Effect of carbohydrate source on fermentation by equine cecal microorganisms and effect of Enogen® Feed Corn on finishing pig growth performance and carcass characteristics

by

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B.S., Pennsylvania State University 2015
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
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Abstract

Commercial horse feeds utilize cereal grains and byproducts; however, their effects on the cecal environment remain poorly characterized. Six cecally cannulated Quarter horses were used in a 6 × 6 Latin square in order to characterize the effect of commonly used feed ingredients on cecal pH and volatile fatty acid (VFA) concentration. On d -2, horses were moved into individual stalls where Smooth bromegrass hay (brome) was offered at 2.0% BW/d split between 2 feedings (0600 and 1800). On d 0, cecal digesta was collected every 2 h for 12 h relative to the 0600 feeding to establish control values for horses consuming only brome (HAY). On d 1, horses began consuming their respective treatments which consisted of beet pulp (BP), maize (M), dehydrated alfalfa (A), oats (OAT), soybean hulls (SBH), or wheat middlings (WM) at 0.25% BW/d split into 2 feedings. On d 7 of each treatment period, cecal digesta was collected every 2 h for 12 h and analyzed for pH and VFA. Data were analyzed using mixed ANOVA with repeated measures, fixed effects of treatment and time, and random effects of horse and period. There was a main effect of hour ($P \leq 0.05$) indicative of post-prandial shifts in cecal metabolites. There were no main effects of treatment on pH or VFA concentration ($P \geq 0.31$). Effects of hour × treatment ($P \leq 0.042$) were observed for all response variables. Regardless of treatment or hour, cecal pH remained well within normal limits. Three horses exhibited signs of lower esophageal choke immediately after consumption of BP pellets. Overall, minimal differences in cecal fermentation parameters were detected when ingredients were fed at a common inclusion level.

Overconsumption of dietary starch is associated with increased risk of hindgut acidosis and subsequent development of gastrointestinal and metabolic disorders. Researchers have suggested that the upper limit of small intestinal starch digestion is 2 to 4 g starch·kg BW^{-1}·meal^{-}

[1]. The objectives of this trial were to determine the effect of increasing levels of dietary starch on the cecal environment and voluntary forage dry matter intake (DMI). Six cecally cannulated Quarter horses (8-13 yr; 524 ± 65.5 kg BW) were used in a dose titration style design. Before the study began, horses were maintained in a single dry lot pen and offered *ad libitum* Smooth bromegrass hay (brome). On d -14, horses were moved to individual stalls and provided brome hay *ad libitum* and a ration balancer (0.0125% BW 4x/day). On d 0, cecal digesta samples were collected every 2 h for 12 h relative to the 0600 feeding. On d 1, pelleted corn (69.4% starch) was offered at 0.5 g starch·kg BW^{-1}·$meal^{-1}$at 0600, 1200, 1800, and 2400 h. Every 8 d thereafter, corn was increased to provide an additional 0.5 g starch·kg BW^{-1}·$meal^{-1}$ until horses were consuming 3.5 g starch·kg BW^{-1}·$meal^{-1}$. Seven days following each increase in dietary starch, the cecal sampling protocol was repeated. Cecal pH was recorded upon sample collection. Cecal digesta was analyzed for volatile fatty acids (VFA) via gas chromatography, and lactate via a colorimetric procedure. Data were analyzed as a randomized complete block design with repeated measures, with fixed effects of treatment and time and random effect of horse. There was an effect of treatment on forage DMI ($P \leq 0.0001$). As dietary starch increased, forage DMI decreased. Greatest total DMI ($P \leq 0.041$) was recorded when horses were offered 3.0 g starch. It should be noted that horses did not consume the full meal for treatments 2.5, 3.0, and 3.5 g starch, and on average consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·$meal^{-1}$ (respectively). Effects of treatment, hour, and treatment × hour were observed ($P \leq 0.032$) for pH, acetate, propionate, acetate:propionate, and total VFA concentration. Cecal pH decreased as dietary starch increased, with lowest pH observed with 2.0 g starch ($P < 0.0001$). Cecal propionate and total VFA concentration were greatest ($P < 0.0485$) for 2.0 and 2.5 g. Acetate:propionate ratio was lower ($P \leq 0.005$) for 2.0 g starch compared to ≤ 1.5 g treatments; however, A:P did not

continue to decrease with additional starch. Cecal acetate, propionate, butyrate, and total VFA concentrations were lowest ($P < 0.0001$) when horses were fed 3.5 g starch. Cecal lactate concentration was greatest ($P < 0.0060$) when horses were fed 2.0 g starch and lowest ($P < 0.0001$) for horses consuming 0 and 0.5 g starch. This trial demonstrates that voluntary forage DMI decreases with increasing levels of starch, as previously observed in ruminants. Consumption of $\geq$ 2.0 g starch·kg BW^{-1}·$meal^{-1}$ may lead to shifts in cecal microbial communities that result in decreased fermentative activity. However, even when horses were offered $\geq$ 2.0 g starch·kg BW^{-1}·$meal^{-1}$ cecal acidosis was not observed.

Enogen® Feed Corn (EFC; Syngenta Seeds, LLC, Downers Grove, IL) hybrids contain a trait for expression of heat-stable alpha amylase in the grain. Alpha amylase is an enzyme responsible for breakdown of starch in the small intestine; supplementation of exogenous alpha amylase to pigs may result in greater starch digestibility and thus improved gain efficiency. A total of 288 pigs (Line 600 × 241, DNA, Columbus, NE; initially 41.6 kg) were utilized in an 82-d trial to determine if replacing conventional yellow dent corn (CONV) with EFC in diets with or without distillers dried grains with solubles (DDGS) influences growth performance and carcass characteristics. Pens of pigs were randomly assigned to 1 of 4 dietary treatments balancing for initial BW. There were 9 pens per treatment with 8 pigs per pen (an equal number of barrows and gilts per pen). Treatments were arranged in a 2 × 2 factorial with main effects of corn source (CONV or EFC) and DDGS (0 or 25%). Experimental diets were fed in meal form in 3 phases: d 0 to 29, 29 to 47, and 47 to 82. Pigs were weighed approximately every 2 wk and at the beginning of each phase. On d 82, pigs were transported to a commercial abattoir for processing and carcass data collection. Data were analyzed using PROC GLIMMIX procedure of SAS with pen as the experimental unit. There were no corn source by DDGS interactions ($P > 0.05$)

observed for overall performance or carcass characteristics. Overall, average daily gain (ADG) was marginally greater ($P < 0.089$) for pigs fed EFC than CONV with no evidence ($P > 0.196$) for difference in average daily feed intake (ADFI), gain efficiency (G:F), hot carcass weight (HCW), or other carcass traits. Addition of DDGS decreased ($P < 0.047$) overall ADG and G:F. Pigs fed DDGS had marginally lower ($P < 0.071$) HCW, less ($P < 0.050$) backfat depth, greater ($P < 0.026$) loin depth, and greater ($P < 0.020$) percentage lean and carcass fat iodine value (IV). In summary, addition of 25% DDGS to the diet decreased ADG and increased carcass fat IV. Pigs fed EFC tended to have improved overall ADG; however, G:F and carcass characteristics were not different between corn sources. These results suggest that EFC, although not beneficial, may be used as a substitute for CONV without any deleterious effects on growth performance. Further research should be conducted to understand if addition of EFC to swine diets could be beneficial in younger pigs exhibiting decreased pancreatic α-amylase secretion following weaning, or whether heat treatment of diets, such as pelleting, may influence the response to EFC.

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James M. Lattimer

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Dedication

I dedicate this work to my partner, Ken Smith. Thank you for your love, patience, and endless encouragement throughout my graduate career.

Preface

Chapter 2, entitled “Caecal fermentation characteristics of commonly used feed ingredients”, was published in the Equine Veterinary Journal (Ochonski, P., J.S. Drouillard, T.L. Douthit, C. Vahl, and J.M. Lattimer. 2020. Eq. Vet. J. 00:1-7. doi:10.1111/evj.13390.); this journal required a numbering format when citing references and is written in British English. Chapter 4, entitled “Evaluation of Enogen® Feed Corn on growth performance and carcass characteristics of finishing pigs”, has been accepted for publication in Translational Animal Science (https://doi.org/10.1093/tas/txab052). Chapters 1 and 3 are written and citations formatted in the style of Journal of Animal Science.

Chapter 1 - Review of the Literature

Introduction

Despite significant energetic contribution of products produced through fermentation of feedstuffs by microorganisms in the equine hindgut (Glinksy et al., 1976; Vermorel et al., 1997) and the role of hindgut dysbiosis in development of disease (Bailey et al., 2009; Kwon et al., 2013; Flores et al., 2011), data characterizing cecal fermentation parameters are sparse in the literature. Furthermore, most published reports regarding effects of diet on hindgut fermentation have been based on *in vitro* rather than *in vivo* techniques, and for both techniques data are commonly derived using fecal samples. Recent data from our laboratory has illustrated that fecal samples are not representative of microbial or fermentative shifts in the cecum (Sorensen et al., 2021). Therefore, characterization of shifts in cecal microbial populations and fermentation parameters, especially in conditions where dysbiosis and subsequent disease may occur, will contribute to advancing equine health and management practices.

Nutritional strategy to improve feed efficiency, thereby improving economic gain, in pigs have ranged from novel ingredients, to feed additives and feed processing techniques. Starch digestibility may be improved through feed processing methods, such as fine grinding and pelleting (Rojas et al., 2016). However, increased grain processing may lead to development of gastric ulcers, which have been reported to occur in 21 to 96% of finishing pigs and contribute to economic loss due to increased mortality or decreased growth performance (De Magalhaes Quieroz et al., 1996; Friendship et al., 2004; Gottardo et al., 2017). Published data regarding α-amylase supplementation in swine diets are relatively limited in comparison to other enzymes, but α-amylase supplementation may provide an alternative method of improving feed efficiency without compromising gastrointestinal health. Furthermore, addition of > 30% distillers dried

grains with solubles (DDGS) to swine diets has yielded negative results; some authors have reported decreased growth performance and impaired carcass characteristics of pigs supplemented with DDGS (Whitney et al., 2006; Lineen et al, 2008). Research is needed in order to assess if feeding DDGS in combination with more novel feed ingredients, such as exogenous enzymes, may mitigate deleterious effects of DDGS on growth performance.

Carbohydrate Digestion and Absorption in Equines

Carbohydrates provided through forage and grains are the predominant source of energy in equine diets. Horses evolved consuming large quantities of forage for up to 18 hours per day (Crowell-Davis et al., 1985; Fleurance et al., 2001), consequently leading to development of a gastrointestinal tract poorly suited for digestion of sugar- and starch-rich feedstuffs. Use of horses as work and performance animals results in increased energy requirements frequently met through dietary supplementation of energy-dense concentrates. This alteration of carbohydrate fractions – from primarily fiber to increased sugar and starch – in equine diets, coupled with modern day management practices have been implicated in development of metabolic and gastrointestinal-related disorders, including gastric ulcers, insulin resistance, laminitis, and colic (Hoffman et al., 2003; Bailey et al., 2009; Kwon et al., 2013; Flores et al., 2011). In order to fully appreciate effects of altering carbohydrate fractions on cecal microorganisms, it is necessary to understand carbohydrate classification, and how these different fractions are digested and absorbed in the gastrointestinal tract.

Carbohydrate Classification

There are many methods of carbohydrate classification, including chemical methods such as degree of polymerization, type of linkage, and character of individual monomers, as well as physical properties related to animal and human health (Englyst and Englsyt, 2005; Cummings and Stephen, 2007). Traditional classification of carbohydrates in animal feeds was derived from time-honored methods of feed analysis developed by Van Soest (1963), which partitions carbohydrates into structural carbohydrates, including neutral detergent fiber (NDF), and non-structural carbohydrate (NSC) fractions. Broadly, structural carbohydrates fractions refer to components that make up plant cell wall and include cellulose, hemicellulose, and lignin while NSC fractions refer to cellular contents and include monosaccharides, oligosaccharides, starches, and fructans (Van Soest et al., 1991; Hall, 2003).

Over the last several decades, new methods to classify carbohydrates in animal feeds have been described. Non-fiber carbohydrates (NFC), a classification that includes all components not found in the NDF portion, has been used by some in lieu of NSC. This classification differs from NSC, as NFC encompasses soluble fibers including pectic substances, galactans, and β-glucans (Hall, 2003; NRC, 2007). Furthermore, determination of NFC is derived mathematically rather than analytically by subtracting 100 – NDF% – crude protein% – ash% – ether extract%. Use of NFC as an all-encompassing term is appropriate in ruminants, as all components that make up this term are fermented by ruminal microorganisms. Due to monogastric digestion of the equine foregut, carbohydrates that fall under the NFC umbrella are not all digested within one location.

Hoffman et al. (2001) proposed a method of classification that more suitably reflects gastrointestinal function of equines. Instead of NDF, NSC, or NFC, the terms hydrolysable

carbohydrates (CHO-H), rapidly fermentable carbohydrates (CHO-F_R), and slowly fermentable carbohydrates (CHO-F_S) were introduced. Digestion of CHO-H occurs predominantly via hydrolysis by mammalian enzymes within the small intestine to produce glucose (Shirazi-Beechey, 2008), whereas CHO-FR and CHO-FS are fermented by cecal and colonic microorganisms to yield volatile fatty acids (VFA; Glinksy et al., 1976; Vermorel et al., 1997). This method of carbohydrate classification is perhaps most useful when discussing gastrointestinal disturbances related to carbohydrate intake. For instance, overconsumption of CHO-H and CHO-FR fractions has been noted to cause unfavorable shifts in cecal microbial communities, decreased cecal pH, and subsequent acidosis (Julliand et al., 2001; Biddle et al., 2013). Additionally, adequate CHO-FS consumption provided through forage has been deemed essential for maintenance of gut health in horses (Daly and Shirazi-Beechey, 2006; Sykes et al., 2015). Despite this, a dietary requirement for fiber has yet to be established for horses (NRC, 2007).

Foregut Digestion and Absorption

Mechanical digestion of carbohydrates begins orally through mastication. Horses possess a hypsodont dentition pattern – defined by continuous eruption of teeth – as an adaptation to wear and tear caused by consumption of coarse forages (Mihlbachler et al., 2011). Relationships between feed form and mastication behavior in horses have been well characterized; in general, forages require greater masticatory activity compared to concentrates (Ellis et al., 2011). Bonin et al. (2007) utilized an optical motion capture system to evaluate motion of the temporomandibular joint of horses and found horses consuming pellets exhibited increased rate of feed intake and decreased occlusal contact compared to forage-fed horses. Decreased grinding

of tooth surfaces through decreased occlusal contact results in greater need for dental care in horses supplemented with concentrates. Furthermore, decreased masticatory rate is correlated with decreased saliva production, as saliva is only secreted from the parotid gland of equines in response to mastication (Alexander, 1966). Equine saliva contains minimal α-amylase compared to other monogastric animals and serves primarily as a buffer to protect nonglandular stomach tissues against acidic secretions of the lower stomach (Eckersall et al., 1985; Al Jassim and Andrews, 2009). Increased concentrate consumption and decreased saliva secretion have been proposed as factors in development of gastric ulcers (Nicol et al., 2002; Hepburn, 2011).

Equine gastric anatomy is divided into 2 regions based on tissue type which are separated by a line of demarcation called the margo plicatus. Stratified squamous epithelial tissues line the dorsal third of the stomach. This region is commonly referred to as the nonglandular region due to absence of secretory glands. This tissue type is similar to tissue lining the esophagus and is relatively unprotected from acidic secretions of the lower stomach. Thus, nonglandular tissues proximal to the margo plicatus are the most common site of gastric ulcers in horses (Al Jassim and Andrews, 2009; Sykes et al., 2015). The lower two-thirds of the stomach consists of a glandular mucosa and is the primary site of secretion of hydrochloric acid, pepsinogen, and mucus (Merritt, 1999; Andrews et al., 2005; Hepburn, 2011).

Aa an evolutionary adaptation of horses who graze almost continuously, hydrochloric acid is secreted continuously, regardless of level of feed intake or presence of digesta in the stomach (Murray, 1994; Murray and Eichorn, 1996). A pH gradient exists within the stomach, with the greatest pH (5 to 7) located dorsally in the nonglandular region and lowest pH (1 to 2) observed at the ventral-most region of the stomach proximal to the pyloric sphincter (Andrews and Nadeau, 1999; Merritt, 2003; Hepburn, 2011). Stratification of gastric contents has been

implicated as vital to maintaining gastric health in horses and can be achieved through adequate forage intake (Hepburn, 2011). Increased homogenization of gastric contents is observed withconsumption of high concentrate meals. Lapinskas (2017) observed increased gastric ulceration of horses consuming forage versus those consuming concentrate. Similarly, Flores et al. (2011) observed increased number and severity of gastric when horses were fed high-grain diets compared to horses fed hay only.

Prior to entering the stomach, hydrolytic breakdown of CHO-H is minimal due to negligible amylase secretion in equine saliva (Eckersall et al., 1985). Although most CHO-H digestion occurs within the small intestine, digestion can also occur to a limited extent within the stomach. Decreased pH, driven by hydrochloric acid secretion, stimulates hydrolysis of CHO-H (Ince et al., 2013; Strauch et al., 2017). Additionally, limited breakdown of CHO-H, CHO-FR, and CHO-FS has been described to occur through fermentation by gastric microorganisms (Argenzio et al., 1974; Nadeau et al., 2009; Perkins et al., 2012). Healy et al. (1995) observed increased gastric lactate and butyrate concentrations in ponies fed a concentrate meal. Similarly, Nadeau et al. (2000) described greater acetate, propionate, and butyrate concentrations in gastric juices obtained from horses consuming an alfalfa and grain-based diet compared to those consuming grass hay. de Fombelle et al. (2003) confirmed presence of lactate-producing and lactate-utilizing bacteria within the stomach of horses fed high-concentrate diets. They observed similar lactate and greater propionate compared to horses only consuming hay, which led them to conclude that lactate had been fermented to propionate by lactate-utilizing microorganisms. Despite this, digestion of carbohydrates and subsequent absorption of products in the stomach is minimal compared to the rest of the equine gastrointestinal tract, largely due to a rapid rate of passage of digesta through the equine stomach (Argenzio et al., 1974).

Published research regarding small intestinal CHO-H digestion in equines are relatively limited compared to other monogastric species. Once in the duodenum, CHO-H are hydrolyzed by pancreatic amylase or brush border membrane disaccharidases to produce monosaccharides (Shirazi-Beechey, 2008). Starch is primarily digested by pancreatic α-amylase in the duodenum of the small intestine. This enzyme cleaves α-1,4 linkages in the starch molecule to produce maltose, maltotriose, and α-limit dextrins (Gray, 1992). These products are further broken down by brush border enzymes to yield glucose, which is transported across the intestinal wall primarily by Na+/glucose co-transporter 1 (SGLT1) and utilized by the host (Moran et al., 2010). Horses are largely unsuited to digest large quantities of starch pre-cecally due to limited pancreatic α-amylase secretion compared to other monogastrics such as pigs (Kienzle, 1994). Interestingly, horses may possess some ability to upregulate glucose absorption in response to greater CHO-H intake. Dyer et al. (2009) observed a 2-fold increase in duodenal and 3-fold increase in ileal SGLT1 expression of horses transitioned from a hay-only to a CHO-H rich diet.

Classical works of Potter et al. (1992) and Kienzle (1994) defined an upper limit of small intestinal starch digestion by feeding ilealy cannulated ponies increasing amounts of corn starch and measuring starch appearance at the distal ileum. A dramatic increase in starch appearance at the distal ileum was noted by both authors when ponies were fed between 2 to 4 g starch·kg BW^{-1}·$meal^{-1}$; this range was thus deemed the upper limit of small intestinal starch digestion. Consumption of > 2 g starch·kg BW-1·meal-1 of starch has been noted to rapidly overwhelm the cecal environment, causing unfavorable shifts in cecal microbial communities, decreased cecal pH, and subsequent acidosis (Potter et al., 1994; Julliand et al., 2001; Biddle et al., 2013).

The rate and extent of starch digestion is highly dependent on starch chemistry, particularly relative ratios of amylose and amylopectin (Svihus et al., 2005). Feed processing

methods, including grinding, pelleting, and extrusion, increase pre-cecal starch digestibility (Julliand et al., 2006). Kienzle et al. (1997) recorded starch appearance at the distal ileum of horses fed whole, crushed or finely ground corn and reported increased pre-cecal starch disappearance with greater degree of feed processing. Others have utilized post-prandial glycemic response as a method to assess pre-cecal starch digestibility. Both Hoekstra et al. (1999) and Nielsen et al. (2010) noted linear increases in glycemic response of horses in response to increased feed processing methods. In both experiments, thermal processing methods such as steam-flaking, pelleting, and extrusion yielded greater post-prandial glycemic response compared to cold-processing methods such as grinding. Pre-cecal starch digestibility is also impacted by botanical origin (Radicke et al., 1991; Potter et al., 1992; Meyer et al., 1995). For instance, Kienzle (1994) observed greater pre-cecal disappearance of oat starch compared to corn starch in ponies. Rosenfeld and Austbø (2009) utilized the mobile bag technique in order to measure pre-cecal and total tract digestibility of cereal grains ground to a similar particle size. Pre-cecal starch digestibility was greatest for oats (94.9%), followed by barley (70.5%), and was lowest for corn (66.3%).

