al., 2004). Collectively, researchers indicate that when horses are maintained on high-fiber diets, relative VFA proportions of acetate, propionate, and butyrate range from 70 to 75, 15 to 20, and 10 to 15 molar percent, respectively. Additionally, lactate concentrations are negligible, typically measuring less than 1 mM (McLean et al., 2000; Moore-Colyer et al., 2000; Julliand et al., 2001; de Fombelle et al., 2003; Al Jassim et al., 2006; Muller et al., 2008; Daly et al., 2012). On the other hand, high-starch diets result in a shift in VFA proportions towards increased propionate and butyrate, which leads to increased luminal lactate concentrations. Greater lactate concentrations, usually correlated with increases in *Lactobacillus* sp. and *Streptococcus* sp., have been reported when rolled barley was included in the diet at 30 and 50% of the total ration or when high-starch diets were fed, presumably resulting from inadequate starch digestion in the small intestine that allowed significant quantities of starch to reach the cecum when fed at these levels (Julliand et al., 2001; Medina et al., 2002). More extensive processing of grains reduces lactate production, possibly as a result of improved small intestinal digestion (McLean et al., 2000; de Fombelle et al., 2003; Al Jassim, 2006). Additionally, feed processing may result in more appropriate VFA production (de Fombelle et al., 2003). However, the extent of this stabilizing effect was limited when processed barley was included at 50% of the total ration, highlighting the limited capacity of the small intestine for starch digestion (Moore-Colyer et al., 2000). Unfortunately, many researchers fail to report the effects of dietary parameters on some of these variables, often excluding lactate and microbial concentrations and sometimes omitting pH and VFA measurements, which limits the scope of comparison between existing reports.

The effects of abrupt dietary changes on the equine hindgut have been studied to an even smaller degree with fewer test subjects. A study conducted by Goodson et al. (1988) was one of the earliest reports on the effects of abrupt dietary changes on the cecal environment. Using a cecally cannulated pony, Goodson et al. (1988) examined the effects of an abrupt change from an alfalfa hay diet to a ground corn-soybean meal concentrate, which was followed by a subsequent abrupt change back to alfalfa hay after 6 wk of acclimation to the concentrate diet. During the concentrate challenge, cecal pH sharply decreased within 5 to 7 h post-feeding and gradually increased to initial values by 24 h. This pH shift was accompanied by considerable alterations in the resident microflora. While the pony was maintained on the alfalfa hay diet, total anaerobic bacterial concentrations were relatively stable with postprandial counts being greater than those prior to feeding. However, 48 h after the abrupt change to the concentrate ration there was an 8-fold increase in total anaerobes. Starch-utilizing bacteria represented 92.2 and 87.5% of the total anaerobes at 24 h and 48 h after this dietary challenge, respectively, compared to 73.1% when the pony was fed the hay diet. Additionally, although slower to respond, lactate-utilizing bacteria increased to represent 69.2% of the total bacteria 7 d after the dietary change. Total anaerobic bacterial counts returned to initial values after 3 d and remained relatively consistent for the remainder of the concentrate phase. Data regarding VFA and lactate concentrations were not reported (Goodson et al., 1988). De Fombelle et al. (2001) examined the effects of abrupt changes in the forage:grain ratio on the ceca and right ventral colons of 3 male ponies. Abruptly changing the diet from 100% meadow hay to a 70:30 meadow hay:rolled barley ration resulted in numerical increases in *Streptococcus* sp. and *Lactobacillus* sp. after 29 h. This microbial shift was accompanied by a moderate increase in propionate concentration (19.81 ± 4.36 to 24.12 ± 5.05 molar %) and a drastic increase in lactate concentration (35.87 ± 13.87 to 305.14 ± 288.67

mg L⁻¹). Unremarkable changes were detected in total anaerobic, cellulolytic, and lactate-utilizing bacterial populations. Insignificant changes in postprandial pH and VFA parameters support this observation. Similar alterations in microbial populations, lactate concentrations, and pH were noted when ponies were switched from a 100% meadow hay to a 50:50 meadow hay:rolled barley ration. Total VFA were increased at 29 h, reflecting a marked increase in propionate in spite of a concurrent decrease in acetate (de Fombelle et al., 2001). Additional research in the same laboratory evaluated the effects of a sudden change in the type of concentrate on the hindgut environment (Respondek et al., 2008), a feeding mistake implicated in the development of colic (Hudson et al., 2001). Cecal pH was unaffected despite increased total lactate concentrations (< 2 mM) 5 h post-feeding. Concentrations of VFA were depressed after 5 h compared to preprandial values, but returned to initial values by 29 h (Respondek et al., 2008). Throughout these studies, small animal numbers resulted in large standard errors and limited the ability of researchers to detect differences in the results.

Cecal motility consists of segmentary haustral contractions and peristaltic mass movements that mix and propel digesta through the cecocolic junction into the colon (Van Weyenberg et al., 2006; Al Jassim and Andrews, 2009). These coordinated actions result in selective retention of larger particles, presumably to promote further microbial fermentation and to optimize fiber digestion. This process, in conjunction with the motility patterns of the large colon, is known as the “Colonic Separation Mechanism” (CSM; Drogoul et al., 2000). On average, cecal MRT is 5 h, ranging from 2 to 7 h and varying with diet (Hyslop et al., 1999; Drogoul et al., 2000). Feeding ground, pelleted forage homogenizes the passage rates of digesta and, as a result, inhibits the CSM’s ability to selectively retain materials in the cecum and ultimately reduces cecal MRT (Drogoul et al., 2000). However, presumably in an effort to

compensate for rapid cecal transit rates, colonic MRT is increased with this type of diet (Drogoul et al., 2000). Thus, no total tract digestibility differences have been observed between chopped and pelleted forages (Drogoul et al., 2000). Drogoul et al. (2001) reported that as barley was added to the diet, total tract MRT increased and fiber digestibility decreased. Additionally, liquid phase markers showed a rapid throughput of digesta through the GIT, suggesting that substantial quantities of starch may have reached the hindgut as the amount of barley increased (Drogoul et al., 2000; Drogoul et al., 2001). While the effects of abrupt dietary changes on cecal motility or digestive passage rates have not been reported, it stands to reason that an abrupt dietary change, particularly to a high-starch diet, which slows the rate of passage and promotes the aforementioned microbial and biochemical responses, could compromise the integrity of the colonic epithelium or prevent the excretion of fermentation gases (Shirazi-Beechey, 2008; White, 2011). Because the cecum is reported to preferentially retain large particulate matter, the effects of abrupt long-stem forage changes may more significantly impact this environment, whereas the impacts of concentrate processing may be more evident in the colon.

The Colon

The large colon is a segmented organ that is divided into 4 compartments in the horse (Al Jassim and Andrews, 2009). As digesta leaves the cecum via the cecocolic junction, it enters the right ventral colon (RVC), and then moves through the sternal flexure into the left ventral colon (LVC). From here, the intestinal contents progress through the pelvic flexure into the left dorsal colon (LDC) and, finally, the digesta flows into the right dorsal colon (RDC) via the diaphragmatic flexure (Al Jassim and Andrews, 2009). The proximal, or ventral, colon is the primary site of microbial fermentation, whereas the distal, or dorsal, colon is largely responsible for VFA, electrolyte, and water absorption. On an all-forage ration, colonic VFA satisfy up to

50% of a horse's energy requirements for maintenance (Glinsky et al., 1976; Vermorel et al., 1997a). As digesta leaves the RDC, it enters the small colon where fecal balls are formed. The rectum acts as a storage site for fecal material prior to excretion (Al Jassim and Andrews, 2009). As a less invasive alternative to cecal and colonic cannulation, feces are often sampled and used as an indicator of hindgut function. However, Dougal et al. (2012) compared the microbial parameters of the cecum, RDC, and feces using 16s rRNA gene terminal restriction fragment length polymorphism and determined that the relative environments of the hindgut differ significantly by region. Although fecal samples provide little information regarding the environment of the proximal hindgut, microbial similarities between the RDC and the feces are such that fecal samples could be used as reasonable indicators of fermentation parameters in the distal regions of the hindgut (Dougal et al., 2012).

Existing reports of dietary impact on the colonic environment primarily focus on the RVC. The pH of the proximal colon is similar to that of the cecum, allowing it to support a similar microbial community (de Fombelle et al., 2003; Sadet-Bourgeteau et al., 2010). However, total microbial populations are greater in the RVC than in the cecum, suggesting that more extensive carbohydrate digestion occurs in this region (de Fombelle et al., 2003). In one study, horses fed a high-fiber pellet had populations of total anaerobic, cellulolytic, *Lactobacillus* sp., *Streptococcus* sp., and lactate-utilizing bacteria of 8.1, 6.0, 6.5, 7.1, and 5.8 \log_{10} c.f.u ml^{-1} , respectively, in the RVC. After acclimating horses to a high-starch pellet, total anaerobic, *Lactobacillus* sp., *Streptococcus* sp., and lactate utilizing bacteria concentrations in this region increased, whereas the concentrations of cellulolytic bacteria decreased. These microbial shifts were accompanied by decreased acetate and increased propionate, butyrate, and lactate concentrations. Although no change in pH was observed following the dietary shift (de

Fombelle et al., 2003), an earlier study using similar diets resulted in a reduction in pH in the RVC when horses were maintained on a high-starch pellet (Medina et al., 2002). Microbial and other biochemical responses were similar between these studies (Medina et al., 2002; de Fombelle et al., 2003) and are consistent with the findings of Julliand et al. (2001) for combined cecal and colonic parameters when rolled barley was incorporated into the diet.

Forage type and physical form seem to have little impact on the fermentative capacity of the RVC. Drogoul et al. (2000) noted a slight decline in VFA concentrations when horses were fed a finely ground hay pellet compared to chopped hay. However, total tract diet digestibility was unaffected, presumably due to a prolonged MRT in the cecum. Muller et al. (2008) also noted unremarkable changes in colonic parameters when horses were fed silage and haylage compared to hay, although there were slight depressions in VFA concentrations with the ensiled forages. Using oligonucleotide hybridization, Daly et al. (2012) observed depressed *Fibrobacter* sp. and Ruminococcaceae populations in the colonic contents of concentrate-fed horses compared to those maintained on forage alone. This change was accompanied by increases in Lachnospiraceae, *Bacteroidetes*, and *Bacillus-Lactobacillus-Streptococcus* populations (Daly et al., 2012). In the same study, similar effects were noted in the microbial communities of horses diagnosed with SCOD colic, emphasizing the potential role of nutrition in the development of gastrointestinal disease (Daly et al., 2012).

The effects of abrupt dietary changes on the colonic environment have been examined, largely in conjunction with cecal observations. Although not significant, de Fombelle et al. (2001) observed numerical increases in *Lactobacillus* sp. and *Streptococcus* sp. with concomitant decreases in lactate-utilizing bacterial populations in the RVC when horses' diets were abruptly changed from 100% meadow hay to a 70:30 meadow hay:rolled barley ration. These microbial

shifts were more exaggerated in the RVC than in the cecum. Abruptly increasing the rolled barley in the ration to 50% resulted in further increases in *Lactobacillus* sp. and *Streptococcus* sp. concentrations that were accompanied by increases in lactate, total VFA, and propionate concentrations in the colonic fluid 29 h after the dietary change (de Fombelle et al., 2001). Respondek et al. (2008) reported increases in total anaerobic, lactate-utilizing, *Lactobacillus* sp., and *Streptococcus* sp. colonic bacterial concentrations when barley was abruptly substituted for the pelleted concentrate meal to which horses were acclimated. A simultaneous decrease in colonic pH and increase in total lactate concentrations and VFA concentrations were also noted (Respondek et al., 2008). Using forages originating from the same crop, Muhonen et al. (2009) reported no differences in microbial or biochemical parameters in colonic digesta when horses were switched to haylage or silage rations from hay. Therefore, different forage conservation methods seem to have little effect on colonic fermentation (Muhonen et al., 2009).

The colonic flexures regulate digestive passage rates under the CSM process to maximize fermentation and absorption (Al Jassim and Andrews, 2009). Colonic MRT is a function of the propulsive and retropulsive actions of haustral contractions, as well as the inhibitory effects of the flexural anatomy (Van Weyenberg et al., 2006). Aside from regulation of ROP, the abrupt changes in luminal diameter at these sites, particularly at the pelvic flexure, also predispose the horse to colonic impactions that may cause colic (Shirazi-Beechey, 2008). Feeding large quantities of poor quality hay may promote this cascade. Colonic MRT has been estimated between 25 to 45 h depending on diet and the calculations used (Drogoul et al., 2000; Van Weyenberg et al., 2006). Feeding a ground hay pellet significantly increases total and colonic MRT when compared to chopped hay, although no differences have been observed for dietary digestibility (Drogoul et al., 2000). Although feed processing seems to have little impact on total

tract MRT, it may selectively affect the ROP of digesta through varying compartments of the GIT and, therefore, impact the amount of soluble carbohydrate that reaches the colon (Drogoul et al., 2000; Rosenfeld and Austbo, 2009). Indeed, a combination of enhanced colonic MRT and increased CHO-H rich digesta reaching the hindgut as a result of consuming high-starch diets may explain the observed relationship between feeding concentrates, altered colonic microbial communities, and the prevalence of colic, specifically SCOD (Daly et al., 2012).

In recent reviews, Shirazi-Beechey (2008) and White (2011) discussed proposed dietary cascades that compromise hindgut function and the mechanisms by which these cascades lead to intestinal diseases, such as colic and laminitis. These authors proposed that dietary factors alter gut microbial characteristics and cause fermentative dysfunction, as evidenced by changes in pH, VFA, and lactate (Shirazi-Beechey, 2008; White, 2011). Specifically, increases in lactic acid-producing bacteria and concomitant decreases in cellulolytic populations lead to altered VFA parameters and decreased intestinal pH (Shirazi-Beechey, 2008). These changes compromise the integrity of colonocytes, reducing the efficacy of their cellular and mucosal barrier function, thus resulting in cellular acidification and potential leaking of endotoxins into systemic circulation (Shirazi-Beechey, 2008; White, 2011). These cellular changes also affect water, electrolyte, and nutrient absorption, which alter the consistency of luminal digesta, potentially increasing the risk of impaction (Lopes et al., 2004). Furthermore, cellular injury initiates inflammatory responses that may be responsible for abdominal pain associated with colic (White, 2011). The dietary factors implicated in the development of gastrointestinal diseases need to be further evaluated to determine whether they indeed elicit the deleterious intestinal and histochemical responses currently proposed by researchers (Shirazi-Beechey, 2008; White, 2001). Existing literature regarding abrupt dietary changes in the equine largely uses pony models, but Dougal et al.

(2012) noted significant differences between hindgut characteristics of the horse and pony, suggesting that ponies may not be valid models for the effects of dietary changes on the general horse population. Therefore, despite reports of sudden dietary alterations and their effects on the hindgut of ponies, much of this work needs to be reevaluated on a larger scale in horses.

Summary

Although dietary effects on feeding behaviors, digestive parameters, and gastrointestinal disease have traditionally been evaluated individually, epidemiological and empirical evidence suggests that researchers may need to consider a more holistic approach when evaluating the effects of various diets and dietary changes on equine health and production. Dietary composition and feeding management heavily influence feeding behaviors, which in turn impact feedstuff digestibility in the small intestine and may ultimately affect hindgut function. High-fiber diets and *ad libitum* feeding practices seem to promote normal feeding behaviors and gastrointestinal health; however, these feeding regimens are not always practical, specifically for intensely managed horses. Aside from the dietary implications, horses subjected to these intensive management strategies also are exposed to situations that depart from the natural equine environment and have been implicated as causal factors related to the development of abnormal behaviors and gastrointestinal disease. These include changes to the animal's housing, turnout and exercise duration, and healthcare regimen. Because the equine digestive tract is predisposed to dietary-induced dysfunction under modern management schemes, further investigation of these relationships is warranted.

Effects of various types of equine diets on microbial populations and fermentation characteristics in the hindgut have been well documented. Yet, very little information is available on the effects of abrupt dietary changes on the equine hindgut. Moreover, experimental diets do

not necessarily reflect diets traditionally fed in the United States or the dietary changes horses are likely to face. Available reports have used small sample populations ($n \leq 4$), often with ponies, that may not accurately represent the effects of abrupt dietary changes on the general horse population. In induced-laminitis studies, horses are dosed with large quantities of either starch or oligofructose to simulate carbohydrate-overload scenarios (Milinovich et al., 2006). While these studies shed light on the pathophysiology of laminitis, their extremity limits researchers' ability to extrapolate results to more moderate dietary challenges. The mechanisms behind the increased colic risk associated with moderate dietary changes need further elucidation at both the molecular and *in vivo* levels. Current research in this area is limited and biologically significant parameters are ambiguous, as most research subjects never develop clinical signs of disease; however, epidemiological evidence suggests that the relationship between the equine diet and gastrointestinal disease are significant. Available information on the effects of various diets and dietary changes on the hindgut, together with the known alterations in prececal digestion, create a more complete picture of the relationship between diet and gastrointestinal health. As such, the effects of relevant dietary changes need to be evaluated on a larger scale. Understanding these basic implications for gastrointestinal health will allow for improved feeding and management strategies to attenuate adverse effects generated by unavoidable sudden changes in the equine diet.

