EFFECT OF ASPERGILLUS NIGER AND ORYZAE ON THE INTAKE AND DIGESTIBILITY OF COASTAL BERMUDAGRASS AND TIFFANY HAY IN HORSES

by

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ABSTRACT

The objective of this study was to compare voluntary dry matter intake and nutrient digestibility of Tiffany (T) and Coastal Bermudagrass (B) hays, and evaluate the effect of a functional feed additive containing a dried *Aspergillus Niger* and *Aspergillus Oryzae* fermentation product, MaxFiber (MF; Provida Supplements, Germany. Fecal samples were collected from 4 mature, stock type geldings in a 4 x 4 Latin Square design experiment with horses receiving all 4 treatments in a 2 x 2 factorial arrangement: B, T, B plus 10 g of MF (B+MF), and T plus 10 g MF (T+MF). Each period consisted of a 3-d acclimation, followed by a 17-d voluntary intake phase, and ending with a 72-h total fecal collection. Samples were analyzed for ash adjusted neutral detergent fiber (aNDFom; Dairyland Laboratories, Inc. Arcadia, WI) and proximate analysis (Servi-Tech Laboratories, Amarillo, TX). Data was analyzed using the Mixed Procedure of SAS v. 9.4 (SAS Institute, Inc., Cary, NC), with horse, period, hay, supplement, and hay by supplement interaction as main effects. Least Squares Means were compared using PDIFF in SAS to compare treatment mean with significance declared at $P < 0.05$ and trends declared at $P < 0.10$.

The overall apparent dry matter (DM) digestibility of T (53.2%) was greater ($P < 0.01$) than that of B (35.9%) regardless of the supplement inclusion. There was an effect of hay ($P < 0.001$) on overall mean apparent CP digestibility with T (75.9%) being greater ($P < 0.001$) than B (45.7%) regardless of supplement. There was a significant effect hay ($P = 0.01$) on overall mean aNDFom. Digestibility of aNDFom was greater ($P < 0.01$) for T (54.0%) compared to B (41.0%). There was an effect of hay ($P < 0.01$) on overall acid detergent fiber (ADF) digestibility. Overall ADF digestibility was greater ($P < 0.01$) for T (46.0%) compared to B (30.2%). There was an effect of hay ($P < 0.001$) on VDMI, with intake levels of T (10.9 kg/d) greater ($P < 0.001$) than intakes of B (8.5 kg/d). These results indicate that there were no significant differences in DM and nutrient digestibilities or VDMI for MF-treated horses, however, Tiffany hay had greater VDMI, DM, CP, NDF, and ADF digestibility.

Key Words: Coastal Bermudagrass, Tiffany, Functional Feed Additive

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CHAPTER I

INTRODUCTION

Forages represent a majority of the diets for large numbers of domesticated horses in the United States (NRC, 2007). There are limited amounts of published data regarding voluntary intake and digestibility of Tiffany (T) grass hay, commonly referred to as "Teff" hay, in horses. McCown et al. (2011) studied palatability of Teff hay when offered alongside Alfalfa and Timothy in horses. The authors reported that horses unfamiliar with Teff hay preferred Alfalfa or Timothy hays. Staniar et al. (2010) analyzed the digestibility of Teff hay at different stages of maturity (boot, early-heading, and late-heading); however, there is minimal information on comparisons of digestibility and intake of Teff compared to other hays commonly fed to horses.

Dried *Aspergillus Niger* (*A. Niger*) and *Aspergillus Oryzae* (*A. Oryzae*) fermentation products have proven effective in other species to aid in digestibility of forages and concentrates. In dairy cattle, using *A. Oryzae* extract increased digestibility and milk production (Wiedmeier et al., 1987). Furthermore, extract from the fungus, *A. Niger*, has been researched extensively in pigs as a digestibility aid and growth performance enhancer. In 2015, Shi et al. observed that *A. Niger* fermented-rapeseed meal when fed to pigs had greater nutritional value than unfermented rape seed. Despite widespread availability and use, scientific peer-reviewed evidence supporting commercial probiotic formulators in horses is limited (Schoster, 2014). Numerous *in* *vitro* studies evaluated probiotics and their possible benefits in the horses; however, the benefits observed have been difficult to confirm *in vivo*. The purpose of the present study was to determine: (1) Compare voluntary dry matter intake (VDMI) and nutrient digestibility of T and B hay in horses, and (2) evaluate the effect of the functional feed additive containing a dried *A. Niger* and *A. Oryzae* fermentation product on VDMI and nutrient digestibility of horses consuming an all-forage diet consisting of T and B hay.

CHAPTER II

REVIEW OF LITERATURE

Forage Characteristics

Forages represent a significant portion, if not all, of the diet for all classes of domesticated horses. A variety of systems exist for describing and classifying forages and their quality (Van Soest, 1982). There is a considerable amount of research on the differences in quality and digestibility of the most commonly fed forages (Sturgeon, 1999). Forage grass hays are divided into two main categories: cool-season and warm-season grasses. Warm-season grasses mature more rapidly than cool-season grasses and tend to have a greater proportion of cell wall and be more highly lignified (NRC, 2007). Depending on stages of maturity, forages consist of leaf, sheath, stem, flowers, and seed heads (Wells, 2015). Each of these plant parts differ in chemical composition and may change substantially during a growing season (NRC, 2007).

Horses are classified as selective grazers, adapted to consume the most immature forage available (NRC, 2007). Forages that are immature have a greater leaf to stem ratio, are higher in protein, water, and minerals, while being lower in crude fiber (Wells, 2015). As plants mature, leaf growth declines, stems elongate, reproductive structures develop, and the cell content to cell wall ratio decreases, ultimately, the readily digestible nutrients of the plant are affected (Sturgeon, 1999; Wells, 2015).

Forage Quality

Forage quality is usually a broad term that includes nutritive value and forage intake (Newman et al., 2006). Descriptions relative to forage quality are not yet wholly satisfactory, especially for horses, and the development and refinement of the descriptive systems is continuing (Van Soest, 1982). Forage nutritive value typically refers to concentration of available energy (total digestible nutrients, TDN) and concentration of crude protein (CP; Newman et al., 2006). Hays of high nutritive value are characterized by a large proportion of leaf, and high protein with low neutral detergent fiber (NDF) contents (NRC, 2007). As plants age, they generally decline in nutritive value as a result of increased lignification and a decreased proportion of leaves to stems (Van Soest, 1982). Concerning forage quality, the amount of dietary fiber a forage contains is one agreed upon factor affecting a forage's classification as high or low quality (Van Soest, 1982). The ultimate test of quality of a forage is animal performance (Newman et al., 2006).

When conserving forages, the feed quality can be no greater than the original sward, thus the principal factors responsible for declining forage nutritive value are stage of maturity at the time of harvest and weathered conditions (Newman et al., 2006; NRC, 2007). Young leafy plants have the highest feed quality, but the lowest yields. As the amount of leaf tissue (the most digestible component of a plant) decreases and the stem tissue increases, forage quality decreases. As the plant ages, stem growth develops as well as deposition of fibrous components (Newman et al., 2006). These stems contain most of the lignin that is considered mostly indigestible by microbes in the hind gut or any mammalian enzymes.

Fiber Definitions

Fiber is a unique and complex entity and has multiple definitions. Simply put, fiber can be defined as the indigestible or slowly fermenting components of feeds, and in livestock is associated with the cell wall components (Jung, 1997; NRC, 2007). Chemically, fiber is a mixture of cellulose, hemicelluloses, some pectins, and lignin (Mertens, 1992; NRC, 2007). Nutritionally, Mertens (1997) defines fiber as "the slowly digestible or indigestible fraction of feeds that occupies space in the gastrointestinal tract of animals." Mammals do not possess the enzymes to hydrolyze the predominant β1-4 linked polysaccharides that occur in cell walls and depend on microorganisms in the gastrointestinal tract to ferment these polysaccharides to absorbable nutrients (Jung, 1997). Differences in amounts and physical properties of fiber can affect the utilization of the diet and the performance of the animal (Mertens, 1997).

All feed evaluation techniques attempt to identify the degree to which individual feedstuffs contribute to the nutritional requirement of the animal (Mould, 2003). Analysis of feed for livestock usually revolves around ratios of indigestible and digestible fiber and can be analyzed as crude fiber (CF), total dietary fiber (TDF), NDF, and acid detergent fiber (ADF; NRC, 2007).