Hindgut Digestion and Absorption

The cecum and large colon, collectively referred to as the hindgut, comprise approximately 60 percent of equine gastrointestinal tract anatomy by volume (Hintz and Cymbaluk, 1994). Rate of passage is faster through the foregut compared to the hindgut, with undigested CHO-H, CHO-FR, and CHO-FS entering the cecum 1.5 to 3 h and exiting via the rectum 24 to 48 h post-prandially (Argenzio et al., 1974; Van Weyenberg et al., 2006; Santos et

al., 2011). Most post-prandial fermentative shifts in the cecum have been reported 4 to 8 h following meal consumption (Jordan et al., 2019; Fehlberg et al., 2020; Ochonski et al., 2020).

The equine hindgut houses a diverse microbial community that is influenced by diet (Costa et al., 2018; Sorensen et al., 2021). Glinsky et al. (1976) observed that approximately 30% of energy requirement is satisfied by VFA production in the cecum of ponies consuming a mixed concentrate/forage diet, whereas for horses consuming an all-forage diet approximately 70% of daily energy requirement was supplied by VFA (Vermorel et al., 1997). Despite the importance of hindgut fermentation to energy status in equines, published data characterizing cecal fermentation characteristics and microbial communities are highly limited (Julliand and Grimm, 2016). Additionally, most experiments designed to evaluate effects of diet on hindgut environment have relied on data derived from fecal samples; which are not indicative of microbial or fermentative shifts in the cecum (Jensen et al., 2013; Murray et al., 2013; Sorensen et al., 2021).

Relative proportions of carbohydrate fractions entering the cecum influence production of individual VFA, particularly acetate, propionate, and butyrate. For instance, fermentation of CHO-FS by fibrolytic bacteria yields acetate (Hintz et al., 1971; Sorensen et al., 2021) while fermentation of CHO-H by amylolytic bacteria primarily yields propionate and butyrate (Medina et al., 2002; Sadet-Bourgeteau et al., 2017). Acetate concentration is always greatest in proportion to other VFA found within the equine hindgut (Hussein et al., 2004; Ochonski et al., 2020; Sorensen et al., 2021). Hydrolysable carbohydrates entering the cecum are rapidly fermented by cecal microorganisms, particularly lactate-producing bacteria Lactobacillus and Streptococcus (Bailey et al., 2003; Al Jassim and Andrews, 2009). Lactate is rapidly metabolized to propionate and butyrate by lactate-utilizing microorganisms in horses that have been adapted

to high-CHO diets (de Fombelle et al., 2003; Millinovich et al., 2007). Douthit et al. (2019) characterized the effect of adding *Megasphera elsdenii*, a prominent lactate-utilizing microorganism, to cultures of cecal fluid containing oligofructose or corn starch. Addition of *M. elsedenii* lessened lactate accumulation in cultures of cecal fluid. Biddle et al. (2013) reported that lactate accumulation and subsequent attenuation of lactate in fecal slurries enriched with starch corresponded to a greater abundance of *M. elsedenii*. Instances of starch overload result in rapid proliferation of lactate-producing microorganisms, leading to lactate accumulation, depressed cecal pH, and subsequent development of cecal acidosis and colic (Bailey et al., 2003; Al Jassim and Andrews, 2009), which is why the role of lactate-utilizing bacteria in modulating cecal pH are of particular interest.

Protonated VFA are predominantly absorbed across cecal and colonic epithelial tissues via passive diffusion (Argenzio et al., 1974; Hintz et al., 1978). Rate of VFA absorption is negatively correlated to pH; lower pH promotes protonation of VFA thereby increasing absorption rate (Rechkemmer et al., 1988; Bergman, 1990). Following absorption, acetate is partially taken up by the liver and can be used directly as an energy source throughout the body via oxidation (Pethick et al., 1993; Janson and Lindberg, 2012). Propionate is the only VFA that is gluconeogenic, and following removal from portal blood by the liver propionate enters the citric acid cycle as succinate (Larsen and Kristensen, 2013). Early work by Ford and Simmons (1985) reported approximately 7% of total glucose production was derived from cecal propionate in ponies fed only hay. Simmons and Ford (1991) went on to report approximately 50 to 60% of blood glucose was derived from colonic propionate of horses fed only hay. Butyrate is metabolized in local epithelial tissues, as it is a vital energy source for cecal and colonic enterocytes (Daly and Shirazi-Beechey, 2006).

Fermentation of Common Feed Ingredients by Hindgut Microorganisms

Despite the fact that equines meet 30 to 70% of their daily energy needs from VFA production (Glinsky et al., 1976; Vermorel et al., 1997), digestible energy (DE) is utilized to the caloric content of equine rations. This method considers energy lost in feces but does not account for heat loss associated with fermentation (NRC, 2007). Characterization of feed ingredients solely in terms of DE does not provide information on pre-cecal absorption or fermentability of these ingredients within the cecum. Published data characterizing fermentation parameters of individual feed ingredients are limited. Most reports regarding effects of feed ingredients on hindgut fermentation have been based on *in vitro* rather than *in vivo* techniques, and for both techniques data are commonly derived using fecal rather than cecal samples (Elghandour et al., 2014; Salem et al., 2015; Kholif et al., 2016). Further research regarding effects of feed ingredients most commonly included in formulation of equine diets – alfalfa, beet pulp, corn, oats, soybean hulls, and wheat middlings – is necessary in order to assist nutritionists and commercial formulators in determining appropriate ingredient alternatives in the formulation of commercial concentrates for different physiological classes of equines.

Alfalfa

Alfalfa, referred to as lucerne in some countries, can be included in horse diets as a long-stemmed forage or processed feed ingredient. This legume has the greatest feed value of all hay types, attributed to greater energy, protein, and organic matter digestibility compared to grass hays (Crozier et al., 1997; LaCasha et al., 1999; Sturgeon et al., 2000). Additionally, alfalfa has been noted to exhibit greater intrinsic buffering capacity compared to other forages due to greater calcium and protein concentrations (Ginger-Reverdin et al., 2002). Increased gastric pH and

decreased incidence and severity of gastric ulceration have been well-documented in horses supplemented with alfalfa hay (Nadeau et al., 2000; Lybert, 2010; Flores et al., 2011; Stowers et al., 2013). However, data characterizing beneficial effects of alfalfa supplementation on hindgut environment are limited and warrant further research.

Julliand et al. (2018) characterized the effect of alfalfa meal versus sunflower meal supplementation at a rate of 0.29% BW/d in combination with rolled barley (0.50% BW/d) and grass hay (1.0% BW/d) on fecal samples. They observed no differences between alfalfa meal and sunflower meal on pH or VFA concentration in fecal samples. Recently, Sorensen et al. (2021) evaluated the effect of Smooth Bromegrass versus alfalfa hay on cecal and fecal characteristics in horses fed *ad libitum* hay. For horses fed alfalfa, cecal samples contained greater VFA concentrations and decreased pH compared to fecal samples indicating that greater fermentation of alfalfa occurs within the cecum. Furthermore, cecal VFA concentration was greater for alfalfa-fed horses compared to brome-fed horses, supporting greater fermentability of this feedstuff.

Beet Pulp

Poor dentition of geriatric horses results in decreased forage intake; therefore, inclusion of alternative fiber sources in the diet is often necessary to maintain gut health (Ralston, 2005; Jarvis, 2009). Additionally, increased evidence associating high CHO-H cereals with development of gastrointestinal and metabolic disorders has resulted in a greater need feedstuffs with reduced energy density which is achieved through inclusion of more fibrous ingredients. Beet pulp is frequently included in equine diets due to its high soluble fiber content that serves as a substrate for microorganisms within the hindgut (Crandell et al., 1999; Rodiek and Stull, 2007).

Due to its beneficial effects on gastrointestinal health, research regarding effects of beet pulp on fermentation by hindgut microorganisms are more abundant compared to other feed ingredients.

Murray et al. (2006) observed greater *in vitro* gas production of fecal microorganisms when high-temperature dried alfalfa was supplemented with beet pulp versus alfalfa alone. Thus, addition of beet pulp may improve fiber degradability when fed in combination with forage, although similar results *in vivo* have yet to be observed. Associative effects of feed ingredients have been well documented in cattle, particularly the ability of ingredients high in soluble fiber to modulate ruminal pH when fed in combination with high-starch ingredients (Huck et al., 1998; Dixon and Stockdale, 1999). Studies regarding the ability of beet pulp to modulate hindgut pH of horses have yielded varying results. Brøkner et al. (2010) utilized a pH electrode inserted through a cecal canula to continuously measure post-prandial pH fluctuations of horses. When fed with barley that provided 2.0 g starch·kg BW^{-1}·$meal^{-1}$, addition of sugar beet pulp at a rate of 0.5% BW stabilized cecal pH compared to barley alone, which was attributed to greater acetate:propionate. Conversely, Jensen et al. (2016) observed no differences in cecal pH of horses were fed barely alone or those supplemented with beet pulp. Ochonski et al. (2020), demonstrated minimal differences in cecal pH and fermentation parameters between beet pulp and other fibrous feed ingredients, such as wheat middlings or soybean hulls, when fed at a rate of 0.25% BW/d. Positive effects of beet pulp supplementation on hindgut microorganisms may be dependent on level of intake and inclusion of other ingredients in the diet; exploration of these relationships requires further investigation *in vivo*.

Corn

Corn is added to equine diets due to its high energy content, which is attributed to greater starch content (69%) compared to other cereal grains (NRC, 2007). It has been well documented that excessive consumption of corn starch leads to shifts in the microbial population of the equine hindgut and development of acidosis (Potter et al., 1992; Kienzle, 1994; Julliand et al., 2001). Horse owners have taken this into consideration when purchasing concentrate feeds, and over the past few decades there has been a consumer push for increased fat and less starch in concentrates. Due to its high starch content, corn has been viewed by some as a less desirable feed ingredient compared to alfalfa, beet pulp, or soybean hulls. However, negative effects associated with consumption of corn are related to level of intake and some researchers support the use of this ingredient when fed at moderate intake (Ochonski et al., 2020; Thorringer et al., 2020).

Fermentation of corn by fecal microorganisms *in vitro* resulted in greater total VFA, propionate, and lactate concentration compared to oats (Harlow et al., 2015). Fermentation of starch by amylolytic bacteria yields production of propionate (Medina et al., 2002; Sadet-Bourgeteau et al., 2017). Similarly, lactate is produced in response to starch fermentation by *Lactobacillus* and *Streptococcus*, and at moderate pH it is metabolized to propionate and butyrate by lactate-utilizing bacteria (Bailey et al., 2003; de Fombelle et al., 2003; Millinovich et al., 2007). It is important to note limitations associated with use of *in vitro* systems. In particular, *in vitro* systems are closed chambers in which organic acids more readily accumulate due to absence of digestive flow and absorption found *in vivo.* Therefore, use of highly fermentable ingredients, such as corn, as substrate in *in vitro* systems may exacerbate fermentation to a greater degree than what may be observed *in vivo*. Results in our laboratory (Ochonski et al.,

2019, 2020) observed similarities in cecal fermentation characteristics of horses fed 0 to 1.5 g starch g starch·kg BW^{-1}·$meal^{-1}$ provided via corn pellet, supporting the use of this feed ingredient when fed at moderate inclusion levels.

Oats

Whole oats have historically been referred to as "the grain of horses" due to their moderate protein and starch content and fibrous hull (Särkijärvi and Saastamoinen, 2006; Lawrence, 2011). Pre-cecal starch digestibility of oats is greater in relation to other cereal grains such as corn or barley (Householder, 1978; Kienzle, 1994; Rosenfeld and Austbø, 2009); therefore, greater intake of oat starch poses a decreased threat to disruption of hindgut microorganisms. Radicke et al. (1991) reported greater cecal pH in horses fed oats (pH = 6.88) versus horses fed corn (pH = 6.32). Similarly, Al Jassim (2006) reported greater fecal pH of horses fed oats (pH = 6.75) versus horses fed sorghum (pH = 6.42) which they attributed to decreased lactate production. An in vitro experiment by Harlow et al. (2015) demonstrated increased pH, decreased lactate concentration, and greater concentration of lactate-utilizing bacteria in fecal inoculum supplemented with oats compared to wheat or corn. Furthermore, oat supplementation yielded greater gas production, indicating greater fermentation by fecal microorganisms. These authors did not indicate whether oats used in the experiment were hulled; supplementation of whole oats may provide additional substrate for fermentation due to presence of the fiber-rich seed hull (Ochonski et al., 2021).

Soybean Hulls

Soybean hulls – a byproduct of soybean processing for oil and meal production – may be included in formulation of equine diets as a source of dietary fiber (Ott and Kivipelto, 2002; Booth et al., 2004). Soybean hulls possess a relatively high proportion of hemicellulose and cellulose and decreased lignin compared to other fibrous byproducts, making them readily fermentable by hindgut microorganisms (Hintz et al., 1971; Miron et al., 2001; Coverdale et al., 2004). Coverdale et al. (2004) evaluated the effect of replacing forage with soybean hulls in cecally-cannulated horses. Linear decreases in pH and linear increases in VFA were observed soybean hull inclusion up to 75% of the total diet (from 0% to 1.35% BW/d). Cecal fermentation characteristics remained well within normal limits for this trial, supporting the fermentability of this feed ingredient. Similarly, Elghandour et al. (2017) observed greater *in vitro* gas production of cecal cultures when corn grain was replaced with soybean hulls. Volatile fatty acid concentrations of fecal samples were similar regardless of level of soybean hull inclusion up to 28% of the total diet in a study by Kabe et al. (2016), contradicting results reported by Coverdale et al. (2004) and Elghandour et al. (2017). As aforementioned, soybean hulls are rich in hemicellulose, cellulose, and soluble carbohydrates; this may lead to greater fermentability of this feedstuff within the cecum. Greater fermentability within the cecum may lead to fewer differences observed in fecal samples. Furthermore, recent research has made it abundantly clear that fecal samples are not indicative of cecal fermentation parameters (Sorensen et al., 2021).

Wheat Middlings

Published data regarding effects of wheat middlings on hindgut microorganisms and fermentation parameters are extremely limited. There are similar shifts in cecal fermentation

characteristics of horses fed wheat middlings to those fed beet pulp and soybean hulls (Ochonski et al., 2020). Harlow et al. (2016) observed similar increases in amylolytic bacteria and decreases in cellulolytic bacteria in feces of horses fed corn or wheat middlings compared to horses fed oats; however, these authors did not assess fecal pH or VFA. Effects of wheat middlings on fermentation by microorganisms have been investigated more extensively in ruminants. Supplementation of wheat middlings to a rolled corn based diet resulted in decreased ruminal pH, increased total VFA concentration, and decreased acetate:propionate in finishing beef cattle (ZoBell et al., 2003). Others have observed no differences in ruminal fermentation parameters of cattle supplemented with wheat middlings (Bowman et al., 2004). As with other feed ingredients, effect on fermentation of cecal or ruminal microorganisms may be highly dependent on level of intake.

Starch Overload and Gastrointestinal Disease in Equines

Gastrointestinal disorders are the second leading cause of death in equines; therefore, research focusing on maintenance of gastrointestinal health is necessary (USDA APHIS, 2005). Horses evolved as a grazing species, almost continuously consuming poor-quality forages for up to 18 h per day. Consequently, this has resulted in limited ability to digest starch pre-cecally due to limited pancreatic α-amylase secretion (Kienzle, 1994). Consumption of > 2.0 g starch·kg BW^{-1}·$meal^{-1}$ surpasses the upper limit of small intestinal starch digestion, with excess starch reaching the cecum. In sufficient quantities this starch causes unfavorable shifts in cecal microbial communities, decreased cecal pH, and subsequent acidosis (Potter et al., 1994; Julliand et al., 2001; Biddle et al., 2013). Accumulation of organic acids, particularly lactic acid, has been associated with release of bacterial lipopolysaccharide (LPS) and damage to intestinal epithelial

cells (Khafipour et al., 2009; Pollit and Visser, 2010). Compromised intestinal epithelial cell integrity results in leaky tight junctions, allowing LPS to permeate the intestinal barrier and induce a systemic inflammatory response (Bailey et al., 2009; Suagee et al., 2015). Chronic inflammation associated with impaired large intestinal integrity has been implicated in pathogenesis of debilitating disorders such as laminitis and colitis (Bailey et al., 2009; Pollit and Visser, 2010; Kwon et al., 2013). This review aims to characterize what is known regarding shifts in hindgut microorganisms, fermentation characteristics, and subsequent development of gastrointestinal disorders.

Effect of Starch Overload on Hindgut Microorganisms and Fermentation Characteristics

Initial fermentation of starch yields propionate and butyrate produced by amylolytic bacteria, thereby increasing total VFA concentration and decreasing acetate:propionate (Medina et al., 2002; Sadet-Bourgeteau et al., 2017). Early culture dependent methods were used by Goodson et al. (1988) to characterize increased amylolytic bacterial populations, decreased cecal pH (5.8 vs. 6.4), and greater propionate concentration in the cecum of ponies abruptly switched from 100% alfalfa to an 87% corn diet. Horses fed increasing dietary starch provided by barley demonstrated increased cecal and fecal propionate and butyrate concentrations (Grim et al., 2017). Additionally, these authors observed shifts in bacterial composition of fecal and cecal samples that were driven by decreased abundances of fibrolytic bacteria and increased abundances of amylolytic bacteria in response to increase dietary starch.

Lactate produced through fermentation of starch is rapidly metabolized to propionate and butyrate by lactate-utilizing microorganisms in horses that have been adapted to high-starch diets

(de Fombelle et al., 2003; Millinovich et al., 2007). In the case of starch overload, large quantities of starch entering the cecum are rapidly fermented by cecal microorganisms, particularly lactate-producing bacteria that include *Lactobacilli* and *Streptococci* (Al Jassim and Andrews, 2009). Excessive lactate production results in rapid decline of cecal pH and inhibition of acid-intolerant fibrolytic species. This alteration of cecal microbial communities causes depressed fiber fermentation, and therefore decreased VFA production (Medina et al., 2002; Shirazi-Beechey, 2008; Biddle et al., 2013).

Bailey et al. (2003) observed greater concentrations of *Streptococcus bovis* and *Lactobacillus* species in cecal inoculum supplemented with corn starch. Similarly, florescent *in situ* hybridization probes were used by Milinovich et al. (2006) to identify increased *Streptococcus* populations in fecal samples obtained from horses subjected to an oligofructose-induced laminitis challenge.

More recently, researchers have utilized next-generation sequencing methods to identify shifts in hindgut microbiome in response to dietary changes. Using an *in vitro* model of starch overload, Biddle et al. (2013) observed increases in the lactate-producing group *Streptococcaecae* and decreases in fibrolytic groups, particularly *Ruminococcacea* and *Lachnospiracaea* within the first 24 h following inoculation of fecal slurries. Additionally, these authors observed greater abundance *M. elsedenii*, a lactate-utilizing microorganism, of fecal slurries where lactate initially accumulated and was subsequently attenuated. Warzecha et al. (2017) characterized the effect of low-starch (0.9 g NSC/kg BW) versus high-starch (1.8 g NSC/kg BW) diets on cecal fermentation parameters and microorganisms. They reported greater starch intake corresponded to decreased cecal pH and increased total VFA concentration. Bacterial diversity was decreased with increasing dietary starch intake, which was attributed to

decreased relative abundances of Fibrobacter and Spirochaetes, both of which are prominent fiber-fermenting bacteria.

In vivo data regarding direct effects of starch overload on cecal fermentation characteristics are lacking, as samples can only be obtained from fistulated or euthanized animals. Most reports regarding effects of diet on hindgut fermentation have been based on *in vitro* rather than *in vivo* techniques, and for both techniques data are commonly derived using fecal rather than cecal samples. Rather than provide starch via concentrate, most experiments regarding carbohydrate overload in horses have involved nasogastric administration of a carbohydrate bolus of corn starch or oligofructose (Budak et al., 2009; Vervuert et al., 2009; Kwon et al., 2013). This method of carbohydrate dosing makes it difficult to extrapolate findings to practical feeding scenarios that horse owners would encounter. Furthermore, data regarding shifts in cecal microorganisms and fermentation parameters in horses slowly adapted to increasing levels of dietary starch, rather than abrupt dietary change, are lacking in the literature.

