ANALYSIS OF SEASONAL EFFECTS ON NUTRITIVE VALUE OF NATIVE FORAGES IN THE SOUTHERN GREAT PLAINS AND ITS RELATIONSHIP TO SAMPLING METHOD

By

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Abstract: The objective was to investigate forage value throughout the year using near infrared reflectance spectroscopy (NIRS) while comparing sample types. Samples were collected from tallgrass native range pastures being grazed by beef cow-calf pairs (n=6) every month for four years. Samples were dried and separated into leaf and whole plant subsamples, ground through a 1 mm screen and analyzed with NIRS. Results were analyzed by a two-way sample type × month interaction, month and sample type. All NIRS components were affected by month ($P < 0.001$). Crude protein (CP) had a sample type × month interaction ($P = 0.001$) and was greatest in May. Acid detergent fiber (ADF), neutral detergent fiber (NDF) and Lignin were not affected by two-way interaction ($P = 0.47$, $P = 0.09$, $P = 0.07$). In May, ADF, NDF and lignin were least. There was no sample effect for ADF ($P = 0.26$). Lignin and NDF were greater in whole samples ($P = 0.052$, $P = 0.027$). In-vitro true dry matter disappearance (IVTDMD) was greater in whole samples during May in a two-way interaction ($P = 0.01$). There was a significant interaction for NDF digestibility (NDFD48) ($P = 0.001$). Fructan had no two-way interactions ($P = 0.18$), but was greatest in May, and not affected by sample type ($P = 0.06$). Sample type × month interactions for Fat were not significant ($P = 0.32$). Fat was greatest in the fall and greater in leaf only samples ($P = 0.003$). Two-way interactions show Starch was greater in leaf samples in June ($P = 0.031$). Digestible energy (DE), metabolizable energy (ME), total digestible nutrients (TDN), net energy for maintenance and net energy for gain were all affected by sample type × month interactions ($P < 0.001$), month ($P < 0.001$), as well as sample type ($P = 0.001$). These results suggest that time of year is a major factor influencing forage nutritive value and energy value, as expected. Sample separation into leaf matter may affect some components depending on time of year, but not to the degree that was expected.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

CHAPTER I

REVIEW OF LITERATURE

INTRODUCTION

In the field of ruminant nutrition, many studies have focused on the importance of forage nutritive value with regard to its effect on grazing livestock production. Cattle and other ruminants have the unique ability to subsist entirely on lower value forages that would be nutritionally inadequate and indigestible to non-ruminant species (Russell and Hespell, 1981). Because of such a niche feeding ability, beef cattle production relies heavily on the availability of forages as a ready feed source for varying stages of the animal's production cycle, especially when it comes to the cow-calf sector of the beef industry (Adams et al., 1996; Bohnert et al., 2011). As Rogers, et al. (2012) detailed and studied, North America's Southern Plains region is heavily utilized for the production of beef cattle due to a large quantity of permanent pasture. Relatively affordable grazing land coupled with sufficient rainfall and proximity to finishing operations creates an ideal region of beef cattle production. Much of the forage in the Southern Plains consists of tallgrass prairie native range, which are almost exclusively warm season perennials, also known as C4 species. These grasses are so named because the first organic product produced within the leaf is a 4-C oxaloacetate (Lambers et al., 1998). Warm season grasses can concentrate CO_2 within the plant cell, which allows the plant to perform

photosynthesis while limiting the need to open stomata for CO_2 intake throughout the middle of the day when temperatures are greatest, preventing the interior of the leaf from being exposed to external conditions. The ability to concentrate carbon dioxide is an adaptation that allows these plants to be productive at much greater temperatures and with less demand for water (Ehleringer and Cerling, 2002). The Southern Plains are known for high average temperatures and are subject to moderate to severe drought periods, making warm season grasses superiorly adapted over introduced C3 forages, otherwise known as cool season species (Lambers et al., 1998; Klein et al., 2006). Warm season grasses are dormant throughout the cooler winter months and become highly productive during warm summer months, with vegetative growth typically beginning around April and peaking in May, and maturing quickly as summer progresses (Miles and Knops, 2009).

Warm season grasses are, however, generally lower in nutritive value than C3 species due to lesser concentrations of nonstructural carbohydrates and crude protein, coupled with a greater fiber content (Barbehenn and Bernays, 1992; Barbehenn et al., 2004). However, due to high relative nutritive value in early summer coupled with a substantially greater biomass output compared to many cool season grasses, cattle can gain as rapidly or greater than cattle grazing cool seasons (Backus et al., 2017). Even though the nutritive value of warm season forages is lower than cool season species, warm season native grasses are often found to be more drought tolerant over long periods of time and therefore better able to subsist during extended dry periods (Hake et al., 1984). Native range is also adapted to fire, allowing for regular burning of pastures to suppress invasive weed and woody plant species and increase vegetative growth (Abrams, 1988; Havill et al., 2015). Drought and fire hardiness has allowed native grasses to remain a prominent forage system on the southern Great Plains. When overall input costs and availability

of warm season native range are compared with improved cool season pasture, many Southern Plains ranchers find it more economical and practical to utilize preexisting native grass for their herds rather than pastures of introduced (non-native) species (Sahs, 2019).

Though many beef cattle producers focus strictly on quantity of forage available to cattle, the often overlooked aspect in the private sector is the nutritive value of the plant matter, whether in improved pastures or on native range. Forages must have sufficient nutrients to provide for the animal's requirements (Undi et al., 2008). Both nutrient demand and intake limitations may differ between enterprises as well as different stages of the production cycle (Fox et al., 1988). It is important to be able to determine which dietary components within native forage suffice to meet an animal's requirements just as it is necessary to know what is lacking in order to supplement with other sources (Krysl and Hess, 1993). Determining deficiencies when native forage is the predominant feed provided to cattle throughout the year, as is often the case on Southern Plains operations, allows producers to target when to supplement. These considerations indicate a need to reliably analyze forage in a manner that can lead to accurate and precise conclusions with regard to nutrient availability throughout the year. Characterizing pasture nutritive value based on season will allow producers to be better advised regarding supplementation strategies without the need to go through their own extensive sampling and analysis.

DEFINING FORAGE NUTRITIVE VALUE

It is generally understood that nutritive value directly relates to whether or not the pasture will provide for grazing cattle. Exactly what nutritive value means, however, is not always as understood when communicating scientific data to the layperson and may even differ

among professional scientists. The terms forage "quality" and forage "value" are often incorrectly used interchangeably, however the two terms are distinct and should be used accordingly (Allen et al., 2011). Barnes and Marten (1979) defined "forage quality" as the form and quantity of digestive nutrients available to the animal over time, stating that quality is a means of expressing the potential for animal performance by utilizing forage nutrients. Other sources state that forage quality is the ability of a forage to cause a desirable response in the animal(s), while treating forage nutritive value as a separate factor, encompassing nutrient concentration, digestibility and how the end products of digestion effect the animal (Barnes et al., 1995). The flexible definition of the term forage quality often leads to the need for individual researchers to define for their specific work what will be considered high- or low-quality forage based on varying factors and forms of analysis, as well as the needs of the group of animals being utilized (Allen et al., 2011). Nutritive value refers to the relative quantity of plant chemical components and digestibility and how these can be used to predict animal response (Philipp et al., 2005; Allen et al., 2011). Forage quality is largely affected by nutritive value but is also impacted by other factors (Allen et al., 2011). Because forage quality is a broader term that includes aspects beyond the available nutrients in the plant, the term nutritive value is more appropriate for discussing concentration of specific nutrient components such as crude protein or available energy (Stokes and Prostko, 1998).