Fiber Fractions

Forages contain structural carbohydrates, originating from the plant's cell walls, and non-structural carbohydrates from the cell contents (NRC, 2007). Carbohydrates are the principal source of energy for horses and originate from forages, concentrates, and grain by-products (NRC, 2007). Non-structural carbohydrates are readily absorbed by the animal, while digestion of structural carbohydrates require microbial fermentation to hydrolyze cellulose and hemicellulose (Van Soest, 1967). The non-structural carbohydrates in plants include simple sugars, glucose, fructose, sucrose, starch, and fructans (NRC, 2007). Structural carbohydrates are a result of the biological functions of the cell wall, such as, preventing disease, pathogens, and protection from predators of the plant. These structural carbohydrates have resulted in a structure that is of variable and often low digestibility by ruminants (Mertens, 1997). Digestibility of structural carbohydrates begins with fermentation by the microflora in the horse's hind gut where the cell wall carbohydrates are fermented by the microbes and converted to volatile fatty acids and then metabolized by the animal's cells to yield ATP (NRC, 2007).

Hemicellulose. Hemicellulose is a polysaccharide that contains various sugars and glycosidic linkages, specifically polymers of arabinose, xylose glucose, fructose, mannose, and galactose (Van Soest, 1994; NRC, 2007). Hemicellulose is a heterogeneous collection of polysaccharides and varies greatly from one plant species to another (Van Soest, 1994). Hemicellulose represents native insoluble polysaccharides and is usually associated with Lignin (Van Soest, 1994). Hemicellulose and lignin together form the encrusting material of the secondary wall thickening that occurs as a plant ages (Van Soest, 1994). Non-ruminants digest more hemicellulose than cellulose due to the hindgut microflora more readily digesting the pectins and polymers of arabinose, galactose, and mannose (Van Soest, 1994; NRC 2007).

Cellulose. The most abundant carbohydrate in the world is cellulose, with dry matter (DM) amounts of 20 to 40% in all plants (Van Soest, 1994). Concerning horses, cellulose and starch are the most common polysaccharides in their diets (NRC, 2007). Furthermore, polysaccharides are the largest and most complex category of carbohydrates in horse feeds (NRC, 2007). Cellulose contains β 1-4 linkages, similar to hemicellulose, and these linkages are only digestible by horses through microbial fermentation (NRC, 2007).

Lignin. Lignin is not a carbohydrate, but a generic term applied to a group of heterogenous compounds derived from the phenylpropanoid pathway (NRC, 2007). As a plant ages, the lignin content increases. This increased lignin content is highly correlated with decreasing degradability of the cell wall carbohydrates, thereby reducing the nutritive value of the forage (Hartley and Jones 1977). Lignin in plants is desirable for plant function as the structural component for strength and rigidity limits water loss and inhibits diseased organisms by reducing permeability of the cell wall (Moore and Jung, 2001). However, Lignin interferes with the digestion of cell-wall polysaccharides by acting as a physical barrier to microbial enzymes (Wells, 2015).

Coastal Bermudagrass Hay

Coastal Bermudagrass hay (*Cynodon dactylon L.*) is a popular forage for feeding equines that is grown in the southern United States. Historically, B hay has been blamed for ileal impaction colic. Observations in a study by Little and Blikslager (2002) concluded that B could contribute to colic, however, authors admitted that the influence of different qualities of B needed further investigation.

Sturgeon (1999) reported that horses consuming B hay had lower DM (51.60%) and CP (60.56%) digestibilities as compared to Alfalfa hay and Matua grass hays. This author also reported that the B had higher NDF values than the Alfalfa and Matua hays, which likely contributed to the results. Another study by Aiken et al. (1989) reported that when consuming B hay, digestibilities of CP and DM were similar in yearling (52.6%, 43.3%) and mature horses (50.7%, 43.0%), respectively. There was a difference in intake as percent of body weight, but this was due to the energy requirements of the yearlings needing to consume more to meet maintenance needs. LaCasha et al. (1999) observed that when yearling horses consumed B, Alfalfa, and Matua grass hays that the percentage NDF would have predicted the relative intake ranking of the 3 hays.

Tiffany Grass Hay

Tiffany (*Eragrostis tef)* hay is a warm season annual grass and well-adapted to drought conditions (Saylor et al., 2018). Tiffany has been reported to have similar composition to Timothy or Alfalfa hays at certain times of cutting (McCown et al., 2011). In fact, T can contain CP levels of 12 to 17%, depending upon maturity at harvest (Miller, 2009). Tiffany hay is an attractive crop to hay producers since this species can be grown in a variety of soils and climates while creating a large amount of product in a relatively short growing season (4 to 7 tons/acre; Miller, 2009). Analysis of T hay reveals high levels of several minerals, such as, Ca, P, Fe, Al, Ba, and the B-Vitamin thiamine. In a study conducted by Staniar et al. (2010), Cu and Zn requirements for mature horses were not met by any T hay maturities (late-heading, early-heading, and boot). However, when baled at boot or early heading maturities, the authors reported that approximately 97% of a mature horse's digestible energy requirements were met.

In a study conducted by McCown et al. (2011), horses unfamiliar with T hay preferred to consume Timothy and Alfalfa hays in a palatability test. In the same study, the authors reported no differences in intakes among the 3 hays. Authors of the study ultimately concluded horses unfamiliar with T will prefer other hays. Saylor (2018) compared productivity of lactating dairy cattle consuming 2 diets. One diet used T as the only source of forage, and the second was a control diet containing corn silage, Alfalfa hay, and prairie hay. The objective of this study was to analyze if the drought-tolerant T hay could aid the dairy industry in water preservation, while maintaining the same levels of productivity as the control diet. These authors reported no significant differences in dry matter intake (DMI), DM digestibility, and neutral detergent fiber digestibility (NDFD) between the 2 treatment diets. Probably most importantly, they reported no differences in milk yield averages due to the treatment diet (Saylor et al., 2018).

Equine Digestive Physiology

Horses are non-ruminant herbivores that have a digestive tract designed to degrade large amounts of forage in a continuous manner over long periods of time (Hussein and Vogedes, 2003). In the small intestine, horses possess the enzymes capable of hydrolyzing α1-6 and α1-4 linkages of starch and maltose. They do not, however, have enzymes to digest the β1-4 linkages found in cellulose or the mixed linkages in hemicellulose, thus the necessity of microbial fermentation (NRC, 2007). During and immediately after birth, the intestinal tract is colonized by a succession of bacteria (Callaway and Ricke, 2012). These bacteria are housed in the large intestine of the horse, where as much as 70 L of undigested food along with the billions of bacteria and protozoa, produce enzymes to break down (ferment) otherwise undigestible plant fiber (Hussein and Vogedes, 2003; Pagan, 2009). Without microbe-produced enzymes, horses would not be able to digest fiber or have a source of energy or micronutrients (Pagan, 2009). Fiber digestion requires 2 events: hydrolysis of polysaccharides; and the conversion of monosaccharides into volatile fatty acids, fermentation gases, and heat

(Van Soest, 1982). Ultimately the fermentations of the volatile fatty acids provide about 30% of the energy a horse needs for maintenance (Hussein and Vogedes, 2003; NRC, 2007).

The key to equine digestion health is linked to the intestinal microbiota in the equine hind gut (Garber et al., 2020). Recent research studies have been centered around the ideal microbial community that allows for optimal equine health. One common theme among this microbial research is analyzing diversity of the microbe species in the hindgut, since increased diversity is more resilient to external stresses (Garber et al., 2020).

A study conducted by Metcalf et al. (2017) reported that domestication of the horse has affected the diversity of the microbes in horses. Domestication is associated with modifications to a species' movement, feeding, protection, environment, and breeding. Researchers compared wild Przewalski horses from Mongolia to domesticated horses. They reported that the Przewalskis had a more diverse microbiome compared to the domesticated horses.

Not surprisingly, diet can also affect hind gut microbe diversity. A study by Hansen et al. (2015), reported horses consuming forage that was supplemented with oats had lower microbe diversity in the hind gut compared to horses consuming an all forage diet. Researchers concluded that higher nutrient availability in a feed leads to a lower microbial diversity. Willing et al. (2009), supported this conclusion when observing racehorses in training consuming either an all-forage diet, or a combination concentrate and forage diet. The horses consuming the all forage diet had lower counts of bacteria in the feces that have been associated with laminitis, thus a more stable microbial population.