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Chapter 2 - The Effects of Abrupt Dietary Changes on the Hindgut Environment of the Horse

Abstract

Abrupt dietary changes are associated with increased risk for gastrointestinal diseases in the horse, particularly colic and laminitis. However, few studies report the effects of abrupt dietary changes on the equine hindgut and published reports often do not reflect the types of dietary changes that domestic horses are likely to encounter. This study evaluated the effects of 4 abrupt dietary changes on the hindgut of the horse. Arranged in a longitudinal design, the experiments examined the effects of an abrupt change from a baseline hay:concentrate ration to a complete pelleted diet, an abrupt change from a baseline ration to a 100% grass hay ration, an abrupt change from a grass hay diet to an alfalfa hay diet, and an abrupt presentation of a large, concentrate meal. For each experiment, 9 cecally cannulated horses were acclimated to the baseline diets for at least 21 d. Beginning on d 14 of each acclimation period, daily preprandial (-1 h) cecal and fecal samples were collected in order to establish baseline fermentation parameters. Final baseline cecal and fecal samples were obtained immediately prior to each dietary change. Cecal and fecal samples were collected at 1, 5, 24, 36, 48, 60, and 72 h following the dietary changes in Experiments 1, 2, and 3. A 21-d washout period began at the conclusion of each collection period and served as the acclimation period for the next experiment. Due to the potential for severe gastric disturbances during Experiment 4, recovery hay meals were provided 12 and 24 h after feeding the large concentrate meal. This abbreviated the cecal and fecal collection period to 36 h. In Experiments 3 and 4, lactate-utilizing bacteria were cultured and the resulting growth curves were used to assess changes in microbial populations. In Experiment 1,

mean cecal pH decreased from 6.87 preprandially to 6.01 five h after the dietary change ($P < 0.0001$). At 24 and 36 h, mean cecal pH was similar to baseline values ($P > 0.05$). However, mean cecal pH decreased again at 48, 60, and 72 h to 6.01, 5.63, and 5.83, respectively ($P < 0.0001$). Mean cecal lactate was increased above baseline values at 48 and 60 h ($P < 0.0001$) following the initial dietary challenge. Mean cecal lactate concentrations exceeded 1 mM in several horses from 5 to 60 h, potentially indicating an increased risk for digestive upset. Fecal pH mirrored cecal trends, although there were no changes in fecal lactate concentrations ($P > 0.05$). However, total VFA in feces were increased 3-fold over baseline values from 36 to 72 h after the dietary change. The dietary change in Experiment 2 elicited few detectable responses in cecal and fecal parameters. Cecal pH decreased ($P < 0.001$) 5 h after the abrupt dietary change and diurnal decreases in cecal pH were noted at postprandial collections obtained 12 h following the morning meal (36 and 60 h; $P < 0.001$). These pH changes were associated with increases in cecal concentrations of acetate and butyrate ($P < 0.05$). Diurnal variation was also noted in fecal pH ($P < 0.05$); however, the effect was less prominent. There were no observed changes in cecal or fecal lactate concentrations ($P > 0.05$). Cecal pH was reduced from baseline values from 36 to 72 h after the dietary change in Experiment 3 ($P < 0.05$). These changes were accompanied by increases in cecal concentrations of lactate ($P < 0.0001$) and VFA ($P < 0.05$). In response to the dietary change in Experiment 3, lactate-utilizing bacteria experienced 1-log increases in inoculation optical density (OD) readings at 24 and 48 h and 4-log increases at 36 and 60 h. Fecal parameters were largely unaffected, although fecal pH increased over time ($P < 0.05$). In Experiment 4, cecal pH was reduced 5 h after the concentrate meal ($P < 0.0025$) and was accompanied by concomitant increases in concentrations of lactate ($P \leq 0.001$) and propionate ($P \leq 0.0006$). In response to the concentrate meal, culturable lactate-utilizing bacteria demonstrated

a 2-log increase in inoculation OD readings at 24 h and returned to baseline values by 36 h. Again, fecal parameters were largely unaffected, although fecal pH decreased over time ($P < 0.05$). Overall, the dietary challenges presented in this series of experiments more closely resemble dietary challenges domestic horses are likely to encounter than those abrupt dietary changes reported previously. Changes in the cecal and fecal parameters reported herein may indicate an increased risk for gastrointestinal illness following these types of abrupt dietary changes. Changes in the amount and type of concentrate, a change in hay type and quality, and abnormal feeding incidents are all investigated in this report. Understanding the implications of this study as they relate to gastrointestinal health should allow for improved feeding and management strategies to prevent and attenuate the adverse effects generated by unavoidable sudden changes in the equine diet.

Key words: cecum, colic, equine, dietary change, lactate-utilizing bacteria

Introduction

Horses have evolved as continuous grazers that thrive on forage-based diets. However, many domesticated horses are confined in stalls with limited pasture access and are provided concentrate-supplemented diets. An increased prevalence of gastrointestinal disease in these horses is likely a consequence of this intensive management (Durham, 2009). In fact, epidemiological studies have reported 5 to 10 colic cases per 100 horses annually, making colic one of the most prevalent diseases within equine populations (Tinker et al., 1997; Traub-Dargatz et al., 2001). Risk assessments have implicated recent dietary changes, including changes in the type or amount of concentrate, changes in hay type or quality, alterations in feeding frequency,

and abnormal feeding incidents in the etiology of colic and other gastrointestinal diseases (Tinker et al., 1997; Cohen et al., 1999; Hudson et al., 2001; Hillyer et al., 2002).

Abrupt dietary changes are of concern due to their impact on the hindgut environment. While the microbial ecosystem of the hindgut will adjust to gradual dietary changes, suddenly changing from forage to a diet containing concentrate, increasing the amount of concentrate in a ration, and changing the concentrate type all lead to increased numbers of total anaerobic and starch-utilizing bacteria, decreased cellulolytic bacteria, and altered lactate-utilizing bacterial populations in the cecum and colon (Garner et al., 1978; Goodson et al., 1988; de Fombelle et al., 2001; Respondek et al., 2008). Decreases in pH and increases in total lactate and propionate concentrations usually accompany these microbial shifts, often within 5 h following the abrupt dietary change (Garner et al., 1978; Goodson et al., 1988; de Fombelle et al., 2001; Respondek et al., 2008). These disruptions in the microbial ecosystem can lead to gastrointestinal and systemic disorders.

Aside from these general trends, little is known about the effects of abrupt dietary changes on the hindgut environment. Furthermore, previous reports do not necessarily reflect the types of diets traditionally fed in the United States or the dietary changes domestic horses are likely to face. Typical scenarios where equines may be exposed to an abrupt dietary change include recovery from colic surgery, a lack of feedstuff availability, and feeding mistakes. Interestingly, horses recovering from colic surgery often encounter an intentional abrupt dietary change when reintroduced to feed. In an effort to prevent further complications and to quickly restore proper gut function, horses are often provided highly digestible feeds or forages during their initial recovery that may not resemble their pre-colic diet. Horses are typically fed small amounts of alfalfa, hand-grazed, or introduced to a complete pelleted ration following a choke or

colic episode. To date, there are no published reports available that have examined the impact of this type of abrupt dietary change on the hindgut environment of the horse.

A lack of feedstuff availability may force owners to suddenly change their horses' rations. Abruptly changing the hay:grain ratio, type of hay, or hay quality have all been implicated as risk factors for colic (Durham, 2009). Yet, no researchers have reported the effects of abruptly increasing the amount of forage provided in the ration, although increasing the fibrous bulk of the diet increases gastrointestinal rate of passage (ROP) and could be linked to an increased risk for intestinal impactions (Van Weyenberg et al., 2006).

Changes in the amount and type of fiber will affect forage digestibility and its gastrointestinal ROP (Van Weyenberg et al., 2006). Additionally, forages will vary in their hydrolyzable carbohydrate (CHO-H) and crude protein (CP) content, which may affect hindgut fermentation parameters (Kagan et al., 2011ab). Muhonen et al. (2009) examined the effects of abrupt changes from hay to silage or haylage on the colon. Although no significant effects were observed, silage and haylage are not popular forages in the U.S. and, thus, the application of these results is limited. Currently, no researchers have examined the effects of abruptly changing from a grass hay diet to an alfalfa hay diet on the hindgut environment.

The effects of carbohydrate challenges on the hindgut environment have been most thoroughly investigated in studies where laminitis is induced with the administration of corn starch, oligofructose, or inulin (Garner et al., 1978; Krueger et al., 1986; Bailey et al., 2003; Milinovich et al., 2006). Goodson et al. (1988) and de Fombelle et al. (2001) evaluated the effects of the abrupt incorporation of novel grain mixtures into the diets of horses previously acclimated to hay alone. Respondek et al. (2008) challenged horses with a novel grain to mimic a feeding mistake and simulate an abrupt change in grain type; however, there are few controlled

studies that describe the effects of abruptly increasing the amount of grain to which horses have previously been acclimated, such as a feeding incident where a horse accidentally ingests more feed than it normally receives.

Thus, the objective of this study was to assess the effects of abrupt dietary challenges on the equine hindgut by evaluating scenarios that domestic horses are likely to encounter. Specifically, experiments included an abrupt change in dietary form and composition, an increase in the amount of dietary fiber, a change in the type and quality of hay, and an increase in the amount of dietary concentrate.

Materials and Methods

Horses

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University. Nine 7-yr-old Quarter Horses (4 mares, 5 geldings) with an initial mean BW of 530 ± 15.4 kg were used in the study. Horses were fitted with cecal cannulae (flexible rumen cannula, #7C; 3.8 cm center diameter and 8.9 cm wall thickness; Bar Diamond, Parma, ID; Beard et al., 2011) 3 years prior to experimentation. Horses were housed individually in 3.05 m x 3.66 m stalls bedded with pine shavings and received 3 to 4 h of daily turnout onto a dry lot. Water was provided *ad libitum*.

Experimental Design and Collection Schedule

This study was arranged into a longitudinal design consisting of 4 sequential experiments. The experimental protocols are summarized in Figures 2.1 and 2.2.

Experiment 1

The effects of an abrupt dietary change from a baseline diet (BL) to a complete pelleted diet (CD) on the hindgut environment were examined in Experiment 1. During a 21-d acclimation period, horses were fed a BL ration consisting of 1.5% BW native prairie grass hay (DM basis) and 0.5% BW textured feed (DM basis; TF; Omolene 200, Purina Mills, LLC, Gray Summit, MO). All TF was fed in the morning (0700 h) and hay rations were divided into 2 feedings per day (0700 and 1900 h). Hay refusals were weighed prior to the morning and afternoon feedings and daily feed intakes were recorded. No TF refusals were observed. Body weights were measured weekly (between 1300 and 1400 h) and diets adjusted accordingly. Sampling of cecal and fecal material began on d 14 to provide baseline information for each horse prior to the dietary change. Cecal and fecal material was collected from each horse 1 h (-1 h) prior to feeding (0 h) for 8 d in order to establish average fermentation parameters for each horse while consuming BL. Throughout all experiments, the data obtained from biological samples collected at -1 h in the days preceding the dietary changes were utilized to establish baseline values for each parameter. Daily sampling was suspended on d 21 of experiment 1 due to the malfunction of laboratory equipment. The acclimation period was extended and horses were maintained on BL until the equipment was replaced. Cecal and fecal collections resumed on d 28 with a ninth collection that allowed for comparisons with previous baseline samples. In total, horses received BL for 28 d. On d 29, cecal and fecal samples were again collected -1 h relative to the morning feeding. At 0700 h (0 h), horses were fed 2% BW of a complete pelleted feed product (CD; Equine Senior, Purina Mills, LLC, Gray Summit, MO). Cecal and fecal samples were collected 1, 5, 24, 36, 48, 60, and 72 h following the initial feeding. Horses were fed CD again at 24 and 48 h, immediately following the respective collections of cecal and fecal material. Feed refusals were collected, weighed, and recorded prior to each feeding. Vital signs

and behaviors were monitored hourly in all horses for signs of colic or laminitis for 24 h following the initial dietary challenge. A 21-d washout period began after the sampling of cecal and fecal material at 72 h during which horses were gradually re-acclimated to and maintained on BL. Concentrate meals were offered in increasing increments of 0.45 kg d⁻¹ until the target amount, as determined by BW, was achieved. Hay was offered at 1.5% BW each day of the washout period.

Experiment 2

The washout period following Experiment 1 also served as the acclimation period for Experiment 2, during which the effects of an abrupt change from BL to a 100% grass hay diet on the hindgut environment were examined. Similarly to Experiment 1, horses were acclimated to BL, consisting of 1.5% BW native prairie grass hay (DM basis) and 0.5% BW TF (Omolene 200, Purina Mills, LLC, Gray Summit, MO) for 21 d. Baseline data collection began on d 14. Cecal and fecal samples were collected 1 h (-1 h) prior to feeding (0 h) for 8 d. Unfortunately, sampling procedures were again suspended on d 18 due to malfunction of laboratory equipment. The acclimation period was extended and horses were maintained on BL until the equipment was replaced. Collections of cecal and fecal material resumed on d 22. The collections for d 4 and 5 were repeated and daily sampling procedures continued until a total of 10 baseline collections had been obtained. In total, horses received BL for 26 d. On the morning of d 27, cecal and fecal samples were again collected -1 h relative to the morning meal. At 0700 h (0 h), horses were fed 2.5% BW native prairie grass hay (GH; DM basis). Cecal and fecal samples were collected 1, 5, 24, 36, 48, 60, and 72 h relative to the initial feeding. Hay rations (2.5% BW) were fed again at 24 and 48 h. Feed refusals were collected, weighed and recorded prior to each feeding. Body weights were measured weekly (between 1300 and 1400 h) and diets adjusted accordingly. Vital

signs and behaviors were monitored hourly in all horses for signs of colic or laminitis for 24 h following the initial dietary change. Following the collection of cecal and fecal material at 72 h, a 21-d washout period began during which horses were maintained on a 2.5% BW GH ration.

Experiment 3

The washout period following Experiment 2 served as the acclimation period for Experiment 3. In this experiment, horses were subjected to an abrupt change from GH to alfalfa hay (*Medicago sativa*; AH). Grass hay rations (2.5% BW; DM basis) were fed once daily (0700 h) throughout the acclimation period and hay refusals were weighed prior to the morning feeding, at which time daily feed intakes were recorded. Body weights were measured weekly (between 1300 and 1400 h) and diets adjusted accordingly. Baseline data collection began on d 14. Cecal and fecal samples were collected 1 h (-1 h) prior to feeding for 8 d. On the morning of d 22, cecal and fecal samples were again collected -1 h relative to the morning feeding. At 0700 h (0 h), horses were fed 2.0% BW AH (DM basis). Cecal and fecal samples were collected 1, 5, 24, 36, 48, 60, and 72 h relative to the initial feeding of AH. Alfalfa hay rations were fed again at 24 and 48 h relative to the initial feeding. Feed refusals were collected, weighed, and recorded prior to each feeding. Vital signs and behaviors were monitored hourly in all horses for signs of colic or laminitis for 24 h following the initial dietary change. Again, following the 72 h collection of cecal fluid and feces, a 21-d washout period began during which horses were gradually re-acclimated to and maintained on BL. Concentrate meals (TF) were offered in increasing increments of 0.45 kg d⁻¹ until the target amount, as determined by BW, was achieved. Grass hay was offered at 1.5% BW on each day of the washout period.

Experiment 4

The washout period for Experiment 3 also served as the acclimation period for Experiment 4. As in Experiments 1 and 2, all TF was fed in the morning (0700 h) and hay rations were divided into 2 feedings d⁻¹ (0700 and 1900 h). Body weights were measured weekly (between 1300 and 1400 h) and diets adjusted accordingly. Baseline data collection began on d 14. Cecal and fecal samples were collected 1 h (-1 h) prior to the morning feeding for 8 d. On the morning of d 22, cecal and fecal samples were again collected -1 h relative to feeding of the morning meal. At 0700 h (0 h), horses were fed 1% BW TF (Omolene 200, Purina Mills, LLC, Gray Summit, MO). Fed at this level, TF supplied horses with approximately 3.8 g starch kg BW, doubling the 1.9 g starch kg⁻¹ BW that horses were acclimated to in BL. Potter et al. (1992) reported that the equine small intestine has the capacity to digest up to 4.0 g starch kg⁻¹ BW. However, de Fombelle et al. (2001) elicited changes in cecal fermentation parameters by presenting ponies with 2.3 g starch kg⁻¹ BW. Here, the experimental diet was designed to mimic a potential feeding mistake that would impact the hindgut environment, but remain within the upper tolerance limit proposed by Potter et al. (1992). Cecal and fecal samples were collected 1, 5, 24, and 36 h following the concentrate meal. Native prairie grass hay was offered at 12 (0.5% BW; DM basis) and 24 h (1.0% BW; DM basis), but no additional concentrate was offered following the meal at 0 h. Following the collection of cecal and fecal material at 36 h, horses were placed on a recovery diet of 1.5% BW native prairie grass hay for 2.5 d. Feed refusals were collected, weighed, and recorded prior to each feeding. Vital signs and behaviors were monitored hourly in all horses for signs of colic or laminitis for 24 h following the initial dietary challenge.

Sampling Protocols

Hay samples were collected prior to starting the trial and frozen (-20°C) for subsequent proximate analysis. A subsample was immediately analyzed for DM to allow dietary calculations

on a DM basis. Purina Animal Nutrition provided estimates of DM in the TF and CD for dietary calculations. Feed samples were obtained periodically throughout the collection period and were frozen (-20°C) for later proximate analysis.

