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Cattle Diet Selection During the Growing Season on Upland Sandhills Rangelands

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CATTLE DIET SELECTION DURING THE GROWING SEASON ON UPLAND
SANDHILLS RANGELANDS

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A THESIS

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CATTLE DIET SELECTION ON UPLAND SANDHILLS RANGELANDS DURING
THE GROWING SEASON

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University of Nebraska, 2021

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The Nebraska Sandhills is diverse and complex ecosystem comprised of different topographic positions (i.e. slopes and interdunes) that influence grazing distribution, plant species composition, and cattle diet selection. The first objective of this study was to evaluate how grazing intensity influences species composition on slopes and interdunes on native rangeland within the Hillside pasture (160 ha) at the UNL Gudmundsen Sandhill's Laboratory. The second objective of this study was to evaluate forage quality of individual species from three plant functional groups (i.e. warm-season grasses, cool-season grasses, and forbs/shrubs) on native rangeland within the pasture. Forage quality samples were taken from 4 warm-season grasses, 5 cool-season grasses, 1 forb, and 2 shrubs. Samples were collected every 7-15 days from mid-May to early August in 2020 and 2021. The third objective of the study was to evaluate diet composition of cattle grazing ($n = 40$) within the pasture during the growing season using fecal DNA barcoding (fDNA). Fecal samples were collected from 7-8 cows every 10-20 days from early June to late-July in 2020 and 2021. Frequency of occurrence of western ragweed (*Ambrosia psilostachya* DC.) was 28 percentage points greater ($P = 0.03$) and Kentucky bluegrass (*Poa pratense* L.) trended to be greater ($P = 0.07$) on high grazing intensity interdunes than low intensity grazing interdunes. When averaged across the growing season, CP of

forbs/shrubs was 3.3 and 2.9 percentage points greater than cool- and warm-season grasses ($P < 0.05$). However, there were no differences ($P > 0.1$) in CP between warm- and cool-season grasses or functional group x collection date interactions. Diet selection as determined by fDNA indicated that cattle obtained most of their dietary protein from cool-season grasses ($43.6\% \pm 1.5$) and forbs ($29.1\% \pm 1.5$), while shrubs ($13.0\% \pm 1.5$) and warm-season grasses ($3.5\% \pm 1.5$) contributed significantly less ($P < 0.01$) to the cattle diets. This research highlights the influence of grazing intensity on species composition and the influence of time during the growing season on forage quality and cattle diet selection within the Sandhills.

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Chapter 1: Literature Review

Nebraska Sandhills

The Nebraska sandhills are a unique ecosystem composed of 4.8 million ha of upland rangeland and sub-irrigated meadows in central and western Nebraska (Barnes and Harrison, 1982; Bleed and Flowerday, 1998). Average max temperatures range from 2° C in January to 32° C in July (35.6° F-89.6° F, respectively) with precipitation of 431-584mm, with 75% of the precipitation occurring during the growing season (April-September) (Volesky et al. 2005). The geomorphic terrain of the dunes found in Sandhills create different topographic positions such as dune tops, interdunes, north-facing slopes, and south-facing slopes, which influence vegetation characteristics (Stephenson et al. 2019). The sand dune and interdune topographic positions support mixed grass plant communities with both warm- and cool-season species (Volesky et al. 2005; Stephenson et al. 2019). Sandhills forage consist mostly of warm-season grasses (60-90%), but also include cool-season grasses and forbs (10-40%) (Volesky et al. 2005). The Sandhills are dominated by sand ecological sites with Valentine fine sand.

Importance of Understanding Forage Quality

Understanding variability of forage quality is essential to improve rangeland management and livestock production on rangelands (Scasta, 2017). Ruminants grazing on rangelands get nutrients (protein, energy, vitamins, and minerals) required for growth, reproduction, and milk production from available forage (Lyons et al. 1996). Increased forage quality is directly linked to positive average daily gain (ADG), reproduction

success, and milk production for grazing livestock and wild herbivores (Lee et al. 2017). Chemical composition and morphology of forage plant species influences palatability and nutritive value which influences efficiency of rumination, amount of forage consumed, and quantity and quality of livestock production variables such as the rates of weight gain, reproduction success, and the quality and volume of milk production (Lee et al. 2017). Forage with low digestibility and crude protein has a slower passage rate which decreases intake, weight gain, and reproduction potential (Soest 1994).

Forage quality is one of the most important variables for financial viability within livestock production systems (Hess et al. 2005). Forage biomass yield, forage quality, and the resulting quality-adjusted yield (biomass yield x forage quality) are economically important variables within forage livestock production systems because increased quality-adjusted yield increases metabolizable energy availability which increases livestock production potential (Schaub et al. 2020). Understanding nutrient supply provided by available rangeland forage and livestock nutritional requirements can reduce supplementation cost and allow for better matching of forage quality availability with livestock demand (Caton and Dhuyvetter, 1997).

Factors Influencing Forage Quality

Nutritional quality of rangeland plant species is affected by the ratio of plant parts, plant maturity, plant functional group, season of growth, weather, soils and range sites, stocking rate, and plant secondary compounds (Lyons et al. 1996; Nichols et al. 1993; Bumb et al. 2016; Powel et al. 1982). Nichols, et al. (1993) found that among species, structural and chemical composition of plant parts contributes to differences in nutritive quality. Plant part cells contain cell solubles (i.e. protein) and cell wall material

(fiber that are included in neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Lyons et al. 1996). Cell soluble contents are highest in active growing tissue and lowest in matured plants, therefore protein content and forage digestibility are typically highest in active growing plants compared to matured or dormant plants. Fruits, flowers, and leaves contain more cell solubles than stems. Additionally, fruits, flowers, and leaves tend to have a greater forage quality because they contain more energy, protein, minerals, and vitamins than structural components of the plant (Lyons et al. 1996, Bumb et al. 2016).

The increase in cell wall material affects the amount of lignin and hemicellulose and reduces the digestibility of the forage (Collins et al. 2018). Lignin and hemicellulose content are influenced by the species plant maturity. Plant maturity had the greatest influence on the declining nutritive value of prairie sandreed and sand bluestem, common warm-season grasses in the Sandhills, as the growing season progressed (Hendrickson et al. 1997). These authors highlighted as plant maturity increased cell wall digestibility decreased as well. The decrease in cell wall digestibility was the major factor that contributed to the reduction of in vitro dry matter digestibility (IVDMD). Lee et al. (2017) found that forage nutritive value decreases with increasing temperatures and the resulting increase in plant maturity in cool- and warm-season species. Plant maturity is the major factor affecting morphology of rangeland forage plant species and largely determines forage quality difference observed during the growing season in the Sandhills (Moser 1994). An increase of plant maturation decreases forage crude protein and increases the fiber in cell wall content (Judy et al. 2015). Powell et al. (1982) found that Nebraska Sandhill range crude protein (CP) was highest in May (16% CP) but decreased

to 6.2% by November. This data is similar to Judy et al. (2015) and Lardy et al. (2004) findings that in ungrazed Nebraska sandhills range pastures peak in May, CP (17.6% and 13.8%, respectively) and in vitro organic matter digestibility (IVOMD, 74.3% and 67.6%, respectively), and declined as the growing season progressed.

Time of growing season effects forage quality among cool-season and warm-season grasses. Cool-season grass growth begins in early spring and forage quality is highest in early spring. Cool-season grass forage quality starts to decrease early in the summer when temperatures increase, and plants mature. Reproductive maturity for most cool-season grass species in the Sandhills is in mid-June. Forage quality of warm-season grasses peaks in early June when growth begins on these species, then steadily declines throughout the remainder of the growing season (Lardy et al. 2004). The difference in when cool-season grasses reach peak nutritive value compared to warm-season grasses is caused by the time of the year these species start to grow and reach maturity. Scasta (2017) found that *Pascopyrum smithii*, and *Agropyron cristatum* (cool-season grasses) had greater lignin content at the end of the growing season than *Bouteloua gracilis* (warm-season grass). Lower lignin content of the warm-season, shortgrass, *Bouteloua gracilis*, contributed to it having greater forage quality later in the growing season.

Forage quality is generally influenced by plant functional group on rangelands. Actively growing forbs and legumes tend to have the highest nutritional quality, with shrubs intermediate, and grasses the lowest (Lyons et al. 1996). Forage quality of grass changes among plant species, plant growth form, and across bioclimatic zones (Lee et al. 2017). Nichols et al. (1993) found that in a mixed sward on subirrigated Sandhills meadows, forage quality was determined by the balance of species prevalent during

different times of the growing season. When comparing grass vs. non grass forage quality, there was a difference in vitro organic matter digestibility (IVOMD) and CP seasonal trends with red clover (a forb) having the highest CP throughout the entire growing season. Additionally, cool-season grasses were greater in crude protein content and digestibility than warm-season grasses (Nichols et al. 1993). Warm season grass digestibility tends to be lower because of greater fiber content needed to support biomass growth and to withstand wilting associated with high temperatures during their growing stage later in the summer (Lyons et al. 1996). However, all functional groups decrease in invitro dry matter (IVDMD) and CP content as the growing season increases.

Stocking rate influences the nutritive value available to grazing animals on rangeland pasture. Lyons et al. (1996) found that pastures that have been previously grazed at greater stocking rates will have greater forage quality as there is less standing dead material and more new growth during the growing season. Cattle tend to select higher quality forage at lower stocking rates because cattle have more options for diet selection (Heitschmidt and Taylor, 1991). Cattle tend to select more leaves than stems and green compared to senescent material (Hardison et al. 1954; Arnold, 1964; Launchbaugh, 1990). An increase of grazing pressure increases evenness in utilization and harvest efficiency across a pasture (Smart et al., 2010). As result, livestock are forced to consume forage across all areas of the pasture (uniform grazing) (Ali and Sharrow, 1994). Therefore, uniform grazing is associated with increasing the efficiency of forage harvest but reduced dietary selectivity and nutrient intake of livestock (Ali and Sharrow, 1994; Hart et al. 1991).

Diet Analysis Methods

Understanding diet composition in grazing animals can improve nutrition, health, and welfare of grazing animals. It can also assist management for the health and resilience of grazed landscapes since it shapes grazing diversity patterns across a given landscape (Holechek et al. 1982; Villalba et al. 2015; Mysterud and Austrheim, 2016; Wang et al. 2018). There are several methods available to estimate diet selection of grazing animals including individual plant utilization, behavioral observation, stomach analysis, fistula analysis (esophageal and rumen), and fecal analyses (microhistology, near infrared spectrum, and DNA barcoding).

Plant utilization techniques include collecting data on the amount of utilization/grazing that individual plant tillers receive during a grazing trial. Therefore, this technique provides fast turn-around data and provides information of where and to what degree a range was utilized. However, it does not provide accurate information on actively growing plants nor on regrowth of defoliated plants and can be difficult in large pastures with diverse grazing patterns (Holechek et al. 1982). Behavioral observations collect information on diet selection of grazing animals by directly observing the species that animals graze. The advantages of this technique are that there is no post processing of forage or fecal samples which eliminates potential digestibility errors. The disadvantages of behavioral observations are that it is labor intensive, not applicable to animals out of sight, and has challenges associated with identifying and quantifying plant species and amount consumed from long distances (Holechek et al. 1982; Garnick et al. 2018).

Stomach and intestinal track analysis is a procedure that involves the sacrifice of animals; therefore, it is restricted to animals being slaughtered (Holechek et al. 1982). The sample size for stomach and intestinal track analysis is typically small as not many rangeland animals are slaughtered for data collection purposes. Animals with esophageal or rumen fistulas allow the observer to analyze naturally grazed samples. However, samples could include contamination by rumen contents, be low in precision of individual species in the diet, and the cost of analysis is high (Holechek et al. 1982). Oslen (1991) found that fistula collection of both methods (rumen and esophageal) affected (content was increased or decreased) contents of nitrogen, organic matter, hemicellulose, and acid detergent lignin due to salivary mineral contamination. In both esophageal and rumen collections, organic matter content of masticated sample decreases because salivary mineral contaminates masticated samples. Additionally, Oslen (1991) results suggest that samples being in the evacuated rumen may alter N and acid detergent lignin content. Nitrogen content increased in rumen collection samples, but nitrogen content did not change in esophageal samples. Nitrogen content increased in rumen samples resulted from ammonia N influx from the blood while acid detergent lignin content increased because of soluble carbohydrate loss through disappearance while sample was in the rumen.

Analyses of fecal material for diet selection of grazing animals provide a less invasive and more easily collected opportunity for researcher to evaluate diet composition on extensive rangelands. Whereas earlier techniques required direct observation of bite counts and invasive procedures to obtain data on diet composition, innovated technology has allowed for diet composition data to be obtained through fecal

analysis (Sanders et al. 1980; Holechek et al. 1982; Lyons and Stuth, 1992; Decruyenaere et al. 2015; Kartzinel et al. 2015; Ottavian et al. 2015; Nunez-Sanchez et al. 2016). Fecal analysis has received greater use for evaluating range herbivore diet composition because it allows for unlimited sampling, collection of samples non-invasively, and comparisons of diets of multiple animals. However, fecal analysis requires collection of plant species and a large learning curve of plant parts to help with identification of plant species found in the feces (Holechek et al. 1982). Garnick et al. (2018) found that when evaluating diet analysis methods with the current trends and accounting for cost, accuracy and precision, resolution, utility of long-term monitoring programs, and appropriateness for both grazers and browsers the three best techniques were microhistology, NIRS, and fDNA. With microhistology, diet composition in the fecal material is identified by morphological characteristics of the plant cells, but for NIRS and DNA barcoding forages are identified by chemical properties found in the sample.

Microhistology has been used in rangelands for many years to evaluate diet composition of livestock (Beck, 1969; Roiere et al. 1975; Havstad and Donart, 1978) and wildlife (Storr, 1961; Johnson et al. 1978; Vara and Holechek, 1980). Plant fragments in the fecal sample are observed under microscope to determine the plant cells of species present in the diet (Vara and Holechek, 1980). Crocker (1959) prepared the slides by diluting the fecal material with water and spreading the samples on two slides. Storr (1961) prepared the slides by boiling, drying, and grounding the samples in a mixture of nitric and chromic acids prior to washing the samples. However, Vara and Holechek (1980) found that the best preparation method was to grind fecal material and to soak the

grinded material in a dilute solution of sodium hydroxide. This preparation method increased the number of identifiable species. Disadvantages of microhistology are that it takes sufficient skill and time to become proficient in this technique. Additionally, accuracy is affected by the differential digestibility of individual plant parts, species, or functional groups consumed. Therefore, it often underestimates the amount of forbs present in the diet (Holechek et al. 1982; Mayes and Dove, 2000; Leslie et al. 1982).

Newer technologies have allowed scientists opportunities to analyze diet composition. Near infrared spectroscopy (NIRS) is a widely used analytic and diagnostic method that uses a combination of physics, math, and engineering to improve our understanding in biology and chemistry. NIRS can be used to determine forage quality using clipped samples, esophageal diet samples, and fecal samples (Holechek, 1982; Barton and Burdick, 1983; Park et al. 1983; Marten et al. 1984; Leite and Stuth 1994). NIRS is based on the principle that reflectance in the near infrared spectrum represents the chemical structure of a sample. This allows NIRS to resolve the presence of target species more quickly than microhistology. However, the disadvantages of NIRS are that it requires independent validation and continual monitoring of calibrations. Additionally, it does not identify individual species within diets well (Dixon and Coates, 2009; Garnick et al. 2018). NIRS can be used as management and research tool to predict digestible dry matter and CP of forage consumed by herbivores grazing rangelands (Lyons and Stuth 1992).

Fecal DNA barcoding (fDNA) evolved as a method to identify plant species when it was discovered that a short chloroplast DNA fragment (P6 loop of the trnL (UAA) intron) can act as a minimalist barcode (Raye et al. 2010). DNA barcoding involves

sequencing target plant DNA found in the fecal material and matching it to a database of known DNA sequences that correspond with the taxonomic origin of the plant (Valentini et al. 2009; Clare, 2014). King and Schoenecker (2019) analyzed fecal samples of feral horses using microhistology and DNA barcoding and found that microhistology underestimated presences of forbs compared to fDNA. The authors contributed this difference to microhistology being known to underestimate forbs in the diet as forbs are digested more completely while fecal DNA barcoding could overestimate forb content due to the high protein content of forbs.

