INFLUENCE OF CONFINEMENT HOUSING ON THE CECAL ENVIRONMENT OF THE HORSE

A Thesis

by

ASHLEY N. WOLFORD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Josie Coverdale
Committee Members,	Robin Anderson
	William Pinchak
	Tryon Wickersham
Head of Department,	H. Russell Cross

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ABSTRACT

Influence of Confinement Housing on the Cecal Environment of the Horse. (December 2011)

Ashley N. Wolford, B.S., Berry College

Chair of Advisory Committee: Dr. Josie Coverdale

Eight cecally cannulated Quarter Horse geldings were utilized in a crossover design with two 28 d periods with a 21 d washout period between. Horses were adapted to dietary treatments from d 1 to 19, dry matter intake was determined from d 20 to 24, and cecal fluid was collected on d 28. Horses were paired by age and body weight (BW) and randomly assigned to treatment. Treatments consisted of housing horses individually in stalls or group housed in a dry lot pen. Regardless of treatment, all horses were individually fed a pelleted concentrate at 1% BW (as fed) offered twice daily 12 h apart. All horses had ad libitum access to coastal bermudagrass hay. Hay was offered to stalled horses initially at 2% BW (as fed) then adjusted based on 120% of a previous 3 d average of voluntary intake.

A dual marker system was utilized for estimation of voluntary intake in all horses using titanium dioxide (TiO_2) as the external marker and acid detergent insoluble ash (ADIA) as the internal marker. Cecal samples were collected 4-h after the morning meal and immediately analyzed for pH. Samples were transported to the USDA/ARS laboratory to enumerate total anaerobic bacteria and lactic acid bacteria, and to determine methane and ammonia activity.

Cecal pH was influenced by housing ($P = 0.02$) with group housed horses having lower cecal pH values when compared to stalled horses (6.52 ± 0.04 and 6.69 ± 0.04, respectively). The cecal pH values of this study are similar to other reported values when

feeding similar diets (5). Populations of cecal total anaerobic bacteria and lactobacillus were not influenced by housing ($P \geq 0.21$). Treatments did not affect the production of acetate, propionate or butyrate ($P \geq 0.15$). Additionally, methane and ammonia production were not affected by treatments ($P \geq 0.17$). Forage intake was greater for group housed horses ($P = 0.04$) than stalled (8.47 ± 0.89 kg DM/d and 5.17 ± 0.89 kg DM/d, respectively). In conclusion, confinement housing did not greatly influence the cecal environment of a horse when similar diets were offered.

DEDICATION

I would like to dedicate this thesis to my family, since they have been my rock in this life. They have always been there for me, supported me and encouraged me. My mother and father have given me strength and wisdom in all of my pursuits. My sisters that have always been there for me and can always make me laugh no matter how stressed out I may be. My family gives me encouragement to get through life's challenges.

I would also like to dedicate this thesis to all of the people that have been a part of my college career and who have guided me and given me the knowledge and passion to progress in academia to accomplish my goals.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Common management practices in the equine industry present multiple challenges to the horse. The most common practices include feeding high-grain diets and confinement housing. Dietary management often consists of feeding horses 2 to 3 large meals per day. Performance horses require a large amount of digestible energy (DE); in order to meet this requirement, horses are supplemented with a high-grain diet that can include a greater percentage of non structural carbohydrates (NSC). Further, horses are housed in stalls at facilities with limited pasture turnout, or horses are housed on small acreage farms that provide inadequate grazing. These two management challenges pose potential problems to the gastrointestinal health of the horse due to the horses' unique digestive tract. Horses have a small stomach relative to their body size and are designed to eat many small meals throughout the day which is in contrast to the 2 to 3 meals typically fed. Feral horses' meals mainly consist of forage with limited amounts of NSC. By confining horses, owners are potentially affecting the horses' voluntary intake which could be caused by multiple factors: for example, an abnormal environment, or social isolation. These management practices are in contrast to the natural behavior of horses.

Cecum

Equids are herbivores that are also known as hindgut fermentors due to their unique anatomical characteristic of an enlarged cecum and colon. The hindgut is the site of fermentation where partially digested feedstuffs are exposed to microbial populations to be

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This thesis follows the style of Journal of Animal Science.

fermented. The cecum is the first segment of the large intestine located between the small intestine and large colon, and it is known as a blind sac due to its structure. In a mature horse, the cecum is approximately 1 m long with a capacity of 25 to 35 L (Frape, 2004). Digesta enters the cecum through the ileocecal value and exits through the base of the cecum at the caeco-ventral colonic valve into the large colon (Argenzio, 1975). Columnar epithelial cells line the cecum which also contains goblet cells that secrete mucus to protect the tissue and lubricate the digesta. Although there are no digestive secretions from the cecum, the cecal walls are thin and well vascularized containing layers of longitudinal and circular muscles (McBee, 1971). Longitudinal and circular muscles contract to mix digesta from either end and are coupled with peristaltic waves running the length of the cecum to move digesta and eventually exiting to the ventral colon (Sellers and Lowe, 1986).

The gastrointestinal tract of equids has evolved to utilize high forage diets due to the availability of fibrous feedstuffs in the wild. Feral horses consume multiple small meals per day allowing for a smaller stomach to digest these frequent small meals. Due to their environment, feral horses have limited access to NSC in their diet. This has permitted horses to have a shorter small intestine where these nutrients could be digested and absorbed when available. Soluble carbohydrates, protein, and fat digestion and absorption occurs primarily in the small intestine. Complete digestion and absorption of all of these nutrients can be limited due to the length of the small intestine and based upon amount of nutrients available in the diet. Therefore, the gastrointestinal tract has developed to contain a small stomach in relation to body size followed by a short small intestine to absorb available nutrients. Digesta that reaches the cecum is primarily fibrous, structural carbohydrates: cellulose and hemicellulose, undigested starch and protein, microorganisms passing from the small

intestine, intestinal secretions, and cellular debris. The content of digesta reaching the cecum depends largely on diet, meal size, and frequency of feeding (Frape, 2004). In general, the cecum is a reservoir that allows cellulose and other undigested feed particles to undergo bacterial fermentation to degrade and utilize these undigested nutrients.

Structural carbohydrates, like cellulose and hemicellulose, are polysaccharides of glucose and other sugars that are connected by β-1,4 linkages. Mammalian species do not produce an enzyme capable of breaking these bonds making these nutrients indigestible in the small intestine. Forages are primarily composed of cellulose, hemicellulose, and lignin; which are structural components of the plant. Since horses primarily consume forages in their diet, these nutrients need to be available to the animal. Horses have overcome this through acquiring a symbiotic fermentative relationship with microbes; this enables them to degrade and digest nutrients that would have been excreted otherwise. Cellulolytic bacterial populations present in the large intestine are capable of fermenting cellulose and hemicelluloses allowing for glucose molecules to be released and either utilized or fermented by other bacterial populations to provide energetic end products. Anaerobic bacteria possess unique extracellular multi-enzyme complexes called cellulosomes (Schwarz, 2001). These enzyme complexes enable bacteria to degrade the crystalline cellulose molecule as well as soluble cellulose through the use of endo-β-1,4-glucanases and exo-β-1,4-glucanases. Endo-β-1,4-glucanases are used to degrade soluble and amorphous regions of cellulose to produce new ends by cutting into cellulose. Exo-β-1,4-glucanase hydrolyze cellulose from both end with the assistance of cellobiohydrolases that specify reducing and non-reducing ends (Schwarz, 2001). Once glucose is available, the molecule proceeds to the Embden-Meyerhof pathway yielding 2 molecules of pyruvate. Pyruvate undergoes anaerobic fermentation

producing acetate through oxidative decarboxylation (Hungate, 1966). Formate is another product of fermentation that will act as a hydrogen source for methane production (Stainer et al., 1986).

The cecum of the horse is often compared to the rumen due to similarities in fermentative capabilities, even though there are differences between the digestive capabilities of horses and ruminants. In the equine, the digesta has been exposed to digestive enzymes and processes of the stomach and absorption in the small intestine leaving only undigested feedstuffs entering the cecum. In contrast, ruminants ferment all substrates due to location of the rumen, and the fermented digesta then proceeds to the remainder of the gastrointestinal tract for available nutrients to be further digested and absorbed. A second opportunity for fermentation in the ruminant system exists in the large intestine. Numerous studies have been performed on the ruminant animal providing more information on the microbial populations of the rumen. Comparative research between steers and ponies fed similar diets of oats and timothy hay indicated that the microbial population of the hindgut and the rumen are similar. Total anaerobic bacteria were 100 fold higher in the rumen when compared to the cecum despite similarities in bacterial populations (Kern et al., 1973). While the cecum of the horse is similar to the rumen in function, research has been limited in equine due to many factors which includes prececal digestion and location of the cecum. Changes in the dietary management of horses has lead to feeding large amounts of NSCs and increased fermentation in the cecum. Accordingly, there was a need for research to determine the effects of additional NSCs in the form of concentrate. The location of the cecum makes it difficult to study the microbial populations of the equine. Fecal samples are often used to estimate populations, but this determination of microbial populations is limited since it is at

the end of the gastrointestinal tract and only a specific species of microbes will be excreted. Researchers have performed cecal cannulation surgery in horses which enable the collection of cecal samples, directly with little difficulty (Wilkins and Lowe, 1993).

Cecal Environment. The cecal environment is important for microbial growth and metabolism. It is an anaerobic environment composed of microbial populations that tolerate a low oxygen environment (Argenzio et al., 1975). Approximately 40 to 45% of cecal microorganisms are strictly anaerobic (Kern et al., 1973). Cecal temperature is close to body temperature, approximately 37˚ C. Normal pH range in the cecum is 6.5 to 7.5; however, this depends on the animal, diet, and meal frequency (Frape, 2004). Data concerning total microbial populations in the cecum and large colon vary based on experimental conditions and method of determination.

Microbial populations in the cecum and colon benefit horses by fermenting structural carbohydrates into products that horses can utilize. Fermentative products produced by microbes include: VFA, microbial protein, and gases. Additionally, microbes synthesize essential amino acids, B vitamins, and vitamin K. Microbial populations produce these products from ingested and endogenous substrates. Fermented carbohydrates (structural and non structural) and proteins provide VFAs that are absorbed and utilized as energy. Dietary protein and endogenous nitrogen containing molecules (i.e. urea) can be utilized by microbes to grow. Urea is hydrolyzed to ammonia and carbon dioxide in the cecum; ammonia either supplies nitrogen required for microbial growth or it is reabsorbed into the bloodstream (Wysocki and Baker, 1975; VanSoest, 1994). Conflicting data exists on the contribution of microbial protein to addressing the animal's requirements, but it is minimal at best. Microbes themselves contribute to the nitrogen content through cell death (Frape, 2004).

Essential amino acids synthesized by microbes do not contribute to the protein needs of the horse significantly due to limited amino acid absorption in the large intestine. However, microbes will produce enough B vitamins and vitamin K to meet the horse's daily requirements.