Fiber Analysis

The objective of laboratory feed analysis is to evaluate nutritional properties of individual feedstuffs. In turn this can estimate an animal's response to certain dietary inputs, and aid in quality control of preserved forages (Van Soest,1967; Van Soest and Robertson, 1979). Forages are typically characterized by their dietary fiber content, which is largely composed of energy-yielding structural carbohydrates and non-structural carbohydrates (NRC, 2007). To evaluate quality of fiber, Van Soest (1963) introduced the use of detergents in feed analysis and the methods are still used today. Even though these methods were originally developed for ruminants, they have since been adapted and applied to several other species of animals.

Fiber analysis systems have gone through many changes over time. In the mid-1800's, animal feeders became concerned with correlating feed quality and quantity with measurements of pound of milk, meat, and various other animal products (NRC, 2007). One of the first systems developed to analyze fiber was the Weende system, or proximate analysis. This system was devised to separate the carbohydrate fraction of animal feedstuff into the less-digestible CF and more readily digested nitrogen-free extract. Unfortunately, there was dissatisfaction with the CF methods and nitrogen-free extract calculations, ultimately leading to the conclusion that the proximate analysis system failed to distinguish between cell wall and cell contents (Van Soest, 1967; Burns, 2011). In the 1960's, Van Soest developed the Detergent Fiber Analysis system with the objective to fraction foods of plant origin into their nutritive availability and fiber content

(Van Soest, 1979). This new system utilized a neutral detergent solution that separated the cell wall from the readily digested cell contents, leaving the insoluble residue termed NDF (Burns, 2011). Additional treatment of the residue, or NDF, by acid detergent resulted in further degradation of the cell wall leaving an insoluble residue, ADF (Burns, 2011). Even though the detergent fiber system quickly replaced the Weende system, 2002 was when Van Soest's methods were officially approved by the Association of Official Analytical Chemists (AOAC; Uden et al., 2005).

Neutral Detergent Fiber. The NDF system was developed to measure the cell wall components of a forage (Van Soest, 1979). For routine feed analysis, the residue remaining after NDF is regarded as being representative of the total cell wall constituents of forages, i.e. the sum of lignin, cellulose, and most of the hemicellulose fractions (NRC, 2007). The NDF system has been shown to establish feed value, however, this does not indicate digestibility accurately in ruminants (Van Soest, 1979). Higher NDF values indicate lower feed quality for ruminants, however, researchers are still unclear how this applies to horses (Hansen and Lawrence, 2017). These authors used simple linear regression on data from 26 in-vivo digestibility trials and reported that the nutrients with the strongest correlation with DM digestibility were NDF ($r^2 = 0.6017$) and CP ($r^2 = 0.5402$; Hansen and Lawrence, 2017).

Acid Detergent Fiber. Residues from the NDF system leave truly indigestible fiber, while the ADF system divides this left-over fiber further into soluble and insoluble fractions (Van Soest, 1979). NDF has traditionally been used as a predictor of forage intake, while ADF has been used as a predictor of forage digestibility (Newman et al., 2006). The ADF system recovers cellulose and the least digestible noncarbohydrate fractions (lignin and pectin) and is generally used as a quick method for determining fiber in feeds (Van Soest, 1979). Higher ADF values are associated with decreased digestibility, thus a low ADF is desired (Newman et al., 2006). However, Van Soest (1994) admits ADF is only related to digestibility on a statistical level in ruminants.

Voluntary Dry Matter Intake

In equines, VDMI is likely controlled by several interacting factors, but usually intakes of all-forage diets average between 2 to 2.4% of BW for grass and alfalfa hays respectively (NRC, 2007). Edouard et al. (2008) stated that equines are adapted for high fiber, low-quality forages; however, this did not account for the variance in intake. The author also went on to state that a horse's response to a lower feed quality is to increase intake to maintain rates of energy and nutrient absorption. St. Lawrence et al. (2001) reported a relationship ($r^2 = 0.67$) between NDF and intake of cool-season grasses, while Reinowski and Coleman (2003) reported a similar correlation of VDMI and the NDF of warm-season grasses. Staniar et al. (2010) reported that when consuming T hay, horses consumed less late-heading as compared to boot or early heading, indicating a preference for the more palatable, immature T grass.

In ruminant nutrition, the objective is to achieve maximum intake of forage together with the minimum allowance of concentrates required so that the whole diet meets the animal's requirements (Jarriage et al., 1986). Jarriage et al. (1986) and Allen (1996) both stated that VDMI of an animal depends on the forage ingestibility and can be limited by restricted flow of digesta through the gastrointestinal tract. In an attempt to measure and predict VDMI in ruminants, Jarriage et al. (1986) suggested applying a Fill

Unit (FU) to feeds, where 1 kg of DM has a certain “fill value” that equals a certain number of fill units, however, limited research has been conducted on this concept.

Over the years horses have been artificially selected for performance as saddle horses, not as production animals where rate of gain is of high importance (Edouard et al., 2008). A possible by-product of this selection is the individual variability of intakes and responses to different diets. Edouard et al. (2008) observed 21 saddle horses that were fed ad libitum, 45 different forages over the course of the study. Researchers reported that average DM digestibility decreased significantly with lower quality forage, lower quality referencing a decrease in CP and an increase in NDF. They did not find a significant relationship between DMI to DM digestibility and CP; they did however find a significant relationship between a decrease in DMI as NDF increased.

Functional Feed Additives

A food is functional if the food contains a component that affects one or a limited number of functions in the body, to have a positive effect on health, and productivity of animals worldwide (Dey and De, 2013). In general, for animal production the goal of functional feed additives is to enhance performance of the animal and decrease risk to the humans consuming the animal or the product produced. In horses, the goal of most functional feed additives is to decrease gastrointestinal tract diseases, such as colic (Chaucheyras-Durand and Durand, 2008). Functional feed additives include probiotics, prebiotics, enzymes, and synbiotics (Hoseinifar et al., 2017).

Probiotics. According to the United States Food and Drug Administration (1995), a probiotic is a “product that is purported to contain live (viable) microorganisms (bacteria and/or yeast)”. Probiotics are also commonly referred to as direct-fed microbials. Organisms classified in this category occur naturally in the gut of a healthy animal and are not genetically engineered (Switzer, 2003). Perhaps a better definition for the use of probiotics concerning nutrition would be, “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989). The definitions of probiotics are very broad and may include specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Elghandour et al., 2015). Most commercially available probiotics for equines are non-spore forming lactic acid bacteria (Weese, 2002). The research on uses of probiotics has been centered on species of animals in food production as an alternative to prevent disease and enhance overall animal health and production. This attention has been largely due to the ban on antibiotics in feed additives in several countries (Callaway and Ricke 2012; Chichlowski et al., 2007; Elghandour et al., 2015).

Probiotics have multiple modes of action, some produce nutrients and growth factors, such as organic acids, which are stimulatory to beneficial microorganisms in the gut microbiota and create favorable ecological conditions (Chaucheyras-Durand and Durand, 2008; Servin, 2004). Still, other probiotics limit pathogenic bacteria by “competitive exclusion,” meaning probiotics can have a higher affinity for nutrients and adhesion sites than the pathogenic bacteria (Chaucheyras-Durand and Durand, 2008; La Ragione et al., 2004). Some modes of action are still not entirely understood, and efficacy is still being researched.

Concerning ruminants, Elghandour et al. (2015) studied the use of fungal probiotics in cattle and reported that they may reduce harmful oxygen from the rumen, prevent excess lactate production, increase feed digestibility, and alter rumen fermentation patterns. The authors also reported probiotics may also compete with and inhibit the growth of pathogens, boost immune system modulation, and modulate microbial balance in the gastrointestinal tract. Concerning dairy cattle, improved dry matter intake, milk yield, fat corrected milk yield, and milk fat content were reported with probiotic administration (Elghandour, 2015). However, the literature reports conflicting results, and the *in vivo* response to probiotics is not consistent and often is affected by numerous variables (i.e. feeding time, dosages, and strains of the direct-fed microbial; Elghandour, 2015).

