Advancements in Forage Management: Grazing Horses on Cover Crops and Exploring Hand-Held NIRS Technology

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Dedication

This thesis is dedicated to my loving Grandma Mary Kay, who did not know a lick about horses or science, but absolutely adored my work and listened to every word.

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CHAPTER ONE: LITERATURE REVIEW

Forages in Equine Nutrition and Management

Horses have evolved to be nomadic grazers that spend a large portion of their time-consuming forage. The relatively small glandular stomach and a specialized hindgut, or cecum, are efficient at utilizing high-fiber diets (Janis, 1976). Forages are comprised of cellular and structural components, both of which can provide valuable nutrients to horses. Cellular components include proteins, vitamins, minerals, and nonstructural carbohydrates (NSC) including simple sugars like glucose and sucrose, and storage carbohydrates like starch and fructan (NRC, 2007). A majority of these nutrients and sugars are digested in the stomach and small intestine of the horse through enzymatic breakdown and subsequent absorption. Simple sugars, therefore, provide readily accessible energy to the horse. Structural carbohydrates make up the cell walls of forages and are comprised of cellulose, hemicellulose, and pectins (NRC, 2007). As forages mature, the structural carbohydrate components, especially cellulose, undergo lignification. Although lignin is not a carbohydrate, its inclusion in the cell walls can decrease digestibility of the non-lignified portions, therefore reducing the quality of the forage (Ralston, 1991; Buxton, 1996). Structural carbohydrates are largely fermented in the horse's large cecum, or hind gut, by the diverse population of microflora. This fermentation generates glucogenic volatile fatty acids, notably proprionic acid, which the horse can utilize for energy; however, this energy is not as immediately available like that developed from simple sugars in the small intestine (Janis, 1976). When consuming high-fiber, mature forages (compared to low-fiber, immature forages) horses may also increase the rate of intake and the subsequent rate of passage to maintain a similar nutrient absorption rate per unit of time (Janis, 1976; Cymbaluk, 1990). This is an important phenomenon that highlights the horse's diverse utilization of feedstuffs compared to ruminants, which have a limited capacity for roughage intake due to rumen fill and the subsequent time needed to ferment the high-fiber feeds in the foregut (Janis, 1976).

In addition to the horse's specialized digestive system, dentition has significantly evolved to accommodate the intake of forages. Notable adaptations in dentition from brachydont molars to hypsodont molars suggest a transition from a frugivorous to a grazing diet over the last 55.5 million years (Janis, 1976; Mihlbachler et al., 2011). Modern equine also exhibit molars with an increased root to crown ratio and greater transverse jaw movements, both suited for abrasive mastication associated with consuming fibrous forage (Janis, 1976). Grazing preference and intake of certain forage species have also been attributed to the differences in dentition between ruminants and horses (Hongo and Akimoto, 2003). The presence of top incisors and mobile lips in horses as opposed to a hardened top pallet paired with a grasping tongue in cattle pose a unique grazing interaction that impacts the intake and preference of each animal.

Forage quality factors such as yield, maturity, the presence of unpalatable compounds, and nutrient composition have also shown to impact horse grazing preference, however consistent trends between pasture characteristics and horse preference are not clearly defined. Grev et al. (2017) and Catalano et al. (2019) reported that preference was negatively correlated to yield, plant maturity, and plant height in annual cool-season grass and legume pastures, respectively. Additionally, McCann and Hoveland (1991) reported a negative relationship between preference and both maturity and the presence of tannins in grass and clover pastures, respectively. Conversely, Allen et al. (2013) did not observe a difference in preference due to maturity in a cool-season grass pasture system, though this may be due to limited varying stages of maturity present. Allen et al. (2013) reported a positive correlation between preference and NSC with positive trends between preference and neutral detergent fiber (NDF) and neutral detergent fiber digestibility; negative correlations were noted between preference and crude protein (CP). Longland and Byrd (2006) also found supporting evidence for a positive relationship between preference and NSC, however Catalano et al. (2019) reported a negative relationship when grazing legumes. Digestible energy (DE) and CP content have also correlated with an increase in preference when grazing legumes (Catalano et al., 2019). Multiple agronomic and nutritional factors can affect horse preference, however there are still not well-defined, consistent parameters that drive this preference (Marten, 1978).

Generally, horses should consume 2% of their body weight (BW) in dry matter (DM) of forage each day to meet basic nutrient requirements (NRC, 2007). To meet the high energy needs of performance horses, concentrates can be fed, but forages should still make up greater than 50% of the ration to support a healthy hind gut environment (Janis, 1976; NRC, 2007). An adult horse weighing 500 kg at maintenance requires 16.7 Mcal of digestible energy and 630 g of CP each day. When fed forage at 2% of BW, the horse would require levels at 1.67 Mcal kg^{-1} DE and roughly 7% CP to meet their needs (NRC, 2007). However, to meet lysine and other amino acid requirements, the minimum CP level is often accepted at 10-12% for adult horses at maintenance. Neutral detergent fiber is an important measure for feed intake; although there are not defined minimum requirements for NDF in horses, levels < 65% are considered optimal for forage intake. Additionally, there are no recommendations for dietary levels of NSC for healthy, adult horses; however, a total diet containing ≤ 12% NSC is suggested for metabolically challenged horses or horses diagnosed with obesity and laminitis (Borgia et al., 2009; Frank, 2009). Finally, calcium (Ca) and Phosphorus (P) are key macro-minerals that serve a variety of functions in the body and are important to balance for maximum utilization and internal homeostasis. Due to P ability to prevent Ca transfer through the body and other inhibitory functions, Ca and P should be balanced in a ratio between 1:1 to 5:1 Ca:P (NRC, 2007), with 2:1 being a common recommendation.

Many horse facilities in the Midwest rely on pastures to provide roughage for a portion of the year, and then utilize preserved forage, normally hay, for the remainder when freezing temperatures and snow cover prevent grazing. Horse pastures in the Midwest often include cool-season perennials like Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.), and timothy (*Phleum pratense* L.; Allen et al., 2013), which yield the best during the spring and fall months. Alfalfa (*Medicago sativa* L.) and both red and white clover (*Trifolium pratense* L.; *Trifolium repens* L.) are common legumes incorporated into horse pastures, and are often mixed with cool-season grasses (Catalano et al., 2019). Warm season species including teff (*Eragrostis tef* Zucc.) and sudan grass (*Sorghum x drummondi* Nees ex. Steud.) have been grazed by horses (DeBoer et al., 2017), however cool-season-legume pastures are predominant in the Midwest. Comparatively, hay is more expensive to feed than pasture due to a variety of management needs including storage, labor, and transportation, but is a necessity for midwestern horse owners during the winter months when pastures are dormant (Martinson et al., 2012b). Nonetheless, pasture utilization should be prolonged in an interest to offset this reliance on preserved forages. Cover crops are annual, cool-season forages that have the potential to extend the grazing season in horse operations.

Cool-Season Annual Grass Cover Crops

Cool-season grasses are common pasture forages across the United States and tend to yield highest during the spring and fall seasons with a decrease in yield mid-summer. Common cool-season grasses utilized in midwestern horse pastures include Kentucky bluegrass , perennial ryegrass, timothy, and orchardgrass (*Dactylis glomerata* L.; Allen et al., 2012). Annual grasses that may be suitable for horse pastures include annual ryegrass (*Lolium multiflorum* L.) and winter rye (*Secale cereale* L.), and are known for their soil-building characteristics, erosion control, and quick growth (SARE, 2012). Annual ryegrass and winter rye can yield between 2,250 and 10,110 kg ha^{-1}, and 3,370 and 11,230 kg ha^{-1}, respectively, which depends on the number of grazing events and the length of the growing season (Kallenbach et al., 2003; Franzluebbers and Stuedemann, 2007; Grev et al., 2017; Brown et al., 2018).

Annual ryegrass is known for its glossy, bright green, fine leaf blades, and is notorious for its quick establishment (SARE, 2012), great emergency grazing potential (Grev et al., 2017), and its ability to scavenge nitrogen (N; Shipley et al., 1992). Annual ryegrass is also an excellent companion crop which can provide shelter and support for slow-germinating crops when interseeded thereby increasing the performance of the slow-germinating crop and potentially producing higher yields (Caballero et al., 1995). When utilized as a forage crop, cutting or grazing can impact annual ryegrass yield, which is important to consider when managing it in a hay field or pasture system (Edmisten et al., 1998).

Winter rye is recognized by its pale-green, wide leaf blades, and is one of the most utilized cover crops in the United States. This grass is a great nutrient catch crop that uptakes excess N from the soil (Shipley et al., 1992) in addition to its weed preventing capabilities (Teasdale et al., 1991), and erosion control (SARE, 2012). Winter rye is known as the "work horse" cover crop because it is adaptable to a wide range of soil types and environments, it has many grazing benefits, and it has subsequent popularity with both crop and animal producers.

Both grass species have been explored in equine grazing systems, therefore their nutrient compositions are better defined for horses than some other cover crops. The equine digestible energy content of both grasses are comparable, averaging at 2.37 and 2.45 Mcal kg^{-1} for annual ryegrass and winter rye, respectively (Grev et al., 2017). Crude protein has been reported between 16.0 and 24.5% for both grasses (Maloney et al., 1999; Kallenbach et al., 2003; Faé et al., 2009; Paulson et al., 2014), but has been observed as low as 10.3% (Brown et al., 2018), and as high as 32.0 and 37.0% for annual ryegrass and winter rye, respectively (Grev et al., 2017). These differences can be attributed to soil nutrient content, fertilizer management, and environmental factors (Buxton, 1996). The NDF content has been reported between 33.1 and 56.5% and 32.1 and 66.8% for annual ryegrass and winter rye, respectively, with most values between 35.0 and 45.0% (Maloney et al., 1999; Kallenbach et al., 2003; Faé et al., 2009; Paulson et al., 2014; Grev et al., 2017; Brown et al., 2018). Neutral detergent fiber is dependent on forage maturity and seasonality, which may explain the range of values (Buxton, 1996; Maloney et al., 1999; Grev et al., 2017). Grev et al. (2017) reported that spring-planted annual grasses had higher NDF concentrations during the summer than summer planted annual grasses had in the fall, suggesting that seasonality plays a role in determining the nutrient content of the grasses. Calcium and phosphorus content has been examined for both annual ryegrass and winter rye in previous horse grazing experiments. Grev et al. (2017) observed Ca:P ratios averaging 0.81:1 and 0.46:1 for annual ryegrass and winter rye, respectively.

Annual ryegrass has been observed to be moderate to highly preferred by horses, while winter rye has been only mildly preferred. Grev et al. (2017) found forage removals averaging between 50 and 88% for annual ryegrass, but only 7 to 16% for winter rye. McCann and Hoveland (1991) found similar grazing preferences by yearlings. Annual ryegrass had 65 to 80% removal while winter rye had roughly 25 to 40% removal. Annual ryegrass and winter rye have also provided sufficient roughage sources for grazing cattle into the late fall, and winter rye has been shown to be an option for early spring grazing (Lesoing et al., 1997). Faé et al. (2009) observed satisfactory average daily gains (ADG) when grazing beef heifers on an oat (*Avena sativa* L.)-winter rye mixed pasture and an annual ryegrass monoculture pasture, and was able to extend the grazing season into early January in Ohio. Cow calf pairs were also able to maintain acceptable ADG into the late fall when grazed on rye (Franzluebbers and Stuedemann, 2007) and on annual ryegrass and winter rye mixtures (Franzluebbers and Stuedemann, 2014) in Georgia. Additionally, Schomberg et al. (2014), observed sufficient heifer ADG while grazing winter rye, and that grazing cattle on cover crops after a cotton harvest had the potential to improve cotton yields in the next harvest, particularly during a dry year.

Annual Clover Cover Crops

Some of the most common clovers incorporated into horse pastures include red and white clover which are perennials that can persist for many years when managed well under horse grazing (Catalano et al., 2019). Annual clovers like crimson (*Trifolium incarnatum* L.) and berseem clover (*Trifolium alexandrinum* L.) can also be utilized in horse pastures, but must be seeded each year (McCann and Hoveland, 1991). Clovers are valuable legumes that can provide both environmental and nutritional benefits when incorporated into horse pasture mixes. Like other legumes, clovers are excellent N scavengers in addition to their ability to fixate atmospheric nitrogen. Clovers can also provide a high yielding, nutrient dense, and preferred forage source when included in a horse pasture (Catalano et al., 2019).

Berseem clover is an annual clover originating in Egypt, and is known for its ability to sustain forage-based animal agriculture in these regions through the winter months (Salama et al., 2020). Berseem clover is most suited for moist, sub-tropical regions with long growing seasons, but has been grown in the northern United States as an annual cover crop (Ross et al., 2005). Unlike many other varieties of clover, the temperature at which berseem clover is winterkilled ($< -6.7^{\circ}C$), and its poor reseeding performance make it an excellent annual, short-term forage without perenniality (Carr et al., 2005). When competition for resources such as light and water is minimal, berseem clover can yield between 6,740 to 11,230 kg ha^{-1} per year, though many have reported yields between 5,270 and 6,530 kg ha^{-1} in the Northern United States and Canada (Fraser et al., 2004; Ross et al., 2005; SARE, 2012). Berseem clover is susceptible to over-shading by other forages and weeds, and is particularly vulnerable during its slow germination stage (Ross et al., 2005). Therefore, it is best grown in monoculture and with stringent weed management practices (e.g., seeded at the right rate for dense establishment, applying selective herbicides when weeds are present) to maximize forage yield and nutrient composition.

