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Forage Based Livestock Systems

FORAGE BASED LIVESTOCK SYSTEMS

Influence of amount and frequency of protein supplementation to ruminants consuming low-quality cool-season forages: efficiency of nitrogen utilization in lambs and performance of gestating beef cows

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Abstract

We evaluated the influence of amount and crude protein (**CP**) supplementation frequency (**SF**) on nitrogen (N) use by wethers and the performance of late-gestation beef cows. In exp. 1, seven Western whiteface wethers (31.8 ± 1.4 kg) were used in an incomplete 7 × 4 Latin square to evaluate intake and N use. Wethers received one of the seven treatments in a 2 × 3 factorial design containing two levels of supplemental soybean meal offered at a rate of 100% (**F**) or 50% (**H**; 50% of F) of the estimated CP requirement daily, once every 5, or once every 10 d, plus a non-supplemented control (**CON**). Low-quality cool-season forage (4.9 % CP; dry matter [**DM**] basis) was provided daily for ad libitum intake. Experimental periods lasted 30 d. In exp. 2, 84 Angus × Hereford cows (560 ± 35 kg) were stratified by age, body condition score (**BCS**), and expected calving date and allocated to 1 of the 21 feedlot pens (three pens per treatment). Pens were randomly assigned to receive the same treatments as in exp. 1 and cows had free access to low-quality cool-season forage (2.9% CP; DM basis). Cow body weight (**BW**) and BCS were measured every 14 d until calving and within 24 h after calving. In exp. 1, supplementation did not alter total DM and organic matter (**OM**) intake ($P \geq 0.26$), but both parameters linearly decreased as SF decreased ($P = 0.02$). Supplementation increased DM, OM, and neutral detergent fiber (**NDF**) digestibility ($P \leq 0.02$). Additionally, F feeding linearly increased DM, OM, and NDF digestibility as SF decreased ($P \leq 0.04$). Digestibility of N, N balance, and digested N retained were greater with supplementation ($P < 0.01$), and N digestibility linearly increased as SF decreased ($P = 0.01$). Mean plasma urea-N concentration was not only greater ($P < 0.01$) for supplemented vs. CON wethers but also greater ($P = 0.03$) for F vs. H. In exp. 2, pre-calving BCS change was greater ($P = 0.03$) for supplemented cows. A linear effect of SF × supplementation rate for pre-calving BCS change was noted ($P = 0.05$), as F-supplemented cows lost more BCS compared with H as SF decreased. When considering supplementation intervals greater than 5 d, reducing the quantity of supplement provided, compared with daily supplementation, may be a feasible management strategy to maintain acceptable nutrient use and animal performance while reducing supplement and labor costs.

Key words: low-quality cool-season forage, nutrient utilization, performance, ruminants, supplementation amount, supplementation frequency

Abbreviations

10D	once every 10 d
5D	once every 5 d
ADF	acid detergent fiber
BCS	body condition score
BW	body weight
CON	non-supplemented control
CP	crude protein
D	daily
DM	dry matter
DMI	DM intake
F	soybean meal offered at a rate of 100% of estimated protein requirement
H	soybean meal offered at a rate of 50% of estimated protein requirement
IADF	indigestible ADF
NDF	neutral detergent fiber
OM	organic matter
PUN	plasma urea N
RDP	rumen degradable protein
SBM	soybean meal
SF	supplementation frequency

Introduction

Grazing livestock in the western United States often consume low-quality forage (<6% crude protein [**CP**]) from late summer through winter (Ganskopp and Bohnert, 2001). Therefore, supplemental CP is often required during this time to maintain livestock body weight (**BW**) and body condition score (**BCS**; Bohnert et al., 2002a; Schauer et al., 2005). Daily supplementation of CP can be costly; therefore, decreasing supplementation frequency (**SF**) is a strategy to reduce labor costs and maintain livestock performance. Previous research indicated that SF can be reduced out to at least 7 d while maintaining adequate performance, likely due to urea recycling and maintenance of N use efficiency (Huston et al., 1999a, 1999b; Bohnert et al., 2002b; Wickersham et al., 2008). Moreover, Schauer et al. (2010) reported that protein supplements could be fed once every 10 d without negative impact on the efficiency of N use and performance of pregnant ewes consuming low-quality forage.