In equines, probiotic distribution has been particularly relevant in cases of stress or when consuming high concentrate diets (Chaucheyras-Durand and Durand, 2008). Probiotic use has been for a variety of reasons, and usually depends on the use of the horse. In gestating mares, the goal of probiotic use is to increase digestibility of nutrients while increasing milk quantity and quality. For foals, probiotics are targeted to aid in growth and limiting diarrhea. For racing horses, purposes of probiotic use is focused on increased digestibility, limiting stress from performance, and hindgut disorders (i.e. colic and laminitis; Chaucheyras-Durand and Durand, 2008).

Prebiotics. A prebiotic is defined by Weese (2002) as "a non-digestible food ingredient that beneficially affects the host by stimulating growth and/or activity of certain bacterial components of the intestinal microflora." Weese (2002) goes on to further state that prebiotics do not require viable microorganisms. McDaniel et al. (1993) observed how a prebiotic containing *A. Oryzae* effected in vitro equine cecal fermentation of soluble starch and amino acids/peptides of Coastal Bermudagrass and alfalfa hay. Researchers observed an increase in volatile fatty acid production, but no increase in digestibility of either forage.

Yeast. Yeast is a common and popular feed additive for livestock. Compounds currently being studied and showing success in efficacy are live yeast cultures derived from the strain *Saccharomyces cerevisiae* (Broadway et al., 2015). In horses, yeast has been evaluated for probiotic properties, however, most yeast supplements act as a nutritional supplement (Weese, 2002). In ruminants, this active dry yeast stabilizes ruminal pH and decreases the risk of acidosis (Chaucheyras and Durand et al., 2008). In horses, live yeast has increased fiber digestibility in the colon (Jouany et al., 2008) and balanced the hind gut microbe community (Medina et al., 2002), thus decreasing the risk of lactic acidosis. Jouany et al. (2008) reported horses consuming a live yeast supplement improved ADF digestibility, and stimulated DM and NDF intakes. Medina et al. (2002) reported horses consuming high starch diets supplemented with yeast had a significantly increased pH and decreased concentrations of lactic acid in the large intestine after feeding.

Aspergillus Niger. For decades this microorganism, *A. Niger,* has been used to produce extracellular (food) enzymes and citric acid (Schuster et al., 2002). This fungus has a wide range of uses, including as an antioxidant, preservative, acidulant, pH-regulator, and flavor additive in foods and beverages (Cairns et al., 2018). Between 1997 and 2017, useful application of enzymes from *A. Niger* was expanding. In 1991, *A. Niger* phytases were marketed to improve the nutritional content of animal feed by generating inorganic phosphorous from phytic acid (Cairns et al., 2018). Other Aspergilli along with *A. Niger* have been used together in the degradation of plant components, whereby cellulose, hemicellulose, and pectin can be broken down into oligosaccharides and monosaccharides (Cairns et al., 2018). Kemme et al. (1998a; 1998b), studied the addition of *A. Niger* and lactic acid to a maize-soybean meal-based diet in growing finishing pigs. They reported an increase in total tract P digestibility and ileal CP and amino acid digestibility (Kemme et al., 1998a; 1998b).

Aspergillus Oryzae. Well known for traditional use in Japanese fermentations industries, *A. Oryzae* has potential for the secretory production of various enzymes, amylases, proteases, and lipase (Machida et al., 2008). Feng et al. (2006) observed that soybean meal fermented with *A. Oryzae* fed to weanling pigs resulted in higher average daily gains and higher DM, CP, and energy digestibility. Gomez-Alarcon et al. (1990) observed that supplementing cattle diets with *A. Oryzae* resulted in increased ruminal NDF and ADF digestibility when animals were fed rations containing either 61 or 70% of concentrate. However, there was no effect reported when animals were fed high forage rations. Miranda et al. (1996) studied the effects *A. Oryzae* and *Saccharamyces cerevisiae* on Holstein heifers consuming diets with 2 different NDF levels (27 and 37%). They reported supplemented cattle had increased *in situ* alfalfa NDF digestion and propionate concentration at 48 h when consuming both levels of NDF (Miranda et al., 1996).

<u>Statement of Problem</u>

Limited scientific information exists on the VDMI and nutritional value of T hay as compared to other popular forage sources for horses. Furthermore, there is limited research on the effect of *A. Niger* and *A. Oryzae* on VDMI and nutrient digestibility of horses consuming an all-forage diet. Therefore, the purpose of the present study was to compare VDMI and nutrient digestibility of T and B hay in horses, and evaluate the effect of the functional feed additive containing a dried *A. Niger* and *A. Oryzae* fermentation product on VDMI and nutrient digestibility of horses consuming an all-forage diet consisting of T and B hay.

CHAPTER III

MATERIALS AND METHODS

Four mature, stock-type geldings, ranging from 7 to 20 yr of age were randomly assigned treatments within a 4 x 4 Latin Square experimental design, arranged as a 2 x 2 factorial. The objectives were to compare VDMI and nutrient digestibility of 2 varieties of hay as well as evaluate the effect of 10 g of a functional feed additive containing a fungal extract (*A. Niger* and *A. Oryzae)*, MaxFiber® (MF; Provida Supplements, Pinneberg, Germany) on digestibility in mature, lightly exercised horses consuming an all-hay diet. Horses were assigned to 1 of 4 dietary treatments: T; T plus additive (T + MF); B; and B plus additive (B + MF). The trial consisted of 4, 23-d experimental periods, which included a 3-d dietary adjustment period, followed by 20-d experimental period. A 72-h fecal collection period was performed during the last 3 d of the period.

Horse Care and Management

All routine care, housing, and sampling procedures applied to horses used in the study were conducted under the guidelines of the West Texas A&M University (WTAMU) Institutional Animal Care and Use Committee (IACUC #2019.07.001). Horses were housed individually in 6 x 20 m dry-lot pens at the WTAMU Horse Center, Canyon, TX. Horses were fed at 0700 and 1700 in feeding bunks at 2.0% BW/d initially (1% BW per feeding), and feed offered was adjusted so there was an ad libitum intake with minimal waste. Hay offered and orts were weighed on a scale (FB1100-2 Series, Fair Banks Scales, Kansas City, MO) and recorded daily. The MaxFiber® was mixed with approximately 75 g of molasses and fed to horses at 0700 in small stainless-steel bowls hung on the side of the stall door. During the 72-h collection period, total feces excreted were collected using a fecal collection harness (Bun-Bag, Sagle, Idaho; Fig 1). Feces excreted were collected about every 2 h or when a horse's fecal bag was full, and a representative sample was placed in a Ziploc freezer bag and frozen at -20ºC for later analysis.

Horses were weighed on a scale (FB1100-2 Series, Fair Banks Scales, Kansas City, MO) at 0600 on d 0 and before the start of each period. Body condition scores were assessed by trained personnel the week prior to the study and before the beginning of each period (Henneke et al., 1983). Water was available ad lib in automatic waterers in each pen. All horses in the study were classified as Moderately exercised (NRC 2007); ridden ever day or every other day in WTAM Equestrian Team practices and Horsemanship classes (NRC, 2007). Prior to the study, horses were dewormed and vaccinated, with hooves routinely trimmed every 6 to 8 wk throughout the trial.

Sample Collections

Hay Collection. Prior to the study, treatment hay samples were collected using a core sampler (Best Harvest, Largo, FL). Approximately 10 bales were chosen at random from each bundle of 31 bales. Core samples were collected and composited for each hay. Additional hay was purchased during periods 3 and 4 due to underestimation of intake, and bales purchased weighing less than expected. Collection bags were labeled by date, correlating collection period, and stored for later analysis. Samples were sent to

Dairyland Laboratories, Inc. (Arcadia, WI) for analysis of DM, CP, ADF, ash free NDF (aNDFom), and lignin.

Fecal Collections. Total fecal collections were conducted for 72 h during the last 3 d of each period. Pens were closed off with panels at 6 x 3 m. Fecal collection harnesses, "Bun-Bags" (Bun-Bag Horse Diapers, Sagle, ID), were attached to horses by 0700 the morning of day 1 of the collection period. Bags were monitored continuously during this time and were emptied as needed. As bags filled, total fecal output was collected and weighed (Smart Weigh Scales, Chestnut Ridge, NY) with a representative sample taken for later compositing and analysis. The representative sample of each collection was placed in a sealed Ziploc plastic bag, labeled with horse ID, period, date, and time. Each sample was stored in a freezer (Whirlpool, Benton Harbor, MI) at -10ºC.