Fiber and Nutritive Value

In the study of forage chemical components, "fiber" is the fraction of the plant largely responsible for the structure of the plant and is predominantly made up of structural carbohydrates including hemicellulose and cellulose (K. Paech, 1955). Crude fiber has long been used in the proximate analysis of feeds in the laboratory. Crude fiber analysis involves the

digestion of nonstructural components, leaving the structural fiber, which includes hemicellulose, cellulose, and lignin, to be measured gravimetrically (Van de Kamer and Van Ginkel, 1952). Fiber can be separated further into neutral detergent fiber (NDF) and acid detergent fiber (ADF) using solvents of different pH, differentiating the sample into hemicellulose, and cellulose and lignin (Van Soest, 1964; Van Soest, 1965). Neutral detergent fiber includes plant structural components remaining after the sample is boiled in a neutral detergent solution, along with an alpha-amylase treatment to remove starch. The remaining components include hemicellulose, cellulose, and lignin, which are then weighed and compared to the original sample weight (Van Soest et al., 1991). Acid detergent fiber further divides structural carbohydrates by using an acidic solution to remove hemicellulose from analyzed samples, leaving behind cellulose and lignin (Raffrenato and Van Amburgh, 2011). Lignin is a class of organic polymers associated with plant structural fiber and is indigestible to the ruminant (Jung and Vogel, 1986). Lignin is measured by exposing the ADF residue to a strong acid (such as 72% sulfuric acid) to remove remaining cellulose (Van Soest et al., 1991; Raffrenato and Van Amburgh, 2011). Neutral detergent fiber is often utilized by nutritionists to predict voluntary intake (Tjardes et al., 2002). Because hemicellulose and cellulose are slower to be degraded by rumen microbes compared to non-structural carbohydrates, high concentrations of NDF and ADF reduce the rate at which feed is fermented within the rumen (Reid et al., 1988; Owens et al., 2010).

In a study conducted by Savage and Heller (1947) on the USDA Southern Plains Experimental Range, forage nutritive value was taken to be denoted largely by the total fiber fraction within a forage when dealing with native grasses of the Southern Great Plains. Fiber was discussed as the key factor effecting both palatability and digestible nutrient availability. The

study went on to investigate crude protein, minerals, lipids and more, but maintained crude fiber as an indicator of forage value. Savage and Heller were far more limited by the technology of the time then similar forage research is today, and thus fiber was a sensible area of emphasis as methods for its analysis have been in use for well over 100 years (Van Soest, 1964).

Fiber content is a common indicator of general forage nutritive value. Reid, et al., (1988) used fiber component analysis to evaluate nutritive value of both cool and warm season grasses and compared intake in sheep and cattle. As with many fiber studies, Reid, et al., (1988) divided fiber into NDF and ADF percentages. Research focused on determining the quantity of NDF that is ideal for maximized intake in grazing cattle has shown that when NDF is greater than 60%, forage intake is significantly reduced (Reid et al., 1988). Both NDF and ADF are generally desired at lower concentrations (Nelson and Moser, 1994).

The issue arising with relying only on fiber to determine nutritive value is that it fails to take into account differing animal requirements beyond digestibility. While it is true that forages high fiber leads to lowered digestibility, among forages with similar fiber quantities, there still must be a way to determine the superior feed source. Voluntary dry matter intake by the grazing animal is one indicator of forage nutritive value that links components such as crude protein and digestible fiber to the dry matter the animal consumes over a given time period (Crampton, 1957). More recently, other means of analysis have led to other considerations when evaluating nutritive value of forages. The digestibility of the forage itself, which is often related to NDF and ADF concentrations, can be used as an indicator. As Vogel, et al. (1999) described, in vitro dry matter disappearance (IVDMD) of forages can be determined utilizing a filter bag system such as those developed by ANKOM Technologies (Macedon, NY). The ANKOM system allows for the simulation of digestion in the animal's rumen to predict the actual digestibility of a feed sample.

Protein and Nutritive Value

Nitrogen within the rumen is a necessary component of microbial growth and activity for the ruminant animal (Maeng et al., 1976). Rumen microbes can use non-protein nitrogen (NPN), such as urea, as well as true protein, to produce microbial protein, which can then be digested and utilized by the animal (Oltjen, 1969). The ability of rumen microbes to utilize NPN is what allows ruminant nutritionists to use crude protein (CP), an adjusted measurement of nitrogen, as an effective indicator of protein content in feed, rather than true dietary protein (Conklin-Brittain et al., 1999). Therefore, most feed value analyses use CP as the means of reporting protein content within forage. Crude protein is often one of the first things nutritionists and producers consider to judge forage nutritive value (Moore et al., 1991).

Due to crude protein's instrumental role in the rumen, many nutritionists consider protein to be a key limiting nutrient in native range pastures. In a study conducted by Lardy, et al. (1999) it was determined that in cow calf herds grazing autumn and winter range, degradable intake protein (DIP) was the first limiting nutrient, followed by undegradable intake protein (UIP). By supplementing beef cows grazing winter range with either high energy, high DIP, a combination of DIP and UIP feeds, or control (no supplement); cattle in the high energy supplement group lost weight similarly to those within the control. Cattle in the high DIP group were less affected by weight loss and had calves that gained quicker. Animals fed both DIP and UIP also fared better than control and energy supplemented animals, but lost more weight than the high DIP group (Lardy et al., 1999).

Several studies have demonstrated how the microbial and animal need for crude protein effects the ruminant animal's ability to utilize roughages (Beaty et al., 1994). The impact crude

protein has on fiber digestion is especially critical in systems where cattle graze low value forages. The dormant warm season native grasses on the Southern Great Plains during the winter are an example. With growing cattle fed low-protein tallgrass native range hay, supplementation with DIP in the form of casein has been shown to increase voluntary intake of this low value forage more so than UIP. Increased intake was due to increased ruminal available nitrogen that was able to meet the needs of fermentative microflora not met by low protein forage (Köster et al., 1996; Bandyk et al., 2001). Increased ruminal degraded intake protein in low-protein prairie hay diets has also been show to increase urea production and kinetics in the rumen when compared to non-supplemented cattle, increasing low value forage utilization again by providing nitrogen sources to microflora (Wickersham et al., 2008). Data from experiments using crude protein to increase fiber disappearance of low nitrogen forages suggests that crude protein content of the diet is a major factor impacting the ruminant's overall dietary value and therefore forage nutritive value (Freeman et al., 1992). It has become widely agreed that the bare minimum crude protein requirement for grazing cattle on pasture is approximately 7% of dry matter to prevent negative effects on cellulose fermentation and forage intake (Pate et al., 1990; Karges et al., 1992). High and low value forage can thus be judged based on the relationship to this concentration of crude protein.

The definition of forage nutritive value can vary depending on the research being conducted and the emphasis placed on different aspects of animal diet as well the forage itself. There are, however, commonalities among most all forage value studies that are taken into account. These typically include fiber fraction, split into NDF, ADF and lignin quantities, and crude protein availability (Oelberg, 1956). It is therefore assumed that forage value can be objectively defined as forage having sufficient crude protein to meet minimum ruminal

requirements, and low enough in fiber fraction that digestibility is not compromised to the point that animal maintenance and growth is adversely affected. Though specific parameters may change from system to system, high value forage is typically that which contains greater crude protein content than the 7% to meet ruminal fermentation needs, and a NDF fraction below 60% to maximize intake and digestion (Reid et al., 1988; Pate et al., 1990). All forage research requires thorough sampling as well as a form of analysis that is both precise throughout many samples, and reliably able to evaluate what nutrients may be lacking to ensure animal health and productivity.

SEASONAL CHANGE IN FORAGE NUTRITIVE VALUE

Warm season native grasses are known to be at a maximum stage of vegetative growth rate during the first stages of summer, when precipitation, temperature and photoperiod are most conducive to forage value (Sims and Gillen, 1999; Benson and Hartnett, 2006; Dillard et al., 2018). It is during the period of rapid growth that warm season grasses are the most nutritious for grazing cattle, when nutrient components are at their peak for the year and digestibility is greatest (Hanna and Sollenberger, 2007). During early summer, typically the time from late April through May, evidence suggests that native forage nutritive value is sufficient for cattle as a single source of feed for most scenarios encountered by commercial beef operations (Haggar and Ahmed, 1971; Stafford et al., 1996). For cow-calf herds that calve in the Spring, this typically means forage is the most productive close to the time the cow's requirement reaches a peak due to lactation demands, as well as when the growing calf will begin to graze a significant amount to supplement nursing (Boggs et al., 1980; Fox et al., 1988). Growing cattle in typical stocker operations may also subsist largely on native grasses during this time of the year. Research

shows that average daily gain of growing cattle then decreases throughout the late summer unless stocking rate is decreased to allow more forage per animal (Beck et al., 2020).