Berseem clover also provides a high plain of nutrition for horses, similar to other legumes. Crude protein has been reported between 14.7 and 24.5%, with a majority of reports between 16.0 and 20.0% (Fraser et al., 2004; Ross et al., 2005; Paulson et al., 2014; Salama et al., 2020). Most of the differences in CP may have been due to differences in climate, irrigation, cutting sequence, and soil fertility and management. Neutral detergent fiber has been measured between 35.0 and 61.5% (Fraser et al., 2004; Ross et al., 2005; Paulson et al., 2014; Salama et al., 2020), but is largely dependent on the maturity of the clover at harvest (Buxton, 1996), hence the large range of values. Although berseem clover has been grazed in preference trials by horses (McCann and Hoveland, 1991), equine DE has not been reported. Calcium:Phosphorus has also not been reported, but is expected to be similar to other legumes, which tend to remain above 2.7:1 as found by Catalano et al. (2019).

Previous studies have found that clovers and legumes are highly preferred pasture species in equine grazing systems, (McCann and Hoveland, 1991; Catalano et al., 2019; DeBoer et al., 2020) and can have removals as high as 98% when allowed sufficient grazing time (Catalano et al., 2019). White clover mixed with either Kentucky bluegrass or meadow fescue (*Festuca pratensis* Huds.) were also highly preferred grass-legume mixtures with removal rates between 63 and 98% (DeBoer et al., 2020). Berseem clover has been found to be highly preferred by horses, alongside crimson and subterranean clovers (McCann and Hoveland, 1991). Although not grazed simultaneously, McCann and Hoveland (1991) also discovered similar grazing preferences for both annual ryegrass and berseem clover (removals > 65%).

Brassicaceae Cover Crops

Plants in the Brassicaceae family are winter and cool season annuals known for their large, penetrating roots and expansive broadleaf canopies. Often used as cover crops after a cash crop (e.g., corn, soybeans), plants in this family excel at preventing erosion, breaking up soil compaction, fighting weeds and pests, and scavenging soil nutrients (Williams and Weil, 2004;

Chen and Weil, 2010; SARE, 2012). Species in this family include mustards (e.g., yellow mustard [*Sinapis alba* L.], brown mustard [*Brassica juncea* L.]), rapeseed or canola (e.g., *Brassica napus* L. and *Brassica rapa* L.), and radishes (e.g., daikon radish [*Raphanus sativus* subsp. *longipinnatus* L.]; SARE, 2012).

Purple top turnip (*Brassica rapa* subsp. *rapa* L.) is a subspecies in the mustard family, and is well known for its edible root that can be consumed by both humans and animals (Hasanuzzaman, 2020). This variety of turnip is recognizable by its expansive, rough, broadleaf canopies and large purple and cream-colored root bulbs, which can measure up to 12.7 to 15.2-cm in diameter when mature. When grown in monoculture, purple top turnip can yield from 4,000 to 10,100 kg ha^{-1} of DM, which is dependent on seeding date and the date of the first killing frost (winterkills at < -4˚C; Yun et al., 1999; SARE, 2012; Paulson et al., 2014; Villalobos and Brummer, 2015). In the Midwest, turnip is often utilized as an annual cover crop. Its ability to quickly cover bare ground, easy winter kill, and quick degradation before spring make it suitable for rotations with other crops (SARE, 2012). Additionally, turnip can reduce weed pressure (Petersen et al., 2001), alleviate soil compaction, and can potentially deter pests such as nematodes and chewing insects (SARE, 2012; Bangarwa and Norsworthy, 2016). Turnip is also a moderate N scavenger and can store up to 33.7 to 134.8 kg ha^{-1} of N (SARE, 2012).

Daikon radish (also called forage radish, icicle radish, and tillage radish) is similar to purple top turnip in both its potential consumption by humans and animals, and its functionality as a cover crop. Daikon radish is recognized by its tall, bright green, broadleaf canopies and long, white roots that can extend to depths of 1.8 m in some soils (SARE, 2012). Daikon radish can yield between 4,500 to 7,900 kg ha^{-1} DM and is excellent at scavenging excess N (56.2 to 224.6 kg ha^{-1}; Yun et al., 1999; SARE, 2012; Paulson et al., 2014; Villalobos and Brummer, 2015). The large, deep-penetrating roots are effective at breaking-up compacted soils, and providing low-resistance root channels for subsequent crops (Williams and Weil, 2004; Chen and Weil, 2010), making them useful for no-till systems and in pastures where hoof traffic leads to soil compaction (Singleton et al., 2000; Bell et al., 2011). When utilized in crop rotations during the fall, radish is effective at reducing the amount of weeds present in both the fall and the following spring before annual crop seeding and has been shown to have no effect on corn yield the following summer (Lawley et al., 2011, 2012). Some varieties of the daikon radish have also shown to be effective at reducing pests like nematodes, which can be common in sugar beet fields found to the upper Midwest (Yun et al., 1999).

Neither purple top turnip nor daikon radish have been explored in equine grazing systems; therefore, some nutritive parameters of interest to equine rations are not well catalogued. Values such as equine DE, NSC, and Ca:P are not well defined, or are not well defined for the herbage versus root components of these forages. Crude protein for purple top turnip and daikon radish have been reported between 17.2 and 19.3% and 20.5 and 22.4%, respectively (Paulson et al., 2014; Villalobos and Brummer, 2015), with herbage containing as low as 11.0 to 13.0% for both species, and turnip roots containing as little as 7.2 to 9.6% (Yun et al., 1999). Lenz et al. (2019) found that the difference in harvest date over the winter impacted the level of CP in both turnip and radish herbage and roots. Herbage CP averaged 19.8 and 24.0% for turnip and radish, respectively, and root CP averaged 14.3 and 16.5% (Lenz et al., 2019). As the winter progressed, CP was reduced in the herbage components, but raised in the root components. The higher CP in radish compared to turnip may have also been attributed to daikon radish's greater ability to scavenge N (Yun et al., 1999; SARE, 2012; Villalobos and Brummer, 2015). Neutral detergent fiber of both species have tended to be between 17.0 to 30% (Paulson et al., 2014; Villalobos and Brummer, 2015), and increased over the winter season with herbage values as high as 46.9% and root values as high as 28.1% in January when not winterkilled (Lenz et al., 2019). Keim et al. (2020) found NSC content averaged 14.1% in turnip leaves and 31.2% in turnip roots. Moate et al. (1999) reported a Ca:P ratio of 5:1 and 7.5:1 for turnip leaves, and 1.9:1 and 1.4:1 for turnip roots fed in a bunk and grazed in a pasture, respectively.

Neither brassicas species have been previously grazed by horses but have been grazed or bunker-fed to cattle and sheep. Dairy cows maintained BW and produced acceptable milk yields (15.6 to 19.7 L/d, in 1999) when fed turnips as a proteinaceous alternative to barley (Moate et al., 1999). Individual cows also showed some preference for consuming the leaves, roots, or both components, but a general trend was not apparent amongst all of the cows (Moate et al., 1999). Beef heifers stocked at different rates on purple top turnips, forage radishes, and annual ryegrass mixed pastures were able to maintain acceptable ADG and DM intake into the fall season (Brunsvig et al., 2017). Yun et al. (1999) found that sheep grazed on turnip and radish produced acceptable market weights and carcass grades, but took longer to reach market weight. Sheep also preferred to eat turnip and radish herbage, and only rarely consumed turnip roots with no radish root consumption reported (Yun et al., 1999). Ried et al. (1994) reported that finishing lambs tended to have better growth when grazing turnips compared to tall fescue or orchardgrass-red clover pastures, and were able to continue grazing into January in the upper Northeast region of the United States.

An additional unique characteristic of Brassicaceae plants is their production of glucosinolates and S-methyl-cysteine sulphoxide (SMCO; Gustine and Jung, 1985; Barry, 2013). Glucosinolates are secondary plant metabolites containing significant levels of sulfur, and are present in a variety of forms depending on the plant variety, season, maturity, and environment (Gustine and Jung, 1985; Tripathi and Mishra, 2007). These compounds are important to consider when providing Brassicaceae as roughage because, upon chewing and digesting, the glucosinolates are broken down into tertiary compounds that effect feed utilization and production efficiency (Gustine and Jung, 1985; Reid et al., 1994; Barry, 2013). Nitriles and iso-thiocyanates are the result of glucosinolate digestion and can reduce voluntary feed intake (VFI) due to the nitrogen content and perceived bitter taste, respectively. Iso-thiocyanates can further break down into oxazolidine-2-thiones and thiocyanates, which are goitrogens, that reduce iodine uptake by the thyroid gland further causing goiter after prolonged consumption (Reid et al., 1994; Barry, 2013). When fermented, SMCO is converted into dimethyl disulphide, which can inactivate hemoglobin, resulting in a state of anemia and reduced VFI and live weight gain in ruminants (Barry et al., 1984; Barry, 2013). Besides their anti-quality characteristics, glucosinolates have a growing application for pest management when Brassicaceae are utilized in crop rotations (SARE, 2012; Bangarwa and Norsworthy, 2016). Bangarwa and Norsworthy (2016) noted that iso-thiocyanates, products of glucosinolate breakdown, have been effective against certain insects, nematodes, and fungi in the laboratory setting. However, SARE (2012) warned that efficient pest management has not been completely developed and reductions in different pests with varying Brassicaceae have not been consistent between years and geographic regions.

Although many other equine grazing studies have explored the use of a wide range of grasses and legumes in pasture systems, there is a need to investigate the use of cover crops. Cover crops are a diverse set of cool season annuals that provide agronomically and environmentally important services when incorporated into crop rotations. Additionally, they are nutrient-dense forages that can be utilized by many livestock to extend the grazing season. Although cover crops have been successfully utilized in other livestock systems (Reid et al., 1994; Moate et al., 1999; Yun et al., 1999; Brunsvig et al., 2017), there is limited research exploring their use in equine pastures. The unique grazing behaviors that horses exhibit (Cymbaluk, 1990; Shingu et al., 2010) makes it challenging to extrapolate research and conclusions from other livestock management systems when managing horse pastures. Therefore, the objectives of the second chapter of this thesis are to examine the yield, forage nutrient composition, and preference of cover crops grazed by horses.

The Use of Preserved Forages and Alfalfa on Equine Farms

Pastures only provide seasonal forage for horse farms in the Midwest and preserved forage is required for the remainder of the year. Long-stem hay is the most utilized preserved forage, but some farms may also use baleage, haylage, or pelleted or cubed forage (NRC, 2007). Long stem hay is baled in round bales, large square bales, and small square bales, and the type used on a farm is largely dictated by farm preference, feeding style, available labor, and market availability. Large round bales are often fed to provide *ad libitum* forage access, which can help to reduce negative behaviors in horses (Marsden, 1993), and require less labor, but do require machinery to move the bales (Martinson et al., 2012b). Large square bales require more labor and can be more difficult to feed but are efficient to store. Small square bales are the easiest to manage when meal feeding, and require minimal machinery, and tend to be preferred by barns offering specialized feeding regimens or separate meals throughout the day. However, small square bales do require more labor to transport and store.

The most commonly utilized legume hay in the horse industry is alfalfa, which is known for its higher CP and DE, and lower NSC content compared to cool-season grass hay (Buxton, 1996; Sleugh et al., 2000; Martinson et al., 2012a). Martinson et al. (2012) and Catalano et al. (2019) reported alfalfa DE levels between 2.14 and 2.77 Mcal kg^{-1}. Crude protein and NSC content has been found to average between 16.7 and 30.5% and 7.8 and 14.2%, respectively, with most CP values averaging between 19.0 and 26.0% (Buxton, 1996; Sleugh et al., 2000; Martinson et al., 2012a; Catalano et al., 2019). Neutral detergent fiber can vary widely, from 30.5 to 55.0% (Buxton, 1996; Sleugh et al., 2000; Martinson et al., 2012a; Catalano et al., 2019), but is heavily impacted by the maturity at harvest, which can differ based on the intended consumer of the forage (Fick and Mueller, 1989; Buxton, 1996). Martinson et al. (2012) and Catalano et al. (2019) have documented high Ca:P ratios, between 3:1 and 4:1, which confirm alfalfa's higher calcium content compared to cool-season grasses and small grains. Preserved forages like alfalfa hay will always be of equal or lessor quality than fresh forage (NRC, 2007); therefore, forage nutrient composition testing using wet chemistry or near infrared reflectance spectroscopy (NIRS) are important to conduct in order to balance rations and provide adequate nutrition for different classes of horses.