Related Gastrointestinal Disorders

Rapid fermentation of starch by cecal microorganisms results in accumulation of VFA and lactic acid, decreased pH, and damage to epithelial cells (Bailey et al., 2004; Khafipour et al., 2009). Although initial damage to epithelial cells is caused by decreased luminal pH, this effect is exacerbated by permeation of LPS released as a result of acid-induced lysis of bacterial cells (Mani et al., 2012). Lipopolysaccharide, commonly referred to as endotoxin, is a glycolipid present in the outer membrane of gram-positive bacterial cells and is a potent stimulator of inflammatory immune response (Raetz and Whitefield, 2002; Bein et al., 2017). Damaged epithelial cells demonstrate increased expression of proteins involved in receptor mediated

endocytosis of LPS, further facilitating its transport and inducing a local inflammatory response (Eckel and Ametaj, 2016). Horses subjected to carbohydrate overload exhibited increased plasma LPS concentrations, indicating that LPS had escaped the lumen of the gastrointestinal tract (Bailey et al., 2009; Senior et al., 2011). Bailey et al. (2009) observed increased plasma LPS 8 h after horses were dosed with an oligofructose bolus in order to induce laminitis. Similarly, Suagee et al., (2015) observed increased plasma LPS in horses consuming high-sugar and high-starch diets. Clinical data collected by Senior et al. (2011) revealed greater plasma LPS concentrations in horses that presented with signs of colic, while LPS was undetectable in plasma of non-colic patients.

Chronic inflammation associated with impaired large intestinal integrity has been implicated in pathogenesis of debilitating disorders such as laminitis and colitis (Bailey et al., 2009; Pollit and Visser, 2010; Kwon et al., 2013), but few reports assessing hindgut permeability in horses are available. Krueger et al. (1986) described damage to epithelial tissues, evidenced by cellular sloughing and increased size of tight junctions, using electron microscopy in horses subjected to carbohydrate-induced laminitis. Others have described the presence of physical damage and ulceration within hindgut epithelial tissues (Pellegrini, 2005).

Concentration of technectium TC99 diethylenetriaminopentaacetate, a radioactive marker used to assess gastrointestinal permeability, was increased in blood and urine of horses subjected to carbohydrate-induced laminitis (Weiss et al., 1998). Incidences of impaired epithelial barrier function in response to acidic damage and LPS have been well characterized in ruminant animals. Liu et al. (2013) subjected goats to a subacute ruminal acidosis (SARA) challenge and observed decreased expression of tight junction barrier proteins and increased expression of pro-

inflammatory markers in ruminal tissues. Similarly, Klevenhusen et al. (2013) noted increased rumen permeability in goats consuming a 60% grain diet.

Although exact pathophysiology is still unknown, it is abundantly clear that systemic inflammation induced by starch overload plays a role in development of laminitis (Belknap et al., 2009; Pollit and Visser, 2010). Laminitis, characterized by inflammation of hoof laminae, is a chronic disorder that can lead to death if left untreated. Leslie et al. (2011) documented increased circulatory concentrations and mRNA expression of pro-inflammatory cytokines in lamellar tissues of horses administered a nasogastric bolus of corn starch. Suagee et al. (2015) described increased plasma concentration of interleukin-1β, a pro-inflammatory cytokine, in post-prandial plasma samples of mares fed a high-starch diet compared to mares fed only hay. Similar patterns of increased inflammatory markers in circulation and hoof laminae in response to starch overload have been confirmed by others (Faleiros et al., 2010; Kwon et al., 2013; Tadros et al., 2013).

Incidence of starch overload and subsequent development of gastrointestinal disorders can be prevented by avoiding high-starch concentrate meals. However, use of horses as performance animals results in greater energy requirements that can only be met through supplementation of starch-rich concentrates. Classical management of horses involves feeding 1 to 2 concentrate meals per day, but increased meal frequency of concentrate feeds may decrease negative effects on hindgut microorganisms and gastrointestinal disturbances (Clarke et al., 1990; Richards et al., 2006; Julliand and Grimm, 2017). Additionally, negative effects of added concentrate may be combated through adequate forage provided at a rate of $\geq$ 1% BW/d dry matter (Daly and Shirazi-Beechey, 2006; NRC, 2007).

Methods to Improve Starch Digestibility in Swine Diets

Starch contained in cereal grains is the greatest source of energy in swine diets; therefore, novel technologies to increase starch digestibility may improve gain efficiency. Small intestinal digestibility of starch is greater than 95% for pigs and is attributed to greater pancreatic α-amylase activity compared to other monogastric species such as horses or rats (Kienzle, 1994; Bach Knudsen, 2001; Bauer et al., 2003). Starch digestibility may be improved through feed processing methods, such as fine grinding and pelleting (Rojas et al., 2016). However, increased grain processing may lead to development of gastric ulcers, which have been reported to occur in 21 to 96% of finishing pigs and contribute to economic loss due to increased mortality or decreased growth performance (De Magalhaes Quieroz et al., 1996; Friendship et al., 2004; Gottardo et al., 2017). Wondra et al. (1995) observed linear increases in gastric keratinization and lesions as particle size was reduced from 1000 to 400 μm. Furthermore, Mößeler et al. (2010) observed increased fluidity and decreased stratification of gastric contents, indicated by similar pH throughout the stomach of pigs fed a finely ground pelleted diet compared to coarse ground or meal diets. Increased homogenization of gastric contents as a result of pelleted diets has been indicated as a contributing factor to development of gastric ulcers in equines (Hepburn, 2011). Supplementation with exogenous enzymes may provide an alternative method of improving feed efficiency without compromising gastrointestinal health.

Amylase Supplementation

Pancreatic α-amylase secretion is decreased following weaning and increases as pigs age and are transitioned to high-starch diets (Jensen et al., 1997); thus, supplementation of exogenous amylase may be most beneficial during the early growing stage. Published data regarding

amylase supplementation in swine diets are relatively limited in comparison to other enzymes. For instance, it has been well-documented that addition of phytase improves phosphorous utilization (Simmons et al., 1990; Adeola et al., 1995; Selle and Ravindran, 2008). Others have documented effects of xylanase (Patience et al., 2002; Mavromichalis et al., 2000; Medel et al., 2002; O'Shea et al., 2014) or cellulase (Kim et al., 1998; Omogbenigun et al., 2004; Long et al., 2020) supplementation on nutrient digestibility and growth performance. Park et al. (2003) observed no improvements in growth performance or carcass characteristics of pigs fed a sorghum-based diet supplemented with exogenous amylase. Supplementation of raw pea-based diets with amylase resulted in greater feed efficiency of weaned pigs, but this was not observed in pigs consuming extruded or micronized diets (Owusu-Asiedu, 2002).

Use of amylase as a feed additive has been studied in broiler chickens. Alpha-amylase supplementation of broilers fed a corn-soybean meal-based diet resulted in improved feed efficiency, greater apparent digestibility of organic matter and starch, and reduced pancreatic weight (Garcia et al., 2003). Onderci et al. (2006) evaluated the effect of adding of an α-amylase producing *Escherichia coli* to drinking water provided toto broiler chickens fed corn-soybean meal-based diets. Similar to previous reports, supplementation of α-amylase producing *E. coli* resulted in improved feed efficiency and organic matter digestibility and decreased pancreatic weight.

In response to α-amylase supplementation, Jiang et al. (2008) observed improved averaged daily gain, decreased mRNA expression of pancreatic α-amylase, and unchanged feed efficiency in broiler chickens, providing evidence that supplementation with exogenous α-amylase results in decreased energy expended to secrete endogenous amylase. The theory of conservation of digestive enzymes has been described by Rothman et al. (2002). They suggest

that a portion of pancreatic enzymes are absorbed into the bloodstream and recycled in enteropancreatic circulation in order to conserve energy that would otherwise be expended to synthesize new enzymes with each meal. Addition of exogenous α-amylase to diets would increase the amount of this enzyme in enteropancreatic circulation and thus further decrease the need for production of endogenous amylase. Reduced pancreatic weight of broiler chickens observed by both Garcia et al. (2003) and Onderci et al. (2006) may be attributed to decreased secretion of endogenous amylase; however, this theory requires further research.

Enogen® Feed Corn as a Technology to Improve Starch Digestibility in Livestock

Initially developed for ethanol production, Enogen® Feed Corn (EFC; Syngenta Seeds, LLC, Downers Grove, IL) are corn hybrids with increased expression of heat-stable alpha-amylase. Recent researchers have recognized the potential for this corn hybrid to improve feed efficiency in livestock animals. An unpublished report from Johnson (2019) described 5.5% improvement in feed efficiency of growing cattle fed EFC compared to cattle fed conventional corn. Horton et al. (2017) observed improved gain efficiency of feedlot heifers fed steam flaked EFC compared to stem flaked mill-run corn. Similarly, Jolly-Breithaupt et al. (2019) noted improved feed efficiency in feedlot cattle fed dry rolled EFC. Ruminants secrete less pancreatic α-amylase than pigs, making EFC a potential candidate for increasing post-ruminal starch digestion (Harmon et al., 2004). Other researchers have indicated that feed form may affect growth performance of cattle fed EFC. For instance, Baker et al. (2019) observed improved feed efficiency when feeding EFC silage but no improvements when feeding EFC grain.

When fed to broiler chickens, Truelock (2020) reported that EFC resulted in greater average daily gain and feed intake, yielding no differences in feed efficiency between EFC and a

conventional corn variety. Williams (2020) evaluated the effects of EFC and conventional corn in meal- or pelleted-based diets on finishing pig growth performance and carcass characteristics. Improved feed efficiency was observed with pelleted diets; however, growth performance and carcass characteristics were similar between corn sources. When fed to lactating sows, EFC yielded similar effects on sow and litter performance when compared to conventional corn (Williams et al., 2021). While supplementation of EFC to finishing pigs may not yield improvements in growth performance, further research should be conducted to understand if addition of EFC to swine diets could be beneficial in younger pigs exhibiting decreased pancreatic α-amylase secretion following weaning.

Distillers Dried Grains with Solubles (DDGS) in Swine Diets

Production of ethanol has also resulted in availability of low-cost byproducts, such as distillers dried grains with solubles (DDGS), which have become a popular ingredient in swine diets due to their low cost and moderate lysine and digestible phosphorus content. A review by Stein and Shurson (2009) determined that growth performance of pigs fed diets containing < 30% DDGS was unaffected compared with those fed a corn-based diet; however, others have observed addition of DDGS to swine diets may negatively affect growth performance and carcass quality because of its high fiber and unsaturated fatty acid content (Whitney et al., 2006; Lineen et al, 2008; Graham et al., 2014).

Whitney et al. (2006) observed decreased feed efficiency in pigs fed 20 and 30% DDGS compared to pigs fed 0 and 10% DDGS. Evaluation of carcass characteristics revealed decreased loin depth and belly thickness and increased iodine value of carcass fat of pigs fed 30% DDGS. Inclusion of DDGS results in greater crude fiber of diets, resulting in decreased nutrient

digestibility and impaired growth performance (Fu et al., 2004; Weimer et al., 2008). Carcass fat iodine value (IV) is used in commercial pork processing plants to characterize the proportion of unsaturated fat, and thus carcass firmness. Corn DDGS contain a high proportion of unsaturated fatty acids, leading to increased deposition of unsaturated fatty acids in adipose tissue (Madsen et al., 1992). Similar to Whitney et al. (2006), Lineen et al. (2008) evaluated the effect of increasing DDGS inclusion from 0 to 30% on finishing pig performance and carcass characteristics. They noted decreased feed efficiency, reduced percentage yield, and reduced backfat thickness in pigs fed ≥ 20% DDGS. Reduced percentage yield with increased DDGS inclusion may be attributed to greater crude fiber intake, which has been demonstrated by others to increase gut fill and gastrointestinal weight (Zhu et al., 1990; Pluske et al., 1998; Asmus et al., 2012). More recently, Graham et al. (2014) observed similar decreases in growth performance and impaired carcass characteristics of pigs fed up to 40% DDGS.

Source and quality of DDGS have been shown to impact growth performance and carcass characteristics, making it difficult to interpret data where these factors are not uniform (Stein and Shurson, 2009). Urriola et al. (2010) indicated differences in apparent ileal digestibility and apparent total tract digestibility of dietary fiber in DDGS sourced from 28 different locations. Methods of processing or fermentation of DDGS in ethanol plants may result in products of greater or lesser quality. Inconsistent results within the literature regarding appropriate level of DDGS inclusion may be attributed to differing sources used by different authors (Fu et al., 2004; Hill et al., 2008; Widmer et al., 2008).

Use of a DDGS withdrawal period prior to marketing may alleviate effects of DDGS on carcass characteristics (Xu et al., 2010). Coble et al. (2017) demonstrated that switching pigs consuming 30% DDGS to a predominantly corn-soybean meal-based diet 5 to 9 d prior to

marketing improved hot carcass weight and carcass yield compared to pigs not subjected to a withdrawal strategy. More recently, Lerner et al. (2020) revealed that longer withdrawal periods result in greater improvement in carcass characteristics; DDGS removal 35 d prior to marketing corresponded with greatest improvements in carcass yield and fatty acid saturation (IV).

Summary

In summary, dietary effects on microorganisms and fermentation characteristics require further investigation. Digestible energy (DE) is utilized to the caloric content of equine rations; this method considers energy lost in feces but does not account for heat loss associated with fermentation (NRC, 2007). Characterization of commonly used feed ingredients – including alfalfa, beet pulp, corn, oats, soybean hulls, and wheat middlings – fed at different inclusion levels will provide invaluable information to assist nutritionists and commercial formulators in creating equine diets for different physiological classes.

Consumption of > 2.0 g starch·kg BW^{-1}·$meal^{-1}$ surpasses the upper limit of small intestinal starch digestion, with excess starch reaching the cecum causing unfavorable shifts in cecal microbial communities, decreased cecal pH, and subsequent acidosis. These sequalae have been implicated in development of leaky gut, colitis, and laminitis in equines. Nevertheless, research to further characterize the effect of high starch diets on changes in cecal environment and resulting colic remain limited.

Experiments regarding exogenous α-amylase supplementation in swine have yielded varying results. Use of EFC, a high-amylase corn hybrid, has proven promising in ruminants, and similar improvements in starch digestibility and growth performance may be observed in swine, particularly around the time of weaning. Meanwhile, inclusion of DDGS as an energy source in

swine diets may result in unfavorable growth performance and carcass characteristics. Strategies to alleviate these effects while preserving economic gain include withdrawal of DDGS prior to marketing and limiting the level of dietary inclusion. Research is needed in order to assess if feeding DDGS in combination with such EFC may mitigate deleterious effects of DDGS on growth performance and carcass characteristics.

The overarching hypothesis of this dissertation is that corn, if fed at appropriate levels, can be fed to both equines and swine without deleterious effects on gastrointestinal health or growth performance. In both species, methods to improve pre-cecal corn digestibility, particularly exogenous α-amylase supplementation, have the potential to improve animal health and efficiency.

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Chapter 2 - Caecal Fermentation Characteristics of Commonly Used Feed Ingredients

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Running title: Caecal fermentation of feed ingredients

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Summary

Background: Commercial horse feeds utilize cereal grains and byproducts; however, their effects on the caecal environment remain poorly characterized.

Objective: Characterize the effect of commonly used feed ingredients on caecal pH and volatile fatty acid (VFA) concentration.

Study design: 6 × 6 Latin square.

Methods: On d -2, 6 caecally cannulated Quarter horses were moved into individual stalls where Smooth bromegrass hay (brome) was offered at 2.0% BW/d split between 2 feedings (0600 and 1800). On d 0, caecal digesta was collected every 2 h for 12 h relative to the 0600 feeding to establish control values for horses consuming only brome (**HAY**). On d 1, horses began consuming their respective treatments which consisted of beet pulp (**BP**), maize (**M**), dehydrated alfalfa (**A**), oats (**OAT**), soybean hulls (**SBH**), or wheat middlings (**WM**) at 0.25% BW/d split into 2 feedings. On d 7 of each treatment period, caecal digesta was collected every 2 h for 12 h and analyzed for pH and VFA. Data were analyzed using mixed ANOVA with repeated measures, fixed effects of treatment and time, and random effects of horse and period.

Results: There was a main effect of hour ($P \leq 0.05$) indicative of post-prandial shifts in caecal metabolites. There were no main effects of treatment on pH or VFA concentration ($P \geq 0.31$). Effects of hour × treatment ($P \leq 0.042$) were observed for all response variables. Regardless of treatment or hour, caecal pH remained well within normal limits. Three horses exhibited signs of lower esophageal choke immediately after consumption of BP pellets.

Limitations: Interactions between ingredients when mixed for formulation of a concentrate warrant further research. Furthermore, shorter adaptation period was chosen to mimic common management practices.

Conclusion: Minimal differences in caecal fermentation parameters were detected when ingredients were fed at a common inclusion level.

Introduction

It has been suggested that inclusion of high-starch feedstuffs, such as maize, in the equine diet may lead to unfavorable shifts in hindgut fermentation parameters and development of gastric disturbances [1,2]. Thus, low-starch ingredients such as beet pulp, dehydrated alfalfa meal, soybean hulls, and wheat middlings have been utilized by horse owners and considered a safer energy source. These ingredients are commonly used in many commercial concentrates; however, their effects on hindgut fermentation parameters remain poorly understood. Most reports regarding effects of feed ingredients on hindgut fermentation have been based on *in vitro* rather than *in vivo* techniques, and for both techniques data are commonly derived using faecal rather than caecal samples [3-6]. However, faecal samples are not necessarily reflective of caecal microbial communities and fermentation parameters [7-9]. Equines meet 30 to 70% of their daily energy needs from volatile fatty acid (VFA) production [10,11,]. Nevertheless, digestible energy (DE) is utilized to the caloric content of equine rations. This method considers energy lost in faeces but does not account for heat loss associated with fermentation [12]. Increased use of horses for leisure riding has resulted in a greater need for lower energy diet formulations, achieved through inclusion of more fiberous ingredients. Characterization of feed ingredients solely in terms of DE does not provide information on the fermentability of these ingredients within the caecum.

Therefore, the objective of this study was to characterize the effects of commonly used feed ingredients on fermentation parameters (pH and VFA concentration) within the caecum. Treatments were chosen to reflect ingredients most often utilized as energy sources in formulation of commercial concentrates and were fed at a standardized dry matter intake as these ingredients are commonly used as alternatives for one another. We hypothesize minimal differences on the caecal environment due to feedstuffs. These novel data are especially useful for nutritionists and commercial formulators when determining appropriate ingredient alternatives in the formulation of commercial concentrates for different physiological classes of

equines. Furthermore, data presented herein provide veterinarians and owners with a deeper understanding of the interaction between feed sources and the digestive physiology of the equine.

Materials and Methods

Subjects and Study Design

All animal protocols were carried out in accordance with relevant guidelines and regulations and approved by the Kansas State University Animal Care and Use Committee. Six Quarter horses (8 to 13 yr; 516 ± 55.1 kg BW) each fitted with a permanent caecal cannula (flexible rumen cannula, #7c; 3.8 cm center diameter and 8.9 cm wall thickness[a]; [13]) were used in a 6 x 6 Latin square across 6 treatment periods. Treatments consisted of beet pulp (BP), maize (M), dehydrated alfalfa (A), whole oats (OAT), soybean hulls (SBH), and wheat middlings (WM). All ingredients were ground to 1000 µm and pelleted at the Kansas State University Feed Technology Innovation Center. Samples of concentrates and hay were sent to a commercial laboratory[b] for analysis prior to start of the trial (Table 2.1). Treatments were fed twice daily for 7 d at a rate of 0.25% BW/d (DM) along with Smooth Bromegrass hay (HAY) at a rate of 2% BW/d (DM) split into 2 feedings (0600 and 1800). Treatment levels were chosen to mirror typical inclusion rates in commercial horse feeds.

Prior to initiation of the study, horses were housed in a dry lot and offered HAY *ad libitum*. For the duration of the trial, horses were housed in individual stalls (3.05 m × 3.66 m) bedded with pine shavings and turned out together in a dry lot (9.75 m × 30.48 m) for approximately 4 h/d where they had *ad libitum* access to water and a salt block. On d -2 horses were moved into individual stalls where only HAY only was offered at 2.0% BW/d (DM) split into 2 feedings (0600 and 1800) and on d 0 caecal digesta was collected every 2 h for 12 h relative to the 0600 feeding to establish control values.

Sample Collection

On d 7 of each 7-d treatment period, caecal digesta was collected every 2 h for 12 h relative to 0600 feeding, with 0 h corresponding to samples obtained immediately before feeding representing fermentation of HAY prior to consumption of concentrate. At least 50 mL of digesta was collected from each horse by removing the cannula plug and allowing digesta to flow into a sample cup (Specimen Storage Containers, #14955117A)[c]. Digesta was immediately analyzed for pH using a portable pH probe (Thermo Scientific™ Orion Star™ A121 Portable pH Meter)[e] then strained through 4 layers of cheesecloth, as previously validated in our laboratory [7, 14-17]. One milliliter of cecal fluid was then transferred in quadruplicate into microcentrifuge tubes and mixed with 0.25 mL 25% meta-phosphoric acid for deproteinization. Samples were then immediately stored at -20º C; samples were completely frozen within 2 h of storage.