The main advantages of fDNA are that it offers fast processing and turnaround of results, especially when the diet cannot be determined morphologically. Additionally, it can generate a more complete list of the species in the diet with fewer samples (Kohn and Wayne, 1997; Soininen et al. 2009; Clare, 2014). Fecal DNA barcoding processing cost per sample is \$80 with a return time of approximately 2 months. This is much cheaper than microhistology (\$200) at a commercial lab which can take a year or more to complete (King and Schoenecker 2019). Also, because it uses common DNA sequencing techniques, more labs are available that can provide this type of analysis.

Fecal DNA barcoding provides more detailed results because it can detect plant species from smaller plant fragments than microhistology. Pegard et al. (2009) and Soininen et al. (2009) found that qualitatively, plant cuticles for herbivores samples analyzed using the trnL DNA barcoding technique were faster and taxonomically more precise than microhistology. King and Schoenecker (2019) reported twice as many genera were detected using fDNA compared to microhistology for wild horse diet composition analysis using fecal samples. Additionally, fDNA is quicker at determining

botanical composition at species level for numerous plant species. Fecal DNA can read numerous DNA sequence at a single time (Pompanon et al. 2012). Scasta et al. (2020) found that fDNA could be used for adaptive grazing management to enhance livestock nutrition and as a proactive tool to increase awareness of potential toxicosis problems before clinical signs or mortalities occur. This can be accomplished by knowing what species are being targeted and adjusting the management plan to avoid overgrazing of certain species. Additionally, knowing when certain species such as cheatgrass are being grazed versus not grazed allows managers to alter their grazing management plan to target graze species such as cheatgrass when they are most palatable. However, this technique has shown a need of improvement and validation.

The disadvantages of fecal DNA barcoding include technological errors, biological errors, significant bias towards detecting undegraded DNA which limits taxonomic identification, and the potential of it for overestimating forbs and shrubs due to their high content of protein. A study conducted by Scasta et al. (2019) using known diets fed to cattle, found that fDNA can significantly overestimate or underestimate the contribution of a given functional group. Additionally, fDNA misidentified some of the species. The fDNA results found species in the diet that were not present in the ration and overestimated forb content. Additionally, cool- and warm-season grass contribution was overestimated when fed with a high digestible legume (i.e., Alfalfa). Therefore, the difference in digestibility among forbs/legumes and grasses contribute to the inaccuracy of fDNA results. To decrease error, it is important for fDNA results to be verified using plant composition data from the site of the data collection (Garnick et al. 2018; Scasta et al. 2019). Using fDNA along with other techniques, such as microhistology, may

improve quality control and give more powerful results as using multiple techniques allows for more results that include the pros and cons of each technique, therefore the results would account for the variability of each technique and produce more powerful results (Scasta et al. 2019).

Variability of Diet Selection Composition

Understanding diet selection of grazing livestock species can help improve grazing management. Livestock and wildlife utilizing rangelands have access to a diversity of forage plants. Their diet selection is influenced by if they are grazers, browsers, or both (Shippy, 1999; Lyons et al.1996). For example, cattle and bison are typically classified as grazers because a majority of their diet is herbaceous plant material. Goats tend to consume more browse plant material from trees and shrubs and sheep are typically intermediate between cattle and goats. Diet selection may vary among species based on forage availability and within individual species preferences (i.e. cattle, sheep, bison, etc.).

Sowers et al. (2019) collected fecal samples of yearling steers and mature ewes in native tallgrass prairie in the Kansas Flint Hills and used microhistological analyses to compare diet selection between livestock species. They concluded that yearling steers diets were dominated by graminoids (greater or equal to 88.4%) while ewes selected approximately equal proportions of graminoids and forbs (58% and 42%, respectively). Diets between steers and ewes overlapped by 65%. In the Sierra Foothills of California when sheep and cattle grazed together, dietary overlapped averaged 86% (Macon 2018). Similarly, Vavra and Sneva (1978) found that dietary overlap between sheep and cattle was 78 to 86% in eastern Oregon. In contrast, Kirby et al. (1988) in western North Dakota

found that sheep and cattle dietary overlap was only 30-35%. The higher dietary overlap of Vavra and Sneva (1978) and Macron 2018 compared to other studies may have been that grasses were the primary forage available and there were limited forb options for sheep to consume. Therefore, diet composition of both livestock species consisted primarily of grasses. In more diverse environments with greater opportunities to select from multiple functional groups, the spread of diet selection may be greater.

Studies conducted in North America and in Europe have analyzed dietary overlap among cattle, horses, and other species (bison and wild ungulates). Cromsigt et al. (2017) analyzed how functionally diverse the diets of European bison, cattle, and horses are through observations. The authors found that in all three species, greater than 80% of their diet was composed of grasses. However, while 20% of bison and cattle diet was composed of woody species, horses did not utilize woody species. Horses tended to supplement their grass diet with sedges and herbs. Additionally, horses were the only species to be observed eating roots. Scasta et al. (2016) conducted a quantitative synthesis of microhistological fecal studies for wild horses, beef cattle, sheep, elk, pronghorn, and mule deer on rangelands in western North America (United States, Canada, and Mexico) to analyze diet composition and potential conflicts of wild horses with livestock and wild ungulates. Scasta et al. (2016) found that the greatest potential of dietary overlap is between wild horses and cattle or elk and sheep. Cattle and wild horse diet composition was similar (66-89% graminoids throughout all seasons) and responded to regional and season variation similarity. Diet selection of horses was similar to sheep and elk in some seasons for a specific plant functional group (e.g., wild horses and elk selected for similar proportions of forbs in the spring).

Breed of cattle may also influence diet selection. Hessle et al. (2008) analyzed the effect of breed (Swedish Vaneko heifers vs. Chrolais heifers) and season on diet selection. Hessle et al. (2008) found no main effect of breed on the composition of plant fragments, but the proportion of grasses was lower for the Vaneko heifers in spring than in summer and autumn and the proportion of Cyperaceae (sedges) was greater in spring than in autumn and summer. For the Charolais heifers, the proportion of woody plants was greater in autumn than in spring and summer. Winder et al. (1996) used fecal microhistology to analyze the effect of cattle breed (Hereford vs. Angus vs. Brangus) on botanical composition. Genetic composition of the animal is an important factor determining utilization of key species on Chihuahuan desert ranges because the breed of cattle may affect the degree a certain plain community is utilized (i.e. Brangus cows utilized *Sporobolus* spp. more than Angus and Hereford cows in January and July). Spiegal et al. (2017) used fecal DNA barcoding to compare Raramuri Criollo (RC) cattle and Angus-Hereford (AH) cattle in the Chihuahuan Desert. The authors concluded that black grama and four-wing saltbush was selected more by AH cattle in the dormant season than RC cattle. The results suggests that AH cattle diet selection differs from RC cattle. Additionally, that the differences in the diet selected are influenced by plant growth phenology.

Topography Influence on Plant Characteristics in the Sandhills

A better understanding on how ecological sites and their characteristics (soil, climate, and topography) influence vegetation is important to ecosystem management (Reynolds et al. 2019). Ecological sites (distinct areas of land that have specific topography, soil type, and climate characteristics) influence the amount of vegetation and

the plant species present in an area (Reynolds et al. 2019). Topographic position on western Great Plains landscapes influences botanical composition and other vegetation characteristics (Pool, 1914; Schacht et al. 2000; Stephenson et al. 2013; Stephenson et al. 2019; Hoover et al. 2021). Topography along with precipitation amount and pattern influence vegetation because of variable hydrology and topoclimate (incoming solar radiant, soil moisture, and temperature) characteristics on slopes and lowlands (Dobrowski, 2011, Liu et al. 2013, Williams et al. 2013; Adams et al. 2014; Hoover et al. 2021).

Topography influences grass species in Nebraska Sandhills. In eastern Nebraska Sandhills, plant production and precipitation use efficiency (PUE) was greater on the interdune lowland positions than slope and dune tops (Stephenson et al. 2019). The authors reported that early spring and above average summer precipitation contributed to a significant increase in biomass of cool-season species on the interdunes compared to the dune positions. These cool-season grasses are more abundant on the interdunes than the dune positions (Schacht et al. 2000; Stephenson et al. 2019). As a result, the amount of total forage available in August in eastern Sandhills during wet and dry years will vary by the relative amount of different topographic positions within a pasture (Stephenson et al. 2019).

Vegetation on dunes, slopes, and ridges is more widely spaced compared to lowlands allowing for an increase amount of bare ground on Sandhills rangelands (Barnes and Harrison, 1982). South-facing slopes and dune tops have greater light intensity causing greater surface temperatures than north-facing slopes. This allows for a better plant habitat for warm-season grasses (i.e. sand bluestem and prairie sandreed) than

cool-season grasses (Schacht et al. 2000; Stephenson et al. 2019). However, north-facing slopes tend to have more little bluestem, needlegrasses, and prairie junegrass (Barnes and Harrison, 1982; Schacht et al. 2000). The differences of grass species found on north and south facing slopes can also be explained by the differences of humidity and evaporation rates among the aspects. Slopes and dunes were found to have different vegetation than interdunes since lowland sites have greater maximum availability of water than dunes and slopes, especially early in the growing season (Barnes and Harrison, 1982). Tolstead (1942) found that in dry years coarse textured soils on dunes provide more available water during summer months than lowlands, but a fine textured soils of lowlands had greater water holding capacity than dunes coarse textured soils in the early spring and summer. This is a significant ecological factor that allows for cool-season grasses to have greater frequency of occurrence in the interdunes (Burzlaff, 1962; Barnes and Harrison, 1982). Gibson and Hulbert (1987) found that in the Konza Prairie in Kansas, upland soils support greater species richness and diversity than lowland soils, especially right after a fire. Schacht et al. (2000) and Barnes et al. (1983) found that in eastern Nebraska Sandhills and mixed prairie of northcentral South Dakota, mean species richness was lower in interdunes topographic positions than ridge/dune tops and slopes. Therefore, species composition and forage production are influenced on topographic positions which can influence the effect of precipitation variability on forage production.

A better understanding in how topographic positions and precipitation/climate variability influences plant production throughout the pasture is essential as it allows producers to better match forage demand with forage availability without significantly overestimating or underestimating forage availability (Andales et al. 2006; Nippert et al.

2011). Overgrazing on heavily selected topographic positions can have negative effects on plant communities and soil properties (Ren et al. 2018). Li et al. (2016) and Collins and Calabrese (2012) found that structural and functioning thresholds of grazing intensity depend on plant traits and species composition. Additionally, the thresholds are mediated by topography since topography influences resource availability and influences species community structure. Topography affects grazing distribution and can alter species composition and soil properties through erosion and nutrient availability differential through dung and urine accumulation (Murray et al. 2010; Kolbl et al. 2011; Collins and Calabrese, 2012; Li et al. 2017; Liu et al. 2017). Li et al. (2016) found that with grazing disturbance, species abundance was reduced in both slope and flat systems. However, the negative influence of grazing intensity on species occurrence was greater on slopes than on flat plains. Slopes were impacted more severely than flat plains because flat plains allow species to recover more easily. Flat plains can recover from grazing pressure quicker as they have greater organic matter and precipitation efficiency than slopes.

Grazing Influence on Rangeland Ecosystems

Dyksterhuis (1949) defined range condition as the “....state of health or productivity of both soil and forage of a given range, in terms of what it could or should be under normal climate.” Overgrazing can deteriorate range condition and can negatively influence forage species composition. A reliable way to detect overgrazing is by recognizing when one plant species cover is replaced by another (Dyksterhuis, 1949; Sampson, 1919). Land degradation and altering species composition have resulted from overgrazing. Based on plant species response to grazing, species have been grouped into 3 categories: decreasers (species that decreases due to grazing), increasers (species that

increase due to grazing), and invaders (non-native species that take over the community due to grazing pressure and disturbance) (Dyksterhuis, 1949; McIntyre et al. 2003). Overgrazing with livestock has contributed and allowed invaders (invasive species) to occupy rangelands while reducing the presence of native species (McIntyre et al. 2003). Additionally, the proportion present of decreases, increasers, and invaders are correlated with range condition since they affect species richness, species diversity, quality of the forage present (Dyksterhuis, 1949). Dyksterhuis (1949) explained that indiagrass, big bluestem, and little bluestem are examples of decreaseers, sand dropseed and hairy grama are examples of increasers, and western ragweed is an example of an invader. These species are commonly found throughout the Nebraska sandhills. Therefore, it is important to understand how grazing influences the presence of these species. Disturbances caused by overgrazing on sandy acidic soils reduces soil organic carbon stock of rangelands (Dlamini et al. 2016). Organic matter impacts nutrient retention, soil structure, moisture retention and availability, degradation of pollutants, and carbon sequestration. Therefore, reducing the soil organic carbon stock can have a negative influence on range productivity and health.

Disturbances, such as wildfires and overgrazing, along with climate variability can stress semiarid ecosystems and allow them to be less resilient to invasive species. Disturbance caused overgrazing of wild horses and livestock has been documented as a contributing factor to the increase of presence of invasive species (Chambers et al. 2007; Chambers and Wisdom, 2009; Chambers et al. 2014; King et al. 2019). Additionally, King et al. (2019) found that wild horses can distribute viable seeds of species in their feces, altering the plant community of rangelands. Overgrazing has resulted in a decline

of biodiversity and ecosystem functioning in grasslands world-wide (Li et al. 2016; Milchunas and Lauenroth, 1993; White et al. 2000; Diaz et al. 2007) and alters C, N, and P pools and stoichiometry of steppe ecosystems through the effects of wind, water erosion, and deposition process (Bai et al. 2012; Hoffman et al. 2008; Kolbl et al. 2011). Additionally, grazing directly affects plant growth and C allocation, soil fertility, and edaphic properties (Bardgett et al. 1998; Mikola et al. 2009; Sorensen et al. 2009; Liu et al. 2015). However, moderate well managed grazing typically has neutral effects and can increase plant diversity and productivity (Milchunas et al. 1988; Cingolani et al. 2005; Li et al. 2017). Grazing influences rangeland ecosystems but the affect grazing has on the ecosystem is also influenced on the topographic position, because topography influences grazing behavior.

What Influences Grazing Behavior

A better understanding on how topography influences grazing behavior can provide land managers with essential information to improve grazing management and livestock performance by informing them what areas of their pastures are being overgrazed and what areas are not being utilized. (Mueggler, 1965; Ganskopp and Vavra 1987; Bailey et al. 2015; Raynor et al. 2021). Livestock grazing behavior is complex because it is influenced by multiple spatial and temporal factors (Senft et al. 1987). During livestock foraging, site selection influences diet selection by increasing or decreasing the number of species available (Bailey et al. 2015). Livestock are selective of their diet based on the availability of preferred forages and satiation (Bailey, 2005; Bailey and Provenza, 2008; Bailey et al. 2015). Grazing distribution is affected by abiotic (terrain and water sources) and biotic factors (Senft et al. 1985; Senft et al. 1987;

Coughenour, 1991; Howery et al. 1998; Bailey, 2005; Bailey et al. 2015). Grazing distribution is highly uneven in larger pastures with minimal water sources and rugged terrain compared to smaller well-watered pastures with gentle terrain (Raynor et al. 2021). This occurs because livestock grazing pressure and concentration increases near water sources and other preferred location (Fuhlendorf and Engle, 2001). Livestock tend to not travel far from water sources and avoid steep slopes for more gentle terrain (Mueggler, 1965; Holechek, 1988; Valentine, 1947). The interaction between grazing and topography contribute to important implications for vegetation dynamics and how rangeland managers achieve desired outcomes from these landscapes (Gersie et al. 2019). Wang et al. (2018) and Firincioglu et al. (2009) found that grazing exclosures (fenced areas to avoid grazing for 6 to 23 years, respectively) decreased species diversity patterns. Additionally, grazing effects interact with topography and soil to impact the abundance of dominant species in desert steppe in China (Wang et al. 2018). Gersie et al. (2019) found that topographic position classes can create models that effectively predict grazing distribution. Raynor et al. (2021) found that in arid and semiarid rangelands, livestock grazing distributions showed a preference for lowlands and flat plains than slopes and uplands. Ren et al. (2018) found that topography interacts with grazing to influence hyphal length density (density of fungi in the roots). Topography mediates the effect of grazing on arbuscular mycorrhizal (AM) fungi. AM fungi are soil microorganisms that colonize approximately 80% of terrestrial plants and are important in semi-arid grassland ecosystems because they increase plant nutrient uptake and resistance to stress (Smith and Read 2008; Brundrett 2009). Therefore, understanding the role of topography on livestock distribution could provide land managers with important

information as it allows for adaptive management. Adaptive management of livestock can address both conservation and production goals from these ecosystems (Raynor et al. 2021).

