

Influence of stocking rate and advancing season on forage intake, digestibility, and ruminal fermentation in steers supplemented with dried distillers grains with solubles while grazing northern Great Plains rangelands¹

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ABSTRACT: The objectives of this study were to evaluate the effects of stocking rate and advancing season on diet chemical composition, intake, digestibility, and ruminal fermentation in steers supplemented with distillers grains with solubles [0.3% of body weight (BW)] while grazing northern Great Plains rangelands. Angus cross beef steers ($n = 188$; 320 ± 30.3 kg initial BW) were used to establish targeted stocking rates, and 12 ruminal cannulated steers (272 ± 20.0 kg initial BW) were used for diet sampling while cograzing with the noncannulated animals on 12 pastures ($n = 3$ per treatment). Stocking rates were set to target 65%, 50%, 35%, and 20% of an average annual above-ground biomass remaining at the end of the grazing season (May–September). Five 10-d collection periods were conducted for May 13–22, June 10–19, July 8–17, August 5–14, and September 2–11. There was no difference in steer BWs or average daily gain during any of the collection periods or between stocking rate ($P \geq 0.10$). Organic matter (OM), neutral detergent fiber, and acid detergent fiber of forage masticate samples were not affected ($P \geq 0.25$) by stocking rate. Crude protein, and all

N fractions of forage masticates also did not differ between stocking rate treatments ($P \geq 0.18$). Forage OM intake (grams per kilogram of BW) increased cubically across the entire grazing season ($P = 0.05$). Organic matter digestibility decreased quadratically ($P < 0.01$) from May to September. Neutral detergent fiber digestibility showed a cubic effect ($P < 0.01$) across the grazing season, increasing from May to June, then decreasing till September. Crude protein digestibility decreased linearly ($P < 0.01$) as the season advanced. Ruminal ammonia and volatile fatty acid (VFA) concentrations were affected by stocking rate \times period interactions ($P \leq 0.02$). Ruminal pH, ammonia, and VFA concentrations were not affected by the stocking rate ($P > 0.13$) but were impacted by the advancing season ($P < 0.01$). Ruminal pH increased quadratically ($P \leq 0.01$) with advancing season (6.3 to 6.6 ± 0.05 from May to September, respectively). The results of this study demonstrate that intake, fermentation, and digestibility of northern Great Plains forages were influenced more by seasonal factors associated with forage maturity than stocking rate under the conditions of this study.

Key words: advancing season, beef cattle, dried distillers grains with solubles, grazing, intake

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INTRODUCTION

The mixed-grass prairies of northern Great Plains are dominated by cool-season grasses,

including nonnative grasses (Toledo et al., 2014), often resulting in greater nutrient quality of forage in the spring and fall of the grazing season. Dietary chemical compositions of grazed forage when coupled with forage intake and digestion are important factors impacting livestock production in rangeland-based cattle production systems. As forage matures, dietary crude protein (CP), digestibility, and intake often decline, while dietary fiber usually increases (Olson et al., 1994b; Johnson et al., 1998; Cline et al., 2009, 2010). Furthermore, as the stocking rate increases, forage quantity becomes limiting, resulting in decreased diet quality or nutrient intake (Cook et al., 1953; Pieper et al., 1959; Bryant et al., 1970) often negatively impacting animal performance (Aiken, 2016). Modifying cattle stocking rates on pasture is a common management tool used to achieve long-term goals of optimizing forage use, livestock production, and agroecosystem sustainability (Hart et al., 1988; Biondini et al., 1998; Derner et al., 2008).

Supplementing grazing livestock may improve forage utilization and help in meeting livestock production goals (Bohnert and Stephenson, 2016). Supplementation of dried distillers grains with solubles (DDGS) to yearling beef calves is one way to offset the potential decline in forage quality, replace portions of forage, and improve average daily gain (ADG; McDonald et al., 2007) with no negative impacts on subsequent feedlot performance (Larson et al., 2019). Information regarding the impact of stocking rate on forage masticate nutrient composition, intake, and digestion by cattle grazing mixed-grass prairie when supplemented with DDGS is lacking. Therefore, the objectives of this study were to evaluate the effects of stocking rate and advancing season on diet chemical composition, intake, digestibility, and ruminal fermentation in steers supplemented with DDGS while grazing northern Great Plains rangelands.

MATERIALS AND METHODS

This study was approved by the North Dakota State University Institutional Care and Use Committee prior to the initiation of the project (Project # A15071).

Study Area

The grazing trial was conducted approximately 18 km northwest of Streeter, ND, at the Central

Grasslands Research Extension Center located on the Missouri Coteau in south central North Dakota. The study site had been divided into 12 pastures of approximately 12.9 ha each in 1989. Cattle grazed from May 15 to September 11, 2015. Total precipitation from May 1 to September 30, 2015 equaled 35.8 cm, which is 7.0% (2.5 cm) more than the 64-yr average (North Dakota Agricultural Weather Network, 2015). During the month of May, this area received 55.5% (8.2 cm) of rainfall above the 64-yr average, while June received 13.5% (1.4 cm) above the 64-yr average rainfall. July, August, and September received less precipitation [28.0% (2.1 cm), 52.6% (3.0 cm), and 44.4% (2.0 cm), respectively, of the 64-yr average rainfall (North Dakota Agricultural Weather Network, 2015)]. The average temperature between May and September 2015 was 17.9 °C, which was 1.7° above the 64-yr average (North Dakota Agricultural Weather Network, 2015).