Fecal and Hay Analysis

Fecal Sample Preparation and Dry Matter Determination. The DM procedure was adapted from the methods outlined by Van Soest and Goering (1970). The process was designed to allow for subsequent analysis of CF, lignin, and acid detergent insoluble nitrogen. Fecal and hay samples were thawed to room temperature for analysis. For DM determination, the weight of an aluminum pie pan was recorded (W1), then the fecal sample was placed in the pie pan and weight recorded (W2). All samples were dried for 72 h at 60°C in a forced air heating room located at the United States Department of Agriculture facility in Bushland, Texas. Samples were removed from the oven and weight recorded (W3). Percent DM was calculated as:

$$\% \text{ Total DM} = [(W3\text{-}W1/W2\text{-}W1)*100]$$

Samples were composited by Horse, Period, and Treatment by a percentage of dry weight of original feces voided represented by that sample. Composited samples were placed in individual plastic bags (WhirlPaks, Nasco, Fort Atkinson, WI), and shipped to Dairyland Laboratories, Inc. (Arcadia, WI) for aNDFom digestibility (DairyLand Laboratories, 2015). Composited samples were also sent to Servi-Tech Laboratories (Amarillo, TX) for proximate analysis. Crude protein analysis was performed according to the methods outlined by AOAC Official Method 990.03 (2002). Acid Detergent Fiber analysis was performed according to the methods outlined by the ANKOM Technology ADF Method 5 (2017).

Hay Sample Preparation and Analysis. Composited hay samples were sent to DairyLand Laboratories, Inc. (Arcadia, WI) to be dried and analyzed for DM (NFTA Method 2.1.4), CP (AOAC 990.03, 2002), aNDFom (DairyLand Laboratories, 2015), and ADF (AOAC 973.18, 2005).

Statistical Analysis

Data for DMIs and nutrient digestibilities were analyzed using the mixed procedure of SAS v. 9.4 (SAS Institute, Inc., Cary, NC), with horse, period, hay, supplement, and hay by supplement interaction as fixed effects. Least Squares Means were compared using PDIFF in SAS to compare treatment mean with significance declared at $P < 0.05$ and trends declared at $P < 0.10$.

CHAPTER IV

RESULTS AND DISCUSSION

Nutrient analysis of hays are shown in Tables 1 and 2. Horse BW averaged 506 ± 23 kg, and BCS scores averaged 5.6 ± 0.5 throughout the study. Overall mean apparent DM digestibility is shown in Table 3. There was an effect of hay ($P = 0.002$) on overall mean apparent DM digestibility. There was a trend for the effect of horse ($P = 0.06$) on overall mean apparent DM digestibility. Mean apparent DM digestibility was greater ($P = 0.002$) for T (53.2%) treatments compared to B (35.9%) treatments. There was no effect (P 0.73) of supplement on overall mean apparent DM digestibility. These data are similar to Staniar et al. (2010) who reported DM digestibility of late-heading T hay of 51.5% in 6 mature (12 ± 3 yr) Quarter Horse mares. These data are also empirically lower than those reported by Sturgeon (1999) who observed an average B apparent DM digestibility of 51.6% in 6 mature horses ranging from 10 to 14 yr of age. Additionally, Aiken et al. (1989) reported overall apparent DM digestibility of B at 43.0% in mature horses.

Crude protein requirements for adult, lightly exercised horses (699 g/CP/d; NRC, 2007) were met for all diets and all periods, except horse 4 in period 1 consuming B (CP DMI was 631 g/d), and horse 2 in period 2 consuming B+MF (CP DMI was 582 g/d).

Table 1. Nutrient analysis of Coastal Bermudagrass hay for each period (DM Basis).

Coastal Bermudagrass hay				
Item, Unit	Period 1	Period 2	Period 3	Period 4
DM, %	89.57	89.57	89.10	89.10
CP, %	7.63	7.63	9.15	9.15
ADF, %	40.74	40.74	36.98	36.98
NDF, %	74.08	74.08	69.54	69.54
Lignin, %	8.52	8.52	6.16	6.16
Ash, %	4.40	4.40	8.98	8.98
Fat, %	1.92	1.92	2.66	2.66
Ca, %	0.35	0.35	0.46	0.46
P, %	0.19	0.19	0.24	0.24

Table 2. Nutrient analysis of Tiffany hay for each period (DM Basis).

	Tiffany grass hay			
Item, Unit	Period 1	Period 2	Period 3	Period 4
DM, %	89.70	89.70	91.34	90.23
CP, %	15.75	15.75	17.58	13.83
ADF, %	37.43	37.43	31.03	35.03
NDF, %	61.49	61.49	53.39	58.96
Lignin, %	7.09	7.09	6.29	7.20
Ash, %	10.25	10.25	10.36	9.70
Fat, %	2.40	2.40	2.98	2.93
Ca, %	0.43	0.43	0.35	0.40
P, %	0.16	0.16	0.27	0.21

Table 3. Overall mean apparent dry matter digestibility (%) in mature horses consuming Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), and Coastal Bermudagrass hay plus functional feed additive (B+MF; $P = 0.01$)

	Treatments				SEM
Item, Unit	T	T+MF	B	B+MF	
DM Digestibility, DM, %	52.6[a]	53.8[a]	35.3[b]	36.5[b]	4.69

[a,b]Means in rows with differing superscripts differ at $P < 0.01$

These lowered intakes occurred when horses consumed B and B+MF treatments immediately after consuming the more palatable T hay in previous periods, thus leading to the deficiencies. There was an effect of hay on overall mean apparent CP digestibility ($P < 0.001$; Table 4). There was a trend for the effect of horse ($P = 0.08$) on overall mean apparent CP digestibility. Overall mean apparent CP digestibility was greater ($P < 0.001$) for T (75.9%) treatments compared to B (45.7%) treatments. There was no effect (P 0.59) of supplement on overall mean apparent CP digestibility. These data agree with Staniar et al. (2010) who reported CP digestibility of T hay (69.1%) at the boot stage of maturity. The NRC (2007) reports the average digestibility of CP in grass as 53% (range 20 to 74). Sturgeon (1999) reported an average apparent CP digestibility in mature horses consuming B as 60.56%, this is empirically lower compared to the average apparent DM digestibility of the B in this study. These data partially agree with Aiken et al. (1989) who reported an average apparent CP digestibility of B as 50.7% in mature horses.

Overall mean ADF digestibility is shown in Table 5. There was an effect of hay on overall mean ADF digestibility ($P < 0.01$), with trends for horse ($P = 0.06$) and period ($P = 0.09$) effects. Overall mean ADF digestibility was greater ($P < 0.01$) for T (46.0%) as compared to B (30.2%) treatments. There was no effect (P 0.84) of supplement on overall mean ADF digestibility. These data partially agree with Staniar et al. (2010) who reported average ADF digestibility of T hay (49.7%) at the late-heading stage. These data are in partial agreement with Aiken et al. (1989) who observed an average ADF digestibility of B hay as 35.7% in mature horses. These data are in partial agreement

Table 4. Overall mean apparent crude protein digestibility (%) in mature horses consuming Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), Coastal Bermudagrass hay plus functional feed additive (B+MF; $P < 0.01$)

	Treatments				SEM
Item, Unit	T	T+MF	B	B+MF	
CP Digestibility, DM, %	75.8[a]	76.0[a]	44.3[b]	47.1[b]	3.77

[a,b]Means in rows with differing superscripts differ at $P < 0.001$

Table 5. Overall mean acid detergent fiber digestibility (%) in mature horses consuming Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), Coastal Bermudagrass hay plus functional feed additive (B+MF; $P = 0.04$)

	Treatments				SEM
Item, Unit	T	T+MF	B	B+MF	
ADF Digestibility, DM, %	45.2[a]	46.7[a]	40.7[b]	30.3[b]	5.58

[a,b]Means in rows with differing superscripts differ at $P < 0.05$

with Aiken et al. (1989) who observed an average ADF digestibility of B hay as 35.7% in mature horses. These data also partially agree with Sturgeon (1999) who reported higher average ADF digestibility of B hay of 43.53%, the B hay in this study had lower ADF digestibility.