We are unaware of research comparing the effects of differing amounts of a CP supplement provided at extended SF on performance and efficiency of N utilization by ruminants consuming a low-quality, cool-season forage. Likewise, Sawyer et al. (2012) suggested that if the efficiency of N utilization is related to protein supply (Chowdhury and Orskov, 1997), reduced amounts of supplemental protein at extended SF could be used with an improved efficiency compared with greater quantities offered at the same SF. Therefore, we hypothesized that reducing the quantity of supplemental CP provided at extended SF to ruminants consuming low-quality, cool-season forage would improve the efficiency of N use. Hence, we conducted two experiments to evaluate the effects of quantity and SF of CP supplements on intake and efficiency of N utilization (experiment 1) and performance (experiment 2) of ruminants consuming low-quality, cool-season forage.

Materials and Methods

All animals utilized in these experiments were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP# 4061).

Experiment 1

Animals and treatments

Seven Western whiteface wethers (initial BW = 31.8 ± 1.4 kg) were used in an incomplete 7 × 4 Latin square (Cochran and Cox, 1957) to evaluate the intake and efficiency of N use. Wethers were randomly allotted to treatments and housed in individual metabolism crates (30 × 7.5 cm) within an enclosed barn with continuous lighting. Wethers received one of the seven treatments in a 2 × 3 factorial design plus a non-supplemented control (**CON**). Treatments consisted of two levels of supplemental soybean meal (**SBM**; Table 1) provided at 100% (**F**) or 50% (**H**) of the daily amount estimated to meet CP requirements for a 30-kg, 4 mo of age, late-maturing lamb gaining 200 g/d (NRC, 2007). In addition, SBM was provided daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) as a loose meal in a separate bunk immediately prior to providing grass seed straw (Chewings fescue; Table 1). Within each level of supplementation, the amount of SBM was the same over a 10-d period, whereas the amount of CP supplied for each animal was approximately 0.144% and 0.072% of BW/d (dry matter [**DM**] basis) for F and H, respectively. Supplements were typically consumed by wethers within 1 h for all treatments except for F10D, which often took up to 24 h to be consumed. Chopped (2.5 cm length) grass seed straw was provided daily at 0830 hours at 120% of the average daily intake for the previous 5 d. Grass straw refusals from the previous day were determined immediately prior to 0830 hours.

Wethers had continuous access to fresh water and received 35 g of a trace mineral salt mix (16% Ca, 8.0% P, 21% NaCl, 2.75% Mg, 1,400 ppm Mn, 5 ppm Cu, 3,000 ppm Zn, 3 ppm Co, 100 ppm I, 20 ppm Se, 227 IU/kg vitamin E, and 113,500 and 11,350 IU/kg vitamins A and D, respectively) that was provided daily to each lamb at 0830 hours. In addition, an intramuscular injection of vitamins A, D, and E (200,000, 20,000, and 600 IU, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each wether at the onset of the trial to safeguard against deficiency.

Sampling

Experimental periods were 30 d with at least 4 d between periods to allow for the removal of wethers from metabolism crates. DM intake was determined from day 19 to 28. Samples of grass seed straw and SBM (approximately 150 g/d) were collected from day 19 to 28, whereas orts were collected and subsampled (20% of total daily refusals; as-fed basis) from day 20 to 29.

Table 1. Nutrient content of feedstuffs used in the present experiments

	Experiment 1		Experiment 2[1]	
Item	Grass seed straw[2]	SBM	Grass seed straw[2]	SBM
Nutrient composition, %DM				
OM	92.3	92.4	—	—
CP	4.9	49.9	2.4	51.7
NDF	79.1	17.0	81.8	15.3
ADF	46.0	4.5	48.2	4.4
IADF	20.8	0.7	—	—

[1]OM and IADF were not analyzed.
[2]Chewings fescue.

Total urine and fecal output were collected daily from day 21 to 30 of each experimental period. Sufficient 6 N HCl (100 mL) was added daily to urinals to maintain urine pH < 3 (verified with pH paper during the urine collection period) to minimize bacterial growth and N loss. Urine was composited daily by wether (25% of total daily output; weight basis) and stored at 4 °C. A subsample of each total daily fecal sample (7.5%; wet-weight basis), in addition to the subsamples of feed and orts, were dried in a forced-air oven at 55 °C for 96 h for the calculation of DM, ground through a Wiley mill (1-mm screen), and then grass seed straw and SBM were composited by period and orts and feces were composited by lamb within period.