The botanical compositions of plant communities at the study site were reported by Patton and Nyren (2014a) and include the grasses Kentucky bluegrass (*Poa pratensis* L.), western wheatgrass (*Pascopyrum smithii* A.), sun sedge (*Carex inops*), green needlegrass (*Nassella viridula*), obtuse sedge (*Carex obtusata* Lilj.), and blue grama (*Bouteloua gracilis*). Patton and Nyren (2014a) also reported that the common forbs in the study area included heath aster (*Symphyotrichum ericoides*), common dandelion (*Taraxacum officinale*), and western yarrow (*Achillea millefolium* L.), while Western snowberry (*Symphoricarpos occidentalis*) was the only common shrub. Forage production and utilization were determined by clipping 0.25-m² plots in a paired plot cage comparison method, which is further described by Patton et al. (2007). Long-term peak biomass production has most recently been reported to average 4,454, 4,178, 3,792, and 2,912 ± 273 kg/ha for light, moderate, heavy, and extreme stocking rate pastures, respectively, across both ecological sites evaluated (Patton and Nyren, 2015). For more detail about plant community dynamics or forage production on these pastures, the reader is referred to the peer-reviewed articles by Biondini et al. (1998), Patton et al. (2007), and Limb et al. (2018).

Animals and Stocking Rate

Angus cross beef steers [$n = 188$; 320 ± 30.3 kg initial body weight (BW)] were used to establish targeted grazing pressures, and 12 ruminal cannulated

steers (272 ± 20.0 kg initial BW) were used for diet sampling while cograzing with the noncannulated steers. A single cannulated steer was placed into each of the 12 treatment pastures ($n = 3$ per treatment). Stocking rates were set to target 65% (light, LT), 50% (moderate, MOD), 35% (heavy, HVY), and 20% (extreme, EXT) of an average annual above-ground biomass remaining at the end of the grazing season. Stocking rates were previously established as part of a long-term grazing intensity study (Biondini et al., 1998; Patton et al., 2007; Patton and Nyren, 2015). Cannulated steers were assigned to pastures at random, each treatment having three pastures with an average stocking rate of 0.61, 0.34, 0.22, and 0.17 ha/animal unit month for the LT, MOD, HVY, and EXT treatments, respectively. All animals were removed at the end of the grazing season when 6 of the 12 total pastures reached targeted forage utilization.

During the trial, all steers had free access to water and trace mineral salt blocks (95.5–98.5% salt, 3,500 mg/kg zinc, 2,000 mg/kg iron, 1,800 mg/kg manganese, 280–420 mg/kg copper, 100 mg/kg iodine, and 60 mg/kg cobalt; American Stockman Hi-Salt with EDDI; North American Salt Company, Overland Park, KS). Steers were fed DDGS daily at sunrise at 0.3% of their BW. Cannulated steers were individually fed their respective DDGS supplements during study periods. All animals were weighed on two consecutive days at the beginning and conclusion of the study, with intermediate single-day weights collected every 28 d to determine gains as the grazing season progressed and to adjust the amount of DDGS fed. All steers were implanted with Revalor-G (40 mg of trenbolone acetate and 8 mg of estradiol; Intervet Inc., Millsboro, DE) 1 d before being turned out on pasture.

Sampling Periods

Five, 10-day collection periods were conducted from May 13 to 22, June 10 to 19, July 8 to 17, August 5 to 14, and September 2 to 11. Sampling periods began with the collection of diet samples on day 0. At sunrise, the cannulated steers were restrained and subjected to total ruminal evacuation. Ruminal digesta was physically removed from each cannulated steer and the rumen was then double rinsed with water to assure the complete removal of contents. Steers were then allowed to graze on their assigned pastures for 30–45 min. Steers were regathered and ruminal masticate samples were collected. If more masticates were required, grazing

and masticate collection procedures were repeated. Once sampling collections were complete, original ruminal contents were returned to each steer. All masticate samples were composited within animals, placed immediately on ice, and immediately transported to North Dakota State University Department of Animal Sciences where they were dried to a constant weight in a forced-air oven (55 °C; The Grieve Corporation, Round Lake, IL) and ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 2-mm screen.

Dacron bags (Ankom, Fairport, NY; 10 × 20 cm; 53 ± 10 µm pore size) containing approximately 5 g of dried ground masticate were placed in the rumen in reverse extraction order and incubated ruminally for 72, 48, 36, 24, 12, 8, 4, and 0 h beginning at 1900 hours on day 5 of each collection period. All bags were heat sealed and ruminally suspended in large unanchored, nylon mesh bags (38 × 46 cm) with a nylon zipper. Blank bags were used to correct for the influx of dry matter (DM), neutral detergent fiber (NDF), and CP. Bags were removed on 0 h at 1900 hours of day 8 of each collection period. Zero-hour bags were submersed intraruminally for approximately 3 s and removed with all other bags at 0 h. All bags were individually rinsed with tap water until the rinse water was clear. Bags, then, were dried to a constant weight in a forced-air oven (55 °C) for 24 h, desiccated, weighed, and stored.

In situ data were used to determine rates of DM, NDF (Mertens and Loften, 1980), and CP (Ørskov and McDonald, 1979; NRC, 1985; Johnson et al., 1998) degradation. The model used for CP calculates a rapidly degraded (solubilized) fraction (fraction A) and a slowly degraded fraction (fraction B). Rates derived from this model were calculated on fraction B protein, which was the slowly degraded fraction (NRC, 1985). Calculated rates were then statistically analyzed as described below.