Cecal samples (approximately 250 mL) were collected via gravity flow into 500-mL containers (Specimen Storage Containers, #14955117A, Fisher Scientific, Pittsburg, PA). Immediately following collection, 0.1 mL subsamples of cecal fluid were allocated in duplicate into prepared Hungate culture tubes filled with 10 mL of a semi-defined lactate (SDL) medium, the composition of which is described in Table 2.1. This medium was prepared in 1 L batches, autoclaved, and flushed with nitrogen (N₂) gas. Prior to aliquoting, the Hungate culture tubes were flushed with N₂ and capped with rubber stoppers to create an anaerobic environment. A 1.0% indigo carmine solution was incorporated into the medium as an anaerobic indicator; in the presence of oxygen, the medium would turn green, in which case affected tubes were discarded prior to inoculation. Culture tubes filled from the same batch of medium were used in each sample collection for all horses. Cecal fluid was analyzed for pH using a portable pH meter (Accumet Portable pH Meter AP62, Fisher Scientific, Pittsburg, PA) immediately following collection. For each timepoint in which cecal material was collected in Experiment 1, four 1-mL subsamples of raw cecal fluid were transferred to microcentrifuge tubes and deproteinized with 250 µl of 6N perchloric acid. Unfortunately, the acid in these samples was not adequately neutralized to allow for VFA analyses. Thus, the deproteinizing agent was subsequently changed to meta-phosphoric acid for remaining experiments. Specifically, four 1-mL subsamples of raw cecal fluid from each horse were transferred to microcentrifuge tubes and deproteinized with 250 µl of a 25% (wt/vol) meta-phosphoric acid solution during each collection timepoint. These samples were frozen (-20°C) for later determination of VFA and total lactate content.

Immediately following each collection of cecal fluid, fecal samples were obtained per rectum. Fecal material (approximately 250 to 400 g) was collected into 500-mL containers (Specimen Storage Containers, #14955117A, Fisher Scientific, Pittsburg, PA). A 100-g subsample was immediately frozen (-20°C) for later analysis of fecal dry matter. For pH determination, approximately 30 g of feces were mixed with 30 mL of deionized water. The pH probe was submerged in the mixture until the pH reading stabilized (Accumet Portable pH Meter AP62, Fisher Scientific, Pittsburg, PA). The mixture was then strained through 4 layers of cheesecloth. Four 1-mL subsamples of the supernatant were transferred to microcentrifuge tubes and deproteinated with 250 µL of a 25% (wt/vol) meta-phosphoric acid solution. These samples were frozen (-20°C) for later determination of VFA and total lactate concentrations.

Microbial Analyses

Following inoculation of cecal fluid into SDL medium, culture tubes were maintained at 38°C until arrival at the laboratory, where initial absorbance readings, or optical densities (OD), were determined using a spectrophotometer set at a wavelength of 600 nm. Culture tubes were then incubated at 38°C for 36 h. Twelve h following inoculation, culture tubes were vortexed and OD were recorded. This process was repeated every 4 h for an additional 24 h. These data were used to construct growth curves for lactate-utilizing bacteria and to characterize the effects of dietary changes on microbial population characteristics.

Chemical Analyses

Feed samples were dried at 55°C in a forced-air oven for 24 h and allowed to air equilibrate. Subsamples were then ground through a 1-mm screen in a Wiley Mill (Model 4,

Thomas Scientific, Swedesboro, NJ) and sent to the Michigan State University Diagnostics Laboratory (Lansing, MI) for proximate analyses.

Deproteinized cecal and fecal samples were thawed and centrifuged at $17,000 \times g$ for 15 min at 4°C. Concentrations of total lactate were measured colorimetrically (Barker and Summerson, 1941). Concentrations of VFA were determined using a Hewlett Packard 5890 gas chromatograph equipped with a 7673A auto-sampler and ChemStation software (Agilent Technologies, Santa Clara, CA). A 2 m x 2 mm Carbowax B-DA glass column (Supelco, Bellefonte, PA) was used. Nitrogen was used as the carrier gas at a flow rate of 24 mL min⁻¹. The oven, injection port, and detector (flame ionization) port temperatures were 175°C, 200°C, and 200°C, respectively.

The 100-g fecal samples were thawed and 1-g subsamples were analyzed in duplicate for DM (error rate < 5%). Samples were weighed in 2.54-cm aluminum pans (Fisherbrand, Fisher Scientific, Pittsburg, PA), dried in a forced-air oven at 105°C for 24 h, allowed to cool in a desiccator for 30 min, and reweighed. Fecal % DM was calculated as:

$$1 - \left(\frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \right) \times 100$$

Statistical Analyses

Body weight and feed intake data were analyzed using the AUTOREG procedure of SAS (Enterprise Guide, SAS version 9.2, SAS Inst. Inc., Cary, NC) to assess changes over time while accounting for serial correlation. The MIXED procedure (Enterprise Guide, SAS version 9.2, SAS Inst. Inc., Cary, NC) was used to compare least squares means (LSMEANS) between feed intake for horses on the BL and experimental diets. Significance was determined at $P \leq 0.05$ for all tests.

Fermentation parameters were analyzed using PROC MIXED (Enterprise Guide, SAS version 9.2, SAS Inst. Inc., Cary, NC) for repeated measures with horse as a random effect. Baseline (-1 h) data was assessed for changes over time and for serial correlation using an autoregressive covariance test with day as the fixed effect. If values remained stable over time ($P > 0.05$), then baseline data were combined and LSMEANS were calculated. Differences between LSMEANS for all collections were assessed using Tukey-adjusted pairwise comparisons. For both analyses, degrees of freedom were determined using the Satterthwaite approximation.

The NONLIN procedure (Enterprise Guide, SAS version 9.2, SAS Inst. Inc., Cary, NC), employing the Marquardt method, was used to obtain parameter estimates for OD at the time of inoculation (β_1), growth rate (β_0), and population maximums (a_1) to describe bacterial growth curves.

Results

Dietary Composition

The feed analyses for the diets utilized are shown in Tables 2.2, 2.3, 2.4, and 2.5 for Experiments 1, 2, 3, and 4, respectively. Upon experiencing the dietary change in Experiment 1, horses encountered an alteration in the physical form of the ration from a mixed hay-concentrate diet to a complete pelleted diet. This dietary change also altered the amount of dietary neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and ether extract (EE) the horses received. Abruptly changing the hay:concentrate ratio in Experiment 2 impacted the amount of dietary fiber, CP and starch in the rations. In Experiment 3, horses encountered a change in hay type and quality, along with changes in the amount of dietary fiber, non-fiber carbohydrates, and CP that were ingested. Upon receiving the concentrate meal in the final experiment, horses encountered a change in the hay:concentrate ratio that doubled the amount of

starch provided. Aside from developing loose stools by the end of the sampling period in Experiment 3, no other apparent signs of digestive distress were observed in any horses during any experimental period.

Intake and Body Weight

Mean dry matter intake (DMI) of BL was 9.9 ± 0.30 kg, 9.6 ± 0.42 kg, and 10.3 ± 0.36 kg for Experiments 1, 2, and 4, respectively, with no changes in DMI during each acclimation period ($P > 0.05$). In Experiment 1, mean DMI of CD was 10.9 ± 0.32 kg, which was not different from BL ($P = 0.46$). While small refusals were noted on the day that CD was introduced, larger refusals were noted on subsequent days. In Experiment 2, DMI was greater for horses while consuming GH (10.5 ± 0.45 kg) than for horses consuming BL ($P = 0.02$). However, GH was fed at a greater rate (2.5% BW vs. 2.0% BW for BL), so this difference is easily explained, considering that there were no feed refusals observed during Experiment 2. For Experiment 3, mean DMI for horses consuming GH at 2.5% BW was 11.7 ± 0.46 kg and there were no changes throughout the acclimation period. Initially, horses readily accepted AH (DMI = 10.1 ± 0.95 kg); however, refusals were greater for the 2 d following the dietary change, reducing daily DMI to 8.0 kg and 9.2 kg, respectively ($P < 0.0001$). Dry matter intake was greater for horses consuming GH than for AH ($P < 0.0001$); in addition to declining consumption rates on days 2 and 3, AH was fed at 2.0% BW compared to 2.5% BW for GH, so decreased DMI is easily explained. Differences in feeding protocols throughout the phases of Experiment 4 precluded meaningful comparisons of DMI between the BL, TF, and recovery hay meals. Horses readily consumed the experimental TF meals and no refusals were observed. Overall, body weights were maintained throughout the study.

Cecal and Fecal Parameters

Experiment 1

Parameters measured in cecal fluid for Experiment 1 are summarized in Table 2.6. Cecal pH was decreased from baseline values (6.87) at 1 (6.59; $P = 0.03$) and 5 h (6.01; $P < 0.0001$) following the abrupt dietary change. By 24 and 36 h, cecal pH values returned to baseline levels ($P > 0.05$). However, at 48, 60, and 72 h, cecal pH again decreased ($P < 0.0001$) to 6.01, 5.63, and 5.84, respectively. Mean concentrations of total lactate in cecal samples were elevated above baseline values at 48 h ($P = 0.04$) and 60 h ($P < 0.0001$) and returned to baseline values by 72 h ($P > 0.05$). There were large standard errors in total lactate, presumably due to individual variation between horses. Because individual tolerance levels are of concern when discussing colic risk, individual cecal concentrations of total lactate are plotted against the group means for each sampling time point following the dietary change in Figure 2.3. Total lactate concentrations exceeded 1 mM in several individuals by 5 h and remained elevated in certain individuals for all measurements through 60 h following the dietary challenge. By 72 h, only 1 horse experienced total lactate concentrations above 1 mM.

Table 2.7 summarizes the fecal parameters measured during Experiment 1. Fecal pH followed a similar trend to cecal pH. Five hours after the dietary change, fecal pH decreased below the mean baseline value (6.60) to 6.04 ($P < 0.0001$). Yet, at 24 and 36 h, fecal pH was elevated above baseline values ($P < 0.001$). By 48, 60, and 72 h, fecal pH again declined, as compared to baseline values, to 6.13, 5.69, and 6.07, respectively ($P \leq 0.0004$). No differences were observed in concentrations of total lactate between fecal samples collected at any time point; however, concentrations of total VFA were elevated in fecal samples collected at 36, 48, 60, and 72 h ($P < 0.01$), with a 3-fold increase over baseline values observed at 48 and 60 h.

While all VFA were elevated above baseline values from 36 to 72 h ($P < 0.05$), the acetate:propionate ratio decreased numerically during this time, indicating a relatively larger increase in propionate present in the feces ($P < 0.05$). No changes were observed in fecal DM content during this experiment.

Experiment 2

Table 2.8 summarizes the parameters measured in cecal fluid collected during Experiment 2. Cecal pH was decreased below baseline 5 h after the abrupt change to GH ($P < 0.001$). Subsequently, diurnal decreases below baseline (-1 h) cecal pH values were noted 12 h following the morning meal on both d 28 and d 29 (36 and 60 h; $P < 0.001$). No changes differing from baseline values in concentrations of total lactate in cecal contents were observed. Figure 2.4 depicts individual concentrations of total lactate in cecal fluid plotted with the group LSMEANS for each time point. Although concentrations of total lactate showed individual variation, all measurements were well below 1 mM. Concentrations of iso-butyrate decreased 5 h following the dietary change ($P < 0.05$), but experienced no other significant changes. Yet, cecal concentrations of butyrate increased above baseline at 5, 36, 60, and 72 h after the dietary change ($P < 0.015$). Post-prandial decreases were noted in the concentrations of 2-methyl-valerate at 5, 36, and 60 h. Compared to baseline values, valerate concentrations were increased at 60 h following the dietary change ($P < 0.05$). Additionally, the acetate:propionate ratio was increased above values obtained at -1 and 1h at 36 h, although there were no other differences in the acetate:propionate ratio noted between samples collected at each time point. No other changes were observed in concentrations of cecal VFA.

Fecal parameters for Experiment 2 are summarized in Table 2.9. Fecal pH remained stable 5 h after the abrupt dietary change to GH; however, pH was elevated above baseline

values at 48 and 72 h following the dietary change ($P < 0.01$). No changes in fecal lactate concentrations were detected. Total VFA concentrations were reduced at 36 h ($P < 0.01$), which coincided with a decrease in acetate concentrations ($P = 0.02$). Propionate concentrations were less than baseline values at 48 and 72 h ($P < 0.05$). Fecal concentrations of butyrate and valerate were reduced below baseline values at 24, 36, 48, and 72 h after the dietary change ($P < 0.05$). The acetate:propionate ratio was increased 72 h following the dietary change ($P = 0.02$). No other differences were observed in fecal concentrations of VFA in this experiment. Fecal DM was reduced at 24, 36, 48, 60, and 72 h compared to baseline means ($P < 0.05$).

Experiment 3

Parameters measured in cecal fluid are summarized in Table 2.10 for Experiment 3. As noted in Experiments 1 and 2, cecal pH again was decreased below the baseline mean (6.72) 5 h after abruptly changing from GH to AH (6.52; $P < 0.01$). Cecal pH again demonstrated a diurnal pattern and was reduced below the baseline mean at 36, 60, and 72 h ($P < 0.05$). Total lactate concentrations exceeded baseline values at 24 and 36 h following the dietary change ($P < 0.05$); however, total lactate measurements produced large standard errors presumably due to individual variation which made it difficult to detect differences. Figure 2.5 illustrates individual concentrations of total lactate plotted against the mean lactate of all cecal samples collected at each time point. Concentrations of total lactate exceeded 2 mM for 1 horse at 5 h. By 24 h, 3 horses exceed this threshold with concentrations of total lactate in cecal samples of 4.5, 5.4, and 8.4 mM. This increase was more pronounced at 36 h, where total lactate concentrations exceed 1 mM for every horse (range: 3.03 to 9.76 mM). By 48 h, total lactate concentrations returned to baseline values for all horses. Total cecal VFA concentrations were increased 5 h after the dietary change and remained elevated above baseline values for the remainder of the sampling

period ($P < 0.05$), which coincided with increased acetate concentrations in cecal fluid collected at each of the same time points ($P < 0.05$). Propionate concentrations were increased at 24, 36, and 60 h compared to baseline ($P < 0.05$). Butyrate concentrations were elevated above baseline at 5, 24, 36, and 60 h after the dietary change ($P < 0.05$). Iso-butyrate and 2-methyl-valerate concentrations exceeded baseline values for each at 24, 36, 48, and 72 h following the dietary change ($P < 0.05$). Three-methyl-valerate concentrations were increased above baseline values at 24, 36, 48, 60, and 72 h ($P < 0.05$). Valerate concentrations initially increased at 5 h ($P < 0.05$). From 24 to 72 h, valerate concentrations exhibited a 4-fold increase over baseline and 1 h values ($P < 0.0001$). Although the acetate:propionate ratio increased at 5 h ($P < 0.01$), it was not different from baseline at other sampling time points due to concomitant increases in acetate and propionate.

Growth curves for lactate-utilizing bacteria obtained from cecal fluid collected during Experiment 3 are presented in Figure 2.7. Specific subsets of these bacterial growth curves have been arranged to show baseline (-1 h on d 14 to 21) curves compared with other samples collected preprandially (-1, 24, 48, and 72 h) and postprandially (1, 5, 36, and 60 h) to the morning meal. The mean bacterial growth curve generated from cecal samples collected prior to the dietary change and used to generate a baseline average (-1 h on d 14 to 21) is nearly parallel to that of the samples obtained 1 h prior to the dietary change through the first 20 h of incubation, at which point the curves begin to overlap. When bacterial growth curves generated while horses were on BL are compared to bacterial growth curves generated from subsequent preprandial cecal fluid samples following the dietary change (24 h, 48 h, 72 h), there is an upward shift in the growth curves due to a greater OD reading at the time of inoculation. Still, the growth rate and population maximums for the curves generated by samples collected 24 and

48 h following the dietary change seem to mirror that of the baseline growth curves. However, the growth curve for samples obtained 72h following the dietary change depicts a seemingly slower growth rate that culminates with a greater population maximum in the stationary phase. When baseline growth curves are compared to those generated from postprandial cecal fluid samples following the dietary change (1 h, 5 h, 36 h, 60 h), there is a visual increase in inoculation OD and a slower growth rate for the growth curve generated from cecal fluid collected 1 h following the dietary change. The growth curve initiated 5 h following the dietary change presents an inoculation OD similar to that of the baseline growth curves and also depicts a slower growth rate. The 36 and 60 h growth curves begin with greater inoculation OD readings and achieved greater population maximums, in spite of apparently slower growth rates, when compared to the baseline curves.

Table 2.11 summarizes the fecal parameters measured for Experiment 3. Fecal pH increased above the baseline mean (6.48) by 48 h (6.99) and remained elevated through 72 h (7.10; $P \leq 0.05$). No differences were observed for total lactate, total VFA, or acetate concentrations in the fecal samples obtained. At 36 h, propionate concentrations were greater than at 48 h ($P = 0.033$), although both values were similar to the baseline mean ($P > 0.05$). Fecal concentrations of iso-butyrate, 3-methyl-valerate, and valerate also increased 36 h following the dietary change ($P < 0.05$). Two-methyl-valerate concentrations were elevated at 24 and 36 h compared to baseline means ($P < 0.05$). The acetate:propionate ratio did not differ between samples. Fecal DM was decreased from baseline in all samples collected 36 to 72 h after the dietary change in this experiment.

Experiment 4

Table 2.12 summarizes the parameters measured in cecal fluid for Experiment 4. As in the previous experiments, cecal pH decreased below the baseline mean (6.96) 5 h after the abrupt dietary change to a large concentrate meal (6.59; $P = 0.003$); however, this parameter returned to baseline values by 24 h ($P > 0.05$). There was a corresponding increase in cecal concentrations of total lactate by 5 h ($P = 0.0004$), which also returned to baseline values by 24 h. Figure 2.6 depicts cecal concentrations of total lactate in individual horses plotted against the group means. One hour after the dietary challenge, 1 horse had a cecal concentration of total lactate in excess of 1 mM (1.54 mM). By 5 h, all but 2 horses had total lactate concentrations over 3 mM. In fact, 3 horses' total lactate concentrations exceeded 10 mM (10.04, 13.94, and 15.76 mM). All individuals had total lactate concentrations below 1 mM by 24 h. Total VFA and acetate concentrations initially decreased 1 h following the dietary change ($P < 0.05$) and later peaked at 5 h, although these values were no different from baseline parameters ($P > 0.05$). Cecal propionate concentrations were increased at 5 h ($P = 0.0004$) and subsequently decreased and returned to baseline values 36 h following the large concentrate meal. Cecal iso-butyrate decreased at 36 h, compared to baseline values ($P < 0.05$). Cecal butyrate concentrations decreased at 1 h and increased at 5 h, as compared to baseline values ($P < 0.05$). Cecal concentrations of valerate were elevated at 5 and 24 h over baseline values ($P < 0.05$). The acetate:propionate ratio in cecal fluid was primarily influenced by changes in propionate. At 1 and 5 h following the dietary change, the ratio decreased, whereas at 36 h the ratio increased, as compared to baseline values ($P < 0.05$).

