# THE APPLICABILITY OF NEAR INFRARED REFLECTANCE SPECTROSCOPY TO PREDICT DRY MATTER INTAKE AND IN-VIVO NEUTRAL DETERGENT FIBER DIGESTIBILITY IN MATURE GELDINGS CONSUMING AN ALL-FORAGE DIET

by

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## ABSTRACT

An experiment was conducted to investigate the applicability of near infrared reflectance spectroscopy (NIR) to predict DMI and in-vivo NDF digestibility in horses. Nine mature, sedentary stock-type geldings, ranging from 10 to 20 yr of age were randomly assigned to one of three treatments within three simultaneous 3 x 3 Latin Squares to compare intakes and digestibilities of alfalfa (A), coastal Bermudagrass (C), and mixed grass hay (MGH). The 51-d study consisted of three 17-d periods. Each period consisted of a 4-d dietary adjustment period, 10-d feeding period, and 3-d total fecal collection period. Total fecal collections were conducted for 72 h, with feces collected and weighed every h. Representative hay and fecal samples were remitted to Dairy One Forage Lab (Ithaca, NY) for analysis.

There was a main effect of treatment (P = 0.02) on overall mean DMI. Horses consuming A had a greater mean DMI (P = 0.02) as compared to horses consuming MGH (7.31 vs. 5.41 kg/d). Overall mean DMI for horses consuming A and C (7.31 vs. 6.79 kg/d) were similar (P = 0.71). Dry matter intake was negatively correlated (r = -0.44) with NIR analysis of NDF with sulfite and ash correction (aNDFom). Dry matter intake was significantly (P = 0.02) related to hay aNDFom; however, the $R^2$ value was 0.17, which indicated that 17% of the variation in DMI could be explained by the aNDFom content of hay. Dry matter intake was negatively correlated (r = -0.45) with

NIR analysis of undigested NDF at 30 h (uNDF30). Dry matter intake was significantly ($P = 0.02$) related to hay uNDF30; however, the $R^2$ value was 0.17, which indicated that 17% of the variation in DMI was explained by the uNDF30 content of hay.

There was a main effect of treatment on in-vivo NDF digestibility ($P = 0.01$). Correlations between in-vivo NDF digestibility and NIR hay analysis were not significant ($P > 0.61$) for aNDFom, uNDF30, or NDF digestibility at 30 h (NDFD30). Significant correlation coefficients between aNDFom content and in-vitro estimates were observed; 0.99 for uNDF30 and -0.68 for NDF digestibility at 30 h. Results from this study indicate that the use of NIR to estimate intake and in-vivo NDF digestibility in horses warrants further research. Perhaps the further collection of data will lead to development of NIR calibration equations that will be robust enough to predict NDF digestibility in horses.

Key Words: Equine, NDF digestibility, forage, NIR, in-vivo, in-vitro

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This thesis is dedicated to my late mother, Kim Anderson, who passed away on August 28th, 2011. She was a caring, loving, and genuine woman with a big heart, and everyday teaches me to be strong.

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# CHAPTER I

# INTRODUCTION

Traditional in-vivo methods of determining digestibility, while accurate, can be costly and time-consuming. As a result, in-vitro methods of determining digestibility have been developed. Utilizing in-vitro technologies often require a microbial inoculum prepared from ruminal or cecal fluid extracted from cannulated animals. This has proven to be a disadvantage and has limited the use of in-vitro methodologies in equine research, as cecally-cannulated horses are not readily available.

The use of near infrared reflectance spectroscopy (NIR) been accepted as a useful tool for analyzing feeds and forages. The application of NIR to predict chemical composition and digestibility coefficients of forages has been observed in multiple species. Park et al. (1998) reported $R^2$ values of 0.85 for DM digestibility and OM digestibility, 0.79 for NDF digestibility, and 0.85 for GE digestibility for NIR to predict digestibility in sheep. Andrieu and Martin-Rosset (1995) observed r = 0.98 correlation between in-vivo OM digestiblity and OM digestibility prediction from NIR in horses.

The NDF analysis provides an estimate of total cell wall constituents of forages, and is highly correlated with intake and rate of digestion in ruminants (Mertens, 1997). While nutritionists have been primarily concerned with fiber digestibility, recent efforts have focused on the concept of “undigestible NDF,” (uNDF) as it sets the extent and rate

of fiber fermentation in the rumen. According to Cotanch et al. (2014), uNDF should be included in routine forage analysis, as uNDF offers a more predictable digestibility and uniformity as compared to NDF.

While data from several studies have successfully predicted digestibility with NIR in other species, research pertaining to the prediction of in-vivo NDF digestibility in horses are minimal. The objective of this study is to investigate applicability of NIR to predict DMI and in-vivo NDF digestibility in mature geldings consuming an all-forage diet.

# CHAPTER II

# REVIEW OF THE LITERATURE

## Forage Classification and Composition

Forages are divided into two categories: cool-season species that are adapted to temperate conditions, and warm-season species that are found in subtropical or tropical environments (NRC, 2007). Additionally, forages belong to either the Graminae (grasses) or the Leguimisae (legumes) family (Van Soest, 1994). Depending on stage of maturity, forages consist of leaf, sheath, stem, flowers, and seed-heads. Each of these different plant parts differs in its chemical composition, and may change substantially during a growing season (NRC, 2007).

According to the NRC (2007), horses are classified as selective grazers, adapted to consume relatively the most immature forage available. Immature alfalfa and grass forages possess greater leaf to stem ratios, are higher in protein, water, and minerals, and are lower in crude fiber. However, as plants mature, leaf growth declines, stems elongate, reproductive structures develop, and the cell content to cell wall ratio decreases. Ultimately, chemical composition is changed and the nutritive value of the forage is decreased with greater maturity (NRC, 2007). Warm-season forages are generally of lower quality than cool-season forages, because warmer temperatures and stronger light exposure increase growth rate and leaf to stem ratios change rapidly (NRC, 2007).

## Forage Quality

Newman et al. (2006) stated that when defining forage quality, a distinction between quality and nutritive value should be made. Forage nutritive value refers to the TDN and concentration of CP that constitutes potential available energy of a forage; whereas forage quality includes both nutritive value and forage intake in livestock. Further, forage quality is the greatest predictor of animal response, and directly affects individual animal performance (Newman et al., 2006). Animal performance is largely determined by the combination of voluntary intake and nutritive value of consumed material (Nelson and Moser, 1994).

Forage quality is affected by differences in species, temperature during growing, stage of maturity, light intensity, harvest and storage methods, water availability, and latitude (Van Soest, 1994). In general, forages grown at high temperatures tend to be of reduced feeding value compared to those grown at lower temperatures (NRC, 2007). The principal factor responsible for declining forage nutritive value is stage of maturity, but plant environment alters the impact of plant maturity. Plant environment includes biotic and abiotic factors that influence growth and development of forages. Year-to-year, seasonal, and variations in environment related to geographical location alter forage quality, even when forages are harvested at similar morphological stages (Buxton and Fales, 1994). The feed quality of a conserved forage can be no greater than the original sward, and thus stage of plant maturity at time of harvest is an important factor influencing the feeding value of the final product (NRC, 2007).

A decrease in the ratio of leaf:stem is the primary reason for a decrease in forage quality, since leaf tissue is the highest quality, most digestible component of forage in

both legumes and grass hays. Lower digestibilities of stems are associated with anatomy, as stems are composed of highly lignified xylem cells, a high concentration of vascular bundles, and other sclerenchyma cells; whereas leaf lamina are made of thin-walled mesophyll cells (Nelson and Moser, 1994). In legumes (e.g. alfalfa), stems are structural organs and leaves are metabolic organs. In grasses, leaves have an important structural function through the lignified midrib. The result, in terms of nutritive value, is that alfalfa leaves better maintain their quality as they age, while grass leaves decline rapidly in quality with maturity (Van Soest, 1994).

Fiber Definitions

Forages are characterized according to their high dietary fiber content (NRC, 2007). The term "fiber" is defined in countless different ways in the literature. Mertens (1989) described fiber as "the indigestible and slowly digesting, or incompletely available, fractions of feeds that occupy space in the gastrointestinal tract". Chemically, fiber is composed of a mixture of hemicellulose, cellulose, and lignin, along with pectins, indigestible proteins and lipids (NRC, 2007). Moore and Hatfield (1994) defined fiber as the components of plant-derived foods that are indigestible by mammalian enzymes. Mammals are unable to hydrolyze the β1-4 polysaccharide linkages present in plant cell walls due to a lack of appropriate enzymes; therefore, fermentable microorganisms present in the gastrointestinal tract are necessary for degradation (Jung, 1997). Physical properties and amount of fiber directly influence utilization by the animal, ultimately impacting performance (Mertens, 1997).

Most methods to measure fiber have been developed and applied for use in ruminant and human nutrition. However, neither the ruminant nor the human system fit

well with the digestive physiology and intermediary metabolism of the horse. Measuring fiber in feeds is very method-dependent due to variability in protocols and type of feed (NRC, 2007). Fiber can be assessed as CF, total dietary fiber (TDF), NDF, and/or ADF. Neutral detergent fiber refers to cell wall components of cellulose, hemicellulose and lignin; whereas ADF represents the least digestible portion of the plant including cellulose and lignin (Mould, 2003).

Fiber Fractions: Hemicellulose, Cellulose, and Lignin

Forages consist of cell contents and cell walls (Pagan, 2009). Cell contents, also known as non-structural carbohydrates, are composed of simple sugars, glucose, fructose, sucrose, and storage carbohydrates such as fructan or starch (NRC, 2007). Plant cell contents are available for degradation by ruminants and non-ruminants (Van Soest, 1967). Cell walls consist of hemicellulose, cellulose, and lignin, which make up the structural carbohydrates of the plant (NRC, 2007). Digestibility of cell wall components are limited or completely unavailable for digestion by mammalian enzymes (Van Soest, 1967). Utilization of plant cell wall components is possible only through microbial fermentation. Cell wall carbohydrates and fructans are converted via fermentation by gut microflora to VFAs, which are then metabolized by the host animal to yield ATP (NRC, 2007). In immature vegetative tissues, cell contents represents up to 66 percent of the total DM; however, with increasing forage maturity, the proportion of cell contents decreases, and in the mature plant represents less than 40 percent of forage DM. The combination of NSC and SC establish the primary energy-yielding portions of forages. The energy value of forage is governed by the types and relative proportions of digestible and fermentable carbohydrate the forage contains (NRC, 2007).

*Hemicellulose*

Hemicellulose is a heterogeneous mixture containing polymers of arabinose, xylose, glucose, fucose, mannose, and galactose, along with pectins (containing β D 1-4 linked galacturonic acid residues, arabinose, and galactose; NRC, 2007). Together, hemicellulose and lignin form the structural encrusting material of secondary cell wall thickening. Hemicellulose is found primarily within lignified walls of forage plants and is generally insoluble in water; however, when de-lignified using acid or alkali becomes soluble. Generally, non-ruminants, including horses, digest more hemicellulose than cellulose; however, ruminants digest equivalent amounts of both carbohydrates (Van Soest, 1994). Unlike cellulose, hemicellulose has a random amorphous structure that is weak, and can be easily digested by hemicellulase enzymes (Coughlan and Hazelwood, 1993). The pectins and polymers of arabinose, galactose, and mannose are readily degraded by hindgut microflora of non-ruminants, whereas those of cellulose and particularly xylan are more resistant to breakdown (Moore-Colyer and Longland, 2000). The amount of hemicellulose in a sample can be calculated by subtracting the percent ADF from percent NDF (Cheeke, 2004).

*Cellulose*

Cellulose is considered the most abundant carbohydrate in the world, and accounts for approximately 20 to 40% of DM of most plants. Cellulose contains β-linked polymers of glucose. When separated from forages, cellulose is composed of β 1-4 glucan and 15% pentosans, along with cutin and silica present in most of the plant tissue (Van Soest, 1994). Cellulose is found in stalks, stems, trunks, and all woody portions of plant tissues (Pond et al., 2005). Cellulose is fibrous, water soluble, and indigestible to

mammalian enzymes; therefore, microbial fermentation involving the breakdown of β 1-4 linkages is required (NRC, 2007).

*Lignin*

According to Van Soest (1994), defining lignin is problematic due to its nomenclature, and appears differently in the literature. Lignin is a polymer formed from monolignols derived from the phenylpropanoid pathway in vascular plants (Moore and Jung, 2001). Lignin is deposited in the cell walls of plants as part of the process of cell maturation (Moore and Jung, 2001). Lignin, along with associated phenolics, is the forage entity most responsible for impairment of digestion (Mould, 2003). Lignin is the only major plant polymer whose subcomponents are not clearly defined. Due to its condensed structure, hydrolysis is not achieved during in-vivo studies (Van Soest, 1994). Core lignin distinguishes the primary lignin entity from extractable phenolics. True lignin is a polymerized product of phenylpropanoid alcohols, and ferulic and para-coumaric acids (NRC, 2007). Lignin interferes with the digestion of cell-wall polysaccharides by acting as a physical barrier to microbial enzymes. Lignification, therefore, has a direct and often negative impact on the DE value of the forage (Moore and Jung, 2001).The amount of lignin in plant tissue affects the bioavailability of cellulose and hemicellulose for microbial use (Pond et al., 2005).

Fiber Analysis

The purpose of fiber analysis is to determine the amount of fiber in a feed. To use as a measurement of nutritive value, fiber must be divided into further components. Fiber analytical methods partition forages by isolating different chemical and nutritional fractions (Mould, 2003).