Chromic oxide (Cr_2O_3) was used to estimate fecal output (FO). Chromic oxide (8 ± 0.02 g) was weighed into gelatin capsules and stored until intraruminally dosed on days 0–9 of each collection period at 0700 and 1900 hours. Rectal grab samples of feces were taken at 0700, 1100, 1500, and 1900 hours on days 5–9. Fecal samples were composited on an equal volume basis for each animal across sampling time and stored frozen (−20 °C). For analysis, fecal samples were dried in a forced-air oven (55 °C) to a constant weight and ground in a Wiley Mill to pass a 2-mm screen.

Chromic oxide was used to estimate forage intake. Total fecal organic matter (OM) output was

estimated by dividing Cr_2O_3 concentration in feces by the original dose of Cr_2O_3 . Forage FO and total FO were then separated by subtracting the indigestible amount of DDGS from total FO. Total intake was determined by dividing estimated daily fecal OM output by in vitro OM indigestibility (Merchen, 1988). Total intake was then adjusted for DDGS intake to provide estimated forage intake utilizing the known quantity of DDGS intake and DDGS in vitro OM digestibility.

On day 6 of each collection period, each steer was dosed intraruminally with 200 mL of cobalt ethylenediamine tetraacetic acid (Co-EDTA; Uden et al., 1980). Samples of whole ruminal contents were taken before Co-EDTA dosing at -2 h and before feeding DDGS at 0 h. Additional samples were also collected postfeeding DDGS at 4, 8, 12, 16, and 24 h. Samples were strained through four layers of cheesecloth, analyzed immediately for pH with a combination electrode (model 2000, Beckman Instruments Inc., Fullerton, CA), acidified with 1 mL of 7.2 N H_2SO_4 /100 mL of ruminal fluid, placed immediately on ice, and later frozen at -20 °C for future analysis.

Laboratory Analyses

Masticate samples used for chemical composition analyses were lyophilized (Genesis 25LL, Virtis, Gardiner, NY). Dry matter, ash, and CP were determined using AOAC (2010) procedures. Neutral detergent fiber and acid detergent fiber (ADF) of diet samples were determined using Ankom procedures (ANKOM, Macedon, NY). Acid detergent insoluble N (ADIN) was calculated as N remaining in the ADF residue. Soluble N (SN) was extracted with 0.15 M NaCl according to the procedure of Waldo and Goering (1979). In vitro OM digestibility was determined with procedures defined by Tilley and Terry (1963). Masticate forage and inoculum collected from each animal were used for in vitro determinations at a field lab near the study site. In situ samples were analyzed for DM and N (CP) using AOAC (2010) procedures. NDF of in situ sample residues were determined by Goering and Van Soest, (1970) as modified by Ankom Technologies.

After thawing at room temperature, ruminal fluid samples were centrifuged at $20,000 \times g$ for 10 min. Atomic absorption spectroscopy using an acetylene flame was used to analyze the separated liquid for Co (Hart and Polan, 1984). This supernatant fluid was analyzed for ammonia concentration by a phenol-hypochlorite colorimetric

procedure by Broderick and Kang (1980), as well as volatile fatty acid (VFA; Goetsch and Galyean, 1983) by gas chromatography (Hewlett-Packard 5890 Series II, Hewlett-Packard Company, Wilmington, DE) using metaphosphoric acid solution mixed with 2-ethylbutyric acid as an internal standard.

Ruminal fluid passage rate was calculated by regressing the natural log of Co concentration in ruminal fluid on time after Co dosing intraruminally. The rate of passage was then determined using the absolute value of the slope of the line (%/h). Ruminal fluid volume was estimated by dividing the extrapolated Co concentration at time 0 by Co dose. Fluid flow was then calculated by multiplying the dilution rate and volume. Turnover time (h) was calculated as 1 divided by the fractional dilution rate. Outflow from the rumen (L/h) was determined by multiplying volume (L) and dilution rate (%/h).

Statistical Analyses

Chemical composition, intake, in situ digestion rates, and digestibility were analyzed using a repeated-measures mixed-model approach in SAS (SAS Inst. Inc., Cary, NY). Effects for stocking rate, sampling period, and stocking rate \times period interactions were included in the model. Contrasts were used to determine linear, quadratic, and cubic responses across the grazing season (sampling period). Intact steer performance and forage production data were analyzed using the mixed-model approach with pasture serving as the experiment unit and stocking rate included in the model. For ruminal fermentation parameters, sampling time was included in the model. Stocking rate \times sampling period \times sampling time interactions were not observed in ruminal fermentation measurements, and data were averaged across sampling time. *P*-values ≤ 0.05 were considered significantly different.

RESULTS AND DISCUSSION

There were no differences in peak forage production, peak forage remaining, estimated total forage production, or estimated total remaining forage during the 2015 growing season between all stocking rates ($P > 0.16$, data not shown). Previously published data from these same research pastures and treatments have indicated that forage responses to grazing were dependent on range site, soil type, and precipitation (Patton et al., 2007). The higher than normal amount of precipitation (35.8 cm) during the growing season make it highly

possible that forage availability was not limited to livestock at any of the stocking rates examined in this year of the study. However, lower than normal precipitation later in the growing season may have contributed to decreasing forage quality. Given that the growth characteristics of the cool season dominate the plant community of the treatment pastures, the precipitation pattern in this growing season likely impacted the results of this study.