Microbial populations present in the equine cecum are divided into the following categories: cellulolytic, amylolytic, or proteolytic bacteria and protozoa. Cellulolytic bacteria primarily degrade structural carbohydrates such as cellulose, hemicellulose, and pectin. A few of the predominant cellulolytic bacterial species that have been identified in the cecum include *Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes* (Julliand et al., 1999). Cellulolytic bacteria represent 3 to 9% of the bacterial population in the cecum depending on diet. Horses that consume a high-fiber diet tend to have a larger population of cellulolytic bacteria; whereas horses that consume a lower-fiber diet have a decrease in available substrate resulting in reduced population of cellulolytic bacteria. Amylolytic bacteria ferment starches through the use of α-amylases. The predominant amylolytic bacterial species include *Streptococcus bovis* and *Lactobacillus* (Kern et al., 1973, Julliand et al., 2001). Proteolytic bacteria degrade proteins that escape digestion and absorption in the small intestine. Proteolytic bacteria have been found to represent 19.7% of the microbial population (Kern et al., 1973). The other major microbial populations in the cecum are protozoa. Protozoa possess β-galactosidasic and lipolytic activities and have some cellulolytic activity (Bonhomme-Florentin, 1969). Protozoa do not affect bacterial populations or cellulose digestibility in horses (Frape, 2004).

Products of Fermentation. Fermentation end products meet a substantial portion of the animal's energy and nutrient requirements. Horses obtain approximately 60 to 70% of

their daily DE requirement from products of microbial fermentation, particularly VFAs (Argenzio et al., 1974a; Glinsky et al., 1976). Regardless of diet, the primary VFAs produced include acetate, propionate, and butyrate with minimal amounts of lactate production. Ratios of individual VFAs produced will depend on the diet. When a high forage diet is fed, acetate and butyrate will be predominately produced. When feeding a high grain diet, a higher percentage of propionate will be produced and a lower percentage of acetate will be produced (Hintz et al., 1971a). Regardless of diet, acetate remains higher than propionate. Absorption of VFAs is thought to be similar to ruminants; VFAs are absorbed in their non-ionized, more lipid soluble form into the blood stream by passive diffusion across the luminal membrane (Argenzio et al., 1975). Absorption of VFAs requires the exchange of H^+ and Na^+ ions across the membrane in addition to HCO_3^- being secreted into the lumen in exchange for Cl^- leading to a net absorption of NaCl. Thus, these two processes determine net water absorption in the large intestine (Stevens and Hume, 1998). Each individual VFA contributes to the energy requirements of the animal; however, their metabolism differs. In ruminants, acetate is converted to acetyl-CoA to be used in the citric acid cycle, and it can be utilized in fatty acid synthesis (Leek, 2004). Butyrate can also be converted to acetyl-CoA, and it is an energy source for intestinal epithelial cells to maintain tissue health (Leek, 2004). Propionate is gluconeogenic providing an alternate source of glucose, and it can be converted to succinyl-CoA and enter the citric acid cycle as succinate to produce oxalacetate (VanSoest, 1994). Therefore, VFAs function as energetic compounds to the animal, but more importantly they allow for the net absorption of water in the large intestine. This is important since 95% of the daily volume of water entering the large intestine is reabsorbed

(Argenzio et al., 1975). Although VFAs are a key end product of fermentation of substrates they are not the only fermentative by-product.

Methane, carbon dioxide, and hydrogen gases are produced from microbial fermentation. Studies to decrease methane production in cattle have been conducted to reduce the environmental footprint concerns of ruminants in addition to decrease the amount of energy lost as methane (Anderson et al., 2010). Methane yield in horses is intermediate to the amounts produced by pigs and cattle, which has been stated as 2 to 3% of the gross energy intake (Crutzen et al., 1986). Peak production of methane in horses is 6 to 8 h after feeding with greater production at high feed intakes (Hintz and Cymbaluk, 1994). Methane production in horses has not been studied as much as in ruminants because less is produced from horses. Methane and propionate production are two ways that bacteria can dispose of reducing equivalents such as hydrogen that can potentially hinder fermentation (Jensen, 1996). When NSCs are incorporated in the diet, lactate production increases causing propionate to be produced instead of methane resulting in a decreased methane production. Previous research has concluded that when cattle and horses are fed a grain diet methane production is decreased (McDaniel et al., 1993; Lana et al., 1998). There is limited research on the production of fermentative gases from the cecum; however, available studies suggest that methane producing bacteria are significantly lower in horses compared to ruminants (Spring, 2000). Ammonia production is similar to methane production as it is affected by feeding a grain diet.

Ammonia in the large intestine comes from protein degradation and/or hydrolysis of urea. Ammonia is an important source of soluble nitrogen for protein synthesis in the ruminant animal to support growth and productivity of microbes; therefore it may be equally

important in the equine large intestine to support microbial growth and function (Argenzio et al., 1975). Cattle fed a high grain diet have lower ammonia concentrations due to increased microbial synthesis and(or) decreased deamination rate (Lana et al., 1998). Therefore, products of microbial fermentation are important aspects to study in order to determine positive and negative impacts to the cecal environment and health and nutritional status of the horse.

Feeding Management

Current management strategies for equine athletes include confinement, being housed in stalls and 2 to 3 large grain meals per day. High-grain diets contain large amounts of NSC to provide the horse with DE needed for performance activities. Forage is often provided in the form of hay due to the constraints of confinement housing and offered with the concentrate portion of the diet as a meal. These management practices are contrary to the natural grazing behavior of the horse. Meal feeding disrupts the continuous meal frequency horses naturally have by grazing, leaving the stomach empty for a majority of the day. While the stomach capacity is small, horses consume frequent meals while grazing that provides a means for forage to pass quickly to the cecum, where structural carbohydrates can be fermented. Therefore, adding concentrates to the diet alters the rate of passage through the gastrointestinal tract affecting digestion and absorption of various feedstuffs (deFombelle et al., 2004). Therefore, common management strategies for confinement housed horses are often not well suited for the gastrointestinal tract of the horse.

Rate of Passage. Rate of passage of a given meal through a horse's gastrointestinal tract is influenced by physical form and particle size of the feedstuff provided. Other important factors that can influence the rate of passage include the quantity, frequency and

composition of a meal (Stevens et al., 1980). These dietary factors cause prececal mean retention time to vary from 1.6 to 9.9 h (deFombelle et al., 2004). Thus, a grazing horse will have a faster rate of passage than one fed long stem hay, and a horse fed a pelleted concentrate will be faster than one fed long stem hay (Hintz et al., 1975; Frape, 2004). Retention time of digesta in the cecum can vary depending on particle size with small particles reaching the ventral colon in 2 h whereas larger particles can take up to 48 h (Argenzio et al., 1974a). Drogoul et al. (2001) determined that mean retention time increased when grain was fed, but fiber digestibility decreased.

Digestibility of Starch. Total tract digestibility of NSC averages 98.6% of intake, and there is little difference in digestion with the source of starch (Potter et al., 1992). However, when starch intake increases, the proportion digested in the large intestine increases (Radicke et al., 1991). When starch is feed in quantities greater than 4 g/kg BW in one meal starch overload occurs and excess starch reaches the cecum (Potter et al., 1992). However, it has been suggested that the upper limit is less than 3.5 g/kg BW to induce starch overload depending on the starch source (Kiensle, 1994). The result of excessive amounts of NSC in the hindgut is rapid fermentation by bacterial populations causing an increase in total VFA concentrations with decreased acetate to propionate ratio, a decrease in pH, and an increase in lactic acid production due to an overgrowth of gram positive bacteria (Radicke et al., 1991). These results are also found in cattle and sheep when fed to induce carbohydrate overload (Allison et al., 1975). Since the equine gastrointestinal tract is not well suited for the large grain meals associated with confinement housing, disorders are becoming increasingly prevalent.

Equine Gastric Ulcer Syndrome (EGUS) is a common gastrointestinal disorder affecting 58 to 100% of horses and is often caused by management strategies of performance horses. Gastric ulcers are caused by stomach acid (hydrochloric acid) reaching unprotected stratified squamous epithelium in the non-glandular region of the stomach. Acid accumulates in the equine stomach due to the continuous production of hydrochloric acid, regardless of whether a meal has been consumed. In meal-fed horses, this results in acid accumulation in an empty stomach between meals. Several risk factors associated with the development of gastric ulcers in horses include exercise, transportation (McClure et al., 2005), consumption of high-grain diets (Murray and Eichorn, 1996), stress (McClure et al., 1999), and confinement in stalls (Murray and Eichorn, 1996). Researchers hypothesize that stalling horses alters their eating behavior increasing risk for ulceration. Horses could potentially be eating less and decreasing salivary flow to the stomach, which acts as an important gastric buffer. The most common risk factor associated with management across the United States tis consumption of high-grain diets, confinement in stalls and stress. While these risk factors have been extensively studied independently and collectively in relation to EGUS, little work has evaluated these risk factors relative to the remainder of the gastrointestinal tract. High grain diets can interfere with microbial ecosystem as stated previously with starch overload; however, there is limited research on the effects of confinement housing in horses related to gastrointestinal health.

Influence of Diet on the Cecal Environment

An appropriate cecal environment is essential for the microbial populations to flourish. Cellulolytic bacteria require with an optimum pH 6.2 to 6.8; whereas, amylolytic bacteria have an optimum pH 5.5 to 6.2 and require a source of ammonia (Leek, 2004). Diet

influences both the cecal and ventral colon microbial ecosystems (Hintz et al., 1971a; Kern et al., 1973; Julliand et al., 2001; Medina et al., 2002). Fluctuations in microbial populations occur when nutrients are fed in excess or when the rate of passage does not allow for adequate digestion and absorption in the small intestine. The dietary alteration of the predominant bacteria will modify the fermentative products produced including the VFAs, methane, and ammonia, shifting the pH (Hintz et al., 1971; deFombelle et al., 2001; Julliand et al., 2001). As a result, the cecal environment can be altered based upon the diet fed.

Changing microbial populations. The effects of cereal grain diets on microbial populations were first mentioned in ruminants. Bryant and Burkey (1953) determined that when cattle were fed an increased concentrate diet, the total bacterial numbers increased along with changes in the numbers and species of bacteria. The increase in total bacterial numbers is recognized as a result of bacterial populations having more substrate available for maintenance, growth and reproduction (Leek, 2004). Additional research showed that *Lactobacillus* populations increase when cattle were fed a high grain diet (Latham et al., 1971). A majority of the research has been performed in ruminants, and due to the limited amount of research in horses researchers have assumed the cecal environment is similar to the rumen. Kern et al. (1973) compared microbial populations in cattle and horses when fed similar diets. The study concluded that horses have a similar proportion of cellulolytic bacteria when compared to the rumen, whether or not oats were included in the diet, and protozoal numbers were not affected by diet in either animal (Kern et al., 1973). Additional studies concluded that horses fed hay diets supplemented with grain had an increase in total cecal bacterial numbers from 10^7 to 10^9 bacteria/g digesta (Kern et al., 1973; Julliand et al., 2001; Medina et al., 2002). The increase in total bacterial numbers with grain diets is due to

the proliferation of amylolytic bacteria primarily *Streptococcus bovis* and *Lactobacillus* spp. (Garner et al., 1978). Kern et al., (1974) stated the number of viable cellulolytic bacteria per gram of ingest was 6 fold greater in the cecum than terminal colon. Therefore, the cecum is the primary site of microbial activity and where the most active fermentation occurs.

Diet impacts cecal microbial populations because of the substrates available to each population. In general, when horses fed a pelleted feed rich in fiber or cereal grain, the cecal total anaerobic bacteria counts were 7.6 $\log_{10}$ cfu and 7.7 $\log_{10}$ cfu, respectively (deFombelle et al., 2003). These values are similar to other studies that have cultured total anaerobic bacteria (Medina et al., 2002; Respondek et al., 2008). Studies incorporating an abrupt diet change from all forage to a mixed diet found that total anaerobic bacterial counts increased, and this also occurs in sheep and cattle along with a decreased pH from concentrate feeding (Grubb and Dehority, 1975; Goodson et al., 1988).