Interest in forage analysis was developed in the mid-1800's. Animal feeders became concerned with correlating feed quality and quantity with measurements of pounds of milk, meat, and other animal products. The first chemical method of analyzing forage was developed at the Weede Experiment Station near Gottingen, Germany in 1864 (Burns, 2011). The Weede system of proximate analyses initially separated the carbohydrate fraction of a forage into two categories based on digestibility (Burns, 2011). The intent of the Weede system was to identify the less digestible carbohydrate fraction (crude fiber, or CF) of DM from the more readily digestible fraction (nitrogen-free extract, NFE). To analyze CF, samples were treated with dilute acid and then with alkali to mimic digestion by gastric secretions (AOAC, 2002). The complete proximate analysis separated forages into NFE, CF, ash, CP, ether extract, and water (Burns, 2011).

Developing a consistent method of characterizing feeds became evident after researchers discovered inaccuracies with the Weede System. The proximate analysis failed to distinguish between cell contents and cell walls of forage, which underestimated the total cell wall content and portions of wall polysaccharides and lignin (Jung, 1997). In the 1960's, Peter Van Soest at the USDA and Cornell University in Ithaca, NY developed the detergent system of feed analysis (Uden et al., 2005). In this system, detergents are used to separate cell contents from cell wall of forages (Burns, 2011). Utilization of a neutral detergent solution to separate cell solubles from the cell wall proper revealed an insoluble residue, known as NDF, which provided an estimate of the plant cell wall (Burns, 2011). Subsequent treatment of the NDF residue by acid detergent solution yielded another insoluble residue, termed ADF, which estimated the least digestible portions of the plant (Burns, 2011). The ADF procedure quickly replaced CF in

several countries, and was approved by the Association of Official Analytical Chemists (AOAC) without major difficulty (Uden et al., 2005). The NDF method was accepted by the AOAC in 2002, effectively replacing the traditional Weede system (Mertens, 2002).

## Methods of Fiber Assessment

### *Neutral Detergent Fiber*

The most common method of measuring the cell wall contents of forages is the Van Soest Detergent System (NRC, 2007). The original method determines the percentage of cell wall material in a feed (NRC, 2007). The NDF method proposed by Van Soest and Wine (1967) exposed forages to a detergent solution with sodium sulfite to remove protein and EDTA in order to chelate Ca and remove pectin. Initially, the use of sodium sulfite was considered optional. However, Hintz et al. (1996) suggested that sodium sulfite was necessary to remove contaminated N from feeds. Mertens (2002) agreed with this conclusion, as sodium sulfite usage was crucial to remove proteins that have been denatured or bound to carbohydrates. To prevent contamination of starch in feeds, the original method was modified using the amylase-treated NDF (aNDF) procedure (NRC, 2007). The updated NDF procedure utilizes both sodium sulfite for protein removal and alpha-amylase to eliminate starch. While the NDF procedure developed by Van Soest measures chemical characteristics of fiber, it does not include measurements of physical properties, such as particle size and the effectiveness of fiber in meeting requirements for the animal (Mertens, 2002).

Because NDF provides an estimate of the total cell wall constituents of forages, NDF is highly correlated with intake, feed density, rate of digestion, and depression of digestibility related to high levels of intake in ruminants (Mertens, 1997). Neutral

detergent fiber is related to space-occupying or ruminal fill effect (Beauchemin, 1996). Oba and Allen (1999) reported that as NDF digestibility increased by 1%, a 0.17 kg increase in DMI by dairy cattle would result. Sales et al. (2013) developed energy digestibility prediction equations using treatment means from a diverse collection of hay digestibility studies in horses. The authors reported that OM digestibility could be predicted from NDF content with a root mean square error (RMSE) of 4.71 and an $r^2$ value 0.51. The authors also reported that NDF was the most accurate single chemical entity for predicting gross energy digestibility (RMSE = 2.95; $R^2$ = 0.63). St. Lawrence et al. (2001) reported a correlation (r = 0.86) between NDF concentration and DMI of 4 cool-season grasses. The study involved a literature review of published studies comparing the relationship between DMI and forage NDF content, with the subsequent development of an empirical prediction equation. The authors concluded from that while NDF content was an important predictor of DMI, it is not the only determinant of intake in horses.

*Acid Detergent Fiber*

Acid Detergent Fiber represents cellulose and lignin, along with variable levels of xylans (Jung, 1997). These values are inversely related to digestibility, so forages with low ADF concentrations are desired (Pinkerton and Cross, 1995). The ADF procedure is widely used as a quick method for estimating fiber in feeds, often substituting for CF as part of a proximate analysis (Van Soest, 1994). Van Soest and Wine (1967) stated that ADF analysis is used as sample preparation to determine the sum of cellulose, lignin, ADIN, AIA, and silica (NRC, 2007). Since ADF excludes hemicellulose, ADF is an inaccurate measurement of total fiber in feed (Mertens, 2002). Estimation of

hemicellulose can be achieved by subtracting ADF from NDF, but the accuracy of the estimations will depend on the sample (e.g. beet pulp, citrus pulp, etc.) and the botanical origin of the sample (NRC, 2007). Increasing ADF levels are associated with decreased digestibility (Newman et al., 2006). However, according to Van Soest et al., (1994), while statistical associations with ADF and digestibility have been obtained, there is no chemical basis for this finding. Van Soest and Wine (1967) observed a reduction in the accuracy of ADF estimation due to heat-damaged protein that contaminated the ADF solution. As a result, the ADF analysis has been modified to include a stronger acid and a longer treatment period to eliminate heat-damaged protein (Givens et al., 1989).

Near Infrared Reflectance Spectroscopy

Obtaining accurate data on the digestibility of forages, while beneficial in diet formulation and economic evaluation of various forages, can be time consuming, costly, and requires copious amounts of test forage (Weiss et al., 1992). The utilization of Near Infrared Reflectance Spectroscopy (NIR) offers a cost effective, rapid, and safe alternative to expensive in-vivo procedures. The purpose of NIR is to relate a feed sample's reflectance of near infrared light to its chemical composition. Developed by the USDA in Beltsville, Md, over the last two decades NIR has been accepted as a useful tool for analyzing feeds and forages due to speed of analysis, minimal operating costs, accuracy, and safety (NRC, 2007). Norris et al. (1976) reported the first applicable use of NIR by evaluating forage quality with greater speed. Because all energy-yielding components of feeds absorb light in the near-infrared region of the spectrum, NIR has potential to be a fast alternative method to predict metabolizable energy content of feeds.

However, mineral analysis should not be considered while utilizing NIR, as minerals do not absorb light energy in the near infrared region (NRC, 2007).

Instead of direct measurements, NIR relies on prediction equations calibrated into the device (NRC, 2007). Shenk et al. (1979) reported a minimum requirement of 50 calibration group samples necessary to develop NIRS equations. Brown and Moore (1987) reported successfully developing NIR equations by analyzing 25 to 113 samples via wet chemistry. These equations were able to accurately predict chemical composition and in-vitro digestion of the forage samples (Brown and Moore, 1987). Near infrared reflectance spectroscopy can also be used to measure NDF in forages and feeds (NRC, 2007).

Over the years, NIR has demonstrated potential use in predicting quality and digestibility coefficients of forages in multiple species. Valdes and Leeson (1992, 1994) reported NIRS accurately measured apparent metabolizable energy in poultry feeds and feed ingredients. Park et al. (1998) reported on the use of NIR on undried samples of grass silage to predict chemical composition and digestibility parameters in sheep. One hundred thirty six grass silages produced on farms across Northern Ireland were offered to 72 wethers in a 17-period changeover design experiment. Four wethers were offered each silage over a 3-wk period, with 8 silages offered as the primary feed for each period. In-vivo DM, OM, N, NDF, and energy digestibilities were calculated. Silages were also analyzed using NIR to calculate correlations with the in-vivo digestibilities. The authors reported the following coefficients of determination: $R^2 = 0.85$ for DM and OM digestibility, 0.79 for N digestibility, 0.79 for NDF digestibility, and 0.85 for GE digestibility.

Brogna et al. (2009) suggested that NIR analysis can provide accurate estimates of in-vitro NDF digestibility in dairy cattle. Calibration equations were successfully developed using 319 samples of alfalfa hay collected over 4 years in Northern Italy. The authors reported an $R^2$ value of 0.87 to predict in-vitro NDF digestibility from NIR. Andrieu and Martin-Rosset (1995) observed a correlation of 0.98 between in-vivo OM digestibility and OM digestibility prediction from NIR in horses. The authors also reported ADF determined according to the methods of Van Soest was the best single chemical predictor of OM digestibility in horses, while NDF determination did not improve precision of OM digestibility estimation.

While several studies have validated the use of NIR to predict digestibility, results from other studies appear to contradict these findings. Andres et al. (2005) used 62 herbage samples harvested in northwest Spain in an experiment to evaluate the ability of chemical composition and NIR to predict in-vitro digestibility and in-situ degradability of NDF. For the in-situ experiment, three Holstein-Friesian cows fitted with rumen cannula were used to incubate herbage samples. Prediction equations for the estimation of NDF digestibility both in-vitro and in-situ were generated. Significant correlation coefficients of NDF content were observed; -0.69 for NDF digestibility, -0.74 for NDF digestibility at 24 h in-situ, and -0.78 NDF digestibility at 48 h in-situ. However, due to low ratio performance deviation statistic values as part of the prediction equations, the authors reported in-vitro NDF digestibility and in-situ kinetic parameters of NDF digestibility could not be predicted accurately by NIR. Mentink et al. (2006) evaluated the ability of NIR to predict nutrient composition and in-vitro digestibility of a total mixed ration (TMR) fed to dairy cattle. One hundred ten TMR samples were scanned using NIR with

spectra retained with NIRS calibration and cross-validation statistics determined using partial least squares regression methods. While nutrient composition of the TMR was well-predicted by NIR ($R^2 > 0.85$), in-vitro NDF digestibility was not well estimated by NIR.

Fiber Digestibility

A simple definition for digestibility in animals is the fraction of a feed or forage that is lost in passage through the digestive tract (Cochran and Galyean, 1986). Equine diet digestibility can be influenced by exercise, method or form of feed processing, and individual variability (Hintz et al., 1985; Pagan et al., 1998). Generally, utilization of forage nutrients varies among different classes of livestock animals (Cymbaluk, 1990). When measuring forage quality in relation to animal performance, fiber digestibility and indigestibility are critical factors (Cotanch et al., 2014).

*Cattle Fiber Digestibility*

True ruminants and tylopods (camels, llamas, and related species) ruminate on forages. Rumination is defined as the postprandial regurgitation of ingesta, followed by remastication, bolus reformation, and re-swallowing (Van Soest, 1994). Prior to digestion in the abomasum and small intestine, feeds and forages are fermented by microorganisms present in the rumen. The end products of fermentation which include VFAs and microbial protein become available to the animal as an energy source (Russell et al., 1992). Ruminants require adequate amounts of course-textured feeds to prevent metabolic disorders (Sudweeks et al., 1981). However, because forages are traditionally lower in digestibility and energy than concentrates and other grains, often fiber is lowered to minimum levels in the diet to achieve higher production. While reducing fiber in cattle

diets can be more cost effective, in order to maintain the long-term health and productivity of the herd, sufficient roughage should be included in the diet (Mertens, 2002).

Without an adequate supply of rough textured feeds presented to the rumen, reduction in chewing activity will occur. Lack of course material also contributes to reduced rumen motility, leading to diminished muscle tone, abomasum displacement, and gastrointestinal epithelial deterioration. Additionally, diets high in concentrates containing rapidly-fermentable carbohydrates lowers the pH of the rumen, leading to ruminal acidosis. Diminished roughage consumption in dairy cattle results in reduced milk fat production (Sudweeks et al., 1981). These metabolic issues attributed to the development of minimum fiber or effective fiber requirements for ruminants (Mertens, 2002). Previous recommendations of the Dairy Cattle NRC (1989) provided limited guidance to nutritionists and producers to meet the minimum fiber and carbohydrate needs of lactating cows (Mertens, 1997).

Mertens (1985) reported that NDF analysis can be a beneficial tool to determine the maximum amount of forage to concentrate ratio of dairy cattle rations. However, while NDF measures chemical characteristics of fiber, it fails to determine physical characteristics such as particle size and density that impact ruminal health, metabolism, and milk production (Mertens, 1997). Due to the importance of these physical fiber characteristics, the NDF method has been modified over the years to measure the additional parameters of physically-effective NDF (peNDF) and effective NDF (eNDF; Mertens, 1997). The eNDF parameter is defined as the additive ability of a feed to replace roughage while maintaining milk fat percentage effectively in dairy cows. The

peNDF measurement of fiber relates to particle size that affect physical properties that stimulate chewing activity and establishes the biphasic stratification of ruminal contents. The peNDF measurement of fiber is a product of its physical effectiveness factor and concentration of NDF. Mertens et al. (1997) reported that a minimum requirement of 22% peNDF was necessary to maintain a ruminal pH of 6.0 and milk fat percentage at 3.4%.

*Indigestible NDF*

Because NDF influences rumen fill, it is the first limiting factor affecting intake. Indigestible fiber takes up space in the rumen, so diets traditionally high in NDF result in lowered intake, preventing the dairy cow from consuming sufficient forage to meet the nutrient requirements for optimal production (Beauchemin, 1996). While nutritionists have primarily focused on fiber digestibility with regard to intake, recent efforts have concentrated on the concept of "indigestible NDF," (iNDF; Cotanch et al., 2014). To allow for the accurate estimation of the potentially digestible fraction and respective digestion rates, iNDF is required as the end point of fermentation (Cotanch et al., 2014). Since iNDF represents the undigested portion of forages which do not provide energy to the animal, iNDF cannot be used to estimate the energy content of forage (Traxler et al., 1998).