There was no difference in steer BWs or ADG between stocking rates or during any of the collection periods ($P \geq 0.10$; data not shown) during this grazing season. Previous years' data from these same pastures and treatments have shown year to year variation of the impacts of stocking rate on livestock gains; however, long-term averages of ADG indicate that, as the stocking rate increased, livestock gains decreased (Patton and Nyren, 2014b). Supplementing DDGS has been shown to improve grazing livestock ADG (MacDonald et al., 2007; Martinez-Perez et al., 2013; Murillo et al., 2016; Larson et al., 2019). This year was the first in which cattle, within the greater long-term grazing study, were supplemented with DDGS; thus, comparisons between the current year's data and that of previous years should be done cautiously.

Diet Composition Analyses

No stocking rate \times period interactions ($P \geq 0.29$; Table 1) were observed, except for in vitro OM digestibility (IVOMD; $P < 0.01$), which are discussed later in this section. Therefore, the main effect means are reported for stocking rate treatment and grazing period. Organic matter, NDF, and ADF of forage masticate samples were not affected ($P \geq 0.25$) by stocking rate. Crude protein, total N, SN, insoluble N (IN), and ADIN of forage masticates also did not differ between stocking rate treatments ($P \geq 0.18$). Forage availability was likely not limited by stocking rate during the growing season and likely contributed to the lack of differences in forage masticate fiber and nitrogen fraction content between grazing treatments.

Neutral detergent fiber content of masticate increased cubically with advancing season ($P < 0.01$), while ADF concentration of masticate increased ($P < 0.01$) with advancing season. Early season increases followed by summer decreases in forage quality of cool-season grasses have been previously detailed (Lardy et al., 2004). Pastures in the current study were dominated by cool-season grasses; while rainfall early in the growing season was above normal, it is likely that the below-average rainfall

and warm temperatures later in the grazing season increased forage masticate fiber content as a result of increasing plant maturity. Increasing fiber content with advancing season has also been reported by Olson et al. (1994a) for south central North Dakota, Johnson et al. (1998) for western North Dakota, and McCollum et al. (1985) for south central New Mexico. The impacts of supplemental DDGS on selective grazing behaviors of steers is unknown in this study; however, previous research has demonstrated that protein supplementation did not change masticate chemical composition (Caton et al., 1988) of steers grazing dormant New Mexico rangelands.

Nitrogen (% of OM) decreased cubically ($P < 0.01$) as the season advanced. Typically, forage masticate N concentrations decline with increasing forage maturity associated with advancing season. Such was the case in our study and the work of others within the region (Olson, et al., 1994a; Johnson, et al., 1998; Cline et al., 2009). Soluble N decreased ($P = 0.03$) in a quadratic fashion, whereas IN declined cubically ($P < 0.01$). Nitrogen, SN, and IN decreased with advancing grazing season (McCollum et al., 1985; Caton et al., 1993) in New Mexico and North Dakota, respectively. Cline et al. (2009) observed an increase in ADIN with the advancing season in western North Dakota, while, in the present study, ADIN was not impacted by advancing season. Forage species between these studies, as well as precipitation quantity and pattern, were different, possibly resulting in different forage growth patterns potentially explaining the observed differences between the two studies.

For IVOMD, there was a stocking rate \times sampling period ($P < 0.01$). In vitro OM digestibility decreased from May to September ($P < 0.01$; 75.9%, 62.1%, 52.8%, and 47.6% \pm 3.35%, respectively). However, there were no differences ($P = 0.82$) in IVOMD due to the stocking rate. The interaction was largely driven by divergence in IVOMD of the masticate samples from LT (48.1% \pm 5.06%) and HVY (76.0% \pm 5.06%) in July compared to 60.5% and 63.9% \pm 5.06% digestibility of MOD and EXT, respectively. This decline in forage digestibility is representative of advancing plant maturity of cool-season grasses. Decreasing IVOMD of forage masticates with the advancing season in North Dakota has also been reported by (Johnson et al., 1998; Schauer et al., 2004; Cline et al., 2010). The impacts of supplemental DDGS on IVOMD of forage masticates are unknown within the confines of the current study. The lack of a nonsupplemented control in the present study

Table 1. Effects of stocking rate and advancing season on forage masticate chemical composition and IVOMD in steers grazing mixed-grass prairie