Specific bacterial populations can be affected based upon substrate available to the microbes. deFombelle et al. (2003) compared *Lactobacillus* populations between two diets; the high-fiber diet resulted in 6.2 $\log_{10}$ cfu and 6.1 $\log_{10}$ cfu for the high grain diet. This is opposite of what would normally be expected from the microbial populations. The cereal grain feedstuff would be expected to increase the amylolytic bacteria compared to a high-fiber feedstuff. This study took samples at 2.5 h after the morning meal, thus the digesta most likely did not have sufficient time to alter microbial populations. Julliand et al. (2001) determined *Lactobacillus* spp. concentrations when 3 levels of concentrate were offered. When a diet of 50:50 hay:barley was fed, *Lactobacillus* populations were 7.34×10^6 bacteria/g of contents compared to the 100% forage diet containing 4.17×10^5 bacteria/g of contents. When increased amounts of cereal grains were fed to horses, it allowed more

starch to reach the cecum and be fermented causing the amylolytic bacteria to predominate. Additional modifications to microbial environment with an abrupt dietary change include an increase in *Lactobacillus* spp. and *Streptococcus* without changing cellulolytic or lactate utilizing bacteria (deFombelle et al., 2001). Increased *Lactobacillus* spp. has been associated with acute laminitis due to the augmented production of lactate from the overgrowth of gram positive bacteria which is then thought to release vasoactive amines causing inflammation of the sensitive laminae in the hoof (Garner et al., 1978; Bailey et al., 2003).

Therefore, gastrointestinal disorders can be caused by changes in the diet; so adaptation periods are critical to allow microbial populations to adjust to the availability of new substrates (Kern et al., 1974; Julliand et al., 2001). Whereas, an abrupt change in the diet does not allow time for bacterial populations to adapt, resulting in intestinal disturbances.

Change to fermentative products. Fermentative products provide a significant amount of the daily energy requirements of horses. The concentration of VFAs produced and absorbed will depend upon the substrates available for each bacterial population. Acetate will be produced with a high fiber diet compared to propionate production with a high starch diet (Willard et al., 1977). Volatile fatty acid levels vary with diet, feeding regime, time of sampling, and site of sample, it is important to note these factors when comparing concentrations of individual VFAs (Argenzio et al., 1974a). Horses eating an all forage diet with cecal samples taken 4 h after the meal have a molar percentage of 80.15 acetate and 15.72 propionate compared to horses fed a concentrate with 68.81 acetate and 25.45 propionate (Willard et al., 1977). Similar trends in the concentration of acetate and propionate have been reported in other studies (Hintz et al., 1971a; Mackie and Wilkins, 1988) which is also consistent with data in ruminants (Hungate, 1966; Hintz et al., 1978).

Horses fed an all forage diet and then gradually adapted to concentrate have little change in total VFA concentrations (Kern et al., 1973; Medina et al., 2002) compared to horses that are abruptly changed from an 100:0 to 50:50 hay:barley diet which experienced an increase in total VFA concentration (deFombelle et al., 2001). By gradually introducing concentrate into the diet, microbial populations have time to adapt to the newly available substrates. It takes approximately 2 weeks for microbial populations to either shift or become established based upon the substrate (Leek, 2004). The end products are not limited to VFAs; gaseous production is dependant upon the microbial ecosystem also.

Methanogenic bacteria require a source of formate, carbon dioxide, and reducing equivalents like H^+ to produce methane. In ruminants, methane accounts for about 30 to 40% of the total gases produced (Leek, 2004). Methane production is a helpful process that is also extremely wasteful for the host animal. Production of methane acts as a hydrogen sink for excessive reducing equivalents in order to reoxidize coenzymes like NAD^+ that are needed in biological processes for energy production. Methane and propionate are the two major hydrogen sinks in microbial fermentation in the rumen and it is believed that both are also hydrogen sinks in the cecum (Hungate, 1966). *In vitro* work has determined the maximal rate of gas production for glucose fermentation occurs between 0 to 4 h compared to cellulose fermentation that occurs between 12 to 20 h with ruminal fluid (Beuvink and Spoelstra, 1992). Glucose is fermented by amylolytic bacteria that have a faster fermentation rate than the cellulolytic bacteria that are slower due to their lower metabolic rate (Leek, 2004). When high-starch diets are fed to cattle, methane production is decreased from 48 to 7 nmol mg/protein/min compared to high-fiber diets that decreased from 14 to 2 nmol mg/protein/min when *in vitro* pH decreased (Lana et al., 1988). McDaniel et al. (1993)

demonstrated a decrease in methane production measured by *in vitro* fermentation of equine cecal fluid with bermudagrass hay and soluble starch resulting in 1.49 mmols and 0.26 mmols, respectively. The decrease in methane production could be caused by the increased number of amylolytic bacteria producing higher amounts of lactic acid then decreasing the pH below 6.2, no longer being optimal for cellulolytic bacteria (Jouany, 2008). Another explanation for the decreased methane production is that more propionate is being produced from starch decreasing the amount of reducing equivalents which decreases the need for methane to be formed as a sink allowing for more dietary energy to be saved (Leek, 2004). These reasons could be individually causing the decrease, or it could be a combination of both explanations. Either way, when NSCs are added to a diet, ammonia production also decreases.

Ammonia is required for cellulolytic and amylolytic bacteria to produce fermentative products. Ammonia is produced when plant proteins are degraded. However, too much accumulation of ammonia can cause toxicity. Feeding concentrate decreases ammonia accumulation. McDaniel et al. (1993) reported that *in vitro* fermentation of bermudagrass hay accumulated 373.7 mg/L of ammonia compared to *in vitro* fermentation of soluble starch that only accumulated 8.8 mg/L of ammonia when equine cecal fluid was utilized. The decrease in ammonia accumulation was believed to be due to an assimilation of ammonia into amino acids and ultimately microbial protein synthesis. Another reason could be a decrease in deamination of amino acids, decreasing the amount of ammonia released into the environment. A decrease in deamination could be caused by a decrease in proteolytic bacteria; which could be due to a decrease in pH caused by the increased NSC. An optimal environment for amylolytic bacteria not proteolytic or cellulolytic bacteria.

Change to cecal pH. Cecal pH can shift based upon the amount of each fermentative product produced. A relationship exists between the amount of VFA produced and cecal pH. An increase in amylolytic bacteria results in additional VFAs produced particularly propionate and lactate. Organic acids have different rates of absorption based on size (number of carbons). Acetate (2C) is absorbed faster than propionate (3C) which is faster than butyrate (4C) into the tissues based upon *in vitro* work (Argenizo et al., 1974a). Frape (2004) discussed the primary reason for a decrease in pH is lactate having a lower pK value, 3.9 compared to other VFAs with a pK value of 4.8. Time of sampling is important in determining pH just as it is with fermentative products. A majority of digesta will be in the cecum 4 to 6 h after a meal when cecal pH is at its lowest (Argenzio et al., 1974b; Willard et al., 1977).

Decreases in pH is dependent upon the amount of NSC in the diet as well as the individual horses' metabolism. Researchers have shown that concentrate diets result in lower cecal pH at 4, 5, and 6 h following a meal than when a hay diet was fed (Willard et al., 1977). Medina et al. (2002) collected cecal samples 4 h after the morning meal and observed horses fed a high-fiber diet had a pH of 7.2 compared to horses eating the high-starch diet with a pH of 6.6. These numbers are higher compared to Julliand et al. (2001) where a 100% forage diet had a pH of 6.74 compared to a 50:50 hay:barley diet with a pH of 6.26. Variation in pH depends on substrate fed, time of sample collection, and the animal. After an abrupt change in a diet of all forage to a forage/concentrate mix, cecal pH decreased from 6.4 to 5.8, when samples were collected 6 h after the morning meal (Goodson et al., 1988). In a study that induced laminitis, pH dropped from 7.18 to 5.72 in 8 h and then steadily declined to 4.14 after 24 h (Garner et al., 1978). When pH is below 6.0, the horse is considered to have

subclinical acidosis that can cause mucosal damage (Clark et al., 1990). In order to avoid digestive upsets and prevent the incidence of gastric disorders, management strategies need to be clearly defined to limit the amount of NSC fed to horses in one meal. Further research needs to determine the role housing has in increasing the incidence of gastric disorders when high grain diets are fed.

Stoichiometric Calculations

Stoichiometric calculations are based upon quantitative relationships of chemical reactions between reactants and products. The calculations used are carbon-hydrogen balancing equations that are based upon measured VFA proportions when the assumed substrate is glucose (Wolin, 1960). The equation is used to estimate the amount of methane and carbon dioxide produced assuming that those are the only products produced. Further equations can then be calculated to determine the amount of carbohydrate fermented along with the fermentative efficiency and the amount of reducing equivalents produced and consumed. These calculations have been widely used in ruminant studies with reasonable results. However, in swine studies, estimated methane production was 3.3 to 3.6 times higher than directly measured values in the hindgut (Zhu et al., 1993).

Influence of Housing

Confinement housing is a common management strategy in the equine industry. Confining horses in stalls reduces the opportunity to get kicked, bitten, sun bleached or injured. For performance horses, these situations are important to avoid in order for the horse to maintain a better body condition for performances. However, there are several negative aspects to stalling horses such as owners often limit access to forage, voluntary exercise, and social interaction. Confinement housing also contributes to the development of behavioral vices (McGreevy et al., 1995). There is limited research on confinement housing

in horses, and most studies that incorporate stalling confound stalling with dietary treatment making it difficult to elucidate the effects of housing.

Behaviorial changes associated with confinement. Feral horses spend approximately half of the day grazing in a herd. Research conducted on time budgets of domestic horses found that 30 to 70% of their time is spent eating, 15 to 50% is spent standing, 2 to 10% is spent lying down, and 4 to 10% is spent moving around (Budiansky, 1997; Crowell-Davis et al., 1985). Confinement in stalls has the potential to alter behavior. Behavioral studies have shown that confinement in stalls increases abnormal and stereotypic behaviors such as cribbing, wood chewing and wind sucking, weaving, or box-walking (McGreevy et al., 1995). When horses are confined, stalls restrict available space to move and limit contact and social interactions with conspecifics. Since this management practice alters the natural tendency to be a social herd animal, there is a possibility that confinement will alter behavior such as eating, drinking, and resting.

When horses were weaned either individually in a stall or in small groups in a paddock the stalled horses had a rise in aberrant behaviors: licking, chewing, and/or kicking the wall, pawing and bucking; whereas, paddock housed weanlings displayed a broader range of behaviors and had a stronger motivation to graze (Heleski et al., 2002). This study did not quantify DM intake in horses, and therefore, the authors were unable to determine the effect of housing on DM intake in horses. Although this study was performed in young horses during a very stressful time period, the same behavioral responses to confinement have been observed in adult horses surveyed by McGreevy et al. (1995). By offering large amounts of hay per day and offering it more frequently, the prevalence of abnormal behaviors can be reduced (McGreevy et al., 1995). Therefore, behavioral stereotypes occur in confined

horses whether out of boredom or lack of forage. The impacts of these altered behaviors, such as the feeding behavior, on the gastrointestinal tract due to confinement housing have not been extensively researched.