*Undigestible NDF*

Although iNDF is the standard term in the literature, Mertens (2013) referred to indigestible NDF as "undigested NDF" (uNDF) to improve the accuracy of the terminology defining fiber fermentation dynamics. As described by Mertens (2013), uNDF is the laboratory measure of indigestible NDF at a specific fermentation time in the

rumen, usually by in-vitro methods. Undigestible NDF represents the fiber fraction that impacts physical effectiveness, digestion and passage rates, and gut fill of forages (Van Amburgh et al., 2015). Using the uNDF fraction, the accuracy of determining NDF digestion is improved by estimating rate of digestion at different time points of in-vitro fermentation (Cotanch et al., 2014). According to Cotanch et al. (2014), uNDF should be included in routine forage and feed analysis, as undigestible NDF offers a more predictable digestibility and uniformity than NDF. The amount of undigested forage present in the rumen affects rumen turn-over and fill, thereby restricting intake. Jones (2014) suggested that uNDF measured at 30 hours of fermentation predicted DMI of forages in dairy cattle. Jones utilized previous data on NDF pools in the rumen, including the percent NDF digested at a certain time point (NDFd), and the percent rate NDF is digested per hour. Using previous in-situ and in-vitro studies along with empirical prediction equations, the authors concluded that when compared to other fermentation time points, uNDF30 best represents ruminal residency time of hays.

## Equine Fiber Digestibility

### *Equine Digestive Physiology*

Anatomically, the horse is a non-ruminant herbivore that possesses a digestive tract designed to degrade large amounts of forage in a continuous manner over time. The horse's stomach comprises less than 10% of the total digestive tract volume, and has a retention time between 2 to 6 h for most feeds (Hussein and Vogedes, 2003; Weyenberg et al., 2006). In contrast to cattle, the primary site of degradation of protein and absorption of amino acids occurs prior to the site of fermentation, in the small intestine (Hussein and Vogedes, 2003). The hydrolysis of α1-6 and α1-4 carbohydrate linkages

present in starch and maltose occur in the small intestine. However, horses, like other mammals, do not possess the enzymes to digest the β1-4 linkages found in cellulose and hemicellulose. Therefore, digestion of feedstuffs containing β1-4 linkages is only possible through microbial fermentation that occur in the hindgut (NRC, 2007).

The horse possesses a voluminous hindgut capable of holding 80 to 90 L. The hindgut contains billions of bacteria and protozoa that break the β1-4 linkages of fiber (Pagan, 2009). To digest fiber, two major steps must occur to break the structural carbohydrates into useful substances: (1) the hydrolysis of polysaccharides; and (2) the transformation of monosaccharides into VFAs, gas production from fermentation, and heat release (Sturgeon et al., 2000). Fermented fiber fractions lead to the production of VFAs that provide the horse energy (Hussein and Vogedes, 2003). Microbial VFA production in the cecum may meet up to 30% of a horse's energy needs at maintenance when consuming forages (NRC, 2007). Therefore, VFA production contributes significantly to total DE utilization, especially for horses provided all-forage diets (NRC, 2007).

Forage in the Equine Diet

Due to the influence forage has on maintaining normal gut function in the horse, forage should remain the foundation of all equine diets. Additional concentrates, mineral or protein supplements should be used to supply the horse nutrients lacking in the forage (Pagan, 2009). According to the NRC (2007), horses at maintenance can usually meet their daily energy requirements by consuming an all-forage diet. Depending on forage quality, forage-based diets can reduce or eliminate the need for concentrates. Feeding a long-stem roughage to horses is generally supported by nutritionists. The benefits of

feeding solely forage-based diets are numerous; however, determining the absolute minimum requirement for long-stem forage in horses is unclear (NRC, 2007). The 1989 NRC committee for horses established a general guideline for minimal intake of long-stem roughage or pasture at 1% of BW/d. Bailey et al. (2003) suggested that insufficient fiber combined with excess starch in equid diets will reduce pH of the hindgut, possibly leading to acidosis. However, the NRC (2007) states that additional studies are needed to quantify the minimum roughage needs of horses.

Providing adequate forages not only aids in maintaining normal gut function, but can also help offset behavioral issues in horses. Continual access to forage that is palatable and high in fiber can reduce boredom and stress. When long-stem forage consumption is restricted, vices such as wood-chewing may result (NRC, 2007). Additionally, forages offer a beneficial buffering capacity in the stomach, potentially preventing ulcers in horses (Pagan, 2009). Murray and Schusser (1993) reported significantly higher mean gastric pH in horses receiving free-choice timothy hay for 24 h as compared to fasted horses.

Forages add bulk to rations, thereby slowing intake. Fiber is more slowly digested than concentrates. Utilizing more fiber rather than starch as an energy source may ultimately reduce the likelihood of developing colic or laminitis (NRC, 2007). There is a large body of circumstantial evidence that suggest insufficient fiber in equid diets can lead to colic (Tinker et. al, 1997) and gastric ulcers (Murray and Schusser, 1993). The peNDF and eNDF concepts that have been developed to maintain production and gastrointestinal health in dairy cattle have not been established in horses. Although research in horses is lacking, there may be a need for a larger particle, slower-digested

fiber to maintain gut homeostasis. Research on the effect of fiber amount and type on digestion is needed (NRC, 2007).

Intake and Rate of Passage

The NRC (2007) states that DMI in horses is controlled by various factors, including DM digestibility, palatability of feed, weather, interaction with other horses, energy requirement, and cell wall content of forage. Rate of passage through the equine gastrointestinal tract can be measured by mean retention time (MRT). A more rapid rate of passage occurs in the stomach and small intestine (about 5 h), while MRT is much longer in the hindgut (35 h on average; Weyenburg et al., 2006). Depending on type of feed, total gastrointestinal MRT in horses have been reported to be as short as 18 to 20 h (Uden et al., 1982) to 45 to 60 h (Cuddeford et al., 1995).

Dulphy et al. (1997) compared the DMI between mature geldings and adult sheep. The authors observed a stronger correlation between forage quality and DMI in sheep ($R^2 = 0.79$) than for horses ($R^2 = 0.63$). The authors concluded that DMI of hay by horses was less influenced by CP, ADF, or NDF content of the hay; but rather affected by organoleptic qualities such as palatability. Aiken et al. (1989) measured the voluntary DMI and digestibility of coastal Bermudagrass hay in yearling and mature horses. The authors observed similar NDF digestibilities between yearling and mature horses. Voluntary DE intakes for yearlings and mature horses were 18.2 Mcal (108% of NRC requirements), and 17.9 Mcal DE (109% of NRC requirements), respectively. The authors suggested that DMI was related more to energy requirement rather than gut fill. These results agreed with that of Frape et al. (1982) who proposed that intake is related more to energy requirement than gut volume in mature horses.

Boulot et al. (1987) reported a 0.65 kg/d greater forage intake by lactating mares compared to pregnant mares. Martin-Rosset et al. (1990) fed grass hay *ad libitum* to dry, pregnant, or lactating draft horse mares, as well as light horse geldings at maintenance level. The authors reported DMI's of 117, 113, and 162 g/kg of BW/d for the mares, respectively. Further, DMI was 100 g/kg of BW/d for the geldings. Edouard et al. (2008) performed a meta-analysis of 45 equine intake and/or digestibility studies. The dataset was analyzed both at group level (to allow comparisons in the literature) and at individual level (to control for individual variability using mixed models). As expected, in both analyses, DM digestibility declined as forage quality decreased. The authors observed at group level, intake had no effect on CP content and DM digestibility, while intake decreased slightly as forage NDF content increased. However, when analyzing individual differences between horses, the authors observed as intake increased, DM and CP digestibilities decreased. In contrast to group level, the individual horse observations showed that intake increased with greater NDF concentrations. Results from this meta-analysis suggested that individual horses may increase their intake as forage quality and digestibility decline, but not all individuals respond the same way to forage quality. Some horses appeared to compensate for a low quality forage by increasing their DMI, while others did not. The authors concluded that horses are selected for traits not associated with feeding behavior and production as in other livestock species, and individual horses are more variable with regard to forage intake.

Digestibility Studies

Digestibility trials are often based upon maintaining a consistent or constant daily feed intake with the goal to minimize day to day variation in fecal excretion (Pond et al., 2005). Digestibility can be simply calculated by measuring the amount of feed consumed and quantity of feces excreted after animals have been accustomed to a diet. The conventional digestion trial is a critical component in determining the digestibility of nutrients in a feed or feed ingredient (Cochran and Galyean, 1986).

$$\% \text{ Nutrient Digestion} = \frac{(\text{nutrient fed (kg)} - \text{nutrient refused (kg)}) - \text{nutrient in feces(kg)}}{(\text{nutrient fed (kg)} - \text{nutrient refused (kg)})} \times 100$$

Some of the earliest digestion trials in horses were conducted by Wolff and others from 1877 to 1895, using few experimental units (Martin-Rosset and Dulphy, 1987). The necessity for more complete information regarding nutritive value and digestibility of various horse feeds was acknowledged by Patterson (1897). Using total-collection trials involving steers, multiple studies were conducted to calculate digestibility of oats, corn, timothy, “new corn product,” and various other feeds. Each trial included a 9-d adaption period followed by a 5-d fecal collection (Patterson, 1897).

Mesochina et al. (1998) used fecal indices to predict digestibility in mature geldings. Prediction equations were developed using data from in-vivo digestibility trials with 4 to 6 horses fed 27 forage-based diets and DM digestibility, OM digestibility, crude ash, CP, and CF contents of diets and feces. The authors observed that fecal CP was the single best predictor of DM digestibility and OM digestibility ($R^2 = 0.59$). Mesochina et

al. (1998) concluded that the most reliable indicator of in-vivo OM digestibility was fecal CP rather than fecal CF.

Total fecal sample collection studies allow digestibility to be measured under different metabolic conditions. Studies utilizing the total collection method are the basis of the French Horse Net Energy System (Miraglia et al., 1999). The influence of physiological state and feeding amount on digestibility rates have been calculated with total fecal sample collection studies (Martin-Rosset et al., 1990). While the NRC (2007) does not report a minimum suggested length for digestion trials, various research groups have established their own protocols. Kentucky Equine Research, Inc., (Versailles, KY) utilizes a standard experimental design involving a 3-wk adjustment period followed by a 5-d total fecal sample collection period (Pagan, 2009). Dependent on the variable measured, different time period requirements for collection studies are reported. Pond et al. (2005) stated that on average feed residues pass through the gastrointestinal tract in $\leq$ 24 to 48 h for most non-ruminants. Consequently, a minimum 3-d collection period is recommended.

In-Vitro Methods

Traditional in-vivo methods of determining digestibility, while accurate, can be costly and time-consuming. As a result, in-vitro methods of determining digestibility have been developed for multiple species (Earing et al., 2010). A two-stage technique for the in-vitro digestion of forage crops was developed by Tilley and Terry (1963) in an attempt to simulate digestion in ruminants. Using a buffer and rumen fluid as the inoculum, feed samples were exposed to inoculum for 48 h to simulate microbial fermentation. Samples were then digested in either pepsin (Tilley and Terry, 1963), or

neutral detergent extraction (Van Soest and Wine, 1967) to remove undigested microbial and feed protein. Meyer et al. (1971) compared four in-vitro methods for predicting in-vivo digestibility of forages in ruminants. Comparisons were made between the two-stage technique of in-vitro digestion of forage crops by Tilley and Terry (1963), the NDF method of Van Soest and Wine (1967), the one-stage in-vitro fermentation method (referred to as the "Wisconsin" method), and the in-vivo artificial rumen method of Fina et al. (1962). The authors reported the methods of Tilley and Terry (1963) and Van Soest and Wine (1967) were the best estimators of in-vivo DM digestibility.

In order to increase efficiency, the Tilley and Terry method has been modified throughout the years to include technology such as the Daisy$^{II}$ Incubator, a batch system that incubates samples (ANKOM, 2005). The Daisy$^{II}$ Incubator employs filter bag technology, which encapsulates samples and prevents filtration errors. Using four digestion vessels rather than test tubes, the Daisy$^{II}$ Incubator also enables batch processing of more than 100 samples at once (ANKOM, 2005). The Daisy$^{II}$ Incubator can determine in-vitro true digestibility, apparent digestibility, rate of digestion, and neutral cellulase gamanase digestibility (ANKOM, 2005).

Utilizing the Daisy$^{II}$ Incubator and other in-vitro technologies require a microbial inoculum prepared from ruminal or cecal fluid extracted from cannulated animals (Lowman et al., 1999). This disadvantage has limited the use of this technique in equine research, as cecally-cannulated horses are not readily available (Earing et al., 2010). However, gut microorganisms in ruminants which are closely associated with plant debris in the rumen are also excreted with plant residues in the feces (Van Soest, 1982; Theodorou et al., 1993). Fecal material remains largely anaerobic after voiding and

the microflora can be viable for several hours after excretion from the digestive tract (Holter, 1991). Therefore, equine feces can also be used as an adequate source of inoculum during in-vitro studies (Lowman et al., 1999).

Results from previous studies have validated the use of the Daisy$^{II}$ Incubator for in-vitro studies with horses. Ringler et al. (2005 a,b) reported in-vitro DM, NDF, and ADF digestibilities using equine fecal inoculum with a Daisy$^{II}$ Incubator. The authors reported digestibilities at 15, 30, 45, and 60 h for alfalfa and timothy hays. The reported means and standard deviation of NDF digestibility for alfalfa were 16.94 $\pm$ 2.84% at 15 h, with subsequent increase until 45 h where NDF digestibility reached a plateau of 40.13 $\pm$ 2.84%. The values for NDF digestibility for the timothy hay were 21.61 $\pm$ 3.28% at 15 h with no significant change at 30 h, then an observed increase to 31.67 $\pm$ 2.84% between 45 and 60 h. The authors observed NDF digestion was significantly greater at 60 h as compared to 45 h.