Overall effect of treatment on mean aNDFom digestibilities are shown in Table 6. There was a significant effect of horse ($P = 0.04$) and hay ($P = 0.01$) on digestibility of aNDFom. Digestibility of aNDFom was greater ($P = 0.01$) for T (54.0%) compared to B (41.0%). There was no effect (P 0.83) of supplement on overall mean aNDFom digestibility. These data partially agree with Staniar et al. (2010) who reported NDF digestibility of T hay (55.7%) at the early stage of maturity. These data are also in partial agreement with Aiken et al. (1989) who reported NDF digestibility in mature horses consuming B hay as 45.5%. These NDF digestibilities are empirically lower than Sturgeon's (1999) report of 51.69% for B hay in mature horses.

The effect of treatment on mean VDMI for horses consuming T, T+MF, B, and B+MF are shown in Table 7. There were significant effects of horse ($P < 0.01$), period ($P = 0.02$) and hay ($P < 0.001$) on VDMI. There was an effect of hay ($P < 0.001$) on VDMI, with intake levels of T (10.9 kg/d) greater ($P < 0.001$) than intakes of B (8.5 kg/d). There was no effect (P 0.79) of supplement on overall mean VDMI. A study by Aiken et al. (1989) reported 4 mature horses consumed B voluntarily at 10.0 kg/d on a DM basis. Staniar et al. (2010) reported VDMI's for horses consuming different maturities of T hay at 9.7 DMI, kg/d (boot maturity), 9.2 DMI, kg/d (early), and 8.1 DMI, kg/d (late).

Table 6. Overall mean ash free neutral detergent fiber digestibility (aNDFom) (%) in mature horses consuming Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), Coastal Bermudagrass hay plus functional feed additive (B+MF; $P = 0.01$)

	Treatments				SEM
Item, Unit	T	T+MF	B	B+MF	
aNDFom Digestibility, DM, %	$53.4^{a,c}$	54.6^{a}	39.3^{b}	$41.3^{b,c}$	5.12

[a,b,c]Means in rows with differing superscripts differ at $P < 0.05$

Table 7. Effect of hay on mean voluntary dry matter intake (*P* <0.0001).

	Treatments			
Item, Unit	T	T+MF	B	B+MF
VDMI, kg/d	10.9[a]	11.0[a]	8.63[b]	8.39[b]

Calcium requirements for adult, lightly exercised horses (30 g/Ca/d; NRC, 2007) were met for all diets and all periods, except horse 2 in period 2 consuming B+MF (27 g/Ca/d). Phosphorous requirements for adult, lightly exercised horses (18 g/P/d; NRC, 2007) were met for all diets and all periods except horse 2 in period 1 consuming T (17 g/P/d), horse 4 in period 1 consuming B (16 g/P/d), and horse 2 in period 2 consuming B+MF (15 g/P/d). These deficiencies are related to lowered intakes that occurred when horses consumed B and B+MF treatments immediately after consuming the more palatable T hay in previous periods. The deficiencies in P for the horse consuming the T hay can also be explained by the average P content of the hay used in this trial. The NRC (2007) reports that the mean P concentration in mature cool season grass hays is 0.26 %. This P concentration may or may not meet the requirements for a lightly exercised, mature horse at intakes of 2.0% of BW.

CHAPTER V

CONCLUSIONS AND IMPLICATIONS

Results and from this study show significant increase in DM, CP, and ADF digestibility in T and T+MF treated horses compared to B and B+MF treated horses. Results from this study suggest that T was more palatable than B. There were no independent statistical differences in NDF digestibility and VDMI. Taking into consideration the effect of horse on NDF digestibility, and the effect of horse and period on VDMI, inferring any conclusions is difficult.

Results from this study also indicate that there were no differences in nutrient digestibilities or VDMI due to MF-treatment, however, empirically, horses consuming the MF treatment had consistently higher VDMI and nutrient digestibilities. Due to the small number of replications and interactions between horse, period, and treatments, a possible Type II error may have occurred in this study concerning the supplement. In any future studies, a limited intake of forage could give a better indication of nutrient digestibility.

Concerning the MF treatment, in previous studies of other animal species, the fungi fermentation products were used in conjunction with yeast or another fermentation product of lactic acid. Gomez-Alacorn (1990) only had improved digestibility when animals were fed diets containing at least 60% concentrate. Possibly studying the effects this supplement on horses who are consuming concentrate should be researched more.

This research project provided additional information on digestibility of T hay for horses in comparison to B hay. This study has also provided more data to add to *in vivo* digestibility studies concerning the use of functional feed additives in equines.

LITERATURE CITED

Aiken, G.E., G.D. Potter, B.E. Conrad, and J.W. Evans. 1989. Voluntary intake and digestion of coastal bermudagrass hay by yearling and mature horses. Equine Vet. Sci. 9:262-264. doi: 10.1016/S0737-0806(89)80084-X

Allen, M.S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74:3063-3075. doi: 10.2527/1996.74123063x

Broadway, P.R., J.A. Carroll, and N.C. Burdick Sanchez. 2015. Live yeast and yeast cell wall supplements enhance immune function and performance in food-producing livestock: a review. Microorganisms 3:417-427. doi: 10.3390/microorganisms3030417

Burns, J.C. 2011. Advancement in assessment and the reassessment of the nutritive value of forages. Crop Sci. 51:390-402. doi: 10.2135/cropsci2010.06.0334

Cairns, T.C., C. Nai, and V. Meyer. 2018. How a fungus shapes biotechnology: 100 years of *Aspergillus niger* research. Fungal Biol. Biotechnol. 5:13. doi: 10.1186/s40694-018-0054-5

Callaway, T.R., and S.C. Ricke. 2012. Direct-fed microbials and prebiotics for animals: science and mechanisms of action. Springer Science. New York, NY.

Chaucheyras, F., and H. Durand. 2008. Probiotics in animal nutrition and health. Beneficial Microbes 1:3-9. doi: 10.3920/BM2008.1002

Chichlowski, M., W.J. Croom, F.W. Edens, B.W. McBride, R. Qui, C.C. Chiang, L.R. Daniel, G.B. Havenstein, and M.D. Koci. 2007. Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and Salinomycin. Poultry Sci. 86:1121-1132. doi: 10.1093/ps/86.61121

DairyLand Laboratories. 2015. Ash Free Blank Adjusted Amylase Neutral Detergent Fiber. DL.ARC.SOP.FEED.WCFEED.NDF.003.

Dey, A., and P.S. De. 2013. Influence of Moringa oleifera leaves as a functional feed additive on growth performance, carcass characteristics, and serum lipid profile of broiler chicken. Indian J. Anim. Res. 47:449-452.

Edouard, N., G. Fleurance, W. Martin-Rosset, P. Duncan, J.P. Dulphy, S. Grange, R. Baumont, H. Dubroeucq, F.J. Pérez-Barbería, and I.J. Gordon. 2008. Voluntary intake and
digestibility in horses: effect of forage quality with emphasis on individual variability. Animal 10:1526-1533. doi: 10.1017/S1751731108002760

Elghandour, M.M.Y., A.Z.M. Salem, J.S.M. Castañeda, L.M. Camacho, A.E. Kholif, and J.C.V. Chagoyàn. 2015. Direct-fed microbes: a tool for improving the utilization of low quality roughages in ruminats. J. Integrative Agriculture 14:526-533.

Feng, J., X. Liu, Z.R. Xu, Y.P. Lu, and Y.Y. Liu. 2006. The effect of *Aspergillus oryzae* fermented soybean meal on growth performance, digestibility of dietary components and activities of intestinal enzymes in weaned piglets. Anim. Feed Sci. Technol. 134: 295-303. doi: 10.1016/j.anifeedsci.2006.10.004

Fuller, R. 1989. A review: Probiotics in man and animals. J. Applied Bacteriology. 66:365-378.