Summer Nutritive Value

As summer continues, digestibility and nutrient density diminishes, often rapidly, so that overall nutritive value is significantly lowered (Abdalla et al., 1988), largely due to an increase in fiber fraction, as well as indigestible lignin in the cell walls of grasses that occurs as forage species mature and reach the reproductive phase (Miller et al., 2021). Following the end of the reproductive phase in which the plant's primary physiological role is to develop and release seeds, the forage will then enter a period of dormancy, which can vary based on yearly precipitation, temperature, soil type and other factors (Benson and Hartnett, 2006). As summer progresses, the digestibility and crude protein content of the leaf and stem material decrease rapidly in native grasses. Some studies have shown that nitrogen fertilization can alleviate this lack of protein and digestibility by promoting vegetative growth across the whole plant in certain native species, demonstrating that maturity is likely the leading factor in declining warm season forage value (Perry Jr and Baltensperger, 1979). However, fertilization is still rarely if ever recommended for use on native range pastures on the Southern Great Plains due to a lack of significant response and a propensity to cause accelerated growth of unwanted weed species (Huffine and Elder, 1960).

Winter Nutritive Value and Dormancy

Dormancy typically lasts from the end of summer to early spring, when conditions are not ideal for warm season grass growth and drought and extreme temperatures are most prevalent (Sarath et al., 2014). During dormancy nutrient content is extremely low, and fiber fraction at its

greatest point of the year (Marston et al., 1993). Crude protein is frequently found to be lower than 3% of dry matter in native grasses, with NDF and ADF in the 70-80% and 50-60% range (respectively) (McBee and Miller, 1990; Köster et al., 1996). Greater fiber content correlate directly to lower digestibility of forage, as well as decreased overall dry matter intake, which can greatly effect growth and performance of cattle (Reid et al., 1988). Cattle will continue to graze low value forage through much of the year despite its decreased nutrient availability (less than 7% CP) (Turner and DelCurto, 1991). Therefore, for optimized production, management practices must change during this time period to reflect the decreased ability of native range forage to support cattle. Management can be altered as was previously mentioned by altering stocking rate to allow animals more access to sufficient forage nutrients (Beck et al., 2020), or through various supplementation regiments (Raleigh, 1970). A combination of these practices can be used to optimize the use of native range in grazing systems.

SUPPLEMENTATION ON NATIVE RANGE

Due to native range's reputation as poor forage during much of the year, grazing cattle on the Southern Great Plains both in cow-calf operations and stocker programs are often supplemented with other feedstuffs, in particular those high in crude protein (Bohnert et al., 2011). Protein is one of the main limiting nutrients in dormant forages (Lardy et al., 1999). Studies suggest that increasing crude protein fed to cattle grazing lower nutritive value forage improves forage intake, leading to improvements in energy available to growing cattle (Lintzenich et al., 1995). Dry matter digestibility of forages may also be improved for supplemented cattle on dormant forage when fed a prepared soybean meal and dry-rolled sorghum mixture, as suggested by DelCurto et al. (1990). Overall bodyweight gain is thus increased for growing cattle despite grazing low nutritive value forages (Bodine et al., 2001).

Protein Supplementation

As was discussed previously, crude protein greatly impacts the ability of grazing cattle to consume and ferment low nutritive value forages. By providing sufficient supplemental nitrogen and DIP that is not found in the pasture during periods of low nutritive value, animals are able to meet the requirements of rumen microflora that are essential for adequate intake, digestion and growth (Beaty et al., 1994; Lardy et al., 1999). Protein can be supplemented to range cattle in several forms, the most common of which are commercially available range cubes typically containing soy products, distillers grains, and other by products high in crude protein that are highly palatable to grazing cattle (Sawyer et al., 2012; Beck et al., 2014). Other supplements include harvested forages high in protein such as alfalfa that are fed to cattle while on pasture (DelCurto et al., 1990; Vanzant and Cochran, 1994). The need for protein supplementation is most apparent in the fall and winter months when warm season forage is exceptionally low in crude protein (Jensen et al., 2002). The winter months are also when the differences between supplemented cattle and non-supplemented cattle become most significant and impactful to operations, most notably the difficultly in maintaining weight (Van Niekerk and Jacobs, 1985; Farmer et al., 2004). Therefore, it is necessary to identify the months when CP, and other nutrients, are too low for optimal performance, and months when forage satisfies the nutritional needs of cattle. The reliable way to derive such information is through sampling and testing the forage in similar native range pastures to establish nutrient trends for use by other operations.

EFFECT OF SAMPLING TECHNIQUE ON FORAGE NUTRITIVE RESULTS

A common misconception when analyzing pastures for forage nutritive value is that the data obtained from forage samples represent the diet being consumed by grazing animals.

However, research shows that the nutrient profile found in collected pasture samples can differ substantially from the forage that is actually consumed by grazing cattle (Cable and Shumway, 1966; Pauler et al., 2020). The disparity between overall nutrient value of a forage stand and the diet the animal takes in from grazing is due to the manner in which ruminants select for the most palatable and nutritious parts of the pasture, while passing over less desirable plant matter (Weir and Torell, 1959). Therefore, one consideration when sampling forage for analysis must be to attempt to determine what the animal would be grazing, effectively mimicking their selection.

When taking samples of forage for any nutritional analysis, researchers have several options. The simplest form is hand sampling by clipping the forage standing within a given area, which can be marked and repeated as necessary (Hughes et al., 2010). Hand clipping allows the researcher to select the height at which a forage stand is sampled as well as the location within pasture. Hand clipping is also the most practical means for producers to collect forage samples to be tested for nutritional evaluation. Other methods include ruminal fistula sampling, in which fistulated animals are allowed to graze a forage stand after having their rumen contents removed and then grazed forage is removed from the rumen (Lesperance et al., 1960). Animals can also be fitted with esophageal fistulae with a bag attached at the neck, preventing samples from being exposed to potential contamination in the rumen (Van Dyne and Torell, 1964). Fistulated animal methods use the animal's own grazing selection to choose where and at what height samples are collected. Research suggests that samples taken from within the rumen of grazing cattle yield greater crude protein and digestibility estimates when compared to clipped forage (Cable and Shumway, 1966). Dubbs et al. (2003) found that hand clipped samples of fescue showed a more consistent quantity of organic matter throughout the year than masticate, whereas hand clipped samples had significantly greater NDF and ADF and lower CP through much of the year.

However, the masticate sampling method is not practical for producers or researchers lacking access to fistulated animals throughout the experimental period and requires more inputs and expense overall.

There are occasions in which hand clipping is the only practical way to sample pastures for research or production purposes. As with any sampling procedure, collected samples must be large enough to be representative of the entire pasture for accurate results. It is possible that the way in which the hand sample is collected could have effects on analysis. The purpose of hand clipping forage is to mimic the animal's grazing, which will allow researchers to estimate dietary value without the use of fistulated cattle. Mimicking grazing cattle requires that those collecting clipped samples attempt to collect only the material the animals themselves would consume. Therefore, thought must be given concerning what part of the plant is being gathered for analysis. It is well understood that the nutrients within forage grasses are not uniform throughout the anatomy of the plant. More mature stem material is significantly greater in fiber and lignin then leaf and vegetative material, and will have a lower density of other nutrients such as protein and nonstructural carbohydrates, as well as overall digestibility (Nelson and Moser, 1994). When samples of big bluestem (*Andropogon gerardii*) were collected and the ratio of stem to leaf matter was measured via hand separation, samples with greater leaf concentrations displayed greater CP content and lower NDF than those with more stem material (Alexander et al., 2001). Disparity between parts of the grass plant will often lead to differences in palatability, both from plant to plant and within an individual grass shoot itself. Grazing cattle will often select leaf material and avoid stems when grasses are abundant enough to allow such selection, and cattle prefer vegetative stands to those more mature and with a greater stem ratio (Pauler et al., 2020). Stems left behind will continue to mature, which leads them to accumulate fiber and become

even less nutritious and palatable to grazing cattle, especially if allowed to leech nutrients throughout the year due to rain fall and sun exposure (McBee and Miller, 1990). As the stems of warm season native grasses mature and increase in quantity, less leaf material is available for grazing per area of pasture, which leads to an overall decrease in CP and digestibility, and an increase in ADF, NDF and lignin within the whole sample (Griffin and Jung, 1983).