Although cattle diets are made up of multiple plant species, they usually focus on a few species (Bailey et al. 2005). Cattle typically make decisions of their diet every 1-3 seconds (Bailey et al. 1999; Bailey et al. 2005; Bailey et al. 2015). Livestock grazing behavior actions are a reaction of the environment using their hierarchy instincts that result in a variation of distribution of the landscape, community, patch, and feeding stations (Senft et al. 1987; Stuth, 1991).

Variation of grazing behavior among individual cows can be influenced by their genetic make-up and the physiological stage of the cow (i.e. dry cows vs. lactating cows) (Bailey et al. 2001). Although Rouda et al. (1990) reported no difference in the distance traveled among lactating and non-lactating cows, but Bailey et al. (2001) and Black Rubio et al. (2008) found that non-lactating cows used steeper slopes than lactating cows and that lactating cows explored smaller areas than non-lactating cows. However, the higher water requirements of lactating cattle and presence of a young calf may influence the distance and terrain used by lactating cows (Bailey et al. 2001). Additionally, in Rouda et al. (1990) lactating and non-lactating cattle grazing behavior may have been the same because they supplemented while on rangelands.

Genetic make-up also influences grazing behavior patterns of cattle grazing on rugged rangelands in the western United States. Bailey et al. (2015) evaluated how genetic makers influences grazing behavior and found that a genetic maker, gene (GMR5) that influences locomotion, memory, and spatial memory, accounted for 24% of

the phenotypic variation in use of slopes in high elevations. The correlation among the genetic marker and the terrain use demonstrate how grazing behavior can be inherited. This genetic variability may explain why grazing patterns can be variable among individual animals with some cows (hill climbers) preferring grazing areas with steeper slopes and higher elevations and some cows (bottom dwellers) preferring grazing areas with gentler slopes and lower elevations (Bailey et al. 2004). Therefore, grazing behavior data of a herd can be used to select cattle that tend to be hill-climbers and low dwellers to better utilize range pastures. Understanding individual cattle patch selection on extensive rangelands can improve grazing management (Walker 1995, Roath and Kruger (1982), Howery et al. (1996), and Howery et al. (1998). Cattle with different grazing behaviors patterns can be grouped together to better utilize pastures. Therefore, individual animal selection through culling and genetic selection has been proposed as management tool to increase uniformity of rugged terrain rangelands pastures.

Bailey et al. (2001) and VanWagner et al. (2006) found that Tarentaise and Piedmontese cows, both breeds developed in mountainous terrain, used steeper slopes and traveled further vertically from water sources than Hereford and Angus cows, which originated from more gentle terrain. Additionally, cows sired by Piedmontese bulls are more willing to utilize areas further from water sources than Angus (VanWagoner et al. 2006). Variation of grazing behavior among different breeds was also seen in a study done by Russel et al. (2012). That study found that spatial movement patterns of Brahman cows differed from Angus and Brangus cows. Herbel and Nelson (1966) found that Santa Gertudis cows (three-eighths Brahman and five-eighth Shorthorn) walked greater distance per day than Hereford cows in southern New Mexico. Heritage breed

such as Raramuri Criollo cows have a greater distribution of than some mainstream improved beef breeds because they tend to explore larger areas of the pasture in the southwest United States (Roacho-Estrada et al. 2008; Peinetti et al. 2011; Spiegel et al. 2019). Hessle et al. (2008) found that a Swedish cow breed, Vanko, heifers had greater activity levels than Charolais heifers indicating that breed of cattle influences activity level and their willingness to travel longer distance to water. However, Russel et al. (2012) found that there was no difference in the average distance traveled to water among Brahman, Angus, and Brangus cows. Therefore, when analyzing variability of grazing behavior, it is important to consider all the factors such as forage availability and quality, breed and genetic influence, stage of lactation, and the ecosystem and topography of the livestock to better understand the variation of grazing behavior.

Summary

The Nebraska Sandhills is an important ecosystem comprised of upland rangeland and sub-irrigated meadows in central and western Nebraska. The Sandhills plant community is mostly warm-season grasses, but cool-season grasses, forbs, and shrubs are also present and important. The growing season in the Sandhills is May-September, therefore forage quality is highest in those months. Different topographic positions (interdunes, north-facing slopes, south-facing slopes, and dunestops) in the Sandhills influence variability of grazing intensity and plant species composition. Topography influences grazing distribution as cattle tend to graze areas closer to water sources and flatter areas heavier than steeper areas and areas further away from water. As a result, cattle can influence plant species availability, composition, and diversity. Forage quality is influenced by species composition, stage of maturity, standing dead material, and

functional group. Cattle tend to be highly selective of their diet as they tend to select for greater quality and more palatable forage when available. Therefore, it is important to understand the species composition throughout the pastures and grazing behavior regimes to have a better understanding of diet selection of cattle in the Sandhills.. Previous studies have not analyzed how grazing intensity influences species composition of slope and interdune sites and how species composition and forage availability may influence forage quality and diet selection. Therefore, our study was created to analyze to better understand how grazing behavior, forage quality, and diet selection influence each other.

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Chapter 2: Influence of grazing intensity on species composition at different topographic positions in the Nebraska Sandhills

Introduction

A better understanding on how topography influences grazing behavior on diverse rangeland ecosystems can provide land managers with important information to improve grazing management and livestock performance (Mueggler, 1965; Ganskopp and Vavra 1987; Bailey et al. 2015; Raynor et al. 2021). Livestock grazing behavior actions are a reaction of the environment and hierarchy instincts that result in a variation of grazing distribution at the landscape, community, patch, and feeding stations level (Senft et al. 1987; Stuth, 1991). As a result, livestock grazing can negatively influence plant communities at highly preferred areas even if pastures are appropriately stocked for a given rangeland (Bailey 2005).

In arid and semiarid rangelands, livestock prefer grazing in lowlands and flat plains more than slopes and uplands. Raynor et al. (2021) found that grazing intensity was 120% greater on lowlands than uplands at six different locations in the western United States. In this study, pasture size, distance to water, stock density, and terrain roughness influenced the uniformity of grazing across the landscape. Livestock tend to not travel far from water sources and avoid steep slopes for more gentle terrain (Mueggler, 1965; Holechek, 1988; Valentine, 1947). However, Bailey et al. (2004) found that grazing patterns are variable among individual animals with some cows preferring to graze areas with steeper slopes and higher elevations, while other cows preferred grazing areas with gentler slopes and lower elevations. Multiple studies have shown that there may be a breed effect on grazing behavior as certain breeds will travel further from water

and use rougher terrain (Roacho-Estrada et al. 2008; Peinetti et al. 2011; Spiegel et al. 2019; Hesse et al. 2008; VanWagoner et al. 2006).

A better understanding in how topographic position influences plant production throughout the pasture is important to better match forage demand with forage availability (Andales et al. 2006; Nippert et al. 2011). Ecological sites (distinct areas of land that have distinct topography, soil type, and climate characteristics) influence the amount of vegetation and the plant species present in an area (Reynolds et al. 2019). Topographic position influences botanical composition and vegetation characteristics of rangeland sites (Pool, 1914; Schacht et al. 2000; Stephenson et al. 2013; Stephenson et al. 2019; Hoover et al. 2021). In eastern Nebraska Sandhills and mixed prairie of northcentral South Dakota, mean species richness was lower in interdunes topographic positions than ridge/dune tops and slopes (Schacht et al. 2000 and Barnes et al. 1983). Since topography influences resource availability and influences species community structure, topography mediates structural and functioning thresholds of grazing intensity (Li et al. 2016; Collins and Calabrese, 2012). Not only does topography have an influence on the plant community but grazing pressure has also shown to also influence the plant community.

Grazing disturbance can reduce species abundance on both slope and flat plains (Li et al. 2016). Overgrazing can deteriorate range condition and can negatively influence forage species composition (Dyksterhuis, 1949; Sampson, 1919). Disturbances caused by overgrazing have been documented as a contributing factor to the increase of presence of invasive species (Chambers et al. 2007; Chambers and Wisdom, 2009; Chambers et al. 2014; King et al. 2019). However, Porensky et al. (2020) reported that in the Great Plains

grasslands, light to moderate levels of grazing intensity may be necessary to resist invasion of annual invasive grasses. Milchunas et al. (1988), Cingolani et al. (2005), and Li et al. (2017) found well managed grazing can increase plant diversity and productivity. Milchunas et al. (1994), Derner and Hart (2007), and Gonzalo et al. (2016) found that for short grass prairie and northern mixed prairie, grazing intensity contributes to shifts in functional group composition. They found that increasing grazing intensity reduced cool-season grass biomass while increasing warm-season grasses biomass.

Understanding how grazing intensity influences rangeland plants communities is important to improve rangeland management and livestock production on rangelands. However, analyses on the effect of grazing intensity on plant frequency and ground cover at different topographic positions are limited in the Nebraska Sandhills (Stephenson et al. 2013). The objectives of this study were to analyze how grazing intensity, as determined by selection of pasture areas with GPS-tracked cattle, influences species composition at different topographic positions (interdunes and slopes). Additionally, this study evaluated plant species composition within a Sandhills pasture to better understand forage plants available within the diet of grazing animals (see chapter III). Scasta et al. (2019) concluded that a thorough understanding of range plant species available to grazing animals was necessary to understand diet selectivity using fecal DNA sequencing techniques. I hypothesized that areas of a pasture that typically received high grazing intensity would have different plant communities (i.e., more grazing tolerant species) and more bare ground than areas with low grazing intensity.

Materials and Methods

Study Site

This study was conducted during the 2020 and 2021 growing seasons at the University of Nebraska Gudmundsen Sandhills Laboratory (GSL) located 11 km northeast of Whitman, Nebraska (lat 42°03'34.9"N, long -101°24'52.1"W, elevation 1,068 m). The 30 year average annual precipitation for GSL is 537.2 mm (21.2 inches) (PRISM Climate Group). In 2020 and 2021 the annual precipitation was 402.1 mm (15.8 inches) and 470.9 mm (18.5 inches), respectively (PRISM Climate Group). The study was conducted in the Hillside pasture at the GSL which consists of 160.3 ha (396 acres) of upland Sandhills rangeland. Common plant species at the study area consisted of a mixture of warm-season grasses [(little bluestem (*Schizachyrium scoparium* [(Michx.) Nash]), prairie sandreed (*Calamovilfa longifolia* [(Hook.) Hack]), sand bluestem (*Andropogon hallii* [(Hack.) Wipff]), switchgrass (*Panicum virgatum* L.), sand dropseed (*Sporobolus cryptandrus* [(Hook.) E.K. Jones & Fasset]), blue grama (*Bouteloua gracilis* [(Kunth.) Lag. Ex Steud]), and hairy grama (*Bouteloua hirsute* Lag.), cool-season grasses [Scribner's rosette grass (*Dichanthelium scribnerianum* [(Hitch. & Chase) Gould]), prairie junegrass (*Koeleria macrantha* [(Vasey ex Burt Davy) Roy L. Taylor & MacBryde]), western wheatgrass (*Pascopyrum smithii* [(Rydb.) Barkworth & D.R. Dewey]), sedges (*Carex spp.*), and Kentucky bluegrass (*Poa pratensis* L.)], and forb and shrub species [(stiff sunflower (*Helianthus pauciflorus* Nutt.) and wild rose (*Rosa arkansana* Porter)].

Study Site Selection Within the Hillside Pasture

Historically, the hillside pasture has been grazed by cows and calves at moderate stocking rates (mean stocking rate 2009 to 2020 = $0.54 \text{ AUMs} \cdot \text{acre}^{-1} \pm 0.18 \text{ SD}$) season-long during the growing season. From 2018 to 2020, the pasture was grazed by 40 May calving cows ($0.61 \text{ AUMS} \cdot \text{acre}^{-1}$) for a study evaluating differences in grazing behavior between upland and meadow environments during the growing season from mid-May to mid-August (Beard et al. 2020). Of the 40 cows, 12 or 13 were randomly fitted with GPS collars in mid-June and tracked until mid-August at 5-minute fix intervals. Data from these cattle were utilized to identify sample areas of the pasture that were grazed at relatively heavy and light intensities based on the amount of time cattle spent grazing on these areas. To identify sample areas, a point density map was created within ArcGIS pro using the Spatial Analyst function. The analysis only used GPS points where cattle were classified as grazing using a classification tree analysis to separate grazing from resting with a 3-way axis accelerometer attached to the collar (Augustine and Derner 2013). Point densities were mapped based on 10 quantile values representing the number of points $\cdot \text{acre}^{-1}$ (Fig. 2.1). Random points were selected within pasture areas with point densities greater than the 8th quantile for sample areas classified as receiving a high intensity grazing. Low intensity grazed areas were selected from areas with point densities lower than the 2nd quantile. Random points were selected with the assumption that the intensity of grazing time from GPS-tracked animals represented longer-term grazing patterns and would reflect consistently heavy and light grazing intensities on areas which have persisted long-term over years. Actual grazing intensity (GPS tracked cow grazing $\text{hrs} \cdot \text{acre}^{-1} \cdot \text{yr}^{-1}$) were evaluated at each of the random points.

Vegetation monitoring transects were established at the randomly identified points on nearby areas that were either slopes or interdunes. Sample areas were selected based on similar topography, elevation, and distance from water between grazing intensities. A fifty-pace transect with 25 data collection readings per transect were placed on six sloped sites (three per grazing intensity treatment) and six interdune sites (three per grazing intensity treatment) in July, 2020. Additional transects were placed on a different set of six sloped sites (three per grazing intensity treatment) and six interdune sites (three per grazing intensity treatment) in July, 2021.

In each reading along the transect, a 40 cm by 40 cm square frame was used to obtain frequency of occurrence plant species. All plant species rooted inside the frame were recorded. The readings per transect line for each individual species were summed and divided by the total number of frame placements along the transect to obtain the percent frequency of occurrence for each species. Additionally, at each reading, using a cover point located on the frame, ground cover is recorded at each frame placement along the transect. Bare ground, litter, and basal ground cover readings were summed and divided by 25 to determine the ground cover % of each class of ground cover.

Statistical Analysis

Grazing intensity at sample sites, frequency of occurrence of plant species, and ground cover data were entered and organized in Microsoft excel. Sample site within the study pasture was treated as the experimental unit. All data were statistically analyzed using the Proc Glimmix statement in SAS 9.4 (Cary, North Carolina, USA). Frequency of occurrence data of the 10 most common perennial plant species at each topographic position was included in the analysis. Sample site grazing intensity (high vs. low) was the

fixed effect and year of data collection was treated as a random variable. Data were analyzed for normality using studentized residual plots and data from individual plant species were transformed if normality assumptions were not met. Data collected on slopes and interdune topographic positions were analyzed separately. Effects were considered significant at a P-value of 0.05, while tests with P-values between 0.05 and 0.10 were considered trending significant.

Results

Grazing Intensity Differences at Samples Sites

Mean relative grazing intensity on heavily grazed samples sites (26.9 cow grazing hours · acre⁻¹ · yr⁻¹ ± 1.4 SE) was 7.6 times greater (P < 0.01) than grazing intensity on the lightly grazed sample sites (3.5 cow grazing hours · acre⁻¹ · yr⁻¹ ± 0.4 SE). The grazing intensities across the sample sites ranged from a low of 1.3 cow grazing hours · acre⁻¹ · yr⁻¹ to a high of 36.5 cow grazing hours · acre⁻¹ · yr⁻¹.

Frequency of Occurrence and Ground Cover on Slopes

Frequency of occurrence of western ragweed and blue grama were 15.3 and 24.3 percentage points greater (P < 0.05) on high intensity grazed slopes compared to low intensity grazed slopes, respectively (Fig. 2.2). Frequency of occurrence of Scribner's rosette grass tended to be greater (P = 0.08) in high intensity grazed slopes than low intensity grazed slopes (Fig. 2.2). Frequency of wild rose was 47.3 percentage points greater on low intensity slopes (P < 0.01) compared to high intensity slopes (Fig. 2.2). Prairie sandreed, little bluestem, sand bluestem, stiff sunflower, sand dropseed, needle grasses, and prairie junegrass were species that were frequently observed on slopes but

not different ($P > 0.1$) in frequency of occurrence between the grazing intensities (Fig. 2.2). There were no differences ($P > 0.1$) in bare ground, litter, or basal ground cover between the different grazing intensities on the slope study sites (Fig. 2.3).