Garber, A., P. Hastie, and J. Murray. 2020. Factors influencing equine gut microbiota: current knowledge. J. Equine Vet. Sci. doi: 10.1016/j.jevs.2020.102943

Gomez-Alcorn, R.A., C. Dudas, and J.T. Huber. 1990. Influence of cultures of *Aspergillus oryzae* on rumen and total tract digestibility of dietary components. J. Dairy Sci. 73:703-710. doi: 10.3168/jds.S0022-0302(90)78723-1

Hansen, N.C.K, E. Avershina, L.T. Mydland, J.A. Næsset, D. Austbø, B. Moen, I. Måge and K. Rudi. 2015. High nutrient availability reduces the diversity and stability of the equine caecal microbiota, Microbial Ecology in Health and Disease, 26:1, 27216, doi: 10.3402/mehd.v26.27216

Hansen, T.L., and L.M. Lawrence. 2017. Composition factors predicting forage digestibility by horses. J. Equine Vet. Sci. doi: 10.1016/j.jevs.2017.08.015

Hartley, R.D., and E.C. Jones. 1977. Phenolic components and degradability of cell walls of grass and legume species. Phytochemistry 16: 1531-1534. doi: 10.1016/0031-9422(77)84017-X

Henneke, D.R., G.D. Potter, J.L. Kreider, and B.F. Yeates. 1983. Relationship between body condition score, physical measurements and body fat percentage in mares. Equine vet. J. 15:371-372. doi: 10.1111/j.2042-3306.1983.tb01826.x

Hoseinifar, S.H., M. Dadar, and E. Ringø. 2017. Modulation of nutrient digestibility and digestive enzyme activity in aquatic animals: the functional feed additives scenario. Aquaculture Research. 48: 3987-4000. doi: 10.1111/are.13368

Hussein, H.S., and L.A. Vogedes. 2003. Review: forage nutritional value for equine as affected by forage species and cereal grain supplementation. Prof. Anim. Sci. 19: 388-397.

Jarriage, R., C. Demarquilly, J.P. Dulphy, A. Hoden, J. Robelin, C. Beranger, Y. Geay, M. Journet, C. Malterre, D. Micol, and M. Petit. 1986. The INRA "fill unit" system for predicting the voluntary intake of forage-based diets in ruminants: a review. J. Anim. Sci. 63:1737-1758. doi: 10.2527/jas1986.6361737x

Jouany, J.P., J. Gobert, B. Medina, G. Bertin, and V. Julliand. 2008. Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet. J. Anim. Sci. 86: 339-347. doi: 10.2527/jas.2006-796

Jung, H.G.J. 1997. Analysis of forage fiber and cell walls in ruminant nutrition. J. Nutr. 127: 810S-813S. doi: 10.1093/jn/127.5.810S

Kemme, P.A., A.W. Jongbloed, Z. Mroz, J. Kogut, and A.C. Beynen. 1998a. Digestibility of nutrients in growing–finishing pigs is affected by Aspergillus niger phytase, phytate and lactic acid levels: 1. Apparent ileal digestibility of amino acids. Livestock Prod. Sci. 58:107-117. doi: 10.1016/S0301-6226(98)00203-6

Kemme, P.A., A.W. Jongbloed, Z. Mroz, J. Kogut, and A.C. Beynen. 1998b. Digestibility of nutrients in growing–finishing pigs is affected by Aspergillus niger phytase, phytate and lactic acid levels: 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livestock Prod. Sci. 58: 119-127. doi: 10.1016/S0301-6226(98)00202-4

LaCasha, P.A., H.A. Brady, V.G. Allen, C.R. Richardson, and K.R. Pond. 1999. Voluntary intake, digestibility, and subsequent selection of matua bromegrass, coastal bermudagrass, and alfalfa hays by yearling horses. J. Anim. Sci. 77:2766-2773. doi: 10.2527/1999.77102766x

La Ragione, R.M., A. Narbad, M.J. Gasson, and M.J. Woodward. 2004. *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. Letters in Applied Microbiology, 38: 197-205. doi: 10.1111/j.1472-765X.2004.01474.x

Little, D., and A.T. Blikslager. 2002. Factors associated with development of ileal impaction in horses with surgical colic: 78 cases (1986-2000). Equine vet. J. 34:464-468. doi:10.2746/042516402776117773

Machida, M., O. Yamada, and K. Gomi. 2008. Genomics of *Aspergillus oryzae*: Learning from the history of Koji mold and exploration of its future. DNA Research 15:173-183. doi: 10.1016/S0301-6226(98)00202-4

McCown, S., M. Brummer, S. Hayes, G. Olson, S.R. Smith Jr., and L. Lawrence. 2011. Acceptability of teff hay by horses. Equine Vet. Sci. 32:327-331. doi: 10.1016/j.jevs.2011.11.008

McDaniel, A.L., S.A. Martin, J.S. McCann, and A.H. Parks. 1993. Effects of *Aspergillus oryzae* fermentation extract on *in vitro* equine cecal fermentation. J. Anim. Sci. 71: 2164-2172. doi: 10.2527/1993.7182164x

Medina, B. I.D. Girard, E. Jacotot, and V. Julliand. 2002. Effect of a preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. J. Anim. Sci. 80: 2600-2609. doi: 10.1093/ansci/80.10.2600

Mertens, D.R. 1992. Critical conditions in determining detergent fiber. Pp. C1-C8 in Proc. NFTA Forage Analysis Wkshp., Denver, CO.

Mertens, D.R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481. doi:10.3168/jds.S0022-0302(97)76075-2

Metcalf, J.L., S.J. Song, J.T. Morton, S. Weiss, A. Seguin-Orlando, F. Joly, C. Feh, P. Taberlet, E. Coissac, A. Amir, E. Willerslev, R. Knight, V. McKenzie, and L. Orlando. 2017. Evaluating the impact of domestication and captivity on the horse gut microbiome. Sci Rep 7: 15497. https://doi.org/10.1038/s41598-017-15375-9

Miller, D. 2009. Teff Grass: A new alternative. In: Proc., 2009 California Alfalfa & Forage Symposium and Western Seed Conference, Reno, NV.

Miranda, R.L.A, M.G.D. Mendoza, J.R. Bárcena-Gama, M.S.S. González, R. Ferrara, C.M.E. Ortega, and P.M.A. Cobos. 1996. Effect of *Saccharomyces cerevisiae* or *Aspergillus oryzae* cultures and NDF level on parameters of ruminal fermentation. Anim. Feed Sci. and Tech. 63: 289-296. doi: 10.1016/S0377-8401(96)01008-5

Moore, K.J., and H.G.J Jung. 2001. Lignin and fiber digestion. J. Range Manage. 54: 420-430. doi: 10.2458/azu_jrm_v54i4_moore

Mould, F.L. 2003. Predicting feed quality-chemical analysis and in vitro evaluation. Field Crop Res. 84:31-44. doi: 10.1016/S0378-4290(03)00139-4

Newman, Y.C., B. Lambert, and J.P. Muir. 2006. Defining forage quality. Texas A&M Univ., College Station, TX. p. 1-13.

NRC. 2007. Nutrient requirements of horses. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Pagan, J.D. 2009. Forages: the foundation for equine gastrointestinal health. Kentucky Equine Research: Adv. In Equine Nutr. Kentucky Equine Res., Versailles, KY. 4: 17-24.

Reinowski, A.R., and R.J. Coleman. 2003. Voluntary intake of big bluestem, eastern gamagrass, Indiangrass and timothy grass hays by mature horses. In: Proc. 8th Equine Nutr. Physiol. Soc. Sym., Lexington, KY. p. 3-5.

Saylor, B.A., D.H. Min, and B.J. Bradford. 2018. Productivity of lactating dairy cows fed diets with teff hay as the sole forage. J. Dairy Sci. 101:5984-5990. doi: 10.3168/jds.2017-14118

Schuster, E., N. Dunn-Coleman, and J.C. Frisvad. 2002. One the safety of *Aspergillus niger* – a review. Appl. Microbiol. Biotechnol. 59:426-435. doi: 10.1007/s00253-002-1032-6

Schoster, A. 2014. Probiotics and dietary supplements in horses: useful or not? In: Annual Forum of the American College of Veterinary Internal Medicine, Nashville, TN. p. 1-3.