Certain forage species, such as alfalfa, possess very distinct leaves that are easily removed for separate analysis regardless of plant maturity. Separated alfalfa analysis shows significantly greater concentrations of nutrients such as crude protein in leaf subsamples (Hakl et al., 2016). However, because it belongs to an entirely different class of forages, results from separating alfalfa samples cannot be assumed to hold true in grass samples. Tall grass species, such as those found in native range stands, are more difficult to effectively separate. Studies that have attempted hand separation of warm season grasses found that, as was seen in alfalfa, leaf material averaged greater crude protein concentrations then whole plant matter, and was lower in ADF and NDF (Haggar, 1970). However, the differences were not as significant between subsamples as was expected. Overall concentrations of nutrients were more alike between leaf samples and whole plant samples from grasses of the same age and maturity then was seen in alfalfa (Haggar and Ahmed, 1971). Some researchers, therefore, have been lead to conclude that maturation of the leaf is the driving factor for rapid decline in nutritive value throughout the year rather than stem growth in warm season species (Perry Jr and Baltensperger, 1979). Limited research is available to compare whole plant analysis to hand separated leaf samples, and how the differences between the two may change from month to month. It is expected that separating forages into leaf subsamples will allow for a better understanding of nutrient availability but may not be necessary or effective across the whole year as grass growth changes.

FORAGE ANALYSIS TECHNIQUES

Analysis must allow researchers and producers to determine accurately the nutrients present within a feedstuff that is used for livestock production. Over time several techniques for forage analysis have developed. To be appropriate, nutrient analysis must be replicable across a wide number of samples, as many forage and grazing studies will deal with many samples taken from multiple plots or pastures, possibly over an extended period. Therefore, the best forage analysis methods, for both scientific research and for producers' information, will also ideally require only a small amount of forage, and be able to be performed on samples that have been stored for a length of time. Expediency is always a benefit when dealing with large sample groups that could number into thousands, but care must be given to decide if faster methods will yield results that are both accurate and precise.

Animal Performance

One form of measurement of forage nutritive value is often performed without the collecting of individual plant samples and may simply be a derivative of the larger purpose. Such a strategy involves allowing animals to graze forage in pasture or via feeding over the given research period or production time frame and monitoring animal performance. Performance can be measured in several ways, including weight gain, milk production, offspring growth, and so on, which allows researchers to compare large samples (pastures, hay cuttings, etc.) without the need for laboratory input (Norris et al., 1976). However, this method is not as exact nor precise as other methods and does not allow for the testing of smaller and more numerous sample groups.

In Vitro and In Situ Analysis Methods

More intensive methods include laboratory analysis techniques, such as the *in vitro* method developed by Tilley and Terry (1963) that has been well used by forage researchers over the years. The Tilley and Terry method involves replicating the digestive tract of a grazing animal and measuring in vitro digestion rates of different forages. Others include the use of detergents to remove digestible components to measures remaining fiber fraction, such as the well-known Van Soest detergent fiber method (Van Soest, 1965). For the measurement of ruminal escape protein content in feeds, *in situ* protein degradation methods allow researchers to determine ruminal escape protein as a percentage of total crude protein in feed using live animals (Wilkerson et al., 1995). The Kjeldahl method of total nitrogen analysis has become a well-accepted and long utilized method in animal feeds, and is considered reliable (Perrin, 1953; Ekpete and Cornfield, 1964). These techniques are capable of producing reliable research results, but demand both training on the part of the researcher as well as laboratory materials that may be difficult to utilize, and may not be efficient if time is a factor. In situ methods involving the use of actual animals as digestion systems also require facilities and often surgical methods that may be more demanding than necessary (Wilkerson et al., 1995). A more rapid technique that can measure multiple nutritional components is needed for large-scale sampling, especially when decisions must be made in a timely manner regarding nutrient availability in a forage (Stuth et al., 2003).

Near Infrared Reflectance Spectroscopy

Near infrared reflectance spectroscopy (NIRS) has become a widely used and accepted method in the industry to analyze animal feeds for multiple nutrients at once. A non-destructive technique, NIRS uses the spectral range of 780 to 2500 nanometers to read vibrational energy changes in covalent bonds which are specific and recognizable depending on the molecular

bonds within a given organic sample, and allow the system to detail the molecules present and to what extent (Cen and He, 2007). In forage analysis, the components desired to be analyzed to determine value have their own vibrational reading that, when processed by the machine, can be quantified as they relate to the total contents of the forage sample (Norris et al., 1976).

Near infrared spectroscopy technology has been used to analyze grain and seeds for nutritional content since the 1960's (Norris, 1965). One of the first works to describe NIRS as a means to analyze nutrient content specifically in forages was conducted by Norris, et al. (1976). Near infrared spectroscopy allows for a more expedient analysis of many forage samples that can be compared to one another or to animal requirements. The NIRS method also allows analysis that is nondestructive, meaning that samples can be analyzed, and then stored and kept for later use. Results can be used along with results from wet chemistry for reliable information on the value of forage (Brown and Moore, 1987), or treated as a complete analysis when properly validated. Reports show that using NIRS to measure forage value has a correlation of 0.90 or greater to laboratory methods for fiber fraction and crude protein and lower standard errors than commonly are reported for conventional laboratory analysis when properly calibrated (Marum et al., 1979). The repeatability of NIRS in determining in vitro true dry matter disappearance was found to be greater than those of the Tilley Terry laboratory method and yielded accurate results when locally calibrated (Tilley, 1963; Aastveit and Marum, 1993). Near infrared reflectance spectroscopy data has been used extensively in range land and pasture research where multiple samples may be required for accurate representation of forage fluctuation in large pastures over a number of months or years (Holechek et al., 1982; Ward et al., 1982). Raw NIRS results can be used as the predominant data source itself, as is often the case in simple feed value studies, or

can be used within summative equations to calculate variables not used by NIRS, such as energy content and digestibility (Lundberg et al., 2004).

Near infrared reflectance spectroscopy calibration equations for plant components such as CP, IVMD and NDF can be validated either with specific forage species for more exact analysis or broad based when origin may be unknown, such as in service laboratories (Brown et al., 1990). Calibration protocols have been developed to ensure that results are consistent and reliable, as well as standardized across the industry (Windham et al., 1989). Calibration consists of developing an equation based on a large set of samples. Sample sets for calibration can include samples of high, medium, and low value forages from an array of conditions, and should include forages similar to those that are being analyzed. More specific calibrations are also used to analyze forages, but broad-based data sets allow for a comparable accuracy to local equations and allow researchers to calibrate without having to go through the effort of creating a calibration set for each NIRS unit (Abrams et al., 1987). Calibration of NIRS requires samples measured by both NIRS and the reference laboratory method (e.g., wet chemistry or in vitro digestibility). Calibrating NIRS for forage analysis can use a wide range of grass samples with different nutritive values. A calibration model predicts the results of the wet chemistry, or reference method using only the NIRS spectrum (Aastveit and Marum, 1993; Andueza et al., 2011). Calibration is essential for reliable and repeatable forage analysis on NIRS and is often specific for types of samples (Windham et al., 1989). Equations can be made for individual studies to allow researchers to customize NIRS calibrations to their specific needs (Kramer et al., 2021), but this can lead to poor reproducibility and large variation between laboratories. For forage nutritive values, research that compares NIRS values for components such as CP, ADF, NDF and NDF digestibility (NDFD48) to laboratory data have found that NIRS machines

processing in the spectra range above 700 – 1070 nm can produce data both precise and accurate in relation to accepted laboratory analysis (Berzaghi et al., 2021). Studies can also choose to utilize widely available calibration sets created using many samples from multiple sources that are updated routinely, such as that provided by the NIRS Consortium (Berea, KY) (Villalba et al., 2016). These consist of calibration sets developed specifically for a group of similar forages, such as grass hay, and updated annually (NIRS Forage and Feed Consortium, Berea, KY). The forage components typically analyzed using such sources include different forms of fiber, CP, digestibility, minerals, nonstructural carbohydrates, and other variables that impact nutritive value. Data from samples of multiple species of both cool and warm season forage varieties are collected from sources across the country to ensure a representative and inclusive data base (McIntosh et al., 2022).