Vegetation and Ground Cover on Interdunes

Frequency of occurrence of western ragweed was 28 percentage points greater ($P = 0.03$) on high grazing intensity interdunes than low grazing intensity interdunes. There was a trend ($P = 0.07$) for greater Kentucky bluegrass on high grazing intensity interdunes compared to low intensity grazing interdunes (Fig. 2.4). Frequency of occurrence for needle grasses, sand dropseed, wild rose, little bluestem, and prairie junegrass was lower ($P < 0.05$) on high grazing intensity interdunes than low intensity grazing interdunes (Fig. 2.4). Prairie sandreed, Scribner's rosette grass, blue grama, stiff sunflower, and sand bluestem were other species that were frequently observed on the interdunes but not different ($P > 0.1$) in frequency of occurrence between the different grazing intensities (Fig. 2.4). Bare ground on high grazing intensity interdunes was 16 percentage points greater ($P < 0.05$) than on low grazing intensity interdunes (Fig. 2.3). In contrast, low intensity grazed interdune sites tended ($P < 0.07$) to have greater vegetation basal ground cover compared to high intensity grazed sites (Fig. 2.3). Similar to the slope sites, no differences were detected in litter ground cover percentage between treatments on the interdune study sites (Fig. 2.3).

Discussion

Non-uniform grazing distribution patterns on the study pasture created areas with different intensities of grazing in the central Nebraska Sandhills. Our data indicates that

grazing intensity influenced species composition on both interdunes and slope study areas. For both topographic positions, western ragweed was more frequently observed on the high intensity grazed areas than the low intensity grazed areas. Western ragweed tends to increase within pastures with greater stocking rates and when grasses are reduced by stressors such as overgrazing (Elder 1951, Sims and Dwyer 1965, Vermeire and Gillen 2000). The increase of western ragweed in our study pasture areas likely resulted from over grazing disturbance on the high grazing intensity sites compared to less preferred and low grazing intensity sites.

Grazing intensity influenced the frequency of occurrence of blue grama on the slope sites. Our data support Hart and Ashby (1998) findings that blue grama biomass increased with increased grazing intensity in the western Great Plains. Additionally, Hyder et al. (1975) found that blue grama biomass increased under summer heavy grazing at United States Department of Agriculture Central Plains Experimental Research Station (CPER) in north-east Colorado, USA. Samuel and Howard (1982) classified blue grama as an increaser, or a species that increases in frequency with increased grazing disturbance. Additionally, Riegel (1903) explained that blue grama is resistant to grazing disturbance and can revegetate successfully. Grazing intensity tended to also influence the frequency of Scribner's rosette grass on the slope sites. Our results support Dyksterhuis (1984) findings that Scribner's rosette grass tends to increase in vegetation under grazing. Additionally, Towne et al. (2005) found that Scriber's rosette grass cover reduced over time in the absence of grazing. The increases in blue grama and Scribner's rosette grass, both grazing tolerant plants, in our study likely resulted from a higher

grazing disturbance on the high grazing intensity sites compared to the low grazing intensity sites.

Grazing intensity influenced the frequency of occurrence in Kentucky bluegrass on the interdune sites. Kentucky bluegrass was observed 2.4 times more frequently in the heavy grazing intensity interdunes compared to low grazing intensity interdunes. The importance of Kentucky bluegrass to the interdune topographic position has been reported in other studies in the Sandhills (Schacht et al. 2000, Stephenson et al. 2019). Patton et al. (2007) found in south-central North Dakota that when compared to no grazing, areas grazed with low to moderate stocking rates can increase the production of Kentucky bluegrass, but overgrazing can reduce production. Otfinowski et al. (2007) and Roath and Krueger (1982) did not see an increase of Kentucky bluegrass in grazed areas vs. nongrazed areas, but they found that grazing did not reduce the abundance of Kentucky bluegrass. Grazing intensity influence on the abundance of Kentucky bluegrass was not measured in the slopes because our data showed that Kentucky bluegrass was typically not observed on slopes, as observed in other studies in the Sandhills (Schacht et al. 2000; Dallman 2018). Additionally, interdunes have greater organic matter which increases water availability in the topsoil in the early spring (Schacht et al. 2000). This contributes to Kentucky bluegrass being abundant in the interdunes, but not the slopes (Stephenson et al. 2019). Diet composition data collected from cattle in the study pasture (see Chapter III) suggest that Kentucky bluegrass is one of the major dietary protein contributors in cattle diets during the growing season.

Little bluestem and other perennial grass species tended to be greater on lightly grazed interdune areas compared to heavy grazed interdunes. Dyksterhuis (1949)

classified little bluestem as an example of a decreaser (i.e., species that decrease in frequency at greater grazing disturbances). However, Dyksterhuis (1949) also explained that sand dropseed was an increaser (species that increases in frequency because of grazing disturbance), but our data showed that greater grazing intensity reduced the abundance of sand dropseed in interdunes that were grazed heavier. This could have resulted because of the ecological threshold not being pushed since the larger pasture area was grazed at moderate stocking rates. Therefore, although the high intensity grazing areas likely had more grazing pressure than the low intensity area, our generally moderate stocking rates may have limited extreme overgrazing to occur at all sample sites. As a result, some species would not have had enough grazing pressure to change their species composition.

There was a grazing intensity effect on frequency of occurrence of needle grasses in the interdune sites as needle grasses frequency of occurrence was significantly lower in interdunes that were grazed with higher intensity. Hart and Ashby (1998) found that needleandthread decreased in biomass as grazing intensity increased. Additionally, Hyder et al. (1975) found that needleandthread biomass decreased under heavy summer grazing on short grass prairie rangelands. Furthermore, Clarke et al. (1947) found that needleandthread was affected by how often it received grazing pressure. Therefore, the decrease in needle grasses in our study likely resulted from a greater grazing disturbance.

There was not a grazing intensity effect on frequency of occurrence of prairie sandreed, little bluestem, sand bluestem, stiff sunflower, sand dropseed, needle grasses, and prairie junegrass on the slopes sites. No differences in these species between the sample locations may have been to cattle generally avoiding these species in their diets.

Fecal DNA diet selection data collected in the study pasture throughout the growing season (see Chapter III) indicates that cattle grazing this pasture from late May to late July obtain only 3.47% of their dietary protein from all warm-season grasses (Fig. 3.11). Therefore, cattle grazing this pasture may not be overgrazing these species, even in preferred areas with higher grazing intensities.

Our results did not show a significant difference between the grazing intensity sample sites for bare ground, litter, or basal ground cover on the slopes, but greater bare ground and less vegetation on the interdunes. This could have resulted because the Hillside pasture (our study area) were grazed by cattle at a moderate stocking rate for the area. In general, interdunes and lowlands tend to more intensively grazed than associated nearby uplands in the Sandhills (Raynor et al. 2021). As a result, the slopes as a whole, were generally not as heavily grazed as the interdunes and ground cover was not reduced by grazing intensity. Naeth et al. (1991) found that in Canadian grasslands areas that were not grazed had the lowest amount of bare ground. Our findings support Grudzinski et al. (2015) findings that areas grazed more intensely are subject to a greater abundance of bare ground. Grazing intensity for both slopes and interdunes did not affect litter cover. Unlike our findings, Naeth et al. (1991) found that in Canadian grasslands, heavy grazing intensity reduced litter and organic matter content.

Management Implications

It is important for managers to understand the grazing patterns within their pastures to improve grazing uniformity and avoid perpetual overgrazing on preferred areas which can reduce site specific rangeland health. Differences in grazing intensity caused by non-uniform grazing in properly stocked season-long grazed pastures can have

both positive and negative influences on different rangeland plant species. An increase of grazing disturbance can increase bare ground and decrease basal vegetation cover, which was observed on interdunes, but not on slopes. Increased grazing intensity influenced species composition and increased the frequency of western ragweed, a species associated with reduced rangeland health, at both slope and interdune sample sites. Other species generally associated with areas receiving high grazing pressure were observed more frequently at the high grazing intensity sites compared to the low grazing intensity samples sites. This data can be used by producers to better understand how increases in grazing intensity can increase or decrease the presence of plant species and ground cover within the same pasture. A better understanding of the influence of topography on grazing behavior and of the influence grazing intensity has on species composition and ground cover can improve grazing management.

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Figures

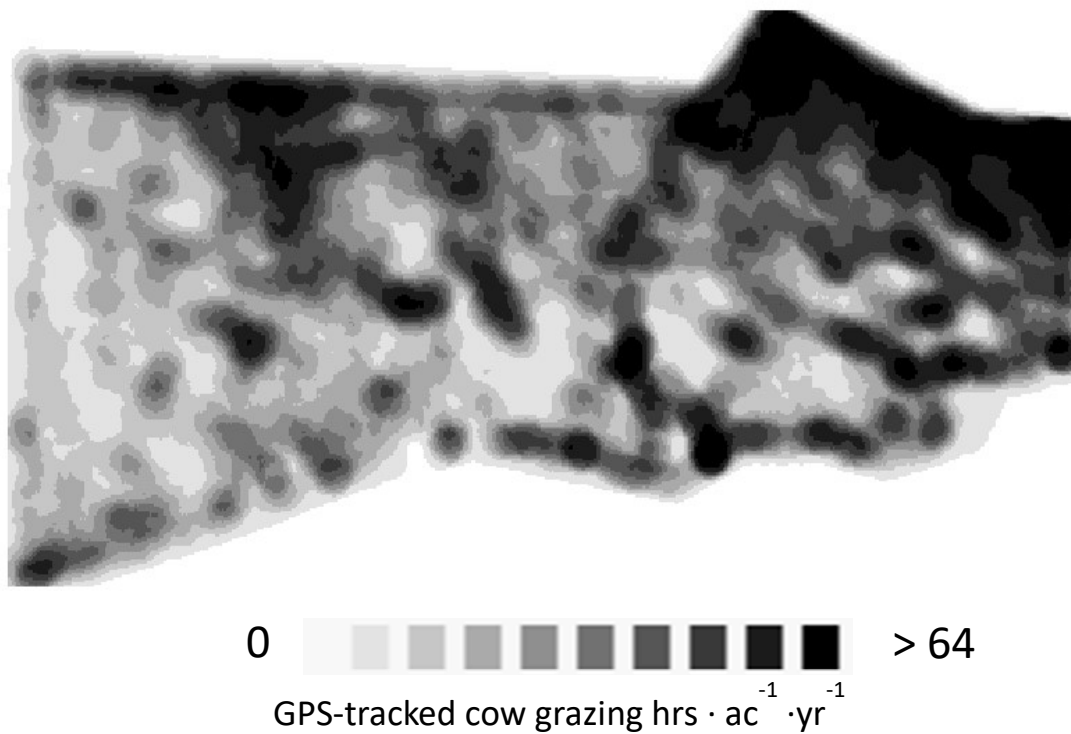


Figure 2.1. A point density map of 12 to 13 GPS-tracked cows grazing on the Hillside pasture (160.26 ha) at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska from 2018 to 2020. High relative grazing intensity areas are shaded by black and low grazing intensity shaded by white.

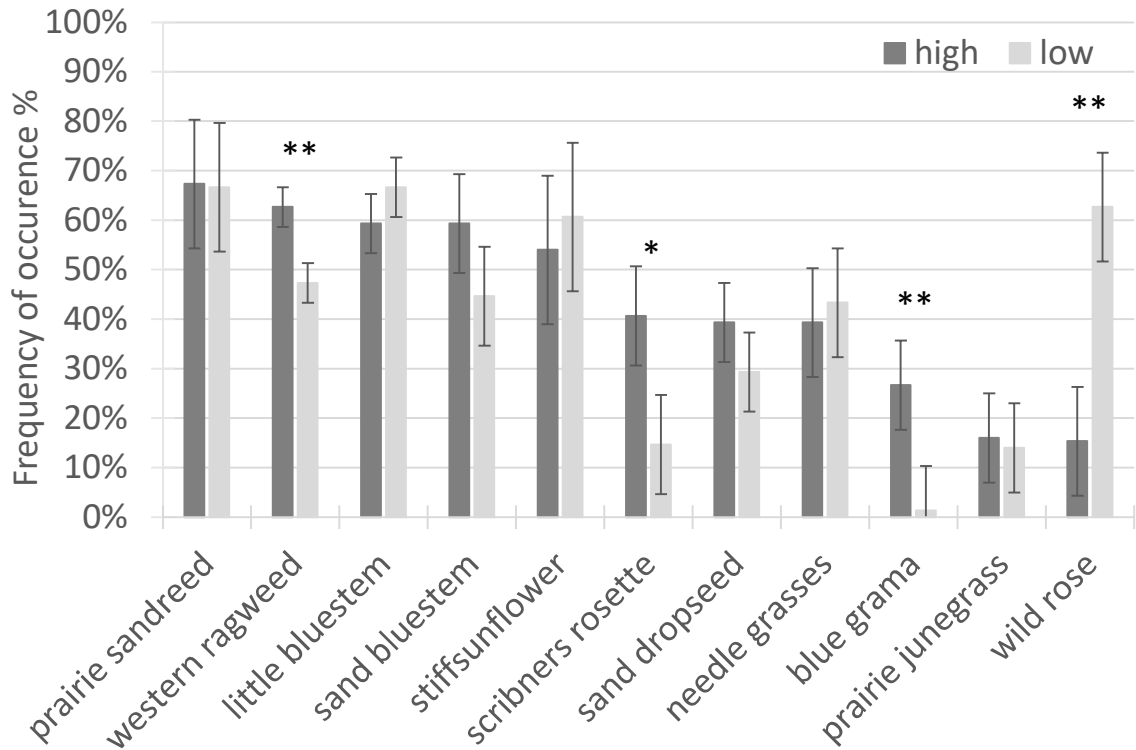


Figure 2.2. Frequency of occurrence (% , \pm SE) of the ten most frequently observed plant species on slope topographic positions grazed at relatively high grazing intensity (high) and low grazing intensity (low) sample sizes based on GPS-tracked cattle data. ** indicates differences at $P \leq 0.05$ and * indicates differences at $P \leq 0.1$ based on least square means simple effects comparison method. Samples were collected in July (2020 and 2021) in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

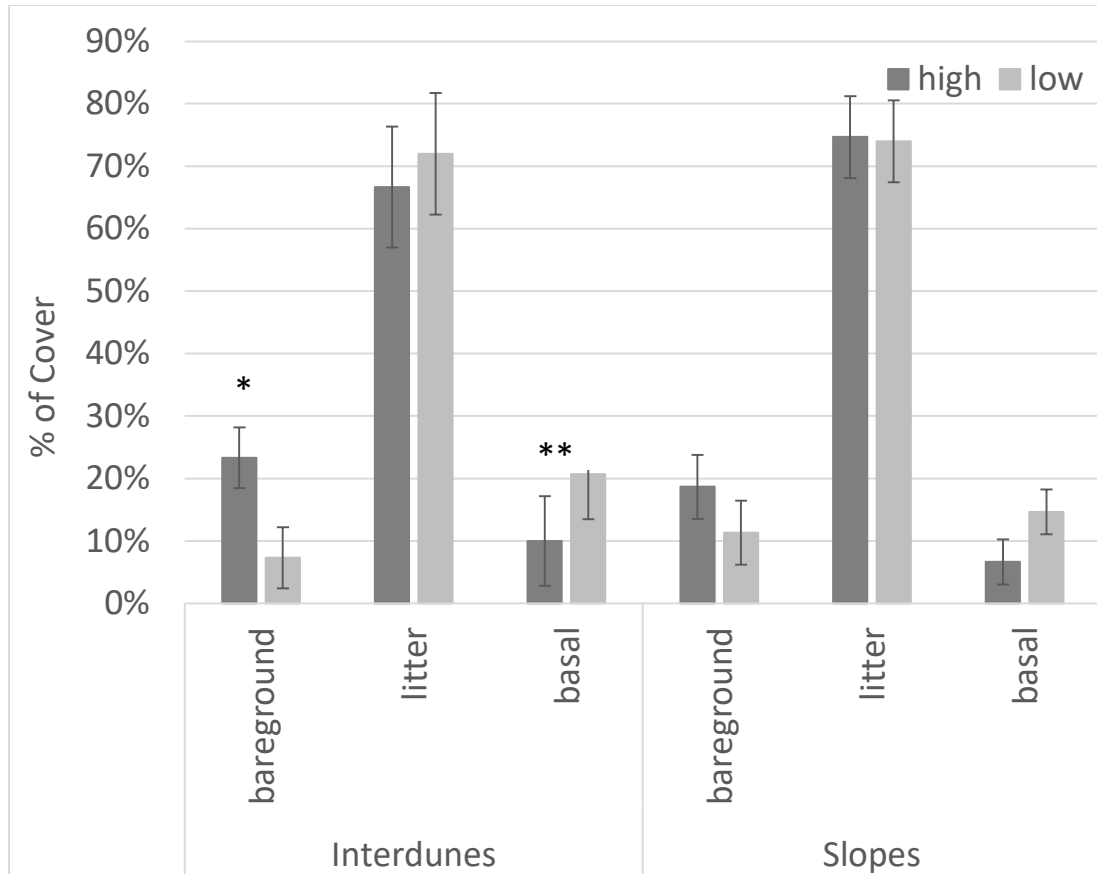


Figure 2.3. Ground cover (% , \pm SE) on high grazing intensity and low grazing intensity sample sites located on interdunes and slopes. ** indicates differences at $P = 0.07$ and * indicates differences at $P = 0.05$ based on least square means simple effects comparison method. Ground cover data were collected in July (2020 and 2021) in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