Volatile Fatty Acids

Methods for analysis of VFA in caecal fluid follow protocols previously established in our laboratory [7, 14-17]. Aliquots of caecal fluid were thawed and centrifuged at 20,000 x g for 20 min. Supernatant was transferred into gas chromatography vials and analyzed for VFA concentrations using an Agilent 7890 gas chromatograph[d] equipped with a Supelco Nukol capillary column (15 m × 0.53 mm; and 0.5 μm film thickness; Supelco columns)[e] with flame ionization detection. Hydrogen was the carrier gas with a flow rate of 35 mL/min and initial oven temperature was 80ºC with increases of 20 ºC/min until a final temperature of 200 ºC was achieved. Inlet and detector temperatures were maintained at 250 ºC. Concentrations of VFA in samples were determined by comparing against commercial standards (Supelco Volatile Fatty Acid Standard Mix)[e] containing acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate.

Statistical Analyses

Data were analyzed using the PROC GLIMMIX function of SAS (version 9.4)[f] as a mixed ANOVA with repeated measures with fixed effects of treatment, hour, and treatment × hour. Day 0 control values (HAY) were considered a treatment in the statistical model. Horse and period were included in the model as random effects. Differences between and within treatment LSMEANS were determined using the PDIFF option in SAS. Significance was declared if $P \leq 0.05$ and tendency declared if $0.05 < P \leq 0.10$.

Results

For all variables measured, there was a main effect of hour ($P \leq 0.05$; Table 2.2) and no main effect of treatment ($P \geq 0.31$). However, interactions of hour × treatment and treatment × hour ($P \leq 0.042$) were observed for all response variables. Forage or concentrate dry matter intake (DMI) did not differ between treatments ($P > 0.1$).

Treatment within Time

Caecal pH: Soybean hulls resulted in greater ($P \leq 0.0324$) caecal pH at h 0 compared to HAY, BP, and WM. At h 12, lower ($P \leq 0.0433$) caecal pH was observed for horses consuming A, OAT, and SBH than horses consuming HAY.

Volatile Fatty Acids: Caecal concentrations of acetate, propionate, and total VFA were greatest ($P \leq 0.038$) for HAY at h 0 and 4. At h 4, cecal propionate concentrations were lower for A ($P = 0.0413$) and OAT ($P = 0.0368$), and tended to be lower ($P \geq 0.0889$) for BP, M, SBH, and WM compared to HAY. Horses consuming OAT had lower ($P \leq 0.0449$) caecal propionate concentration than those consuming BP, M, A, and SBH and the greatest ($P \leq 0.0061$) acetate:propionate (A:P) at h10. Acetate:propionate was greater ($P = 0.0054$) for M

than OAT at h 2, greater ($P \leq 0.0488$) than HAY, BP, and A at h 4, and greater ($P = 0.0221$) than DEHY at h 12. Butyrate was greater ($P = 0.0211$) for M compared to OAT at h 12.

Time within Treatment

Cecal pH: Caecal pH was decreased ($P \leq 0.0499$) in horses consuming HAY and WM at h 6 compared to h 0, 2, 10, and 12. At h 2 and 12, caecal pH was greater ($P \leq 0.0146$) than at h 4, 6, 8, and 10 for horses consuming BP. The greatest ($P \leq 0.0291$) caecal pH was observed at h 0 for M and SBH compared to all other timepoints. For A, caecal pH at h 0 was greater ($P \leq 0.0396$) than at h 2, 4, 6, 8, and 10. Caecal pH was greater ($P \leq 0.0049$) at h 0 and 2 compared to h 4, 6, 8, and 10 in OAT-fed horses.

Volatile Fatty Acids: Within treatment, horses consuming HAY exhibited greater ($P \leq 0.0001$) acetate, butyrate, and total VFA concentrations at h 0 and 4 compared to all other time points. For BP, acetate concentration was decreased ($P \leq 0.0056$) at h 8 compared to h 0, 2, 4, and 6. There were no differences ($P \geq 0.10$) in acetate or propionate concentration for horses consuming M. Acetate:propionate was greatest ($P \leq 0.0482$) at h 6 in BP-fed horses. Butyrate concentration was lowest ($P \leq 0.0473$) at h 12 for HAY, BP, M, and WM treatments. Total VFA concentration was lowest ($P \leq 0.0013$) at h 12 for HAY-fed horses.

Discussion

Regardless of treatment or hour, caecal pH remained well within normal limits [18,19]. Consistent with previous work, pH nadir occurred 4 to 8 h following meal consumption for all treatments [14, 16, 17], corresponding to typical transit time of digesta [20]. Presumably, after the majority of digesta was fermented in the caecum, caecal pH began to increase and stabilizes by h 12 post prandially. Accordingly, there was increased caecal pH at h 10 for HAY, BP, M, and WM compared to h 4 to 8(7.02, 6.91,

6.92, and 6.89, respectively). However, caecal pH at h 10 for A, OAT, and SBH treatments did not increase (6.82, 6.83, and 6.82, respectively). Of all ingredients, SBH and A possessed the greatest neutral detergent fibre (NDF), potentially requiring greater time to ferment compared to less fibrous ingredients [21]. Cloverdale et al. [18] observed linear decreases in caecal pH and increased VFA concentration of horses fed hay supplemented with increasing SBH (13.1% CP; 60.6% NDF) from 0% to 1.35% BW/d, supporting the fermentability of this feedstuff.

Prolonged fermentation of OAT, as indicated by a decreased pH, may be attributed to greater lignin content compared to SBH [22]. Whole oats were ground and pelleted, thus the resulting pellet consisted of a mixture of oat seed and hull. Greater A:P at 10 h indicates oat hulls were being fermented at this time point. It has been well-characterized that fermentation of high-fiber feedstuffs drives production of acetate, whereas fermentation of high-starch feedstuffs results in greater propionate and butyrate production [23-25]. While limited data are available on fermentation of oat β-glucans by equine caecal microorganisms, *in vitro* fermentation of oat β-glucans with human fecal inoculum demonstrated increased production of propionate [26]. Caecal propionate concentration for OAT was not different or lower for all time points compared to all other treatments; these data indicate that the amount of β-glucan fermented was insufficient to significantly shift cecal fermentation parameters. However, it is important to note that data presented here are indicative of VFA concentration in caecal digesta at a given time point rather than a measure of VFA production by caecal microorganisms.

This trial took place during June and July in Kansas, with daytime temperatures often exceeding 32ºC. Anecdotally, outdoor temperature during the HAY treatment was greater compared to all other treatment periods. Voluntary feed intake has been shown to decrease in response to heat stress in cattle and swine [27, 28]. Although average daily forage DMI was not different between treatments ($P > 0.34$), it is possible that horses were driven to consume most HAY during late nighttime or early morning hours when temperatures were cooler. This may explain greater total VFA concentration, driven by greater acetate concentration, at h 0 compared to h 2,

6, 8, 10 and 12. A similar increase in total VFA and acetate concentration was observed at h 4 as a result of typical post-prandial shifts in response to fermentation of the morning meal by caecal microorganisms.

It is largely agreed upon that excessive consumption of starch leads to shifts in the microbial population of the equine hindgut and development of acidosis [1,2]. Horse owners have taken this into consideration when purchasing concentrate feeds, and over the past few decades there has been a consumer push for increased fat and less starch in concentrates. Due to its high starch content, M has been viewed by some as a less desirable feed ingredient compared to BP or SBH. In this trial, fermentation parameters responded similarly to M and the more fibrous ingredients. Researchers in our laboratory have observed depressed caecal pH and increased VFA concentration in horses consuming 2.0 g starch·kg BW^{-1}·$meal^{-1}$. Feeding $\geq$ 2.0 g starch·kg BW^{-1}·$meal^{-1}$ resulted in impaired production of VFAs [17]. Treatments in the current trial were fed at a rate of 0.25% BW, with the M treatment providing on average 0.92 g starch·kg BW^{-1}·$meal^{-1}$ and caecal pH ranged from 6.81 to 7.37. These values are similar to those reported by Ochonski et al. [17] where average daily caecal pH for horses consuming 0.5 and 1.0 g starch·kg BW^{-1}·$meal^{-1}$ were 7.04 and 6.85, respectively. Limited changes in caecal acetate and propionate concentrations indicate that majority ofM was digested pre-caecally. The results of this trial clearly indicate that inclusion of M in equine concentrates poses low risk for caecal acidosis when fed at 0.25% BW.

Beet pulp is a commonly-used feed ingredient with high soluble fiber content that is rapidly fermented by microorganisms in the hindgut [29]. Consumption of long-stem forage becomes difficult in geriatric horses with poor dentition. Inclusion of water-soaked BP in the diet provides a palatable, low starch source of energy [30]. Total caecal VFA concentration did not differ between horses consuming BP compared to the other feed ingredients, and caecal pH was similar to horses fed HAY only. Lower-cost fibrous ingredients such as SBH or WM provide similar hindgut responses and thus could be used as alternatives to beet when formulating commercial

concentrates. Previous researchers demonstrated BP improves fiber degradability when fed in combination with fibrous forage *in vitro* [31]. Studies regarding ability of BP to modulate caecal pH when fed in combination with high-starch ingredients such as barley have yielded varying results. When fed with barley that provided 2.0 g starch·kg BW^{-1}·$meal^{-1}$, addition of sugar BP at a rate of 0.5% BW stabilized cecal pH compared to barley alone [32]. A similar trial was conducted by Jensen et al. [33] who reported no differences in caecal pH when barley was fed alone or in combination with BP. Associative effects of feed have been well-documented in cattle [34-36]. Similarities in rumen and caecal environments suggest associative effects occur in equines as well, and warrant further investigation to broaden understanding of caecal utilization of feed ingredients.

Signs of acute gastrointestinal disturbances were observed in 3 of 6 horses after consuming BP and included rolling, looking at flank, pawing, pacing, and elevated heart and respiratory rate. In each case, signs were observed 30 sec to 1 min following consumption of the evening meal indicating lower esophageal obstruction/choke. Dried BP has greater water holding capacity compared to other ingredients such as ground maize or wheat middlings [37]. Additionally, Grimm et al. [38] demonstrated that pelleted BP is consumed faster compared to shredded and soaked BP. To date, few incidences of BP-induced lower esophageal obstruction have been reported in the literature, and it is widely recommended to soak BP prior to feeding [39]. Once signs presented, horses were hand walked for approximately 30 min until no signs of acute pain were evident. Horses exhibiting this syndrome were well adapted to the BP treatment and did not exhibit any further signs of pain or discomfort for the remainder of the trial.

This study is the first to directly compare the post-prandial effects of BP, M, SBH, WM, A, and OAT on caecal pH and VFA concentrations *in vivo* when fed at typical inclusion levels for commercial concentrates. There were minimal differences in caecal fermentation parameters between these commonly fed ingredients when fed at a rate of 0.25%

BW/d. These results provide valuable information in understanding fermentative physiology of commonly used ingredients within the equine cecum. However, these data are limited to shifts in cecal metabolites and give no indication of postprandial glycemic and insulinemic responses; further research is warranted to characterize glycemic response to ingredients fed at this inclusion level. The shorter adaptation period may be viewed as a limitation to data presented herein. A 1 wk adaptation period was chosen due to the minuscule nutritional changes that occurred when switching treatments, attributed to horses only consuming 0.25% BW/d of each treatment. Additionally, this study did not characterize fermentation parameters within the large and small colon. However, the minimal differences observed in cecal samples indicate a low probability of differences in later sections of the gastrointestinal tract. Data presented here are indicative of individual ingredients rather than mixtures, such as those in commercial concentrates. Future research should focus on investigating possible associative effects of these ingredients *in vivo*.

Declaration of Interests

Authors declare no competing interests.

Ethical Animal Research

All procedures were approved by Kansas State University Institutional Animal Care and Use Committee (Protocol #4079).

Owner Informed Consent

Not applicable.

Data Accessibility

Source of Funding

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Authorship

P. Ochonski contributed to the study design, study execution, data analysis and interpretation, and preparation of the manuscript. J.S Drouillard, T. Douthit, and J.M. Lattimer contributed to the study design, data analysis and interpretation, and preparation of the manuscript. C. Vahl contributed to statistical analyses of the data.

Manufacturers' Addresses

[a]Bar Diamond, Parma, ID, USA.

[b]Dairy One, Ithaca, NY, USA.

[c]Fisher Scientific, Pittsburgh, PA, USA.

[d]Agilent Technologies, Santa Clara, CA, USA.

[e]Sigma-Aldrich, St. Louis, MO, USA.

[f] SAS Institute Inc., Cary, NC, USA.

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Tables

Table 2.1 Composition (dry matter basis) of hay and concentrates

		Concentrate†					
Item, %	HAY‡	BP	M	A	OAT	SBH	WM
Dry matter	91.10	90.90	88.30	90.30	89.70	90.90	91.80
Crude protein	8.90	8.30	9.20	17.90	14.10	12.20	17.20
Acid detergent fiber	39.90	21.20	2.70	34.80	6.70	41.40	15.00
Neutral detergent fiber	66.50	33.10	7.90	46.30	14.10	59.00	37.40
Non-fiber carbohydrates	15.00	50.70	77.10	23.80	59.60	21.20	36.40
Starch	0.80	4.00	73.80	4.30	46.70	4.70	23.20
Crude Fat	2.90	1.60	4.30	2.30	7.80	2.50	4.40
Calcium	0.49	0.80	0.29	1.34	0.17	0.70	0.23
Phosphorus	0.16	0.13	0.33	0.28	0.41	0.15	1.06

†BP – beet pulp; M – maize; A – dehydrated alfalfa; OAT – whole oats; SBH – soybean hulls; WM – wheat middlings. Concentrates were ground to 1000 µm and pelleted at the Kansas State University Feed Technology Innovation Center (Manhattan, KS), and fed at a rate of 0.25% BW/d (DM) in two feedings (0700 and 1700h)
‡HAY -- Smooth Bromegrass hay fed at a rate of 2% BW/d (DM) in two feedings (0700 and 1700).

Table 2.2 Effect of hay or ingredient inclusion and sampling time on cecal pH and VFA concentration

			Concentrate†							
Item	Time, h	HAY‡	BP	M	A	OAT	SBH	WM	SEM	*P*-value§
pH	0	7.06^{A,ac}	7.04^{A,abd}	7.37^{AB,a}	7.36^{AB,a}	7.34^{AB,a}	7.49^{B,a}	7.09^{A,ad}		
	2	7.07^{ac}	7.25^{bd}	7.22^{c}	7.02^{bcd}	7.22^{a}	6.98^{b}	6.98^{abd}		
	4	6.92^{abc}	6.87^{a}	6.81^{c}	6.94^{bcd}	6.84^{bc}	6.81^{b}	6.76^{bc}		
	6	6.81^{b}	6.75^{c}	6.88^{c}	6.84^{c}	6.71^{b}	6.77^{b}	6.70^{c}	0.064	H, T × H
	8	6.85^{ab}	6.83^{ac}	6.81^{c}	6.77^{c}	7.74^{b}	6.94^{b}	6.83^{abc}		
	10	7.02^{A,c}	6.91^{AB,ac}	6.92^{AB,c}	6.82^{B,c}	6.83^{B,bc}	6.82^{B,b}	6.89^{AB,ab}		
	12	7.10^{c}	7.23^{d}	6.99^{bc}	7.12^{ad}	7.01^{ac}	6.91^{b}	7.16^{d}		
Acetate, mM	0	119.27^{A,a}	54.48^{B,abc}	50.25^{B}	54.94^{B,a}	37.04^{B,abc}	41.29^{B,abc}	57.89^{B,ab}		
	2	83.80^{b}	47.19^{abc}	47.57	49.92^{a}	45.52^{ab}	51.58^{abc}	46.89^{abcd}		
	4	115.88^{A,a}	57.21^{B,a}	46.81^{B}	50.58^{B,a}	47.11^{B,a}	53.92^{B,ab}	59.08^{B,a}		
	6	62.02^{c}	56.26^{ac}	43.68	48.73^{a}	52.82^{a}	57.98^{a}	59.57^{ab}	7.992	H, T × H
	8	45.52^{d}	39.96^{b}	46.11	46.13^{a}	38.69^{a}	46.52^{abc}	45.89^{bc}		
	10	35.99^{e}	43.23^{cd}	40.11	41.99^{ab}	29.53^{bc}	42.09^{bc}	39.46^{cd}		
	12	28.03^{AB,e}	34.35^{AB,d}	36.13^{AB}	33.14^{AB,b}	27.51^{A,c}	39.22^{B,c}	35.51^{AB,d}		
Propionate, mM	0	29.83^{A,a}	13.88^{BC,abc}	14.30^{BC}	14.52^{BC,a}	8.84^{B,abc}	10.33^{BC,a}	14.79^{C,ab}		
	2	19.03^{bc}	11.75^{abcd}	13.12	12.13^{ab}	9.48^{abc}	13.23^{ab}	11.43^{abc}		
	4	23.70^{A,b}	12.80^{AB,abc}	11.95^{AB}	10.82^{B,ab}	10.41^{B,ac}	13.51^{AB,ab}	13.43^{AB,ab}		
	6	14.42^{cd}	14.50^{a}	11.18	10.94^{ab}	12.50^{a}	15.32^{b}	14.86^{a}	2.069	H, T × H
	8	11.55^{AB,de}	10.59^{AB,bcd}	12.56^{AB}	11.47^{AB,ab}	8.55^{A,ac}	13.09^{B,ab}	11.94^{AB,abc}		
	10	10.22^{AB,ef}	10.87^{B,cd}	11.69^{B}	10.97^{B,ab}	6.60^{A,b}	12.40^{B,ab}	10.42^{AB,bc}		
	12	7.62^{f}	9.10^{d}	10.51	8.34^{b}	7.94^{bc}	10.69^{a}	9.72^{c}		

†BP – beet pulp; M – maize; A – dehydrated alfalfa; OAT – whole oats; SBH – soybean hulls; WM – wheat middlings. Concentrates were ground to 1000 µm and pelleted at the Kansas State University Feed Technology Innovation Center (Manhattan, KS), and fed at a rate of 0.25% BW/d (DM) in two feedings (0700 and 1700h)

‡HAY -- Smooth Bromegrass hay fed at a rate of 2% BW/d (DM) in two feedings (0700 and 1700).

§H = main effect of sampling time (hour); T = main effect of treatment; T × H = interaction between time and treatment.

^{ABC}Means within the same row without a common superscript are different ($P \leq 0.05$).

^{abcdef}Means within the same column without a common superscript are different ($P \leq 0.05$).