Housing and behavior in other species. In other animal industries, housing situations serve two objectives: either increasing production status of the animal or decreasing costs of operation. For example in swine, producers are trying to maximize available housing space to increase production by increasing the amount of pigs. Studies have concluded that pigs can be successfully group housed as long as they have adequate space to move around and lie down (McGlone and Newby, 1994). However if growing pigs are crowded, feed intake is reduced causing a decline in body weight and an increase in behavioral problems (Gehlbach et al., 1966). Thus, it becomes increasingly important to understand the limitations of each species to ensure the animal welfare when living contrary to their natural environment. For example, it is known that sheep have a difficult time habituating to confinement housing when compared to other farm animals. This is seen through an increase in heart rate and cortisol levels with long term physiological disturbances when placed in confinement housing systems (Pearson and Mellor, 1976). It has also been observed that when sheep are taken from a pasture housing system to an indoor housing system, there is an increase in the incidence of stereotypic behaviors (Done-Currie et al., 1984). Casamassima et al. (2001) performed a study that observed indoor versus outdoor housing with lactating ewes; sheep housed outdoors had an increase in locomotor activities with less time spent idle compared to indoor housed sheep. The plasma urea and glucose concentrations were lower in sheep housed outdoors compared to indoor; although the milk composition did not differ between the different housing systems (Casamassima et al., 2001). The authors stated that the welfare

and productivity of lactating ewes was not substantially affected by the housing systems utilized in the study. Although these industries do not have the same objectives as the equine industry, there are advantages and disadvantages to any operation that confines animals.

The dairy industry has focused their research primarily on cow comfort in an effort to increase milk yield. It has utilized confinement housing and pasture housing throughout history, and researchers have been observing the positive and negative features of both systems. An advantage to pasture housing cows is an increase in reproductive capabilities with fewer services per conception and shorter calving intervals, along with a decrease in mastitis, better hoof health and cow health in general (Washburn et al., 2002; Legrand et al., 2009). Disadvantages of pasture housing systems include a decreased BW and BCS due to the energy expenditure of making trips to the parlor twice daily along with time spent grazing in the pasture allowing for less energy for productive purposes (Washburn et al., 2002; Fontaneli et al., 2005). In confinement housing, dairy cows have access to a high quality and consistent diet composed of a total mixed ration with more time spent lying down and ruminanting and protection from environmental extremes (Legrand et al., 2009). O'Connell et al. (1989) noted that confinement altered the distribution of feeding times and activity levels of dairy cows. The cows spent more time feeding when on pasture than indoors, and indoors the cows were more restless with their lying behavior affected which disrupted rumination (O'Connell et al., 1989). Studies observing cow preferences have concluded that cattle have a strong preference for access to pasture at night and indoor housing during the day when temperatures and humidity are high (Legrand et al., 2009). The main disadvantage to confinement housing is the increased prevalence to disease particularly mastitis. Washburn et al. (2002) reported that cows in confinement have 1.8 times more clinical

mastitis compared to pasture housed cows. Economically both housing systems cost the same when milk income and feed costs were considered (Fontaneli et al., 2005). Therefore, the dairy industry can utilize both systems and not affect the overall health of the cows. However, their feeding behavior was shown to be greater when housed on pasture. From a production stand point, the dairy industry does not have as many similarities to the equine industry as the beef industry.

Beef cattle producers focus on the growth of steers by increasing live weight gain along with maintaining an economically efficient cost. Beef producers started adding concentrate feeds to supplement the lower nutrient dense forage diet of cattle on pasture. The addition of concentrate feeds to a forage based diet makes the beef industry more comparable to the equine industry. Concentrate feeds increase the amount of feedstuffs available to be fermented into VFAs particularly propionate; these additional energy sources can be utilized to produce more muscle or stored it as fat, producing a larger, more profitable animal. However, there is limited research available on the effects of confinement housing in beef cattle as well. The limited information that is available is also confounded by house type or dietary treatments (DelCurto et al., 1990; Lowe et al., 2001). Tayler and Wilkinson (1972) completed a study feeding steers varying levels of concentrate with similar forage being offered when housed either individually in a stall or group housed in a pasture. They concluded that the forage to concentrate ratio differs slightly with housing shown by pasture housed steers voluntarily consuming more grass, compared to when confined to stalls and offered the same fresh cut forage (Tayler and Wilkinson, 1972).

Babu et al. (2004) conducted an experiment based on rearing systems in crossbred calves that was similar to the behavioral study with group housed versus stalled weanlings

(Heleski et al., 2002); this study utilized calves that were either stalled individually or group housed in pens. Group housed calves spent more time eating solid feeds high in DM compared to stalled counterparts who spent more time lying and standing idle (Babu et al., 2004). Housing does influence behavior at the time of weaning in both cattle (Babu et al., 2004) and horses (Heleski et al., 2002). In cattle, DMI was affected by housing (Babu et al., 2004) which provides reasonable evidence that this trend could also occur in horses that are individually stalled versus in a paddock.

Based upon the available information in sheep and dairy cattle, the welfare and productivity of an animal in confinement will not be affected, but behavioral aspects can be affected by confinement housing. In other species and horses, confinement housing is observed in respect to behavioral or physiological responses not digestive health. Since confinement housing is already a proven risk factor for equine gastric ulcer syndrome, it could also cause potential problems in the hindgut environment as well as the stomach. There is no previous information concerning the influence of confinement housing on the hindgut environment. Thus, further research is needed to determine the effects of housing on the gastrointestinal health of the horse.

Voluntary Intake

Forages are an important part of any herbivore's diet; however, to meet the increased energy demands of stalled performance horses, owners have diminished the amount of forage in the diet to allow for greater intake of concentrate. There are negative aspects to decreasing forage in any herbivores diet, particularly in confinement that can lead to behavioral problems. As dietary fiber is restricted and social restrictions start to increase in stalled horses, more time is spent standing rather than eating/grazing (Kiley-Worthington, 1997),

leading to an increased incidence of stereotypic behaviors such as wood chewing, cribbing, and coprophagy (Willard et al., 1977). Forage needs to be a predominant feedstuff in a horses' diet to maintain gastrointestinal health and natural foraging behaviors. The minimum amount of forage in a horse's diet should be 0.75% BW (NRC, 2007). Pasture housed horses spend up to 18 h/d grazing and browsing on forages (Goodwin et al., 2002); this amount is often restricted in confined horses or horses with limited turnout space. Therefore, confinement housing is an additional management challenge that disrupts the natural foraging behaviors of horses. With restricted access to forage in stalls, horses must rely solely on humans for the selection and delivery of their diet. Most management practices include meal feeding hay with a concentrate ration. Meal feeding horses inevitably causes horses to fast for long periods of time between meals since a majority of stalled horses are provided a low fiber diet. Since horses do not voluntarily fast for more than 3 to 5 h (Ralston, 1984); there could be an alteration in the physical and mental health of the horse due to intake patterns.

Voluntary forage intake. Voluntary intake in horses is influenced by multiple factors including but not limited to palatability, digestibility, rate of passage and chemical composition of the forage, in addition to the physical characteristics of each forage. Research data from ruminant animals clearly indicates factors that influence VDMI, but these relationships in horses are not clearly established. In ruminants, voluntary intake is affected by gut fill (Allen, 1996). However, Frape et al. (1982) and Aiken et al. (1989) indicated that intake was regulated by energy requirements rather than gut volume. Digesta passage in horses might be slowed down but is not limited by particle size as it is in ruminants, since ruminants have a reticulo-omasal orifice that decreases the rate of passage allowing digestion

of forages to occur before moving further down the tract (Poppi et al., 1981). Horses do not have an analogous structure to the reticulo-omasal orifice, so horses do not retain digesta in the gastrointestinal tract in the same way making it difficult to affect intake through gastric or intestinal filling (Cuddeford, 2004). An advantage in horses is the large intestine contains multiple flexures and continuous sacculations that allow for a decreased rate of passage in order to ferment undigested nutrients.

A factor that could control voluntary intake is the absorption of nutrients after or during a meal causing metabolic responses to either decrease or increase satiety. Ralston and Baile (1983) demonstrated that intake decreases after an absorptive state when fed glucose and cellulose, for glucose it was within 10 to 15 min whereas cellulose takes 4 to 6 h. Based upon the diet, meal frequency could be altered and regulated by digesta that is present in the digestive tract or that is being absorbed (Ralston, 1984).

The type of forage offered or available to horses can affect the voluntary intake. Commonly fed forages include alfalfa, orchardgrass hay, timothy hay, coastal bermudagrass hay, brome grass hay, and many others based upon region. Coastal bermudagrass hay (*Cynodon dactylon*) is a widely used grass hay in the southern United States. Aiken et al. (1989) determined that yearlings consume 2.5% BW and mature geldings voluntarily consume 2% BW of Coastal bermudagrass hay, when hay was the sole source of nutrients. Voluntary forage intake in horses could be related to energy requirements based upon yearlings consuming more based on BW when compared to mature horses (Aiken et al., 1989). In a study comparing various types of forages, yearlings consumed 10.9 kg/d DM of alfalfa compared to 10 kg/d DM of Coastal bermudagrass hay (LaCasha et al., 1999). Preferential selection of forages is not understood completely, although the higher intake of

legumes could be related to the palatability or chemical composition. Cattle also show the same preference by consuming more legumes than grasses when digestibilities are similar (Ulyatt, 1981). More recent research observed the effects of offering multiple forages to horses in confinement housing, since stalled horses have limited access to forage. Goodwin et al. (2002) concluded that confined horses offered multiple forages spent more time foraging than when offered a single forage. The addition of this management practice to stalled horses could decrease the amount of time spent in stereotypic behavioral patterns. Multiple forages also decreased the amount of time horses spent searching for food (Thorne et al., 2005). In ruminants housed in pens, there was a higher total hay intake when several types of hay were provided compared to when a single hay was offered (Reid and Jung, 1965). Essentially, stalled horses with access to forage throughout a majority of the day or are offered ad libitum access to forage are able to engage in frequent meal eating patterns similar to horses on pasture (Ralston, 1984).

The quality of forage has the potential to affect voluntary intake as well. Ruminants will decrease forage intake as the forage quality decreases (Edouard et al., 1997), and horses could be similar. The cell wall content of plants relates to the digestibility as well as the intake in ruminants. The more mature a plant is the higher the amount of structural carbohydrate content will be, causing a decrease in digestibility. The cell wall content is helpful in estimating intake in ruminants with the amount of NDF in the cell wall, and ADF is useful in predicting the digestibility of forages (VanSoest, 1965). With these two measures of cell wall content, estimates can be made based upon the chemical composition of the forage. For instance, as NDF increases intake will decrease and as the ADF increases in a forage, the digestibility will decrease. Conversely, a decrease in ADF will increase the

digestibility allowing for an increased rate of passage which could increase voluntary intake in animals (Allison, 1985). Although, horses seem to be less sensitive to the cell wall contents of forages compared to ruminants which could be caused by the differences in rate of passage or retention time for fermentation of the forages (Cymbaluk, 1990; Dulphy et al., 1997). The digestive tract could be allowing for a faster transit rate of digesta which would decrease the amount of cell wall contents digested when compared to ruminants. Martin-Rosset and Doreau (1984) found no significant relationship between forage quality and intake in horses. Therefore, it is more difficult to estimate voluntary intake in horses than cattle.

Voluntary forage intake is influenced by confinement housing in beef cattle. When growing cattle were offered similar amounts of supplemental concentrate, cattle housed on pasture consumed an increased percentage of their diet as forage compared to those offered fresh cut forage in stalls (Tayler and Wilkinson, 1972). As stated previously, horses will voluntarily consume 2% BW of Coastal bermudagrass hay (Aiken et al., 1989; LaCasha et al., 1997). Dulphy et al. (1997) stated that horses voluntarily consume 22.2 g/kg DM/kg LW of grass hays when fed twice daily in comparison to sheep that voluntarily consume 18.2 g/kg DM/kg LW. Although horses consume a similar amount of DM as ruminants, the meal frequency is not similar.