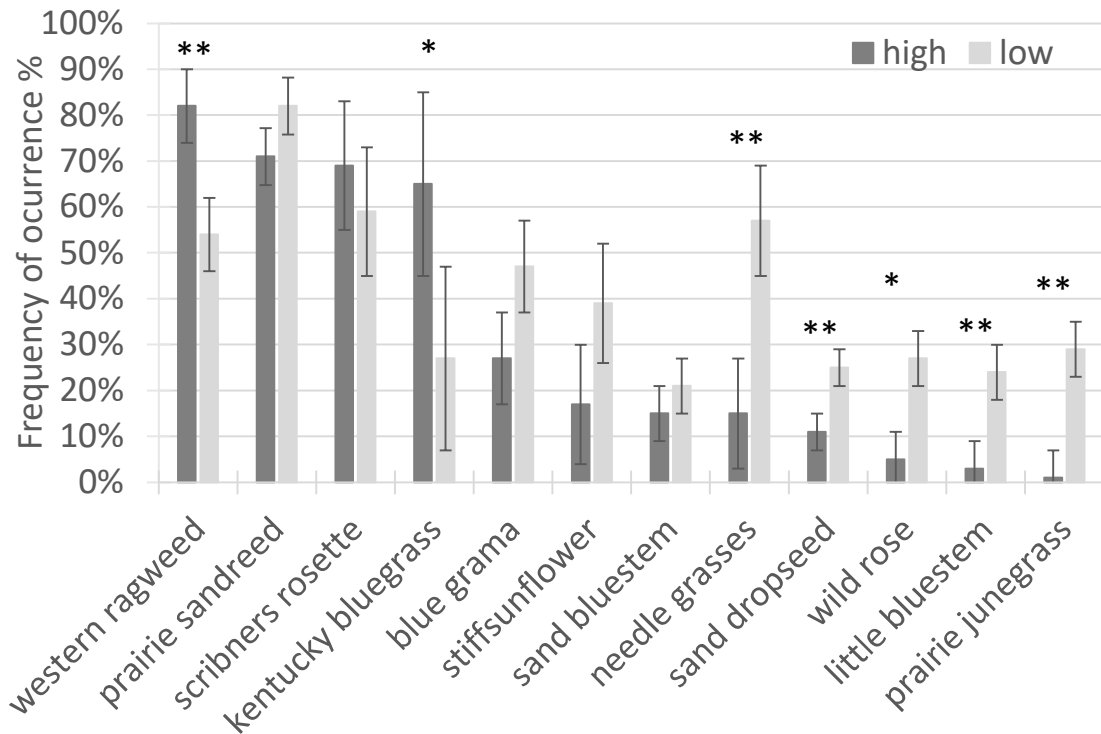


Figure 2.4. Frequency of occurrence % on high grazing intensity and low grazing intensity interdunes. Bars represent frequency of occurrence % means of the respective functional groups averaged across the growing season and years. Standard error bars are \pm SE of least square means. ** indicates differences at $P \leq 0.05$ and * indicates differences at $P \leq 0.1$ based on least square means simple effects comparison method. Frequency of occurrence data were collected in July (2020 and 2021) in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

Chapter 3: Forage quality and cattle diet selection during the growing season in the Nebraska Sandhills

Introduction

Understanding forage quality and diet selection of grazing cattle can help improve grazing management and livestock production. A better understanding of the forage quality can reduce supplementation cost and improve livestock performance (Lardy et al. 2004). Forage quality is directly linked to positive average daily gain (ADG), reproduction success, and milk production for grazing livestock and wild herbivores (Lee et al. 2017). Forage with low digestibility and crude protein has a slower passage rate which decreases intake, weight gain, and reproduction performance in livestock (Soest 1994). Chemical composition and morphology of different plant species influences palatability and nutritive value which influences diet selection, efficiency of rumination, amount of forage consumed, and quantity and quality of milk produced (Givens et al. 2000). Baumont et al. (2000) found that the relationship between nutritive value and palatability influence intake of small ruminants. Welch and Smith (1970) explained that relative intake potential is one of the important variables in forage quality.

There are several methods that can be used to analyze diet selection. However, for our study Fecal DNA barcoding (fDNA) was used as it has evolved as a method to identify plant species when it was discovered that a short chloroplast DNA fragment (P6 loop of the trnL (UAA) intron) can act as a minimalist barcode (Raye et al. 2010). DNA barcoding involves sequencing target DNA and matching it to a database of known plant sequences to identify the taxonomic origin (Valentini et al. 2009; Clare, 2014). This technique has a fast turn around and can generate a more complete list of species found in

the diet with fewer samples compared to other methods of determining diet composition livestock and wildlife (Kohn and Wayne, 1997; Soininen et al. 2009; and Clare, 2014).

Using fDNA, Craine et al. (2016) reported that 45% of cattle dietary protein intake came from grasses in a study evaluating cattle diets across the Great Plains, a lower proportion of the diet than generally expected. Other studies have reported 80 or more percent of the diets of cattle are graminoid species in the Great Plains (Sowers et al. 2019) and western US (Scasta et al. 2016). Scasta et al. (2019) fed heifers known rations of cool-season grass hay, warm-season grass hay, and alfalfa to validate fDNA and found that this technique can misidentify some of the species. Additionally, when feeding cool- and warm-season grasses with high digestible legumes, cool- and warm-season grass contribution was overestimated. The difference in digestibility among forbs/legumes and grasses contribute to some inaccuracy of fDNA results (Scasta et al., 2019). Scasta et al. (2019) recommended 3 ways to reduce errors using fDNA. First, having a reference library of the DNA sequences of the species found in the area/pasture can improve the accuracy of fDNA results. Second, having an understanding that a DNA sequence can be the same or similar for multiple species, therefore interpretation must be compared and crossed checked with other sources/evidence to improve the accuracy of the results. Lastly, verify fDNA results with field-derived plant composition data to assure that the results are accurate. Additionally, being trained to able to identify different species under the same/similar exact DNA sequence is crucial to reduce misinterpretations.

The objectives of this study were to collect forage and fecal samples throughout the growing season to evaluate forage quality and diet selection among plant functional groups and individual plant species for cattle grazing on Sandhills upland rangelands

during the growing season of 2020 and 2021. I hypothesized that forbs and shrubs would have the greatest CP and TDN during the growing season compared to grasses and that cool-season grasses would have greater forage quality early in the growing season, but warm-season grasses would have greater forage quality later in the growing season. Additionally, I hypothesized that diet selection would follow the forage quality curve, with cattle selecting for higher forage quality species throughout the growing season. Lastly, I hypothesized that on average across the growing season, grasses would have a greater contribution to the dietary protein of cattle diet than forbs and shrubs.

Materials and Methods

Study Site

This study was conducted during the 2020 and 2021 growing seasons at the University of Nebraska Gudmundsen Sandhills Laboratory (GSL) located 11 km northeast of Whitman, Nebraska (lat 42°03'34.9"N, long -101°24'52.1"W, elevation 1,068 m). The 30 year average annual precipitation for GSL is 537.2 mm (21.2 inches) (PRISM Climate Group). In 2020 and 2021 the annual precipitation was 402.1 mm (15.8 inches) and 470.9 mm (18.5 inches), respectively (PRISM Climate Group). The Nebraska Sandhills obtains approximately 75% of its yearly precipitation during the growing season (April-September) (Wilhite & Hubbard, 1990; Volesky et al. 2005). The Nebraska Sandhills are comprised of sand dunes and interdunes that support mixed grass plant communities (Volesky et al. 2005; Stephenson et al. 2019). The Nebraska Sandhills is a unique ecosystem that consists mostly of warm season grasses (60-90%), but also include cool-season grasses, forbs, and shrubs (10-40%) Volesky et al. (2005). The study area was dominated by warm-season grasses (little bluestem (*Schizachyrium scoparium*

[(Michx.) Nash]) and prairie sandreed (*Calamovilfa longifolia* [(Hook.) Hack])), cool-season grasses Scribner's rosette grass (*Dichanthelium scribnerianum* [(Hitch. & Chase) Gould]), and forb and shrub species (stiff sunflower (*Helianthus pauciflorus* Nutt.) and wild rose (*Rosa arkansana* Porter)). Other warm-season grasses found in the study site were sand bluestem (*Andropogon hallii* [(Hack.) Wipff]), switchgrass (*Panicum virgatum* L.), sand dropseed (*Sporobolus cryptandrus* [(Hook.) E.K. Jones & Fasset]), blue grama (*Bouteloua gracilis* [(Kunth.) Lag. Ex Steud]). Additionally, other cool-season grasses at the study site prairie junegrass (*Koeleria macrantha* [(Vasey ex Burt Davy) Roy L. Taylor & MacBryde]), western wheatgrass (*Pascopyrum smithii* [(Rydb.) Barkworth & D.R. Dewey]), sedges (*Carex* spp.), and Kentucky bluegrass (*Poa pratensis* L.) (see chapter II).

Study Site Selection Within the Hillside Pasture

The study was conducted in the Hillside pasture at the GSL which consists of 160 ha (396 acres) of upland Sandhills rangeland. Historically, the hillside pasture has been grazed by cows and calves at moderate stocking rates (mean stocking rate 2009 to 2020 = 0.54 AUMs per acre \pm 0.18 SE) season-long during the growing season.

To evaluate forage quality of different plant species, samples of five cool-season grasses, four warm-season grasses, one forb, and two shrubs were collected throughout the pasture (Table. 3.1). Forage samples were collected every 7-15 days from late-May to early-August. Forage samples were generally collected within 2 or 3 days of the same date in each year and mean collection dates ranged from May 24th to August 9th in both years. Each forage sample was clipped from 10-15 different plants per species at approximately 2.54 cm above ground level equaling approximately 10-15 grams of

current year growth of stems, leaves, and flowers for each species. Plant samples were placed into a paper sack, dried at 60° C for 48 hours, and mailed to Ward Labs (Kearney, Nebraska) for analysis. At the lab, samples were ground to 1 mm and analyzed using a wet chemistry analysis. The data obtained included crude protein (CP) and total digestible nutrient (TDN) on a dry matter basis so that all forages could be equally compared.

Fecal samples were collected from 7 to 8 cows that grazed the study site from early-June to late July. Fecal samples were collected every 10-20 days with mean collection period being every 15 days. Fecal samples were generally collected within a 2 to 3 days of the same date in each year and mean collection dates ranged from June 4th to July 21st in both years. The fecal samples were collected directly from each cow when they were processed through a chute. Following collection, fecal samples were frozen in a freezer at -12.3° C until they were processed for analysis. At processing, fecal samples were thawed in a walk-in cooler for 12-16 hrs at the University of Nebraska Lincoln Panhandle Research Experimental Center in Scottsbluff, Nebraska. A small sample of the fecal material for each cow was placed in barcoded vial provided by Jonah Ventures Lab (Boulder, Colorado). Once fecal samples were transferred to the vials, the vials were refroze and sent to Jonah Ventures Lab for analysis. Jonah Ventures Lab conducted fecal DNA metabarcoding analysis to obtain species present in the fecal material and the relative diet composition of species found in the fecal sample (See Craine et al. 2016 for a description of DNA barcoding analysis). Using a list of known species found in the study site along with ESV codes provided by the lab, species found in the diet were identified. The data were organized by matching ESV codes with species found in the

study pasture (See chapter II). Fecal samples were collected in the same study area and on similar dates as the forage quality samples to better analyze the influence of forage quality throughout the growing season on diet composition and to have a better understanding of the diet selection of cattle throughout the growing season with a low stocking rate.

Statistical Analysis

All data were analyzed using the proc glimmix statement in SAS 9.4 (Cary, North Carolina, USA). The forage quality data were analyzed using a repeated measure analysis of variance (ANOVA) with plant functional groups (cool-season grasses, warm-season grasses, and forb /shrub) and sample date as fixed effects. Year was treated as a random variable. Effects for the tests were considered significant at a P-value of 0.05, while tests with P-values between 0.05 and 0.10 were considered trending significant. The data were statically analyzed by functional group to obtain the CP and TDN per collection date throughout the growing season to analyze the influence of forage quality on diet selection.

The fDNA data were analyzed using a similar repeated measure ANOVA test. Plant functional group and sample date were fixed effects and year was treated as the random variable. Cow was treated as the individual experimental unit. Effects for the tests were considered significant at a P-value of 0.05. The data were statically analyzed by functional group to obtain the percentage of each functional group that contributed to the dietary protein per collection date throughout the growing season to analyze how diet selection changes throughout the growing season.

Results

Forage Quality

When averaged across the growing season, CP of forbs/ shrubs was 3.3 and 2.9 percentage points greater than cool-season grasses and warm-season grasses, respectively (Fig. 3.1 and 3.2). There were no significant differences between cool-season grasses and warm season grasses for CP or TDN and no date*plant functional group interactions (Fig. 3.1 and 3.2). For all functional groups, CP and TDN decreased as the growing season progressed ($p < 0.01$) (Fig. 3.1 and 3.2).

When comparing individual cool-season grasses, Scribner's rosette grass and western wheatgrass tended to be greater in CP and TDN and hold onto their forage nutritive value later in the growing season compared to needle grasses, prairie junegrass, and Kentucky bluegrass (Fig. 3.3 and 3.4). Scribner's rosette grass and western wheatgrass raised mean cool-season grass CP by 1.9 percentage points and TDN by 3.2 percentage points. When comparing cool-season grasses without Scribner's rosette grass and western wheatgrass with the warm-season grasses, forage quality was greater in the warm-season grasses (9.6% CP, 57.4% TDN) than the cool-season grasses (7.3% CP and 53.7% TDN). Therefore, if these two species were removed from the cool-season grass functional group in the analysis, cool-season grasses had a lower average CP during the growing season compared to warm-season grasses and forb/shrubs (Fig. 3.5 and 3.6). For warm-season grasses, all species had similar CP and TDN at all dates during the growing season (Fig. 3.7 and 3.8). Individual forbs and shrubs had variable CP and TDN through the growing season (Fig. 3.9 and 3.10). For all individual species, CP and TDN was

greater at the beginning of the growing season (May) than at the end of the growing season (August).

Diet Composition

The number of species found in the diets that contributed to at least 0.5% of the dietary protein was 28 species in 2020, which contributed to 92.6% of the total dietary protein, and 24 species in 2021, which contributed to 95.5% of the total dietary protein (Fig. 3.11). Cool-season grasses contributed significantly ($P < 0.01$) more to the cattle diets compared to other functional groups throughout the whole growing season (Fig. 3.12). Forbs contributed significantly ($P < 0.01$) more than shrubs and warm-season grasses contributed the least to the dietary protein in the diet selection. There were seven species that contributed a majority of the cattle diets (i.e., 55% and 63% of cattle diets in 2020 and 2021, respectively). Six out of those seven species were the same in both years of the study. The top six species that contributed to the dietary protein in both 2020 and 2021 were Scribner's rosette grass, Kentucky bluegrass, needle grasses, prairie sandreed, and wild rose (Fig. 3.13 and 3.14).