Table 2.2 (continued)

Item	Time, h	HAY	Concentrate						SEM	*P*-value
			BP	M	A	OAT	SBH	WM		
A:P	0	4.01^{acde}	3.82^{a}	3.75^{abc}	3.89^{ac}	4.16^{ab}	3.93^{ab}	3.96^{ab}		
	2	4.40^{AB,abce}	4.03^{AB,a}	3.61^{A,ab}	4.33^{AB,abc}	4.72^{B,a}	4.13^{AB,a}	4.28^{AB,ab}		
	4	4.91^{A,b}	4.77^{A,b}	3.86^{B,abc}	4.89^{A,b}	4.65^{AB,a}	4.20^{AB,ab}	4.57^{AB,a}		
	6	4.40^{AB,abc}	4.00^{AB,a}	4.19^{AB,a}	4.56^{A,b}	4.36^{AB,a}	3.85^{B,ab}	4.10^{AB,ab}	0.224	H, T × H
	8	4.00^{AB,cde}	3.99^{AB,a}	3.72^{B,bc}	4.05^{AB,c}	4.54^{A,a}	3.78^{B,ab}	3.86^{B,b}		
	10	3.69^{A,d}	3.89^{A,a}	3.55^{A,c}	3.83^{A,c}	4.54^{B,a}	3.61^{A,b}	3.87^{A,ab}		
	12	3.67^{AB,de}	3.75^{AB,a}	3.50^{A,abc}	4.4^{B,abc}	3.63^{A,b}	3.94^{AB,ab}	3.78^{AB,ab}		
Butyrate, mM	0	6.96^{a}	4.59^{a}	4.01^{ab}	4.37	3.23^{ab}	3.60	4.82^{acd}		
	2	4.61^{b}	3.63^{ab}	4.28^{ab}	3.43	3.31^{ab}	3.41	3.49^{bde}		
	4	6.32^{a}	4.33^{a}	4.59^{ab}	3.88	3.40^{ab}	3.41	5.10^{ac}		
	6	3.80^{b}	4.77^{a}	4.22^{ab}	4.08	3.87^{ab}	4.27	6.00^{c}	1.029	H, T × H
	8	3.33^{b}	3.57^{ab}	4.89^{a}	4.05	3.57^{a}	3.59	4.26^{abde}		
	10	2.44^{c}	4.01^{a}	3.87^{b}	3.69	2.78^{ab}	3.31	3.80^{de}		
	12	2.05^{B,c}	2.60^{AB,b}	3.52^{A,b}	3.20^{AB}	2.32^{B,b}	2.74^{AB}	3.23^{AB,e}		
Total VFA, mM	0	156.09^{A,a}	72.94^{B,abc}	68.55^{B,ab}	73.82^{B,a}	49.19^{B,abc}	55.23^{B,abc}	77.50^{B,abc}		
	2	107.44^{b}	62.56^{abcd}	64.97^{ab}	65.49^{ab}	58.31^{abc}	68.22^{abc}	61.82^{abcd}		
	4	145.90^{A,a}	74.34^{B,a}	63.35^{B,ab}	65.28^{B,a}	60.92^{B,a}	70.84^{B,ab}	77.60^{B,a}		
	6	80.24^{c}	75.53^{a}	59.07^{ab}	63.75^{ab}	69.19^{a}	77.58^{a}	80.43^{ab}	10.748	H, T × H
	8	60.40^{d}	54.12^{bcd}	63.56^{a}	61.65^{a}	50.80^{ac}	63.19^{abc}	62.08^{bd}		
	10	48.68^{e}	58.10^{cd}	55.67^{ab}	56.64^{ab}	38.90^{b}	57.79^{bc}	53.68^{cd}		
	12	37.36^{e}	46.05^{d}	50.16^{b}	44.68^{b}	37.77^{c}	52.65^{c}	48.46^{d}		

Chapter 3 - Changes in Cecal Fermentation Characteristics and Forage Intake of Horses Fed Increasing Amounts of Starch

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Abstract

Overconsumption of dietary starch is associated with increased risk of hindgut acidosis and subsequent development of gastrointestinal and metabolic disorders. Researchers have suggested that the upper limit of small intestinal starch digestion is 2 to 4 g starch·kg BW^{-1}·$meal^{-1}$. The objectives of this trial were to determine the effect of increasing levels of dietary starch on the cecal environment and voluntary forage dry matter intake (DMI). Six cecally cannulated Quarter horses (8-13 yr; 524 ± 65.5 kg BW) were used in a dose titration style design. Before the study began, horses were maintained in a single dry lot pen and offered *ad libitum* Smooth bromegrass hay (brome). On d -14, horses were moved to individual stalls and provided brome hay *ad libitum* and a ration balancer (0.0125% BW 4x/day). On d 0, cecal digesta samples were collected every 2 h for 12 h relative to the 0600 feeding. On d 1, pelleted corn (69.4% starch) was offered at 0.5 g starch·kg BW^{-1}·$meal^{-1}$at 0600, 1200, 1800, and 2400 h. Every 8 d thereafter, corn was increased to provide an additional 0.5 g starch·kg BW^{-1}·$meal^{-1}$ until horses were consuming 3.5 g starch·kg BW^{-1}·$meal^{-1}$. Seven days following each increase in dietary starch, the cecal sampling protocol was repeated. Cecal pH was recorded upon sample collection. Cecal digesta was analyzed for volatile fatty acids (VFA) via gas chromatography, and lactate via a colorimetric procedure. Data were analyzed as a randomized complete block design with repeated measures, with fixed effects of treatment and time and random effect of horse. There was an effect of treatment on forage DMI ($P \leq 0.0001$). As dietary starch increased, forage DMI decreased. Greatest total DMI ($P \leq 0.041$) was recorded when horses were offered 3.0 g starch. It should be noted that horses did not consume the full meal for treatments 2.5, 3.0, and 3.5 g starch, and on average consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·$meal^{-1}$ (respectively). Effects of treatment, hour, and treatment × hour were observed ($P \leq 0.032$) for pH, acetate,

propionate, acetate:propionate, and total VFA concentration. Cecal pH decreased as dietary starch increased, with lowest pH observed with 2.0 g starch ($P < 0.0001$). Cecal propionate and total VFA concentration were greatest ($P < 0.0485$) for 2.0 and 2.5 g. Acetate:propionate ratio was lower ($P \leq 0.005$) for 2.0 g starch compared to ≤ 1.5 g treatments; however, A:P did not continue to decrease with additional starch. Cecal acetate, propionate, butyrate, and total VFA concentrations were lowest ($P < 0.0001$) when horses were fed 3.5 g starch. Cecal lactate concentration was greatest ($P < 0.0060$) when horses were fed 2.0 g starch and lowest ($P < 0.0001$) for horses consuming 0 and 0.5 g starch. This trial demonstrates that voluntary forage DMI decreases with increasing levels of starch, as previously observed in ruminants. Consumption of ≥ 2.0 g starch·kg BW^{-1}·$meal^{-1}$ may lead to shifts in cecal microbial communities that result in decreased fermentative activity. However, even when horses were offered ≥ 2.0 g starch·kg BW^{-1}·$meal^{-1}$ cecal acidosis was not observed.

Key words: horse, cecum, acidosis, starch

Introduction

Horses evolved as grazing animals equipped with gastrointestinal anatomy suited for digestion of fibrous forages and are largely unable to digest large quantities of starch pre-cecally due to limited secretion of pancreatic α-amylase compared to other monogastrics such as pigs (Kienzle,1994). Potter et al. (1992) and Kienzle (1994) defined an upper limit of small intestinal starch digestion by feeding ilealy cannulated ponies increasing amounts of corn starch and measuring starch appearance at the distal ileum. A dramatic increase in starch appearance at the distal ileum was noted by both authors between 2 to 4 g starch·kg BW^{-1}·$meal^{-1}$; this range was thus deemed the upper limit of small intestinal starch digestion.

The equine hindgut houses a diverse microbial community that is influenced by diet (Costa et al., 2018; Sorensen et al., 2021). Starch fed in excess of 2 g starch·kg BW^{-1}·$meal^{-1}$ has been noted to cause unfavorable shifts in cecal microbial communities, decreased cecal pH, and subsequent acidosis (Julliand et al., 2001; Biddle et al., 2013). Accumulation of organic acids, particularly lactic acid, has been associated with release of bacterial lipopolysaccharide (LPS) and damage to intestinal epithelial cells (Khafipour et al., 2009; Pollit and Visser, 2010). Compromised intestinal epithelial cell integrity results in "leaky" tight junctions, allowing LPS to permeate the intestinal barrier and induce a systemic inflammatory response (Bailey et al., 2009; Suagee et al., 2015). Inflammation associated with impaired large intestinal integrity has been implicated in pathogenesis of debilitating, sometimes fatal, disorders such as laminitis and colitis (Bailey et al., 2009; Pollit and Visser, 2010; Kwon et al., 2013).

Despite advancements in knowledge of equine nutrition and physiology over the last several decades, colic remains second to old age as a leading cause of death (31.2%) in equines (USDA APHIS, 2015). Nevertheless, research to further characterize the effect of high starch

diets on the cecal environment and remain limited. Majority of research focused on effects of diet on hindgut environment relies on data derived from fecal rather than cecal samples, but it has been well established that fecal samples are not indicative of microbial or fermentative shifts in the cecum (Jensen et al., 2013; Murray et al., 2013; Sorensen et al., 2021). Rather than provide starch via concentrate, most experiments regarding carbohydrate overload in horses have included nasogastric administration of a carbohydrate bolus of cornstarch or oligofructose (Budak et al., 2009; Vervuert et al., 2009; Kwon et al., 2013). This method of carbohydrate dosing makes it difficult to extrapolate data obtained to a practical feeding scenario.

Physiological and behavioral responses to consumption of high starch diets have been best characterized in ruminants. Olson et al. (1999) observed depressed voluntary forage dry matter intake (DMI) of beef steers supplemented with increasing amounts of dietary starch. Similarly, Souza et al. (2010) noted decreased forage DMI and depressed neutral detergent fiber (NDF) digestibility of cattle supplemented with starch. It has been hypothesized that forage DMI is reduced due to gut fill effect stimulated by accumulation of VFA and decreased pH (Dixon and Stockdale, 1999). Depressed NDF digestibility is attributed to the "carbohydrate effect", wherein fibrolytic and amylolytic bacteria compete for essential nutrients (Costa, 2008). Amylolytic bacteria preferentially digest starch, leading to decreased pH and inhibition of fibrolytic bacteria (Arroquy et al., 2005). Similar effects of starch supplementation causing depressed fibrolytic bacterial concentrations have been reported in equines (Medina et al., 2002; Biddle et al., 2013); however, characterization of forage DMI in response to increased starch supplementation has not been documented in equines.

Therefore, the objectives of this trial were to determine the effects of increased dietary starch on cecal pH, VFA, and lactate and voluntary forage DMI. Dietary starch, provided via

corn pellets, was gradually increased by 0.5 g starch·kg BW^{-1}·$meal^{-1}$ every week to avoid sudden disruption of cecal microorganisms and allow for proper adaptation. We hypothesized that an initial shift in fermentation characteristics and voluntary forage DMI would occur at or around 2.0 g starch·kg BW^{-1}·$meal^{-1}$, the lower end of small intestinal threshold described by Potter et al. (1992) and Kienzle (1994). However, we hypothesize that clinical manifestation of acidosis may not occur until horses are consuming > 2.0 g starch·kg BW^{-1}·$meal^{-1}$.

Materials and Methods

All procedures were approved by Kansas State University Institutional Animal Care and Use Committee (Protocol #4149).

Horses

Experimental animals consisted of six Quarter horses, 3 mares and 3 geldings between the ages of 8 and 13 yr (524 ± 65.5 kg BW), each fitted with a permanent cecal cannula (#7c, Bar Diamond, Parma, ID; Beard et al., 2011). Horses were housed on a dry lot and offered *ad libitum* Smooth Bromegrass hay (brome) prior to the study.

Study Design and Treatments

Study design consisted of a randomized complete block design with each horse serving as its own control and all horses received dietary treatments in the same sequence. Dietary starch was increased by 0.5 g starch·kg BW^{-1}·$meal^{-1}$ every 7 d via a corn pellet (69.4% starch, Table 3.1). A ration balancer (Table 3.1) was fed at 0.0125% BW/meal to meet the NRC requirements for protein, lysine, vitamins, and minerals for a mature, idle horse. Meals were offered daily at 0600, 1200, 1800, and 2400 h. Brome hay (Table 3.1) was offered *ad libitum.* Feed refusals were recorded on d 6 and 7 of each period to calculate DMI.

On d -14, horses were weighed via a calibrated electronic commercial livestock scale and moved into individual stalls (3.05 m x 3.66 m) equipped with automatic waters and a salt block. On d 1, 8, 15, 22, 29, and 36, and 43 horses were acclimated to the new treatment over 24 h by increasing starch level by 0.125 g starch·kg BW^{-1}·$meal^{-1}$. Body weights were obtained on d 7, 14, 21, 28, and 35, and 42 to determine amount of starch (g/meal) fed at the subsequent treatment.

Horse health was monitored daily by recording body temperature, heart rate, respiration rate, and capillary refill time, and pain was evaluated using an Equine Comfort Assessment Scale developed by Blossom et al. (2007; Appendix A). Any horse exhibiting elevated vitals, scoring $\geq$ 2 on the Equine Comfort Assessment Scale, and/or displaying signs of gastrointestinal discomfort was immediately removed from the study.

Sample Collection

Cecal digesta samples were collected every 2 h for 12 h relative to the 0600 h feeding on d 0, 7, 14, 21, 28, 35, 42, and 49 with 0 h samples corresponding to samples obtained immediately before feeding. At least 100 mL of digesta was collected from each horse by removing the cannula plug and allowing digesta to flow into a sterile cup (Specimen Storage Containers, #14955117A, Fisher Scientific, Pittsburg, PA). Digesta was immediately analyzed for pH using a portable pH probe (Thermo Scientific™ Orion Star™ A121 Portable pH Meter, Fisher Scientific, Pittsburgh, PA) then strained through 4 layers of cheese cloth. One milliliter of cecal fluid was transferred in quadruplicate into microcentrifuge tubes and mixed with 0.25 mL 25% meta-phosphoric acid for deproteinization. Samples were stored at -20º C for later analysis of volatile fatty acids (VFA) and lactate.

Volatile Fatty Acids

Methods for analysis of VFA in cecal fluid followed protocols previously established in our laboratory (Douthit et al., 2019; Ochonski et al., 2020; Sorensen et al., 2021). Aliquots of cecal fluid were thawed and centrifuged at 20,000 x g for 20 min. Supernatant was transferred into gas chromatography vials and analyzed for VFA concentrations using an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a Supelco Nukol capillary column (15 m × 0.53 mm; and 0.5 μm film thickness; Supelco Columns, Sigma-Aldrich, St. Louis, MO, USA) with flame ionization detection. Hydrogen was the carrier gas with a flow rate of 35 mL/min. Initial oven temperature was 80ºC with increases of 20 ºC/min until a final temperature of 200 ºC was achieved. Inlet and detector temperatures were maintained at 250 ºC. Concentrations of VFA in samples were determined by comparing against commercial standards (Supelco Volatile Fatty Acid Standard Mix; Sigma-Aldrich, St. Louis, MO, USA) containing acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate.

Lactate

Cecal fluid was analyzed for D- and L-lactic acid using a colorimetric procedure adapted from Barker and Summerson (1941) previously used for analysis of cecal fluid in our laboratory (Douthit et al., 2019). Aliquots of deproteinized cecal fluid were diluted with deionized (DI) water 25:1 and compared against standard solutions containing 0, 10, 20, 30, 40, and 50 mg/dL lactate. Prepared in triplicate, 0.5 mL of diluted cecal fluid or standard were combined with 4 mL DI water, 0.5 mL 20%-$CuSO_4 \cdot 5H_2O$, and 0.5 g $Ca(OH)_2$ powder. Tubes were vigorously vortexed for at least 10 sec then incubated at room temperature for 30 min with occasional shaking. Following incubation, tubes were centrifuged for 10 min at 1000 × g, 0.5 mL of

resulting supernatant was transferred to a separate glass tube containing 25 µL 4%-$CuSO_4$, and vortexed. Following addition of 0.5 mL sulfuric acid, tubes were again vortexed and then placed into a boiling water bath for 5 min followed by a cold water bath until sample temperature reached 20°C. After desired sample temperature was reached, 50 µL p-hydroxydiphenyl was added to tubes and quickly vortexed. Tubes were then placed in a 30°C water bath for 30 min with gentle mixing after 10 and 20 min. Tubes were immediately transferred again to a boiling water bath for 1.5 min, then removed and cooled in a cold water bath until reaching room temperature. After reaching room temperature, tubes were vortexed and 30 µL of sample transferred to a 96-well plate. Absorbance of the plate was measured at 560 nm using a plate reader (PowerWave XS; BioTek Instruments Inc., Winooski, VT). Standard values were used to calculate a standard curve with a minimum of $r^2 \geq 0.98$ that was then used to determine lactate concentration of cecal fluid.

Statistical Analysis

Data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure or SAS (Version 9.4; SAS Institute Inc., Cary, NC). Analyses of pH, VFA, and lactate included fixed effects of treatment and time, and random effect of horse. Analyses of total and forage DMI included random effect of horse and fixed effect of treatment. Differences between and within treatment LSMEANS were determined using the PDIFF option in SAS. Significance was declared if $P \leq 0.05$ and tendency declared if $0.05 < P \leq 0.10$.

Results

For all cecal fermentation characteristics measured there was a main effect of hour ($P \leq 0.05$), treatment ($P \leq 0.0043$; Table 3.2), and hour × treatment interaction ($P \leq 0.0462$; Table 3.3). It should be noted that for 2.5, 3, and 3.5 g starch treatments horses did not consume the full

allotment of corn pellets and instead consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·$meal^{-1}$. Additionally, removal of horses from the trial due to gastrointestinal upset resulted in n = 5 for 2.0 g starch treatment, n = 4 for 2.5 g starch treatment, and n = 3 for 3.0 and 3.5 g starch treatments.

Cecal pH

Overall, cecal pH decreased ($P < 0.0001$) as dietary starch increased up to 2.0 g starch (Table 3.2). Horses fed 0, 0.5, and 1.0 g starch exhibited greater ($P \leq 0.0459$) cecal pH than those fed ≥ 1.5 g starch·kg BW^{-1}·$meal^{-1}$ at h 0. At h 2, 4, and 6, cecal pH was lowest ($P \leq 0.0427$) with 1.5 and 2.0 g starch treatments. Cecal pH at h 8 of horses fed 0 and 0.5 g starch was greater ($P \leq 0.0104$) than when 1.0, 2.0, 2.5, or 3.0 g of starch were fed. Greatest ($P \leq 0.0069$) cecal pH for h 10 was observed with 0 and 0.5 g of starch, and lowest ($P \leq 0.0335$) with 2.0 g starch. At h 12, lowest ($P \leq 0.0344$) cecal pH was observed with 2.0, 2.5, and 3.0 g starch.

Volatile Fatty Acids

Cecal acetate, propionate, butyrate, and total VFA were lowest ($P < 0.0001$) when horses consumed 3.5 g starch (Table 3.2). Similar ($P \geq 0.1387$) acetate concentration was observed with 0, 0.5, 1.5, and 2.5 g starch. Acetate:propionate (AP) decreased ($P < 0.0001$) at ≥ 2.0 g starch. Cecal propionate and total VFA were greatest ($P \leq 0.0485$) with 2.0 and 2.5 g starch.

Cecal concentrations of acetate were lowest ($P \leq 0.0484$) with 3.5 g starch treatment at h 0, 2, and 4. Similarly, cecal propionate ($P \leq 0.0371$) and total VFA ($P \leq 0.0468$) were lowest for horses offered 3.5 g starch at h 0. Horses offered 3.0 and 3.5 g starch had less cecal acetate ($P \leq 0.0114$), propionate ($P \leq 0.0384$), butyrate ($P \leq 0.0367$), and total VFA ($P \leq 0.0415$) at h 6, 8, 10, and 12 compared to all other time points. Acetate:propionate was greatest ($P \leq 0.0354$) for

horses consuming 0 and 0.5 g starch at h 0. At h 6 and 8, A:P was decreased ($P \leq 0.0480$) for 2.0, 2.5, 3.0, and 3.5 g starch compared to 0 and 0.5 g starch treatments.

Lactate

Cecal lactate concentration was greatest ($P \leq 0.0060$) for horses consuming 2.0 g starch and undetectable for horses consuming 0 and 0.5 g starch (Table 3.2). Cecal lactate concentration was lower ($P \leq 0.204$) for horses consuming 0 and 0.5 g starch compared to 1.5, 2.0, and 2.5 g starch treatments. Horses consuming 2.0 g starch had greatest ($P \leq 0.0439$) cecal lactate concentration at h 4, 8, and 10 compared to all other timepoints. At h 12, greatest ($P \leq 0.0259$) lactate concentration was observed with 1.0 and 2.0 g starch treatments.

Total and Forage Dry Matter Intake

Total DMI was greatest ($P \leq 0.0411$) for horses offered 3.0 g starch and similar ($P \geq 0.3624$) for horses offered 0, 0.5, 1, and 2 g starch. An overall decrease ($P < 0.0001$; Figure 3.1) in forage DMI was observed as dietary starch was increased. Forage DMI was greatest ($P \leq 0.0158$) when horses were fed 0 and 0.5 g starch and lowest ($P \leq 0.0268$) when horses were fed 3.5 g starch. Horses fed 1.5, 2.0, 2.5, and 3.0 g starch possessed similar ($P \geq 0.2826$) forage DMI.

Discussion

Results of this trial confirm the upper limit of small intestinal starch digestion defined by Potter et al. (1992) and Kienzle (1994). As expected, cecal pH decreased as dietary starch increased but only up to 2.0 g starch·kg BW^{-1}·$meal^{-1}$. This can be attributed to greater concentration of cecal propionate, total VFA, and lactate. Cecal pH did not continue to decrease and instead appeared to stabilize at > 2.0 g starch·kg BW^{-1}·$meal^{-1}$. This is likely attributed to adaptation of both pancreatic enzymes and cecal microogranisms to increasing dietary starch.

Horses in this trial consumed four meals/d, supporting previous research that greater concentrate intake may be achieved through increased meal frequency (Clarke et al., 1990; Richards et al., 2006; Julliand and Grimm, 2017).

Starch entering the cecum is rapidly fermented by cecal microorganisms, particularly lactate-producing bacteria such as *Lactobacillus* and *Streptococcus* (Bailey et al., 2003; Al Jassim and Andrews, 2009). In cases of hindgut overload of starch, excessive lactate production results in a rapid decline of cecal pH and inhibition of acid-intolerant fibrolytic species. This alteration of cecal microbial communities supresses fiber fermentation, and subsequent VFA production (Medina et al., 2002; Shirazi-Beechey, 2008; Biddle et al., 2013). Interestingly, cecal lactate concentration was greatest for horses consuming 2.0 g starch·kg BW^{-1}·$meal^{-1}$; however, cecal pH corresponding to cecal acidosis (< 6) was not observed for this experiment, and recorded values were similar to those previously reported (Jorden et al., 2019; Fehlberg et al., 2020; Ochonski et al., 2020). It has been well-documented that fermentation of fibrous feeds by fibrolytic bacteria primarily yields acetate, while fermentation of starch by amylolytic bacteria primarily yields propionate and butyrate (Hintz et al., 1971; Medina et al., 2002; Sadet-Bourgeteau et al., 2017). Total VFA concentration was greatest in the cecum of horses fed 2.0 and 2.5 g starch·kg BW^{-1}·$meal^{-1}$, and cecal acetate concentration for horses fed 2.0 g starch·kg BW^{-1}·$meal^{-1}$ was not different compared to the 0 and 2.5 g starch treatments; thus, fermentation by acid-intolerant species may not have been impaired. As expected, cecal propionate concentration was greater for 2.0 and 2.5 g starch·kg BW^{-1}·$meal^{-1}$, corresponding to fermentation of greater concentrations of starch (Medina et al., 2002; Sadet-Bourgeteau et al., 2017).