Growth curves generated by lactate-utilizing bacteria collected from the cecum of horses in Experiment 4 are presented in Figure 2.8. Curves have been organized in the same manner as presented in Experiment 3. Baseline growth curves (the average of samples collected 1 h prior to

the morning feedings on d 14 to 21 and samples collected 1 h prior to the administration of the large concentrate meal) overlap through 12 h of incubation, exhibiting similar inoculation ODs and lag phases. After 12 h, the growth curves diverge during the log phase of growth. Nonetheless, the populations of lactate-utilizing bacteria in these samples seemed to reach similar maximums, although a distinct stationary phase was not observed in these samples. In spite of the limited dietary disruption window used in this experiment, the bacteria cultured from cecal samples collected 5 and 24 h following the dietary disruption demonstrated visible shifts in growth patterns compared to the bacterial growth curves generated from baseline cecal fluid collections. In examining the pre-prandial curves, there was an upward shift in the inoculation OD when bacteria were collected in cecal fluid 24 h following the dietary disruption compared to the inoculation OD for bacteria collected in baseline cecal samples. While the growth rates appear to be similar, the population maximum is greater for bacteria collected at 24 h compared to that of bacteria collected from baseline sampling periods. In the postprandial cecal fluid samples, the inoculation OD is increased 5 h after the concentrate meal was fed. Yet, the 5 h curve appears to have a slower growth rate and reaches a population maximum similar to that of the baseline growth curves.

Fecal parameters for Experiment 4 are summarized in Table 2.13. Fecal pH remained stable until 36 h following large concentrate meal, at which time it fell below the baseline mean ($P < 0.01$). No changes in fecal measurements of total lactate, VFA concentrations, or DM content were detected in this experiment.

Discussion

Experiment 1

The dietary change from the baseline ration to a complete pelleted diet presented in the first experiment exposed horses to an abrupt alteration in dietary composition, as well as the physical form of the feed, and was sufficient to elicit a notable response in hindgut fermentation parameters. The initial changes in cecal pH are similar to the postprandial effects noted by Goodson et al. (1988), who evaluated the effects of an abrupt change from an all-forage diet to an all-concentrate diet. Given that mean prececal ROP is 6.8 ± 1.2 h in the horse (Julliand et al., 2006; Van Weyenberg et al., 2006), the initial decrease in cecal pH noted in this experiment at 5 h was likely the result of a stimulatory effect of feeding that prompts the rapid transit of liquid phase digesta through the small intestine (Goodson et al., 1988; Drogoul et al., 2000). Native prairie grass hay is a mixed-grass forage and is often of fairly low-quality (Olson et al., 2008). The proximate analysis of native prairie grass hay used in this study revealed that the hay used was lesser in DE and CP and greater in NDF, ADF, and ash when compared to reference values for mature mixed-grass hay (NRC, 2007); however, in comparison to proximate analysis values for tallgrass-prairie hays collected from the central plains region (Olson et al., 2008), the hay was greater in DM and ash and lesser in NDF, ADF, and DE, potentially indicating less plant maturity at the time of harvest. Textured feed and CD compositions were similar to the guaranteed analyses provided by the manufacturer. Although several components of the proximate analyses of the diets were comparable, the degree of feed processing in the CD may have increased the quantity of fine particles reaching the cecum. The reduced NDF and ADF of the CD diet, in addition to the smaller particle sizes, may have led to increased microbial fermentation in the cecum, resulting in decreased pH values and increased total lactate concentrations. At 5, 48, 60, and 72 h following the dietary change, cecal pH values were less than or approximately equal to 6.0, which is considered to be indicative of subclinical

fermentative acidosis (Radicke et al., 1991). On the other hand, cecal pH was similar to baseline values at 24 and 36 h after the dietary change. This is not entirely unexpected, as all baseline measurements were obtained immediately prior to the morning meal, and the 24 and 36 h measurements were also obtained immediately prior to the morning meal. Some of the differences in cecal pH noted throughout this experiment may be primarily a reflection of preprandial versus postprandial cecal environments. Reduced passage rate, as a result of reduced particle size, coupled with greater CP content and presumably increased bicarbonate secretions in response to increased VFA absorption (Shirazi-Beechey et al., 2008), could have temporarily buffered the cecal environment at these time points in horses following the dietary alteration, but these data are difficult to interpret without knowing specific effects of the dietary change on MRT throughout the hindgut and without measuring bicarbonate secretions (Van Weyenberg et al., 2006). Larger feed refusals were noted for CD meals fed 24 and 48 h following initial dietary change, which may be a behavioral product of hindgut disturbances. Horses have been shown to develop taste aversions to feeds that have previously made them ill (Houpt et al., 1990; Matsuoka et al., 1996; Raymond et al., 2003). Although no apparent signs of digestive distress were noted in this experiment, it is plausible that the reduced DMI observed for the CD diets may represent a learned feed aversion due to acidotic conditions in the cecum following the dietary change.

The alterations in fecal pH in the first experiment mirrored cecal pH trends. According to Drogoul et al. (2000) and Van Weyenberg et al. (2006), colonic MRT ranges from 25 to 45 h, depending on dietary characteristics. Thus, alterations in pH and other fermentation parameters should not be evident in the feces until many hours after the disturbance has occurred in the cecum. The decrease in fecal pH observed at 5 h may represent normal postprandial parameters,

whereas the pH shifts after 48 h may reflect the effects of the dietary change. More frequent fecal sampling and obtaining postprandial fecal samples while horses were consuming BL may have aided in further elucidating the effects of the dietary change on the relationship between cecal and fecal parameters. The stable lactate concentrations in the feces could indicate that the lactate produced in the cecum and colon was mostly metabolized prior to excretion, being that similar concentrations have been previously reported in the equine (Berg et al., 2005; Muller et al., 2008). The fecal concentrations of VFA obtained in this experiment while horses were fed BL were less than those reported by Al Jassim (2006) and Dougal et al. (2012) for horses maintained on a mixed grass hay and concentrate ration. The reduced concentrations of fecal VFA in these horses probably reflects the reduced digestibility and fermentability of the native prairie hay used in the BL diet. Feces collected 36, 48, and 60 h after the dietary challenge had greater total VFA concentrations, which may reflect the “better quality” digesta resulting from CD finally passing through the gastrointestinal tract (GIT; Drogoul et al., 2000). If so, it would appear that a minimum of 2 d are required for the effects of an abrupt dietary change from a hay:concentrate ration to a complete pelleted meal to be observed in fecal material.

Current industry practice is to place horses on a CD following colic surgery (regardless of the nature of the horses’s pre-colic diet) in an effort to provide an easily digested, “gentle” ration to horses with a disturbed GIT. Exposing horses recovering from colic surgery to this type of dietary change may predispose them to additional GIT complications by inducing subclinical cecal acidosis. These acidic conditions impair normal cellular function in the intestinal lumen which could lead to epithelial necrosis and, ultimately, and ironically, colic (Shirazi-Beechey, 2008). Although not evaluated in this study, an increase in feed quality, which may occur with the switch to a CD, also could result in increased gas production resulting from increased

microbial fermentation within the equine GIT, which may lead to further abdominal discomfort. The horses used in this study were cecally cannulated, which may have allowed excess gas to escape, thus circumventing intestinal distention and signs of abdominal discomfort that may have occurred with intact horses.

Experiment 2

In the second experiment, abruptly changing the hay:grain ratio to include more hay and eliminate the concentrate did not elicit biologically significant responses. An initial decrease in cecal pH at 5 h was noted; however, this postprandial decrease in pH has been observed in other studies, regardless of diet, and may be the result of digesta passing from the small intestine into the cecum (Goodson et al., 1988; Drogoul et al., 2000ab). A similar decrease in cecal pH also may have been noted 5 h following the morning meal if similar postprandial samples had been obtained while horses were consuming BL, thus the decline noted in this experiment cannot necessarily be attributed to the dietary change. Postprandial effects on cecal pH were also noted in samples collected 36 and 60 h following the initial dietary change. This may correlate to diurnal variations in forage intake and fermentation rates (Fleurance et al., 2010), although these parameters were not evaluated in the current study. Increases in cecal butyrate, as were observed in this experiment and that might have resulted from increased dietary forage, in fact, are likely beneficial for colonic health (Argenzio et al., 1974). Colonocytes, epithelial cells lining the large intestine, preferentially absorb acetate and propionate into the bloodstream (Argenzio et al., 1974). Acetate is metabolized by peripheral tissues for energy production, whereas propionate is taken up by the liver for gluconeogenesis (Argenzio et al., 1974). Butyrate, on the other hand, is retained and metabolized by the colonocytes to help regulate cellular function (Argenzio et al.,

1974; Shirazi-Beechey, 2008). So, forage-based diets that lead to an increase in cecal butyrate concentrations, as seen with this dietary change, may promote intestinal health.

Diurnal effects were also noted in fecal parameters observed during the second experiment. Preprandial pH values obtained from the feces increased over time following the dietary change, providing evidence that a buffering effect of an all forage diet on the GIT tract may have occurred. Fecal DM decreased 24 to 72 h after the dietary change, although this decrease was not sufficient to induce diarrhea or any abnormal fecal output. An increase in the water holding capacity of the digesta resulting from increased fiber content or increases in alimentary secretions due to greater DM content of the hay may partially explain this change (Van Weyenberg et al., 2006). As evidenced by the proximate analysis, the native prairie hay used in this study was of fairly poor quality. It has been reported that feeding poor quality forages may increase the risk of colonic impactions (Hudson et al., 2001); however, no measurable or visible disruptions in the hindgut environment were detected as the hay content of the diet increased in this experiment. Thus, when feedstuff availability is limited, the GIT of horses may tolerate an abrupt increase in the amount of conserved forage or a decrease in the concentrate portion of their ration fairly well.

Experiment 3

An abrupt change from a GH ration to an AH ration in the third experiment elicited significant changes to the hindgut environment. Cecal pH and lactate concentrations were similar to observations reported by Respondek et al. (2008) after a sudden change in grain type to barley, indicating that an abrupt change in hay type and quality may be just as radical to the hindgut as the introduction of a novel grain. While horses consumed the initial meal of AH in its entirety, refusals were noted for most horses at subsequent feedings, which again may result from a

learned feed aversion in response to dietary distress resulting from the initial dietary change (Houpt et al., 1990; Matsuoka et al., 1996; Raymond et al., 2003). Proximate analysis revealed that the composition of the AH used in this experiment was typical of reference values listed for immature legume hay (NRC, 2007) and, therefore, horses experienced an abrupt reduction in dietary fiber and an increase in dietary CP and non-fiber carbohydrates as a result of the dietary change from poor quality GH to very good quality AH. Forage type and physical form, as well as high-fiber alternatives, have been reported to cause little variation in cecal parameters of horses adapted to forage-based diets (Drogoul et al., 2000; McLean et al., 2000; Moore-Colyer et al., 2000; Coverdale et al., 2004); however, the abrupt change in hay type and quality in this experiment was associated with increased cecal lactate and VFA concentrations which simultaneously led to reduced cecal pH. Cecal pH, however, never approached the levels noted in the first experiment; therefore, it appears that a change in forage type and quality – although disruptive - is better tolerated by the hindgut than the abrupt introduction of a CD. As with the first experiment, these shifts within the hindgut environment may also predispose horses to excessive gas production and intestinal dysfunction (Shirazi-Beechey, 2008).

Fecal parameters were largely unaffected by the dietary change in Experiment 3, which may be due, in part, to the selective retention mechanisms in the hindgut. Larger particles are preferentially retained in the cecum and ventral colon, potentially confining the effects of the dietary alteration to the proximal colon. Additionally, increased MRT in the colon may have provided more time for fermentation parameters to equilibrate (Drogoul et al., 2000); however, 4 horses developed loose stools by the end of the experiment, providing further evidence that these horses may have experienced a digestive disturbance. Changes in the water holding capacity due

to forage type, increases in proximal alimentary secretions, and changes in electrolyte and water absorption all could impact water content of the feces (Van Weyenberg et al., 2006).

Overall, an abrupt change from a low-quality grass hay to a high-quality legume hay elicited alterations in cecal metabolites, supporting an increased colic risk. The data indicate that the risk is greatest during the first 36 h after the abrupt introduction of AH, although these data must be interpreted with caution as postprandial samples were not obtained while horses were consuming GH. However, because pH remained fairly stable in the cecum and increased in the feces from 48 to 72 h as the horses continued to consume AH, the buffering effect of increased CP may be sufficient to offset the observed increases in cecal lactate and VFA concentrations. Increases in cecal populations of lactate-utilizing bacteria may have also mitigated the effects of increased lactate production as the microbial ecosystem adapted to the new diet, further stabilizing the pH of the cecum. As with Experiment 1, cecal cannulation may have allowed excess gas to escape from the cecum and attenuated intestinal distention and abdominal pain; however, reduced DMI of AH and reduced fecal DM may have been indicative of digestive distress. Therefore, horse owners are advised to avoid, as much as possible, abrupt changes in the type and quality of hay fed to their horses when faced with limited forage availability.

Experiment 4

Although dietary starch challenges have been conducted in horses (Goodson et al., 1988; de Fombelle et al., 2001; Respondek et al., 2008), previous researchers have not necessarily examined diets traditionally fed in the United States or the dietary changes that domestic horses are likely to face. The abrupt change from BL to a large concentrate meal in this experiment elicited a significant response in cecal parameters measured at 5 h that may have been mitigated by the consumption of a hay meal 12 and 24 h following the large concentrate meal. Thus,

feeding hay may be beneficial in instances where excessive grain consumption has occurred. The postprandial decrease in cecal pH was accompanied by a marked increase in total lactate and VFA concentrations. The magnitude of the increase and the variability in cecal lactate may be biologically relevant when evaluating an individual's risk for gastrointestinal disease, as it may be indicative of the reaction of the fermentative bacteria in his or her individual hindgut to dietary changes. The cecal concentrations of total lactate for this dietary challenge were greater than those reported by Respondek et al. (2008) in the colonic contents of horses exposed to a novel grain source; however, they are less than the cecal lactate concentrations reported by Moore et al. (1979) in horses following a severe starch-overload challenge used to induce laminitis. In this experiment, changes in VFA concentrations were marked by an increase in propionate at 5 h that subsequently decreased by 24 h and returned to baseline values. Similar shifts in VFA concentrations have been reported for other grain challenges (Goodson et al., 1988; de Fombelle et al., 2001), indicating that the amount of dietary starch provided in this experimental diet (3.8 g starch kg⁻¹ BW) was sufficient to exceed the small intestine's capacity for starch digestion and thus altered the cecal environment. Starch digestion and monosaccharide absorption in the horse are limited by low alpha-amylase activity in the small intestine (Richards et al., 2004), which in turn limits the expression of monosaccharide transporters along the epithelium (Dyer et al., 2009). During this dietary challenge the capacity for starch digestion and absorption in the small intestine may have been overwhelmed; therefore, a portion of undigested starch and unabsorbed monosaccharides likely passed to the cecum where they were fermented by resident microbes to produce the observed alterations in cecal parameters. Previously, researchers have suggested that the small intestine has a digestive capacity of up to 4.0 g starch kg⁻¹ BW (Potter et al., 1992), although an upper tolerance limit of 2.0 g starch kg⁻¹ BW may be

more accurate when horses encounter an abrupt increase in dietary starch (Radicke et al., 1991; Kienzle et al., 1992). Nevertheless, researchers agree that many factors influence the degree of starch digestion and absorption in the small intestine, including starch source, degree of grain processing and mastication, feeding frequency, rate of ingestion, acclimation to the diet, and individual variation (Radicke et al., 1991; Potter et al., 1992; Kienzle et al., 1992; Meyer et al., 1995; McLean et al., 2000). In light of the adaptive capacities of the equine GIT for CHO digestion and absorption (Shirazi-Beechey et al., 2008; Dyer et al., 2009), extended acclimation periods, in addition to small, frequent meals, may increase the tolerance of the GIT to dietary starch in horses that require concentrate supplementation.

Fecal parameters were largely unaffected by the dietary challenge in Experiment 4. By 36 h following the large concentrate meal, fecal pH decreased, which may be attributed to “starch-rich” digesta from the cecum and colon finally passing through the gut. Although not significant, fecal DM numerically increased over time. Lopes et al. (2004) suggested that increased fecal DM in response to a high-starch diet was a product of compromised water and nutrient absorption in the colon which alters the electrolyte balance of the lumen. According to these researchers, dehydration of colonic contents may predispose horses to intestinal impaction and other gastrointestinal complications (Lopes et al., 2004). Overall, an abrupt increase in the amount of concentrate offered to a horse from 0.05% BW to 1.0% BW elicited concentrations of cecal lactate greater than those reported with a novel grain source and may, in fact, increase a horse’s risk for colic. While sudden increases in dietary concentrates also are a known causative factor in the development of laminitis in horses (Pollit and Visser, 2010), the increase provided in this experiment did not induce any observable laminitic changes. Recovery hay meals fed 12 and 24 h following the dietary challenge may have attenuated further deterioration of the hindgut

environment, as cecal pH observations never approached the values reported for Experiment 1. Thus, in the event of an unavoidable sudden increase in the grain or concentrate component of the diet, horse owners are advised to provide long-stem forage, in addition to cryotherapy of the legs and feet (Mitchell et al., 2014), as a precaution until the veterinarian arrives.