Item	Treatment ^a												Period ^b												P-value ^c				Contrasts ^d																			
	LT			MOD			HVI			EXT			SEM ^e			MAY			JUN			JUL			AUG			SEP			SEM ^f			Treat-ment × Period			L			Q			C					
	15	15	15	15	15	15	15	15	15	15	15	15	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02						
<i>n</i>	15	15	15	15	15	15	15	15	15	15	15	15	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02						
OM, %	81.3	80.6	80.6	82.2	80.6	80.6	82.3	82.3	82.3	1.49	1.49	1.49	74.6	74.6	74.6	82.4	82.4	82.4	83.9	83.9	83.9	83.7	83.7	83.7	83.2	83.2	83.2	3.08	3.08	3.08	0.83	0.83	0.83	0.44	0.44	0.44	0.02	0.02	0.02	0.02	0.02	0.02	0.16	0.16	0.16			
% of OM																																																
NDF	67.5	69.6	70.7	65.6	65.6	65.6	65.6	65.6	65.6	2.05	2.05	2.05	58.4	58.4	58.4	69.9	69.9	69.9	67.5	67.5	67.5	70.7	70.7	70.7	75.2	75.2	75.2	3.81	3.81	3.81	0.34	0.34	0.34	0.89	0.89	0.89	<0.01	<0.01	<0.01	0.32	0.32	0.32	0.01	0.01	0.01			
ADF	38.5	40.2	41.5	36.1	36.1	36.1	36.1	36.1	36.1	1.85	1.85	1.85	37.2	37.2	37.2	38.5	38.5	38.5	37.1	37.1	37.1	39.5	39.5	39.5	43.1	43.1	43.1	4.05	4.05	4.05	0.25	0.25	0.25	0.87	0.87	0.87	<0.01	<0.01	<0.01	0.33	0.33	0.33	0.44	0.44	0.44			
CP	18.7	17.7	17.8	19.6	19.6	19.6	19.6	19.6	19.6	0.63	0.63	0.63	29.9	29.9	29.9	18.3	18.3	18.3	16.9	16.9	16.9	14.9	14.9	14.9	12.3	12.3	12.3	0.86	0.86	0.86	0.18	0.18	0.18	0.63	0.63	0.63	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N ^g	2.99	2.84	2.85	3.14	3.14	3.14	3.14	3.14	3.14	0.10	0.10	0.10	4.78	4.78	4.78	2.92	2.92	2.92	2.70	2.70	2.70	2.38	2.38	2.38	1.97	1.97	1.97	0.14	0.14	0.14	0.18	0.18	0.18	0.63	0.63	0.63	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
SN ^h	0.70	0.73	0.69	0.82	0.82	0.82	0.82	0.82	0.82	0.06	0.06	0.06	1.08	1.08	1.08	0.81	0.81	0.81	0.66	0.66	0.66	0.57	0.57	0.57	0.55	0.55	0.55	0.09	0.09	0.09	0.36	0.36	0.36	0.29	0.29	0.29	<0.01	<0.01	<0.01	0.03	0.03	0.03	0.73	0.73	0.73			
IN ⁱ	2.28	2.11	2.16	2.32	2.32	2.32	2.32	2.32	2.32	0.08	0.08	0.08	3.70	3.70	3.70	2.11	2.11	2.11	2.05	2.05	2.05	1.81	1.81	1.81	1.42	1.42	1.42	0.12	0.12	0.12	0.22	0.22	0.22	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
ADIN	0.48	0.42	0.44	0.47	0.47	0.47	0.47	0.47	0.47	0.04	0.04	0.04	0.52	0.52	0.52	0.37	0.37	0.37	0.47	0.47	0.47	0.44	0.44	0.44	0.45	0.45	0.45	0.06	0.06	0.06	0.77	0.77	0.77	0.26	0.26	0.26	0.93	0.93	0.93	0.31	0.31	0.31	0.15	0.15	0.15			
IVOMD	60.4	62.6	63.4	60.6	60.6	60.6	60.6	60.6	60.6	2.62	2.62	2.62	75.9	75.9	75.9	70.3	70.3	70.3	62.1	62.1	62.1	52.8	52.8	52.8	47.6	47.6	47.6	3.35	3.35	3.35	0.82	0.82	0.82	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.97	0.97	0.97	0.14	0.14	0.14			

^aStocking rate treatments were LT = light, MOD = moderate, HVI = heavy, and EXT = extreme.^bGrazing period collections were MAY = May 11–22, JUN = June 10–19, JUL = July 8–17, AUG = August 5–14, and SEP = September 2–11.^cObserved significance level of the *F*-test for treatment effects of items considered significant, *P* < 0.05.^dContrasts for period main effects; L = linear, Q = quadratic, and C = cubic.^eMost conservative SEM values were used, *n* = 15.^fMost conservative SEM values were used, *n* = 12.^gN = % nitrogen.^hSN = % soluble nitrogen.ⁱTN = % insoluble nitrogen.

does not allow for any direct comparisons of the impacts of DDGS on IVOMD of forage masticates. Supplementing DDGS to steers consuming moderate-quality forage improved microbial efficiency (Leupp et al., 2009). However, at the level of DDGS provided in the current study, Leupp et al. (2009) reported nearly identical apparent and true ruminal digestion values compared to non-supplemented steers. Future research focusing on the impacts of DDGS supplementation on forage selectivity under increasing grazing pressure and advancing season would certainly be warranted.

Intake and FO

Forage DM intake (DMI), OM intake (OMI), and FO were unchanged (Table 2; $P \geq 0.31$) across stocking rate treatment, and stocking rate \times seasonal period interactions ($P \geq 0.07$) were observed. Therefore, the main effects of the period will be discussed. As detailed earlier, there were no differences in forage production or estimated forage remaining across the stocking rate in this year of the study. This fact coupled with the supplemental DDGS may explain why forage DMI, OMI, and fecal DM output (FO) were similar across the stocking rates evaluated in this study.

Forage DMI (g/kg of BW) increased from May to June, then remained constant from June to September, resulting in a quadratic increase across the entire grazing season ($P < 0.01$). Forage OMI (g/kg of BW) increased cubically across the entire grazing season ($P = 0.05$). Olson et al. (1994a) also observed an increase in OMI (g/kg BW) from late June to late August. Other researchers in the northern Great Plains observed that OMI (g/kg BW) remained constant across the season (Adams et al., 1987; Silcox, 1991; Hirschfeld, 1992; Cline et al., 2010). Additionally, forage intake has also been observed to decline across the season as forage matures (Johnson et al., 1998; Cline et al., 2009). Typically, intake will peak alongside the early stages of forage growth and will decline steadily the remainder of the season (Kartchner, 1980; Hirschfeld et al., 1996). Forage OMI intake in the current study are similar to other data supplementing DDGS to grazing cattle (McDonald et al., 2007). Total OMI from the current study (20.9 g/kg BW) when compared with the 20.8 g/kg BW (Cline et al., 2009) appear to show that OMI (g/kg BW) was similar assuming that the DDGS intake of 2.2 g/kg (OM basis; average across June–September) supplied in the current study replaced forage OMI on an equal forage:DDGS basis. While this comparison is

highly subjective and influenced by location, time, and class of cattle, the relative relationship between the data warrants continued research.