Horses consume 11.9 meals/d compared to ruminants consuming 5.8 meals/d (Dulphy et al., 1997). Horses consume meals throughout the day; whereas ruminants consume a meal and then spend a majority of time ruminating. The addition of concentrates to a diet could cause meal frequency to change. However, it has been reported that Thoroughbred horses fed concentrate with hay consumed 11 meals/d (Doreau, 1978). This study shows that meal

patterns in horses stay relatively consistent with alterations in diet portions. Previous research has determined that horses will consume approximately 2% BW in DM per d based upon physiological status. Mature horses fed only hay diets consume 2% BW in hay (Aiken et al., 1989); the addition of supplemental grain causes the voluntary forage intake to decrease keeping the total dry matter intake per d similar (Moore et al., 1999). Therefore, VDMI is approximately 2% BW regardless of diet; horses are able to modify their voluntary forage consumption when grain is supplemented. This principle is also true in beef cattle that were supplemented with grain (Tayler and Wilkinson, 1974).

Voluntary forage intake is directly measured in stalled animals making it easy to determine. However, there is limited information on voluntary forage intake when horses are housed on a pasture or allowed ad libitum hay in group-fed housing. There are many difficulties associated with determining voluntary intake of grazing animals to overcome. Previous studies have utilized inert markers to determine estimated intake rather than a direct measurement; since it is nearly impossible to determine the type, quantity, or quality of forage consumed when horses are housed on pasture. A study estimating intake in pasture horses used alkanes as an inert marker to assess the digestibility of forages consumed, but the results were unreliable and overestimated forage intake (Friend and Nash, 2000). Although markers are commonly utilized to estimate intake in ruminants and horses, most studies have validated markers based upon its recovery rate compared to total fecal collections (Titgemeyer et al., 2001; Patterson et al., 2001). There is limited research with actual intake values compared to estimated marker intake.

Estimations utilizing markers. Forage intake in many species is either to difficult to directly measure or it is not practical to measure. Thus, indirect methods have been created

to estimate intake indirectly by dividing total fecal excretions by indigestible nutrients from the diet (Dove and Mayes, 1991). The most common indirect method used is inert markers. External and internal markers can be utilized to estimate digestibility, rate of passage, retention time, and fecal output. Ideally, markers should not be absorbed or affected by the digestive tract, the microbial population or the digesta; markers should flow parallel with the digesta and should not have any laxative or toxic effects on the experimental animal, and markers should be easy to analyze in the laboratory (Glindemann et al., 2009). External markers must be indigestible substances that are not naturally present in the animals' environment, and they are administered orally or through a fistula. Therefore, markers are either added or bonded to the feed (Marais, 2000). Common markers that have been utilized in farm animals include transition metals like chromium oxide, Co-EDTA, titanium, along with rare earth metals, polyethylene glycol, and even chain n-alkanes.

Internal markers are naturally present in the diet as a part of either the forage or concentrate consumed by the animal. For this reason, internal markers are inexpensive and convenient and do not require dosing the animal. Therefore, an advantage of internal markers includes using them with free ranging animals. However, there are only a few internal markers that are accepted and repeatable (Marais, 2000). Common internal markers include alkanes, ADL, acid detergent insoluble ash (ADIA; Marais, 2000). Depending on the markers properties, some can be used as a liquid marker for rate of passage or a solid marker that can be used to determine retention time or digestibility. For instance, certain external markers incorporate into feedstuffs better than others making each marker have a given estimation based upon experimental objectives. Whereas, internal markers are better at estimating retention rates and apparent digestibility of forages (Marais, 2000).

Chromic oxide is an external marker that works independent of particulate and fluid phases making it difficult to estimate rate of passage, but this marker is commonly used in a dual marker system to overcome these limitations. A dual marker system involves administration of an external marker along with an internal marker to account for any limitations of either marker. The external marker must be given at a known concentration and allowed time to reach a steady state. Since the digestibility of forages is calculated as the difference in forage consumed and excreted in the feces, total fecal collections are required to obtain the samples which can be labor intensive with large herbivores. By incorporating an external marker into the diet with the internal marker, total collections can be replaced by fecal grab samples to estimate apparent digestibility which can save on overall costs and labor expenses (Marais, 2000). Therefore, utilizing a dual marker system is beneficial for decreasing labor costs and decreasing the stress placed on the animal during total collections.

The most commonly used external marker, chromic oxide, has been used as a digestibility marker in ruminants and horses (Frape et al., 1982; Patterson et al., 2001). However, chromic oxide is a carcinogen, and it is not approved by the FDA as a feedstuff. A suggested alternative to chromic oxide is titanium dioxide (TiO_2). Titanium dioxide is not harmful to the animal and can be legally added to feedstuffs as long as it does not exceed 1% of the finished product (AAFCO, 1996). Titanium dioxide has been used as an alternative digestibility marker in cattle and sheep (Titgemeyer et al., 2001; Myers et al., 2006; Glindemann et al., 2009), pigs (Jagger et al., 1992) and chickens (Short et al., 1996). Hafez et al. (1988) concluded TiO_2 has a 99% fecal recovery rate when fed to dairy cows, and Titgemeyer et al. (2001) found that fecal recovery averaged 93% when fed with forage and grain. Although TiO_2 has been observed to be an alternative to chromic oxide in other

species, it has not been validated as an external marker in horses. Recent research utilizing TiO_2 as an external marker in horses has been completed with adequate results (Spurgin et al., 2011; Winsco et al., 2011); however, further research validating TiO_2 as an external marker in horses needs to be conducted.

Acid detergent fiber has been utilized to estimate digestibility in ruminants. Fractions of the cell wall remaining after an ADF procedure are cellulose and lignin. Lignin is an indigestible portion of forage whereas cellulose digestibility will depend upon the microbial degradation in the rumen or cecum. Therefore, as the amount of ADF in forage increases, the lower the digestibility of that forage will be. In a sequential order using the ANKOM fiber system, ADF can be ashed in an ash oven to create ADIA. Acid detergent insoluble ash can estimate digestibility and fecal output in ruminants. By incorporating an external marker with either of these two markers, fecal output can be estimated. Sampaio et al. (2011) utilized a dual marker system and determined that fecal excretion estimates with internal markers have a higher precision when compared to external markers. The same study observed fecal recoveries of chromic oxide, TiO_2 in cattle to be 99.5%, and 101.95%, respectively (Sampaio et al., 2011). The external markers were dosed through an esophageal fistula which could be a reason for the higher recovery rates than the previous studies mentioned where TiO_2 was added to the feedstuff. Again further research utilizing a dual marker system with TiO_2 and ADIA to estimate voluntary intake in horses needs to be continued (Spurgin et al., 2011; Winsco et al., 2011).

Conclusion

The information available in ruminants and horses regarding the influence of diet on cecal microbial populations allows equine researchers to apply this data to other experimental

variables. Concentrate feeding is a major part of the horse industry along with confinement housing. Through determination of the effects of these two factors on the cecal environment will assist barn owners to understand how management strategies are important in maintaining a healthy cecal environment. Also by determining the voluntary forage intake of horses in confinement compared group housed situations, equine managers will have insight into the foraging behaviors of horses and can understand more fully the amount of forage a horse will consume when housed in different locations. Therefore, the objectives of the current study were to determine influence of housing on cecal environment and voluntary forage consumption of horses fed a high grain diet.

CHAPTER II

MATERIALS AND METHODS

All procedures were approved by the Texas A&M University Institutional Animal Care and Use Committee.

Horses, Diet, and Housing Treatments

Eight previously cecally cannulated Quarter Horse geldings (7 to 10 yr; 510 to 666 kg BW) from the Texas A&M University Horse Center were used in a crossover design with 2 treatment periods. Horses were paired by age and BW and randomly assigned to treatment. Treatments consisted of housing horses individually in stalls (3.9 m x 4.4 m) or group housed in a dry lot paddock (97.5 m x 27.1 m). Treatment periods were 28 d with a 21 d washout period between. Days 1 to 19 allowed for adaptation to dietary treatments, d 20 to 24 were used for determination of dry matter intake, and on d 28 cecal fluid was collected for enumeration of hindgut bacteria, determination of pH, and VFA concentrations.

Diets were similar between the two treatment groups. All horses were individually fed a pelleted concentrate at 1% BW (as fed) offered twice daily. Forage was provided in the form of coastal bermudagrass (*Cynodon dactylon*) hay. Nutrient composition of grain and hay are found in Table 1. Group housed horses had *ad libitum* access to hay as round bales and stalled horses were offered hay individually that was collected daily from the same round bale. Hay was offered to stalled horses initially at 2% BW (as fed) then adjusted based on 120% of a previous 3 d average of voluntary intake. Bodyweights were recorded on 0 d of each treatment period and amount of feed offered adjusted accordingly. Horses were fed twice daily, at 0600 and 1800, in individual stalls or tied and observed individually when

group housed. All horses had *ad libitum* access to water. Stalled horses were allotted 1 h of free exercise per day.

Grain and hay refusals were weighed and recorded daily. Grain samples were obtained weekly and hay samples were obtained through grab samples from each round bale offered. Samples obtained throughout the trial were analyzed by a commercial laboratory (SDK Laboratories, Hutchinson, KS; Table 1) for CP, starch, ADF and ADIA, NDF, Ca and P.

Table 1. Nutrient components of concentrate and forage (DM basis) fed to mature Quarter Horse geldings

Item	Concentrate[1]	Hay[2]
ADF, %	15.74	37.18
ADIA, %	1.61	2.03
NDF, %	28.43	68.00
CP, %	16.79	9.10
Starch, %	18.7	2.70
Ca, %	1.29	0.33
P, %	0.87	0.17

[1]Diets consisted of 1% BW (as fed) per day in commercially pelleted concentrate.
[2]Hay consisted of coastal bermudagrass (*Cynodon dactylon*).