Bacterial Growth Curves

In Experiments 3 and 4, microbial growth curve data were used to observe the effects of the abrupt dietary changes on the lactate-utilizing bacterial populations of the cecum. These bacteria convert lactate to butyrate and other intermediate VFA (Hobson and Stewart, 1997) and the growth of these populations often follows rapid proliferation of lactate-producing bacteria, such as *Lactobacillus* sp. and *Streptococcus* sp., which ferment starch and other dietary sugars to lactate. Monitoring lactate-utilizing bacterial populations provides an indicator for an animal's tolerance for abrupt increases in lactate production within the hindgut.

Bacterial growth curves typically display four distinct phases of growth, including a lag phase, log phase, stationary phase, and death phase, which present as a sigmoid curve when plotted on a graph (Novick, 1955). The lag phase occurs after inoculation and is the period in which the bacteria adjust to the conditions of the media. The log phase is a period of exponential growth. As bacterial numbers reach the maximal population that can be sustained by the medium, the bacteria deplete the medium of nutrients and metabolic by-products accumulate. These factors limit bacterial growth and lead to the stationary phase, where the growth rate and death rate are equivalent. The death phase presents when the bacterial population is no longer supported by the medium and the death rate exceeds the growth rate.

These phases were observed for each sampling timepoint in Experiments 3 and 4 using optical density (OD), or turbidity, readings. As lactate-utilizing bacterial populations grew in the

SDL medium, the broth became more opaque which increased the OD reading. When plotted over time, the OD readings presented the sigmoid curve typical of bacterial growth curves. Comparisons of the growth curves of bacteria collected from each sampling timepoint allowed for inferences regarding the effects of the dietary challenges on the microbial ecosystem of the hindgut. More specifically, shifts in the OD at the time of inoculation, growth rates (lag and log phases), and population maximum (stationary phase) OD provide insight into microbial population dynamics, especially when examined in combination with cecal pH, VFA, and lactate shifts. In Experiment 3, the increased inoculation OD at 1 h, and thus presumed increase in the number of bacteria, may have contributed to the increased cecal VFA concentrations noted at 5 h. The rapid ROP of digesta through the small intestine may have stimulated fermentation and bacterial growth in the cecum, resulting in the visual increase of the inoculation OD noted at 1 h (Julliand et al., 2006; Van Weyenberg et al., 2006). This increase in the microbial population observed via inoculation OD is not specific to lactate-utilizing bacteria, however, as the growth rate appears slower than the baseline curves and the population maximum was not different from baseline values. The SDL medium was selective for bacterial populations that utilize lactate for growth and proliferation, although it was possible that other types of bacteria also grew in the culture as there were other potential energy substrates in the media. As cecal populations of lactate-utilizing bacteria increase, it is expected that the growth curve will shift upward and be characterized by faster growth rates in the media. The absence of this shift at 1 h supports the assumption that the proportion of lactate-utilizing bacteria has not yet changed in the cecal fluid. The abrupt decrease in cecal pH and increase in total VFA concentrations at 5 h may have temporarily created suboptimal conditions for resident microbial populations, as the microbial inoculation OD at this timepoint is similar to that of the baseline curves. Based on previous work

in our laboratory with *Megasphaera elsdenii*, we estimate that a 4 h difference along the x-axis between the inoculation OD of a growth curve and data points on the baseline growth curve reaching the same OD is indicative of a 1-log increase in the population (J. Drouillard, personal communication). If this same growth timeline is applied to the mixed culture obtained in these experiments, there is approximately a 1-log increase in lactate-utilizing bacteria 24 h following the abrupt introduction of AH and a 4-log increase by 36 h following the dietary change. Although population parameters return to similar values as those observed at 24 h by 48 h following the dietary change, which could be a diurnal effect of fasting, cecal conditions were still supporting a thriving lactate-utilizing bacterial population by 60 h, as evidenced by the 2-log increase from baseline. The lack of a traditional S-shaped growth curve from bacteria collected at 36 and 60 h following the dietary change likely indicates that lactate-utilizing bacteria represent a significant proportion of the total bacterial population. The growth curves at these time points appear to bypass the lag phase of growth and immediately enter the log phase, which occurs when large bacterial populations are present in the inoculum (J. Drouillard, personal communication). By 72 h following the dietary change, microbial populations appeared to have acclimated to the experimental diet with a reduction in the concentration of lactate-utilizers, as the lag phase is reestablished in the growth curves generated.

In Experiment 4, no changes in lactate-utilizing bacteria were observed up to 5 h following the administration of the large concentrate meal, where the growth rates were slower than those of the baseline curves and the population maximums were no different from baseline values. If we apply a timeline similar to that of *Megasphaera elsdenii* to our mixed culture, total microbial populations experienced a 1-log increase at the 1 h sampling timepoint and a 4-log increase at 5 h following the dietary change. Lactate-producing bacteria, such as *Streptococcus*

sp. and *Lactobacillus* sp., have population turnover rates of about 27 min (Hobson and Stewart, 1997). Therefore, the initial increase in bacteria, as evidenced by the increase in the inoculation OD, and slower growth rate noted at 1 and 5 h following the dietary change is probably an increase in these populations in response to the starch challenge. In bacteria cultured from cecal fluid collected 24 h following the dietary disruption, there was a 1-log increase in what were presumably lactate-utilizing bacteria, due to the greater inoculation OD and the upward shift in the population maximum. By 36 h, there appears to be a 4-log increase in total bacterial numbers based on an increased OD reading at the time of inoculation; however, due to the longer lag phase it would seem that lactate-utilizing bacteria were a smaller proportion of the total microbial population at this time point. This decrease in lactate-utilizing bacterial numbers was probably due to decreased substrate availability due to the passage of time following the carbohydrate meal without any subsequent concentrate provided to the horses, and could be a sign that the microbial populations were re-stabilizing.

The most recently reported research regarding abrupt dietary changes in the equine monitored fermentation and microbial parameters for 29 h following the dietary change (de Fombelle et al., 2001; Respondek et al., 2001; Muhonen et al., 2009). As evidenced by our bacterial growth curves and cecal and fecal parameters, 29 h may not be long enough to distinguish relevant shifts in lactate-utilizing bacterial populations. In Experiment 3, growth curves generated by bacteria obtained 36, 60, and 72 h following the dietary disruptions indicate that lactate-utilizing bacteria experience notable growth during this time, and these effects may have gone unnoticed in previous studies. De Fombelle et al. (2001) reported no significant alterations in lactate-utilizing bacterial populations when barley was abruptly incorporated into the diets of ponies. The authors indicated that their dietary challenge provided 2.3 g starch kg⁻¹

BW per meal, and they concluded that their challenge was not sufficient to elicit the more extreme responses noted in induced-laminitis studies (Garner et al., 1978; de Fombelle et al., 2001). These observations coincide with the upper digestive capacity of the small intestine of 3.5 to 4.0 g starch kg⁻¹ BW proposed by Potter et al. (1992). Respondek et al. (2008), on the other hand, noted a 1-log increase in colonic lactate-utilizing bacteria 29 h after changing the type of grain in the diet while providing 2.8 g starch kg⁻¹ BW. In their study, colony forming units were counted on roll tubes and petri plates after a 48 h incubation period. Their increase in the lactate-utilizing bacterial populations noted is similar to what was observed in the current study at 24 h. Had Respondek et al. (2008) continued to monitor lactate-utilizing bacterial populations beyond 29 h, they may have noted further increases in bacterial populations consistent with those observed in this study. These data coincide with a small intestinal digestive capacity of 2.0 g starch kg⁻¹ BW proposed by Kienzle et al. (1992) and supported by Julliand et al. (2006). Muhonen et al. (2009) indicated no significant changes in lactate-utilizing bacterial populations when diets were abruptly changed from hay to either haylage or silage. However, in Experiment 3, growth curves of bacteria generated from cecal fluid following an abrupt change from GH to AH depict a 1-log increase in bacterial populations at 24 h and a 4-log increase at 36 h. Additionally, ponies served as the experimental models in previous reports (de Fombelle et al., 2001; Respondek et al., 2008; Muhonen et al., 2009). Horses may be more or less sensitive to dietary alterations than the pony; thus caution should be exercised in extrapolating data obtained from ponies to draw conclusions regarding the horse.

Summary

The dietary alterations investigated in this study were designed to mimic abrupt dietary changes that domesticated horses are likely to encounter in the United States. The dietary

alterations in these experiments produced hindgut conditions similar to those reported in previous studies that utilized dietary changes thought to be more extreme. Overall, the results from the current experiments support the epidemiological evidence that associates increased colic risk with abrupt dietary changes in type or amount of concentrate, changes in hay type or quality, and abnormal feeding incidents. More specifically, it appears that exposing horses recovering from colic surgery to a complete diet may predispose them to additional GIT complications by inducing subclinical cecal acidosis. Additionally, abruptly increasing the proportion of the current hay in the diet is of less concern than changing the type of hay. Lastly, abnormal feeding incidents that abruptly increase the amount of concentrate in the diet are likely to negatively alter the conditions of the hindgut. Understanding the implications of this study as they relate to gastrointestinal health should allow for improved feeding and management strategies to prevent and attenuate the adverse effects generated by unavoidable sudden changes in the equine diet.

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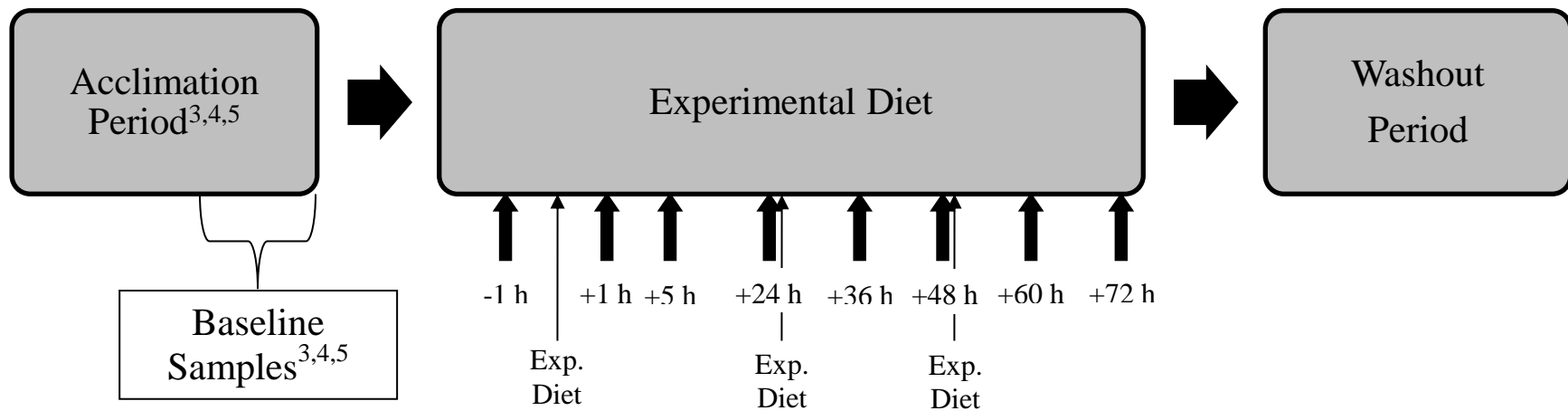
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Figure 2.1. Experimental protocol during Experiments 1, 2, and 3^{1,2}



¹Short, thick vertical arrows denote the time of collection of cecal and fecal material relative to the abrupt introduction of the experimental diet

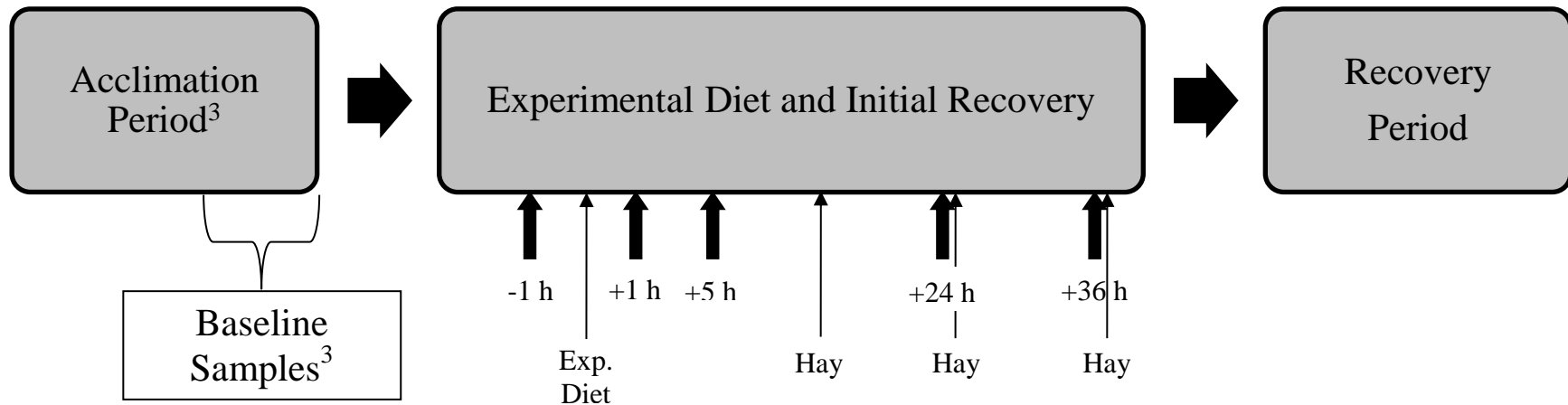
²Long, thin vertical arrows denote the feeding schedule of the experimental diets (Exp. Diet); meals were fed at 0 h and immediately following the 24 h and 48 h collections of cecal and fecal material

³Experiment 1: Horses were acclimated to a baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed) for 28 d where cecal and fecal material was collected an hour prior to feeding (-1 h) during each of the last 9 d of the acclimation period. On d 29, diets were abruptly changed to a complete pelleted feed (2.0% BW). A washout period followed the 72 h collection protocol and served as the acclimation period for Experiment 2

⁴Experiment 2: Horses were acclimated to a baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed) for 26 d where cecal and fecal material was collected an hour prior to feeding (-1 h) during each of the last 10 d of the acclimation period. On d 27, diets were abruptly changed to a 100% native prairie hay diet (2.5% BW). A washout period followed the 72 h collection protocol and served as the acclimation period for Experiment 3

⁵Experiment 3: Horses were acclimated to a 100% native prairie hay ration (2.5% BW) for 21 d where cecal and fecal material was collected an hour prior to feeding (-1 h) during each of the last 8 d of the acclimation period. On d 22, diets were abruptly changed to 100% alfalfa hay (2.0% BW). A washout period followed the 72 h collection protocol and served as the acclimation period for Experiment 4

Figure 2.2 Experimental protocol for Experiment 4^{1,2}

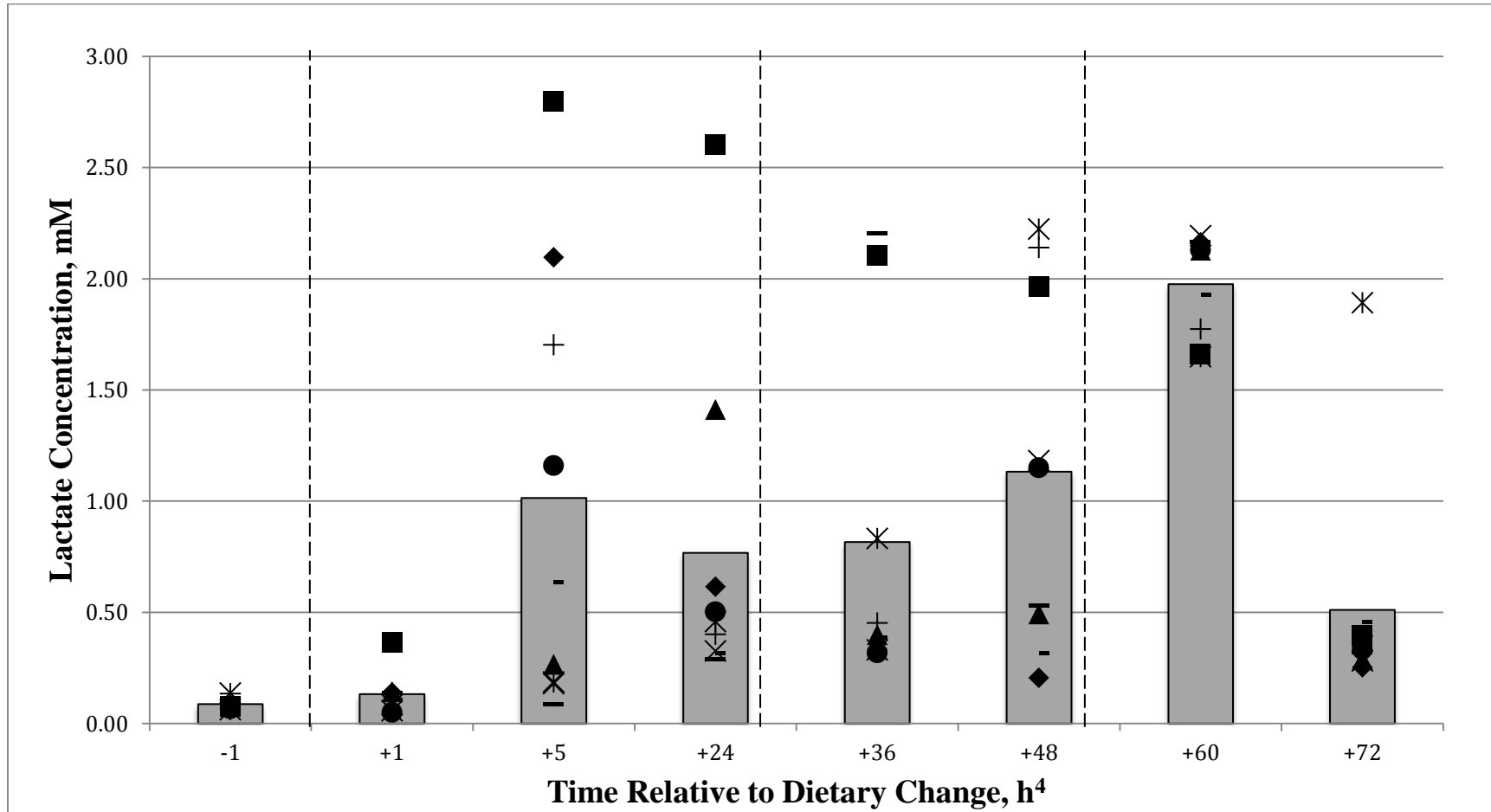


¹Short, thick vertical arrows denote the time of collection of cecal and fecal material relative to an abrupt dietary change