Lattimer et al. (2007) conducted two experiments to evaluate the use of the Daisy$^{II}$ Incubator in determining the effects of yeast culture preparation on in-vitro microbial populations, diet digestion, and fermentation patterns in horses. In Exp. 1, 4 mature horses were fed a pelleted concentrate and alfalfa cubes in a 50:50 (percent, as-fed) ratio. Fecal samples were taken from each horse to form the inoculum and placed in separate incubation vessels. A 48-h in-vitro fermentation period was used to determine DM, NDF, and ADF digestibilities. In Exp. 2, fecal samples were taken from 4 mature horses consuming either a high concentrate or high fiber diet. Filter bags containing the diets along with respective inoculums were added to the 4 vessels. Yeast culture was added to 2 vessels and all samples were incubated at 24 and 48 h. Data from Exp. 1

indicated that the 0.25 g sample size provided a more accurate estimate of DM digestibility. The authors observed that using both equine feces and the Daisy$^{II}$ Incubator provided an effective and practical method to estimate DM digestibility in horses.

Earing et al. (2010) compared in-vitro digestibility estimates between a Daisy$^{II}$ Incubator using equine feces as inoculum and in-vivo equine DM digestibility and NDF digestibility (NDFD). The study used 4 mature geldings in a 4 x 4 Latin square design experiment and a 2 x 2 factorial arrangement of dietary treatments (timothy hay, alfalfa hay, timothy hay plus oats, and alfalfa hay plus oats). Horses were fed for four 26-d periods. Each period consisted of a 7-d wash-out phase, a 14-d adaptation phase, and a 5-d collection period. Collected feces were composited and evaluated using the Daisy$^{II}$ Incubator. Three incubation periods (30, 48 and 72 h) were evaluated. In-vitro NDFD for alfalfa were 13.1, 23.8, and 37.9% at 30, 48, and 72 h, respectively. In-vitro NDFD estimates for the alfalfa-oat diet were slightly lower. In-vitro NDFD for the timothy diet were 5.1, 21.5, and 31.0% at 30, 48, and 72 h, respectively. The authors reported $R^2$ values for in-vitro DM digestibility of 0.71, 0.70, and 0.63 for 30, 48, and 72 h of incubation, respectively, compared to in-vivo estimates. However, $R^2$ values for in-vitro NDFD were 0.18, 0.06, and 0.06 at 30, 48, and 72 h, respectively, compared to in-vivo estimates. The authors stated that when using Daisy$^{II}$ Incubator for estimating digestibility for diets higher in fiber, a longer incubation period may be necessary.

The authors concluded that accurately predicting in-vivo NDFD based on data from in-vitro digestion experiments in horses was difficult. The authors reported that it was unclear how close predictions of DM digestibility in-vitro and in-vivo could be

developed, but not reasonable estimates of NDFD, given that NDF constitutes a large proportion of the DM in each of the diets.

In-Vivo Methods

*Indigestible Marker Usage*

The use of indigestible markers in horses permit the measurement of digestibility in pasture or grazing studies. Lignin, digestible fibers, and acid insoluble ash have been used as internal markers in equine studies (Miraglia et al., 1999). An ideal digestibility marker is one that mimics a feed's passage through the gastrointestinal tract, but remains inert (Saastamoinen, 2012). Marker studies use multiple nutrient digestibility calculations with small amounts, increasing the room for error. Marker methods are ideal if few samples of feces can be collected (e.g. grazing animals), but the proportions of recovered marker vary. As the accuracy of total feces voided is much higher, the calculations are therefore much more reliable in total collection studies.

Miyaji et al. (2014) used 8 adult TB horses assigned a high (2.0 KgDM/100kg BW) or low (1.3 Kg DM/100 KG BW) intake of Timothy hay intake to study fiber digestion and digesta retention time in the hindgut of horses on an all-forage diet. Total mean retention time was measured from d 11 to 15 using Co-EDTA and Yb-labeled marker. Horses were euthanized on d 17, gastro intestinal tracts removed, and ingesta measured. Digesta were collected from the ileum, cecum, right ventral colon, left ventral colon, left dorsal colon, right dorsal colon, and small colon. Fiber digestibility, the MRT in each hindgut segment, VFA concentration, and fibrolytic activity were determined on the extracted digesta. Digestibilities of NDF and ADF in the total gastrointestinal tract were lower for high intake as compared to low intake. The digestion of NDF and ADF

mostly occurred in the cecum, right ventral colon, and left ventral colon, which accounted for 90% of total tract digestion. There were no differences reported in VFA concentrations or proportions in any part of the gastro intestinal tract due to treatment. Fibroytic activity was highest in the cecum and right ventral colon, but decreased significantly beyond the left ventral colon. Results indicated there was a reduction in total mean retention time with higher feed intake and that the cecum and right ventral colon are the primary sites of fiber digestion. The authors suggested that high hay intake may increase the amount of digesta that bypasses the cecum and flows directly into the right ventral colon from the ileum without cecal digestion, thus decreasing fiber digestibility.

*Equine Total Fecal Collection Trials*

Prior to the 1960's, numerous forage digestion studies involving ruminants had been conducted; however, research in equine forage digestibility was needed. Fonnesbeck et al. (1967) evaluated the digestibility of several forages by equines. Pennscot red clover, Lincoln bromegrass, Atlantic alfalfa, Reed Canarygrass, Alta fescue, Midland Bermudagrass, Timothy, and Orchardgrass were fed to mature horses in two 6 x 6 Latin square design experiments. The authors observed that horses consuming legume hays had significantly greater DMI and CP digestibility as compared to horses consuming grass hays. Additionally, the authors reported legume hays contained more digestible nitrogen free extract, resulting in more digestible TDN and DM. However, in comparison to the other forages, alfalfa contained significantly less digestible NDF and ether extract. The authors reported the nutritive value and quality of legume hays was much greater than that of grass hays.

Cymbaluk (1990) compared intake and forage digestibility between cattle and horses. Two trials were conducted using 6 growing beef steers and 6 crossbred, mature geldings in a replicated 6 x 6 Latin square design. In Exp. 1, digestibilities between cattle and horses were compared using the following 6 hays: Altai wildrye, smooth bromegrass, crested wheatgrass, Kentucky bluegrass, oat hay, and reed canarygrass. In Exp. 2, DMI's and nutrient digestibilities were compared in the same animals consuming either alfalfa hay or alfalfa pellets. In Exp. 1, the 27-d period consisted of an ad-libitum feeding period for 14-d, a 9-d restricted feeding period in which animals were restricted to 85% of voluntary intake, and a 4-d total collection period. Feed and feces were analyzed for DM, GE, CP, ADF, NDF, Ca, and P. The authors reported that cattle digested more DM, GE, ADF, NDF, and P, but less Ca from hays used in Exp. 1 than horses. In Exp. 1, cattle digested 28 to 82% more NDF and ADF from grass and oat hay than horses. In Exp. 2, cattle digested more DM from alfalfa hay than from the dehydrated alfalfa pellets. However, in Exp. 2, both species digested equivalent amounts of NDF from pelleted and long alfalfa, and ADF from dehydrated alfalfa. The authors reported that the lower fiber digestibilties observed in horses may be due to the reduced cellulolytic activity of equine cecal microflora compared to the microflora in the bovine rumen as reported by Kern et al. (1974). The authors reported DMI of hay by cattle was related to NDF content; however, no single nutrient predicted DMI by horses.

Crozier et al. (1997) measured the digestibility, mineral absorption, and voluntary intake of alfalfa, Tall Fescue, and Caucasian Bluestem fed to 6 mature Arabian geldings. The geldings were blocked by weight and used in a replicated Latin square design experiment. To determine intake, geldings were fed ad libitum for 5 d before feeding a

restricted diet. After the 15 d dietary adjustment phase, geldings were fitted with collection harnesses for a 5 d total fecal collection period. Feed, orts, and feces were weighed and analyzed for CP, NDF, ADF, cellulose, and lignin. In-vitro DM digestibility was determined on feed samples using rumen fluid from an Angus steer (Tilley and Terry, 1963 as modified by Barnes, 1966). The authors reported that DMI of alfalfa hay was greater than the mean of the grass hays, and that DMI between the grasses was not different. Both apparent and in-vitro DM digestibility were higher for alfalfa than the mean of the grass hays. The authors observed that NDF digestibilities of alfalfa did not differ compared to the grass hays, and NDF digestibilities did not differ between the grasses.

Aiken et al. (1989) compared the voluntary intake and digestibility of Coastal Bermudagrass hay between yearling and mature horses. Four yearlings and 4 mature geldings were fed at 90% of ad libitum intake during a 4 d adjustment period, followed by a 4 d total collection period. While DMI was slightly higher in yearlings as compared to mature horses, there was no significant difference in DM digestibility observed between the 2 age groups. There were no observed differences in DM, CP, or NDF digestibilities between the two groups. Due to the typical high cell wall content (cellulose, hemicellulose, and lignin) and degree of lignification of coastal Bermudagrass hay, the authors anticipated low ADF digestibilities for both age groups. The authors reported that yearling and mature horses were similar in their ability to digest coastal Bermudagrass hay, and concluded that hay digestibility values attained from mature horses could be applied to yearlings.

LaCasha et al. (1999) measured voluntary intake and digestibility of OM, CP, and fiber components of Matua bromegrass, coastal Bermudagrass, and alfalfa hay in yearling horses. Eighteen Quarter Horse yearlings were blocked by sex and weight, and used in a randomized complete block design experiment. Horses were fed ad libitum during a 15 d trial period followed by a 5 d total urine and fecal collection. Feed and fecal samples were analyzed for CP, NDF, ADF, cellulose, lignin, and AIA. Voluntary intake of DM was greater for alfalfa (10.9 kg/d) than for the mean of the grasses, and intake of Matua (10.0 kg/d) was greater than that of coastal Bermudagrass (7.4 kg/d). Apparent digestibility of OM was greater for alfalfa (74%) than for the mean of the grasses, but did not differ between Matua (64%) and coastal Bermudagrass (60%). The authors reported that the apparent NDF digestibility for alfalfa was lower than the mean of the grasses. The authors reported that the difference may be related to low total cell wall content of the alfalfa hay, reducing rate of passage.

Sturgeon et al. (2000) studied the digestibility and mineral availability of Matua, coastal Bermudagrass, and alfalfa hay in mature horses. Six mature stock-type geldings were utilized in 2 simultaneous 3 x 3 Latin square design experiments. Horses were housed in pairs and fed at 2% BW/d. Experimental periods consisted of a 10 d dietary adjustment period followed by a 4 d total sample collection period. Body weight of horses was measured every other day, and amount of hay offered and refused was measured and recorded daily. Feed and feces were analyzed for DM, CP, Ca, P, Mg, ADF, and NDF. The authors reported that horses consuming alfalfa had greater apparent DM digestibility (63.40%), compared to coastal Bermudagrass (51.60%). However, Sturgeon et al. (2000) reported DM digestibility of alfalfa and Matua hay (63.40% and

58.70%) were not different. A similar NDF digestibility was observed for geldings consuming coastal Bermudagrass hay and alfalfa (51.69 and 50.14%).

## Statement of the Problem and Objective

Traditional in-vivo digestibility procedures, while accurate, can be relatively costly and time consuming. Previous studies have validated the use of NIR to predict digestibility in multiple species. Very few studies have reported the successful application of NIR to predict intake and NDF digestibility in horses. Therefore, the objective of the current study was to investigate the applicability of NIR to predict DMI and in-vivo NDF digestibility in mature geldings consuming an all-forage diet.

# CHAPTER III

# MATERIALS AND METHODS

## Horse Care and Management

Nine mature, sedentary stock-type geldings, ranging from 10 to 20 yr of age were randomly assigned treatments within 3 simultaneous 3 x 3 Latin square design experiments to compare intakes and digestibilities of mixed grass hay (MGH), coastal Bermudagrass hay (C), and alfalfa hay (A). Horses were housed in 3 groups of 3 in 60 x 20 m dry-lot pens at the West Texas A&M University Horse Center, Canyon, TX.

Horses were fed at 0700 and 1900 in 2 x 5 m individual feeding bunks. Horses were allowed 3 h to consume rations before being turned out into 6 x 20 m pens for both morning and evening feedings. Horses were turned out in a 40 x 60 m dry-lot paddock between morning and evening feedings 3 times per wk. Horses were fed hay initially at 3.0% BW/d for the first 2 d of the trial, and feed offered was adjusted on d 3 based on avg intakes. In order to minimize hay wastage, the amount of hay offered was adjusted to achieve no more than 0.45 to 0.91 kg of orts per feeding.

Horses were weighed prior to the morning feeding on d 0 and every other day thereafter. Body condition score was assessed the week prior to the study and weekly thereafter. Water was available ad libitum using buckets hung in individual feeding bunks and automatic waterers in group pens. Routine deworming, farrier care, and vaccinations

were administered prior to the study. All horses in the study were classified under "Maintenance" according to the NRC (2007). Experimental protocol for this trial was approved by the WTAMU Institutional Animal Care and Use Committee.

Experimental Treatments

The 51-d study consisted of three 17-d periods. Each period consisted of a 4-d dietary adjustment period, a 10-d feeding period, and a 3-d total fecal collection period. Horses were fed individually in 2 x 5 m concrete bunks, separated using sheet-metal cut to fit so that horses could not share feed. Hay offered and orts were weighed daily and weights recorded. Feed, orts, and feces were weighed using a M3315 Multifunctional Scale (Escali Products, Burnsville, MN).

Nutrient analysis for treatment hays are listed in Table 1. Hays were selected based on availability and differing sulfite and ash corrected NDF (aNDFom) concentrations. Alfalfa hay was harvested and baled in Deming, NM, while C was obtained from a producer in Patagonia, AR. Both A and C were in the form of small 2 string square bales that were approximately 22.7 kg. The MGH was composed of 4 different types of grasses, Side Oats Grama, Timothy, Orchard, and Brome grasses. Round bales of the MGH were obtained from a producer in Kansas. To meet Equine NRC (2007) requirements for CP and DE, 0.68 kg of ground soybean meal (Hi Pro 47.5% Bagged Soybean Meal, Hi Pro Feeds, Friona, TX, USA) was fed daily to horses receiving MGH in plastic flat-back 18.9 L buckets hung from the feed bunk rail.