Laboratory Methods for Estimating Forage Nutrient Composition

There are a variety of methods for determining forage nutrient composition that can assist horse owners and nutritionists when balancing rations. The two most common methods are wet chemistry and near infrared reflectance spectroscopy, which are readily available at commercial laboratories, University research laboratories, and through agricultural entities. Trade-offs exist with each method and can impact consumer decisions. Wet chemistry is considered the gold standard analysis method and is widely accepted in the research arena; however, there is a time lapse of multiple days between sampling and receiving the results. Near infrared reflectance spectroscopy may only take a day or two when utilizing a commercial laboratory, and only minutes when utilizing NIRS at a local entity. Although the lapse in time may not be of concern for most horse owners evaluating their hay or pasture, animal nutritionists and forage producers may require a faster turnaround to make quick ration formulation decisions like on dairy operations or to make forage management decisions, respectively. Another drawback of wet chemistry is the need for both laboratory space and skilled technicians. Although this is not likely a primary concern for commercial laboratories, local entities offering forage analysis services may benefit more from utilizing an NIRS unit due to its smaller footprint in the laboratory and ease of operation. Lastly, cost can be a deciding factor for horse owners when selecting an analysis type. Wet chemistry typically costs much more than NIRS, though the price is largely dependent on the number of nutrient components that the consumer is interested in. Overall, both wet chemistry and NIRS have been developed to accurately predict multiple forage nutrient compositions and are invaluable resources for horse owners when balancing rations.

Wet chemistry includes a series of steps conducted on the sample to determine different nutrients of the feed. Both accuracy and precision are important at every step to ensure that the final estimation is correct. Many of the processes also require multiple measured values which are then applied to an equation that predicts the outcome of interest. Digestible energy is a complex measurement and is calculated differently for each livestock species due to differences in digestion and nutrient availability. Digestible energy for dairy cattle (Mcal kg^{-1}), for example, is equal to:

(truly digestible non-fibrous carbohydrates/100) * 4.2 + (truly digestible NDF/100) * 4.2 + (truly digestible CP/100) * 5.6 + (fatty acids/100) * 9.4 – 0.3 (NRC, 2001).

In comparison, the equation developed for equine DE (Mcal kg^{-1}) is equal to:

$$2{,}118 + 12.18 * \%CP - 9.37 * \%\text{acid detergent fiber (ADF)} - 3.83 * \%\text{hemicellulose} + 47.18 * \%\text{fat} + 20.35 * \%NSC - 26.3 * \%\text{ash}$$ (Pagan, 1998).

Both equations require different inputs, and each input is derived from its own equation or raw analysis. See tables 1.1 and 1.2 for a list of secondary equations that feed into the DE equations or that estimate other nutrient values. Components not defined by an equation (e.g., nitrogen content) are determined using a chemical analysis procedure unique to that component and a list of common analyses used to evaluate forage nutritive values is included in table 1.3.

Near infrared reflectance spectroscopy gathers near infrared light reflectance from the sample and utilizes equations to predict forage nutrient composition based on the gathered spectra. Laboratory NIRS technology was first explored in animal nutrition and forages in 1964 (Norris, 1964) on various grasses and legumes in different feed forms (e.g., hay, silage). Initially, NIRS was effective at predicting forage DM, CP, and NDF (Norris et al., 1976), and has since evolved to provide more predictions like energy values (Valdes and Leeson, 1992) and animal performance components like neutral detergent fiber digestibility at 48 hours (NDFD48; Abrams et al., 1987). Although a majority of NIRS forage calibrations are related to production livestock and performance, calibrations for equine organic matter digestibility (Martin-Rosset et al., 1994; Andrieu et al., 1996) and hydrolysable carbohydrates (Hoffman et al., 2001; Jafari et al., 2003) have been developed for use when developing horse rations. Despite the advances of NIRS, the technology and physics of the equipment itself does offer limitations to analyses. For example, minerals such as Ca and P may not be accurately predicted because minerals do not effectively absorb light in the near infrared region, which is the mechanism of analysis for NIRS (Shenk and Westerhaus, 1994).

The NIRS prediction equation for each forage nutritive component has been developed and improved upon for many decades. Near infrared reflectance spectroscopy technology was first applied to forages in 1964 (Norris, 1964), and the first component analyzed was moisture content (or DM). Moisture content is an important parameter in a variety of products including lumber (Kobori et al., 2013), fruits (Pissard et al., 2018; Santos Neto et al., 2018), and grains (Mutlu et al., 2011; Lin et al., 2019) and can be an indicator of quality and ripeness. Moisture, or water content, is one of the simplest parameters to measure because the spectral output for water presents with large spikes in the O–H marker zones (960 nm, 1450 nm, and 1940 nm) of the near infrared region (Choppin and Violante, 1972; Kobori et al., 2013). Because of its signature spectral fingerprint, moisture has been accurately predicted by NIRS units for decades. However, the accuracy of prediction equations for all forage nutrient parameters, including moisture content, have improved over time as more samples are added to individual laboratory databases with which the equations are calibrated from.

Over time, spectral markers and corresponding prediction equations have been identified and developed for other forage parameters such as CP, NDF, and NDFD48. Some parameters are more accurately predicted than others depending on the spectral fingerprints associated with the compound and the complexity of the compound. Crude protein, for example, is comprised of hydrogen (H), carbon (C), oxygen (O), and nitrogen atoms (some amino acids also include sulfur). Of these bonded atoms, the N-H bonds produce very distinct spectra (Ozaki, 2012), which resulted in CP also being one of the early forage parameters predicted using NIRS. Performance parameters like NDFD48 are not only comprised of forage nutrient parameters but also include a prediction of the animal's performance (e.g., digestibility). The complexity of performance parameters makes them more difficult to predict using an NIRS and can even be inconsistently measured using standard wet chemistry analysis (Rymer et al., 2005). Therefore, there can be differences in the accuracy of prediction equations for some forage nutrient parameters when analyzed by NIRS; however, time and the accumulation of samples measured via wet chemistry can improve the accuracy of NIRS predictions.

In-Field Methods for Estimating Forage Nutrient Composition

Methods for in-field forage nutrient composition estimation have been developed with the goal of maximizing forage quality at harvest. These processes can generate quick results but tend to not produce as accurate of estimates compared to laboratory methods. One of the quickest estimations of forage nutrient composition is determining the field's maturity stage (e.g., 50% bloom) as developed by Kalu and Fick (1981) and further described by Fick and Mueller (1989). Each of the ten maturity stages is associated with a nutrient composition estimate (e.g., early bloom stage will have higher NDF content than early vegetative stage, but early vegetative stage will have higher CP content; Fick and Mueller, 1989). However, forage nutrient composition is also largely dependent on environmental and management factors such as fertilizing, time of the season, precipitation, and temperature during the different growth stages (Ralston, 1991; Buxton, 1996).

Mean stage by weight (MSW) and mean stage by count (MSC) are refined methods that use mathematical equations and forage maturity to predict forage nutrient composition (Kalu and

Fick, 1981; Fick and Onstad, 1988; Mueller and Fick, 1989). Although MSW and MSC calculations can generate more accurate estimates than maturity estimations, they are often time consuming and impractical for large fields with natural variation. Additionally, developed equations can be region-specific and may provide different results based on location (Sanderson, 1992), which highlights the impact of environmental factors on forage quality (Ralston, 1991; Buxton, 1996).

Hintz and Albrecht (1991) further developed estimation equations (predictive equations for alfalfa quality, or "PEAQ") based on both plant maturity and plant morphological factors (e.g., nodes per plant, plant height; Owens et al., 1995). They concluded that their estimation equations provided more accurate results than either MSW or MSC alone. Additionally, the ease of measuring some of the physiological factors (e.g., maturity of the most mature stem in the sample, tallest stem height in the sample) could be easier to measure for producers in a large field, however the number of samples required for accurate estimates may be time prohibitive (Hintz and Albrecht, 1991).

The growing degree day (GDD) model is another method for determining alfalfa maturity and nutrient composition, and accounts for past and present temperature and environmental factors (Sharratt et al., 1989; Sulc et al., 1999). The main limitation when using the GDD model is its rough estimation of alfalfa forage parameters, similar to maturity evaluations.

Recently, methods using advanced remote sensing technologies combined with portable NIRS units have been evaluated for in-field use. Noland et al. (2018) assessed a method for determining both alfalfa nutrient composition and yield using remote sensing technology and air temperature. Their study found that models incorporating remote sensing NIRS, canopy reflectance, and growing degree units provided reasonable yield and nutrient composition predictions, but could be improved with higher spectral resolution and continued technological growth in this sector (Noland et al., 2018). Knox et al. (2012) also utilized remote sensing technology paired with ancillary ecological data to predict forage nutrient composition in a grassland-savanna setting in Africa. Similar to Noland et al. (2018), Knox et al. (2012) concluded that combining ecological data with NIRS reflectance data produced the best predictions of forage nutrient composition, particularly when plant species and soil class were considered.

Current Applications for Hand-Held NIRS Devices

Hand-held NIRS units have been developed for a variety of agricultural and industry products and tend to be highly individualized. In the meat processing industry, Silva et al. (2020) evaluated the use of a hand-held NIRS unit when quantifying the amount of beef, pork, and chicken in ground meat samples and concluded that beef composition was accurately evaluated by the device. de Lima et al. (2018) assessed the ability to predict milk lactose content with a hand-held NIRS device with an interest in discriminating between lactose-free and regular milk to ensure lactose-intolerant consumers are guaranteed a lactose-free product. They concluded that the hand-held NIRS could accurately detect differences in milk lactose content while in the field. Also in the milk-production sector, de la Roza-Delgado et al. (2017) developed methodologies for utilizing a hand-held NIRS unit for predicting milk composition parameters while on-farm. The NIRS unit could accurately predict milk fat, protein, and non-fat-solids and performed similarly to a laboratory NIRS. Lastly, in the chocolate processing industry, Gatti et al. (2021) assessed the use of a hand-held NIRS device in predicting the amount of fat bloom in chocolate. Fat bloom occurs due to drastic changes in temperature during shipping and is often initially undetectable to the naked eye and only becomes visually apparent over time. They concluded that the hand-held NIRS unit could accurately assess the amount of fat bloom in chocolate exposed to different temperatures, which can be utilized in the field to ensure chocolate quality for consumers.

In addition to the food production industry, forage production and animal nutrition sectors have also started developing hand-held NIRS units for use in the field. Berzaghi et al. (2021) investigated the performance of three hand-held and one laboratory NIRS devices when predicting forage nutrient composition of preprocessed, ground alfalfa, and grass samples. The narrow spectral range (740-1070 nm) of one of the hand-held devices restricted the unit's ability to capture and predict some values compared to the laboratory NIRS. However, the hand-held units with moderate spectral range (950-1650 nm) performed similar to the laboratory NIRS unit when the laboratory NIRS was restricted to the same range. The authors concluded that the spectral range for each of the hand-held units played the largest role in determining the accuracy of the predictions, with spectral ranges between 950 to 1650 nm producing the most informative and accurate predictions for hand-held devices. It is important to note that portable NIRS devices can be restricted to producing limited spectral ranges due to their size as wide spectral ranges produce more heat and require sufficient ventilation and climate control to ensure accurate predictions (Beć et al., 2021). Rukundo et al. (2021) compared the performance of two laboratory and two hand-held NIRS devices when assessing forage nutrient composition of perennial warm-season grasses. Both total N and in vitro DM digestibility were similarly predicted by the hand-held devices and the laboratory NIRS units, however fiber values (e.g., NDF, ADF, and acid detergent lignin) were poorly predicted. Both studies have shown that hand-held NIRS devices can accurately predict some forage nutrient composition parameters, however there is a need to more widely investigate the use of hand-held NIRS devices, especially on fresh alfalfa while in the field.

The use of wet chemistry and laboratory NIRS procedures to determine forage nutrient composition can be costly and time intensive. Field prediction methods utilizing maturity, morphological data, and weather data can be useful, but can also be time-prohibitive and are not as accurate as wet chemistry or NIRS. Field applications using both environmental data and NIRS reflectance data have been shown to improve predictions (Knox et al., 2012; Noland et al., 2018), but there is still room for improvement when utilizing these methods. Both Berzaghi et al. (2021) and Rukundo et al. (2021) demonstrated that hand-held NIRS units could accurately predict select forage parameters for different dried forages in a laboratory setting. However, hand-held NIRS devices and prediction equations are often species-specific and can be negatively impacted by fluctuating outdoor environmental conditions. Therefore, the objective of the third chapter of this thesis was to develop and validate nutrient prediction equations for fresh alfalfa using a hand-held NIRS device.

Table 1.1: Analysis equations for dairy cattle nutrition (NRC, 2001).