²Long, thin vertical arrows denote the feeding schedule of the experimental diet (Exp. Diet) and hay; hay meals were fed at 12 h and immediately following the 24 h and 36 h collections of cecal and fecal material

³Experiment 4: Horses were acclimated to a baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed) for 21 d where cecal and fecal material was collected an hour prior to feeding (-1 h) during each of the last 8 d of the acclimation period. On d 22, horses were fed a large meal consisting only of textured feed (1.0% BW). A 2.5 d recovery period followed the 36 h collection protocol during which horses were maintained on a grass hay diet (1.5% BW native prairie hay)

Figure 2.3 Individual concentrations of total lactate in cecal fluid compared to the group mean in response to an abrupt dietary change from BL to CD¹ in Experiment 1^{2,3}



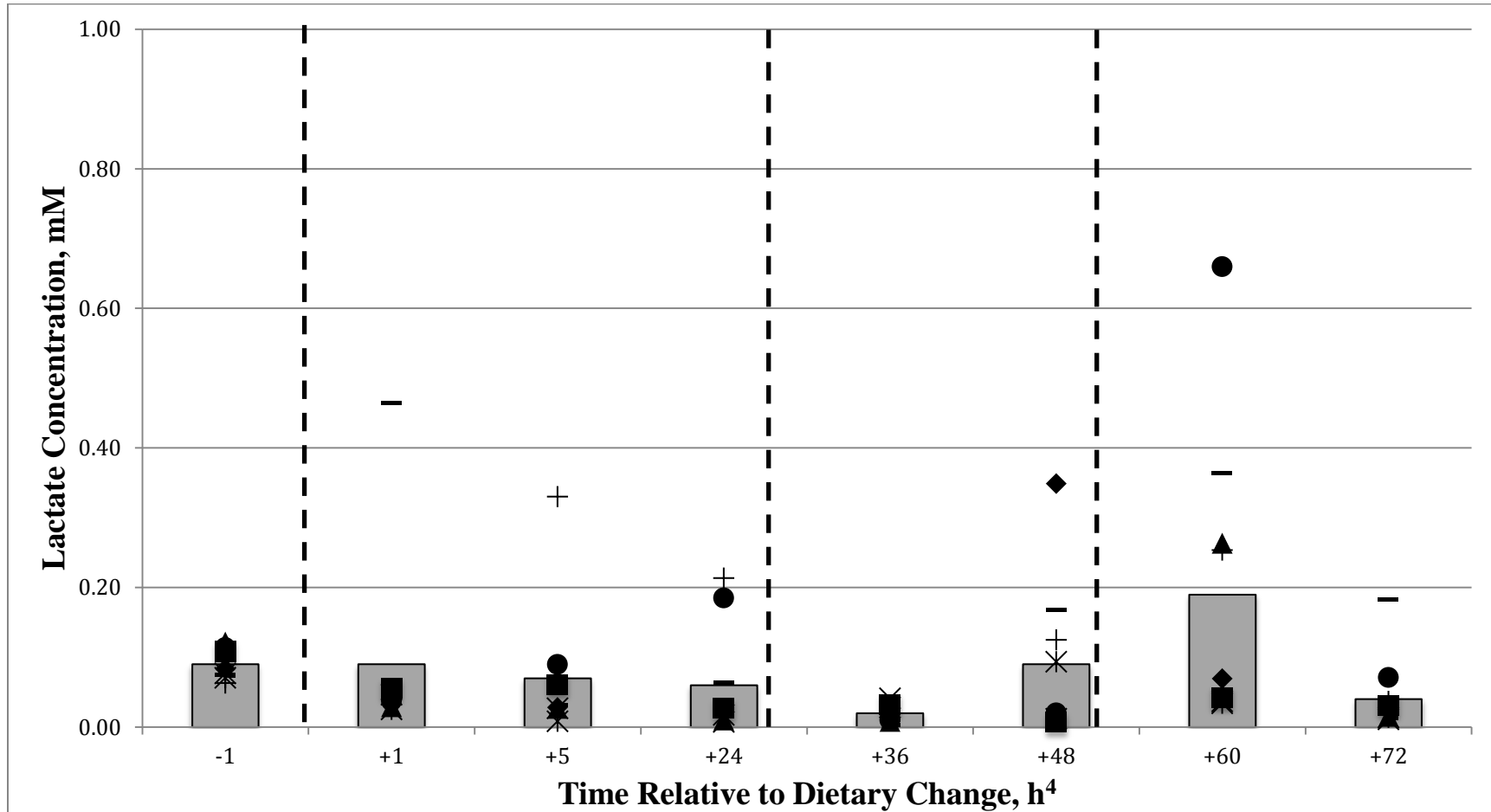
¹BL = Baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed); CD = Complete pelleted diet (2.0% BW)

²Each symbol represents values obtained from individual animals; bars represent the group LSMEANS

³Dashed vertical lines represent CD meal offerings at 0 h and immediately following the 24 h and 48 h collection of cecal fluid

⁴The “-1” timepoint represents the LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 21 and on d 28 and 29 while horses were consuming BL

Figure 2.4 Individual concentrations of total lactate in cecal fluid compared to the group mean in response to an abrupt dietary change from BL to GH¹ in Experiment 2^{2,3}



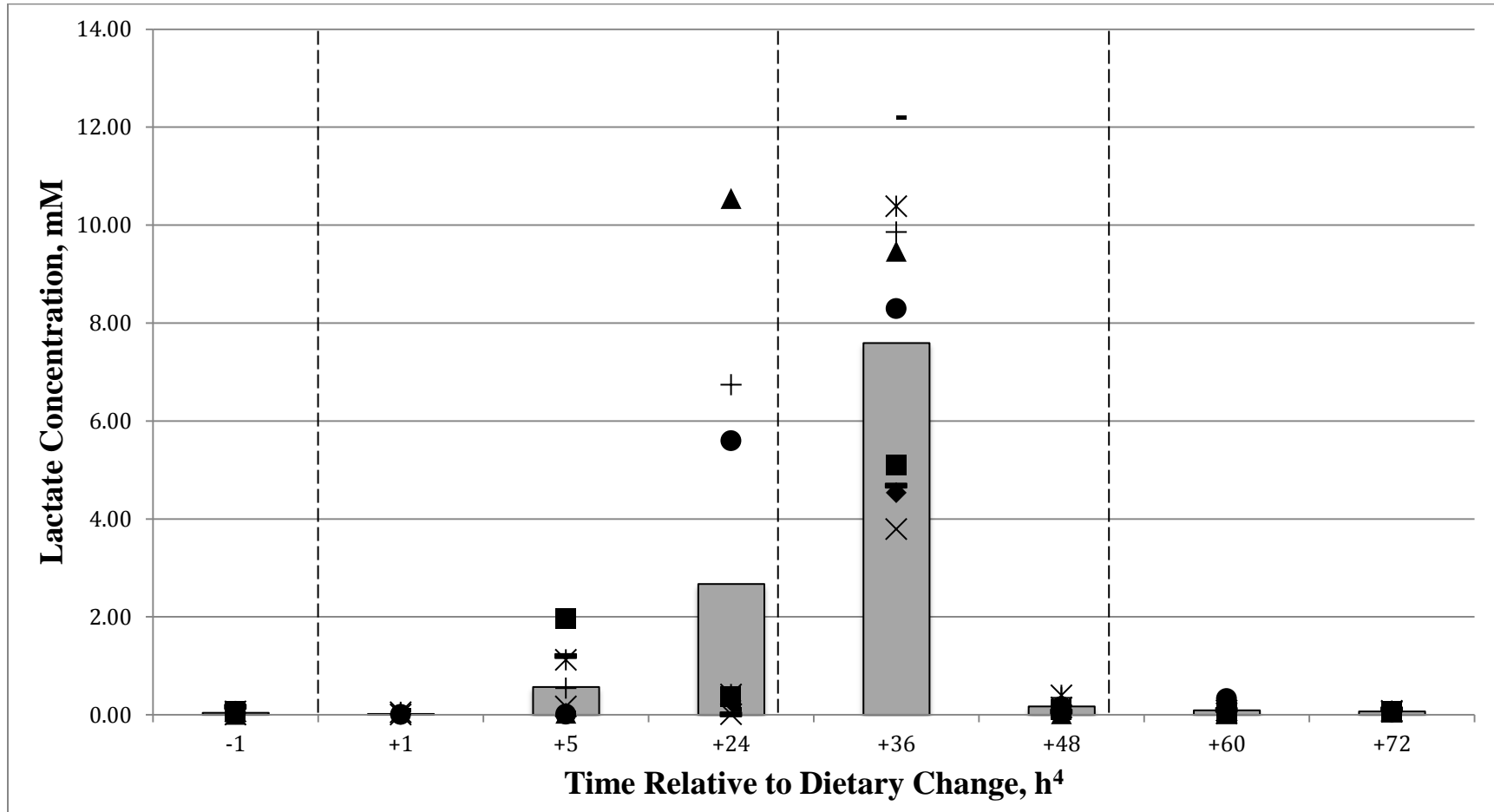
¹BL = Baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed); GH = Grass hay (2.5% BW native prairie hay)

²Symbols represent values obtained from individual animals; shaded bars represent the LSMEANS for the group

³Dashed, vertical lines represent GH meal offerings at 0 h and immediately following the 24 h and 48 h collection of cecal fluid

⁴The “-1” timepoint represents the LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 18 and 22 through 27 while horses were consuming BL

Figure 2.5 Concentrations of total lactate in cecal fluid of individual horses compared to the group mean in response to an abrupt dietary change from GH to AH¹ in Experiment 3^{2,3}



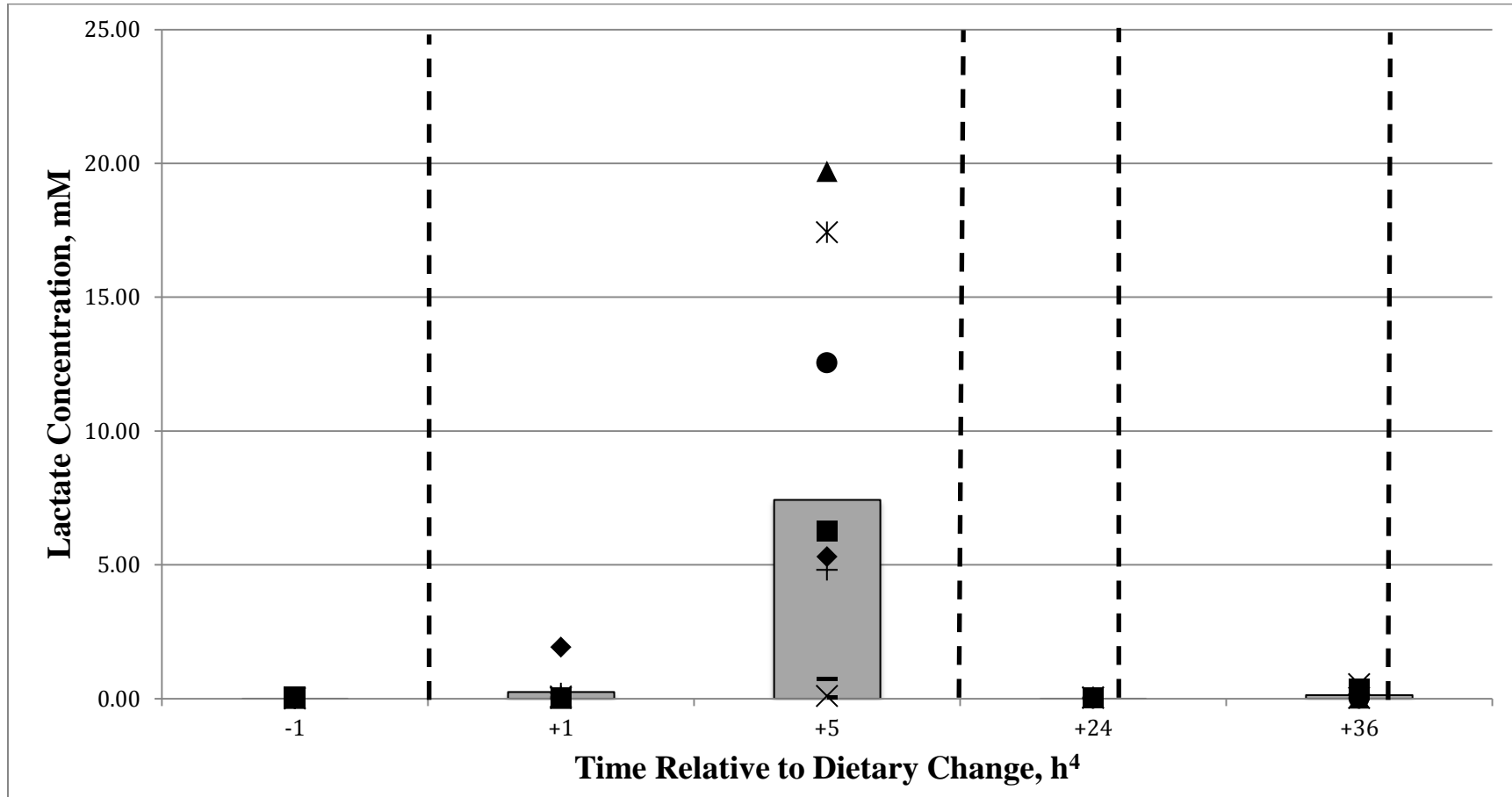
¹GH = Grass hay (2.5% BW native prairie hay); AH = Alfalfa hay (2.0% BW)

²Symbols represent values obtained from individual animals; shaded bars represent the LSMEANS for the group

³Dashed, vertical lines represent AH meal offerings at 0 h and immediately following the 24 h and 48 h collection of cecal fluid

⁴The “-1” timepoint represents the LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 22 while horses were consuming BL

Figure 2.6 Individual concentrations of total lactate in cecal fluid compared to the group mean in response to an abrupt dietary change from BL to TF¹ in Experiment 4^{2,3}



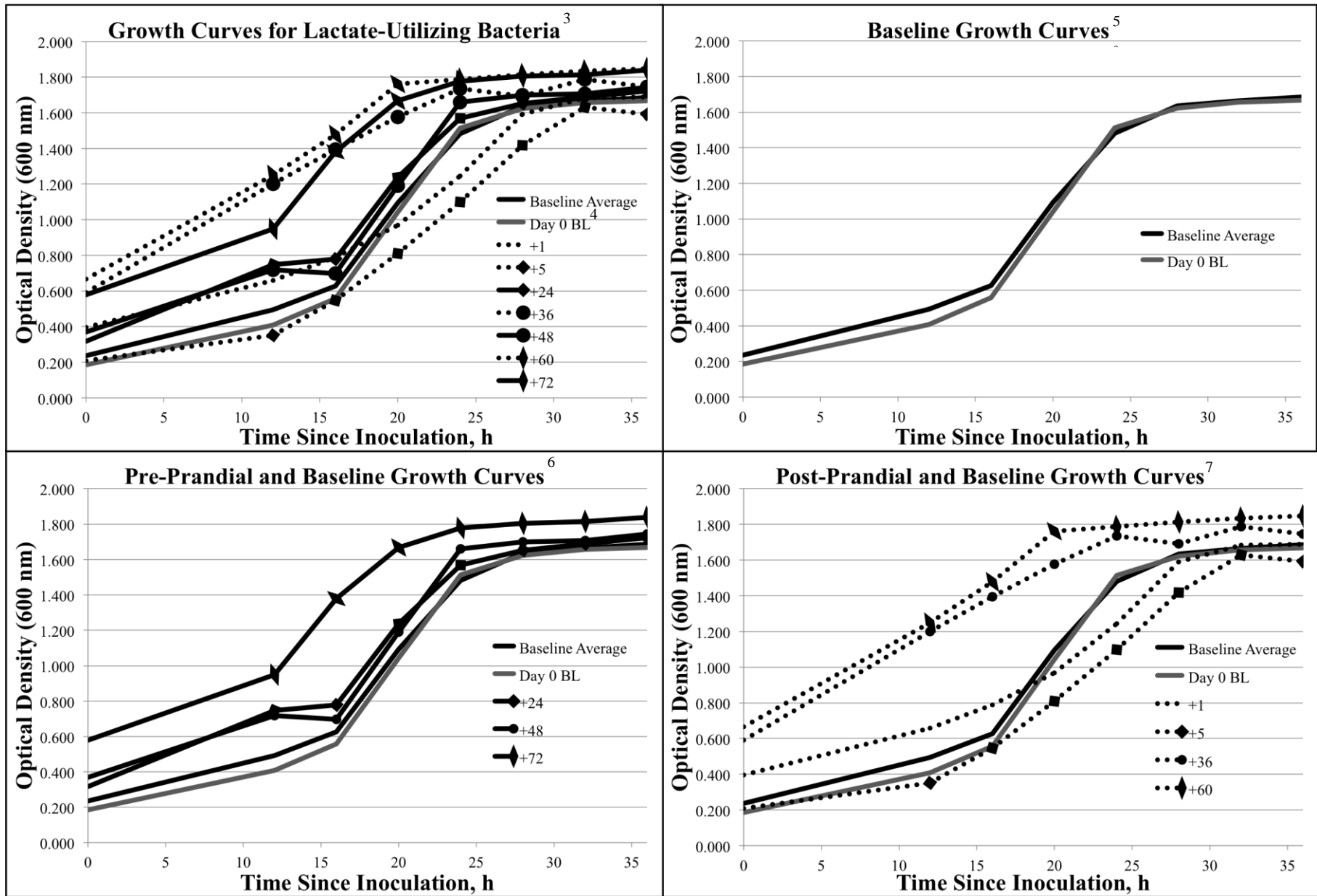
¹BL = Baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed); TF = Textured feed (1.0% BW; Omolene 200, Purina Mills, LLC, Gray Summit, MO)