## Sample Collection

### *Hay Collection*

Prior to the study, treatment hay samples were collected using a Penn State Forage Sampler, (Scientific Systems, Inc., Ft. Atkinson, WI.). Multiple core samples of each hay were collected and composited. Samples were sent to Dairy One Forage Lab (Ithaca, NY) for analysis. During the fecal collection period, orts were weighed and a representative sample was taken per feeding. At the end of each collection, composite samples of orts were developed using the quartering method (Zhang and Redfearn, 2015). Composite orts samples were labeled by horse, time, period, and treatment.

### *Fecal Collection*

Total fecal collections were conducted for 72 h during the last 3 days of each period. Rubber mats and plastic tarps (2 x 2 m) were placed under each horse and total feces excreted were collected and weighed. Mats were sloped or angled so that minimal contamination of feces with urine occurred. Total feces were collected every h, weighed, and mixed together thoroughly with previous feces in a 18.9 L bucket for 24 h. At the end of each 24 h period, feces were mixed thoroughly in a large plastic container and a representative sample of 0.64 kg was taken. The samples were placed in Ziploc freezer bags and labeled with horse, period, time, and treatment, then frozen at -20°C for later analysis. At the end of each 72-h collection period, the three 24-h fecal samples were thawed to room temperature and thoroughly mixed to obtain a 72-h composite sample for each horse and collection period.

Laboratory Analysis

*Hay Sample Preparation*

Laboratory procedures were performed at Dairy One Forage Lab (Ithaca, NY) and the Ruminant Nutrition Lab, Killgore Research Center, West Texas A&M University (Canyon, TX). Representative composite samples from each collection period were weighed prior to placement in a drying oven (Model D-2631-Q, Big M Electric CO., Blue Island, IL) at 55°C for 48 h. Dried samples were ground through a 2-mm screen (Wiley-Mill Model 4, Thomas-Wiley Mill, Philadelphia, PA). Hay samples remitted to Dairy One Forage Lab were ground through a 1- mm screen using a UDY cyclone Mill (UDY Corporation, 201 Rome Court, Fort Collins, CO 80524). Ground hay samples were scanned using a Foss NIRSystems Model 6500 NIR with Win ISI II v1.5 (Foss NIRSystems, 7703 Montpelier Road, Suite 1, Laurel, MD 20723) to determine component concentrations.

*Dry Matter Determination*

The DM Procedure was adapted from methods outlined by Van Soest (1967). Fecal samples were thawed at room temperature. The weight of a weigh boat was recorded ($W_1$), then the fecal or hay sample was placed in the weigh boat and the weight recorded ($W_2$). All samples were dried in a forced-air oven at 55°C for 48 h. Samples were removed from the oven and the weight was recorded ($W_3$). The percent DM was then calculated as:

$$\% \text{ Total DM} = [((W_3-W_1)/(W_2-W_1)) * 100]$$

*Fecal Sample Preparation*

After weighing, dry composite fecal samples were mixed thoroughly for each horse to prevent particle segregation and potential sample bias. Fifteen grams of fecal sample were placed into a Ziploc bag, labeled by horse, treatment, period, and date. Duplicate samples were also prepared and stored for future use. Samples were mailed to Dairy One Forage Lab, Ithaca, NY. Upon arrival, fecal samples were ground through a 1 mm screen using a UDY cyclone Mill (UDY Corporation, 201 Rome Court, Fort Collins, CO 80524).

*Analysis by Near Infrared Reflectance Spectroscopy*

Hay components that were analyzed by NIR included DM, CP, soluble protein (SP), RDP, Acid Detergent Insoluble Protein (ADI-CP), Neutral Detergent Insoluble Crude Protein (NDI-CP), ADF, amylase and sodium sulfite treated Neutral Detergent Fiber (aNDF), lignin, starch, Water Soluble Carbohydrates (WSC), Ethanol Soluble Carbohydrates (ESC), fat, ash, Ca, P, Mg, K, S, Cl, NDF Digestibility (NDFD) at 24, 30, and 48 h, starch digestibility, aNDF on an OM, ash free basis (aNDFom); undigestible NDF on OM, ash free basis at 30, 120, and 240 h (uNDFom); and NDF Digestibility on an OM, ash free basis at 30, 120, and 240 h (NDFDom).

*Neutral Detergent Fiber Analysis of Orts*

To determine if sorting occurred, ort samples were tested for NDF using the Filter Bag Technique (Method 6; ANKOM Technology, 2011). A filter bag was labeled with a solvent-resistant marker and weighed (W1). Duplicate 0.45 to 0.55 g samples (W2) were weighed for each ort sample and placed into filter bags and sealed with a heat sealer 4-mm from the top of the bag. One blank bag was weighed and included in the sample

analysis to determine blank bag correction ($C_1$), to serve as an indicator of particle loss. The samples were placed into the bag suspender, and all 9 trays were used. To ensure submergence, the bag suspender was placed into the fiber analyzer vessel with a weight placed on top.

After filling the analyzer with samples, 2,000 mL of neutral detergent solution, 20 g $Na_2SO_3$ (sodium sulfite) and 4 mL of alpha amylase were added to the vessel. Sodium sulfite was added to the vessel to remove protein (Mertens, 2002). The addition of alpha amylase was used for the prevention of starch contamination (Mertens, 2002). The agitate and heat buttons were turned on and the timer was set for 75 min. The drain valve was then opened slowly and hot solution was exhausted after 75 min.

Once the solution was completely emptied, the exhaust valve was closed and lid opened. To rinse the detergent solution from the samples, 1900 mL of 70 to 90°C of rinse water and 4.0 mL of alpha amylase were added to the analyzer vessel. The agitate and heat was turned on and timer set for 5 min. The process was repeated 2 more times, with the final rinse excluding the alpha amylase. After the final rinse, the samples were removed and excess water was pressed out of the bags. Bags were placed into a 250 mL beaker and acetone was added with enough to cover the bags for 3 to 5 min. A rubber cork was placed on top to ensure submergence. The bags were removed and placed on a wire screen to air-dry for 45 min. The bags were then placed in an industrial drying oven (Model D-2631-Q, Big M Electric CO., Blue Island, IL) at 55° C for 3 to 4 hrs. Bags were removed from oven and placed into a collapsible desiccant pouch to flatten, remove air, and cool. Dried sample bags were weighed and recorded ($W_3$). The percent NDF fraction was calculated by the following equation:

$$\% \text{ NDF} = \frac{(W_3 - (W_1 \times C_1))}{W_2} \times 100$$

*Neutral Detergent Fiber on an Organic Matter, Ash Free Basis (aNDFom) on Ort and Fecal Samples*

Samples were subsequently analyzed for aNDFom using the addition of an ashing step to remove inorganic materials including minerals, soil, and silica. Following the NDF extraction, the fiber residue was ashed for 450°C for 8 h to burn off OM, leaving the ash behind. The residual ash was subtracted from the fiber residue to determine NDF on an ash-free, or OM, basis.

*Fecal Analysis*

To determine dry matter, the 1 mm ground fecal samples were scanned using a Foss NIRSystems Model 6500 NIR with Win ISI II v1.5 (Foss NIRSystems, 7703 Montipelier Road, Suite 1, Laurel, MD 20723).

*Neutral Detergent Fiber on Fecal Samples*

Fecal samples were tested for NDF using the Filter Bag Technique that was similar to the method for orts described above (Method 6; ANKOM Technology, 2011). Samples were weighed individually at 0.5 g into filter bags and digested for 75 min in 2,000 mL of neutral detergent solution in the ANKOM$^{200}$ Digestion Unit. Four mL of alpha amylase and 20 g of $Na_2SO_3$ were added to each vessel. Samples were rinsed 3 times with 1900 mL of 70 to 90°C of rinse water for 5 min. Four mL of alpha amylase were added to the analyzer vessel for the first and second rinses. Following the water rinses, samples were soaked in acetone for 3 min and then dried at 105°C for 2 h.

*Analysis of Fecal Undigested Neutral Detergent Fiber on an Organic Matter, Ash Free Basis (uNDFom)*

Three sets of 3 fecal samples were weighed individually at 0.5 g into 9 filter bags to determine uNDFom at 30, 120, and 240-h time points. Samples were subsequently analyzed with the addition of an ashing step to remove inorganic materials including minerals, soil, and sand by burning the undigested fibrous residue at 550°C for 2 h. Undigested NDF is expressed on an OM (ash free) basis as a percentage of the DM at 30, 120, and 240-h time points to reflect rates of undigested NDF digestibility.

*Neutral Detergent Fiber Digestibility on an Organic Matter, Ash Free Basis (NDFDom) on Fecal Samples*

The aNDFom and uNDFom values are used together to calculate the NDFDom. The results of NDFom are expressed on an OM (ash free) basis as a percentage of the aNDFom at 30, 120, and 240-h time points that reflect rates of NDF digestibility. The following formula was used to calculate NDFDom:

$$NDFDom = ((aNDFom - uNDFom)/aNDFom) * 100$$

*Neutral Detergent Fiber Digestibility (NDFD) on Fecal Samples*

To develop in-vivo digestibility estimates, fecal samples were tested for in-vivo true digestibility using the Daisy$^{II}$ Incubator (ANKOM Technology Method 3, ANKOM Technologies, Macedon, NY). Filter bags were pre-rinsed in acetone for 3 to 5 min and air dried. The acetone rinse removes a surfactant that inhibits microbial digestion. Each F57 filter bag was weighed and recorded ($W_1$). Dried, 1-mm ground fecal samples were

weighed at 0.25 g ($W_2$) directly into filter bags. Filter bags were then sealed using Impulse bag sealer (1915/1920 Heat Sealer, Uline, 12575 Uline Drive, Pleasant Prairie, and WI 53158). Samples were placed in the Daisy$^{II}$ Incubator digestion jar. Samples were evenly distributed on both sides of the digestion jar divider. One sealed blank bag was used for a correction factor ($C_1$). For each digestion jar, 2 buffer solutions (labeled buffer solution A and B) were combined. Buffer solution A contained 10 g of $KH_2PO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.1 g $CaCl_2 \cdot 2H_2O$, and buffer solution B contained 0.5 g Urea (reagent grade), 15 g $Na_2CO_3$, and 1 g $Na_2S \cdot 9H_2O$.

Buffer solution was pre-warmed to 39°C. In a separate container, 266 mL of buffer solution B was added to 1,330 mL of buffer solution A in a 1:5 ratio. The exact amount of A to B was adjusted to obtain a final pH of 6.8 to 39°C. After combining both the A and B solutions, 1,600 mL of combined solution was added to each digestion jar. The digestion jars were added to the Daisy$^{II}$ Incubator and the heat and agitation switches were turned on. Temperature of digestion jars were equilibrated for at least 20 to 30 min.

The inoculum used was rumen fluid collected from a total mixed ration fed, high producing, ruminally-cannulated dairy cow from a farm 8 mi from the Dairy One Forage Lab. At least 2,000 mL of rumen inoculum was poured into 2 pre-heated thermos bottles. Approximately 2 "fistfuls" of fibrous mat from the rumen were added to the thermos bottles. The thermos contents were added to a blender preheated at 39°C. The blender container was purged with $CO_2$ gas and contents were blended at high speed for 30 s. The blending action served to dislodge microbes that are attached to the mat and assured a representative microbial population for the in-vitro fermentation. The blended digesta were then filtered through 4 layers of cheesecloth into a 5 L flask that was pre-heated to

39° C. The remaining rumen fluid in the other thermos was filtered through 4 fresh layers of cheesecloth into the same 5 L flask. Extra cheesecloth was used around the edges of the flask to facilitate squeezing contents of filtered mat. The flask was continually purged with $CO_2$ during the transfer of the inoculum.

One digestion jar was removed from the Daisy$^{II}$ Incubator and 400 mL of inoculum was added to the buffer solution and samples. The digestion jar was purged with $CO_2$ gas for 30 s prior to securing the lid. The process was repeated for all digestion jars used. The fecal samples were incubated at 24, 30, and 48 h at 39.5°C. Once incubation was complete, jars were removed and liquid drained. The filter bags were rinsed with cold tap water until the water was clear. Samples were placed in the ANKOM$^{200}$ Fiber Analyzer and the aNDF procedure was used to remove bacterial contamination. The remaining residue was undigested fibrous material and used to determine in-vitro true digestibility and NDF digestibility.

Following the aNDF procedure, samples were weighed and recorded as $W_3$. The following calculations were performed to determine in-vitro true digestibility on an as received basis and in-vitro true digestibility on a DM basis:

$$\% \text{ IVTD (as received basis)} = \frac{100 - (W_3 - (W_1 \times C_1)) \times 100}{W_2}$$

$$\% \text{ IVTD}_{DM} \text{ (DM basis)} = \frac{100 - (W_3 - (W_1 \times C_1)) \times 100}{(W_2 \times DM)}$$

Where:

$W_1$ = Bag tare weight
$W_2$ = Sample weight
$W_3$= Final bag weight after In Vitro and sequential ND treatment
$C_1$ = Blank bag correction (final oven-dried weight/original blank bag weight)

Statistical Analysis

Data for DMIs and digestibilities were analyzed using the Mixed Procedure of SAS v. 9.4 (2014; SAS Institute, Inc., Cary, NC), with period and treatment as main effects. The effect of horse was included in the statistical model as a random effect. All means were compared using least squares means with a Tukey PDIFF adjustment to detect differences in treatment means. Simple correlations were calculated between DMI, in-vivo NDF digestibility, fecal aNDFom content, and NIR estimates using PROC CORR procedure of SAS. Prediction model data for DMI and NIR predictions were developed using PROC REG procedure of SAS. Significant differences were declared at $P < 0.05$, while trends were declared at $P < 0.10$.