Category	Component	Equation
Energy	DE[a]	(tdNFC/100) * 4.2 + (tdNDF/100) * 4.2 + (tdCP/100) * 5.6 + (FA/100) * 9.4 – 0.3
	ME	1.01 * DE – 0.45
	NE_m	$1.37 * ME – 0.138 * ME^2 + 0.0105 * ME^3 – 1.12$
	NE_g	$1.42 * ME – 0.174 * ME^2 + 0.0122 * ME^3 – 1.65$
	NE_l	0.703 * ME – 0.19
Digestibility	TDN	tdNFC + tdCP + tdFat + tdNDF - 7
	tdNFC	0.98 * (100 – [NDF – NDICP] + CP + EE + Ash)
	tdCP[b]	CP * exp(-1.2 * [ADICP/CP])
	tdNDF	$0.75 * ([NDF – NDICP] – L) * (1 – [L/\{NDF – NDICP\}]^{0.667})$
	tdFat	(Fat – 1) * 2.25
Protein	CP	%N * 6.25
	ADICP	ADIN * 6.25
	NDICP	NDIN * 6.25
Fats	FA	EE - 1

[a]DE, cattle digestible energy; tdNFC, truly digestible non-fibrous carbohydrates; tdNDF, truly digestible neutral detergent fiber; tdCP, truly digestible crude protein; FA, fatty acids; ME, metabolizable energy; NE_m, net energy for maintenance; NE_g, net energy for gain; NE_l, net energy for lactation; TDN, total digestible nutrients; tdFat, truly digestible fat; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; CP, crude protein; EE, ether extract; ADICP, acid detergent insoluble crude protein; L, acid detergent lignin; N, nitrogen; ADIN, acid detergent insoluble nitrogen; NDIN, neutral detergent insoluble nitrogen; FA, fatty acid

[b]tdCP is presented for forages. Other equations exist for concentrates (e.g. grains).

Table 1.2: Analysis equations for equine nutrition (Pagan, 1998; NRC, 2007).

Category	Component	Equation
Energy	DE[a]	2,118 + 12.18 * CP – 9.37 * ADF – 3.83 * %hemicellulose + 47.18 * fat + 20.35 * NSC – 26.3 * ash
Carbohydrates	Hemicellulose	ADF – NDF
	NSC[b]	100 – NDF – Fat – Ash – CP *OR* WSC + starch
Protein	CP	%N * 6.25
	ADICP	ADIN * 6.25
	NDICP	NDIN * 6.25

[a]DE, equine digestible energy; CP, crude protein; ADF, acid detergent fiber; NSC, non-structural carbohydrates; NDF, neutral detergent fiber; WSC, water-soluble carbohydrates; ADICP, acid detergent insoluble crude protein; ADIN, acid detergent insoluble nitrogen; NDICP, neutral detergent insoluble crude protein; NDIN, neutral detergent insoluble nitrogen
[b]NSC can be calculated by two different methods (Longland and Byrd, 2006; NRC, 2007).

Table 1.3: Common methods for determining nutrient components.

Category	Component	Method Source or AOAC Method Number
Structural Carbohydrates	TDF[a]	991.43 (AOAC International, 2005a)
	NDF	2002.04 (AOAC International, 2005b)
	ADF	973.18; 989.03 (NIRS Method) (AOAC International, 2005c; d)
Nonstructural Carbohydrates	WSC	(Hall et al., 1999)
	Starch	(Hall et al., 1999)
Protein	NDIN	(Licitra et al., 1996)
	ADIN	(Licitra et al., 1996)
	CP / N	990.03; 976.05; 976.06; 989.03 (NIRS Method) (AOAC International, 2005e; f; d; AOAC, 2010a)
	AA[b]	994.12 (AOAC International, 2005g)
Fat	EE	920.39 (AOAC International, 2005h)
Lignin	L	973.18 (AOAC International, 2005c)
Ash	Ash	942.05 (AOAC International, 2008)
Moisture	DM	991.01 (NIRS method) (AOAC International, 2005i)

[a]TDF, total dietary fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrates; NDIN, neutral detergent insoluble nitrogen; ADIN, acid detergent insoluble nitrogen; CP, crude protein; N, nitrogen; AA, amino acid; EE, ether extract; L, lignin; DM, dry matter

[b]This method lists procedures for determining the levels of multiple amino acids.

CHAPTER TWO: FORAGE CHARACTERISTICS AND GRAZING PREFERENCE OF COVER CROPS IN EQUINE PASTURE SYSTEMS

Chapter Summary

Cover crops are commonly used to provide environmental benefits and can extend the grazing season, but have not been explored in horse pastures. The objectives of this research were to evaluate forage mass, forage nutrient composition, and preference of annual ryegrass, winter rye, berseem clover, purple top turnip, and daikon radish under horse grazing. Cover crops were seeded in monoculture and mixtures in August 2018 and 2019 as a randomized complete block with four replicates and grazed by four adult horses. Prior to grazing, forages were sampled to determine herbage and root mass and nutrient composition. After grazing, forages were visually assessed for the percentage of removal on a scale of 0 to 100% to estimate preference. Data was analyzed using an analysis of variance and linear regression; significance was set at $P \leq 0.05$. Berseem clover was the lowest producing forage (590 to 1,869 kg ha^{-1} dry matter; $P \leq 0.001$), while minimal differences in herbage mass were observed among the other cover crops. All forages met digestible energy (>2.17 Mcal kg^{-1}) and crude protein (>19%) requirements for idle, adult horses. Berseem clover was most preferred (>73% removal) while turnip and radish were the least preferred (<19% removal; $P \leq 0.001$). Winter rye and annual ryegrass in monoculture and when seeded with berseem clover were moderately preferred (20 to 68% removal). Placing a priority on preference, berseem clover, annual ryegrass, and winter rye appear to be suitable cover crops to extend the grazing season in horse pastures.

Introduction

The climate in the Midwestern United States is comprised of warm summers and cold winters that limit access to pastures for a significant part of the year. Consequently, most horse operations rely on preserved forage, primarily in the form of hay, during the late fall, winter, and early spring months. To offset this reliance on more costly preserved forages, pastures can be planted with various forages that are productive into the late fall, including cover crops. In the Midwestern United States, cover crops are planted in the late summer before or after a cash-crop is harvested to provide vegetative cover, erosion control, and weed suppression into the winter, and nutrient recycling in the spring when incorporated into the soil (SARE, 2012; Schipanski et al., 2014; Finney et al., 2016; Adler and Nelson, 2020; Koehler-Cole and Elmore, 2020).

Commonly used cover crops include annual ryegrass (*Lolium multiflorum* L.), winter rye (*Secale cereale* L.), berseem clover (*Trifolium alexandrinum* L.), purple top turnip (*Brassica rapa* subsp. *rapa* L.), and daikon radish (*Raphanus sativus* subsp. *longipinnatus* L.). Annual ryegrass and winter rye are utilized because of their relatively high germination and establishment rates and ability to efficiently cover bare soil (SARE, 2012), which are desirable qualities for weed suppression and forage production. Legumes, like berseem clover, can improve soil fertility due to their nitrogen fixation properties and are often utilized as green manure or as a source of high-quality forage for livestock (Fraser et al., 2004; SARE, 2012; Salama et al., 2020). Turnip and radish are tap-rooted species that provide a biological means to alleviate soil compaction and are excellent scavengers of fall residual nitrogen (N) (Williams and Weil, 2004; Chen and Weil, 2010; SARE, 2012). Most of these cover crops have been explored in other livestock production systems and have been used to successfully extend the grazing season for ruminants (Reid et al., 1994; Lesoing et al., 1997; Yun et al., 1999; Faé et al., 2009; Franzluebbers and Stuedemann, 2014; Schomberg et al., 2014; Sulc and Franzluebbers, 2014; Brunsvig et al., 2017) while producing acceptable average daily gains (ADG) (Reid et al., 1994; Franzluebbers and Stuedemann, 2007, 2014). Specifically, sheep were able to graze purple top turnip until early January in the Northeastern United States (Reid et al., 1994) and net return on beef cattle was higher when backgrounded on winter rye versus confinement-fed (Franzluebbers and Stuedemann, 2007). Due to their environmental benefits and success in ruminant grazing systems, cover crops are potential forages that can be incorporated into horse pastures.

However, limited research exists on using cover crops in horse grazing systems. Berseem clover has been reported to be highly preferred by horses (McCann and Hoveland, 1991), and Grev et al. (Grev et al., 2017) found that annual ryegrass produced acceptable forage mass under horse grazing, was preferred by horses, and successfully extended the grazing season. However, other commonly grown cover crops have not been explored under horse grazing. Additionally, the unique selective grazing behavior displayed by horses (Cymbaluk, 1990; Shingu et al., 2010) makes it challenging to extrapolate research from other livestock species when managing horse pastures. Therefore, the objectives of this research were to determine forage mass, forage nutrient composition, and preference of cover crops grazed by horses. It was hypothesized that grasses and clover would be more suitable cover crops for horse pastures compared to turnip and radish.

Materials and Methods

The experiment was conducted at the Minnesota Agricultural Research Station in Saint Paul, MN (44.98829, -93.17563) on a Waukegan silt loam soil (fine-silty over sandy or sand-skeletal, mixed superactive, mesic Typic Hapludolls) with a soil pH, phosphorus, and potassium of 6.6, 18 mg kg^{-1}, and 85 mg kg^{-1}, respectively. No soil amendments or fertilizers were added, and irrigation was not required due to sufficient rainfall. Cover crops were planted into a prepared (e.g., tilled) seedbed following a grass-legume pasture in 1.8-m by 6.1-m plots on 9 August 2018 and 8 August 2019 using a drill seeder. The experimental pastures were arranged in a randomized complete block design with 14 treatments replicated four times. Cover crops included annual ryegrass, winter rye, berseem clover, purple top turnip, and daikon radish planted in monoculture and binary, quaternary, and complete mixtures (Table 2.1). A separate pasture area (0.06 ha) was seeded with a mixture of all five cover crops and was used as an acclimation pasture.

To determine forage mass, three 30-cm by 30-cm quadrats totaling 0.27 m^2 were sampled in each plot prior to grazing on 24 October 2018, 16 September 2019, and 14 October 2019. Annual ryegrass, winter rye, and berseem clover were hand-harvested to a 5-cm stubble height, while turnip and radish were completely removed (e.g., herbage and roots). Cover crops and weeds (e.g., nonplanted species) were separated and weighed to determine botanical composition. Turnip and radish were separated into herbage and root components, and after washing, roots were sliced into smaller sections to facilitate drying. Samples were dried in forced-air ovens at 60˚C until they maintained a constant mass (approximately 72 hours) to determine dry matter (DM) content.

To determine forage nutrient composition, samples were collected from the five cover crops planted in monoculture by hand-harvesting random, single 1-m^2 areas to a height of 5 cm prior to grazing on 23 October 2018, 15 September 2019, and 13 October 2019 between 1600 and 1800 hours and immediately placed in onsite driers using the methods described above. Turnip and radish roots were also harvested, washed, sliced, and dried before nutrient analysis. All dried forage and root samples were ground through a 5-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) followed by a 1-mm screen in a Cyclotec (Foss, Hillerød, Denmark). Samples were mixed thoroughly and analyzed for forage nutrient composition using near-infrared spectroscopy (NIRS) and wet chemistry at a commercial forage testing laboratory (Equi-Analytical, Ithaca, NY). Annual ryegrass, winter rye, and berseem clover were analyzed using NIRS due to existing equations for grasses and clovers. Turnip and radish were analyzed using wet chemistry due to a lack of existing NIRS equations.

Specifically, equine digestible energy (DE) was calculated using the equation established by Pagan (Pagan, 1998). Crude protein (CP) was determined by multiplying the percent N by 6.25 (AOAC, 2010b). Neutral detergent fiber (NDF) was measured using filter bag techniques (Ankom Technology, 2017). Starch and water-soluble carbohydrates were measured using the procedures detailed by Hall et al. (Hall et al., 1999) and were summed to estimate nonstructural carbohydrates (NSC; (Longland and Byrd, 2006)). Calcium (Ca) and phosphorus (P) levels were measured after microwave digestion (Microwave Accelerated Reaction System, CEM, Mathews, NC).

All experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (1905-37032A).

Four adult (18 ± 3 years) stock-type mares with an average bodyweight (BW) of 553 ± 29 kg and body condition score (BCS) of 6.25 ± 0.25 (Henneke et al., 1983) grazed the cover crops for two consecutive fall seasons. Horses had *ad libitum* access to water throughout the study. When the horses were not grazing cover crops, they were housed in a dry lot overnight and grazed perennial cool-season grass pastures during the day. While in the dry lot, horses had *ad libitum* access to a grass-legume mixed hay comprised of cool-season grass and alfalfa (*Medicago sativa* L.) fed in the form of a large round bale covered with a slow-feed net (Hay Chix, Taylors Falls, MN). Horses were fed a daily ration balancer (Enrich Plus Ration Balancing Horse Feed, Purina, St. Louis, MO) at 0.1% BW to meet vitamin and mineral requirements for adult horses at maintenance (NRC, 2007).

Horses were acclimated to grazing all species of cover crops over a four-day period prior to data collection and were monitored for gastric upset. During acclimation, grazing time started at one hour and increased by one hour each day until four consecutive hours were reached. Horses grazed the experimental pastures, which included all species, on 25 and 26 October 2018 when turnip and radish reached a late vegetative stage and had ≥5 cm of root protruding from the soil. Due to low intake of late vegetative turnip and radish in 2018, grazing was initiated earlier in 2019 on 19 and 20 September when turnip and radish were in an earlier vegetative stage with no root protrusion. The second grazing in 2019 occurred on 17 and 18 October when turnip and radish reached a late vegetative stage and had ≥5 cm of root protruding from the soil. For all grazing events, grass and clover species were in a vegetative growth stage; however, specific growth stage was not recorded at the time of grazing for these species. During each two-day grazing event, horses grazed two of the four replicates for four hours per day. The grazing duration was selected to allow for the determination of preference while achieving a minimum average residual height of 5 cm of the most preferred species to avoid overgrazing (Marten, 1978). The area grazed during each four-hour period was 0.09 ha with a stocking rate of 24,616 kg ha^{-1}. Turnip and radish are not commonly mowed after grazing due to limited regrowth post-mowing [1] and plot layout did not allow for mowing of grass and legume species after grazing. Therefore, pastures were not mowed between grazing periods in 2019. Manure was manually removed following each grazing period and the grazing season was concluded each year when a killing frost terminated the forages.