CONCLUSION

In the fields of ruminant nutrition and commercial beef production, an understanding of forage nutritive value is critical in an industry that relies on the ruminant animals' ability to produce on forages. Forage nutritive value is extremely relevant on the Southern Great Plains of North America. Millions of cattle, both in the cow-calf and stocker/backgrounder sector, are grazed on native range pastures, where forage is known to be of unreliable value through much of the year. In order for native forage nutritive value to be understood and put to use both in research and in production, the aspects that make a forage high or low value must be decided upon for each scenario.

Seasonal changes in native range nutritive value, regardless of how it is being defined in a given study or operation, plays a role as well. Seasonal fluctuation, once understood through

effective sampling and analysis, will decide how grazing systems will be maintained, stocked, and supplemented throughout the year. The need to make these decisions is especially true for tallgrass native range systems where nutritional components are lacking throughout a long dormant period. Supplementation and stocking programs are best performed when the change in value is well known and understood. Forage nutritive value analysis also allows producers as well as researchers to know what exact nutrients require supplementation. For analyses to be done properly and in a representative manner, the way in which samples are collected and treated must be considered. As leaf matter may differ from an entire plant sample and be more representative of the nutrients actually available and ingested by the grazing animal, it may be a beneficial step to obtain the best results.

Though much research has been done regarding the analysis of forages throughout the year, as well as comparing masticate samples from hand clipped samples, little has been done to investigate how hand separated clipped grass samples change from month to month. Data such as this can be used to determine if forage analysis is improved when samples are separated into leaf only material in order to view how nutrients available to the grazing animal changes during the year. Though many methods of forage analysis exist and are readily used, NIRS analysis allows for the most expedient and encompassing look at the multiple nutrient components that may impact native forage value results.

CHAPTER II

ANALYSIS OF SEASONAL EFFECTS ON NUTRITIVE VALUE OF NATIVE FORAGES IN THE SOUTHERN GREAT PLAINS AND ITS RELATIONSHIP TO SAMPLING METHOD

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Abstract: The objective of this study was to investigate the effect of month and leaf vs. whole plant samples on forage nutritive value using near infrared reflectance spectroscopy (NIRS). Samples were collected from tallgrass native range pastures being grazed by beef cow-calf pairs (n = 6) every month for four years. Samples were dried and separated into leaf and whole plant subsamples, ground through a 1-mm screen and analyzed with NIRS. Results were analyzed for month, sample type and a two-way interaction. All NIRS components were affected by month (P < 0.001). Crude protein (CP) had a sample type × month interaction (P = 0.001) and was greatest in May. Acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin were not affected by the interaction (P = 0.47, P = 0.09, P = 0.07). In the month of May, ADF, NDF and lignin were the least. There was no sample type effect for ADF (P = 0.26). Lignin and NDF were greater in whole samples (P = 0.052, P = 0.027). In-vitro true dry matter disappearance (IVTDMD) was greater in whole samples in May in a two-way interaction (P = 0.001).

There was a significant interaction for NDF digestibility (NDFD48) ($P = 0.001$), in which NDFD48 was greatest in May. Fructan had no two-way interactions ($P = 0.18$), but was greatest in May, and not affected by sample type ($P = 0.06$). Sample type × month interactions for Fat were not significant ($P = 0.32$). Fat was greatest in the fall and greater in leaf only samples ($P = 0.003$). Two-way interactions show starch was greater in leaf samples in June ($P = 0.031$). Digestible energy (DE), metabolizable energy (ME), total digestible nutrients (TDN), net energy for maintenance and net energy for gain were all affected by sample type × month interactions ($P < 0.001$), month ($P < 0.001$), as well as sample type ($P = 0.001$). These results suggest that time of year is a major factor influencing forage nutritive and energy value, as expected. Sample separation into leaf matter may affect some components depending on time of year but not to the degree expected.

Key words: native range, grazed, forage analysis, month, sample type

INTRODUCTION

Forages provide the predominant source of nutrients for cow-calf production in the United States (Short, 2001; Bohnert et al., 2011). In the Southern Great Plains, many cattle are maintained on native range throughout the year (Sims and Gillen, 1999). Native range in this region consists predominantly of warm-season species that peak in nutritive value in late spring and early summer, when it is most nutritious for grazing cattle (Hanna and Sollenberger, 2007). Nutrient density and overall value decrease as fiber increases in late summer, with crude protein (CP) and digestibility remaining low throughout fall and winter. It is during the months from late

summer until the following spring in which native forages may not provide sufficient energy and protein for many classes of livestock (Griffin and Jung, 1983).

While the nutrient content of grasses changes as they mature, cattle may be able to select more nutrient-dense portions of plants (Hughes et al., 2010). Cable and Shumway (1966) demonstrated that native range consumed by cattle and collected through a rumen fistula had greater CP than samples clipped from the same pasture. Sampling differences indicates that collecting forage samples by simply clipping the entire plant from the ground may not be an effective representation of the nutrients a grazing ruminant would obtain from the pasture, despite this being the method most often utilized by producers. Further processing of the clipped forage may be better able to mimic the grazing methods of cattle in pasture, allowing a better understanding of the nutritive value being eaten by cattle.

Monthly forage nutritive value information is critical for private operations which may rely entirely on native range, if much of the grazing season their cattle are not receiving adequate nutrition through grass alone (Raleigh, 1970; Martin and Hibberd, 1990). Forage nutritive value data, in turn, can then be used to determine when to supplement cattle grazing native pasture and what form of supplement is needed to ensure productivity (Stafford et al., 1996; Dubbs et al., 2003). Research conducted on native range forages show that leaf matter, in general, is greater in crude protein and digestibility concentrations compared to stem matter (Haggar and Ahmed, 1971; Alexander et al., 2001; Mitchell et al., 2001). Greater nutritive value in leaf material is linked to less fiber and lignin accumulation in the leaf fraction of the plant as the grass matures then is found in the stem (Griffin and Jung, 1983). The changing concentration of multiple forage nutrients throughout the year has been established in tallgrass native range, though little work has also taken into account sampling methods by analyzing the differences between the leaf

of the forage and the entire plant (Abdalla et al., 1988; Miller et al., 2021). Other studies have demonstrated that hand separating forage samples to replicate selection of a better value diet of mostly leaf matter by cattle leads to greater crude protein and digestibility results when compared to unseparated samples (Mowat et al., 1965; Tremblay et al., 2002). The objective of this experiment was to measure forage nutritive value in native range throughout the year to determine if hand separating forage samples to mimic animal selection demonstrates a significant effect of both sampling month and plant component to better reveal months in which overall nutrient availability and energy content are too low for optimal production.

MATERIALS AND METHODS

Animals were not directly used in this experiment but were present in the pastures that were sampled. All procedures involving animals were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Animals and Facilities

The experiment was conducted from 2013 to 2017 at the Oklahoma State University's Range Cow Research Center located near Stillwater, OK. Separate pastures (n = 6) of tallgrass native range were selected and grazed by commercial beef cow-calf pairs. Pastures are predominantly loamy bottomland and upland, and sandy loam savannah soils (NRCS, 2018). Native forage species were typical of the Northern Cross Timbers tallgrass prairie species including predominantly Big Bluestem (*Andropogon gerardii*), Little Bluestem (*Schizachyrium scoparium)*, Indiangrass (*Sorghastrum nutans*), and Switchgrass (*Panicum virgatum*) (NRCS, 2018). In April of each year, cattle were removed briefly while pastures were burned using a

prescribed burning method. Cattle were provided hay during extreme winter weather, as well as a protein supplement from October to March.