Dry Matter Intake Determination

A dual marker system was utilized for estimation of voluntary intake in both stalled and dry lot horses using titanium dioxide (TiO_2) as the external marker and acid detergent insoluble ash (ADIA) as the internal marker. Voluntary forage intake was measured directly and indirectly in stalled horses; whereas in dry lot horses it was only measured indirectly. Titanium dioxide was offered beginning on d 13, in the form of a grain cookie at 10 g/d separated in two 5 g doses offered prior to each grain meal. Fecal samples were collected

twice daily at 12 h intervals on d 20 to 24 with collection times advancing by 3 h each d to account for diurnal variation and provide a composite sample for a 24 h period. For example: samples were collected on d 20 at 0600 and 1800, d 21 at 0900 and 2100, d 22 at 1200, d 23 at 0000 and 1500, d 24 at 0300. Fecal samples were dried at 60˚C for 72 h and ground through a 1 mm screen (Wiley Mill, Thomas Scientific, New York, NY) then composited into a representative 24 h sample. All fecal, hay, grain, and cookie samples were analyzed for TiO_2 marker recovery using a UV Vis spectrophotometer (DU Series 700, Beckman Coulter, Fullerton, CA) by colorimetric assay (Short et al., 1996) and ADIA was analyzed using an ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY) and an ashing furnace (StableTemp Cole-Parmer, Hanwell, London; Llewellyn et al., 2006). Titanium dioxide concentration was used to estimate daily fecal production; while, ADIA was used to estimate dietary intake. Both values determined the estimated forage intake. The formulas used to calculate the voluntary forage intake through the dual marker system is as follows:

1. *Feces per d (kg):*

$$\frac{\left(\frac{g\ TiO2}{[\%\ TiO2]/_{1000}}\right)}{1000}$$

2. *Feces per d (kg DM):*

$$\frac{kg\ of\ feces\ per\ d}{\%\ DM}$$

3. *ADIA in Feces (kg):*

$$(kg\ of\ feces\ per\ d)\ X\ (\%\ ADIA\ in\ feces)$$

4. *ADIA in grain (kg):*

$$(grain\ intake\ per\ d\ kg\ DM)\ X\ (\%\ ADIA\ in\ grain)$$

5. *Forage intake per d (kg):*

$$\frac{(kg\ ADIA\ in\ feces - kg\ ADIA\ in\ grain)}{\%\ ADIA\ in\ hay}$$

Microbial Enumeration

Cecal samples were taken on d 28 of each treatment period approximately 4 h after the morning meal, when cecal pH begins to decline (Willard et al., 1977). Cecal cannulas were opened and 350 mL insulated containers were filled with approximately 100 to 200 g of cecal contents (liquid and solid). Approximately 50 mL of cecal fluid was strained through cheesecloth, immediately analyzed for pH with a handheld pH meter (Thermo Orion, West Chester, PA) then frozen at -20˚C for later analysis of VFA concentrations. After cecal samples were collected, insulated containers were transported to the Food and Feed Safety Unit of the Southern Plains Agricultural Research Unit, USDA/ARS facility for further analysis.

Samples were prepared for microbial analysis in the Bovine Microbiology Laboratory at Southern Plains Agricultural Research Unit within 45 min of collection. The insulated containers were placed in a Bactron Anaerobic/Environmental Chamber (Sheldon Manufacturing, Inc. Cornelius, OR) with a 90% N_2-5% H_2 atmosphere. Samples were prepared in a series of 10-fold serial dilutions in an anaerobic mineral solution (Bryant and Burkey, 1953) from 10^{-1} to 10^{-9}. Dilutions were inoculated onto one of three specific media for bacteria enumeration. One media was Anaerobic Brucella Blood agar plates (Anaerobe Systems, Morgan Hill, CA) to enumerate total culturable anaerobes (Mangels and Douglas, 1989). Previous research indicated a serial dilution of 10^{-4} to 10^{-10} provided an ideal range

for growth on this media (Wilson et al., 2009). Plates were inoculated with 0.1 mL/plate in the Bactron Chamber and incubated at 37.5˚C for 96 h. The bacterial colonies were counted and recorded.

Lactobacilli anaerobes were determined using two different selective media: Rogosa agar (Rogosa et al., 1951) and DeMan Rogosa Sharpe (MRS) agar (de Man et al., 1960). Previous research indicated that a dilution set of 10^{-1} to 10^{-8} was ideal for sufficient growth on this media (Wilson et al., 2009). Difco™ Rogosa SL agar (Becton Dickson and Company, Sparks, MD) was prepared in petri dishes prior to sample collection. A set of the media was inoculated with serial dilutions 10^{-1} to 10^{-8} with 0.1 mL/plate and incubated anaerobically in the Bactron Anaeobic Chamber at 37.5˚C for 96 h then bacterial colonies were counted and recorded. Difco™ Lactobacilli MRS Agar was prepared in petri dishes also prior to sample collection. A set of media was inoculated with serial dilutions 10^{-1} to 10^{-8} with 0.1 mL/plate and incubated aerobically in a Steri-CultCO_2 Incubator (Forma-Scientific, Mariett, OH) with a 5% CO_2 atmosphere at 35˚C for 72 h then bacterial colonies were counted and recorded.

Methane and Ammonia Activity

Methane-producing activity was determined using a procedure described by Anderson et al. (2006) through an *in vitro* incubation of cecal fluid. The procedure includes 2 g of cecal contents, 8 mL of anaerobic dilution solution (Bryant and Burkey, 1953) that contains 60mM sodium formate, and 0.2 g finely ground alfalfa to be mixed in 18 x 150 Hungate culture tubes that were capped under a H_2:CO_2 (50:50) gas phase. Each sample was performed in triplicate with two sets prepared: 3 h sample and 24 h sample. Samples were incubated for the respective time at 39˚C. A 1 mL sample of each tubes headspace was

analyzed for composition using gas chromatography (Gow-Mac Instrument Co., Bethlehem, PA). After the composition reading, the remaining headspace was determined to see the amount of gas produced in the allotted time.

Ammonia-producing activity was determined using a colorimetric assay that uses a catalyzed indophenol reaction (Chaney and Marbach, 1962). The samples were read by a SpectraMax 340 PC spectrophotometer (Molecular Devices, Sunnyvale, CA) at a wavelength of 630 nm. The absorbance of each sample was compared to known standards using the SoftMax Pro software (Molecular Devices, Sunnyvale, CA) to calculate a value in μmol/mL.

VFA Analyses

Cecal samples were thawed in preparation for analysis. Metaphosphoric acid (3.125 M) was added to cecal samples at a ratio of 1 to 4. Samples were frozen again and shipped to Kansas State University for VFA analysis. The VFA concentrations were determined using gas chromatography (Model 5890, Hewlett Packard, Avondale, PA) with a flame ionization detector, a procedure described by Vanzant and Cochran (1994).

Stoichiometric Calculations

Stoichiometric calculations were used to estimate the amount of hexose fermented utilizing the measured VFA concentrations according to Demeyer (1991):

Hexose fermented = ½ acetate + ½ propionate + butyrate

These equations are also used to estimate the amount of methane and carbon dioxide produced using the results from VFA analysis according to a series of fermentation balance equations from Wolin (1960). The amount of reducing equivalents generated and consumed can also be calculated based upon equations described by Demeyer (1991) along with the fermentative efficiency as described by Chalupa (1977):

Reducing equivalents generated =

$$(2\ acetate)+(propionate)+(4\ butyrate)+(2\ valerate)+(2\ isovalerate)$$

Reducing equivalents consumed =

$$(2\ propionate)+(2\ butyrate)+(valerate)+(4\ CH_4)$$

Fermentative efficiency =

$$\frac{(0.62\ acetate + 1.09\ propionate + 0.78\ butyrate)}{(acetate + propionate + butyrate)}(100)$$

Statistical Analyses

Data were analyzed using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The model contained effects for horse, period, and treatment with $P \leq 0.05$ considered significant and $P \leq 0.10$ considered a trend towards significance.

CHAPTER III

RESULTS AND DISCUSSION

Concentrate Intake

Concentrate intake was not influenced by housing ($P > 0.17$). Concentrate consisted of 18.7% starch (Table 1) which provided an average of 0.86 g starch/kg BW/meal. According to previous research, intake of 2 to 4 g starch/kg BW results in starch overload to the hindgut and causes significant alterations to cecal fermentation (Radicke et al., 1991; Potter et al., 1992; Kiensle, 1994). It is unlikely that starch overload occurred in the current study based upon the calculated starch intake of the diet. However, the current diet consisting of 1% BW (as fed) in concentrate per day is similar to that provided to horses performing light to moderate workloads (NRC, 2007).

Forage Intake

Forage intake was affected by treatment with group housed horses consuming greater amounts of forage ($P = 0.04$) compared to stalled horses (Figure 1) despite similar concentrate consumption. The dual marker estimated forage intake averaged 8.46 kg DM/d (1.41% BW) when horses were group housed in a dry lot and 5.17 kg DM/d (0.90% BW) when horses were stalled individually ($P = 0.05$).

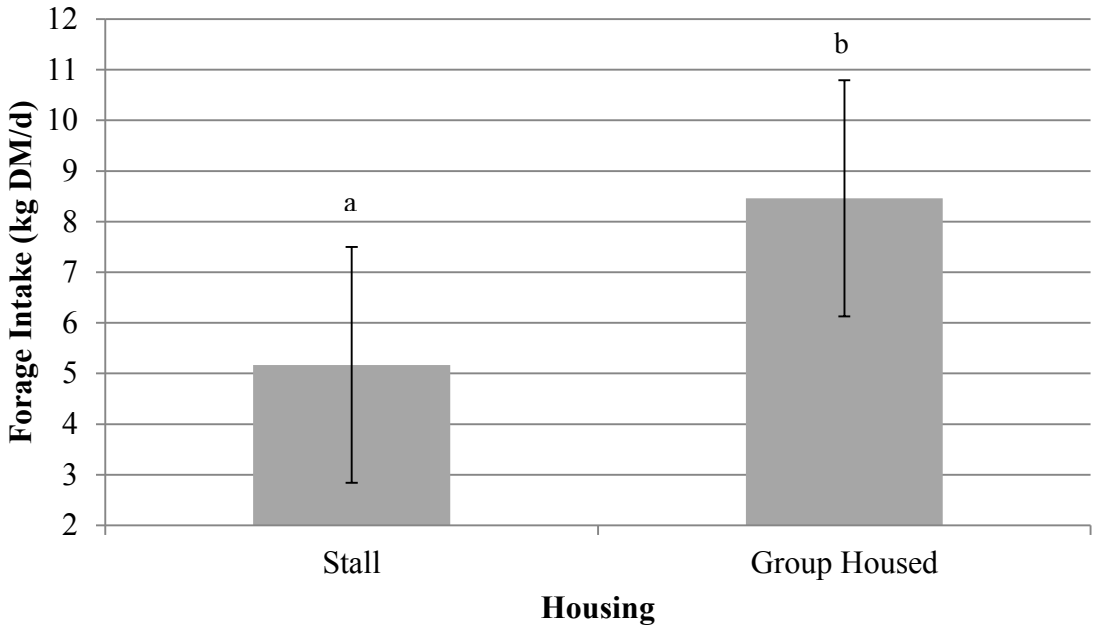

Figure 1. Estimated voluntary forage intake in mature Quarter Horse geldings that were group housed in a dry lot or individually in a stall. Titanium dioxide and ADIA were utilized together in a dual marker system to estimate intake. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with *ad libitum* coastal bermudagrass hay. Values are means ± SE (n = 8). Means without a common letter (a, b) are different ($P < 0.05$).

Reductions in intake of forage by individually housed horses, in the current study agrees with previous research in beef cattle (Tayler and Wilkinson, 1974). Decreased forage consumption in confinement can relate to a horse's natural behaviors, primarily the social interactions within a herd. Being a herd animals, horses follow other conspecifics whether that is foraging, drinking, or sleeping; so when other horses are near, foraging might be a more constant activity compared to when confined with limited contact (visual or physical) with other horses (Sweeting et al., 1985). Group housed horses could have possessed a competitive behavior while consuming foraging than stalled. Since the group housed horses were consuming forage from one round bale; whereas, stalled horses had *ad libitum* access to forage with no competition.

In a previous voluntary forage intake study, mature horses fed only coastal bermudagrass hay consumed 2.0% BW (Aiken et al., 1989). Horses in the current study were fed concentrate at 1% BW (as fed) with minimal refusals, total forage and concentrate intake averaged 2% BW/d in stalls and 2.4% BW/d when group housed. When feeding a mixed diet of forage and concentrate, horses will decrease the % BW consumed of forage in order to consume concentrate (Vermorel et al., 1997).

Table 2. Digestibility estimate from ADF (DM) in mature Quarter Horse geldings based on housing[1]

	Housing			P value		
Item	Stall	Group Housed	SEM	Treatment	Period	Horse
DDM, %	57.72	56.79	0.92	0.48	0.67	0.42

[1] Horses were group housed in a dry lot or individually in a stall. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (n = 8).