It is important to emphasize that data presented herein is indicative of pH and organic acid concentration within cecal samples at a given time point rather than a measure of organic

acid production by cecal microorganisms. For instance, cecal lactate concentration is typically low due to the activity of lactate utilizing microorganisms that convert lactate to propionate or butyrate (Milinovich et al., 2006; Al Jassim and Andrews, 2009). Slow adaptation of horses to high-starch diets, as done in this experiment, may allow for greater colonization of lactate utilizing bacteria (Bailey et al., 2002; de Fombelle et al., 2003). Adaptation of cecal microorganisms may explain observed decreases in lactate and stabilization of pH in horses offered > 2 g starch·kg BW^{-1}·$meal^{-1}$. Samples of cecal digesta from this experiment are still awaiting microbiome analysis, which will undoubtedly provide further insight into shifts of cecal microbial communities and fermentation products.

Consistent decreases in acetate, propionate, butyrate, and total VFA concentrations for 3.0 and 3.5 g starch·kg BW^{-1}·$meal^{-1}$ treatments may be attributed to differences in forage and total DMI. Voluntary forage DMI for horses offered 3.0 g starch·kg BW^{-1}·$meal^{-1}$ was similar to 1.5, 2.0, and 2.5 g starch·kg BW^{-1}·$meal^{-1}$treatments; total DMI was greatest when horses were offered 3.0 g starch·kg BW^{-1}·$meal^{-1}$ indicating that greatest starch intake was achieved during this treatment. Unexpectedly, A:P for horses consuming 3.0 g starch·kg BW^{-1}·$meal^{-1}$ was numerically greater, but not statistically different compared to 2.0, 2.5, and 3.5 g starch·kg BW^{-1}·$meal^{-1}$ treatments. Fermentation of greater quantities of starch would yield greater cecal propionate concentration, thereby reducing A:P (Medina et al., 2002; Sadet-Bourgeteau et al., 2017). Furthermore, decreased total VFA concentration for this treatment despite greatest total DMI may indicate initial deleterious shifts in cecal microorganisms. Previous reports in equines and ruminants have described depressed VFA concentration in animals subjected to acidosis induced by starch overload (Nagaraja et al., 1981; Nagaraja and Titgemeyer, 2007; Shirazi-Beechey, 2008). Ruminal pH < 5.6 promotes protonation of VFA, thereby increasing absorption

rate and decreasing concentration of organic acids within the rumen (Bergman, 1990). Horses consuming 3.0 g starch·kg BW^{-1}·$meal^{-1}$ maintained cecal pH > 6.59, therefore it is unlikely that decreased VFA concentration can be attributed to increased VFA absorption. As aforementioned, data obtained from this experiment represents organic acid concentrations within a sample at a given timepoint and may not be indicative of organic acid production or representative of cecal environment as a whole. Decreased total VFA concentration may be attributed to shifts in cecal microbial communities, particularly decreases in fibrolytic and lactate-utilizing microorganisms (Medina et al., 2002; Shirazi-Beechey, 2008; Biddle et al., 2013).

Horses offered 3.5 g starch·kg BW^{-1}·$meal^{-1}$ exhibited decreased forage DMI compared to all other treatments. Decreased cecal total VFA concentration, particularly acetate, may be the result of decreased availability of fiber as a substrate for fermentation by cecal microorganisms. Forage intake of horses offered 3.5 g starch·kg BW^{-1}·$meal^{-1}$ was 0.72% BW/d, which is lower than the current forage recommendation for mature horses of > 1.0% BW/d. In addition to providing substrate for hindgut microorganisms, adequate forage intake is necessary for maintenance of gut health. Butyrate, an organic acid produced by fermentation of feedstuffs by hindgut microorganisms, is a vital energy source for cecal and colonic enterocytes (Daly and Shirazi-Beechey, 2006). Therefore, decreased butyrate production may negatively affect health and maintenance of hindgut epithelial tissues. Lowest cecal butyrate concentration was observed with the 3.5 g starch·kg BW^{-1}·$meal^{-1}$ treatment corresponding to lowest voluntary forage DMI, however, analyses of cecal tissues were not performed for this experiment. Results of this trial indicate that feeding 3.0 or 3.5 g starch·kg BW^{-1}·$meal^{-1}$ negatively affect cecal fermentation parameters with possible negative implications for gut health.

Similar to what has been observed in ruminants (Olson et al., 1999; Souza et al., 2010), horses in this trial exhibited decreased forage DMI with increasing dietary starch intake. Regulation of satiety in ruminants is stimulated by accumulation of VFA and decreased ruminal pH (Dixon and Stockdale, 1999). In this experiment, lowest forage intake corresponded to lowest cecal total VFA concentration. Hormonal regulation of satiety in equines has been attributed to post-prandial glycemic response, which occurs in response to pre-cecal digestion of starch (Julliand et al., 2008; Cuddeford, 2013). Therefore, it is possible that greater starch intake, and thus greater post-prandial glycemic response, would result in decreased forage DMI. Despite being offered both concentrate and forage at the same time, horses in this experiment consumed concentrate first followed by forage. This experiment is the first to characterize effect of increasing dietary starch on forage DMI in horses. In order to better understand this effect, relationships between glycemic response and forage DMI must be further explored.

Treatment × hour interactions were analyzed for this experiment in order to assess post-prandial shifts of fermentation parameters in response to increasing amounts of dietary starch. Samples of cecal fluid corresponding to 0 h were obtained immediately prior to feeding the 0600 h meal. At this time, cecal pH of horses consuming > 1.5 g starch·kg BW^{-1}·$meal^{-1}$was lower; these results indicate that cecal environment of horses consuming > 1.5 g starch·kg BW^{-1}·$meal^{-1}$ was overall more acidic, attributed to greater consumption of starch. Fermentation of starch by cecal microorganisms predominantly results in production of propionate and lactate, which decrease cecal pH (Medina et al., 2002; Sadet-Bourgeteau et al., 2017). Indeed, at h 0 cecal lactate concentration was greater and A:P was lower for horses consuming > 1.0 g starch·kg BW^{-1}·$meal^{-1}$.

Corresponding to typical transit time of digesta, pH nadir was expected to occur around 4 to 8 h follow consumption of the 0600 h meal (Jordan et al., 2019; Fehlberg et al., 2020; Ochonski et al., 2020). Cecal pH of horses fed 0 and 0.5 g starch·kg BW^{-1}·$meal^{-1}$at h 6 was greater compared to all other treatments, attributed to lower cecal propionate concentration. Similarities in VFA and lactate concentrations between 0 and 1.0 g starch·kg BW^{-1}·$meal^{-1}$treatments indicate that most starch provided up through 1.0 g starch·kg BW^{-1}·$meal^{-1}$treatment was digested pre-cecally.

Colic, an umbrella term to describe abdominal pain, can be broadly divided into 4 categories: impaction, incarceration, displacement, and spasmodic (Archer and Proudman, 2006). Of these categories, spasmodic colic is most frequently associated with consumption of high-starch diets and subsequent deleterious shifts in hindgut microorganisms and fermentation products (Medina et al., 2002; Shirazi-Beechey, 2008). Of the 6 horses enrolled in this experiment, 3 had to be removed due to clinical signs of spasmodic colic. Horse 5 was removed when transitioned to 2.0 g starch·kg BW^{-1}·$meal^{-1}$, horse 1 was removed when transitioned to 2.5 g starch·kg BW^{-1}·$meal^{-1}$, and horse 3 was removed when transitioned to 3.0 g starch·kg BW^{-1}·$meal^{-1}$. Horses displayed moderate signs of colic, including looking at flank, rolling, and refusal to eat, elevated heart and respiratory rates, and a score of 2 on the Equine Comfort Assessment Scale (Blossom et al., 2007; Appendix A). In all cases, horses were administered flunixin meglumine (1.1 mg/kg BW; Merck Animal Health, Madison, NJ, USA) intravenously and hand walked for 30 min to 1 h until acute pain was no longer evident. Cecal pH for all horses was recorded to be > 6.4 at time of colic, well within normal limits for healthy horses (Ochonski et al., 2020; Sorensen et al., 2021). It is important to note that collection of cecal pH data for this experiment was not continuous and data presented herein only represent pH within a sample at a

given time point. Therefore, additional changes in cecal pH may have occurred but were not characterized due to limitations in methodology used for this experiment. Horses removed from the trial displayed differing patterns of cecal pH and lactate concentration compared to horses that persisted for the duration of the experiment (Figure 3.2). Horses removed exhibited a sharp numerical increase in cecal lactate concentration and decreased cecal pH prior to exhibiting signs of colic. On the contrary, horses that did not display signs of colic had lactate remain at lower concentrations with only moderate fluctuations in cecal pH corresponding to treatment. As aforementioned, horses adapted to high starch diets possess greater concentrations of lactate-utilizing bacteria compared to un-adapted horses (Bailey et al., 2002; de Fombelle et al., 2003). These microorganisms convert lactate to propionate and butyrate, thereby modulating cecal pH and attenuating risk of cecal acidosis. Recent *in vitro* research in our laboratory (Douthit et al., 2019) characterized the effect of adding *Megasphera elsdenii*, a prominent lactate-utilizing microorganism, to cultures of cecal fluid containing oligofructose or corn starch. Addition of *M. elsedenii* lessened lactate accumulation in cultures of cecal fluid. Biddle et al. (2013) reported lactate accumulation and subsequent attenuation in fecal slurries enriched with starch corresponded to a greater abundance of *M. elsedenii*. Novel data presented herein indicate that individual horse variation in microbiome may play a role in susceptibility to spasmodic colic. Horses that experienced lactate accumulation and ensuing colic may possess fewer lactate-utilizing microorganisms.

Conclusion

In summary, horses fed 2.0 g starch·kg BW^{-1}·$meal^{-1}$ demonstrated decreased pH and shifts in fermentation parameters consistent with greater appearance of starch in the cecum, in agreement with the upper limit of small intestinal starch digestion defined by Potter et al. (1992)

and Kienzle (1994). Horses in this trial consumed 4 meals/d, supporting previous research that greater concentrate intake may be achieved through increased meal frequency and slow adaptation. Even those horses with the greatest lactate concentrations never had cecal pH values consistent with cecal acidosis (< 6). Consumption > 2.0 g starch·kg BW^{-1}·$meal^{-1}$ may lead to shifts in cecal microbial communities and depressed voluntary forage DMI that result in decreased fermentative activity, as evidenced through marked decreases in VFA concentration of horses offered 3.0 and 3.5 g starch·kg BW^{-1}·$meal^{-1}$. This experiment is the first to demonstrate decreased forage DMI as starch is increased, as previously observed in ruminants. Differences in cecal lactate concentration were noted for horses removed from the experiment due to colic versus horses that remained for duration of the experiment, indicating differences in individual horse microbiome may play a role in susceptibility to spasmodic colic.

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Tables and Figures

Table 3.1 Proximate analysis of Smooth Bromegrass hay, pelleted corn, and ration balancer[1,2,3]

Item, %	Smooth Bromegrass hay[4]	Corn pellet[5]	Ration balancer[6]
Dry matter	91.6	91.5	89.7
Crude protein	12.3	8.8	19.8
Acid detergent fiber	36.2	3.5	27.6
Neutral detergent fiber	63.2	7.1	37.9
Starch	0.8	69.4	5.9
Crude Fat	3.9	3.4	1.8
Calcium	0.41	0.02	3.07
Phosphorus	0.18	0.28	1.58

[1]Proximate analysis using wet chemistry (Dairy One Forage Lab, Ithaca, NY).
[2]Dry matter basis.
[3]Feed was delivered at 0600, 1200, 1800, and 2400 h.
[4]Smooth Bromegrass hay was offered *ad libitum*.
[5]Dietary starch was increased by 0.5 g starch·kg BW^{-1}·$meal^{-1}$ every 7 d via corn pellet until horses were offered 3.5 g starch·kg BW^{-1}·$meal^{-1}$.
[6]Ration balancer was fed at 0.0125% BW/meal to meet the NRC requirements for protein, lysine, vitamins, and minerals for a mature, idle horse.

Table 3.2 Main effect of increasing dietary starch on cecal pH, volatile fatty acid (VFA), and lactate

	Starch[1], g starch·kg BW^{-1}·meal^{-1}									
Item	0[2]	0.5	1.0	1.5	2.0	2.5[3]	3.0[3]	3.5[3]	SEM	*P*-value
pH	7.00^{a}	7.04^{a}	6.85^{b}	6.73^{c}	6.58^{d}	6.72^{c}	6.71^{c}	6.72^{c}	0.05	< 0.0001
Acetate, mM	102.02^{ab}	94.19^{a}	93.07^{a}	99.41^{ab}	109.85^{b}	104.47^{ab}	57.72^{c}	29.21^{d}	5.75	< 0.0001
Propionate, mM	30.50^{a}	28.16^{a}	30.56^{a}	31.93^{a}	43.31^{b}	45.30^{b}	22.94^{c}	12.07^{d}	2.79	< 0.0001
Acetate:Propionate	3.43^{a}	3.42^{a}	3.18^{a}	3.28^{a}	2.63^{b}	2.53^{b}	2.80^{b}	2.75^{b}	0.144	0.0001
Butyrate, mM	8.66^{a}	7.99^{a}	8.59^{a}	9.23^{a}	9.68^{a}	9.03^{a}	5.90^{b}	3.08^{c}	0.081	< 0.0001
Total VFA, mM	142.18^{a}	131.28^{a}	133.22^{a}	141.88^{a}	164.51^{b}	160.43^{b}	88.26^{c}	45.50^{d}	8.82	< 0.0001
Lactate, mM	0.00^{a}	0.00^{a}	1.02^{b}	1.43^{b}	6.13^{c}	1.99^{b}	1.11^{b}	0.09^{b}	0.006	0.0043

[1]Dietary starch provided via corn pellet was increased by 0.5 g starch·kg BW^{-1}·meal^{-1} every 7 d and fed every 6 h (0600, 1200, 1800, and 2400 h).
[2]Smooth Bromegrass hay fed *ad libitum* throughout the experiment.
[3]Horses did not consume the full meal of corn pellet for treatments 2.5, 3.0, and 3.5 g starch, and, on average, consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·meal^{-1}, respectively.
[abcd]Means within the same row without a common superscript are different ($P \leq 0.05$).

Table 3.3 Effect of increasing dietary starch and sampling time on cecal pH, volatile fatty acid (VFA), and lactate concentration

		Starch[1], g starch·kg BW-1·meal-1									
Item	Time, h	0[2]	0.5	1.0	1.5	2.0	2.5[3]	3.0[3]	3.5[3]	SEM	*P*-value[4]
pH	0	7.07^{ab}	7.29^{a}	7.28^{a}	6.88^{b}	6.65^{c}	6.55^{c}	6.59^{c}	6.47^{c}		
	2	6.96^{acd}	7.13^{ade}	6.98^{acd}	6.68^{b}	6.74^{b}	7.13^{d}	6.86^{acd}	6.67^{e}		
	4	7.01^{ac}	6.96^{ad}	6.81^{cd}	6.63^{b}	6.58^{b}	6.93^{c}	6.86^{acd}	6.70^{d}		
	6	6.96^{ab}	7.02^{ab}	6.87^{b}	6.50^{c}	6.54^{c}	6.73^{e}	6.68^{e}	6.64^{de}	0.048	T, H, T × H
	8	6.99^{ac}	6.92^{a}	6.45^{b}	6.83^{c}	6.46^{b}	6.46^{b}	6.66^{d}	6.88^{ac}		
	10	6.99^{a}	6.99^{a}	6.79^{b}	6.76^{b}	6.50^{c}	6.67^{d}	6.75^{b}	6.88^{a}		
	12	7.03^{ab}	7.00^{ab}	6.75^{c}	6.85^{bc}	6.61^{e}	6.53^{e}	6.60^{e}	6.78^{c}		
Acetate, mM	0	97.31^{b}	74.49^{a}	88.45^{ab}	113.32^{cd}	103.59^{bd}	136.51^{d}	129.22^{d}	35.06^{e}		
	2	82.10^{a}	93.53^{ab}	109.07^{abc}	125.36^{b}	112.09^{ab}	81.30^{c}	103.53^{abc}	27.42^{d}		
	4	117.53^{ac}	106.44^{abcd}	89.15^{bde}	104.70^{bc}	126.98^{d}	69.62^{e}	62.50^{e}	32.32^{f}		
	6	105.74^{a}	90.04^{a}	76.21^{a}	84.63^{a}	99.17^{a}	91.82^{a}	32.02^{b}	25.55^{b}	9.124	T, H, T × H
	8	88.88^{a}	103.51^{a}	117.63^{a}	81.49^{a}	97.40^{a}	103.74^{a}	27.10^{b}	28.62^{b}		
	10	109.84^{a}	108.65^{ac}	87.07^{b}	83.87^{b}	101.07^{bc}	112.10^{c}	28.26^{d}	24.15^{d}		
	12	112.74^{ac}	82.68^{b}	83.94^{b}	102.53^{ab}	128.61^{ac}	136.16^{c}	21.44^{d}	31.34^{d}		
Propionate, mM	0	27.65^{a}	23.14^{a}	30.52^{ab}	38.79^{b}	35.93^{bd}	62.70^{c}	52.61^{c}	15.18^{e}		
	2	24.79^{a}	30.90^{ab}	37.41^{bc}	43.94^{c}	40.70^{bc}	35.64^{ab}	39.90^{abc}	11.25^{d}		
	4	35.62^{ac}	34.62^{ac}	28.89^{acd}	35.25^{ac}	50.94^{b}	33.86^{ac}	24.58^{cd}	13.74^{d}		
	6	29.57^{abc}	21.53^{acd}	23.79^{acd}	24.44^{acd}	43.03^{b}	35.26^{ab}	12.94^{d}	11.11^{d}	3.115	T, H, T × H
	8	27.24^{ac}	30.29^{ab}	40.22^{bd}	24.87^{c}	39.98^{bd}	40.44^{d}	11.41^{e}	11.56^{e}		
	10	34.46^{ab}	32.87^{ab}	28.23^{a}	25.15^{a}	42.14^{bc}	46.27^{c}	11.23^{d}	9.32^{d}		
	12	34.19^{a}	23.78^{a}	24.83^{a}	31.06^{a}	50.42^{b}	62.92^{bc}	7.92^{d}	12.35^{d}		

[1]Dietary starch provided via corn pellet was increased by 0.5 g starch·kg BW^{-1}·meal^{-1} every 7 d and fed every 6 h (0600, 1200, 1800, and 2400 h).
[2]Smooth Bromegrass hay fed *ad libitum* throughout the experiment.
[3]Horses did not consume the full meal of corn pellets for treatments 2.5, 3.0, and 3.5 g starch, and, on average, consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·meal^{-1}, respectively.
[4]T = main effect of treatment; H = main effect of sampling time (hour); T × H = interaction between time and treatment.
[abcdef]Means within the same row without a common superscript are different ($P \leq 0.05$).