Discussion

Forage Quality

Forage quality (CP and TDN) varied among functional groups. CP and TDN were greater in forbs and shrubs than all grasses (cool-season and warm-season). Lyons et al. (1996) found similar results with Texas range nutritional quality tending to be highest in actively growing forbs, with shrubs intermediate, and grasses the lowest. Our results did not find a significant difference in CP and TDN in cool-season grasses versus warm-

season grasses when all cool-season species were included in the analysis. However, other studies have found a difference in forage quality among cool-season grasses and warm-season grasses because of their growing season pattern. Lardy et al. (2004) found that there was a time of the growing season effect on forage quality among cool-season grasses and warm-season grasses. Additionally, Nichols et al. (1993) found that forage quality of warm-season vs. cool-season grasses on Sandhills subirrigated meadows were different due to a difference in seasonal growth trends. Cool-season grasses tend to be greater in crude protein content and digestibility than warm-season grasses because warm season grasses tend to have greater fiber content to withstand wilting associated with high temperatures during their growing stage (Lyons et al. 1996).

In our study, there was no significant difference between cool-season and warm season grasses when analyzed across the whole collection period/growing season (May-August). However, when Scribner's rosette grass and western wheatgrass (two species who had greater forage quality later in the growing season) were removed from the analysis, cool-season grass forage quality was lower than warm-season grass forage quality. Species selection may have an important contribution to declines in forage quality between cool- and warm-season grasses as the growing season progresses. While western wheatgrass is not as common as Scribner's rosette grass in the study pasture, both of these greater quality cool-season species are present and available for cattle to consume. While Western wheatgrass was not especially common in the diet, Scribners rosette grass was an important species that appeared in the diet composition using fDNA.

All functional groups had the greatest forage quality (CP and TDN) at the beginning of the growing season (May) and the lowest forage quality at the end of the

growing season (August). Powell et al. (1982) found that Nebraska Sandhill range CP in May was 16% but in November it was only 6.2%. Our data would support Judy et al. (2015) and Lee et al. (2017) findings that forage nutritive value decreases with increasing plant maturity which reduces CP concentration and increases fiber content. Scasta (2017) found that the cool season grasses (*Pascopyrum smithii*, and *Agropyron cristatum*) had greater crude protein peak early in the growing season while warm season shortgrasses (*Bouteloua gracilis*) peaked in crude protein later in the growing season. Lyons et al. (1996) found that pastures with less standing dead would have greater forage quality than pastures with more standing dead material because this plant material had less CP and TDN.

When we visualized individual species within functional groups (cool-season grasses and forbs/shrubs) results suggest that some species had a greater CP and TDN. Additionally, among the forbs and shrubs, lead plant seemed to have a greater CP, but wild rose seemed to have a greater TDN. However, none of the warm-season grasses had significant greater or lower quality than the rest of the warm-season grasses. Therefore, there was more variability among individual cool-season grasses and forbs/shrubs than there was among warm-season grasses. This data suggests that within a functional group species forage quality may vary. More years of data collection is needed to fully analyze forage quality at the species level to develop a better understanding of individual species forage quality variability.

Diet Composition

The data from our study suggest that cattle obtain most of their dietary protein from cool-season grasses (43.6%) and forbs (29.1%). Shrubs (13.01%) and warm-season grasses (3.47%) accounted for a smaller portion of the dietary protein when averaged across the growing season. Craine et. al. (2016) reported that 45% of cattle dietary protein intake came from grasses in a study evaluating cattle diets across the Great Plains. Craine (2021) reported that bison obtained 38.2% of their dietary protein from all grasses. Volesky et al. (2005) found that most of cattle diet composition collected from esophageal fistulated cows came from cool-season grasses early in the growing season (April to late-may). In their study cool-season grasses contributed 74% of the diet composition of cattle grazing Sandhills rangelands. However, the contribution of grasses to the diet of cattle in our study was much lower than other studies (Cromsigt et al. 2017; Mphinyane et al. 2015; Sowers et al. 2019). Other studies have reported 80 or more percent of the diets of cattle are graminoid species in the Great Plains (Sowers et al. 2019) and western US (Scasta et al. 2016).

The data in our study suggest that 3.47% of the dietary protein of cattle grazing in the Hillside pasture came from warm-season grasses. The low amount of warm-season plant species in the cattle diets in our study was unexpected, but not outside what other studies have suggested. Craine (2021) reported that Bison obtained 11.5% of their dietary protein from warm-season grasses. Additionally, Craine (2021) found that warm-season grass dietary protein intake for bison peaked in September (16.2 %) and was lowest in July (10.2 %). However, Northup (1993) found yearling cattle grazing on small (1 ha) pastures consisting of dunes and slopes in the Sandhills selected mostly warm-

season grasses in their diet throughout the growing season. The low amount of warm-season grasses observed in the diet of cattle in our study compared to other studies in the Sandhills may be the result of cattle having access to more ecological sites, topographic positions, and species at the larger pasture scale compared to small study pastures. Cattle used in our study also had access to interdunes and lowlands which tend to have greater amounts of cool season species such as Kentucky bluegrass (see Orozco 2021 chapter 2) and utilization was observed on these species throughout the growing season.

Additionally, moderate stocking rates, low stock density, and continuous grazing during the summer would allow cattle to be more selective. Cattle grazing at lower stocking rates are more selective in their diets on Sandhills rangelands compared to cattle grazing at greater stocking rates (Judy et al. 2015).

Our study suggest that forbs contributed 29.1% of the dietary protein of cattle grazing in the Sandhills. Our results would agree with Craine (2021) who found that in bison, forbs contributed 20.9-27.7% of the June dietary protein and 16.7-21.3% of the September dietary protein in both years. Sowers et al. (2019) found that in the summer, forbs contribute 33% of the dietary protein of sheep grazing in the Kansas Flinty Hills. Additionally, Scasta et al. (2016) found that forbs contributed 9-21% of the dietary protein of cattle year-round. However, Mphinyane et al. (2015) evaluated cattle, sheep, and goats diets that grazed communal rangelands in the Central District of Botswana using microhistology. They found that forbs contributed to 2%, 5%, and 2% of the diet composition of cattle, sheep, and goats, respectively. However, the difference in forb contribution to the diet could have resulted from the difference in diet analysis methods.

Greater CP content may explain why forbs and shrubs contributed more to the dietary protein in the fDNA for cattle diets. However, the high contribution of forbs and shrubs to the dietary protein of the diet could be a lab error as fDNA can overestimate forb content due to their greater protein content. Scasta et al. (2019) used known diets to validate the fDNA method. Their data found that fDNA can over- or under-estimate the contribution of a given functional group. Additionally, their data showed that species with similar ESV's can be misidentified. King and Schoenecker (2019) analyzed fecal samples of feral horses using DNA barcoding and microhistology and found that DNA barcoding could overestimate forb content due to the high protein content of forbs while microhistology underestimates forb content due to forbs being more digestible. More validation is needed to the fDNA method to improve this method accuracy and validate results of species that are showing up in greater amounts in the cattle diets.

Our data indicated that 10.7% of the dietary protein in the diet came from other species, or species that were not identifiable or species such as ponderosa pine, maple tree, etc. that are not found in nor nearby the study area. Additionally, there were small contributors that had multiple species options, so they were classified in the other category. Our results would validate Scasta et al. (2019) and Garnick et al. (2018) findings that to decrease error it is important for fDNA results to be verified using plant composition data from the site of the data collection. Although more validation is needed, the results found in our study can help improve grazing management and livestock performance by informing managers the main species being consumed by cattle grazing sandhills rangelands.

Our results suggest that cattle grazing in the Sandhills would obtain most (52%) of their dietary protein from Scribner's rosette grass, Kentucky bluegrass, needle grasses, prairie sandreed, stiff sunflower, and wild rose in both years data were collected. Visual observations made while collecting the species composition data showed that Kentucky bluegrass was highly utilized in the interdunes of the study pasture. because the Kentucky bluegrass was grazed throughout the growing season, it was kept short which allowed it to have a greater leaf to stem ratio. Studies have shown that grazing can influence forage quality and likely cattle selectivity. Michunas et al. (1995) found that nitrogen concentration and digestibility increase with defoliation in light grazed areas. Additionally, Clark et al. (2000) found that Idaho fescue crude protein was 1.3 percentage points greater in grazed plots than in ungrazed plots. This could result in a greater forage quality in plants that were grazed vs. plants that weren't grazed. Therefore, Kentucky bluegrass forage quality may be greater in the grazed interdune areas and more highly selected for by the cattle.

Management Implications

A better understanding of the Nebraska Sandhills forage quality of individual species and functional group and cattle diet selection throughout the growing season can improve grazing management and livestock production. Meeting cattle nutritional requirements is important to improve livestock performance and production. Our data found that forbs and shrubs have greater CP and TDN than cool-season grasses and warm-season grasses throughout the growing season. We did not find a significant difference in quality of cool-season grasses and warm-season grasses when all species were included in the analyses. However, a few cool-season species (i.e., Scribner's

rosette grass and western wheatgrass) maintained greater forage quality throughout the growing season compared to the other cool-season grasses. Therefore, when Scribner's rosette grass and western wheatgrass were excluded from the data, warm-season grasses had significantly higher CP than cool-season grasses. Additionally, our data suggests that all functional groups had greater CP and TDN in the May (beginning of the growing season) than in August (end of the growing season). Fecal DNA barcoding data showed that cattle grazing on Sandhills range in our study consumed most of their dietary protein from cool-season grasses (43.6%) and that warm-season grasses (3.47%) typically did not provide a large portion of the diet. More validation for the fDNA method is needed to confirm these results under different management scenarios in the Sandhills before strong conclusions can be made. Additionally, more validation is needed to provide a comparison of fDNA results to other techniques that can be used to improve the understanding of how forage quality and diet selection changes throughout the growing season in the Sandhills. Our results provide key insight into species that could be monitored to avoid overgrazing and highlights important differences that could help in development of grazing management plans to target and manage species that are being less utilized.

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Tables and Figures

Table 3.1. Forage quality samples were collected in Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska. Samples were collected from 5 cool-season grasses (needle grass, Scribner’s rosette grass, Kentucky bluegrass, prairie junegrass, and western wheatgrass), 4 warm-season grasses (little bluestem, sand bluestem, prairie sandreed, and switch grass, 1 forb (stiff sunflower), and 2 shrubs (lead plant and wild rose) from May 24th – August 9th every 7-15 days in 2020 and 2021.

Functional Group	Common Name	Scientific Name
Cool-season grass	needle grasses	<i>Hesperisotpa comata</i> or <i>spartea</i>
	Scribner’s rosette grass	<i>Dichantheium scribnerianum</i>
	Kentucky bluegrass	<i>Poa pratensis</i>
	prairie junegrass	<i>Koeleria macrantha</i>
	western wheatgrass	<i>Pascopyrum smithii</i>
Warm-season grass	little bluestem	<i>Schizachyrium scoparium</i>
	sand bluestem	<i>Andropogon hallii</i>
	prairie sandreed	<i>Calamovilfa longifolia</i>
	switchgrass	<i>Panicum virgatum</i>
Forb	stiff sunflower	<i>Helianthus pauciflorus</i>
Shrub	lead plant	<i>Amorpha canescens</i>
	wild rose	<i>Rosa arkansana</i>

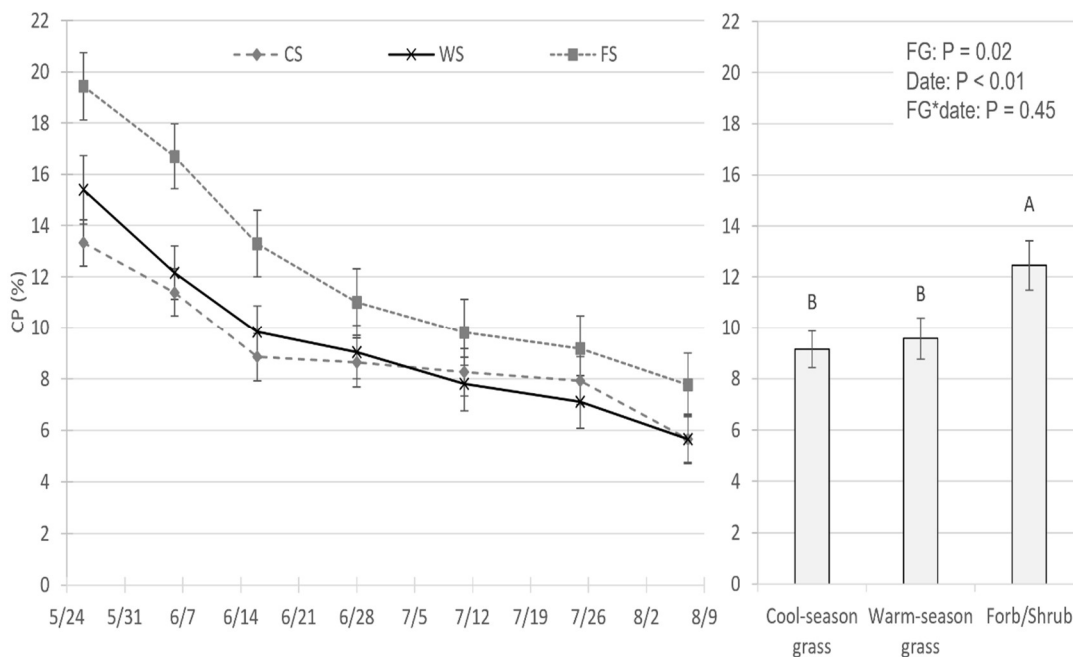


Figure 3.1. Crude Protein % (CP%) of cool-season grasses (CS), warm-season grasses (WS), and forb and shrubs (FS). Bars represent CP% means of the respective functional groups averaged across the growing season and years. Standard error bars are ± 1 SE of least square means. Different letters across grazing intensities indicates differences at $P \leq 0.05$ based on least square means simple effects comparison method. Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

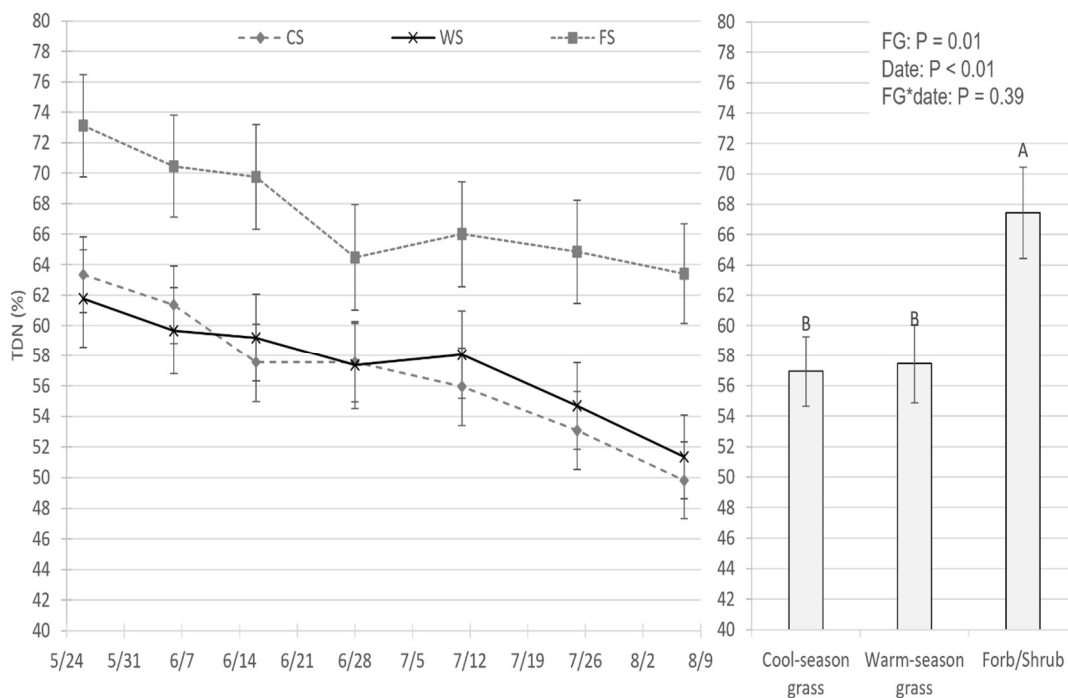


Figure 3.2. Total digestible nutrients % (TDN %) of cool-season grasses (CS), warm-season grasses (WS), and forb and shrubs (FS). Bars represent TDN% means of the respective functional groups averaged across the growing season and years. Standard error bars are ± 1 SE of least square means. Different letters across grazing intensities indicates differences at $P \leq 0.05$ based on least square means simple effects comparison method. Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