Servin, A.L. 2004. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiology Reviews 28:405-440. doi: 10.1016/j.femsre.2004.01.003

Shi, C., J. He, J. Yu, B. Yu, X. Mao, P. Zheng, Z. Huang, and D. Chen. 2015. Amino acid, phosphorous, and energy digestibility of *Aspergillus niger* fermented rapeseed meal fed to growing pigs. J. Anim. Sci. 93:2916-2925. doi:10.2527/jas2014-8326

Staniar, W.B., J.R. Bussard, N.M. Repard, M.H. Hall, and A.O. Burk. 2010. Voluntary intake and digestibility of teff hay fed to horses. J. Anim. Sci. 88:3296-3303. doi: 10.2527/jas.2009-2668

St. Lawrence, A.C., L.M. Lawrence, and R.J. Coleman. 2001. Using an empirical equation to predict the voluntary intake of grass hays by mature equids. In: Proc. 17th Equine Nutr. Physiol. Soc. Sym., Lexington, KY. p. 99-100.

Sturgeon, L.S. 1999. The digestibility and mineral availability of matua, bermudagrass, and alfalfa hay in mature horses. Masters thesis. West Texas A&M Univ., Canyon.

Switzer, S.T. 2003. The effect of yeast culture supplementation on nutrient digestibility in aged horses. Masters thesis. West Texas A&M Univ., Canyon.

Uden, P., P.H. Robinson, and J. Wiseman. 2005. Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. Anim. Feed Sci. and Tech. 118: 181-186. doi: 101016/j.anifeedsci.2004.11.011

United States Food and Drug Administration. 1995. Compliance Policy Guide 689.100: Direct-Fed Microbial Products. Center of Veterinary Medicine.

Van Soest, P.J. 1963. Use of detergents in the analysis of fibrous feeds. 1. Preparation of fiber residues of low nitrogen content. J. Assoc. Off. Agric.Chem. 73: 487-491. doi: 10.1093/jaoac/73.4.487

Van Soest, P.J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci. 26:119-128. doi: 10.2527/jas1967.261119x

Van Soest, P.J, and H.K. Goering. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications).

Van Soest, P.J. and J.B. Robertson. 1979. System of analysis for evaluating fibrous feeds. In: Proc. of a standardization of analytical methodology for feeds, Ottowa, Canada.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O and B Books, OR. USA.

Van Soest, P.J. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University, Ithaca, NY.

Weese, J. S. 2002. Probiotics, prebiotics, and synbiotics. In: N. E. Robinson, author, Current therapy in equine medicine, 5th ed. Saunders St. Luis, MO. p.357-360.

Wells, L.A. 2015. The applicability of near infrared reflectance spectroscopy to predict dry matter intake and in-vivo neutral detergent fiber digestibility in mature gelding consuming an all-forage diet. Masters thesis. West Texas A&M Univ., Canyon.

Wiedmeier, R.D., M.J. Arambel, and J.L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 70:2063-2068. doi:10.3168/jds.S0022-0302(87)80254-0

Willing, B., A. Vörös, S. Roos, C.Jones, A. Jansson, and J.E. Lindberg. 2009. Changes in faecal bacteria associated with concentrate and forage-only diets fed to horses in training. Equine vet. J. 41: 908-914. doi: 10.2746/042516409X447806

APPENDIX TABLES

Table A-1. Effect of treatment on mean body weight (BW), body condition score (BCS), and voluntary dry matter intake (VDMI) on mature gelding consuming Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), Coastal Bermudagrass hay plus functional feed additive (B+MF).

	B	B+MF	T	T+MF	SEM	*P*-value
Average BW, kg	498[b]	495[b]	518[a]	513[a]	7.04	0.03
VDMI, DM, kg/d	8.63[a]	8.39[a]	10.88b	10.98[b]	0.25	< 0.0001
BCS	5	5.4	6	5.5		

Table A-2. Calcium and Phosphorous requirements according to the NRC (2007) for lightly exercised, mature horses, compared to horses on study who were deficient by treatment.

Calcium Intake		**NRC Requirement**
Horse 2, Period 2, B+MF	27 g/d	30 g/d
Phosphorous Intake		
Horse 2, Period 1, T	17 g/d	18 g/d
Horse 4, Period 1, B	16 g/d	18 g/d
Horse 2, Period 2, B+MF	15 g/d	18 g/d

APPENDIX FIGURES

Figure B-1. Horse in study wearing fecal collection harness from Bun Bags (www.bunbags.com)

Figure B-2. Mean voluntary dry matter intake (kg/d) of horses consuming treatments of Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), Coastal Bermudagrass hay plus functional feed additive (B+MF).

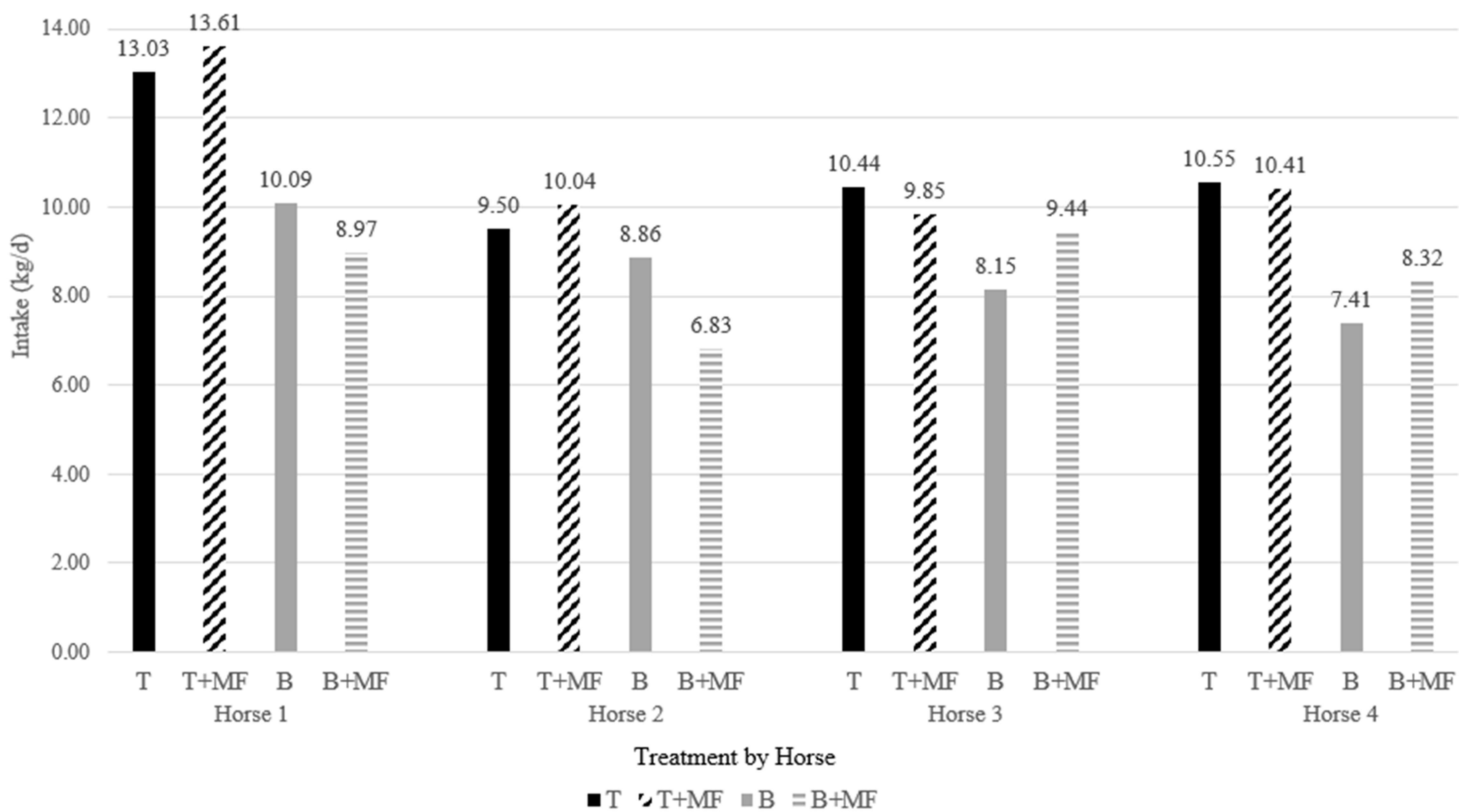

Figure B-3. Effect of treatment on average body weight (BW) of horses consuming treatments of Coastal Bermudagrass hay (B), Tiffany hay (T), Tiffany hay plus functional feed additive (T+MF), Coastal Bermudagrass hay plus functional feed additive (B+MF).