Fecal output (g/kg of BW) increased quadratically ($P = 0.03$) across the grazing season. Similar observations were reported by other studies (Olson et al., 1994a; Johnson et al., 1998). Fecal output also closely reflected changes in DMI and OMI early in the season but continued to increase later in the season as forage matured. These data also agree with OM digestibility data presented later in this manuscript. The lowest FO values coincided with the highest IVOMD values of grazed diets. This pattern was also observed in other studies in the northern Great Plains (Olson et al., 1994a; Hirschfeld et al., 1996; Johnson et al., 1998) and was also the case in southwestern blue grama rangeland (McCollum and Gaylean, 1985). Increasing the supply of DDGS to grazing livestock did not result in any differences in fecal OM output (Martinez-Perez et al., 2013). Combined with the masticate nutrient content data presented earlier, these data appear to indicate that the advancing season resulted in lower forage digestibility and, subsequently, greater FO as a result of increased plant maturity.

Digestibility

There were no stocking rates or stocking rate \times grazing period interactions with respect to apparent total tract nutrient digestibility values (Table 2; $P \geq 0.21$). Therefore, grazing period main effects are discussed. As discussed earlier, forage availability, selective grazing, and DDGS supplementation likely impacted total tract diet digestibility between stocking rates. Had forage become limiting, we would have anticipated lower forage digestibility with increasing stocking rate; however, this was not the case in the current study.

Organic matter digestibility (% of DM) showed a quadratic decline ($P < 0.01$) from May to September. Organic matter digestibility decreasing with advancing season agrees with a number of studies on North Dakota rangelands (Olson et al., 1994b; Johnson et al., 1998; Cline et al., 2009, 2010). Organic matter digestibility data were initially lower in the present study than that observed in other portions of North Dakota (Caton et al., 1993; Johnson et al., 1998). As the grazing season progressed, our data and that of Caton et al. (1993) became more closely related, while OM digestibility values reported by Johnson et al. (1998) remained higher than those observed

Table 2. Effects of stocking rate and advancing season on DMI (grams) and apparent total tract digestibility in steers grazing mixed-grass prairie^a

Item	Treatment ^b										Period ^c				P-value ^d				Contrasts ^e						
	LT		MOD		HVY		EXT		SEM ^f		MAY	JUN	JUL	AUG	SEP	SEM ^g	Treatment	Period	Treatment	Period	Treatment	Period	L	Q	C
	7,725	7,331	7,747	7,278	451.8	4,546	7,730	7,737	8,863	8,727	347.9	0.82	<0.01	0.17	<0.01	0.06									
Forage intake	21.7	20.2	21.5	21.4	1.1	15.8	23.5	21.6	23.5	21.6	0.9	0.74	<0.01	0.07	<0.01	0.06									
g/kg BW	8,575	8,200	8,601	8,086	471.3	5,262	8,446	8,599	9,804	9,717	357.5	0.82	<0.01	0.21	<0.01	0.09									
Total intake	24.1	22.5	23.9	23.7	1.1	18.3	25.6	24.0	26.0	24.1	0.9	0.74	<0.01	0.07	<0.01	0.09									
g/kg BW	3,129	3,043	3,373	3,052	165.4	1,644	2,76	3,039	3,884	4,419	114.4	0.49	<0.01	0.10	<0.01	0.10									
Forage FO	8.7	8.2	9.3	8.9	0.4	5.7	8.4	8.5	10.3	10.9	0.3	0.31	<0.01	0.08	<0.01	0.13									
g/kg BW	3,383	3,304	3,629	3,294	170.1	1,859	2,976	3,297	4,166	4,715	116.6	0.50	<0.01	0.11	<0.01	0.13									
Total FO	9.4	8.9	10.0	9.6	0.4	6.5	9.0	9.2	11.0	11.7	0.3	0.30	<0.01	0.08	<0.01	0.19									
g/kg BW	6,322	6,099	6,373	6,023	394.5	3,489	6,365	6,488	7,417	7,261	317.0	0.90	<0.01	0.14	<0.01	0.07									
Forage OMI	17.7	16.7	17.6	17.6	1.0	12.1	19.3	18.1	19.6	18.0	0.8	0.87	<0.01	0.25	<0.01	0.05									
g/kg BW	7,107	6,901	7,163	6,770	412.6	4,151	7,028	7,285	8,286	8,176	325.3	0.90	<0.01	0.17	<0.01	0.09									
Total OMI	19.9	18.9	19.9	19.8	1.0	14.4	21.3	20.3	21.9	20.2	0.9	0.87	<0.01	0.26	<0.01	0.09									
g/kg BW	Digestibility, % of OM																								
DM	59.6	58.7	56.7	58.4	1.4	62.3	64.0	61.3	55.9	49.4	1.2	0.55	<0.01	0.21	<0.01	0.28									
OM	60.8	59.9	57.1	60.8	2.0	59.4	65.6	63.1	59.9	51.3	1.6	0.55	<0.01	0.39	<0.01	0.27									
NDF	55.9	57.0	53.7	54.7	2.0	49.3	63.2	59.0	55.3	49.8	1.8	0.69	<0.01	0.63	<0.01	<0.01									
ADF	49.8	48.9	47.1	47.2	2.2	44.9	57.5	48.9	47.2	42.7	1.9	0.78	<0.01	0.65	<0.01	<0.01									
CP	63.6	59.7	62.6	64.4	1.9	75.2	68.1	64.4	60.0	45.2	2.0	0.35	<0.01	0.28	<0.01	0.08									