In general, markers have a tendency to underestimate intake due to external markers overestimating fecal output and internal markers underestimating digestibility. Titanium dioxide has been shown to overestimate fecal output in cattle (Titegemeyer et al., 2001), whereas, ADIA has been shown to have a 99.3% recovery rate when bermudagrass hay was fed to growing steers (Bodine et al., 2002). In the current study, ADIA estimated DMD similar to previously reported data (Table 2; NRC, 2007). The actual fecal output was not determined in the current study, so it cannot be determined whether or not TiO_2 overestimated fecal output. Even though the dual marker system underestimated intake in

the stalled horses, further research is needed to validate this dual marker system as an adequate system to determine forage intake in horses.

Cecal pH

Cecal pH was influenced by housing with group housed horses having lower cecal pH values when compared to stalled horses ($P = 0.02$; Figure 2). There was a tendency for cecal pH to be influenced by period with values obtained in the second period being lower than the first period ($P = 0.06$). This variation between periods suggests the washout period between treatment periods may have been inadequate. The washout period of 21 d should have allowed sufficient adjustment time for the microbial populations. Cecal pH values of this study are similar to those reported previously when horses were fed similar diets (Kern et al., 1973; Goodson et al., 1988). Julliand et al. (2001) observed cecal pH values averaging 6.74 at 5 h post meal when horses were fed hay only diets compared to 6.41 when barley was included in the diet for a 70:30 ratio of hay to grain.

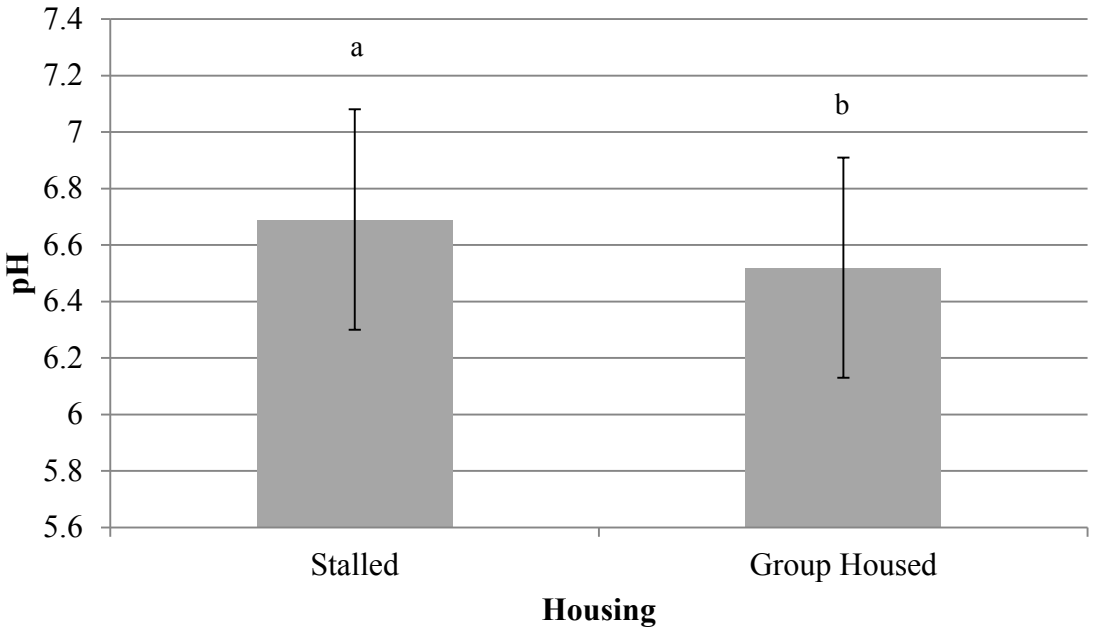

Figure 2. Cecal pH obtained 4 h after meal in mature Quarter Horse geldings that were group housed in a dry lot or individually in a stall. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Values are means ± SE (n = 8). Means without a common letter (a, b) are different ($P < 0.05$).

The pH values in the current study are more similar to the hay only diet, based upon forage intake in the current study, our horses were consuming a higher proportion of forage in their diet. Inclusion of barley in diets utilized by Julliand et al. (2001) caused a rapid decline in cecal pH compared to other cereal grains due to its resistance to small intestinal starch digestion. Medina et al. (2002) observed that a high starch diet produced an average cecal pH of 6.6 using a barley mixture as the starch diet which was designed to cause starch overload in the cecum. When comparing cecal pH values between experiments, it is important to note the influence of diet and timing of cecal samples. The current study measured cecal pH at 4 h post meal while the lower values obtained on barley based concentrate diets were obtained between 5 and 7 h post meal (Julliand et al., 2001).

Additionally, gut motility is directly linked to feeding activity (Ruckebusch, 1984). Rate of passage can be altered based upon frequency of meals and rate of intake which will directly affect the flow of digesta through the gastrointestinal tract. The difference in cecal pH between treatments in the current study could be attributed to alteration in rate of concentrate meal intake caused by group housed horses consuming the concentrate portion of their diet in a defined time period. Group housed horses were given access to concentrate until the entire meal was consumed or 1 h had passed, and in contrast stalled horses had free access to concentrate for 12 h between meals. Offering concentrate to group housed horses in this manner likely increased their rate of consumption and potentially increased rate of passage. Rate of passage could be increased due to the fact that group housed horses had been conditioned to consume their portion of concentrate quickly based upon previous conditioning when all horses were group fed in the same paddock before the current study began. If the group housed horses consumed their concentrate quicker than stalled horses did , the substrates from the concentrate meal would reach the cecum quicker causing the pH to be lower at 4 h after the morning meal.

It is important to note that even though there was a decline in cecal pH, the average cecal pH remained above 6.0, regardless of treatment. A pH value lower than 6.0 is indicative of subclinical lactic acidosis and has been link to increased incidences of colic and laminitis (Radicke et al., 1991).

Microbial Populations

Cecal microbial populations of total anaerobic bacteria and *Lactobacillus* spp. were not influenced by housing or period ($P \geq 0.12$) despite the alterations in cecal pH (Table 3). Even though microbial populations did not differ among treatments, total anaerobic bacteria

values in the current study were similar to values reported in previous studies utilizing similar diets (Kern et al., 1974; Julliand et al., 2001). In additional studies, total anaerobic bacteria values were 7.6 $\log_{10}$ c.f,u./mL when fed a barley concentrate (deFombelle et al., 2003) and 7.9 $\log_{10}$ c.f.u./mL when fed a high fiber diet (Medina et al., 2002). Current values fall within this range suggesting that housing does not cause an alteration in the total bacterial populations when a concentrate and forage diet is offered.

Lactobacillus spp. counts were similar to values previously reported (deFombelle et al., 2001) along with previous data from our laboratory (Wilson et al., 2009). Little previous data exists for the use of MRS agar with equine cecal fluid. However, data from Texas A&M University presented a range of 7.04 to 8.00 $\log_{10}$ c.f.u./mL of *Lactobacillus* spp. counts with MRS agar when horses were fed varying concentrations of dietary starch (Wilson et al., 2009). Results from the current study using MRS bacterial counts were similar (Table 3). Researchers have previously utilized Rogosa agar medium to enumerate equine cecal fluid for *Lactobacillus* spp. and observed bacterial counts of 6.2 $\log_{10}$ c.f.u./mL when feeding a pelleted diet rich in fiber and 6.3 $\log_{10}$ c.f.u./mL when fed a pelleted diet rich in cereals (deFombelle et al., 2003). Another study determined *Lactobacillus* spp. counts to be 6.4 $\log_{10}$ c.f.u./mL when fed a high fiber diet compared to a high starch diet at 7.7 $\log_{10}$ c.f.u./mL when utilizing Rogosa agar (Medina et al., 2002). Values in the current study are lower than these reported values with the same agar. Given the lower starch content in the concentrate portion of the current diet, it is not surprising. Amylolytic bacteria proliferate at an optimum pH range of 5.5 to 6.6 which is lower than the cecal pH values observed in the current study (Leek, 2004).

Table 3. Microbial populations in the cecum of mature Quarter Horse geldings based on housing[1]

Item	Housing		SEM	P value		
	Stall	Group Housed		Treatment	Period	Horse
Total Anaerobes, log_{10} c.f.u./mL	7.56	7.86	0.15	0.21	0.45	0.67
Lactobacillus, log_{10} c.f.u./mL						
MRS[2]	7.40	7.80	0.24	0.29	0.12	0.59
Rogosa[3]	6.05	5.77	0.12	0.16	0.43	0.09

[1] Horses were group housed in a dry lot or individually in a stall. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (n = 8).
[2] Difco™ Lactobacilli MRS Agar (Becton Dickson and Company, Sparks, MD) requires a carbon dioxide atmosphere to limit the amount of oxygen available allowing only strict anaerobes to grow.
[3]Difco™ Rogosa SL agar (Becton Dickson and Company, Sparks, MD) is incubated aerobically.

Volatile Fatty Acid Concentrations

Cecal VFA concentrations were not influenced by treatment or period ($P \geq 0.15$; Table 4). Housing treatments had no influence on cecal acetate concentration ($P = 0.33$; Table 4). Cecal concentrations of acetate in the current study are similar to values reported by Kern et al. (1973) when ponies were fed timothy hay without oats producing acetate concentrations of 39.2 *mM*. However, these values are lower than concentrations reported by Medina et al. (2002) and deFombelle et al. (2003). Medina et al. (2002) observed acetate concentrations to be 50.9 and 43.4 *mM* when fed a high-fiber and high-starch diet. Additionally, deFombelle et al. (2003) reported acetate to be 54.1 *mM* for a pelleted feed rich in fiber and 81.3 *mM* for a pelleted feed rich in cereals. The differences between studies could be related to the variations in DMI or components of the concentrate fed. Variation in

diet, sampling time, and rate of passage could have caused lower cecal acetate concentrations in this study.

Cecal propionate concentration was not affected by treatment ($P = 0.15$; Table 4). Concentrations from the current study are similar to concentrations reported by Kern et al. (1973) when ponies were fed timothy hay and oats. Propionate concentrations of the current study are lower than previous studies when starch content in the diet increases. Although starch was not fed in excess in the current study, the additional substrates from the concentrate portion of the diet did not significantly increase the propionate concentration to values closer to 12.8 or 19.7 that were reported by others using mixed diets (Medina et al., 2002; deFombelle et al., 2003).

Production of butyrate was not affected by treatment ($P = 0.20$; Table 4). Concentrations of butyrate in the current study are similar to concentrations ranging from 3.5 and 3.64 reported by Kern et al. (1973) and Medina et al. (2002), respectively when fed 100% timothy hay or a high-fiber diet.

Cecal valerate concentrations tended to be affected by treatment ($P = 0.07$; Table 4) with group housed horses having a higher concentration than stalled horses. In ruminants, valerate and branched chain VFAs are required for optimal growth of celluloytic bacteria (Hungate, 1966). Therefore, the increased valerate production in group housed horses could have been caused by a higher forage intake when compared to stalled horses. Kern et al. (1973) observed valerate production to be 0.2 mM when ponies were fed timothy hay with oats. The branched chain VFAs, isobutyrate and isovalerate, were not affected by treatment ($P \geq 0.10$) and values are similar those reported previously (Kern et al., 1973).