Preference was immediately assessed after each grazing event by visually estimating the percent of forage grazed to a 5-cm height from each plot on a scale from 0 (no grazing activity) to 100% (all available forage grazed)(Marten, 1978; Allen et al., 2013).

Analysis was conducted using R software (version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria) running RStudio (version 1.2.5019) and statistical significance was set at $P \leq 0.05$. The estimated marginal means from the linear models were compared using Tukey's honest significant difference test. Forage mass was analyzed using a linear mixed effects model where treatment, year, and the interaction between treatment and year were set as fixed effects, and the grazing period and replicate were considered random effects. To represent total season forage mass, data from both grazing periods in 2019 were combined in monocultures and mixtures containing only grasses and clover as these cover crops exhibited regrowth after grazing. However, due to minimal removal of turnip and radish during the first grazing in 2019, data containing turnip or radish are presented for the October grazing events in both years and represent total season mass. Weeds, or non-sown plants, were excluded from total mass.

To compare the relative abundance of individual species within mixtures, aboveground biomass of each species was analyzed as a percent of the total mixture biomass. Percent biomass was analyzed using mixed effects models that fit species as a fixed effect, and year, grazing period, and replicate as random effects. Because year and grazing period did not significantly impact composition, data are presented as an average of all three grazing periods.

Forage nutrient parameters (equine DE, CP, NDF, NSC, and Ca:P) were analyzed using mixed effects models where treatment, year, grazing period, and the interaction between treatment and year were set as fixed effects. Replicate was considered a random effect. For these models, there was a significant interaction between treatment and year; therefore, data are presented separately by year. Since the number of grazing periods varied by year, data are also presented separately by grazing period for these variables.

Horse preference was analyzed using a linear mixed effect model where treatment, grazing period, and the interaction between treatment and grazing were set as fixed effects, and the year and replicate were considered random effects.

Results and Discussion

Temperature and Precipitation

Monthly average air temperatures in August, September, and October appeared similar to the 30-year average in both 2018 and 2019 (Figure 2.1). However, the November average monthly temperatures were 4.6˚C and 3.2˚C less than the 30-year historical average in 2018 and 2019, respectively. Total precipitation was higher in 2018 and 2019 compared to the 30-year historical average. Precipitation exceeded the 30-year historical average by 7 and 16 cm in 2018 and 2019, respectively.

Herbage and Root Mass

A year by cover crop interaction ($P = 0.0012$) was observed for herbage mass. Herbage mass was higher in 2019 compared to 2018 (Table 2.2; $P < 0.001$), which can be explained by the two grazing events that occurred in 2019, which allowed for forage regrowth, compared to one grazing event in 2018. Berseem clover tended to result in the lowest herbage mass each year. There were minimal differences among the remaining cover crops grown in monoculture or mixtures when herbage mass was considered ($P > 0.05$). However, in 2018, the herbage mass of annual ryegrass and the annual ryegrass and berseem clover mixture were less compared to the cover crops with the greatest herbage mass ($P \leq 0.05$).

Cover crop herbage mass was comparable to previous research, except for berseem clover. The relatively high herbage mass of purple top turnip and daikon radish in the current study are similar to other reported herbage masses for these species in monoculture (SARE, 2012); however, some studies have reported lower herbage mass (Yun et al., 1999). Villalobos and Brummer (Villalobos and Brummer, 2015) reported turnip herbage mass as high as 5,503 kg ha^{-1} and radish herbage mass as high as 6,165 kg ha^{-1} when seeded in late July. In addition, Yun et al. (Yun et al., 1999) reported turnip and radish herbage mass between 1,800 and 5,600 kg ha^{-1}. Regarding annual ryegrass and winter rye, Grev et al. (Grev et al., 2017) found fall herbage mass ranged from 2,300 to 5,900 kg ha^{-1}, which are similar to values reported here. In the current study, berseem clover in monoculture resulted in the least amount of herbage mass; however, previous research reported forage mass averaged between 6,532 (Fraser et al., 2004) and 7,364 kg ha^{-1} (Ross et al., 2005). The differences in forage mass between current and past studies can be attributed to differences in weather, seeding rates, soil type and fertility management, grazing management (Deak et al., 2009), number of harvests (Salama et al., 2020), planting and harvest dates (Yun et al., 1999; Villalobos and Brummer, 2015), and the impact of horse grazing pressure and selectivity versus mechanical harvesting (Marten, 1978; Cymbaluk, 1990; Shingu et al., 2010).

Purple top turnip and daikon radish root mass were also harvested. Root mass for these species only differed in September 2019 ($P \leq 0.05$; data not shown). During that grazing period, radish produced the most root mass in monoculture (1,039 kg ha^{-1}) and when grown with annual ryegrass (1,179 kg ha^{-1}). Additionally, average total root mass across all monocultures and mixtures were lower in October 2018 (981 kg ha^{-1}) compared to October 2019 (2,234 kg ha^{-1}; $P <$ 0.001). Lastly, both turnip and radish produced less root mass (552 to 934 kg ha^{-1}) when planted in quaternary and complete mixtures compared to monoculture.

Observed root mass was not consistent with past results. Previous sheep grazing research found that turnip roots produced as much as 3,836 kg ha^{-1} when grazed in monoculture (Reid et al., 1994), and Yun et al. (Yun et al., 1999) reported root mass ranged between 1,976 to 4,290 kg ha^{-1} and 1,168 to 2,313 kg ha^{-1}, for turnip and radish, respectively. Saturated soils, due to higher-than-average rainfall during the fall, could have contributed to the lower root mass reported in the current study. It has been reported that both cover crops perform inefficiently in waterlogged soils (SARE, 2012). The differences in root mass between the current and previous studies could also be credited to differences in weather, soil type and fertility management, and time until harvest (Yun et al., 1999).

Botanical Composition

Although the seeding rates of turnip and radish were reduced in 2019 in an attempt to produce more uniform mixtures compared to the previous year (Table 2.1), there were no differences in composition between the years for most mixtures ($P > 0.05$). Herbage botanical composition differed among the cover crops (Figure 2.2; $P < 0.001$). Berseem clover performed the poorest in cover crop mixtures and comprised ≤ 3% in all mixtures ($P \leq 0.05$). Annual ryegrass and winter rye were dominant in binary mixtures with berseem clover but were minor components of mixtures including turnip or radish. Annual ryegrass composed only 9 to 14% of binary mixtures with turnip or radish, while winter rye comprised between 26 and 30%. Likewise, in quaternary mixtures, annual ryegrass and winter rye averaged only 3% and 7% of the mixture, respectively. In mixtures where both turnip and radish were included, turnip was more prevalent than radish, averaging 66% and 27%, respectively ($P \leq 0.05$). Consequently, turnip and radish comprised the greatest amount in mixtures and were the dominant cover crops.

Managing mixed pastures comprised of diverse forage species can be challenging and producing a uniformly-mixed pasture takes special consideration. While berseem clover has been successfully grown in mixtures with triticale and oats (Salama, 2020), the low composition of berseem clover in all mixtures during the current study is likely due to a difference in seeding ratios and the quicker establishment of the grasses combined with its susceptibility to over shading by other forages (Ross et al., 2005). Additionally, when berseem clover was planted in monoculture, weeds or non-sown plants invaded the area and comprised up to 50% of each plot (data not shown). Therefore, when considering berseem clover as a cover crop to extend the grazing season, managers should be aware of potential weed establishment and berseem clover's poor performance in a mixture. Subsequently, turnip and radish were the dominant forages in mixtures due to their quick germination and early growth of large, broadleaf canopies (SARE, 2012).Therefore, the authors suggest an even lower seeding rate of turnip and radish may be necessary if a more proportional mixture is desired.

Forage Nutrient Composition

Forage nutrient composition of the herbage differed (Table 2.3; $P \leq 0.05$) among the cover crops. There were no consistent trends for equine DE among the years. In 2018, turnip and radish had the greatest equine DE, while annual ryegrass and winter rye had the lowest ($P \leq 0.05$). In both grazing events of 2019, berseem clover had the highest equine DE while daikon radish had the lowest ($P \leq 0.05$). There was no difference ($P > 0.05$) in CP concentration among the cover crops in September 2019; however, differences were observed in October of each year. During the October grazing events, radish had the highest concentration of CP, while annual ryegrass had the lowest ($P \leq 0.05$). There were consistent trends in NDF, with turnip and radish containing lower concentrations, and annual ryegrass and winter rye containing higher concentrations ($P \leq 0.05$). In both years, berseem clover had a moderate amount of NDF. Differences in NSC among cover crop herbage were only found in October 2018 when annual ryegrass, winter rye, and berseem clover had higher amounts of NSC compared to radish ($P \leq 0.05$). In general, the Ca:P was inverted for both grass species. Turnip, radish, and berseem clover had Ca:P ratios ≥1.8:1 ($P \leq 0.05$).

Because purple top turnip and daikon radish have not been researched in horse pasture systems, equine DE had not been previously reported. However, equine DE for annual ryegrass and winter rye were similar to previous reports which averaged 2.32 and 2.47 Mcal kg^{-1}, respectively (Grev et al., 2017). Crude protein for all cover crop species were higher than previously reported (Maloney et al., 1999; Paulson et al., 2014; Villalobos and Brummer, 2015), and were likely a result of the relatively high soil fertility values at this research site, which have been documented previously (Allen et al., 2013; Grev et al., 2017; Catalano et al., 2019). All cover crops had similar NDF values as previously reported (Villalobos and Brummer, 2015; Grev et al., 2017), except berseem clover. Neutral detergent fiber values have been reported to be between 22 to 26% for turnip and radish (Villalobos and Brummer, 2015) and 38 to 45% for annual ryegrass and winter rye (Grev et al., 2017). In the current study, berseem clover had lower NDF concentrations than Fraser et al. (Fraser et al., 2004), who reported NDF values averaging 42%. These differences are likely due to maturity at the time of harvest (Buxton, 1996; Martinson et al., 2012a; Grev et al., 2020). For grass species, NSC concentration was similar, or slightly higher, than Grev et al. (Grev et al., 2017) who found NSC ranged from 9.6 to 18.4%. Nonstructural carbohydrate content had not been previously reported for berseem clover, purple top turnip, and daikon radish. This is not surprising since NSC tends to be an interest when formulating horse rations, but not necessarily with other livestock species. The Ca:P of the grasses was also comparable to previous reports (Grev et al., 2017). Similar to NSC, the Ca:P of the other cover crops had not been documented previously.

Herbage from all of the cover crops met or exceeded the daily requirements of 16.7 Mcal/d DE and 630 g/d CP for an adult 500 kg horse at maintenance when fed at 2% BW in forage DM (NRC, 2007). There are no defined NDF requirements for horses (NRC, 2007), but NDF is a key contributor to forage quality and an indicator of maturity (Martinson et al., 2012a). To maximize livestock forage intake, it is generally accepted that forages contain ≤65% NDF (DeBoer et al., 2017; Grev et al., 2017; Catalano et al., 2019). In the current study, all cover crops were ≤47% NDF, indicating no major barriers to forage intake or palatability based on NDF. Although there are no recommended dietary levels of NSC for healthy, adult horses, a total diet containing ≤12% NSC is suggested for metabolically challenged horses (Frank, 2009) or ≤10% NSC for horses diagnosed with polysaccharide storage myopathy (Borgia et al., 2009). Based on this recommendation, only cover crops grazed in September 2019 could potentially be utilized by these types of horses. However, due to variability in NSC during other grazing periods, these forages may not consistently be suitable for horses with metabolic concerns. The Ca:P is an important consideration when feeding all classes of horses, and most agree a Ca:P of 2:1 is ideal to maintain optimum calcium absorption (NRC, 2007). Both annual ryegrass and winter rye tended to have inverted Ca:P. However, this imbalance could be amended by supplementing Ca to the diet, or by adding forages higher in Ca to the mixture (e.g., berseem clover).

Due to the potential that horses could consume turnip and radish roots, the nutrient composition profiles of the root components were also evaluated. Turnip and radish root nutrient composition only differed in September 2019 (Table 2.3; $P \leq 0.05$). During this grazing, turnip roots had greater amounts of DE, CP, and NDF compared to radish ($P \leq 0.05$); no differences in NSC and Ca:P were observed. There is limited previous research on the nutrient composition of turnip and radish roots. In the current study, root CP concentrations were higher than those reported by Yun et al. (Yun et al., 1999) who found turnip and radish roots averaging 14% CP. It should be noted that both cover crop roots contained high amounts of NSC in October of both years, which may be problematic for some horses if consumed (Shirazi-Beechey, 2008). However, in the current study there was no evidence of horses ingesting turnip and radish roots, although cattle have been reported to readily consumed turnip roots (Moate et al., 1999).