# CHAPTER IV

## RESULTS AND DISCUSSION

Nutrient content of hays are listed in Table 1. Horses consuming A and C met NRC requirements for DE, CP, Ca, and P. To ensure similar DE and CP intakes, horses on MGH were also supplemented ground soybean meal at 0.68 kg per day. Horse BW averaged 500 $\pm$ 10 kg, and BCS scores averaged 5.0 $\pm$ 1.0 throughout the study (Table 2; Fig. A-1, A-2, and A-3). Least squares means and differences in least squares means for BW are listed in Tables B-1 and B-2, respectively. There was no main effect of treatment ($P$ = 0.95) on overall mean BW or ($P$ = 0.23) BCS.

Overall mean DMI and DMI as a percentage of BW for Alfalfa (A), Coastal Bermudagrass (C) and Mixed Grass Hay (MGH) are listed in Table 2. Least squares means and differences in least squares means for DMI are listed in Tables B-3 and B-4, respectively. It is important to note that in the current study, horses were not fed ad-libitum due to a restriction of time; therefore, DMI data may be confounded.

There was a main effect of treatment ($P$ = 0.02) on overall mean DMI. Horses consuming A had a greater mean DMI ($P$ = 0.02) as compared to horses consuming MGH (7.31 vs. 5.41 kg/d). Overall mean DMI for horses consuming A and C (7.31 vs. 6.79 kg/d) were similar ($P$ = 0.71). There was a trend for horses consuming C to have greater ($P$ = 0.09) DMI than for horses consuming MGH (6.79 vs. 5.41 kg/d). There was

a main effect of treatment ($P = 0.02$) on overall mean DMI as a % of BW. Horses consuming A and C had a greater mean DMI as a percent of BW ($P = 0.01$) as compared to horses consuming MGH (1.4 vs. 1.0% /kg BW, respectively). Dry matter intakes as a percentage of BW for horses consuming A and C (1.3 vs. 1.0% /kg BW) were similar ($P = 0.58$). These data are in agreement with LaCasha et al. (1999), who reported yearlings consuming alfalfa had greater DMI ($P = 0.01$) as compared to those consuming Matua Bromegrass and coastal Bermudagrass hays.

Intake data from the current study showed significant variation within treatments, which agree with the NRC (2007), which states DMI is controlled by various factors that can include palatability of feed, DM digestibility, weather conditions, interaction with other horses, energy requirement, and cell wall content. Data from the current study partially agree with Edouard et al. (2008) who suggested that DMIs among horses were dependent on individual horse differences rather than forage quality. The authors concluded that horses compensate for reduced forage quality by increasing their DMI. Data from the current study indicate that horses actually decreased DMI in response to decreased forage quality; however, differences in DMI between individual horses were observed. Results from the current study suggest that while NDF content of forage influences DMI in horses, other factors including palatability and individual preferences may also impact DMI.

Overall mean DM digestibilties for A, C, and MGH are shown in Table 2. The least squares means and differences in least squares means tables for DM digestibility are shown in Tables B-5 and B-6, respectively. There was a main effect of treatment ($P = 0.01$) on overall mean DM digestibility. Horses consuming A had a greater overall mean

DM digestibility ($P$ = 0.01) than horses consuming MGH (60.81 vs. 34.31%). These data are similar to values reported by LaCasha et al. (1999), who observed a 63.0% apparent DM digestibility of alfalfa when fed to yearling horses. Horses consuming A had a greater DM digestibility ($P$ = 0.04) than horses consuming C (60.81 vs. 49.60%). These data are in agreement with Sturgeon et al. (2000), who reported that mature stock type geldings consuming alfalfa had greater apparent DM digestibility (63.40%), compared to Bermudagrass (51.60%). Horses consuming C had a greater DM digestibility ($P$ = 0.01) than horses consuming MGH (49.60 vs. 34.31%). Coastal Bermudagrass hay DM digestibility was similar to that reported by Aiken et al. (1989), who reported apparent DM digestibilities of coastal Bermudagrass hay when fed to yearlings and mature horses (43.3 and 43.0%, respectively). Data are also comparable to LaCasha et al. (1999), who reported a 46.0% apparent DM digestibility of coastal Bermudagrass hay when fed to yearlings.

Overall mean in-vivo NDF digestibilities are listed in Table 2. The least squares means and differences in least squares means tables are shown in Table B-7 and B-8, respectively. There was a main effect of treatment ($P$ = 0.01) on NDF digestibility. Horses consuming C had greater ($P$ = 0.04) overall mean NDF digestibility as compared to horses consuming A (47.59 vs. 39.58%). Horses consuming C also had greater overall mean NDF digestibility ($P$ = 0.02) as compared to horses consuming MGH (47.59 vs. 38.50%). The lower NDF digestibility of A compared to C in the current study could be explained by differences in rate of passage, as a greater retention time increases digestibility (Mertens and Ely, 1982). Passage rate is influenced by water holding capacity of the plant (Weyenberg et al., 2006). It was observed that horses consuming B

had greater water consumption than horses consuming A. Grass hay has a greater hemicellulose content and absorbs more water than alfalfa (Cuddeford et al., 1992). Treatment C contained more hemicellulose and absorbed more water, which possibly slowed rate of passage; therefore, NDF digestibility increased.

There were no differences in overall mean NDF digestibility ($P = 0.94$) between horses consuming A and horses consuming MGH (39.58 and 38.50%). These data partially agree with Crozier et al. (1997) who reported that NDF digestibilities in Arabian geldings consuming alfalfa did not differ when compared to those consuming Tall Fescue and Caucasian Bluestem, and NDF digestibilities did not differ between the grasses. The observed differences in NDF digestibilities of the grasses in the current study may be attributed to the differences in quality and NDF content of C and MGH. The CP content (3.7%) and NDF with a sulfite and ash correction (aNDFom) content (71.4%) of the MGH indicated an extremely low quality forage. The data also partially agree with LaCasha et al. (1999), who reported NDF digestibilities of alfalfa, Matua Bromegrass, and Coastal Bermudagrass as 24, 47, and 52%, respectively, when fed to yearling horses.

Correlations between DMI and NIR hay analysis are listed in Table 3. Dry matter intake was negatively correlated ($P = 0.02$) with aNDFom (r = -0.44) and undigested NDF at 30 h (uNDF30) (r = -0.45), indicating that as aNDFom and uNDF30 of the hay increased, DMI decreased. Dry matter intake was positively correlated ($P = 0.01$) with in-vitro NDF digestibility at 30 h (NDFD30) (r = 0.50), which suggest that DMI increased as NDF digestibility increased. This data is in agreement with Oba and Allen (1999) who reported that as NDF digestibility of a forage increased by 1%, a 0.17-kg increase in DMI in dairy cattle would result. These data partially disagree with Cotanch et al. (2014), who

reported that DMI was unaffected by daily uNDF intake in forages fed to dairy cattle. The authors determined that DMI was driven more by potentially digestible NDF and rate of digestion rather than uNDF; however, uNDF can be useful to calculate potentially digestible NDF. The current study did not calculate potentially digestible NDF; however, future research is needed to determine the effect uNDF has on fermentation to reach a better understanding of fiber digestion rates in horses.

Prediction equations using simple regression for DMI in relation to NIR hay analysis are listed in Table 4. The linear regression graphs with corresponding equations and $R^2$ values are shown in Fig. A-4 and A-5. At a 5% significance level, the slopes for both aNDFom and uNDF30 were different from 0. Dry matter intake was significantly ($P = 0.02$) related to aNDFom with $R^2 = 0.17$, which indicated that 17% of the variation in DMI was explained by aNDFom content of hay. This data partially agrees with St. Lawrence et al. (2001) who observed a strong relationship ($r = 0.86$, $P = 0.01$) between DMI and NDF content of grass hays consumed by mature horses.

Dry matter intake was significantly ($P = 0.02$) related to hay uNDF30 with $R^2 = 0.17$, which indicated that 17% of the variation in DMI was explained by uNDF30 content of hay. Data in the current study partially disagree with Jones (2014), who determined that uNDF30 predicted DMI of forages in dairy cattle. Differences in data between previous and current studies are likely due to variances in rate of passage between horses and cattle, as well as differences in factors affecting DMI in horses. Additionally, differences in particle retention time for grasses vs. legumes can cause predicted uNDF30 to not correspond to actual uNDF30 (Jones and Siciliano-Jones,

2014). Because legumes have a lower cell wall content and a greater rate of passage, the use of alfalfa in the current study possibly impacted these results.

Correlations between in-vivo NDF digestibility, aNDFom content of feces, and NIR predictions from hays are listed in Table 3. Correlations between in-vivo NDF digestibility and NIR analysis of hays were not significant for aNDFom ($P = 0.61$), uNDF30 ($P = 0.65$), or NDFD30 ($P = 0.88$). Correlation coefficients for in-vivo NDF digestibility were r = 0.10 for aNDFom, r = 0.09 for uNDF30, and r = 0.02 for NDFD30. Overall, the hay NIR analyses were poor predictors of in-vivo NDF digestibility. However, aNDFom content was highly correlated with uNDF30 and in-vitro NDFD30. Correlation coefficients for aNDFom content of hay were r = 0.99 for uNDFom30 and r = -0.68 for NDFD30. These data suggest that as aNDFom content of the hay increased, uNDFom content increased, while in-vitro NDF digestibility decreased. These data agree with Andres et al. (2005), who reported correlations between NDF content and in-vitro NDF digestibility (r = -0.69), in-situ NDF digestibility at 24 h (r = -0.74), and in-situ NDF digestibility at 48 h (r = -0.78) in dairy cattle.

Correlations between aNDFom content of feces and NIR predictions from hays were significant for aNDFom ($P = 0.01$), uNDF30 ($P = 0.01$), and NDFD30 ($P = 0.01$). Correlation coefficients were r = 0.79 for aNDFom, r = 0.78 for uNDF30, r = -0.68 for NDFD30. The data suggest that as aNDFom and uNDF30 content of hay increased, aNDFom content of feces also increased. The data also suggest that as NDF digestibility increased, NDFom content of feces decreased. Data from the current study agree with Van Amburgh et al. (2015), who reported a 1:1 relationship between uNDF intake and uNDF excretion in dairy cattle.

Data from the current study partially disagree with Park et al. (1998), who reported $R^2 = 0.79$ prediction for NDF digestibility compared to in-vivo NDF digestibility in sheep. Differences in correlation coefficients between studies these may be explained by treatment differences (the previous study used 36 grass silages), greater replication (72 wethers were used), and greater predictability of in-vivo NDF digestibility in ruminants, possibly related to slower rate of passage in ruminants. Data in the current study partially disagree with work by Andrieu and Martin-Rosset (1995), who observed $r = 0.98$ correlation between in-vivo OM digestibility and OM digestibility prediction from NIR in horses. Organic matter digestibility includes digestible cell contents and digestible cell wall of forages, and excludes water and inorganic minerals (Forejtova et al., 2005). The greater correlation coefficient generated in this study may be attributed to a greater ability of NIR to predict OM digestibility, and that multiple regression was performed in the previous study.

Data in the current study partially agree with Mentink et al., (2006) who reported that in-vitro NDF digestibility using a total mixed ration (TMR) was not predicted well by NIR. While the $R^2$ value was 0.59 for NIR to predict in-vitro NDF digestibility, the standard error of calibration and standard error of prediction were relatively high (3.18 and 3.81, respectively). The authors attributed the inability for NIR to predict in-vitro NDF digestibility to reference method error, due to a high repeatability standard deviation of the reference method of 3.2%. Because the TMR contained a mixture of forages and grains, it was more difficult to predict in-vitro NDF digestibility with NIR as compared to a single forage. Data in the current study partially disagree with Brogna et al. (2009), who observed that NIR could provide accurate predictions of in-vitro NDF

digestibility using alfalfa. The authors reported an $R^2$ value of 0.87 for the prediction of in-vitro NDF digestibility by NIR with a lower standard error of calibration of 2.81. Differences between these two studies are likely due to variation in sample size (n = 110 TMR; n = 319 alfalfa) and differences in validation statistics for the NIR calibration equation. The current study did not result in construction of an NIR calibration equation.

To apply the data in the current study in the field, perhaps the development of NIR calibration equations will improve the accuracy of NIR to predict NDF digestibility in horses. Equation development would require the construction of an extensive database using hundreds or thousands of samples, and would take several years to implement. As uNDF30 content of forage contributes to intake in horses, further research is warranted to develop an NIR equation using uNDF30. This could help direct equine nutritionists toward a better understanding of the relationship between forage quality and intake.

# CHAPTER V

# CONCLUSIONS AND IMPLICATIONS

Results from this study indicate that the Near Infrared Reflectance Spectroscopy (NIR) analyses were poor predictors of in-vivo NDF digestibility in horses. While accurate predictions of OM digestibility, DM digestibility, and NDF digestibility from NIR have been observed in other species, additional research is required in equine. Perhaps further collection of data will lead to development of NIR calibration equations that will be robust enough to predict NDF digestibility in horses. This would require a large database from hundreds or thousands of observations, and would take many years to develop. However, the development of NIR calibration equations may be a useful tool for application by industry personnel to create a better understanding of the relationship between digestibility and energy availability in forages fed to horses.

Data from the current study illustrate significant correlations between NIR predictability parameters and DMI in horses; however, $R^2$ values were low ($R^2 = 0.17$). Results from the current study suggest that while sulfite and ash corrected NDF (aNDFom) and undigested NDF at 30 h (uNDF30) content of forage influence DMI in horses, other factors including palatability and individual preferences may also impact DMI. However, horses in the current study were not fed under true ad-libitum conditions

due to time restriction; therefore, some DMI data may be confounded. More studies are needed to fully elucidate contributors of intake in horses.