Table 3.3 (Continued)

		Starch, g starch·kg BW-1·meal-1									
Item	Time, h	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50	SEM	*P*-value
Acetate:Propionate	0	3.66^{a}	3.33^{a}	3.07^{b}	3.03^{b}	2.90^{b}	2.45^{c}	2.42^{c}	2.45^{c}		
	2	3.36^{a}	3.08^{ab}	2.93^{ab}	2.96^{ab}	2.78^{b}	2.62^{b}	2.61^{b}	2.70^{b}		
	4	3.38^{a}	3.08^{abc}	3.11^{ac}	3.18^{ac}	2.51^{b}	2.58^{c}	2.89^{abc}	2.59^{c}		
	6	3.68^{ab}	4.25^{a}	3.33^{bd}	3.55^{b}	2.57^{c}	2.64^{c}	2.73^{cd}	2.57^{c}	0.582	T, H, T × H
	8	3.36^{a}	3.41^{a}	3.04^{ac}	3.40^{a}	2.44^{b}	2.60^{bc}	2.63^{bc}	2.75^{bc}		
	10	3.21^{ac}	3.31^{a}	3.23^{ac}	3.52^{a}	2.50^{b}	2.58^{bc}	2.82^{abc}	3.31^{ac}		
	12	3.39^{a}	3.57^{a}	3.52^{a}	3.32^{ab}	2.68^{b}	2.23^{c}	3.48^{ab}	2.90^{abc}		
Butyrate, mM	0	7.42^{ae}	6.33^{ace}	8.19^{ae}	11.77^{b}	8.92^{abc}	12.26^{cd}	14.73^{d}	4.16^{e}		
	2	6.93^{bc}	8.70^{c}	10.53^{bc}	12.64^{a}	10.01^{ab}	6.56^{c}	10.62^{abc}	2.99^{e}		
	4	9.97^{b}	9.81^{b}	7.78^{ab}	9.18^{a}	12.41^{a}	5.23^{b}	5.99^{a}	3.66^{a}		
	6	8.24^{a}	6.37^{a}	6.45^{a}	7.25^{a}	9.71^{a}	6.98^{a}	3.26^{b}	2.67^{b}	1.848	T, H, T × H
	8	8.04^{ae}	8.61^{ace}	11.24^{b}	6.48^{c}	8.43^{b}	7.99^{b}	2.55^{d}	2.82^{d}		
	10	10.08^{a}	9.46^{a}	7.94^{b}	6.95^{b}	8.21^{bc}	10.32^{a}	2.44^{d}	2.25^{d}		
	12	9.94^{ab}	6.67^{a}	8.01^{ab}	10.37^{b}	10.07^{b}	13.89^{c}	1.69^{d}	3.04^{d}		
Total VFA, mM	0	133.26^{abc}	104.68^{a}	128.37^{abc}	165.55^{bce}	149.95^{ce}	213.53^{d}	199.68^{e}	55.66^{f}		
	2	114.53^{ac}	134.23^{abc}	158.41^{abc}	183.84^{b}	164.55^{ab}	124.91^{c}	156.66^{abc}	42.79^{d}		
	4	164.26^{ab}	152.06^{abc}	126.69^{acd}	150.67^{abcd}	192.42^{b}	109.82^{cd}	94.82^{d}	50.96^{e}		
	6	144.35^{a}	118.67^{b}	107.21^{b}	117.32^{b}	153.83^{a}	135.41^{ab}	49.41^{c}	40.43^{c}	14.594	T, H, T × H
	8	124.95^{a}	143.41^{ab}	170.29^{b}	113.62^{a}	147.31^{ab}	153.62^{ab}	42.20^{c}	44.12^{c}		
	10	155.81^{a}	152.07^{a}	124.06^{b}	116.84^{b}	152.83^{a}	170.60^{a}	43.01^{c}	36.74^{c}		
	12	158.08^{ad}	113.85^{bc}	117.53^{cd}	145.32^{d}	190.66^{e}	215.14^{e}	32.03^{f}	47.73^{f}		

Table 3. 3 (Continued)

		Starch, g starch·kg BW-1·meal-1									
Item	Time, h	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50	SEM	*P*-value
Lactate, mM	0	0.000^{a}	0.000^{a}	0.000^{a}	1.497^{b}	1.343^{b}	0.804^{b}	0.088^{ab}	0.090^{ab}		
	2	0.000^{a}	0.000^{a}	0.000^{a}	1.172^{b}	1.206^{b}	7.533^{c}	0.258^{a}	0.073^{ab}		
	4	0.000^{a}	0.000^{a}	0.271^{b}	2.341^{c}	9.158^{d}	1.265^{c}	4.494^{c}	0.067^{a}		
	6	0.000^{a}	0.000^{a}	1.019^{b}	1.793^{b}	1.586^{b}	0.438^{c}	2.665^{b}	0.083^{a}	0.007	T, H, T × H
	8	0.000^{a}	0.000^{a}	0.693^{b}	1.214^{b}	8.338^{c}	2.451^{b}	0.092^{a}	0.065^{a}		
	10	0.000^{a}	0.000^{a}	0.107^{b}	0.798^{b}	12.002^{c}	1.882^{b}	0.078^{a}	0.085^{a}		
	12	0.000^{a}	0.000^{a}	5.048^{c}	1.212^{b}	9.258^{c}	0.526^{b}	0.091^{a}	0.088^{a}		

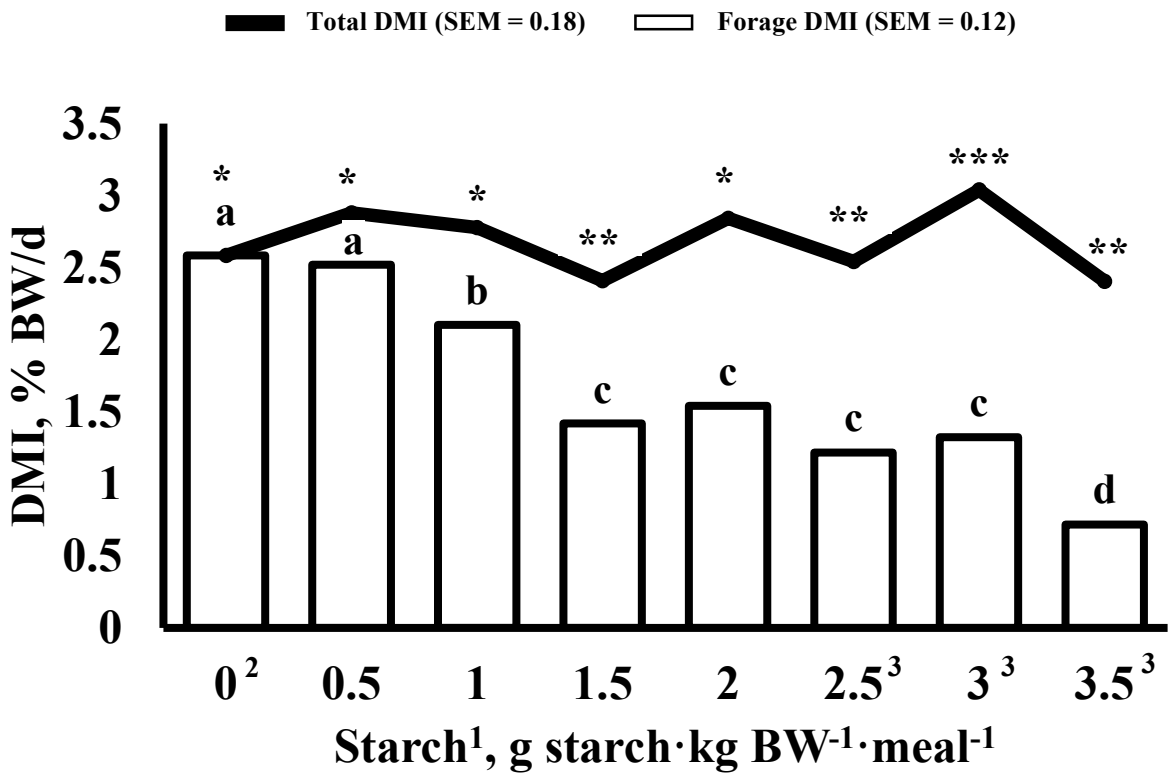

Figure 3.1 Effect of increasing dietary starch on total and voluntary forage dry matter intake (DMI)

[1]Dietary starch provided via corn pellets was increased by 0.5 g starch·kg BW^{-1}·$meal^{-1}$ every 7 d and fed every 6 h (0600, 1200, 1800, and 2400 h).
[2]The 0 g starch·kg BW^{-1}·$meal^{-1}$ corresponds to Smooth Bromegrass hay, provided *ad libitum*.
[3]Horses did not consume the full meal of corn pellet for treatments 2.5, 3.0, and 3.5 g starch·kg BW^{-1}·$meal^{-1}$, and, on average, consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·$meal^{-1}$, respectively.
[***]Means without a common superscript are different, $P \leq 0.05$.
[abcd]Means without a common superscript are different, $P \leq 0.05$.

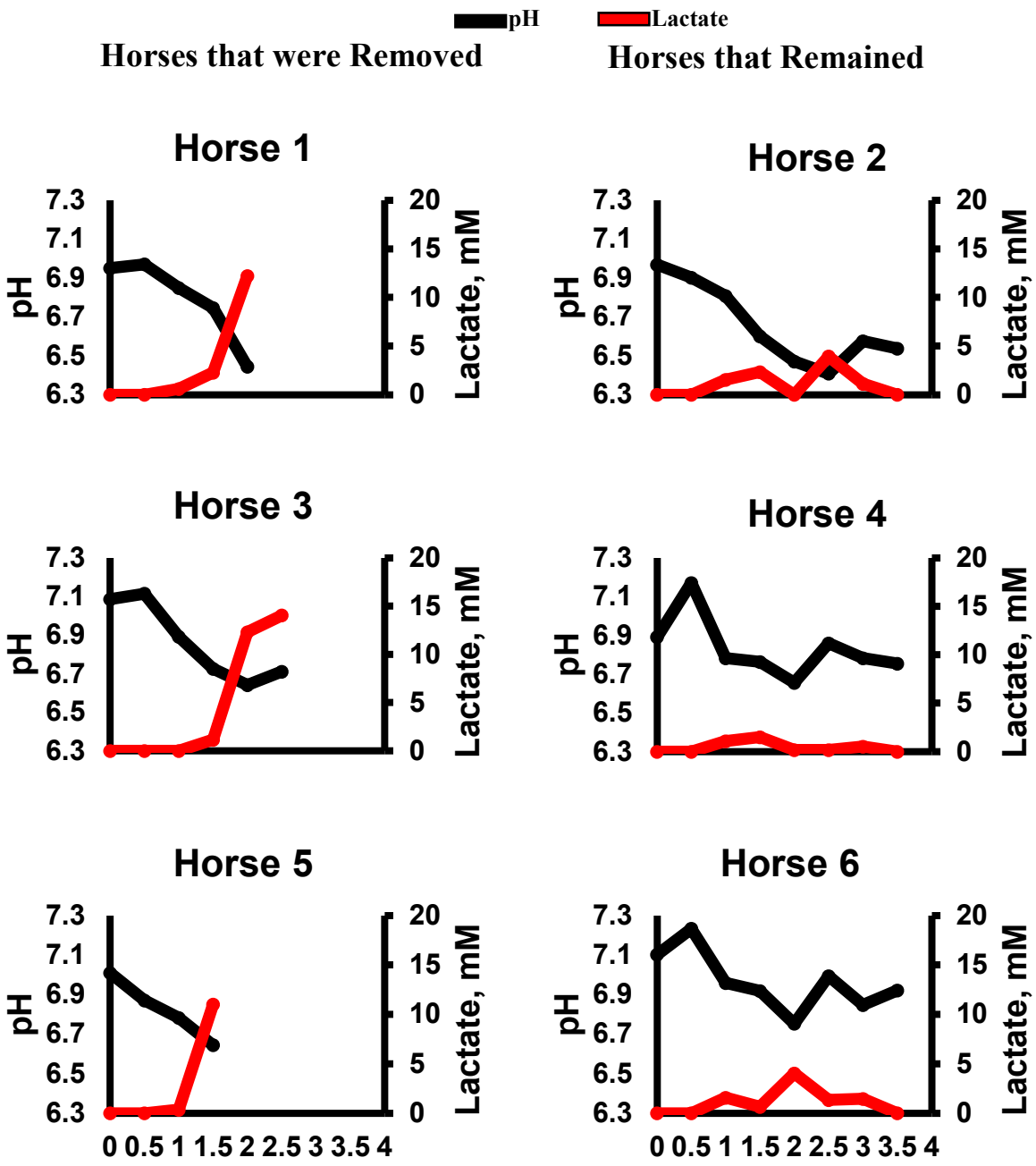

Figure 3.2 Changes in cecal pH and lactate in individual horses in response to increasing dietary starch[1,2,3]

[1]Dietary starch provided via corn pellets was increased by 0.5 g starch·kg BW^{-1}·$meal^{-1}$ every 7 d and fed every 6 h (0600, 1200, 1800, and 2400 h).
[2]The 0 g starch·kg BW^{-1}·$meal^{-1}$ corresponds to Smooth Bromegrass hay, provided *ad libitum*.
[3]Horses did not consume the full meal of corn pellets for treatments 2.5, 3.0, and 3.5 g starch·kg BW^{-1}·$meal^{-1}$, and, on average, consumed 2.1, 2.72, and 2.68 g starch·kg BW^{-1}·$meal^{-1}$, respectively.

Chapter 4 - Evaluation of Enogen® Feed Corn on Growth Performance and Carcass Characteristics of Finishing Pigs

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Abstract

Enogen® Feed Corn (EFC; Syngenta Seeds, LLC, Downers Grove, IL) hybrids contain a trait for expression of heat-stable alpha amylase in the grain. Alpha amylase is an enzyme responsible for breakdown of starch in the small intestine; supplementation of exogenous alpha amylase to pigs may result in greater starch digestibility and thus improved gain efficiency. A total of 288 pigs (Line 600 × 241, DNA, Columbus, NE; initially 41.6 kg) were utilized in an 82-d trial to determine if replacing conventional yellow dent corn (CONV) with EFC in diets with or without distillers dried grains with solubles (DDGS) influences growth performance and carcass characteristics. Pens of pigs were randomly assigned to 1 of 4 dietary treatments balancing for initial BW. There were 9 pens per treatment with 8 pigs per pen (an equal number of barrows and gilts per pen). Treatments were arranged in a 2 × 2 factorial with main effects of corn source (CONV or EFC) and DDGS (0 or 25%). Experimental diets were fed in meal form in 3 phases: d 0 to 29, 29 to 47, and 47 to 82. Pigs were weighed approximately every 2 wk and at the beginning of each phase. On d 82, pigs were transported to a commercial abattoir for processing and carcass data collection. Data were analyzed using PROC GLIMMIX procedure of SAS with pen as the experimental unit. There were no corn source by DDGS interactions ($P > 0.05$) observed for overall performance or carcass characteristics. Overall, average daily gain (ADG) was marginally greater ($P < 0.089$) for pigs fed EFC than CONV with no evidence ($P > 0.196$) for difference in average daily feed intake (ADFI), gain efficiency (G:F), hot carcass weight (HCW), or other carcass traits. Addition of DDGS decreased ($P < 0.047$) overall ADG and G:F. Pigs fed DDGS had marginally lower ($P < 0.071$) HCW, less ($P < 0.050$) backfat depth, greater ($P < 0.026$) loin depth, and greater ($P < 0.020$) percentage lean and carcass fat iodine value (IV). In summary, addition of 25% DDGS to the diet decreased ADG and increased carcass fat IV.

Pigs fed EFC tended to have improved overall ADG; however, G:F and carcass characteristics were not different between corn sources. These results suggest that EFC, although not beneficial, may be used as a substitute for CONV without any deleterious effects on growth performance. Further research should be conducted to understand if addition of EFC to swine diets could be beneficial in younger pigs exhibiting decreased pancreatic α-amylase secretion following weaning, or whether heat treatment of diets, such as pelleting, may influence the response to EFC.

Key words: DDGS, high amylase corn, finishing pig, yellow dent corn

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CONV	conventional yellow corn
DDGS	distillers dried grains with solubles
EFC	Enogen feed corn
G:F	gain:feed ratio
HCW	hot carcass weight
IV	iodine value
RFID	radio frequency identification

Introduction

Starch contained in cereal grains is the greatest source of energy in swine diets; therefore, novel technologies to increase starch digestibility may improve gain efficiency. Small intestinal digestibility of starch is greater than 95% for pigs and is attributed to greater pancreatic alpha-amylase activity compared to other monogastric species such as horses or rats (Kienzle et al., 1994; Bach Knudsen, 2001; Bauer et al., 2003).

Initially developed for ethanol production, Enogen® Feed Corn (EFC; Syngenta Seeds, LLC, Downers Grove, IL) are corn hybrids with increased expression of heat-stable alpha-amylase. Alpha amylase is an enzyme secreted into the small intestine that hydrolyzes starch to produce maltose, maltotriose, and α-limit dextrins. These products are further broken down into glucose, which is absorbed across the intestinal wall and utilized as an energy source for the pig. Therefore, supplementation of alpha amylase may improve starch digestion, allowing for greater glucose absorption and utilization, translating to improved growth performance. Supplementation of alpha amylase through EFC has been demonstrated to be beneficial in ruminant animals. Feeding EFC to both growing and finishing beef cattle has been shown to improve gain efficiency, attributed to greater starch breakdown and availability of glucose for growth (Horton et al., 2017; Johnson et al., 2019; Jolly-Breithaupt et al., 2019). These data indicate the potential for EFC to be used in livestock diets; however, research on the effect of feeding this hybrid to swine is limited.

Production of ethanol has resulted in availability of byproducts, such as distillers dried grains with solubles (DDGS), which have become a relatively common ingredient in swine diets due to their low cost, and moderate lysine and digestible phosphorus content. A review by Stein and Shurson (2009) determined that growth performance of pigs fed diets with up to 30% DDGS

was unaffected compared with those fed a corn-based diet. However, some studies have observed adding DDGS to swine diets may negatively affect growth performance and carcass quality because of its high fiber and unsaturated fatty acid content (Whitney et al., 2006; Lineen et al, 2008; Graham et al., 2014). Due to DDGS being included in swine diets primarily at the expense of corn, it is important to assess whether any interaction exists between corn variety and DDGS inclusion rate. We hypothesize that addition of exogenous amylase from EFC may increase starch digestibility and mitigate deleterious effects of DDGS on growth performance. Therefore, the objective of this trial was to determine whether replacing conventional yellow dent corn (CONV) with EFC in diets with or without DDGS influences growth performance and carcass characteristics of finishing pigs.

Materials and Methods

All procedures used in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. This study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Pigs were housed in a fully enclosed, environmentally regulated barn containing 36 pens with slatted concrete floors. Pens were equipped with a two-space single sided feeder (Farmweld, Teutopolis, IL) and a cup waterer, and pigs were allowed access to feed and water *ad libitum*. The floor space allowance per pig was maintained at 0.73 m^2. An automated feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record feed to each pen.

Study Design

A total of 288 pigs (Line 600 × 241; DNA, Columbus, NE) initially weighing 41.6 kg were utilized in an 82-d trial. There were 9 pens per treatment and 8 pigs per pen with an equal number of barrows and gilts. Pens were randomly assigned to dietary treatments and balanced

based on pen weight at the start of the study. Dietary treatments (Table 4.1) were arranged in a 2 × 2 factorial with main effects of corn source (CONV or EFC) and DDGS (0 or 25% of the diet). Diets were formulated to meet or exceed nutrient requirements (NRC, 2012). The diets with and without DDGS in each phase were formulated to the same standard ileal digestible (SID) lysine, threonine, and tryptophan concentration with all other amino acids above minimum ratios relative to lysine. The DDGS was assumed to contain 2,387 kcal/kg of net energy and 0.484% SID lysine in diet formulation (Graham et al., 2014). The experimental diets were fed in 3 phases: d 0 to 29, d 29 to 47, and d 47 to 82. Diets were fed in meal form and both corn sources were ground to a similar particle size, approximately 700 microns for the experiment. Both corn varieties and DDGS were sourced from one location and lot for the duration of the experiment. Pens of pigs and feed refusals were weighed approximately every 2 weeks and at the end of each phase change in order to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain efficiency (G:F; Table 4.4). During the study, 6 pigs died or were removed due to illness or injury unrelated to the dietary treatments. On d 82, 282 pigs were individually weighed, ear tagged with a radio frequency identification (RFID) tag and tattooed for individual carcass identification and data measurements at the packing plant. All pigs were transported to a commercial packing plant (Triumph Foods, St. Joseph, MO) on the same day for processing and collection of hot carcass weight (HCW), loin depth, backfat depth, and percentage lean in the carcass. Carcass yield was calculated as HCW divided by the individual final live weight measured at the farm. Loin depth and backfat depth were determined using an optical probe (Fat-O-Meter, SFK, Herlev, Denmark) placed between the third and fourth (counting from the ham end of the carcass) rib at a distance approximately 7 cm from the dorsal midline. Percentage lean was estimated using propriety equations from the packing plant. Belly fat samples anterior to the

manubrium were obtained prior to carcass chilling and analyzed for fat iodine value (IV) using near-infrared spectroscopy.

Chemical Analysis

Corn samples were collected at time of feed manufacturing, and feed samples were collected approximately two days after each feed delivery. Samples were stored at -20°C until analysis. Pooled samples for each phase were sent to a commercial laboratory (Ward Laboratories, Inc., Kearney, NE) and analyzed in duplicate for dry matter (method 935.29; AOAC International, 1990), starch (method 920.40; AOAC International, 1990), crude protein (method 990.03; AOAC International, 1990), crude fiber (method 978.10; AOAC International, 1990), fat (method 920.39; AOAC International, 1990), calcium (method 985.01; AOAC International, 1990), and phosphorus (method 985.01; AOAC International, 1990; Tables 4.2 and 4.3).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC) with pen serving as the experimental unit. The main effects of corn source, DDGS, and their interactions were tested using orthogonal contrasts. For analyses of loin depth, backfat depth, and percentage lean, HCW was used as a covariate. Results were considered significant at $P \leq 0.05$ and marginally significant at $P \leq 0.10$.