Horse Preference

Horse preference was consistent among the grazing events. Horses preferred berseem clover in monoculture compared to all other cover crops in monoculture and mixtures (Table 2.4; $P \leq 0.001$). Treatments containing purple top turnip or daikon radish, in monoculture or mixtures, were least preferred. Annual ryegrass and winter rye in monoculture and in mixture with berseem clover resulted in moderate horse preference.

It is well known that horses are selective grazers and that many factors can affect animal preference, including available species, familiarity with species, agronomic management, geographic location, and weather conditions (Marten, 1978; Cymbaluk, 1990; Martinson et al., 2016). McCann and Hoveland (McCann and Hoveland, 1991) also observed that berseem clover was highly preferred by horses, with removal ≥74%. Additionally, Grev et al. (Grev et al., 2017) found horses had a moderate preference for annual ryegrass and winter rye with removal ranging between 35 to 88% and 5 to 33%, respectively. Although there is no previously published research exploring purple top turnip and daikon radish in horse grazing systems, both sheep and beef cow calf pairs have shown the ability to sustain bodyweight while grazing these species long term (Reid et al., 1994; Yun et al., 1999; Brunsvig et al., 2017). Dairy cattle showed individual preference for turnip leaves and roots, but herd trends for specific components were not consistent (Moate et al., 1999). Yun et al. (Yun et al., 1999) noted that sheep preferred to consume turnip and radish herbage, with removals between 50 and 80%, but did not readily consume turnip or radish roots. In the current study, however, there was no observed consumption of turnip and radish roots by the horses. The authors noted a sulfurous smell associated with turnip and radish herbage and roots. The smell combined with the novelty of these species in a horse grazing system could have contributed to the low preference ratings. Selecting forages that will be readily consumed is the goal of any livestock grazing system. Additionally, when planting mixtures, selecting forages with similar preferences should lead to more efficient utilization of a pasture. Therefore, when planting cover crops for horse grazing, purple top turnip and daikon radish should be avoided.

Although not measured in the current study, previous research has investigated the levels of anti-quality factors like glucosinolates and S-methyl cysteine sulfoxide in turnip (Gustine and Jung, 1985) and radish (Malik et al., 2010). These compounds have been suspected to reduce ADG when grazed for long periods and could lead to reduced voluntary feed intake in ruminants (Barry, 2013). The conversion of glucosinolates into iso-thiocyanates produces a bitter flavor (Barry, 2013), which may have also contributed to the extremely low voluntary intake of turnip and radish by the horses in the current study.

Fitting Cover Crops into a Horse Grazing System

While cover crops may not be suitable for every horse pasture system, they can be utilized to achieve a diversity of pasture management related goals. Grass cover crop species can be seeded into overgrazed or bare pasture areas (e.g., due to flooding or winter kill) to quickly provide forage, cover exposed soil to help prevent erosion, ward off the invasion of non-sown plants or weeds, and build soil organic matter (SARE, 2012). Legume cover crops can provide soil enriching benefits due to their N-fixation qualities and may reduce the need for fertilizer in some situations (Fraser et al., 2004; SARE, 2012; Salama et al., 2020). Due to their large, penetrating taproots, turnip and radish have been shown to alleviate soil compaction (Williams and Weil, 2004; Chen and Weil, 2010; SARE, 2012) and could be used in high-traffic areas prior to perennial pasture establishment. Furthermore, both turnip and radish are excellent scavengers of fall residual N and provide a slow-releasing source of N in the spring (SARE, 2012). While the current research is intended to add resources to the horse owner and manager's pasture management toolbox, it also highlighted the need for future research. Future research opportunities include exploring no-till planting of cover crops into older pastures, the impact of mowing on cover crop regrowth, establishing seeding rates that result in uniform mixtures, and exploring environmental benefits of cover crop in horse pasture systems.

Conclusions

Differences in herbage mass, forage nutrient composition, and grazing preference were observed among the cover crops and are important to collectively consider when planting cover crops for horse grazing. Although cover crops tend to be utilized as a short-term forage to extend the grazing season in the Midwestern United States, horse preference should still be a key consideration. Placing a priority on preference, berseem clover, annual ryegrass, and winter rye appear to be the best suited cover crops to extend the grazing season in horse pastures. These species met the DE and CP needs of adult horses at maintenance. However, the grasses tended to have an inverted Ca:P and berseem clover produced lower forage mass both in monoculture and mixtures, which are important tradeoffs to consider. Still, berseem clover, annual ryegrass, and winter rye appear to be suitable for horse pastures because of their acceptable forage mass, nutrient composition, and preference.

Neither the herbage nor the roots of purple top turnip and daikon radish were preferred by the horses, and therefore were considered undesirable as horse pasture species. However, both turnip and radish produced high amounts of forage mass, were nutrient dense, and tended to dominate in mixtures. Purple top turnip and daikon radish should be further investigated in pastures for their environmental benefits and potential in preparing a seedbed for new pastures. Further research should also investigate seeding rates of turnip and radish that result in more uniform mixtures for livestock pastures.

Table 2.1: Seeding rates (kg ha^{-1}) of annual ryegrass (A), winter rye (W), berseem clover (C), purple top turnip (T), and daikon radish (R) planted in monoculture, binary, and quaternary mixtures and grazed by horses in the fall of 2018 and 2019 in St. Paul, MN.

	Treatment	Annual Ryegrass	Winter Rye	Berseem Clover	Purple Top Turnip[a]	Daikon Radish[a]
				kg ha^{-1}		
Monocultures	A	22.5	-	-	-	-
	W	-	112.3	-	-	-
	C	-	-	13.5	-	-
	T	-	-	-	7.9	-
	R	-	-	-	-	13.5
Annual Ryegrass Mixtures	AC	9.0	-	7.9	-	-
	AT	11.2	-	-	3.9	-
	AR	11.2	-	-	-	6.7
	ACTR	6.7	-	5.4	2.4	4.0
Winter Rye Mixtures	WC	-	44.9	8.1	-	-
	WT	-	56.2	-	3.9	-
	WR	-	56.2	-	-	6.7
	WCTR	-	33.7	5.4	2.4	4.0
Complete Mixture	AWCTR	6.7	33.7	5.4	2.4	4.0

[a]Purple Top Turnip and Daikon Radish seeding rates were reduced in 2019 to 2.2 kg ha^{-1} in all mixtures in an attempt to produce more uniform botanical mixtures.

Table 2.2: Total above-ground forage dry matter mass (kg ha^{-1}) of cover crops planted in monoculture and mixtures and grazed by horses in the fall of 2018 and 2019 in St. Paul, MN.

	Treatment[1]	Year 2018	Year 2019
		kg ha^{-1}	
Monocultures	A	1,672^{de}	5,828^{a}
	W	1,855^{bcd}	5,438^{a}
	C	590^{e}	1,869^{b}
	T	2,963^{abc}	5,569^{a}
	R	3,074^{a}	4,805^{ab}
Annual Ryegrass Mixtures	AC	1,766^{cde}	4,511^{ab}
	AT	2,590$^{a\text{-}d}$	5,369^{a}
	AR	2,576$^{a\text{-}d}$	5,078^{a}
	ACTR	2,952^{abc}	6,382^{a}
Winter Rye Mixtures	WC	1,834$^{a\text{-}e}$	4,796^{ab}
	WT	3,038^{ab}	5,197^{a}
	WR	2,562$^{a\text{-}d}$	4,073^{ab}
	WCTR	2,730$^{a\text{-}d}$	5,353^{a}
Complete Mixture	AWCTR	2,236$^{a\text{-}d}$	6,002^{a}

$^{a\text{-}e}$Means within a column without a common letter differ based on Tukey's HSD test ($P \leq 0.05$).
[1]Annual Ryegrass (A); Winter Rye (W); Berseem Clover (C); Purple Top Turnip (T); Daikon Radish (R).

Table 2.3: Herbage and root nutrient composition (on a dry matter basis) for cover crops grown in monoculture and grazed by horses in the fall of 2018 and 2019 in St. Paul, MN.

	Forage Nutrient[1]				
Species	DE (Mcal kg^{-1})	CP (%)	NDF (%)	NSC (%)	Ca:P
			Herbage		
			October 2018		
Annual Ryegrass	2.63^{c}	19^{d}	41^{a}	25^{a}	1.2:1^{b}
Winter Rye	2.53^{c}	22^{cd}	43^{a}	20^{ab}	0.7:1^{b}
Berseem Clover	2.81^{b}	25^{c}	28^{b}	20^{ab}	3.7:1^{a}
Purple Top Turnip	3.01^{a}	29^{b}	21^{c}	17^{bc}	4.3:1^{a}
Daikon Radish	2.94^{ab}	35^{a}	22^{c}	12^{c}	3.2:1^{a}
			September 2019		
Annual Ryegrass	2.43^{ab}	26	46^{a}	12	0.8:1^{c}
Winter Rye	2.42^{ab}	27	47^{a}	11	0.7:1^{c}
Berseem Clover	2.62^{a}	29	33^{b}	12	3.3:1^{a}
Purple Top Turnip	2.30^{bc}	32	22^{c}	12	2.9:1^{ab}
Daikon Radish	2.17^{c}	33	22^{c}	10	1.8:1^{bc}
			October 2019		
Annual Ryegrass	2.64^{b}	25^{b}	39^{a}	19	0.9:1^{c}
Winter Rye	2.64^{b}	28^{ab}	38^{a}	17	0.8:1^{c}
Berseem Clover	2.80^{a}	26^{ab}	28^{b}	17	2.5:1^{b}
Purple Top Turnip	2.47^{c}	28^{ab}	21^{c}	18	4.1:1^{a}
Daikon Radish	2.31^{d}	31^{a}	22^{c}	16	2.7:1^{b}
			Roots		
			October 2018		
Purple Top Turnip	3.21	22	16	42	1.1:1
Daikon Radish	3.18	21	17	41	1.2:1
			September 2019		
Purple Top Turnip	1.97^{a}	16^{a}	19^{a}	16	1.2:1
Daikon Radish	1.11^{b}	11^{b}	13^{b}	9	1.0:1
			October 2019		
Purple Top Turnip	2.66	16	20	44	1.0:1
Daikon Radish	2.37	15	14	38	1.0:1

[a-d]Means within a column, grazing period (September 2019, October 2018, October 2019), and herbage or root forage component without a common letter differ based on Tukey's HSD test ($P \leq 0.05$).

[1]DE, digestible energy for equine; CP, crude protein, NDF, neutral detergent fiber; NSC, nonstructural carbohydrates; Ca, calcium; P, phosphorus.

Table 2.4: Horse preference (% removal) of cover crops grown in monoculture and mixtures grazed in the fall of 2018 and 2019 at St. Paul, MN.

Treatment[1]	October 2018	September 2019	October 2019
		% Removal[2]	
A	55^{b}	21^{b}	43^{b}
W	20^{cd}	28^{b}	53^{b}
C	100^{a}	73^{a}	89^{a}
T	4^{cd}	0^{c}	0^{c}
R	3^{d}	0^{c}	0^{c}
AC	68^{b}	24^{b}	41^{b}
AT	3^{d}	4^{c}	5^{c}
AR	3^{d}	8^{c}	15^{c}
ACTR	3^{d}	0^{c}	1^{c}
WC	24^{c}	25^{b}	53^{b}
WT	3^{d}	3^{c}	11^{c}
WR	3^{d}	5^{c}	19^{c}
WCTR	6^{cd}	3^{c}	3^{c}
AWCTR	4^{cd}	0^{c}	1^{c}

[a-d]Means without a common letter within a column differ based on Tukey's HSD test ($P \leq 0.05$).
[1]Annual Ryegrass (A); Winter Rye (W); Berseem Clover (C); Purple Top Turnip (T); Daikon Radish (R).
[2]Preference assessed as visual removal of available forage after 4 h of horse grazing, ranging from 0 (no evidence of grazing) to 100 (100% of vegetation grazed to a height of 5-cm).

Figure 2.1: Monthly air temperature (˚C), precipitation (cm), and 30-year historical average for St. Paul, Minnesota in the fall of 2018 and 2019. Weather data obtained from http://www.dnr.state.mn.us/climate/historical/index.html.