Feed, orts, and fecal samples were analyzed for DM, organic matter (**OM**; AOAC, 2006), neutral detergent fiber (**NDF**; Robertson and Van Soest, 1981), and acid detergent fiber (**ADF**; Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Feed, orts, fecal, and urine samples were also analyzed for N (TruMac Series; Leco Corporation, St. Joseph, MI). Nitrogen retention was calculated as the difference between N intake and N excretion (feces and urine), whereas digested N retained was calculated according to the formula (Bohnert et al., 1999):

$$[(\text{daily N retention, g/kg BW}/\text{daily N digested, g/kg BW}) \times 100]$$

Blood samples were collected daily 4 h after grass seed straw feeding from day 21 to 30 for the determination of plasma urea-N (**PUN**) concentration. All blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 United States Pharmacopeis (USP) units of freeze-dried sodium heparin. After collection, blood samples were placed on ice, transported to lab, kept in a cooler (–2 °C) for 2 h, subsequently centrifuged (3,640 × *g* for 20 min; 8 °C) for plasma harvest, and stored at –20 °C on the same day of collection until further laboratorial analysis. PUN concentration was determined using quantitative colorimetric kits (#B7551; Pointe Scientific, Inc., Canton, MI). The intra- and inter-assay coefficients of variation were, respectively, 4.8% and 8.3%.

Statistical analysis

All data were analyzed using the PROC MIXED procedure of SAS (version 9.4; SAS Inst., Cary, NC) with wether as the experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects of treatments. For all analyses, wether was used as the random variable. DM and OM intake, total tract nutrient digestibility (DM, OM, NDF, and N), N balance, and digested N retained were analyzed as an incomplete 7 × 4 Latin square (Cochran and Cox, 1957). The model statement contained the effects of treatment and period as independent variables. The model statement used for DM and OM intake, and PUN, included treatment, day, and the resultant interaction as well as period as an independent variable. The specified term for the repeated statement was day, whereas lamb(period × treatment) was included as the subject. The covariance structure was first-order autoregressive, which provided the smallest Akaike Information Criterion, and hence the best fit for the variables analyzed. Because the treatment structure consisted of a 2 × 3 factorial plus a negative control, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were: 1) CON vs. protein supplementation, 2) F vs. H of required daily supplementation amount, 3) linear effect of SF, 4) quadratic effect of SF, 5) linear effect of SF × CP level, and 6) quadratic effect of SF × CP level. The same contrasts denoted above were used to partition treatment sums of squares. Significance was set at $P \leq 0.05$ and tendencies were determined if $0.05 < P \leq 0.10$. Results are reported according to main effects or according to the highest-order significant interaction detected.

Experiment 2

Animals and treatments

Eighty-four multiparous Angus × Hereford cows (initial BW = 560 ± 35 kg; initial BCS = 4.83 ± 0.39; initial age = 7.7 ± 0.8 yr) estimated to be entering the last third of gestation were utilized in this study. Pregnancy status was verified by detecting a fetus via rectal palpation approximately 190 d after the end of a 50-d breeding season and only cows confirmed as pregnant were enrolled in the study. On day 0, all cows were stratified by age and BCS (1 = emaciated and 9 = obese; Herd and Sprott, 1996) and allocated to 1 of the 21 feedlot pens (three pens per treatment; four cows per pen; 8 × 20 m). Pens were randomly assigned to receive the same supplements as described in exp. 1; however, the level of supplementation for the F and H treatments was based on 100% or 50%, respectively, of the daily amount estimated to meet rumen degradable protein (**RDP**) requirements assuming a microbial efficiency of 10% (NRC, 2000; model 1). According to the supplementation level (F or H), cows received the same amount of supplemental CP over a 10-d period. Moreover, supplements were provided based on the average BW of the animals within a pen and according to the allocated SF schedule (D, 5D, or 10D) and allocated RDP level (F or H) at 0800 hours. Supplements were typically consumed by cows within 30 min for all treatments except for F10D, which often took up to 12 h to be consumed. Grass seed straw (Chewings fescue; Table 1) was offered for ad libitum consumption from large bales (1.22 × 0.91 × 2.44 m) daily at 0830 hours. Also, cows had ad libitum access to water and a mineral–vitamin mix (Cattleman's Choice, Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E throughout the experimental period. Cows were provided treatments for 83 ± 1.5 d (days from study initiation to calving; data not shown).