The ratio of primary VFAs in the current study was approximately 40:10:3 acetate:propionate:butyrate. Mackie and Wilkins (1988) observed that grass fed horses have an acetate: propionate: butyrate ratio of 85:10:3. Although the propionate and butyrate proportions are similar, the acetate ratio was lower in the current study. Kern et al. (1973) observed a lower acetate concentration of 34.6 *mM* as well when ponies were fed 75% timothy hay and 25% oat diet. Total VFA expressed as individual percentages in this study averaged 73% acetate, 20% propionate, and 5% butyrate which is similar to estimated values given by Hutjens (2008).

The acetate to propionate ratio was 3.8 to 1 for stalled and 3.6 to 1 for group housed horses, which is in the range observed by Medina et al. (2002) with a ratio of 3.97 when horses were fed a high fiber diet, and Hintz et al. (1971b) with a range of 2.38 to 3.85 when horses were fed various hay:grain ratios. Given the higher ratio and the estimated forage intake, both treatments consumed a higher percentage of forage in their diet.

Table 4. Volatile fatty acid concentration in the cecum of mature Quarter Horse geldings based on housing[1]

Item	Housing		SEM	P value		
	Stall	Group Housed		Treatment	Period	Horse
Acetate, *mM*	37.93	40.16	1.47	0.33	0.49	0.06
Propionate, *mM*	9.97	11.03	0.45	0.15	0.43	0.09
Butyrate, *mM*	3.03	3.34	0.15	0.20	0.44	0.04
Isobutyrate, *mM*	0.12	0.12	0.01	0.88	0.72	0.12
Isovalerate, *mM*	0.10	0.12	0.01	0.74	0.17	0.10
Valerate, *mM*	0.23	0.27	0.01	0.07	0.34	0.17

[1] Horses were either group housed in a dry lot or individually in a stall and fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (n = 8).

Cecal Methane and Ammonia Activty

Calculated cecal gas production including methane, carbon dioxide and hydrogen were not affected by treatment ($P \geq 0.55$; Table 5). However, methane, carbon dioxide, and hydrogen production were influenced by period ($P \leq 0.01$). It is important to note that hydrogen was included to the tubes in this procedure which could have caused an alteration in actual concentration. Additionally, it is difficult to determine what caused the variation between periods. Due to the inverse relationship of methane and propionate, there was not more propionate produced in one period over another to cause an alteration in methane production. In previous studies, methane production has been reported as 0.16 L/d/kg BW (Crutzen et al., 1986). McDaniel et al. (1993) observed *in vitro* fermentation with soluble starch to be 0.26 *mM* of methane. Although these values are higher than current values, methane production was not affected by housing which could be related to the minimal changes observed in the bacterial populations.

Ammonia production was not affected by treatment ($P = 0.46$; Table 4). The lack of differences between treatments could be related to the minimal changes observed with bacterial populations as seen with methane production. Ammonia production was similar to values reported in literature 66.9 mg/L (Medina et al., 2002). However, these values are considerably lower than McDaniel et al. (1993) who reported *in vitro* fermentation of cecal fluid with coastal bermudagrass hay to be 373.7 mg/L. The reason for lower levels of ammonia accumulation could be from the addition of concentrate into the ration which has been seen to decrease ammonia concentration in steers (Lana et al., 1998).

Table 5. *In Vitro* production using cecal fluid collected from Quarter Horse geldings based on housing[1]

Item	Housing		SEM	P value		
	Stall	Group Housed		Treatment	Period	Horse
Methane, µmol/mL/h						
3 h	0.0007	0.0006	0.0002	0.67	0.01	0.56
24h	0.0007	0.0008	0.0001	0.67	<0.01	0.56
Carbon Dioxide, µmol/mL/h						
3 h	0.120	0.130	0.011	0.55	<0.01	0.54
24 h	0.016	0.018	0.004	0.70	<0.01	0.55
Hydrogen, µmol/mL/h						
3 h	0.110	0.120	0.009	0.57	<0.01	0.34
24h	0.005	0.006	0.002	0.68	0.01	0.55
Ammonia, µmol	0.170	0.160	0.007	0.46	0.08	0.03

[1] Horses were either group housed in a dry lot or individually in a stall and fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (n = 8).

Stoichiometric Calculations

Stoichiometric calculations have been used in studies observing the effects of propionate enhancers like monensin sodium and chloral starch (Chalupa, 1977). Although, this study did not observe the effects of these enhancers; it is valuable to observe the stoichiometric calculations with equine cecal VFA data, since there is very limited research in this area. In the current study, the amount of hexose fermented was not influenced by treatment or period ($P \geq 0.24$); although, there was a tendency for the amount to be influenced by horse ($P = 0.06$). A previous study from Texas A&M University performed stoichiometric calculations with equine cecal VFAs and concluded similar results to the

current study (Wilson et al., 2009). Wilson et al. (2009) observed the amount of hexose fermented be to in the range of 20.15 ± 2.54 *mM* to 29.01 ± 2.54 *mM*. These values are similar to the current study (Table 6). These values are proportional to the VFA concentrations at that sample time. In general, the values are lower than those reported in ruminants, but the prececal digestion of NSC in horses might account for the lower hexose amounts available to be fermented in the cecum compared to the rumen.

Fermentative efficiency was not influence by treatment or period ($P \geq 0.15$); however, it was affected by individual horse variation ($P = 0.02$). The fermentative efficiency for stalled and group housed horses was 72.18% and 72.45%, respectively. In ruminants, the fermentative efficiency has been reported as 76.9% (Anderson et al., 2010) and 73.4 % to 77.4% (Chapula, 1977). Although horses are fermenting less hexose, they are still able to be as efficient as ruminants in relation to VFA concentrations. This also suggests that utilizing the fermentation balance equations to estimate fermentation products in horses could be useful. Thus, further research in this area is needed.

Reducing equivalents generated were not influenced by treatment or period ($P \geq 0.26$). The reducing equivalents consumed were also not influenced by treatment or period ($P \geq 0.30$). Both were affected by individual horse variation ($P = 0.05$). Information gathered from the stoichiometric calculations, in the current study, regarding reducing equivalents generated and consumed is listed in Table 6. According to data in ruminants, hydrogen generated and consumed, fermentative balance equations have determined the amount of hydrogen generated to be 79.3 µmol/mL and the amount of hydrogen consumed to be 71.9 µmol/mL (Anderson et al., 2010). These values are lower than the values estimated

in the current study. However, the values are similar and suggest that the fermentation balance equations can potentially be utilized to estimate fermentative products in horses.

Table 6. Stoichiometric calculations estimating fermentative products using equine cecal VFA concentrations in mature Quarter Horse geldings based on housing[1]

Item	Housing		SEM	P value		
	Stall	Group Housed		Treatment	Period	Horse
Carbon Dioxide, *mM*	30.00	32.27	1.21	0.24	0.45	0.05
Methane, *mM*	13.97	14.56	0.53	0.47	0.54	0.04
Hexose Fermented, *mM*	27.21	29.20	1.08	0.24	0.45	0.06
Fermentative efficiency, %	72.18	72.45	0.11	0.15	0.72	0.02
Reducing equivalents consumed, *mM*	82.14	87.27	3.19	0.30	0.48	0.05
Reducing equivalents generated, *mM*	98.39	105.25	3.89	0.26	0.46	0.05

[1]Horses were either group housed in a dry lot or individually in a stall and fed a diet consisting of 1% BW (as fed) pelleted concentrate with ad libitum coastal bermudagrass hay. Cecal contents were collected 4 h after morning meal. Values are means (n = 8).

CHAPTER IV

CONCLUSIONS

In conclusion, confinement housing did not significantly affected the cecal environment measured as cecal pH, enumeration of microbial populations, and fermentation products in horses offered similar diets. However, confinement housing decreased voluntary forage intake as estimated by a dual marker system.

This study was designed to ensure that there were no confounding variables, such as diet, to determine the influence of housing, and the results concluded that housing does not alter microbial populations in the cecum. Further research is needed to determine any long term effects of a reduced forage intake in stalled horses. Additionally, further research is required to more accurately describe behavioral modification that occurs in horses adapting to individual housing when all other variables remain constant.

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APPENDIX

A unique aspect of the current study allowed for a comparison between estimated forage intake using the dual marker system and direct measurements of forage intake from the stalled horses. The dual marker system estimated an average forage DM consumption of 6.98 kg compared to a measured consumption of 5.40 kg DM for stalled horses (Figure 3). Information available on forage intake in horses on pasture is limited and results have been inconsistent when markers are utilized (Friend and Nash, 2000; McMeniman, 2000). Further research is needed to validate TiO_2 as an external marker in horses.

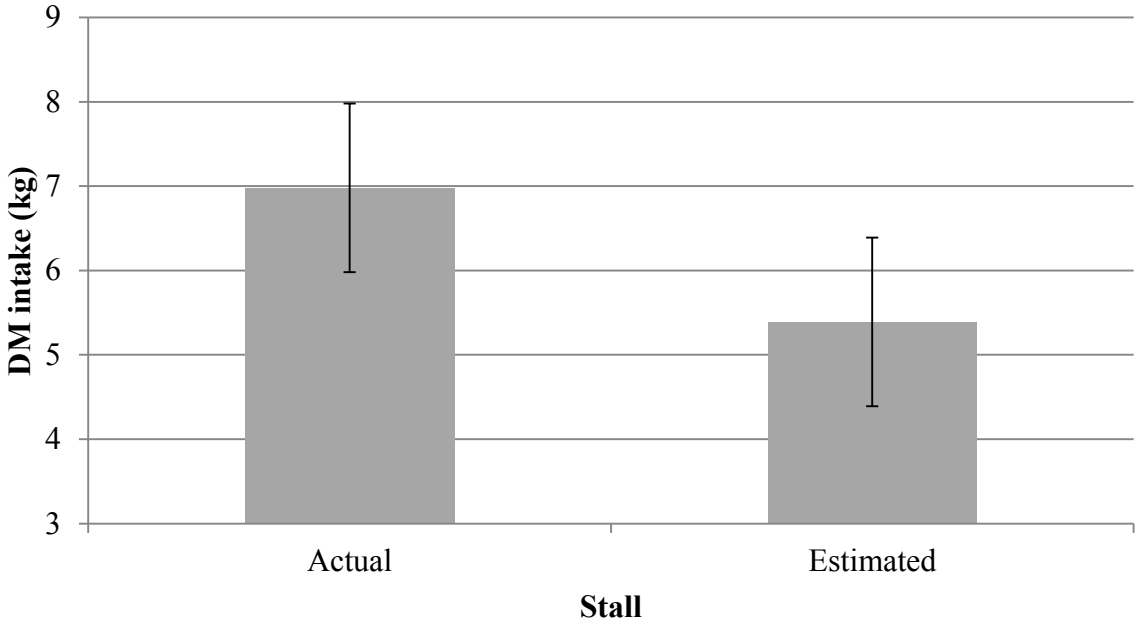

Figure 3. Voluntary forage intake measured directly or indirectly in mature Quarter Horse geldings that were group housed in a dry lot or individually in a stall. Direct measurements of forage intake were collected from stalled horses, and TiO_2 and ADIA were utilized as markers to estimate intake. All horses were fed a diet consisting of 1% BW (as fed) pelleted concentrate with *ad libitum* coastal bermudagrass hay. Values are means ± SE (n = 8).

VITA

Name: Ashley N. Wolford

Address: 2471 TAMU
Texas A&M University
College Station, TX 77843

Email Address: ashley.wolford88@gmail.com

Education: B.S., Animal Science, Berry College, 2009
M.S., Animal Science, Texas A&M University, 2011