²Symbols represent values obtained from individual animals; bars represent the LSMEANS for the group

³Dashed, vertical lines represent TF (0 h) and recovery hay (12 h, 24 h, 36 h) meal offerings

⁴The “-1” timepoint represents the LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 22 while horses were consuming BL

Figure 2.7 Lactate-utilizing bacterial growth curves in response to an abrupt dietary change from GH to AH¹ in Experiment 3²



¹GH = Grass hay (2.5% BW native prairie hay); AH = Alfalfa hay (2.0% BW)

²Obtained from mixed cecal contents and incubated in a semi-defined lactate medium in anaerobic Hungate culture tubes at 38°C for 36 h

³Graph depicts bacterial growth curves from all cecal samples collected at each time point. The mean growth curve generated from bacteria collected during the baseline cecal collections (Baseline Average: -1 h relative to feeding on d 14 to 21) is represented by the solid, black line. The baseline collection taken 1 h prior to horses consuming the novel meal on the morning of the dietary change is represented by the solid, grey line and labeled Day 0 BL. Other growth curves generated from cecal samples collected preprandially on each day following the dietary change are represented by solid, black lines and markers as follows: 24 h (■), 48 h (●), and 72 h (◆). Growth curves generated from postprandial cecal collections are represented by dotted lines and markers as follows: 1 h (◦), 5 h (■), 36 h (●), and 60 h (◆)

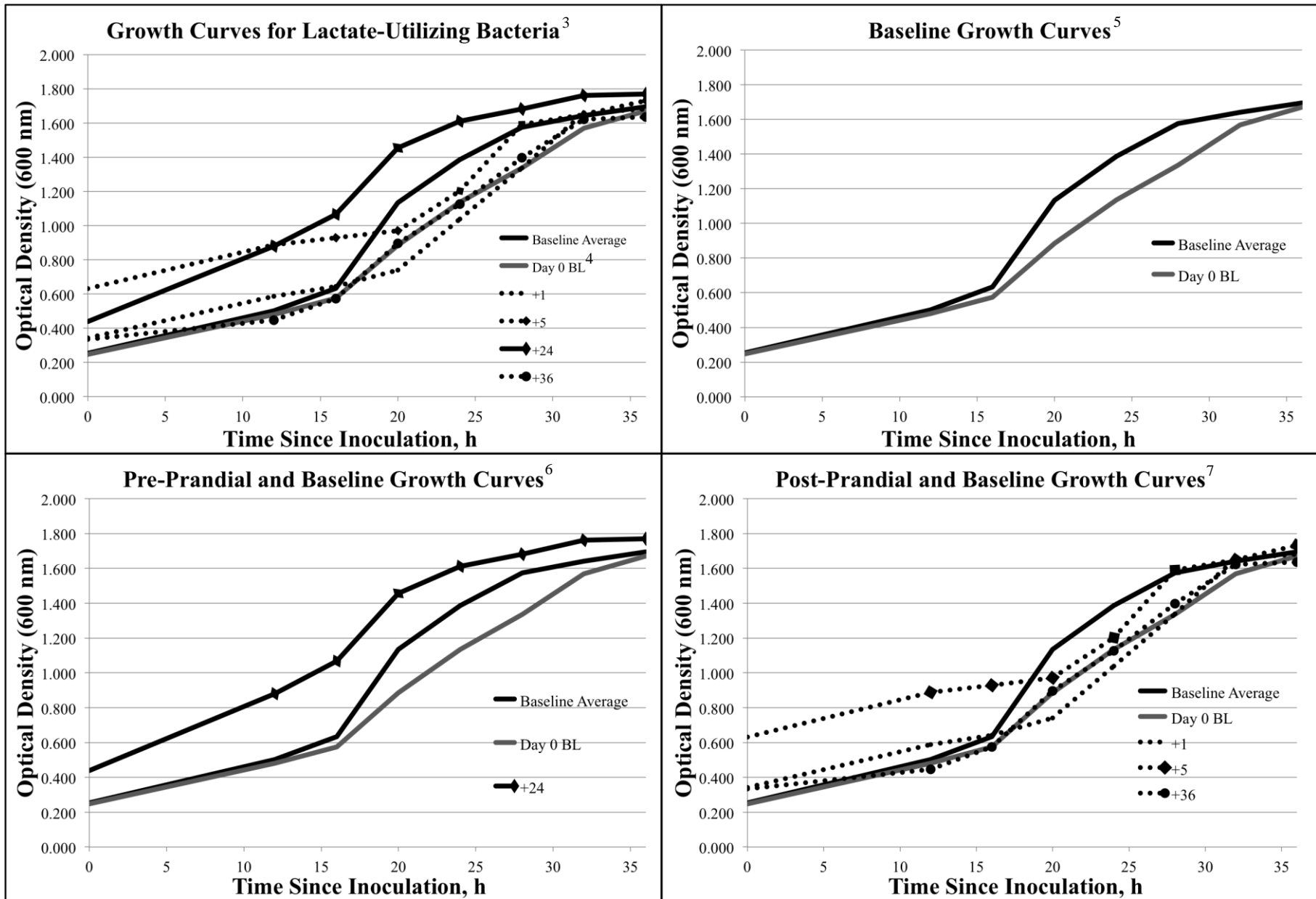
⁴BL = Baseline collection; reflects bacterial growth obtained from cecal fluid collected 1 h prior to the dietary change

⁵Graph isolates the mean baseline bacterial growth curves from all cecal samples collected 1 h prior to the morning meal on d 14 to 21 and just prior to the dietary change (-1 h) on d 22

⁶Graph compares the growth curves generated by bacteria obtained from cecal fluid collected preprandial to the morning meal on each of the days following the dietary change (24 h, 48 h, 72 h) to the baseline growth curves

⁷Graph compares the growth curves generated by bacteria obtained from cecal fluid collected 1 h, 5 h, and 12 h postprandial to the morning meal (36 h and 60 h) to the baseline growth curves

Figure 2.8 Lactate-utilizing bacterial growth curves in response to an abrupt diet change from BL to TF¹ in Experiment 4²



¹BL = Baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed); TF = Textured feed (1.0% BW)

²Obtained from mixed cecal contents and incubated in a semi-defined lactate medium in anaerobic Hungate culture tubes at 38°C for 36 h

³Graph depicts bacterial growth curves from all cecal samples collected at each time point. The mean growth curve for the baseline collections (d 14 to 21) is represented by the solid, black line. The baseline collection taken 1 h prior to horses consuming the novel meal on the morning of the dietary change is represented by the solid, grey line. Other cecal samples collected preprandially are represented by solid, black lines and markers as follows: 24 h (◆). Postprandial collections are represented by dotted lines and markers as follows: 1 h (•), 5 h (■), and 36 h (●)

⁴BL = Baseline collection; reflects bacterial growth obtained from cecal fluid collected 1 h prior to the dietary change

⁵ Graph isolates the mean baseline growth curves from all cecal samples collected 1 h prior to the morning meal on d 14 through 21 and just prior to the dietary change (-1 h) on d 22

⁶ Graph compares the growth curves generated by bacteria obtained from cecal fluid collected preprandial to the morning meal (24 h) to the baseline growth curves

⁷ Graph compares the growth curves generated by bacteria obtained from cecal fluid collected 1 h, 5 h, and 12 h postprandial to the morning meal (36 h) to the baseline growth curves

Table 2.1 Semi-defined Lactate Medium¹

Ingredient	% in medium
Sodium lactate (60% w/v)	2.0
Yeast extract	0.5
Peptone	0.5
Mineral solution 3²	2.5
Trace mineral solution³	0.05
Indigo carmine solution (1.0%)	0.1
Distilled water	93.9
Vitamin solution 2⁴	0.2
Autoclaved L-cysteine solution (2.5%)⁵	0.2
Autoclaved sodium sulfide solution (2.5%)⁵	0.2

¹Prepared under N₂; weight/volume unless otherwise specified; final pH 5.5

²KH₂PO₄, 0.6%; (NH₄)₂SO₄, 0.6%; NaCl, 1.2%; MgSO₄·7H₂O, 0.25%; CaCl₂·2H₂O, 0.16%

³ZnSO₄·7H₂O, 0.044%; MnSO₄·H₂O, 0.031%; CoCl₂·6H₂O, 0.008%; CuSO₄·5H₂O, 0.008%; Na₂MoO₄·2H₂O, 0.005%; KI, 0.001%

⁴Biotin Solution, 1.0% (NH₄CO₃, 1.06%; Biotin, 1.0%); Pyridoxine Hydrochloride, 0.2%; Calcium D Pantothenate, 0.2%; solution added after autoclaving medium

⁵Prepared as a sterile solution (autoclaved at 121°C for 10 min) which was added after autoclaving medium

Table 2.2 Proximate analyses of the dietary ingredients, as well as the dietary totals, utilized in Experiment 1

Item	Proximate Analyses			Dietary Totals ¹	
	GH ²	TF ³	CD ⁴	BL ⁵	CD
Dry Matter, %	93.5	93.3	95.3	93.5	95.3
Neutral Detergent Fiber, %	67.3	18.6	39.9	55.1	39.9
Acid Detergent Fiber, %	40.3	7.6	25.0	32.1	25.0
Non-fiber Carbohydrates, %	16.0	52.9	30.7	25.2	30.7
Starch, %	0.9	37.0	11.3	9.9	11.3
Water Soluble Carbohydrates, %	9.3	8.2	8.7	9.0	8.7
Ether Soluble Carbohydrates, %	5.5	6.4	4.4	5.7	4.4
Crude Protein, %	6.9	16.8	16.6	9.4	16.6
Ether Extract, %	2.1	6.7	7.0	3.3	7.0
Ash, %	10.8	6.8	9.8	9.8	9.8
Digestible Energy, Mcal kg ⁻¹	1.81	3.42	2.69	2.20	2.69

¹Dietary totals for BL were calculated using proximate analyses results and relative proportions of each dietary ingredient fed during the pertinent phase of the experiment. The dietary total for CD was the same as the proximate analyses as that was the only dietary ingredient fed.

²GH = Native prairie hay

³TF = Textured feed (Omolene 200, Purina Mills, St. Louis, MO)

⁴CD = Complete pelleted diet (2.0% BW; Equine Senior, Purina Mills, St. Louis, MO)

⁵BL = Baseline diet (1.5% BW GH, 0.5% BW TF)

Table 2.3 Proximate analyses of the dietary ingredients, as well as the dietary totals, utilized in Experiment 2

Item	Proximate Analyses		Dietary Totals ¹	
	GH ²	TF ³	BL ⁴	GH ⁵
Dry Matter, %	93.5	93.3	93.5	93.5
Neutral Detergent Fiber, %	67.3	18.6	55.1	67.3
Acid Detergent Fiber, %	40.3	7.6	32.1	40.3
Non-fiber Carbohydrates, %	16.0	52.9	25.2	16.0
Starch, %	0.9	37.0	9.9	0.9
Water Soluble Carbohydrates, %	9.3	8.2	9.0	9.3
Ether Soluble Carbohydrates, %	5.5	6.4	5.7	5.5
Crude Protein, %	6.9	16.8	9.4	6.9
Ether Extract, %	2.1	6.7	3.3	2.1
Ash, %	10.8	6.8	9.8	10.8
Digestible Energy, Mcal kg ⁻¹	1.81	3.42	2.20	1.81

¹Dietary totals for BL were calculated using proximate analyses results and relative proportions of each dietary ingredient fed during the pertinent phase of the experiment

²GH = Native prairie hay

³TF = Textured feed (Omolene 200, Purina Mills, St. Louis, MO)

⁴BL = Baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed)

⁵GH = Grass hay (2.5% BW native prairie hay)

Table 2.4 Proximate analyses of the grass hay and alfalfa hay utilized in Experiment 3

Item	Proximate Analyses	
	GH¹	AH²
Dry Matter, %	93.5	92.9
Neutral Detergent Fiber, %	67.3	37.2
Acid Detergent Fiber, %	40.3	29.6
Non-fiber Carbohydrates, %	16.0	31.1
Starch, %	0.9	2.1
Water Soluble Carbohydrates, %	9.3	10.0
Ether Soluble Carbohydrates, %	5.5	7.7
Crude Protein, %	6.9	22.9
Ether Extract, %	2.1	2.0
Ash, %	10.8	10.8
Digestible Energy, Mcal kg⁻¹	1.81	2.45

¹GH = Grass hay (2.5% BW native prairie hay)

²AH = Alfalfa hay (2.0% BW)

Table 2.5 Proximate analyses of the dietary ingredients, as well as the dietary totals, utilized in Experiment 4

Item	Proximate Analyses		Dietary Totals¹	
	GH²	TF³	BL⁴	TF
Dry Matter, %	93.5	93.3	93.5	93.3
Neutral Detergent Fiber, %	67.3	18.6	55.1	18.6
Acid Detergent Fiber, %	40.3	7.6	32.1	7.6
Non-fiber Carbohydrates, %	16.0	52.9	25.2	52.9
Starch, %	0.9	37.0	9.9	37.0
Water Soluble Carbohydrates, %	9.3	8.2	9.0	8.2
Ether Soluble Carbohydrates, %	5.5	6.4	5.7	6.4
Crude Protein, %	6.9	16.8	9.4	16.8
Ether Extract, %	2.1	6.7	3.3	6.7
Ash, %	10.8	6.8	9.8	6.8
Digestible Energy, Mcal kg⁻¹	1.81	3.42	2.20	3.42

¹Dietary totals for BL were calculated using proximate analyses results and relative proportions of each dietary ingredient fed during the pertinent phase of the experiment. TF represents the proximate analysis of a single meal. GH was offered at 12 (0.5% BW), 24 (1.0% BW), and 36 h (1.5 % BW) following the dietary change.

²GH = Native prairie hay

³TF = Textured feed, Omolene 200, Purina Mills, St. Louis, MO

⁴BL = Baseline diet (1.5% BW native prairie hay, 0.5% BW textured feed)

Table 2.6 Cecal pH and concentrations of total lactate relative to the abrupt dietary change from BL to CD¹ in Experiment 1^{2,3}

Parameter Measured	Time of Collection ⁴								SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	+48 h	+60 h	+72 h	
pH	6.87 ^a	6.59 ^b	6.01 ^c	7.09 ^a	7.10 ^a	6.01 ^c	5.63 ^c	5.83 ^c	0.10
Total Lactate, mM	0.09 ^{a,b}	0.13 ^a	1.01 ^{b,c}	0.77 ^{a,b,c}	0.82 ^{a,b,c}	1.13 ^{c,d}	1.98 ^d	0.51 ^{a,c}	0.22

¹BL = Baseline diet [1.5% BW native prairie hay, 0.5% BW textured feed (Omolene 200, Purina Mills, LLC, Gray Summit, MO)];

CD = Complete pelleted diet (2.0% BW; Equine Senior, Purina Mills, LLC, Gray Summit, MO)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from BL to CD [$n = 9$ on each of 10 d prior to dietary change for a total $n = 90$ at -1 h; $n = 9$ for each timepoint (+1 h through +72 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 21, d 28 and d 29 while horses were consuming BL

Table 2.7 Fecal pH, concentrations of total lactate and VFA, and DM relative to the abrupt dietary change from BL to CD¹ in Experiment 1^{2,3}