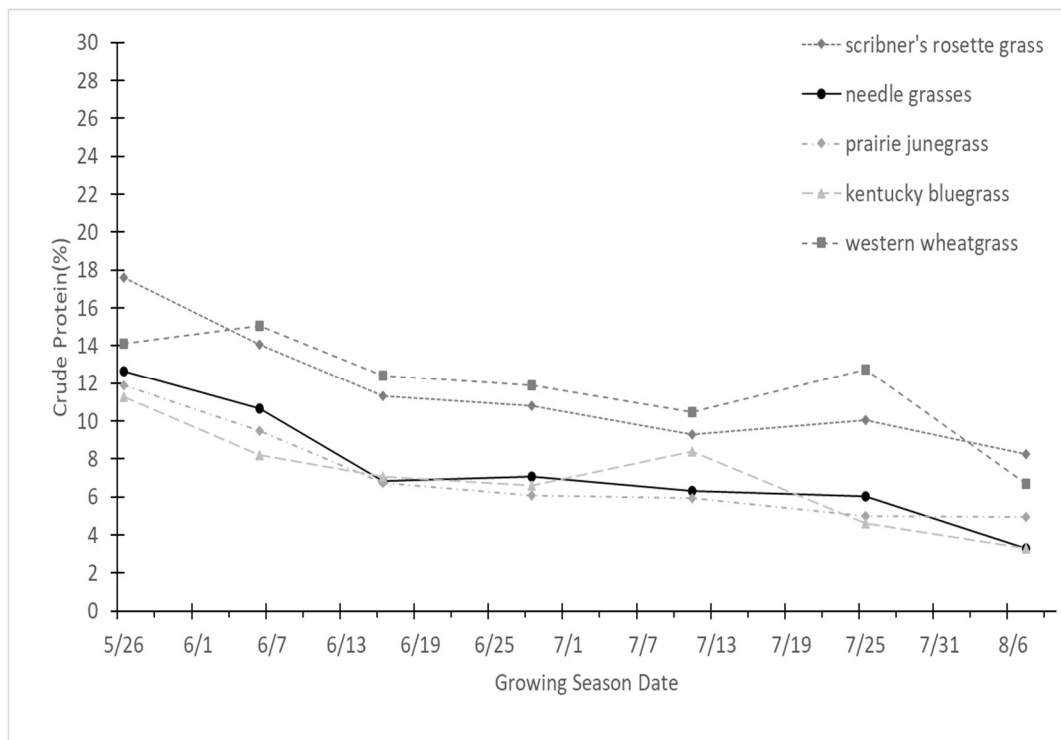


Figure 3.3. Crude Protein % (CP%) of cool-season grasses (Scriber's rosette grass, needle grass, prairie junegrass, Kentucky bluegrass, and western wheatgrass). Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

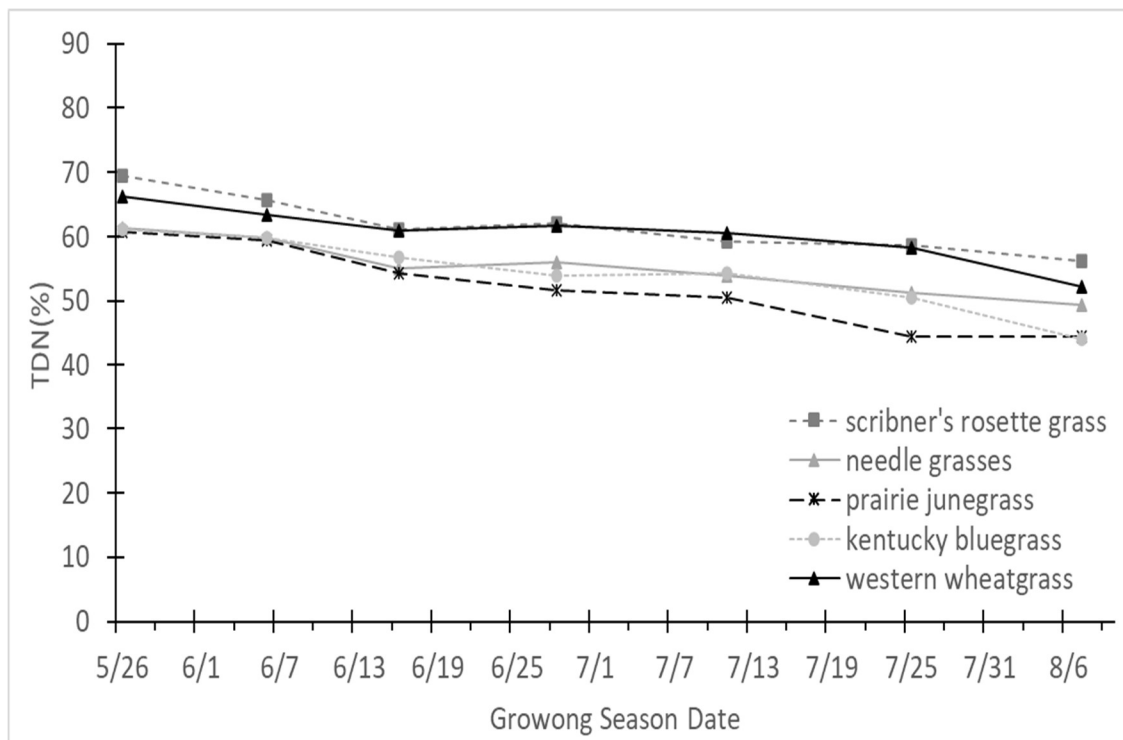


Figure 3.4. Total Digestible Nutrients % (TDN%) of cool-season grasses (Scriber's rosette grass, needle grass, prairie junegrass, Kentucky bluegrass, and western wheatgrass). Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

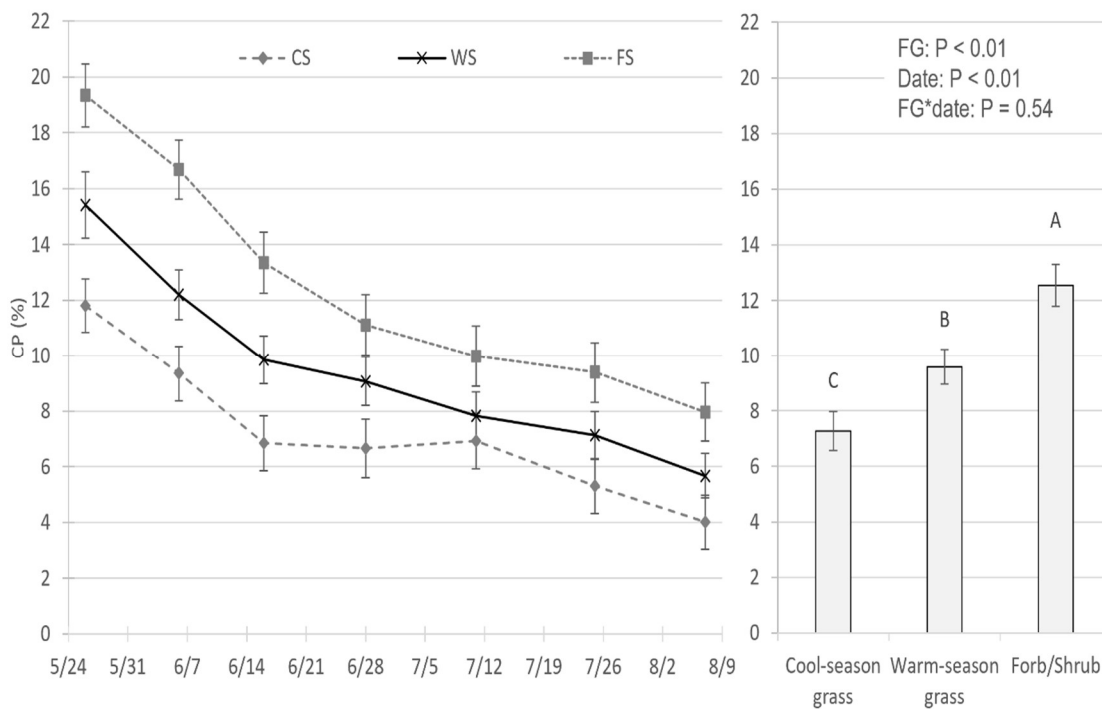


Figure 3.5. Crude Protein % (CP%) of cool-season grasses (CS) (excluding Scribner's rosette grass and western wheatgrass), warm-season grasses (WS), and forb and shrubs (FS). Bars represent CP% means of the respective functional groups averaged across the growing season and years. Standard error bars are ± 1 SE of least square means.

Different letters across grazing intensities indicates differences at $P \leq 0.05$ based on least square means simple effects comparison method. Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

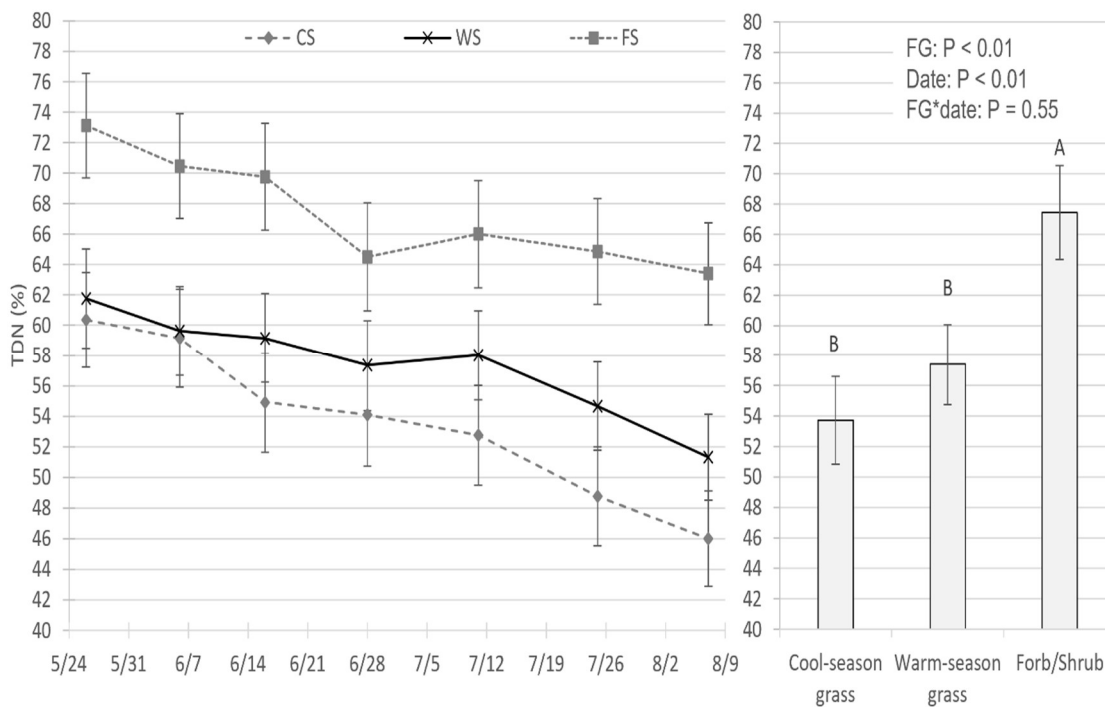


Figure 3.6. Total Digestible Nutrients % (TDN%) of cool-season grasses (CS) (excluding Scribner's rosette grass and western wheatgrass), warm-season grasses (WS), and forb and shrubs (FS). Bars represent CP% means of the respective functional groups averaged across the growing season and years. Standard error bars are ± 1 SE of least square means. Different letters across grazing intensities indicates differences at $P \leq 0.05$ based on least square means simple effects comparison method. Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

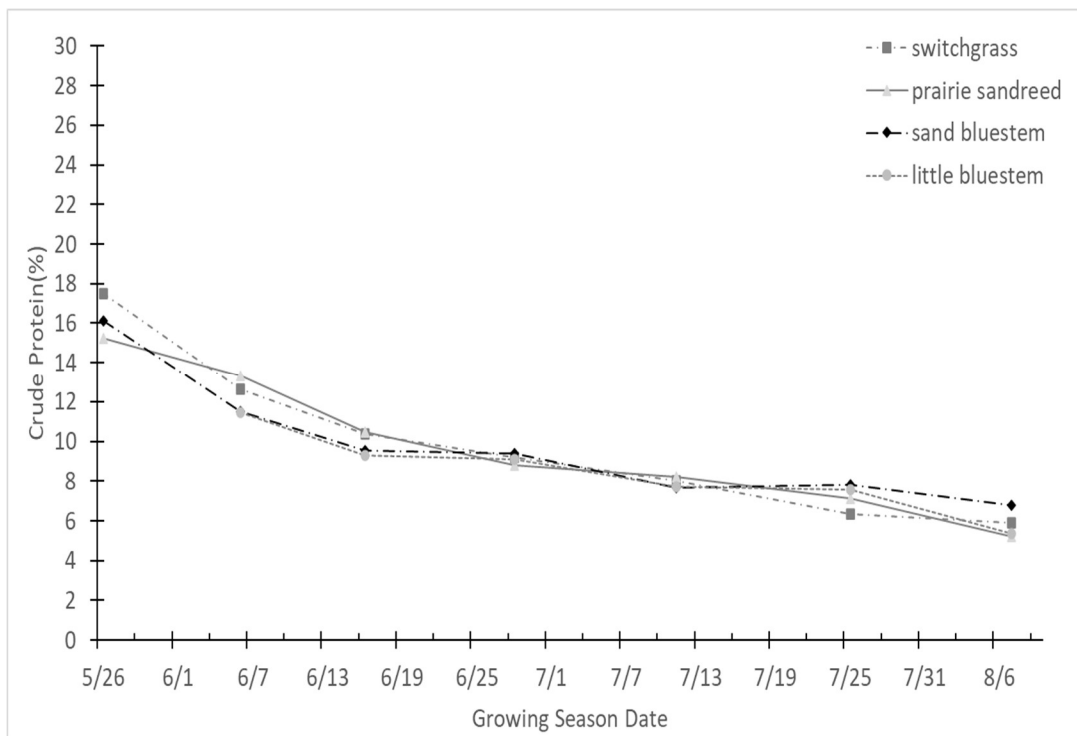


Figure 3.7. Crude Protein % (CP%) of warm-season grasses (switchgrass, prairie sandreed, sand bluestem, and little bluestem). Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

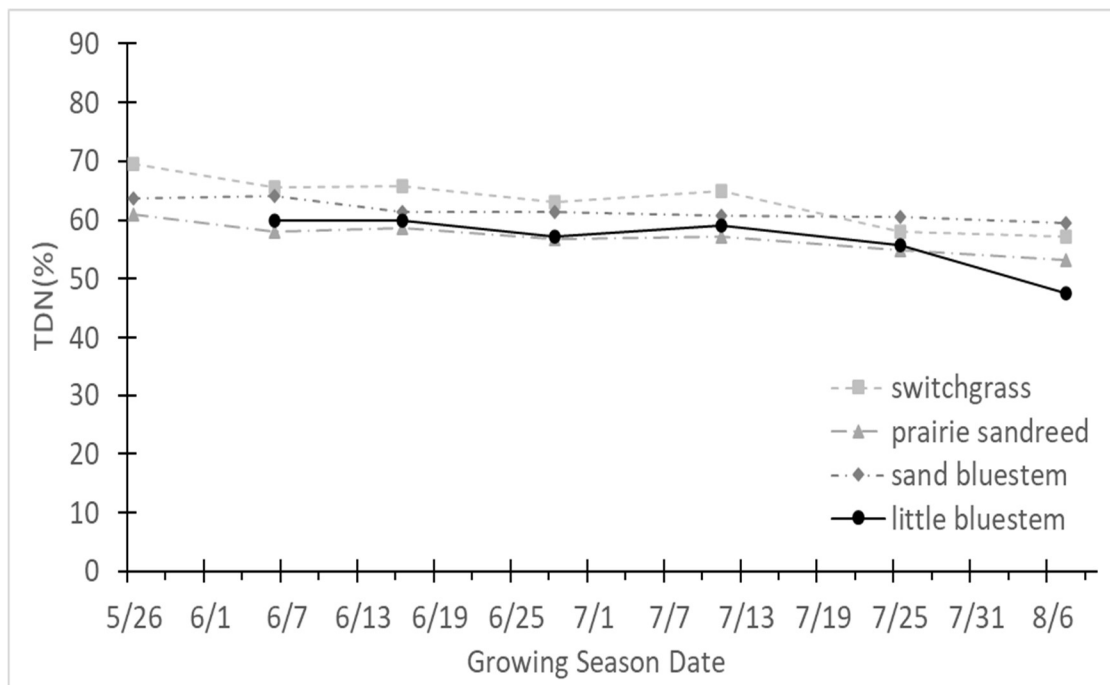


Figure 3.8. Total Digestible Nutrients % (TDN%) of warm-season grasses (switchgrass, prairie sandreed, sand bluestem, and little bluestem). Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