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# APPENDIX FIGURES

**Figure A-1.** Effect of treatment on mean body condition score in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

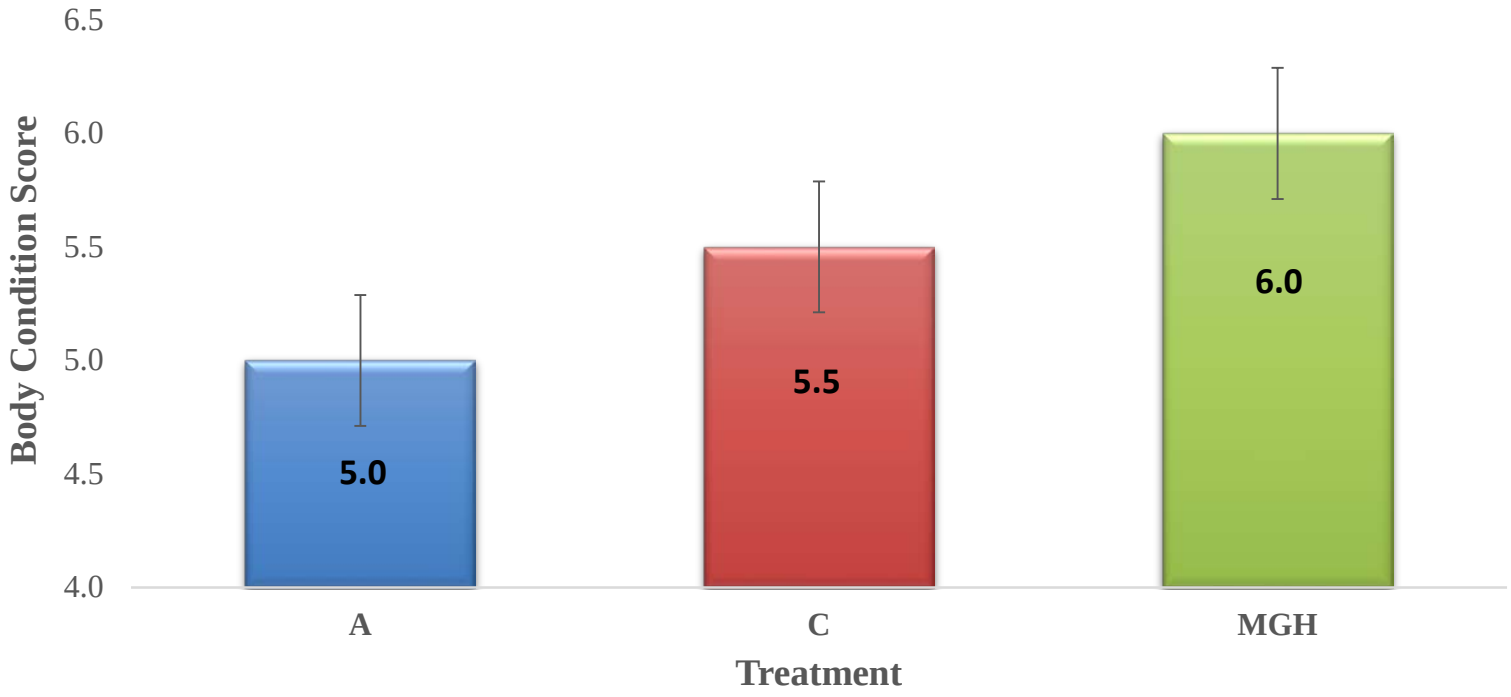


**Figure A-2.** Mean horse BW per treatment, kg in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

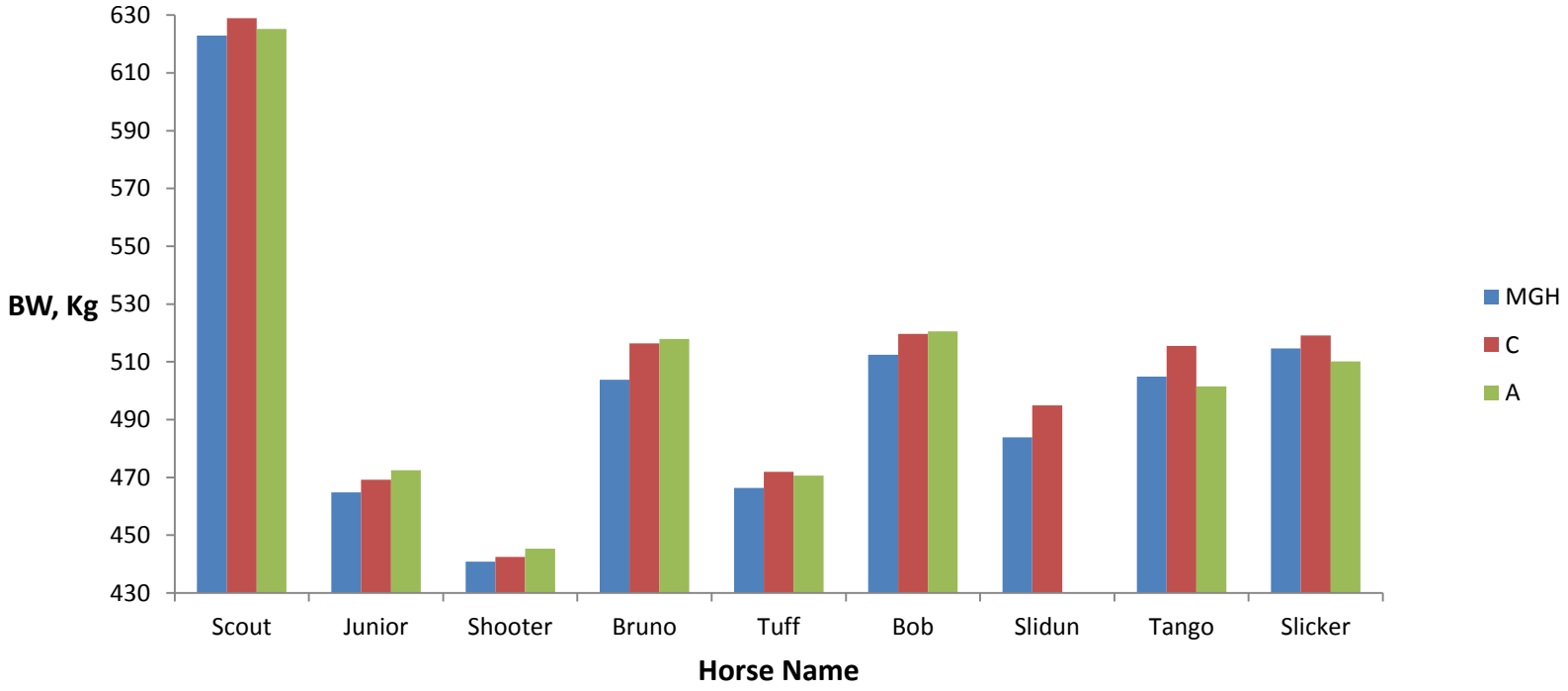


**Figure A-3.** Mean individual horse BW per period, kg in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

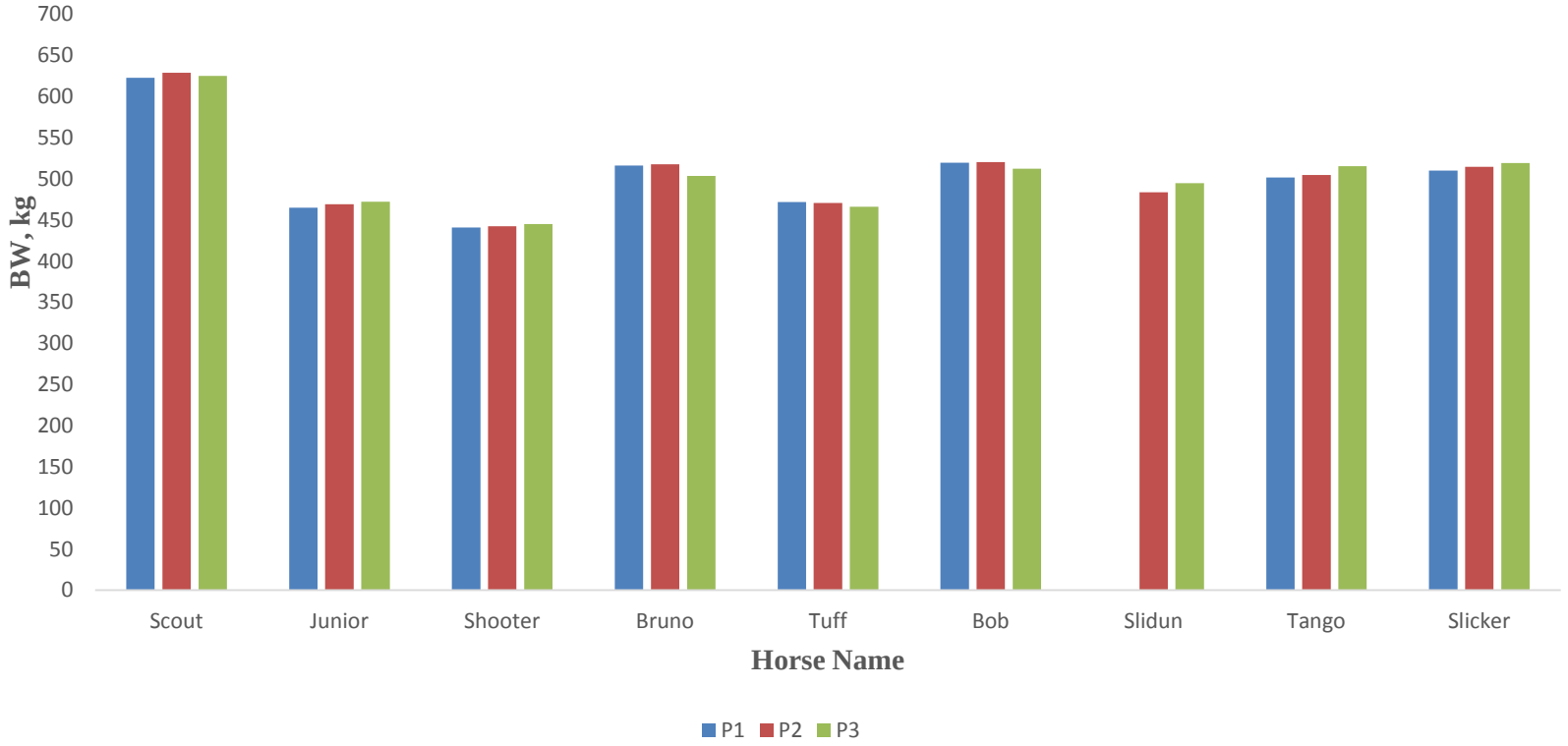


**Figure A-4.** Regression of dry matter intake (kg/d) from aNDFom(%) content of hay from NIR analysis in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay. (n=26)