Forage Sampling and Analysis

Two to four locations in each pasture were chosen and the GPS coordinates were recorded for subsequent sampling. A 0.93 m^2 quadrat was placed upon the forage cover monthly within each sampling location and all plant matter within the quadrant was clipped to 2 cm, collected, and labeled. The quadrat was not placed on the same 0.93 m^2 clipped area from the previous month. Samples in April were taken prior to prescribed burning each year. Forage samples were dried at 60° C for 72 hours in a forced-draft oven. All samples were divided equally into two subsamples, one subsample was retained as whole plant matter and the other subsample was processed to separate leaf matter from stems of each forage piece by hand. The leaf material was retained, and the stem material was discarded. Both leaf only and whole plant subsamples were then ground in a Fritsch Mill (Fritsch Milling and Sizing, Pittsboro, North Carolina) to pass a 1-mm screen.

Samples were analyzed using near infrared reflectance spectroscopy (NIRS; FOSS DS2500, Eden Prairie, MN). The spectrum was subjected to analysis through the NIRS Consortium (Berea, KY) 2020 grass hay calibration. Samples that produced Global H and/or Neighborhood H values greater than 4.00 or 1.80, respectively, were removed from the data set. Before statistical analysis, 0.8% of samples analyzed were removed due to high Global- or Neighborhood-H values. Components used included the following: crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, in vitro true dry matter disappearance (IVTDMD), neutral detergent fiber digestibility (NDFD48), fructan, fat, and

starch. The NDFD48 value is calculated by the NIRS calibration and represents the fraction of NDF that digestible in a 48-hour in vitro digestibility assay and is reported as a percentage of NDF. All other components are reported on a dry matter basis. Digestible energy (DE) and total digestible nutrients (TDN) were calculated using formulas from Weiss and Tebbe (2018). Metabolizable energy (ME), net energy for maintenance (NEm), and net energy for gain (NEg) were calculated using equations from NASEM (2016).

Samples that were more than two standard deviations from the mean for any variable within a given month were considered outlies and removed from the data set. Data from 285 samples were removed from an original 1,396. Data were analyzed using a mixed model analysis (SAS 9.4; SAS Inst., Cary, NC) with month, sample type (i.e., leaf vs. whole plant), and the sample type × month interaction as main effects and year, replicate (i.e., pasture location) and pasture as random effects. Month within year was analyzed as a repeated measure. Covariance structures which resulted in the least Akine Information Criteria were utilized in the repeated measures statement and included autoregressive 1, heterogeneous autoregressive 1, compound symmetry, heterogeneous compound symmetry, unstructured, and antedependence 1. The residual plot for all models was evaluated for normality. All variables displayed a normal distribution of residuals except for crude protein (CP) which was log transformed to achieve a normal distribution. Means were separated using a Tukey post-hoc test. Variables with $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Crude Protein

There was a sample type × month interaction for CP ($P = 0.001$; Table 1), in which whole plant samples averaged 11.62% in May, whereas leaf material averaged 9.40%. However,

throughout the rest of the year, leaf samples showed a greater CP concentration than whole plant samples, as was expected. In May, when native forage is typically the most vegetative, immature elongating stem material has been shown to contain a greater amount of non-protein nitrogen (NPN), despite leaves containing greater true protein (Haggar and Ahmed, 1971). Because CP encompasses both NPN and true protein, CP was greater in stem samples with a greater total nitrogen ratio. Following prescribed burning, at this point in the growth cycle native grasses are highly vegetative and the entire plant is lush and dense in leaf material and the immature stems are not easily distinguishable from leaves (Benson and Hartnett, 2006). As the summer continues and grasses mature, the delineation between stem matter and leaf becomes more evident and the nutrient components of each begin to differ substantially, leading to greater CP in leaf matter in subsequent months. For both sample types, CP was greatest in May and decreased in the following months and was the least in December. From October to March, CP remained between 2 and 3% in both sample types. Low CP is not uncommon for dormant native range grasses in the Southern Great Plains region (Marston et al., 1993; Vanzant and Cochran, 1994; Bandyk et al., 2001). Crude protein of clipped samples is typically lower then ruminal masticate samples from the same pasture throughout the year, indicating that the CP the animal receives is not necessarily as low as hand clipped samples suggest (Cable and Shumway, 1966; Dubbs et al., 2003). Marston et al., (1993), utilizing cannulated animals to sample Oklahoma midgrass prairie and Old World Bluestem, found greater CP values ranging from 6.8% to 11.2% of DM ($P <$ 0.01) in December and February. Higher crude protein values for samples collected via a fistula in winter suggests again the ability of the grazing ruminant to select forage with a higher nutritive value then may otherwise be collected. Hand separation of whole plants may not be 100% effective at mimicking animal selectivity. However, an interaction between sampling type

and month suggests that separating hand collected native grasses into leaf samples could allow for a more representative analysis of crude protein cattle are ingesting, which would allow producers to target specific months in which cattle may require supplementation of protein.

Fiber components

There was not a sample type × month interaction for ADF (data not shown; $P = 0.47$), NDF ($P = 0.09$), or lignin ($P = 0.07$). Lignin, ADF, and NDF were all affected by month ($P < 0.001$; Table 2). All three were least in May. Forage fiber and lignin increased throughout the year to a maximum in December and February, which also follows expected patterns of warm-season grasses, particularly in the case of NDF, which remained above 70% of DM from August through the winter (Marston et al., 1993). There was no difference in leaf ADF compared to whole plant ADF ($P = 0.26$; Table 3*)*, which was not expected (Cable and Shumway, 1966). Neutral detergent fiber, however, was affected ($P = 0.027$; Table 3*)*, in which NDF was greater in whole plant then in leaf. Lignin was also lesser ($P = 0.052$; Table 3*)* in leaf samples than in whole plant samples. Although this affect did follow the assumption that NDF and lignin fraction would be greater in whole plant than leaf only matter, the mean NDF and lignin percentage between the two sample types were, overall, more similar than anticipated which could again be due to the difficulty in obtaining separate leaf only subsamples for many months of the year. During the vegetative phase in May and following burning, the entire forage plant is lower in fiber (Benson and Hartnett, 2006) than when forages begin to mature throughout the summer. By October, ADF and NDF reached an average of 49.58% and 75.61% of total plant matter, respectively. Greater fiber within stems potentially lead cattle to select for remaining leaf matter, leaving behind a plant that consisted of mostly stem material when sampled (Coleman and Barth, 1973). In ungrazed pastures, greater stem to leaf ratio has been associated with increasing

quantities of NDF and ADF in Switchgrass and Big Bluestem grasses (Mitchell et al., 2001). However, in this study, some hand-clipped samples from pastures that were being grazed contained little leaf matter and became difficult to separate for analysis, which prevented some samples from furnishing enough leaf matter to be analyzed as an individual sample. Samples that could not be effectively separated were analyzed as whole plant samples. A lack of interaction for fiber fraction components may indicate that separation of forage into leaf only samples is not a necessary means to analyze fiber fluctuations across the year in grazed pastures when hand clipped samples are being compared.

Digestibility

In vitro true dry matter disappearance exhibited a sample type × month interaction (P = 0.001*;* Table 1) and was greatest in whole plant samples in May (79.22% vs. 75.44 %), while leaf only samples were greater in IVTDMD in the remaining months. In extremely vegetative forages, IVTDMD has been found to be equal in whole plant samples and leaf samples, and in some cases slightly greater in whole plant material by previous studies, before decreasing more rapidly than in leaves (Haggar and Ahmed, 1971; Tremblay et al., 2002). In vitro true dry matter disappearance was greatest in both sample types in May and least in February, where it began to increase once again, as was suggested by previous work (Mowat et al., 1965).

Neutral detergent fiber digestibility, (NDFD48, % of NDF), was affected by sample type × month (P = 0.001; Table 1). Whole plant samples were greatest in May. Leaf only samples were greatest at 56.54% in the same month. Though whole plant samples were greatest in NDFD48 in May, in all other months NDFD48 was greater in leaf samples. The relationship of fiber digestibility to month concurs with previous studies in warm-season grasses, in which

digestibility parameters are greatest in early summer months and decrease in the fall (Haggar, 1970). Significant effects for IVTDMD and NDFD48 two-way interactions suggest that separation may be an effective way to analyze native forage for a more representative analysis of animal digestibility by using material similar to what a ruminant would graze.