^aSupplement intake was 0.3% BW (May, 717 g/hd/d average; Jun, 717 g/hd/d average; Jul, 862 g/hd/d average; Aug, 940 g/hd/d average; Sep, 990 g/hd/d average).^bStocking rates treatments were LT = light, MOD = moderate, HVY = heavy, and EXT = extreme.^cGrazing period collections were MAY = May 11–22, JUN = June 10–19, JUL = July 8–17, AUG = August 5–14, and SEP = September 2–11.^dObserved significance level of the *F*-test for treatment effects of items considered significant, *P* < 0.05.^eContrasts for period main effects; L = linear, Q = quadratic, and C = cubic.^fAverage SEM values were used, *n* = 15.^gAverage SEM values were used, *n* = 12.

in the current study. It is likely that plant composition and the presence of warm season and cool-season grasses resulted in the greater values reported by [Johnson et al. \(1998\)](#) when compared to the current study. Organic matter digestibility values of the present study were also higher than those observed in steers supplemented with DDGS in New Mexico ([Martinez-Perez et al., 2013](#)); however, geographic location, plant species, and precipitation need to be considered in this comparison. The addition of DDGS has been shown to improve OM digestibility of lower quality forages. [Leupp et al. \(2009\)](#) showed a 3% improvement in total tract diet OM digestibility with a supplementation of 0.3% DDGS to steers fed brome grass hay. The hay provided by [Leupp et al. \(2009\)](#) was similar in nutrient content to the lowest quality forage (September) in the current study. However, DDGS supplementation (0–0.6% BW) had a less pronounced effect in grazing steers ([Martinez-Perez et al., 2013](#)).

Neutral detergent fiber digestibility (% of OM) showed a cubic effect ($P < 0.01$) across the grazing season, increasing from May to June, then decreasing till September. This was also the case for ADF digestibility (% of OM). Neutral detergent fiber digestibility and ADF digestibility in May was 49.3% and 44.9% OM, respectively. This increase and then decrease may have been due to precipitation late in the season, extending range plant growth and increasing forage digestibility. The greatest digestibility values for NDF and ADF were observed in June, with 63.2% and 57.5%, respectively. Other researchers working with smooth brome pasture also observed the greatest total tract fiber digestibility in June ([Caton et al., 1993](#)). Data from [Caton et al. \(1993\)](#) also showed a decrease from June to July while remaining fairly constant through August. Supplementation of DDGS has been shown to improve NDF digestibility ([Leupp et al., 2009](#); [Martinez-Perez et al., 2013](#)). Data from the current study indicated that NDF digestibility decreased with advancing season regardless of the fact that DDGS was supplemented. This may indicate that DDGS supplied was not sufficient to improve fiber digestibility with declining forage quality as the season advanced.

Crude protein digestibility (% of OM) decreased linearly ($P < 0.01$) as the season advanced. The highest values of CP digestibility were observed in May (75.2 % of OM) with the lowest values of CP digestibility at the end of the grazing season in September (45.2 % of OM). [Johnson et al. \(1998\)](#) also reported a similar decline in CP digestibility

across the season, and [Caton et al. \(1993\)](#) observed a decline in total tract N digestibility throughout the grazing season. Other researchers have suggested that the decline in CP digestibility resulted from increases in ADIN as forages mature ([Caton et al., 1993](#); [Johnson et al., 1998](#)). However, our data do not support this conclusion, but the decline in digestible CP may have been due to declines in SN across the grazing season, in turn, reducing available CP.

Ruminal Fill and Fluid Kinetics

Ruminal fill was not impacted by stocking rate ([Table 3](#); $P = 0.53$); however, ruminal DM fill (g/kg of BW) increased linearly ($P < 0.01$) from May to September (11.3 to 21.8 ± 1.13 g/kg of BW, respectively). Other studies have reported similar increases in ruminal fill with advancing forage maturity ([McCullum and Galyean, 1985](#); [Johnson et al., 1998](#)).

Fluid dilution rate (FDR) was affected by stocking rate \times grazing period interaction ($P < 0.01$). Fluid dilution rate (%/h) changed in a cubic fashion ($P = 0.03$) with advancing forage maturity but was unaltered by the stocking rate ($P = 0.47$). These data do not support previous research in south central North Dakota ([Olson et al., 1994b](#)) and other studies in New Mexico ([McCullum et al., 1985](#); [Funk et al., 1987](#)). Increasing rates of supplemental DDGS has been shown to improve FDR in steers fed moderate-quality forage ([Leupp et al., 2009](#)) and may explain discrepancies between the current and previous studies.

In Situ Disappearance

No stocking rate \times advancing season interactions were present for in situ disappearance data ([Table 3](#); $P \geq 0.13$). Rate of DM disappearance was not affected ($P \geq 0.50$) by stocking rate but decreased quadratically ($P = 0.05$) with advancing season (11.7%, 9.7%, 9.1%, 7.1%, and $8.5\% \pm 0.75\%/h$ for May, June, July, August, and September, respectively). The rate of masticate NDF disappearance was not affected by stocking rate or advancing season ($P \geq 0.18$) and averaged $4.6\% \pm 0.34\%/h$. The rate of masticate CP disappearance was not affected by stocking rate or advancing season ($P \geq 0.17$) and averaged $5.4\% \pm 0.53\%/h$ across both stocking rate and advancing season.