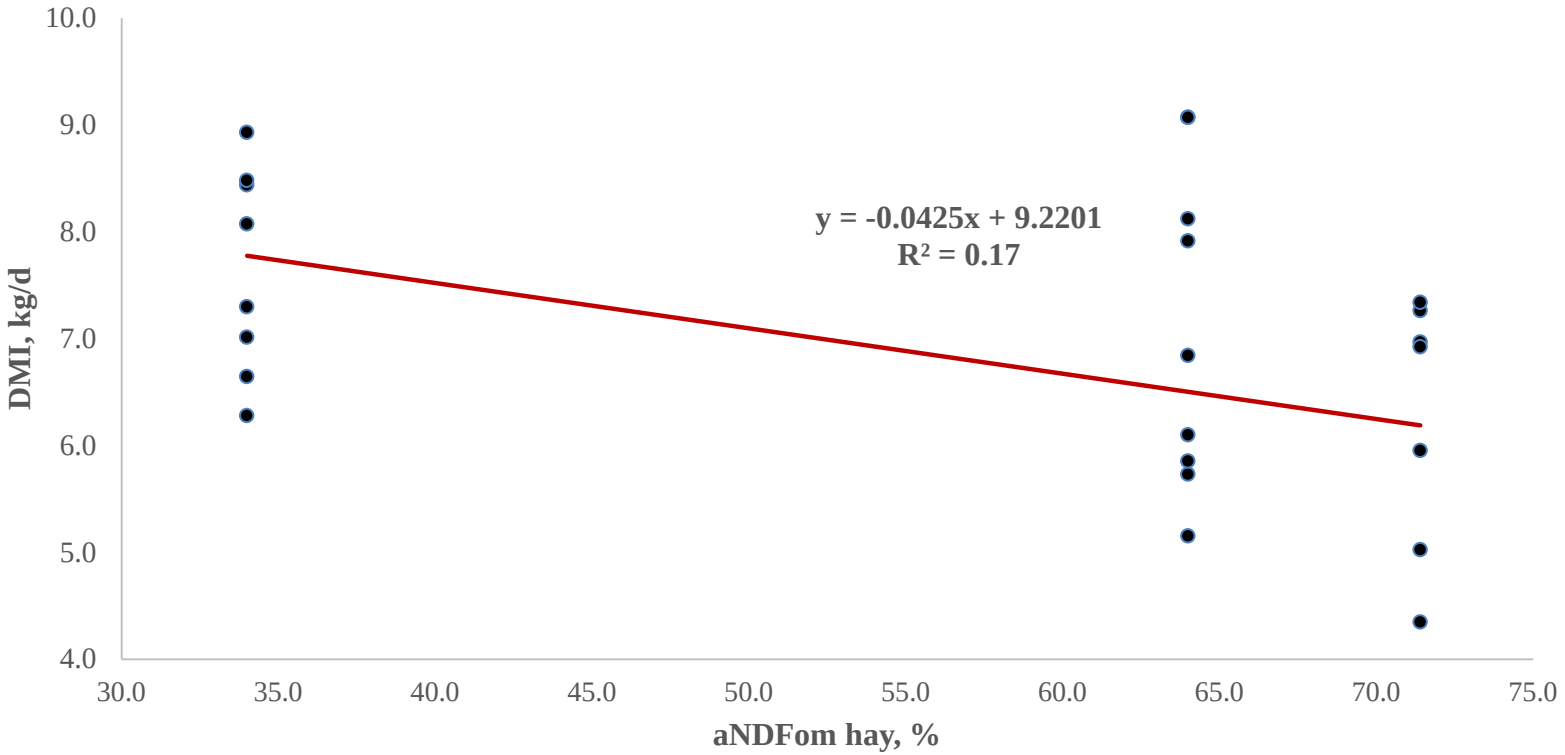
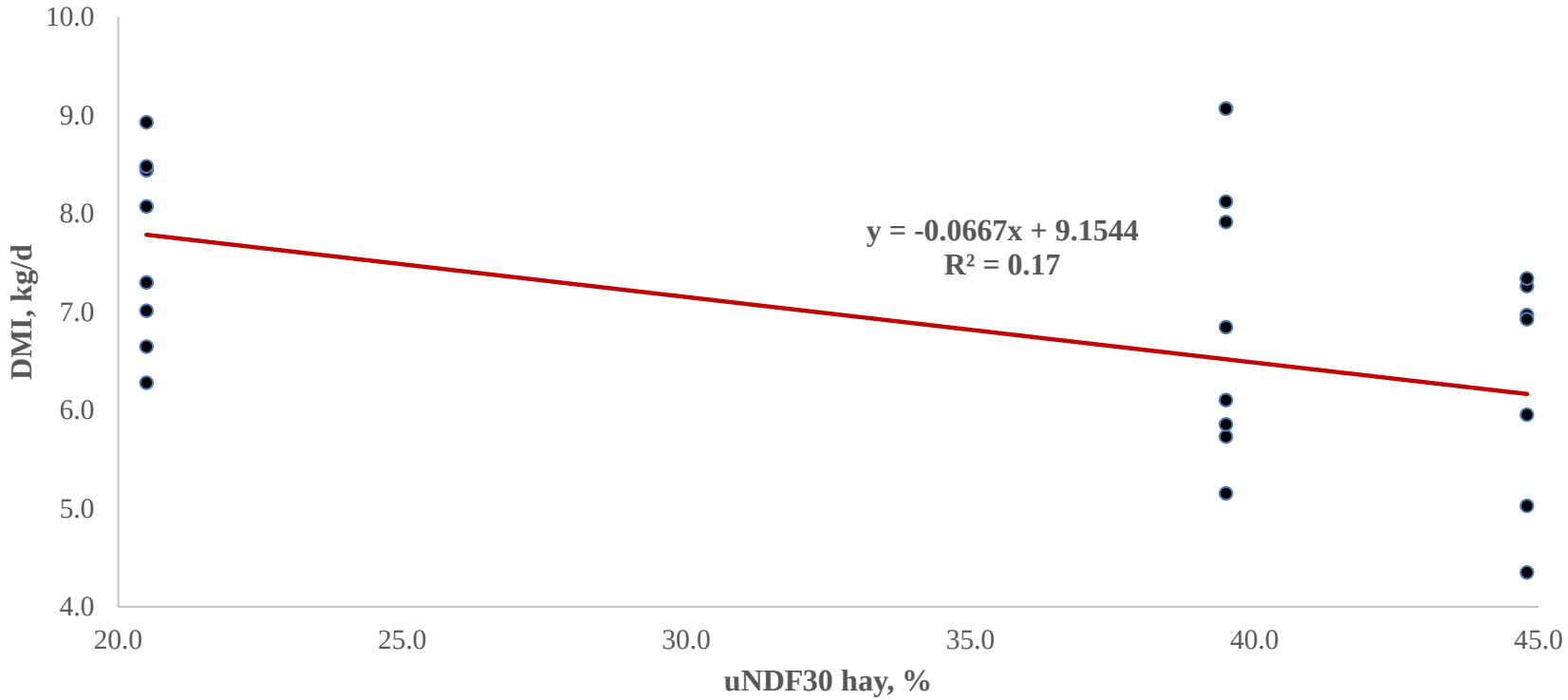


**Figure A-5**. Regression of dry matter intake (kg/d) from uNDF30(%) content of hay from NIR analysis in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay. (n = 26)



# APPENDIX TABLES

Table 1. Nutrient analysis of alfalfa, coastal Bermudagrass, and mixed grass hay (DM Basis).

| Item, Unit | Alfalfa | Coastal | Mixed Grass |
|---|---|---|---|
| DM, % | 89.7 | 90.7 | 92.9 |
| CP, % | 22.7 | 6.9 | 3.7 |
| ADF, % | 28.7 | 38.2 | 49.1 |
| aNDFom, % | 34.0 | 64.0 | 71.4 |
| Lignin, % | 6.9 | 6.4 | 6.2 |
| NFC, % | 30.6 | 20.2 | 14.4 |
| Ash, % | 10.20 | 6.56 | 8.75 |
| Ca % | 1.49 | 0.35 | 0.34 |
| P % | 0.28 | 0.19 | 0.04 |
| DE (Mcal/Kg) | 2.89 | 2.40 | 2.18 |
| %uNDFom 30hr | 20.5 | 39.5 | 44.8 |
| %uNDFom120hr | 18.7 | 27.8 | 23.8 |
| %uNDFom 240hr | 17.9 | 25.7 | 23.3 |
| | | | |
| %NDFDom 30hr | 39.8 | 38.3 | 37.2 |
| %NDFDom120hr | 45.0 | 56.6 | 66.7 |
| %NDFDom 240hr | 47.5 | 59.9 | 67.4 |

[a] Undigested NDF on an ash free, OM basis at 30 h
[b] Undigested NDF on an ash free, OM basis at 120 h
[c] Undigested NDF on an ash free, OM basis at 240 h
[d] NDF Digestibility on an ash free, OM basis at 30 h
[e] NDF Digestibility on an ash free, OM basis at 120 h
[f] NDF Digestibility on an ash free, OM basis at 240 h

Table 2. Effect of treatment on mean body weight (BW), body condition score (BCS), dry matter intake (DMI), DMI (%BW), DM digestibility (DMD; %), and NDF digestibility (NDFD; %) in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | Alfalfa | Coastal | Mixed-grass | SEM | *P*-value |
|---|---|---|---|---|---|
| Average BW, kg | 508 | 509 | 502 | 27.0 | 0.95 |
| BCS | 5.0 | 5.0 | 6.0 | 0.25 | 0.23 |
| DMI, kg/d | 7.31[b] | 6.79[d] | 5.41[a,c] | 0.65 | 0.02 |
| DMI, % BW | 1.4[b] | 1.3[b] | 1.0[a] | 0.13 | 0.02 |
| TDMD, % | 60.81[c] | 49.60[b] | 34.31[a] | 4.17 | 0.01 |
| NDFD, % | 39.58[a] | 47.59[b] | 38.50[a] | 3.15 | 0.01 |

[a] Means without common superscripts differ ($P < 0.05$).
[c,d] Means without common superscripts differ ($P < 0.10$).

Table 3. Simple linear correlations between dry matter intake, in-vivo NDFD, aNDFom content of feces, and NIR hay analysis in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | DMI | IVNDFD | aNDFomfeces | aNDFom | uNDF30 | NDFD30 |
|---|---|---|---|---|---|---|
| DMI | 1.0 | - | - | -0.44* | -0.45* | 0.50* |
| IVNDFD | - | 1.0 | - | 0.10 | 0.09 | 0.02 |
| aNDFomfeces | - | - | 1.0 | 0.79* | 0.78* | -0.68* |
| aNDFom | -0.44* | 0.10 | 0.79* | 1.0 | 0.99* | -0.96* |
| uNDF30 | -0.45* | 0.09 | 0.78* | 0.99* | 1.0 | -0.97* |
| NDFD30 | 0.50* | 0.02 | -0.68* | -0.96* | -0.97* | 1.0 |

*Correlations declared significant ($P < 0.05$) when different from 0.
[a] aNDF reported on an ash free, OM basis
[b] Undigested NDF measured at 30 h in-vitro
[c] NDF digestibility measured at 30 h in-vitro

Table 4. Prediction of dry matter intake (kg/d) from aNDFom and uNDF30 (%) content of hay from NIR analysis in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | | | Slope | | Intercept | |
|---|---|---|---|---|---|---|
| Item | Equation | $R^2$ | SE | *P*-value | SE | *P*-value |
| aNDFom | y = -0.04x + 9.22 | 0.17 | 0.02 | 0.02 | 1.03 | <0.01 |
| uNDF30 | y = -0.07x + 9.15 | 0.17 | 0.03 | 0.02 | 0.99 | <0.01 |

Table B-1. Least squares means for effect of treatment on body weight for mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | Estimate | Standard error | DF | t Value | *P*-value |
|---|---|---|---|---|---|
| Alfalfa | 508.02[a] | 19.70 | 21 | 25.79 | 0.01 |
| Coastal | 508.69[b] | 18.48 | 21 | 25.53 | 0.01 |
| Mixed-Grass | 501.63[c] | 18.48 | 21 | 27.15 | 0.01 |

[a,b,c] Means without common superscripts differ ($P < 0.05$).

Table B-2. Differences in least squares means of effect of treatment on body weight (kg) in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| Item | Estimate | Standard error | DF | t Value | *P*-value | Adjustment | Adj *P*-value |
|---|---|---|---|---|---|---|---|
| A vs. C[a] | -0.6749 | 27.0 | 21 | -0.02 | 0.98 | Tukey | 0.99 |
| A vs. MGH[b] | 6.3840 | 27.0 | 21 | 0.24 | 0.82 | Tukey | 0.97 |
| C vs. MGH[c] | 7.0589 | 26.1 | 21 | 0.27 | 0.79 | Tukey | 0.96 |

[a] Alfalfa vs. coastal Bermudagrass hay
[b] Alfalfa vs. mixed grass hay
[c] Coastal Bermudgrass hay vs. mixed grass hay

Table B-3. Least squares means of effect of treatment on dry matter intake (kg/d) in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | Estimate | Standard error | DF | t Value | *P*-value |
|---|---|---|---|---|---|
| Alfalfa | 7.31[a] | 0.56 | 20 | 12.95 | 0.01 |
| Coastal | 6.79[b] | 0.56 | 20 | 12.07 | 0.01 |
| Mixed-Grass | 5.41[c] | 0.56 | 20 | 9.62 | 0.01 |

[a,b,c] Means without common superscripts differ ($P < 0.05$).

Table B-4. Differences in least squares means of effect of treatment on dry matter intake in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| Item | Estimate | Standard error | DF | t Value | *P*-value | Adjustment | Adj *P*-value |
|---|---|---|---|---|---|---|---|
| A vs. C[a] | 0.52 | 0.65 | 20 | 0.80 | 0.08 | Tukey | 0.71 |
| A vs. MGH[b] | 1.90 | 0.65 | 20 | 2.93 | 0.01 | Tukey | 0.02 |
| C vs. MGH[c] | 1.38 | 0.63 | 20 | 2.20 | 0.04 | Tukey | 0.01 |

[a] Alfalfa vs. coastal Bermudagrass hay
[b] Alfalfa vs. mixed grass hay
[c] Coastal Bermudgrass hay vs. mixed grass hay

Table B-5. Least squares means of effect of treatment on in-vivo dry matter digestibility (%) in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | Estimate | Standard error | DF | t Value | *P*-value |
|---|---|---|---|---|---|
| Alfalfa | 60.81[c] | 3.04 | 20 | 19.98 | 0.01 |
| Coastal | 49.60[b] | 2.85 | 20 | 17.38 | 0.01 |
| Mixed-Grass | 34.31[a] | 2.85 | 20 | 12.02 | 0.01 |

[a,b,c] Means without common superscripts differ ($P < 0.05$).

Table B-6. Differences in least squares means of in-vivo dry matter digestibility in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| Item | Estimate | Standard error | DF | t Value | *P*-value | Adjustment | Adj *P*-value |
|---|---|---|---|---|---|---|---|
| A vs. C[a] | 11.20 | 4.17 | 20 | 2.69 | 0.01 | Tukey | 0.04 |
| A vs. MGH[b] | 26.50 | 4.17 | 20 | 6.35 | 0.01 | Tukey | 0.01 |
| C vs. MGH[c] | 15.30 | 4.04 | 20 | 3.79 | 0.01 | Tukey | 0.01 |

[a] Alfalfa vs. coastal Bermudagrass hay
[b] Alfalfa vs. mixed grass hay
[c] Coastal Bermudgrass hay vs. mixed grass hay

Table B-7. Least squares means of effect of treatment on in-vivo NDF digestibility (%) in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| | Estimate | Standard error | DF | t Value | *P*-value |
|---|---|---|---|---|---|
| Alfalfa | 39.58[a] | 3.01 | 20 | 13.15 | 0.01 |
| Coastal | 47.59[b] | 3.07 | 20 | 15.51 | 0.01 |
| Mixed-Grass | 38.50[a] | 3.07 | 20 | 12.55 | 0.01 |

[a,b,c] Means without common superscripts differ ($P < 0.05$).

Table B-8. Differences in least squares means of effect of treatment on in-vivo NDF digestibility in mature geldings consuming alfalfa, coastal Bermudagrass, and mixed grass hay.

| Item | Estimate | Standard Error | DF | t Value | *P*-Value | Adjustment | Adj *P*-Value |
|---|---|---|---|---|---|---|---|
| A vs. C[a] | -8.01 | 3.15 | 20 | -2.54 | 0.02 | Tukey | 0.05 |
| A vs. MGH[b] | 1.08 | 3.15 | 20 | 0.34 | 0.73 | Tukey | 0.94 |
| C vs. MGH[c] | 9.09 | 3.04 | 20 | 2.99 | 0.01 | Tukey | 0.02 |

[a] Alfalfa vs. coastal Bermudagrass hay
[b] Alfalfa vs. mixed grass hay
[c] Coastal Bermudgrass hay vs. mixed grass hay