Fructan and Starch

Fructan was not affected by a sample type × month interaction ($P = 0.18$). Fructan was greatest in May and least in April ($P < 0.001$; Table 2*)*. Concentrations of fructan decreased and increased month to month in late summer and early fall. According to previous studies, fructan concentrations tend to accumulate in relation to periods of environmental stress, such as drought and falling temperatures, which could explain these fluctuations (Pollock and Jones, 1979; Yoshida and Tamura, 2011), although ambient temperature and rainfall were not recorded as part of this experiment. Fructan was not different between sampling types in the forages when analyzed ($P = 0.06$; Table 3). Therefore, it is possible to conclude that, while month of year does alter fructan concentration as expected, separation of samples into leaf only material does not seem a necessary method for the analysis of fructan in warm season native range grass.

There was a sample × month interaction for starch ($P = 0.031$*;* Table 1). Through most of the year, starch was greater in whole plant samples then leaf samples. McBee and Miller (1990) found that sorghum (a warm-season annual species) had greater nonstructural carbohydrates in stem matter than was found in leaves, particularly in reproductive grasses with visible seed heads. However, in the month of June, starch was greater in leaf only samples (1.25% vs 0.92%). Starch was also greater in leaf samples in August (0.85% vs 0.72%), which could be due to reproductive material present in leaf samples during these months as grasses completed the

reproductive phase (Benson and Hartnett, 2006). The greatest means for starch for leaf only were 1.54% in September, and whole plant at 1.75% May. High leaf starch in September was possibly due to grasses accumulating starch and other nonstructural carbohydrates in periods of dropping temperature in preparation for winter (White, 1973). Data indicate that separation into anatomical parts of the plant may need to be considered when analyzing native range for starch in order to obtain a result representative of what cattle would consume while grazing.

Fat

Fat was not affected by sample type by month interactions ($P = 0.32$) in the experiment. Crude fat was greatest in fall months from September to November ($P < 0.001$*;* Table 2), with a maximum at 1.59% in September. Fat decreased starting in December and was least in April at 0.93%. Fat was greater in leaf only samples at 1.46% then in whole plant samples, which were 1.37% ($P = 0.003$*;* Table 3).

Energy Values

There was a sample type × month interaction on DE ($P < 0.001$; Table 4*)*, with a greatest DE found in whole plant matter in May at 2.75 Mcal/kg, and 2.62 Mcal/kg in leaf only samples. In the months of April, May and June, whole plant samples had a greater DE concentration then leaf samples. However, from July to March, DE concentration was greater in leaf samples. Leaf samples were least in DE concentration in February, whereas whole plant samples were least in January. Due to remaining energy values being calculated based on the results for DE obtained through analysis of samples, the trends exhibited by these values followed those of DE and were all significant. Metabolizable energy (ME) sample type × month interaction showed ME was greatest in whole plant samples and leaf samples in the month of May (Table 4). As with DE,

ME concentration was greatest in whole plant samples from April to June, and greatest in leaf samples from July to March. Concentration of ME was least in February for leaf samples and least in January for whole plant samples. Sample type × month interaction showed TDN was greatest at 59.32% in May leaf samples and 62.32% in May whole samples (Table 4). Total digestible nutrient concentration decreased in the following months until reaching the least concentration in leaf samples in February at 47.77%, and the least concentration in whole samples in January at 46.03%. Following January and February, TDN began to increase again in both sample types. Leaf samples had a greater TDN concentration than whole samples from July to March, and a lesser TDN concentration from March to June. Sample type × month results for NEm showed that May leaf samples were lower, at 1.28 Mcal/kg, than whole samples, at 1.39 Mcal/kg. Throughout the summer, from April to August, NEm remained above 1.00 Mcal/kg in both sample types. Beginning in September, NEm decreased through the fall and was least in January in whole plant samples and least in February in leaf samples (Table 4). The concentration of NEm was greater in leaf samples from July to March and greater in whole plant samples through April, May, and June. Lastly, net energy for gain (NEg) was 0.71 Mcal/kg in leaf only May samples and 0.80 Mcal/kg in whole May samples in sample type × month interactions (Table 4). The concentration of NEg was greatest in the summer for both sample types, and lowest in February for leaf samples and in January for whole samples. As was seen in all previous energy values, NEg was greater in concentration in whole samples from April to June, and greater in leaf samples in all other months.

Due to the greater concentration of CP and digestibility in whole samples through April, May, and June, energy calculations based on these NIRS values were also greater in these months. However, in all other months, energy values were greater in leaf samples, which follows

with work showing greater overall nutritive value in leaf material compared to stem (Griffin and Jung, 1983; Alexander et al., 2001). As was mentioned previously in regard to CP and digestibility, this result is likely due to the fact that any stem material in early summer samples would have been very vegetative and immature following burning, and difficult to distinguish from leaf matter, leading to greater overall nutrients in whole plant analysis (Benson and Hartnett, 2006). As grasses mature and stems begin to lignify, the energy content of leaf matter became greater than whole plant samples containing stems. Following the trend of digestible energy, all remaining energy calculations show a greatest concentration in May in both sample types and a least in January in whole samples and February in leaf, which follows expectations that energy would be greatest in May and least in the winter during the dormant period of native range grasses (Waterman et al., 2007). Energy content was expected to be greater in leaf samples than whole plant samples for all calculations. Though the difference was not as great as was anticipated, the trend observed followed previous results for other factors used to calculate energy values, including CP and fiber (Haggar and Ahmed, 1971; Alexander et al., 2001). When analyzing native range forage samples for energy parameters, separation of samples may be a practical method to gain a true results of forage energy available to the grazing ruminant.

CONCLUSIONS

This experiment determined that the separation of native forage samples into leaf only matter for nutritive value analysis does affect NIRS results for certain components. Crude protein, IVTDMD, NDFD, starch, and calculated energy values demonstrated an interaction between sampling method and month of the year. However, not all expected components were affected by this interaction, notably the fiber components ADF, NDF and lignin. Fiber fractions are often denoted as a marker of nutritive value in range forage samples (Nelson and Moser,

1994). Therefore, separation may not be necessary when analyzing native range grasses for overall nutritive value, particularly in pastures that are burned and grazed regularly, when hand clipped samples are being used. A lack of a difference in separated samples could be due to a lack of highly distinguishable stem matter in vegetative growth immediately following burning, when all fractions of the plant would be high in nutritive value compared to other times of the year (Benson and Hartnett, 2006). This experiment determined that month of year has a significant influence on the nutritive value of native range grass samples analyzed using NIRS. Testing forages across the year is a viable way for grazing cattle operations to determine when forage is sufficient to support grazing animals, as well as months in which certain dietary components are below requirement parameters at different stages of growth and production.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

Table 1. Effect of month × sample type interaction on NIRS results from native range grasses in central Oklahoma