Contrary to the current study, the rate of NDF disappearance decreased with the advancing season ([Johnson et al., 1998](#)) or increased

Table 4. Effects of stocking rate and advancing season on ruminal pH, ammonia, and VFA concentrations in steers grazing mixed-grass prairie

Item	Treatment ^a										Period ^b				P-value ^c				Contrasts ^d											
	LT		MOD		HVY		EXT		SEM ^e		MAY		JUN		JUL		AUG		SEP		SEM ^f		Treatment × Period		L		Q		C	
pH	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	0.07	6.3	6.5	6.5	6.5	6.5	6.6	6.6	6.6	6.6	6.6	0.05	0.99	<0.01	0.18	<0.01	<0.01	<0.01	<0.01	0.79	
Ammonia, mM	5.8	5.7	5.3	7.1	7.1	4.8	6.7	7.1	7.1	0.5	4.8	6.7	7.1	7.1	6.4	4.9	4.9	4.9	4.9	4.9	0.31	0.13	<0.01	0.01	0.94	<0.01	<0.01	0.28		
Total VFA, mM	162.1	159.4	157.7	162.2	162.2	163.2	164.4	145.3	145.3	7.26	163.2	164.4	145.3	152.1	176.8	176.8	176.8	176.8	176.8	4.48	0.96	<0.01	0.02	0.12	<0.01	<0.01	<0.01	<0.01		
Acetate:propionate	2.7	2.7	2.7	2.7	2.7	2.3	2.7	2.9	2.9	0.05	2.3	2.7	2.9	2.9	2.9	2.9	2.9	2.9	2.9	0.03	0.98	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.85		
mol/100 mol																														
Acetate	44.4	44.7	44.9	43.6	43.6	42.2	42.9	42.8	42.8	0.52	42.2	42.9	42.8	47.3	46.8	46.8	46.8	46.8	46.8	0.42	0.38	<0.01	<0.01	<0.01	<0.01	0.10	<0.01	<0.01	<0.01	
Propionate	16.4	16.5	16.6	16.2	16.2	18.2	16.1	14.9	16.3	0.62	18.2	16.1	14.9	16.3	16.5	16.5	16.5	16.5	16.5	0.17	0.72	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Butyrate	17.9	17.5	17.6	18.2	18.2	19.9	18.5	18.1	18.5	0.61	19.9	18.5	18.1	18.5	17.0	17.0	17.0	17.0	17.0	0.39	0.86	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	

^aStocking rate treatments were LT = light, MOD = moderate, HVY = heavy, and EXT = extreme.

^bGrazing period collections were MAY = May 11–22, JUN = June 10–19, JUL = July 8–17, AUG = August 5–14, and SEP = September 2–11.

^cObserved significance level of the *F*-test for treatment effects of items considered significant, $P < 0.05$.

^dContrasts for period main effects; L = linear, Q = quadratic, and C = cubic.

^eMost conservative SEM values were used, $n = 105$.

^fMost conservative SEM values were used, $n = 84$.

between the two increased quadratically ($P < 0.01$) with advancing season and ranged from 2.3 to 2.9 from May to September, respectively. McCracken et al. (1993) observed no change in total VFA, while other studies have reported a decrease in total VFA values (McCullum et al., 1985; Adams et al., 1987; Park et al., 1994). Other studies observed increased acetate proportions at the beginning of the season but decreased as the season progressed, resulting in an overall decrease in acetate from the beginning to the end of the grazing season (Caton et al., 1993; McCracken et al., 1993). Caton et al. (1993) and McCracken et al. (1993) both observed increases in propionate proportions as forage matured. However, in the case of Caton et al. (1993), mid-season propionate values were at their greatest, while McCracken et al. (1993) reported that mid-season propionate quantities were at their lowest. In the present study, our greatest concentrations of propionate were observed in May (18.2/100 mol), while our lowest concentrations were observed in July (14.9/100 mol). McCracken et al.'s (1993) acetate:propionate proportions decreased across the season but reported the highest ratio mid-season. Butyrate proportions decreased cubically ($P < 0.01$) with advancing season across the stocking rate. Other studies observed no change in butyrate proportions with the advancing season (Caton et al., 1993; McCracken et al., 1993; Park et al., 1994). The impacts of DDGS to these findings in the current study likely compound effects observed in direct comparison; however, with the positive response seen in our study, perhaps DDGS provide additional benefits to the ruminant animal through greater energy via VFAs.

In conclusion, the results of this study demonstrate that forage intake, fermentation, and digestibility in beef cattle grazing in the northern Great Plains and supplemented with DDGS were influenced more by seasonal factors than stocking rate. The results of this study further demonstrate that forages grazed by beef cattle in northern Great Plains rangelands increase in fiber and decrease in N as the season advances. The stocking rates evaluated had little impact on grazed forage nutrient composition and intake of steers supplemented with DDGS grazing northern Great Plains rangelands when forage availability was not limited. It is further possible that supplemental protein and energy provided by DDGS may have mitigated the impact of stocking rate through forage substitution or improved forage digestibility if forage had become limiting. However, further research would be required to fully evaluate this response.

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