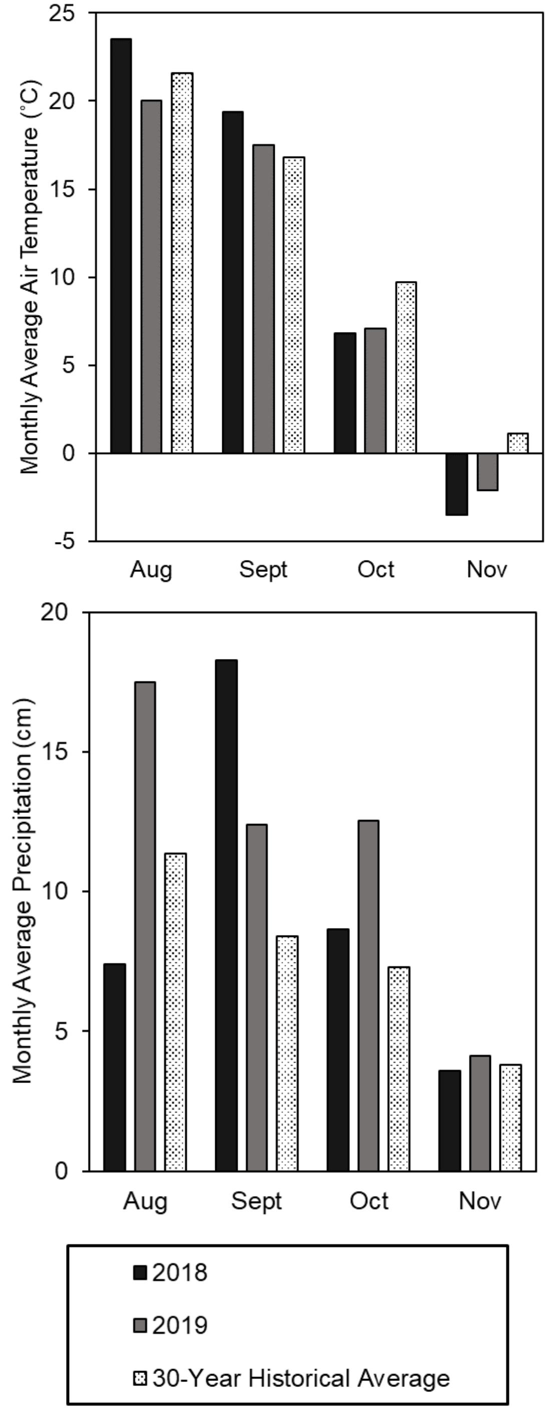
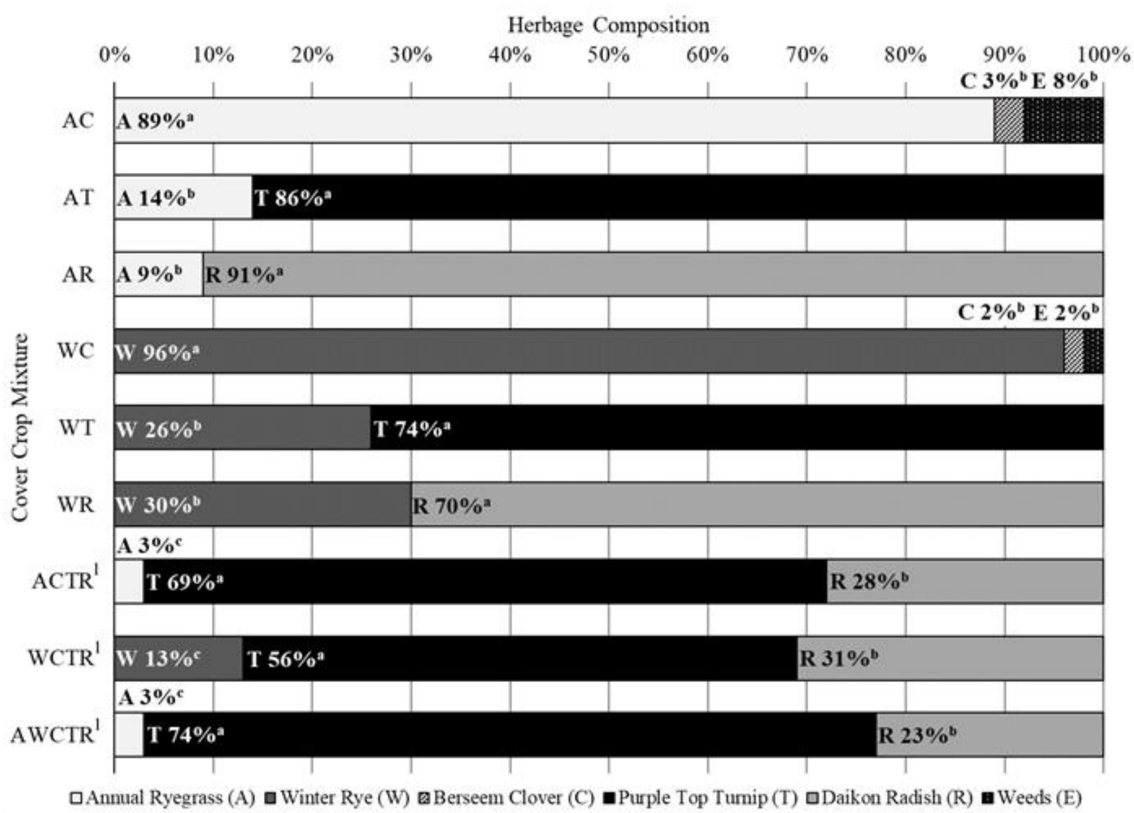


Figure 2.2: Mean percent of above-ground dry matter by forage species or weeds in each cover crop mixture. Proportions are averaged over all three grazing events.



[a-c]Means without a common letter within a treatment differ based on Tukey's HSD test ($P \leq 0.05$).
[1]Forage or weed species composing less than 1% are not presented.

CHAPTER THREE: DEVELOPING AND VALIDATING NEAR INFRARED SPECTROSCOPY PREDICTION EQUATIONS FOR FRESH ALFALFA USING A HAND-HELD DEVICE

Chapter Summary

Near infrared spectroscopy (NIRS) is a staple in laboratories used to determine forage quality in an affordable and efficient manner compared to standard wet chemistry analysis. The use of hand-held, portable NIRS technology is an emerging technology that could be used as a tool to measure forage quality at harvest. The objectives of this research were to formulate and validate NIRS predictive equations for fresh alfalfa (*Medicago sativa* L.) using a hand-held NIRS device (NIR4 Farm, AB Vista, Marlborough, England). In 2019, alfalfa was harvested from an existing stand, sorted by maturity, and chopped to 2.5-cm lengths (n=100 samples). Fresh alfalfa was scanned by the NIRS device in a climate-controlled room to obtain spectral data. Samples were then analyzed using wet chemistry and predictive equations were developed. In 2020, alfalfa was harvested from different existing stands, sorted by maturity, and chopped to 2.5-cm lengths (n=102 samples). Samples were scanned in the field immediately after harvesting using the NIRS device containing previously developed equations. Samples were then analyzed using wet chemistry and benchtop NIRS. Data was analyzed using R (version 3.6.2) and significance was set at $P < 0.05$. Calibration statistics were generated based on the developed prediction equation for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and NDF digestibility at 48 hours (NDFD48). A linear model and pairwise analysis were then used to determine differences between the wet chemistry, benchtop NIRS, and hand-held NIRS values for CP. Calibration statistics showed that the hand-held device could predict DM ($R^2 = 0.718$), CP ($R^2 = 0.84$), and NDF ($R^2 = 0.676$) with acceptable accuracy. There was no difference between wet chemistry and hand-held NIRS values for CP ($P = 0.98$; $R^2 = 0.89$; SEP = 0.77) and both the benchtop and the hand-held NIRS units performed similarly ($P = 0.17$). These results suggest that the hand-held NIRS accurately estimated CP concentrations of fresh alfalfa, and indicates other forage parameters may also be predicted using a hand-held NIRS.

Introduction

Near-infrared reflectance spectroscopy (NIRS) is utilized in a variety of areas to determine the chemical composition of products (Shenk and Westerhaus, 1994). The advantages that NIRS provides over standard wet chemistry analysis include speed, minimal sample preparation, the ability to analyze multiple components during one operation, and that samples are not consumed (Marten et al., 1989). Benchtop NIRS systems have been developed and calibrated to affordably predict nutritive value and leaf concentration of forages, as well as ethanol traits of biofuel crops (Corson et al., 1999; Halgerson et al., 2004; Vogel et al., 2011). Additionally, predicted nutritive values have been used when balancing mixed rations to maximize animal performance (e.g., milk production, meat production, reproductive efficiency). The recent development of low cost and portable hand-held NIRS devices poses new opportunities for both forage producers and animal nutritionists.

Similar to benchtop NIRS, hand-held NIRS units are utilized by a variety of industries. Because of their diverse applications and portability, there is a variety of hand-held NIRS units on the market (Beć et al., 2021). Units vary in size, sensor design, mobile connectivity, and program platform, with units tailored to the needs of the specific industry. One major difference between benchtop and hand-held NIRS units is that hand-held units typically use only a portion of the reflected light spectrum used in the benchtop units (Berzaghi et al., 2021). By narrowing the light spectrum, hand-held units can be limited in what spectra they capture, therefore, limiting the accuracy and scope of the predictions. However, Berzaghi et al. (2021) demonstrated that some hand-held units (NIR range 950 – 1650 nm) predicted forage nutritive composition better than others (NIR range 740 – 1070 nm) based on the selected NIR spectral range, and predictions for those units were similar to a benchtop NIRS restricted to the same NIR spectral range.

Hand-held NIRS units have been used in a variety of human food production industries including fruit production (Cayuela and Weiland, 2010; Tardaguila et al., 2017; Entrenas et al., 2019), meat processing (Silva et al., 2020), chocolate production (Gatti et al., 2021), and milk quality (de la Roza-Delgado et al., 2017; de Lima et al., 2018). The use of hand-held NIRS devices is an emerging tool in the forage industry. Rukundo et al. (2021) recently found that hand-held NIRS units can measure total nitrogen (N) and *in vitro* dry matter digestibility (IVDMD) in perennial warm-season grasses. Additionally, Berzaghi et al. (2021) demonstrated that some hand-held NIRS units could accurately predict a variety of nutrient parameters including crude protein (CP), fibers, and digestibility values for preprocessed and ground alfalfa (*Medicago sativa* L.) and grass samples.

Although there is evidence that hand-held NIRS units can accurately measure forage nutrient composition in ground alfalfa samples, the use of a hand-held NIRS unit has not been developed and validated for use on fresh alfalfa. Due to the unique nature of hand-held NIRS devices, forage species and product differences, and the specialized equations necessary for nutrient analysis, it is challenging to extrapolate results from other research when estimating nutritive values of alfalfa alfalfa. Therefore, the objectives of this research were to develop and validate nutrient prediction equations for fresh alfalfa using a hand-held NIR unit. It was hypothesized that the hand-held NIRS unit would produce similar results compared to benchtop NIRS and wet chemistry analytical methods.

Materials and Methods

The NIRS equation development experiment was conducted in 2019 at the Minnesota Agricultural Research Station in St. Paul, MN in 2019 (44.98829, -93.17563) on a Waukegan silt loam soil (fine-silty over sandy or sand-skeletal, mixed superactive, mesic Typic Hapludolls) with a soil pH, phosphorus, and potassium of 6.6, 18 mg kg-1, and 85 mg kg-1, respectively. Alfalfa was sampled from a 3-year-old stand consisting of three, randomized replicates of 24 cultivars arranged in 1.8-m by 6.1-m plots. Samples were obtained from all three replicates and consisted of three cultivars (‘Magnum 7’, ‘440HVX.RR’, and ‘SW4107’). Cultivars were chosen to represent genetically different alfalfa groups (conventional, reduced lignin, and dairy quality, respectively). Random, 1-m^2 areas from each plot were hand harvested to a 5-cm stubble height to obtain a minimum sample wet weight of 400 grams. Harvests occurred weekly for three weeks in June to obtain samples (n=56) representing diverse alfalfa maturity stages. Plots were mowed with a flail harvester (Carter Manufacturing Company, Inc., Brookston, IN) to a 5-cm stubble height and allowed to regrow. Plots were re-sampled over a three-week period in July once the majority of alfalfa reached the late vegetative stage (n=60 samples; Fick & Mueller, 1989).

Samples were placed into plastic bags and immediately transported to a climate-controlled lab for processing. Stems from each sample area were sorted by maturity and chopped to 2.5-cm lengths using gardening shears (n=86) or mechanically chopped to 1-cm lengths using a Master Prep® Ninja® blender (SharkNinja Operating LLC, Needham, MA; n=30) before scanning with a hand-held NIRS spectrometer (NIR4 Farm, AB Vista, Marlborough, England; 950 – 1750 nm). All samples (n=116) were individually placed in the NIRS unit scanning bowl with a minimum 5-cm depth and reflectance readings were obtained from five scans repeated over six replicates with scans being averaged by replicate. Between replicates, samples were mixed in the bowl and the NIRS prob was wiped with a disposable lens cleaner towelette and recalibrated. Spectral data was stored on the provided tablet and was also instantly uploaded into a cloud file system accessible to the researchers and the NIRS unit provider. Since no equations existed for fresh alfalfa in the device, samples were scanned to gather spectral data and no prediction output was generated.

After scanning, samples were dried in forced-air ovens at 60˚C until they maintained a constant mass (approximately 72 hours). Dried samples were then ground through a 5-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) followed by a 1-mm screen in a Cyclotec (Foss, Hillerød, Denmark). Of the 116 samples, 16 samples were excluded due to improper drying or an insufficient amount of sample to perform wet chemistry.

Ground samples (n=100) were scanned using a benchtop NIRS spectrometer (Model DA 7200; Perten Instruments, Springfield, IL) and were then sent to a commercial forage testing laboratory (Dairy One, Ithaca, NY) to be analyzed for forage nutritive composition using wet chemistry. Samples were analyzed for DM, CP (N x 6.25; AOAC, 2010), NDF (Goering and Van Soest, 1970; Van Soest et al., 1991), and NDFD48 (Hoffman et al., 1993).