Results

Nutrient composition of analyzed diets (Table 4.3) was reasonably consistent with formulated values considering expected analytical variation. As expected, treatments containing 25% DDGS possessed greater crude fat and crude fiber compared to diet without DDGS. Corn

varieties used in this trial (Table 4.2) were consistently similar to each other and standard values documented in the literature (NRC, 2012).

There was no main effect ($P \geq 0.204$) of corn type was observed during phase 1 or 2. Pigs fed EFC tended to have greater ADG during phase 3 ($P = 0.077$) and overall ($P = 0.089$) than pigs fed CONV. No differences were observed ($P \geq 0.196$) in ADFI, G:F, HCW, yield, backfat depth, loin depth, percentage lean, or carcass fat IV between corn sources.

A main effect of DDGS was observed when pigs fed 25% DDGS had decreased ADG during phase 1 ($P = 0.045$), phase 2 ($P = 0.005$), and overall ($P = 0.026$). There was no detectable difference ($P \geq 0.151$) during any phase or overall for ADFI between pigs fed 0 and 25% DDGS. Overall, pigs fed diets containing 25% DDGS had poorer ($P = 0.047$) G:F. Pigs fed 25% DDGS had a marginally lower ($P = 0.071$) HCW, less ($P = 0.050$) backfat, greater ($P = 0.026$) loin depth, and greater ($P = 0.020$) percentage lean and carcass fat IV ($P < 0.0001$) than pigs fed diets without DDGS.

During phase 1 (days 0 to 29), there was a corn source by DDGS interaction ($P < 0.05$) observed for ADG and ADFI (Table 4.4). Pigs fed the diet containing EFC without DDGS had greater ($P = 0.019$) ADG than pigs fed the EFC with 25% DDGS diet; whereas there were no differences between the CONV treatments with or without DDGS. Average daily feed intake (ADFI) was greater ($P = 0.035$) for pigs fed CONV with DDGS compared to pigs fed CONV without DDGS, with no differences between EFC treatments. Furthermore, there was no evidence of a corn source by DDGS interaction ($P = 0.664$) for G:F. There was no evidence for interactions observed between corn source and DDGS inclusion for phase 2 (days 29 to 47). During phase 3 (days 47 to 82), there was a corn source by DDGS interaction ($P < 0.05$) observed for ADG. Average daily gain was greater ($P = 0.041$) for pigs fed EFC diets containing

25% DDGS compared to the 25% DDGS diet with CONV; there were no detectable differences between corn sources when fed without DDGS. Additionally, during phase 3 ADFI was marginally ($P = 0.081$) A marginal increase ($P = 0.053$) in carcass fat IV was observed in pigs fed EFC without DDGS compared pigs fed CONV without DDGS, with no differences observed for pigs fed DDGS. There were no interactions observed between corn source and DDGS for overall performance or any of the remaining carcass measurements ($P \geq 0.10$).

Discussion

Starch is primarily digested by pancreatic α-amylase in the small intestine. This enzyme cleaves α-1,4 linkages in the starch molecule to produce maltose, maltotriose, and α-limit dextrins (Gray, 1992). These products are further broken down by brush border enzymes to yield glucose, which is transported across the intestinal wall primarily by Na^+/glucose co-transporter 1 (SGLT1) and utilized by the host (Moran et al., 2010). The rate and extent of starch digestion is highly dependent on starch chemistry, particularly crystallinity and relative ratios of amylose and amylopectin (Svihus et al., 2005). Evaluation of glucose absorption kinetics identified an inverse relationship, as pigs exhibited decreased rates of glucose uptake as starches of increased amylose concentration were fed (Regmi et al., 2010). Although total starch digestibility has been observed as greater than 95% in pigs (Bach Knudsen, 2001), there is still potential for improvements through feed processing (Rojas et al., 2016) or addition of exogenous enzymes (Kim et al., 2003; Kerr and Shurson, 2013).

Pancreatic α-amylase secretion is decreased following weaning and increases as pigs age and are transitioned to high-starch diets (Jensen et al., 1997). Nevertheless, a main effect of corn type was not observed during phase 1. An interaction of corn type and DDGS was observed during phase 1, with pigs consuming EFC without DDGS having a greater ADG than pigs

consuming EFC with DDGS; this can be attributed to pigs consuming EFC without DDGS having a greater ADFI. This response was not observed for phases 2 or 3. There was an interaction of corn type and DDGS observed during phase 3, with pigs consuming EFC with DDGS having greater ADG than pig consuming CONV with DDGS. Addition of EFC to late finishing swine diets may mitigate deleterious effects of DDGS on growth performance through increased starch digestibility attributed to additional amylase. Additionally, there was tendency for greater ADG during phase 3 and overall for pigs consuming EFC. Data have demonstrated substitutional effects of exogenous α-amylase supplementation in poultry. Jiang et al. (2008) observed improved ADG, decreased mRNA expression of pancreatic α-amylase, and unchanged gain efficiency in broiler chickens, suggesting that supplementation with exogenous α-amylase results in less energy expended to secrete endogenous amylase. This theory of conservation of digestive enzymes has been described by Rothman et al. (2002). Briefly, this mechanism suggests that a portion of pancreatic enzymes are absorbed into the bloodstream and recycled in an enteropancreatic circulation in order to conserve energy that would otherwise be expended through the synthesis of new enzymes with each meal. Addition of exogenous amylase would increase the amount of this enzyme in enteropancreatic circulation and thus further decrease the need for production of endogenous amylase. The tendency for improved phase 3 and overall ADG may be attributed to decreased endogenous secretion of pancreatic α-amylase, however, further research is needed in order to determine the effect of EFC on endogenous enzyme secretion at both the protein and mRNA level in swine.

All previously published data on EFC have been in beef cattle, and a majority of results demonstrates its ability to improve gain efficiency when fed to ruminants (Horton et al., 2017; Johnson et al., 2018; Baker et al., 2019; Jolly-Breithaupt et al., 2019). Ruminants secrete less

pancreatic α-amylase compared to pigs, making EFC a potential method to increase post-ruminal starch digestion (Harmon et al., 2004). In this experiment, data indicate that inclusion of EFC did not enhance digestibility of starch enough to observe consistent improvements in gain efficiency. However, it is important to note that ileal starch digestibility was not measured for this experiment.

Corn DDGS are often included in swine diets due to their favorable economics, high availability, and moderate amino acid profile, but in some cases has been reported to decrease growth performance (Whitney et al., 2006; Lerner et al., 2020). Inclusion level of DDGS required to observe these effects vary and may be attributed to source and quality of the ingredient (Stein and Shurson, 2009; Urriola et al., 2010). In this experiment, pigs consuming 25% dietary DDGS exhibited decreased ADG and G:F, and thus tended to have lower HCW. Diets with DDGS contained greater crude fiber content, which has been demonstrated to decrease nutrient digestibility and subsequently growth performance (Fu et al., 2004; Weimer et al., 2008). The marginally lower back fat and percentage lean may be attributed to the increased fiber and lower energy intake of pigs consuming DDGS, although the observed greater loin depth remains unclear.

Carcass fat IV is used in commercial pork processing plants to characterize the proportion of unsaturated fat, and thus carcass firmness. Corn DDGS contain a high proportion of unsaturated fatty acids, leading to increased deposition of unsaturated fatty acids in adipose tissue (Madsen et al., 1992). Consistent with some previous research, pigs consuming 25% DDGS exhibited greater carcass fat IV, indicating a higher concentration of unsaturated fatty acids and presumably less fat firmness (Hill et al., 2008; Lineen et al., 2008; Graham et al., 2014; Lerner et al., 2020). Unexpectedly, a marginally greater carcass IV was observed for pigs

consuming EFC with no DDGS compared to pigs consuming CONV with no DDGS; the reasons for this observation remain unclear.

In summary, the results of this trial suggest that EFC can be fed to pigs without observing deleterious effects on growth performance, as no evidence of differences in gain efficiency and carcass characteristics were observed between corn sources. Consistent with previous research, the addition of DDGS decreased growth performance and most carcass measurements. Further research should be conducted to understand if addition of EFC to swine diets could be beneficial in younger pigs exhibiting decreased pancreatic α-amylase secretion following weaning, or whether heat treatment of diets, such as pelleting, may influence the response to EFC. Furthermore, data regarding endogenous α-amylase secretion and starch digestibility in pigs fed EFC are needed in order to further evaluate the efficacy of this product.

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Tables

Table 4.1 Diet composition (as fed basis)[1]

	0% DDGS[2]			25% DDGS		
Item	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Ingredient, %						
Corn[3]	75.45	81.90	85.25	59.95	65.65	68.20
Soybean meal, 46% crude protein	21.80	15.65	12.35	12.30	6.95	4.50
Corn DDGS, 7.5% oil	---	---	---	25	25	25
Calcium carbonate	0.93	0.85	0.85	1.08	1.03	1.03
Monocalcium P (21% P)	0.55	0.40	0.35	0.20	---	---
Salt	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.30	0.30	0.30	0.50	0.48	0.45
DL-Methionine	0.07	0.03	0.02	0.02	---	---
L-Threonine	0.09	0.10	0.11	0.09	0.09	0.09
L-Tryptophan	0.01	0.02	0.02	0.04	0.04	0.04
Trace mineral premix	0.15	0.13	0.10	0.15	0.13	0.10
Vitamin premix	0.15	0.13	0.10	0.15	0.13	0.10
Phytase[4]	0.02	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100	100
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lysine	0.95	0.80	0.72	0.95	0.80	0.72
Isoleucine:lysine	62	61	60	60	60	61
Leucine:lysine	139	148	154	165	181	193
Methionine:lysine	32	31	30	31	32	34
Methionine and cysteine:lysine	58	58	58	58	62	65
Threonine:lysine	63	65	68	63	65	68
Tryptophan:lysine	18.6	18.5	18.8	18.5	18.3	18.4
Valine:lysine	69	70	70	72	75	78
Histidine:lysine	42	43	43	43	44	46
Total lysine, %	1.07	0.90	0.82	1.11	0.94	0.86
Net energy, Mcal/kg	2.48	2.52	2.55	2.47	2.51	2.53
SID lysine:NE, g/Mcal	3.82	3.16	2.82	3.83	3.18	2.84
Crude protein, %	17.0	14.6	13.3	18.4	16.3	15.3
Calcium, %	0.59	0.51	0.48	0.59	0.51	0.49
Phosphorus, %	0.47	0.41	0.38	0.47	0.40	0.39
STTD[5] P, %	0.33	0.28	0.26	0.33	0.28	0.27

[1]The experimental diets were fed in 3 phases: d 0 to 29, d 29 to 47, and d 47 to 82.

[2]DDGS = distillers dried grains with solubles; one lot used for entire experiment.

[3]Enogen® Feed Corn (Syngenta Seeds, LLC, Downers Grove, IL) or conventional (yellow dent corn); one lot of each variety used for entire experiment.

[4]HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ) providing 891 phytase units (FTU)/kg and an estimated release of 0.08% available P.

[5]STTD P= standardized total tract digestible phosphorus.

Table 4.2 Chemical analysis of corn varieties (as fed basis)[1]

Item, %	CONV[2]	EFC[3]
Dry matter	87.1	87.4
Starch	60.6	60.8
Crude protein	7.2	7.9
Ether extract	3.1	3.1
Crude fiber	1.7	1.4
Ca	0.05	0.06
P	0.20	0.22

[1]Corn samples were collected at time of feed manufacturing and pooled for analysis (Ward Laboratories, Inc., Kearney, NE).
[2]CONV = Conventional yellow dent corn, one lot used for entire experiment.
[3]EFC = Enogen® Feed Corn (Syngenta Seeds, LLC, Downers Grove, IL), one lot used for entire experiment.

Table 4.3 Chemical analysis of experimental diets (as fed basis)[1]

	Phase 1[2]				Phase 2				Phase 3			
	CONV[3]		EFC[4]		CONV		EFC		CONV		EFC	
Item, % DDGS,[5] %:	0	25	0	25	0	25	0	25	0	25	0	25
Dry matter	88.6	88.9	88.1	88.1	87.8	88.3	87.6	88.4	89.2	89.5	86.9	88.2
Starch	48.4	37.5	44.1	36.3	48.3	38.4	46.5	39.0	46.5	41.5	53.1	39.9
Crude protein	16.2	18.9	16.3	18.6	13.4	16.6	14.2	16.5	13.6	15.7	11.3	15.8
Ether extract	2.3	4.3	2.6	4.1	3.0	4.6	3.4	4.4	3.3	4.4	1.8	4.3
Crude fiber	1.4	3.7	2.2	3.4	2.2	3.4	2.2	3.6	2.9	3.6	1.7	3.5
Ca	0.59	0.68	0.78	0.63	0.64	0.60	0.54	0.53	0.55	0.47	0.61	0.64
P	0.38	0.46	0.43	0.44	0.36	0.44	0.39	0.39	0.36	0.39	0.33	0.40

[1]Feed samples were collected approximately 2 days after each feed delivery, pooled within treatment for each phase, and analyzed. (Ward Laboratories, Inc., Kearney, NE).
[2]The experimental diets were fed in 3 phases: d 0 to 29, d 29 to 47, and d 47 to 82.
[3]CONV = Conventional yellow dent corn; one lot used for entirety of experiment.
[4]EFC = Enogen® Feed Corn (Syngenta Seeds, LLC, Downers Grove, IL); one lot used for entirety of experiment.
[5]DDGS = distillers dried grains with solubles; one lot used for entirety of experiment.

Table 4.4 Effects of corn variety and dried distillers grains with solubles (DDGS) on growth performance and carcass characteristics of finishing pigs[1]

	CONV[2]		EFC[3]			Probability, P =		
Item DDGS, %:	0	25	0	25	SEM	Corn	DDGS	Corn × DDGS
Body weight, kg								
Day 0	41.6	41.5	41.6	41.5	0.682	1.000	0.887	0.924
Day 29	71.2^{ab}	71.3^{ab}	71.8^{a}	70.4^{b}	0.891	0.632	0.071	0.036
Day 47	90.2	89.4	90.6	88.9	0.918	0.956	0.020	0.403
Day 82	130.4	128.6	130.3	129.9	0.873	0.353	0.097	0.305
Phase 1 (days 0 to 29)[4]								
ADG, [5] kg/d	1.02^{ab}	1.03^{ab}	1.04^{a}	0.99^{b}	0.011	0.580	0.045	0.019
ADFI, [5] kg/d	2.30^{a}	2.38^{b}	2.40^{b}	2.34^{ab}	0.043	0.434	0.879	0.035
G/F[5]	0.44	0.43	0.43	0.42	0.015	0.224	0.092	0.664
Phase 2 (days 29 to 47)								
ADG, kg/d	1.06	1.00	1.07	1.03	0.014	0.204	0.005	0.571
ADFI, kg/d	3.00	2.96	3.04	2.96	0.049	0.606	0.151	0.652
G/F	0.35	0.34	0.35	0.35	0.025	0.527	0.206	0.385
Phase 3 (days 47 to 82)								
ADG, kg/d	1.15^{ab}	1.10^{a}	1.14^{ab}	1.17^{b}	0.018	0.077	0.541	0.041
ADFI, kg/d	3.61^{x}	3.52^{y}	3.54^{y}	3.71^{z}	0.073	0.430	0.566	0.081
G/F	0.32	0.31	0.32	0.32	0.031	0.547	0.277	0.971
Overall (days 0 to 82)								
ADG, kg/d	1.08	1.05	1.09	1.08	0.009	0.089	0.026	0.304
ADFI, kg/d	3.01	3.00	3.02	3.06	0.440	0.328	0.760	0.444
G/F	0.359	0.350	0.361	0.353	0.018	0.814	0.047	0.999
Carcass characteristics								
HCW,[6] kg	97.9	95.9	97.4	96.6	0.080	0.883	0.071	0.448
Carcass yield, %	75.1	74.6	74.8	74.4	0.230	0.651	0.139	0.863
Backfat,[7] mm	16.26	15.49	16.26	16.00	0.279	0.196	0.050	0.448
Loin depth,[7] mm	64.26	65.02	63.75	65.28	0.584	0.767	0.026	0.420
Lean,[7] %	54.37	54.80	54.24	54.65	0.160	0.400	0.020	0.951
Iodine value,[8] mg/g	64.41^{x}	72.48^{y}	66.12^{z}	71.78^{y}	0.600	0.400	<0.0001	0.053

^{ab}Means within the same row without a common superscript are different ($P \leq 0.05$).

^{xy}Means within the same row without a common superscript are different ($0.05 < P \leq 0.10$).

[1]A total of 288 pigs (DNA Line 600 × 241; initially 41.6 ± 1.9 kg) were enrolled in an 82-d trial. There were 9 pens per treatment with 4 barrows and 4 gilts per pen.

[2]CONV = Conventional yellow dent corn.

[3]EFC = Enogen® Feed Corn (Syngenta Seeds, LLC, Downers Grove, IL).

[4]The experimental diets were fed in 3 phases: d 0 to 29, d 29 to 47, and d 47 to 82.

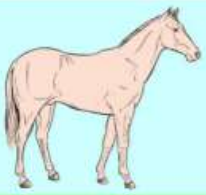
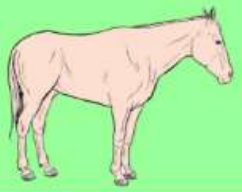
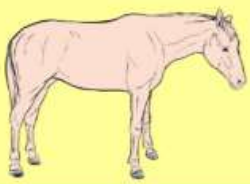
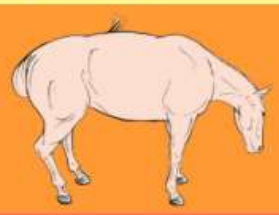
[5]ADG = average daily gain. ADFI = average daily feed intake. G/F = gain efficiency.

[6]HCW = hot carcass weight.

[7]HCW used as a covariate in statistical analysis.

[8] Belly fat samples anterior to the manubrium were obtained prior to carcass chilling and analyzed for fat iodine value (IV) using near-infrared spectroscopy.

Appendix A - Equine Comfort Assessment Scale

Pain Score		Behavior	Clinical Assessment	Postural Features
0		❑ Responds with interest to gate opening, approach by observer ❑ Takes care in movements around people ❑ Head above withers ❑ Attentive ❑ Moving freely, calmly ❑ Resting comfortably	❑ HR:_____ (usually ≤ 40 bpm) ❑ Eyes: relaxed, normally responsive ❑ Normal muscle tension ❑ No focal areas of heat ❑ Palpation not aversive	❑ No lameness perceptible, bears weight equally ❑ Moves with ease of stride
1		❑ Head at or above withers ❑ Facing forward and watching ❑ Performs normal behaviors less frequently than expected ❑ Responds with quiet interest to gate opening, approach by observer ❑ Takes care in movements around people	❑ HR: _____ (may be ≤ 40 bpm) ❑ Mild muscle tension ❑ Mild focal areas of heat ❑ Slightly steps, leans or pulls away from palpation, +/- muscle twitching	❑ Lameness difficult to observe, inconsistently apparent ❑ Mild injury or stiffness in movement
2		❑ Head level with withers ❑ Moving slowly about with bedding undisturbed ❑ Mild but more frequent restlessness ❑ Responds to approach ❑ Less enthusiastic, less interested, less interactive ❑ Less careful about movements around people	❑ HR:_____(may be ≥ 48 bpm) ❑ Tachypnea +/- RR:_____ ❑ Moderate muscle tension ❑ Increasing areas of heat ❑ Palpation more aversive	❑ Lameness apparent only under certain circumstances, favors leg(s) occasionally ❑ Obvious stiffness in movement
3		❑ Head level or below withers ❑ May face back or corner of stall ❑ More vigorous signs of restlessness ❑ Eyes distracted, far away, weary ❑ Minimally reacts to interaction ❑ Stands in one position ❑ Beginning to become internalized ❑ Less careful about movements around people	❑ HR:_____(may be ≥ 60 bpm) ❑ Tachypnea +/- RR:_____ ❑ Sweating ❑ Severe muscle tension ❑ Widespread areas of heat ❑ Vigorously aversive response to palpation	❑ Moderate lameness, able to bear weight but clearly favors one or more limbs ❑ Obvious discomfort, weight shifting ❑ Arched back ❑ Very stiff movements ❑ Abnormal standing posture
4		❑ Head often below withers ❑ Stands in corner or faces wall ❑ Ears back, eyes weary ❑ Frequent signs of severe agitation ❑ Extremely uncomfortable, panicky OR ❑ Extremely internalized/withdrawn ❑ Unwilling to rise ❑ Careless about movements around people	❑ HR:_____(may be ≥ 70 bpm) ❑ Tachypnea +/- RR:_____ ❑ Profuse sweating ❑ Extreme muscle tension / rigidity +/- fasciculation ❑ Widespread areas of heat ❑ Extremely aversive response to palpation, possibly aggressive	❑ Unable or unwilling to bear weight ❑ May not be able to move ❑ Constant shifting of weight ❑ Very abnormal standing posture OR ❑ In sternal or lateral recumbency

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