Parameter Measured	Time of Collection ⁴								SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	+48 h	+60 h	+72 h	
pH	6.60 ^a	6.46 ^a	6.04 ^b	7.29 ^c	7.31 ^c	6.13 ^b	5.69 ^d	6.07 ^b	0.07
Total Lactate, mM	0.17	0.24	0.22	0.22	0.13	0.25	0.10	0.14	0.08
Total VFA, mM	11.63 ^a	13.49 ^a	17.32 ^{a,b}	10.77 ^a	26.67 ^{b,c}	36.81 ^d	35.86 ^{c,d}	26.18 ^{b,c}	2.44
Acetate, mM	8.48 ^a	9.82 ^a	12.34 ^{a,b}	7.79 ^a	18.34 ^{b,c}	24.80 ^c	23.42 ^c	16.80 ^b	1.52
Propionate, mM	1.93 ^a	2.23 ^a	2.88 ^{a,b}	1.76 ^a	4.57 ^{b,c}	6.47 ^{c,d}	6.71 ^d	5.46 ^{c,d}	0.61
Iso-butyrate, mM	0.16 ^a	0.19 ^a	0.26 ^a	0.16 ^a	0.49 ^b	0.68 ^b	0.69 ^b	0.50 ^b	0.06
Butyrate, mM	0.78 ^a	0.96 ^a	1.38 ^{a,b}	0.76 ^a	2.21 ^b	3.36 ^{c,d}	3.49 ^d	2.26 ^{b,c}	0.31
2-methyl-valerate, mM	0.08 ^a	0.08 ^a	0.14 ^a	0.09 ^a	0.32 ^b	0.44 ^b	0.45 ^b	0.33 ^b	0.05
3-methyl-valerate, mM	0.10 ^a	0.10 ^a	0.17 ^a	0.11 ^a	0.36 ^b	0.47 ^b	0.49 ^b	0.37 ^b	0.05
Valerate, mM	0.10 ^a	0.10 ^a	0.17 ^a	0.09 ^a	0.38 ^b	0.60 ^c	0.62 ^c	0.47 ^{b,c}	0.05
Acetate:Propionate	5.00 ^{a,b,c}	5.46 ^{a,b}	5.08 ^{a,b,c}	5.58 ^a	4.21 ^{a,b,c}	4.07 ^{a,b,c}	3.63 ^{b,c}	3.39 ^c	0.63
DM, %	28.34	29.06	29.41	29.55	29.68	28.85	27.39	28.37	0.81

¹BL = Baseline diet [1.5% BW native prairie hay, 0.5% BW textured feed (Omolene 200, Purina Mills, LLC, Gray Summit, MO)];

CD = Complete pelleted diet (2.0% BW; Equine Senior, Purina Mills, LLC, Gray Summit, MO)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from BL to CD [$n = 9$ on each of 10 d prior to dietary change for a total $n = 90$ at -1 h; $n = 9$ for each timepoint (+1 h through +72 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 21, d 28 and d 29 while horses were consuming BL

Table 2.8 Cecal pH and concentrations of total lactate and VFA relative to the abrupt dietary change from BL to GH¹ in Experiment 2^{2,3}

Parameter Measured	Time of Collection ⁴								SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	+48 h	+60 h	+72 h	
pH	6.87 ^a	6.78 ^a	6.55 ^b	6.81 ^a	6.61 ^b	6.85 ^a	6.53 ^b	6.78 ^a	0.03
Total Lactate, mM	0.09 ^{a,b}	0.09 ^{a,b}	0.07 ^{a,b}	0.05 ^{a,b}	0.02 ^a	0.08 ^{a,b}	0.19 ^b	0.04 ^{a,b}	0.04
Total VFA, mM	49.26 ^{a,b,c}	45.33 ^b	56.04 ^{b,c}	49.59 ^{a,b}	60.25 ^c	49.02 ^{a,b}	59.78 ^c	52.37 ^b	2.52
Acetate, mM	37.27 ^{a,b}	34.40 ^b	42.40 ^{a,b}	37.54 ^{a,b}	46.71 ^a	36.99 ^{a,b}	45.14 ^a	39.02 ^{a,b}	2.19
Propionate, mM	8.74	7.81	9.24	8.41	8.12	8.08	8.82	8.82	0.73
Iso-butyrate, mM	0.17 ^a	0.13 ^{a,b}	0.11 ^b	0.12 ^{a,b}	0.12 ^{a,b}	0.13 ^{a,b}	0.12 ^{a,b}	0.15 ^{a,b}	0.01
Butyrate, mM	2.95 ^a	2.86 ^a	4.19 ^{b,c}	3.41 ^{a,b}	5.17 ^{b,c,d}	3.69 ^{a,b}	5.56 ^d	4.23 ^b	0.24
2-methyl-valerate, mM	0.03 ^a	0.03 ^{a,b}	0.00 ^b	0.02 ^{a,b}	0.00 ^b	0.00 ^{a,b}	0.00 ^b	0.01 ^{a,b}	0.01
3-methyl-valerate, mM	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.05	0.01
Valerate, mM	0.06 ^{a,b}	0.05 ^a	0.07 ^{a,b}	0.05 ^a	0.10 ^{b,c}	0.08 ^{a,b,c}	0.11 ^c	0.10 ^{b,c}	0.01
Acetate:Propionate	4.32 ^a	4.53 ^a	5.51 ^{a,b}	4.64 ^{a,b}	5.78 ^b	4.62 ^{a,b}	5.19 ^{a,b}	4.48 ^{a,b}	0.32

¹BL = Baseline diet [1.5% BW native prairie hay, 0.5% BW textured feed (Omolene 200, Purina Mills, LLC, Gray Summit, MO)];

GH = Grass hay (2.5% BW native prairie hay)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from BL to GH [$n = 9$ on each of 11 d prior to dietary change for a total $n = 99$ at -1 h; $n = 9$ for each timepoint (+1 h through +72 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 18 and days 22 through 27 while horses were consuming BL

Table 2.9 Fecal pH, concentrations of total lactate and VFA, and DM relative to an abrupt dietary change from BL to GH¹ in Experiment 2^{2,3}

Parameter measured	Time of Collection ⁴								SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	+48 h	+60 h	+72 h	
pH	6.39 ^{a,b}	6.20 ^a	6.24 ^a	6.59 ^{b,c,d}	6.55 ^{b,c,d}	6.78 ^{c,d}	6.49 ^{a,b,c}	6.79 ^d	0.09
Total Lactate, mM	0.11	0.06	0.05	0.10	0.05	0.05	0.07	0.12	0.04
Total VFA, mM	12.03 ^{a,b}	12.80 ^a	11.25 ^{a,b,c}	10.16 ^{a,b,c}	8.39 ^c	8.53 ^{b,c}	11.34 ^{a,b,c}	9.93 ^{a,b,c}	0.76
Acetate, mM	8.95 ^{a,b}	9.38 ^b	8.09 ^{a,b,c}	7.92 ^{a,b,c}	6.30 ^c	6.61 ^{a,c}	8.80 ^{a,b}	7.95 ^{a,b,c}	0.60
Propionate, mM	1.85 ^{a,b}	2.14 ^b	1.89 ^{a,b}	1.37 ^{a,c}	1.37 ^{a,c}	1.17 ^c	1.53 ^{a,b,c}	1.11 ^c	0.19
Iso-butyrate, mM	0.16	0.16	0.16	0.17	0.13	0.15	0.19	0.17	0.02
Butyrate, mM	0.81 ^{a,b}	0.90 ^b	0.85 ^b	0.47 ^c	0.45 ^c	0.46 ^c	0.61 ^{a,c}	0.51 ^c	0.05
2-methyl-valerate, mM	0.07	0.06	0.08	0.09	0.05	0.06	0.08	0.10	0.02
3-methyl-valerate, mM	0.08	0.07	0.08	0.09	0.05	0.05	0.07	0.05	0.01
Valerate, mM	0.10 ^a	0.09 ^a	0.10 ^a	0.06 ^b	0.04 ^b	0.04 ^b	0.07 ^{a,b}	0.04 ^b	0.01
Acetate:Propionate	5.56 ^{a,b}	5.42 ^{a,b}	4.61 ^a	6.52 ^{a,b}	5.18 ^{a,b}	5.94 ^{a,b}	6.21 ^{a,b}	7.70 ^b	0.81
DM, %	29.25 ^a	29.24 ^{a,b}	29.83 ^a	26.63 ^c	27.07 ^{b,c}	26.32 ^c	26.77 ^c	26.24 ^c	0.67

¹BL = Baseline diet [1.5% BW native prairie hay, 0.5% BW textured feed (Omolene 200, Purina Mills, LLC, Gray Summit, MO)];

GH = Grass hay (2.5% BW native prairie hay)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from BL to GH [$n = 9$ on each of 11 d prior to dietary change for a total $n = 99$ at -1 h; $n = 9$ for each timepoint (+1 h through +72 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 18 and days 22 through 27 while horses were consuming BL

Table 2.10 Cecal pH and concentrations of total lactate and VFA relative to the abrupt dietary change from GH to AH¹ in Experiment 3^{2,3}

Parameter Measured	Time of Collection ⁴								SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	+48 h	+60 h	+72 h	
pH	6.72 ^a	6.71 ^a	6.52 ^{b,c}	6.63 ^{a,c}	6.49 ^{b,c}	6.60 ^{a,c}	6.40 ^b	6.51 ^{b,c}	0.05
Total Lactate, mM	0.04 ^a	0.01 ^{a,b}	0.57 ^{a,b}	2.67 ^b	7.59 ^c	0.17 ^{a,b}	0.09 ^{a,b}	0.07 ^{a,b}	0.63
Total VFA, mM	52.17 ^a	49.03 ^a	76.22 ^b	86.23 ^b	87.47 ^b	74.23 ^b	78.68 ^b	73.76 ^b	4.92
Acetate, mM	39.08 ^a	36.95 ^a	59.70 ^b	64.81 ^b	65.17 ^b	55.74 ^b	58.60 ^b	55.48 ^b	3.49
Propionate, mM	9.02 ^a	8.74 ^a	10.90 ^{a,b}	14.18 ^{b,c}	15.55 ^c	12.51 ^{a,c}	13.75 ^{b,c}	12.42 ^{a,c}	1.06
Iso-butyrate, mM	0.11 ^a	0.10 ^a	0.14 ^{a,c}	0.24 ^b	0.21 ^{b,c}	0.20 ^{b,c}	0.16 ^{a,c}	0.19 ^{b,c}	0.01
Butyrate, mM	3.83 ^{a,b}	3.14 ^b	5.28 ^c	6.49 ^c	6.03 ^c	5.32 ^{a,c}	5.76 ^c	5.23 ^{a,c}	0.52
2-methyl-valerate, mM	0.02 ^{a,c}	0.02 ^c	0.03 ^{a,c}	0.08 ^b	0.07 ^b	0.07 ^b	0.05 ^{a,b}	0.06 ^b	0.01
3-methyl-valerate, mM	0.04 ^a	0.03 ^a	0.04 ^{a,b}	0.10 ^c	0.10 ^c	0.10 ^c	0.08 ^{b,c}	0.09 ^c	0.01
Valerate, mM	0.07 ^a	0.06 ^a	0.13 ^b	0.34 ^c	0.36 ^c	0.29 ^c	0.29 ^c	0.29 ^c	0.03
Acetate:Propionate	4.40 ^a	4.23 ^a	5.48 ^b	4.55 ^a	4.30 ^a	4.57 ^a	4.46 ^a	4.57 ^a	0.20

¹GH = Grass hay (2.5% BW native prairie hay); AH = Alfalfa hay (2.0% BW)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from GH to AH [$n = 9$ on each of 9 d prior to dietary change for a total $n = 81$; $n = 9$ for each timepoint (+1 h through +72 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 22 while horses were consuming GH

Table 2.11 Fecal pH, concentrations of total lactate and VFA, and DM relative to the abrupt dietary change from GH to AH¹ in Experiment 3^{2,3}

Parameter Measured	Time of Collection ⁴								SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	+48 h	+60 h	+72 h	
pH	6.48 ^{a,c}	6.36 ^{a,c}	6.20 ^c	6.69 ^{a,b}	6.53 ^{a,c,e}	6.99 ^{b,d}	6.87 ^{b,d,e}	7.10 ^d	0.09
Total Lactate, mM	0.10	0.12	0.08	0.16	0.18	0.11	0.05	0.06	0.06
Total VFA, mM	15.36	12.40	15.36	11.68	17.99	7.83	10.44	8.54	2.33
Acetate, mM	11.64	9.09	11.42	8.09	11.66	5.23	6.86	5.73	1.58
Propionate, mM	2.40 ^{a,b}	2.20 ^{a,b}	2.69 ^a	1.60 ^{a,b}	2.72 ^a	1.18 ^b	1.71 ^{a,b}	1.34 ^{a,b}	0.38
Iso-butyrate, mM	0.22 ^a	0.19 ^a	0.22 ^a	0.27 ^a	0.52 ^b	0.27 ^a	0.33 ^{a,b}	0.23 ^a	0.06
Butyrate, mM	0.75 ^{a,b}	0.63 ^a	0.73 ^{a,b}	0.77 ^a	1.58 ^b	0.72 ^a	1.00 ^{a,b}	0.72 ^{a,b}	0.21
2-methyl-valerate, mM	0.12 ^a	0.10 ^a	0.10 ^a	0.62 ^{b,c}	0.64 ^c	0.16 ^{a,b}	0.19 ^{a,b,c}	0.17 ^{a,b,c}	0.11
3-methyl-valerate, mM	0.12 ^a	0.10 ^a	0.11 ^a	0.19 ^a	0.46 ^b	0.16 ^a	0.20 ^{a,b}	0.18 ^a	0.07
Valerate, mM	0.10 ^a	0.09 ^a	0.10 ^a	0.14 ^a	0.42 ^b	0.13 ^a	0.16 ^a	0.15 ^a	0.06
Acetate:Propionate	5.37	4.82	5.20	5.24	4.71	4.83	4.33	4.57	0.71
DM, %	27.24 ^b	27.48 ^{a,b}	26.38 ^{a,b,c}	25.10 ^{a,b,c}	23.57 ^{a,c,d,e}	22.14 ^{c,d,e}	21.38 ^{d,e}	21.74 ^e	0.97

¹GH = Grass hay (2.5% BW native prairie hay); AH = Alfalfa hay (2.0% BW)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from GH to AH [$n = 9$ on each of 9 d prior to dietary change for a total $n = 81$; $n = 9$ for each timepoint (+1 h through +72 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 22 while horses were consuming GH

Table 2.12 Cecal pH and concentrations of total lactate and VFA relative to the abrupt dietary change from BL to TF¹ in Experiment 4^{2,3}

Parameter Measured	Time of Collection ⁴					SEM
	- 1 h ⁵	+1 h	+5 h	+24 h	+36 h	
pH	6.96 ^a	7.03 ^a	6.59 ^b	7.07 ^a	7.13 ^a	0.09
Total Lactate, mM	0.04 ^a	0.23 ^a	7.43 ^b	0.00 ^a	0.10 ^a	1.15
Total VFA, mM	47.35 ^{a,c}	40.46 ^b	58.27 ^c	39.64 ^{a,b}	35.90 ^{a,b}	3.98
Acetate, mM	35.16 ^{a,c}	29.11 ^b	39.13 ^c	28.10 ^{a,b}	27.88 ^{a,b}	2.89
Propionate, mM	9.04 ^a	8.65 ^{a,c}	14.95 ^b	8.34 ^{a,c}	5.37 ^{a,c}	0.93
Iso-butyrate, mM	0.15 ^a	0.15 ^a	0.17 ^a	0.19 ^a	0.09 ^b	0.02
Butyrate, mM	2.86 ^a	2.38 ^b	3.75 ^c	2.77 ^{a,b,c}	2.48 ^{a,b}	0.31
2-methyl-valerate, mM	0.04 ^{a,b,c}	0.04 ^{a,b}	0.04 ^{a,b}	0.05 ^b	0.02 ^c	0.01
3-methyl-valerate, mM	0.06 ^{a,b}	0.06 ^{a,b}	0.07 ^a	0.08 ^a	0.03 ^b	0.01
Valerate, mM	0.07 ^a	0.07 ^a	0.16 ^c	0.12 ^b	0.04 ^a	0.01
Acetate:Propionate	3.94 ^a	3.40 ^b	2.66 ^c	3.69 ^{a,b}	5.62 ^d	0.24

¹BL = Baseline diet [1.5% BW native prairie hay, 0.5% BW textured feed (Omolene 200, Purina Mills, LLC, Gray Summit, MO)]; TF = Textured feed (1.0% BW; Omolene 200, Purina Mills, LLC, Gray Summit, MO)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from BL to TF [$n = 9$ on each of 9 d prior to dietary change for a total $n = 81$; $n = 9$ for each timepoint (+1 h through +36 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 22 while horses were consuming BL

Table 2.13 Fecal pH, concentrations of total lactate and VFA, and DM relative to the abrupt dietary change from BL to TF¹ in Experiment 4^{2,3}

Parameter Measured	Time of Collection ⁴					SEM
	-1 h ⁵	+1 h	+5 h	+24 h	+36 h	
pH	6.56 ^a	6.55 ^a	6.42 ^{a,b}	6.38 ^{a,b}	6.15 ^b	0.10
Total Lactate, mM	0.12	0.10	0.18	0.16	0.15	0.05
Total VFA, mM	11.08	9.46	9.27	11.13	12.20	1.15
Acetate, mM	8.05	6.85	6.63	7.87	8.67	0.81
Propionate, mM	1.91	1.64	1.74	2.11	2.40	0.29
Iso-butyrate, mM	0.15	0.16	0.13	0.15	0.17	0.02
Butyrate, mM	0.69	0.57	0.59	0.75	0.75	0.08
2-methyl-valerate, mM	0.08	0.08	0.06	0.07	0.07	0.02
3-methyl-valerate, mM	0.09	0.09	0.06	0.08	0.07	0.02
Valerate, mM	0.10	0.08	0.07	0.12	0.07	0.02
Acetate:Propionate	4.91	4.34	4.53	4.21	4.54	0.69
DM, %	28.78	29.50	31.16	30.01	30.45	1.21

¹BL = Baseline diet [1.5% BW native prairie hay, 0.5% BW textured feed (Omolene 200, Purina Mills, LLC, Gray Summit, MO)]; TF = Textured feed (1.0% BW; Omolene 200, Purina Mills, LLC, Gray Summit, MO)

²Data presented as LSMEANS

³Within row, values with different superscripts differ ($P < 0.05$)

⁴Relative to abrupt dietary change from BL to TF [$n = 9$ on each of 9 d prior to dietary change for a total $n = 81$; $n = 9$ for each timepoint (+1 h through +36 h) following the dietary change]

⁵Represents LSMEANS of all baseline measures taken 1 h prior to feeding on days 14 through 22 while horses were consuming BL