Component[1]	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	SEM[2]	*P*-value
logCP, % DM[3]														
Leaf	0.42^{IJK}	0.37^{JK}	0.49^{GHIJ}	0.52^{FGHI}	0.97^{A}	0.85^{B}	0.77^{CD}	0.65^{E}	0.58^{EFG}	0.40^{IJK}	0.45^{HIJK}	0.37^{IJK}	0.04	
Whole	0.39^{IJK}	0.34^{K}	0.44^{HIJK}	0.49^{GHIJ}	1.07^{A}	0.83^{BC}	0.74^{D}	0.65^{EF}	0.55^{GH}	0.33^{K}	0.39^{IJK}	0.32^{K}	0.04	0.001
CP, % DM[4]														
Leaf	2.60	2.36	3.10	3.28	9.40	7.10	5.95	4.44	3.81	2.52	2.83	2.36	—	
Whole	2.44	2.19	2.76	3.08	11.62	6.84	5.50	4.42	3.54	2.13	2.43	2.08	—	—
IVTDMD, % DM[5]														
Leaf	58.97^{IJKLM}	58.45^{KLM}	61.04^{GHI}	63.42^{DEFG}	75.44^{AB}	72.19^{B}	69.92^{C}	66.38^{DE}	63.34^{F}	60.12^{HIJK}	59.92^{IJKL}	58.83^{IJKLM}	1.11	
Whole	57.74^{LM}	57.51^{M}	60.74^{GHJI}	63.14^{EFGH}	79.22^{A}	72.05^{B}	69.05^{C}	66.24^{D}	62.64^{FG}	58.85^{IJKLM}	58.71^{JKLM}	57.91^{M}	0.87	0.001
NDFD48, % NDF[6]														
Leaf	49.42^{EFGH}	49.16^{EGHI}	51.39^{CDE}	53.38^{BCD}	56.54^{AB}	53.20^{BC}	51.49^{CDF}	50.89^{DE}	49.83^{EFGHI}	48.89^{HI}	49.75^{EFGHI}	49.60^{DEGHI}	1.07	
Whole	47.53^{IJ}	47.73^{IJ}	50.34^{DEG}	52.84^{BCDE}	59.25^{A}	52.91^{C}	50.83^{DE}	49.85^{EGH}	48.93^{GHI}	46.75^{J}	48.77^{GHI}	48.08^{HIJ}	1.06	0.001
Starch, % DM														
Leaf	0.93^{DEFG}	1.01^{DEFG}	0.75^{FG}	0.84^{DEFG}	1.44^{ABCDEF}	1.25^{ABCDE}	1.25^{ABCDE}	0.85^{DEFG}	1.54^{ABC}	1.07^{DEFG}	1.00^{DEFG}	0.78^{EFG}	0.20	
Whole	1.22^{BCDE}	1.19^{BCDEF}	1.07^{DEFG}	0.98^{DEFG}	1.75^{A}	0.92^{DEFG}	1.32^{ABCD}	0.72^{G}	1.62^{AB}	1.34^{ABCD}	1.23^{CD}	1.06^{DEFG}	0.14	0.031

[1]All components are % of dry matter except for NDFD48 which is % of NDF
[2]Standard error of the mean represents greatest monthly SEM for each component
[3]Log of crude protein
[4] Crude protein means represent back transformations of logCP data
[5]In vitro true dry matter disappearance
[6]Neutral detergent fiber digestibilitY

Table 2. Effect of month on NIRS results from native range grasses in central Oklahoma

Component, % of DM	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	SEM[1]	*P*-value
ADF[2]	52.43^{BC}	53.46^{A}	52.40^{BC}	53.14^{AB}	39.53^{J}	41.36^{I}	43.19^{H}	45.09^{G}	46.72^{F}	49.58^{E}	50.49^{D}	51.58^{C}	0.61	<0.001
NDF[3]	76.07^{AB}	77.53^{A}	76.25^{BC}	75.30^{C}	60.64^{H}	65.75^{G}	68.31^{F}	71.20^{E}	73.19^{D}	75.46^{C}	75.61^{C}	77.65^{A}	0.67	<0.001
Lignin	6.15^{AB}	6.23^{A}	5.83^{BC}	5.92^{ABC}	4.24^{G}	4.33^{G}	4.53^{FG}	4.68^{F}	4.93^{E}	5.63^{CD}	5.45^{D}	5.91^{ABC}	0.22	<0.001
Fructan	0.94^{G}	1.26^{D}	1.06^{F}	0.91^{G}	1.55^{A}	1.39^{BC}	1.34^{C}	1.15^{F}	1.42^{B}	1.23^{DE}	1.37^{BC}	1.14^{EF}	0.10	<0.001
Fat	1.36^{C}	1.40^{C}	1.25^{D}	0.93^{E}	1.42^{C}	1.53^{AB}	1.52^{AB}	1.46^{BC}	1.59^{A}	1.54^{AB}	1.57^{AB}	1.41^{C}	0.07	<0.001

[1]Standard error of the mean represents greatest monthly SEM for each component
[2]Acid detergent fiber
[3]Neutral detergent fiber

Table 3. Effect of sample type on NIRS results from native range grasses in central Oklahoma

Component, % of DM	Leaf	Whole	SEM[1]	*P*-value
ADF[2]	48.14[A]	48.35[A]	0.44	0.26
NDF[3]	72.58[B]	73.08[A]	0.41	0.027
Lignin	5.23[A]	5.40[A]	0.18	0.052
Fructan	1.28[A]	1.18[A]	0.10	0.06
Fat	1.46[A]	1.37[B]	0.07	0.003

[1]Standard error of the mean represents greatest monthly SEM for each component
[2]Acid detergent fiber
[3]Neutral detergent fiber

Table 4. Effect of month × sample type interaction on energy values calculated from NIRS results from native range in Central Oklahoma

Component	Sample Type	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	SEM[1]	*P*-value
DE[2], Mcal/Kg DM	Leaf	2.12^{HIJ}	2.11^{HIJK}	2.22^{FG}	2.24^{EFG}	2.62^{B}	2.48^{C}	2.37^{D}	2.32^{DE}	2.23^{FG}	2.15^{GHIJ}	2.17^{GHI}	2.14^{GHIJ}	0.04	< 0.001
	Whole	2.03^{K}	2.06^{JK}	2.18^{GH}	2.27^{DEFG}	2.75^{A}	2.50^{BC}	2.32^{DE}	2.28^{DEF}	2.18^{GH}	2.07^{JK}	2.11^{HIJK}	2.08^{IJK}	0.04	
ME[3], Mcal/Kg DM	Leaf	1.74^{HIJ}	1.73^{HIJK}	1.82^{FG}	1.84^{EFG}	2.14^{B}	2.03^{C}	1.94^{D}	1.91^{DE}	1.83^{FG}	1.76^{GHIJ}	1.77^{GHI}	1.76^{GHIJ}	0.03	< 0.001
	Whole	1.66^{K}	1.69^{JK}	1.79^{GH}	1.86^{DEFG}	2.25^{A}	2.05^{BC}	1.90^{DE}	1.87^{DEF}	1.79^{GH}	1.70^{JK}	1.73^{HIJK}	1.71^{IJK}	0.03	
TDN[4], % of DM	Leaf	48.11^{HIJ}	47.77^{HIJK}	50.39^{FG}	50.89^{EFG}	59.32^{B}	56.22^{C}	53.65^{D}	52.72^{DE}	50.56^{FG}	48.74^{GHIJ}	49.10^{GHI}	48.59^{GHIJ}	0.87	< 0.001
	Whole	46.03^{K}	46.73^{JK}	49.52^{GH}	51.42^{DEFG}	62.32^{A}	56.65^{BC}	52.63^{DE}	51.80^{DEF}	49.55^{GH}	46.98^{JK}	47.93^{HIJK}	47.19^{IJK}	0.82	
NEm[5], Mcal/kg DM	Leaf	0.90^{HIJ}	0.89^{HIJK}	0.98^{FG}	1.00^{DEFG}	1.28^{B}	1.18^{B}	1.09^{C}	1.06^{CD}	0.99^{EFG}	0.92^{GHIJ}	0.94^{GHI}	0.92^{GHIJ}	0.03	< 0.001
	Whole	0.82^{K}	0.85^{JK}	0.95^{GH}	1.02^{CDEFG}	1.39^{A}	1.20^{B}	1.06^{CDE}	1.03^{CDEF}	0.95^{GH}	0.86^{JK}	0.89^{HIJK}	0.87^{IJK}	0.03	
NEg[6], Mcal/kg DM	Leaf	0.36^{HI}	0.35^{HIJ}	0.43^{FG}	0.45^{DEFG}	0.71^{B}	0.62^{B}	0.54^{C}	0.51^{CD}	0.44^{EFG}	0.38^{GHI}	0.39^{GH}	0.37^{GHI}	0.03	< 0.001
	Whole	0.28^{J}	0.31^{IJ}	0.40^{GH}	0.46^{CDEFG}	0.80^{A}	0.63^{B}	0.51^{CDE}	0.48^{CDEF}	0.41^{GH}	0.32^{IJ}	0.35^{HI}	0.32^{IJ}	0.03	

[1]Standard error of the mean represents greatest monthly SEM for each component
[2]Digestible energy
[3]Metabolizable energy
[4]Total digestible nutrients
[5]Net energy for maintenance
[6]Net energy for gain

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