Wet chemistry results were compared to the NIRS spectra generated by the hand-held NIRS unit. Wet chemistry results were used to develop NIRS predictive equations for DM, CP, NDF, and NDFD48. Calibration statistics were generated based on the performance of the developed equations and include: the coefficient of determination (R^2), standard error of calibration (SEC), and the standard error of cross validation (SECV). Coefficients of determination were assessed using the guidelines developed my Williams (2001) to determine the accuracy of the predictions. Similar and low SEC and the SECV values also represented acceptable prediction accuracy.

The validation experiment was conducted in an adjacent field at the Minnesota Agricultural Research Station in Saint Paul, MN in 2020. The alfalfa was harvested from an existing, 1-year-old stand containing four replicates comprised of four cultivars seeded at differing seeding rates for a total of 112 plots that plots measured 0.9-m by 6.1-m. Samples were collected from three cultivars (‘SW 5511’, ‘54VR10’, and ‘55VR08’) and plots were randomly selected at each harvest to produce 2 or more replicates of the three cultivars of interest. Samples were harvested bi-weekly until alfalfa reached an early-seedpod maturity when they were mowed with a flair harvester and allowed to regrow as previously described. This process was repeated four times between June and October and produced 163 samples.

Samples were sorted by stem for maturity while in the field and immediately chopped to 2.5-cm lengths using garden shears. While in the field, samples were scanned with the NIRS unit using the previously developed equations on the updated device. After scanning in the field, samples were dried and ground as previously described. Ground samples were scanned using a benchtop NIR spectrometer before being sent to forage laboratories (CP, Dairy One, Ithaca, NY;

DM, NDF, NDFD48, USDA-ARS Plant Science Research Laboratory, St. Paul, MN) to be analyzed for forage nutritive value using wet chemistry.

Of the 163 samples, 102 were selected to be included in the validation analysis primarily to balance both maturity stage and variety. Samples were selected against based on poor scanning performance in extreme weather when using the hand-held unit, and to balance the number of samples per maturity level. Wet chemistry analysis results were compared to the hand-held NIRS unit predictions. Validation statistics were generated based on the performance of the forage nutritive composition prediction equations and include the coefficient of determination (R^2) and the standard error of prediction (SEP).

Pairwise analysis comparing analysis type (e.g., wet chemistry, hand-held NIRS unit, and benchtop NIRS) were conducted using R (version 3.6.2) running RStudio (version 1.2.5019) and statistical significance was set at $P \leq 0.05$. Averages are presented as mean ± standard deviation. A linear model was used with forage nutrient parameter as the response variable and analysis type as the predictor variable. Estimated marginal means were compared using Tukey's honest significance difference test.

Results and Discussion

Development of NIRS Predictive Equations

The predictive equations built using the NIR spectra were able to predict DM, CP, and NDF, but not NDFD48 (Table 3.1). This determination was concluded based on the scale developed by Williams (2001) for interpreting R^2 values where different R^2 values would determine if the accuracy of a prediction equation was suitable for different analysis purposes (e.g., quality control, research, not useful).

Both DM and CP have been accurately predicted by NIRS instruments for decades due to their unique spectral fingerprints (Choppin and Violante, 1972; Ozaki, 2012; Kobori et al., 2013). Dry matter is a derivative of moisture content, which is spectrally recognized by its large markers in the O–H regions (960 nm, 1450 nm, and 1940 nm) of the NIR spectra (Choppin and Violante, 1972). Similarly, CP is recognized by the large markers in the N–H regions (1700 – 2500 nm) of the spectra due to the N in the amino acid backbone (Williams, 2001; Ozaki, 2012). Therefore, it is not surprising that these two compounds are predicted with acceptable accuracy by the hand-held NIRS device. However, NDFD48 is more complex to predict. Because NDFD48 is a performance measure with derivatives stemming from both nutrient content and bioavailability to the animal, NDFD48 is difficult to predict using NIRS units. Inconsistencies in evaluations have even been shown using wet chemistry (Rymer et al., 2005). Variations in sample preparation procedures and environmental factors impacting the rumen microbiota used for wet chemistry analysis of NDFD48 may be underlying factors preventing consistent predictions of NDFD48. These inconsistencies thereby limit the accuracy when using NIRS devices calibrated with the wet chemistry predictions (Rymer et al., 2005). Therefore, it is understandable that NDFD48 is not accurately predicted by the hand-held NIRS device.

When analyzing perennial warm-season grasses using two different hand-held NIRS units, Rukundo et al. (2021) found acceptable predictability for total N (R^2: 0.824, 0.873; SEC: 2.02, 1.72 g kg^{-1}; SECV: 2.43, 2.06 g kg^{-1}) but found poor performance when predicting NDF (R^2: 0.539, 0.622; SEC: 42.46, 38.44 g kg^{-1}; SECV: 51.33, 46.20 g kg^{-1}). Berzaghi et al. (2021) compared the performance of three hand-held devices when evaluating preprocessed, ground alfalfa and grass samples. Both CP and NDF were accurately predicted by two of the hand-held devices when analyzing grasses ($R^2 > 0.86$) and alfalfa ($R^2 > 0.91$). Neutral detergent fiber digestibility at 48 hours was less accurately predicted by these devices ($R^2 < 0.78$), but was still suitable for screening purposes such as in a production setting or for non-research purposes (Williams, 2001). The third device performed poorly when predicting NDFD48 ($R^2 < 0.52$) due to the limited spectral range of the device. The limited spectral range of some hand-held NIRS devices can lead to differences in prediction accuracies depending on both the width and location of the spectra that is covered. The unit utilized for the current study had a similar spectra range (950 – 1700 nm) to the two better-performing units investigated by Berzaghi et al. (2021) suggesting that the unit is capable of accurately predicting not only CP and NDF, but acid detergent fiber and acid detergent lignin as well. Benchtop NIRS units are climate-controlled and closely monitored, which allows the units to utilize the full NIRS spectra (780 – 2500 nm). Due to the small size of hand-held NIRS units and the inability to accommodate intricate climate control methods such as fans, many hand-held NIRS units have a restricted spectral range (Beć et al., 2021).

Validation of NIRS Predictive Equations

Using the developed NIRS prediction equations, the hand-held unit predicted CP with acceptable accuracy (R^2: 0.89; SEP: 0.77 g kg^{-1}). There was no difference between the hand-held NIRS and the wet chemistry values for CP (Figure 3.1; $P = 0.98$), nor was there a difference between the hand-held and benchtop NIRS predicted values for CP ($P = 0.17$).

When validating a hand-held NIRS device for use on warm-season grasses, Rukundo et al. (2021) observed similar results when predicting total N and when the hand-held devices were compared to the benchtop NIRS units. Berzaghi et al. (2021) also concluded that CP could be accurately predicted by hand-held NIRS units.

However, both Rukundo et al. (Rukundo et al., 2021) and Berzaghi et al. (Berzaghi et al., 2021) faced similar limitations when analyzing preprocessed forages including sample type and scanning environment when validating the hand-held NIRS devices. The samples scanned by both the benchtop and the hand-held NIRS were dried and ground to 2-mm lengths before scanning in a climate-controlled laboratory. Although this was necessary for developing predictive equations for use on the hand-held devices, validation of devices developed for field-use should be completed in the field while exposed to a diverse range of environmental conditions. Additionally, the samples were processed (e.g., dried and ground) and not representative of fresh field samples. Therefore, conclusions based on the accuracy of hand-held NIRS devices should take into consideration the environment and sample characteristics. This study, however, has shown the ability of hand-held NIRS units to accurately predict CP while scanning fresh samples in the field. Therefore, it is likely that other forage parameters may be accurately predicted under similar conditions.

In-Field Performance Observations

In addition to developing and validating predictive equations, general unit characteristics were noted during field use. The hand-held NIRS unit took approximately 6.5 minutes to scan each sample, which was not impacted by climate, unit battery life, or total time spent scanning (e.g., 15 minutes versus 3 hours scanning time). Scanning time included the six replicate scans comprised of 5 sub-scans each in addition to six calibration scans. However, climate did impact performance in 2020 when the unit was used in the field. When ambient air temperature was >30˚C, predicted forage nutrient values, especially CP, appeared exaggerated and were generally unusable. Additionally, the hand-held device was unusable when ambient air temperatures were <5˚C. This was due to the expansion of the metal NIR probe and the inability to consistently attach the probe to the cap for the necessary calibration scans.

These observations are expected and are important to consider when using hand-held NIRS technology in the field. Both technological design and climate conditions play roles in the accuracy of sensitive NIRS devices, which are largely controlled in the laboratory setting. However, the portability of hand-held NIRS devices and the outdoor, uncontrolled climate in which they are intended for can be major barriers to their success. Unit design (e.g., spectral range, light source, NIR probe design), sample traits (e.g., forage maturity, cultivar, particle size, population size), and scanning procedure can all impact the accuracy of NIRS predictions (Roberts and Workman Jr, 2004; Rukundo et al., 2021).The hand-held unit used in this study accounted for some of these limitations in the design. For example, the scanning probe was used by gliding it over the sample surface area, making it susceptible to light pollution. To correct this, a large disk surrounding the sensor end was included and prevented light from penetrating near the scanning area. Additionally, each sample was had six replicate scans and five sub-scans with calibrations occurring between replicates, which accounted for inconsistencies in use. Finally, the metal NIR probe, though designed and important for integrity and protection of the fragile light source within, was susceptible to cold ambient air temperatures and expanded enough to become unusable in the field. Despite these features, portable NIRS devices cannot account for every unique environment encountered in the field, which was apparent in the performance under different climactic conditions. Therefore, design and climate are important considerations, and perhaps limitations, when using hand-held NIRS devices in the field.

Conclusions

This research has shown that predictive equations for use on a hand-held NIRS device can be developed for fresh alfalfa including DM, CP, and NDF. However, predictive equations for NDFD48 were not able to be accurately developed. Additionally, predictive equations for CP were validated which suggests that the other forage parameters could also be accurately predicted using the hand-held NIRS device when evaluating fresh alfalfa. However, the hand-held NIRS unit was susceptible to climate conditions and sample preparation was required, which may inhibit wide-scale, outdoor use in the forage industry.

Table 3.1: Calibration performance of the hand-held NIRS device for quantifying forage nutrient parameters.

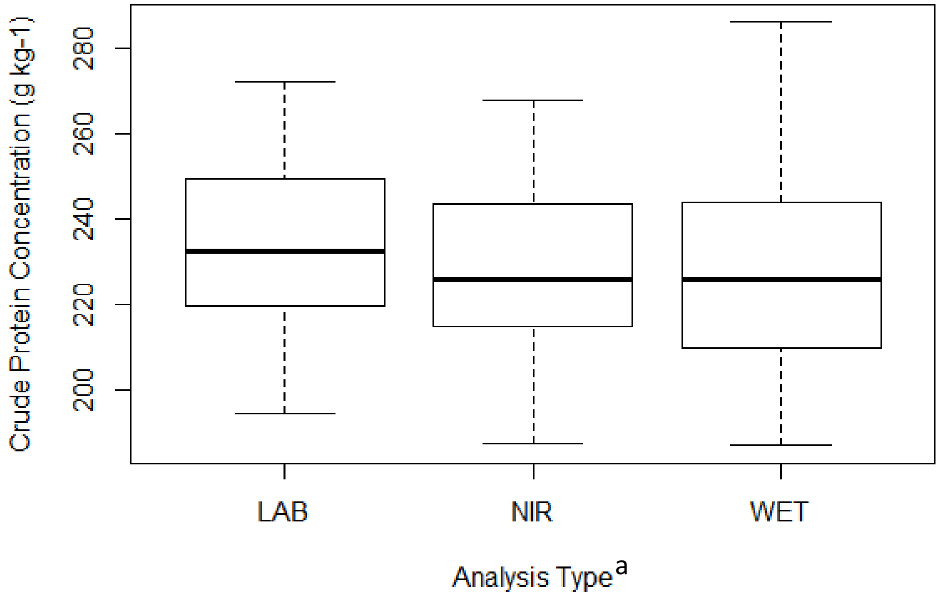
Forage Parameter[a]	Calibration Performance[b]			
	N	R^{2c}	SEC	SECV
			g kg^{-1}	
DM	100	0.718	0.348	0.376
CP	100	0.841	1.449	1.559
NDF	100	0.676	2.320	2.456
NDFD48	100	0.375	3.649	3.846

[a]DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; NDFD48, neutral detergent fiber digestibility at 48 h.

[b]Calibration performance was evaluated based on the number of samples (N), coefficient of determination (R^2), standard error of calibration (SEC), and standard error of cross validation (SECV).

[c]Applicability of prediction equations based on the R^2 evaluation scale developed by Williams (2001): 0.92 – 1.00 = quality control; 0.83 – 0.90 = research; 0.66 – 0.81 = screening/basic analysis; 0.50 – 0.64 = rough screening; 0.00 – 0.50 = poor/not useful.

Figure 3.1: Crude protein concentration (g kg^{-1}) predictions by analysis type.



[a]LAB, benchtop NIRS; NIR, hand-held NIRS; WET, wet chemistry

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