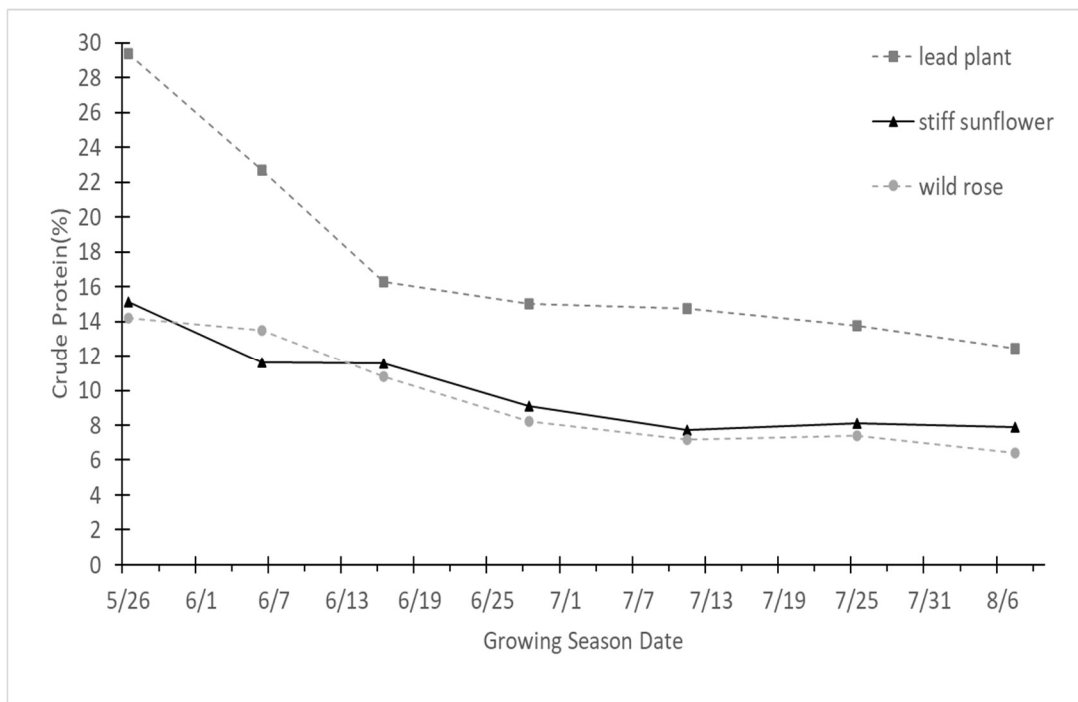


Figure 3.9. Crude Protein % (CP%) of forb and shrubs (stiff sunflower, lead plant, wild rose). Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

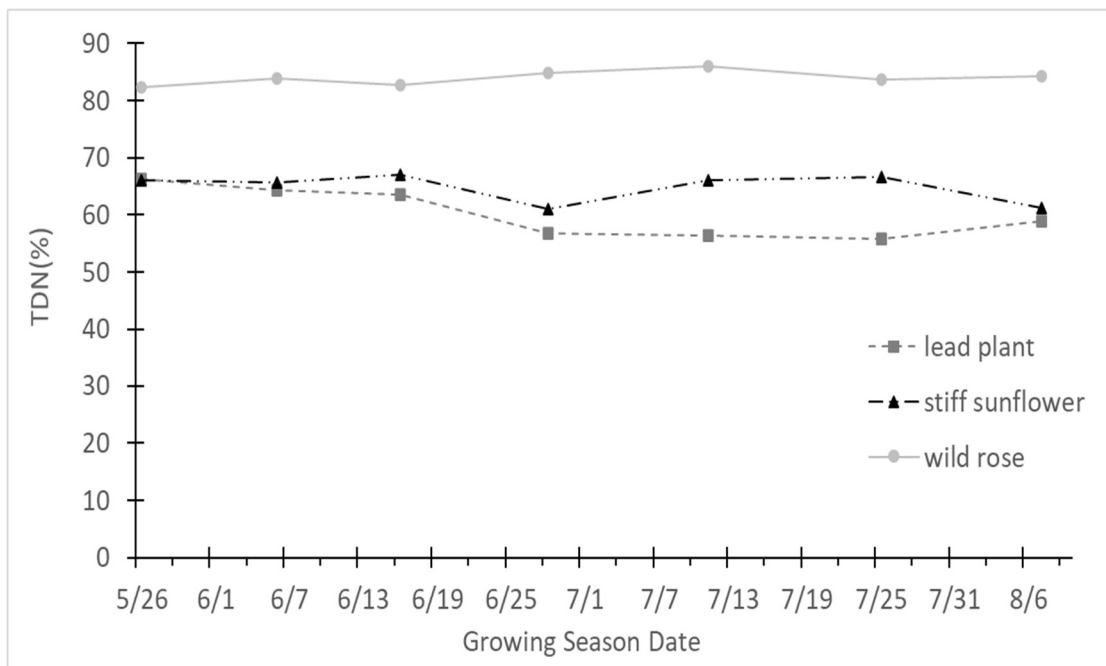


Figure 3.10. Total Digestible Nutrients (TDN%) of forb and shrubs (stiff sunflower, lead plant, wild rose). Samples were collected every 7-15 days from late- May to mid-August in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

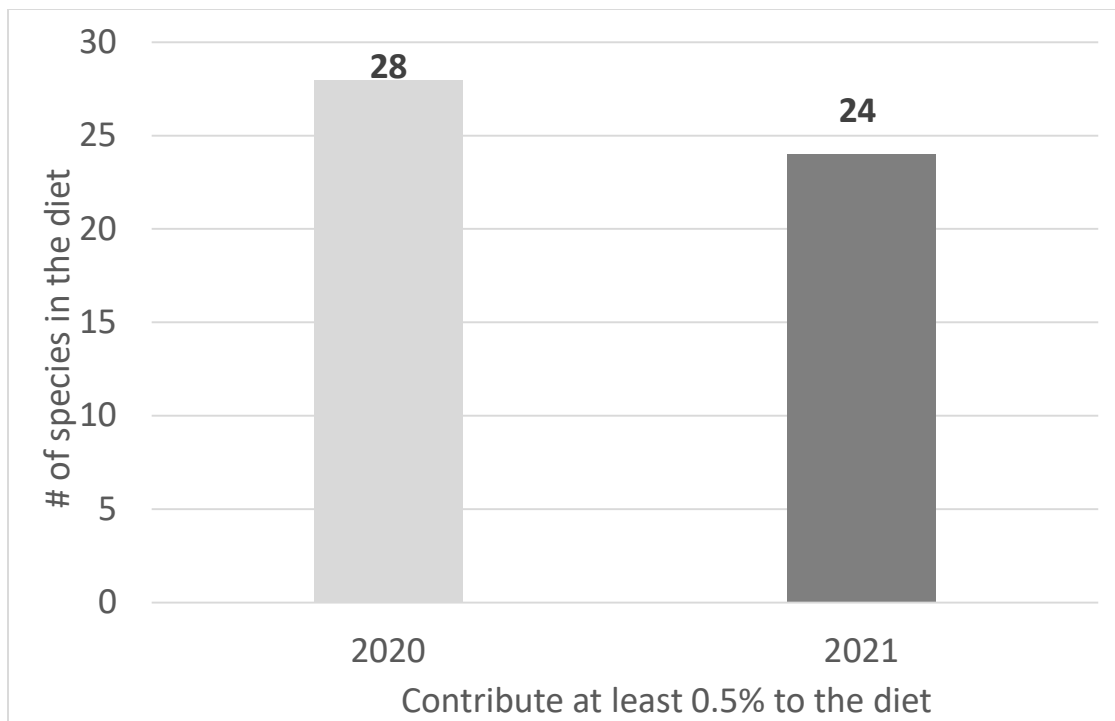


Figure 3.11. Number of species that contributed to at least 0.5% of the dietary protein in 2020 and 2021. In 2020, there were 28 species that contributed to 92.6% of their dietary protein. In 2021, there were 24 species that contributed to 95.5% of their dietary protein. The fecal samples analyzed were collected in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

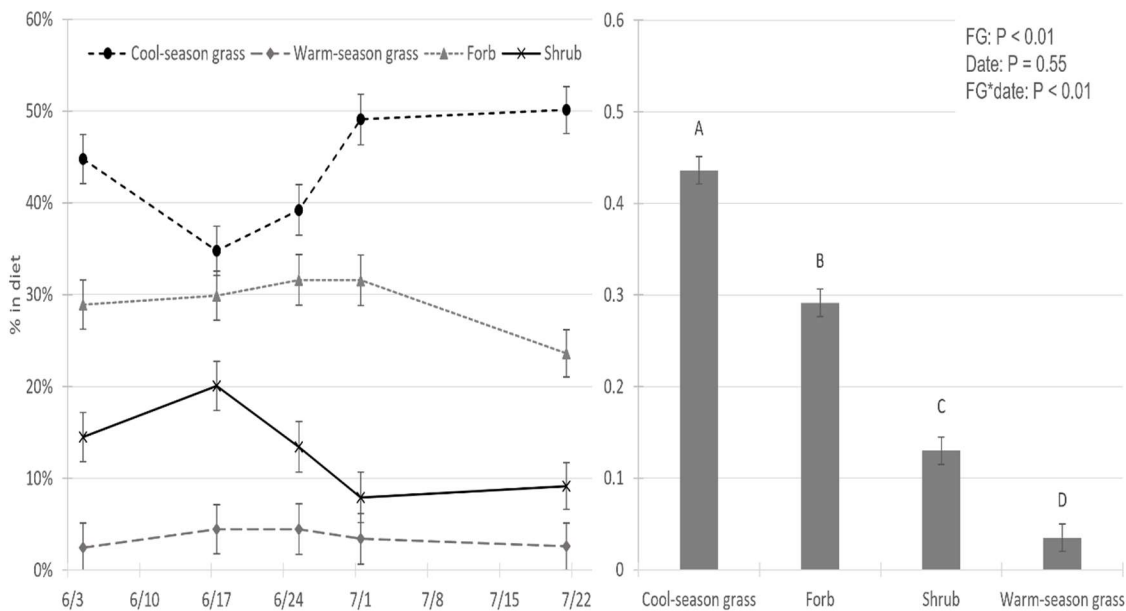


Figure 3.12. Diet selection of different functional groups (% in diet). Bars represent dietary protein % in cattle diets. The means of the respective functional groups were averaged across the growing season and years. Standard error bars are ± 1 SE of least square means. Different letters across grazing intensities indicates differences at $P \leq 0.05$ based on least square means simple effects comparison method. Samples were collected every 10-20 days from early-June to late-July in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

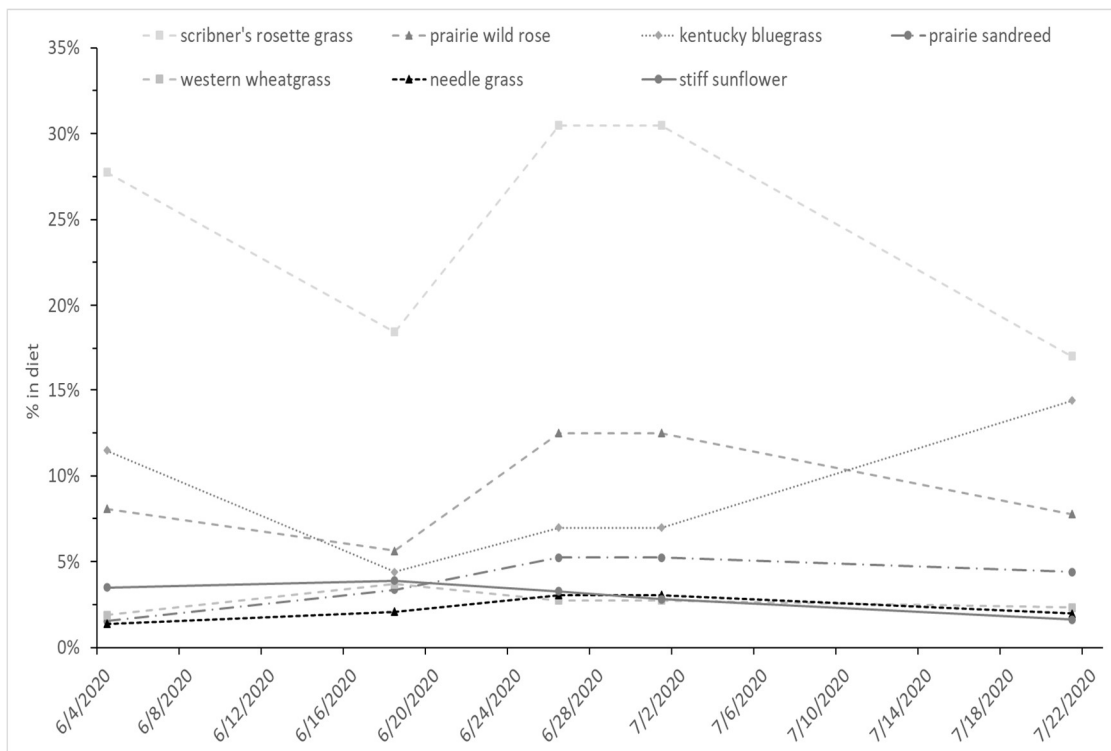


Figure 3.13. Top 7 species contributing to dietary protein % in cattle diet composition in 2020. Fecal samples were collected every 10-20 days from early-June to late-July in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.

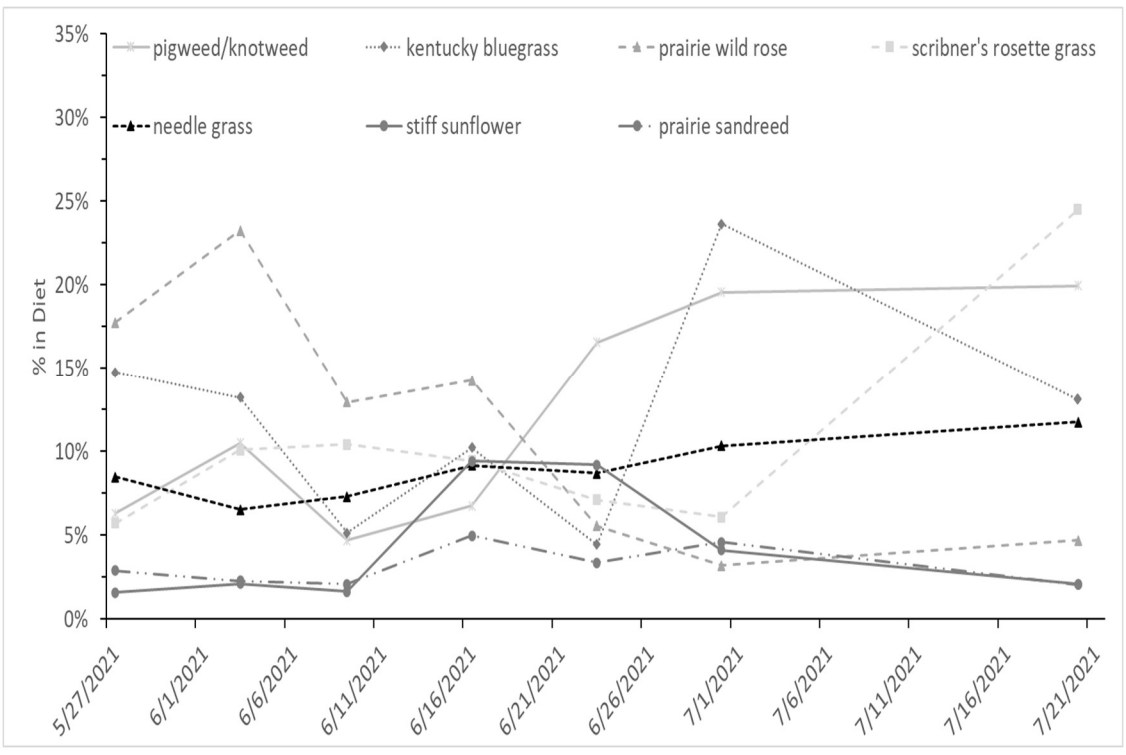


Figure 3.14. Top 7 species contributing to dietary protein % in cattle diet composition in 2021. Fecal samples were collected every 10-20 days from early-June to late-July in the Hillside pasture at University of Nebraska Lincoln Gudmundsen Sandhills Laboratory in Whitman, Nebraska.