Sampling

Cow BW and BCS were measured every 14 d until calving (to obtain pre-calving weight and BCS that was ≤14 d before calving, weights and BCS obtained greater than 14 d prior to calving, with the exception of initial values, were not used in analyses or reported herein) and within 24 h after calving (reported as post-calving weight and BCS). All individual weights were obtained prior to supplement and hay feeding, and cow BCS was evaluated independently by three technicians throughout the experimental period. In addition, calf weights were obtained within 24 h of birth. Samples of hay and SBM were collected weekly, pooled across all weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY).

Statistical analysis

All data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Cary, NC), using pen as the experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects of treatments. The model statement used for BCS and BW changes as well as calf birth date and weight contained the effect of treatment. Pre-calving changes

were calculated by subtracting the pre-calving weight/BCS from the initial weight/BCS. Likewise, post-calving changes were calculated by subtracting the post-calving weight/BCS from the initial weight/BCS. Data were analyzed using pen(treatment) as the random variable. Because the treatment structure consisted of a 2 × 3 factorial plus a negative CON, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were the same as noted for exp. 1. Significance was set at $P \leq 0.05$, and tendencies were denoted if $0.05 < P \leq 0.10$. Results are reported according to main effects or according to the highest-order significant interaction detected.

Results

Experiment 1

Treatment × day interactions ($P \leq 0.01$) were observed for forage and total DM intake (**DMI**) over the 10-d supplementation period; however, after considering the nature of the interactions, we concluded that discussing treatment means (Table 2) while providing the daily forage and total DMI data (Figure 1) would aid in the interpretation of the observed response. Forage and total DM and OM intakes were not affected by CP supplementation ($P \geq 0.26$; Table 2).

Linear decreases in forage and total DM and OM intakes were detected as SF decreased ($P = 0.02$) for both F and H supplement amounts (Table 2). Interestingly, the greatest daily DMI reduction herein was observed on the third day following supplementation with the 5D and 10D SF for both F and H supplementation amounts (Figure 1). Tendencies were noted for the linear effect of SF × supplementation amount ($P = 0.08$) for forage and total DM and OM intake, which decreased to a greater extent with F compared with H as SF decreased.

Supplementation had no effect on NDF and indigestible ADF (**IADF**) intake ($P \geq 0.78$; Table 2); however, similar to DM and OM, NDF and IADF intake linearly decreased as SF decreased ($P = 0.02$). Tendencies for SF × supplementation amount were observed for both parameters ($P \leq 0.09$), which decreased to a greater extent as SF decreased for the F treatments compared with H.

As a result of CP supplementation, DM, OM, and NDF digestibility increased ($P \leq 0.02$) and ADF digestibility tended to increase ($P = 0.08$; Table 2). A linear effect of SF × amount of supplement provided interaction ($P \leq 0.04$) was observed for all these parameters. As SF decreased, DM, OM, NDF, and ADF digestibility increased for lambs fed F while being maintained or decreased for H. Intake of N, urinary N, N balance, N digestibility, and digested N retained were increased with CP supplementation ($P < 0.01$), while fecal N did not change ($P = 0.36$; Table 3). Feeding F vs. H increased ($P < 0.01$) N intake, urine N, and N digestibility and tended to increase ($P = 0.09$) N balance (Table 3). However, as SF decreased, urine N and N digestibility increased, whereas N intake linearly decreased ($P \leq 0.05$). A linear SF × amount of supplement-provided interaction was detected on fecal N ($P = 0.05$) and tended to occur for N digestibility ($P = 0.07$), because as SF decreased, fecal N decreased and N digestibility increased to a greater extent with F compared with H supplemented wethers (Table 3).

A treatment × day interaction was detected on circulating concentrations of PUN ($P < 0.01$); however, after considering the nature of the interaction, we decided discussing treatment means while providing the time × treatment figure would facilitate the interpretation of the data. PUN was increased ($P < 0.01$) with supplementation and for F compared with H (Table 3). It is worth noting that PUN peaked the day following

Table 2. Intake and digestibility parameters in lambs consuming low-quality, cool-season forage and receiving SBM or CON D, 5D, or 10D in differing amounts (F and H)

	Treatments[1]							Contrasts[2]					
Item	CON	FD	F5D	F10D	HD	H5D	H10D	SEM	CON vs. Supp	F vs. H	SF L	SF Q	L vs. Amt	Q vs. Amt
DMI, g/kg BW														
Forage	18.8	21.6	19.5	14.0	20.2	19.3	18.7	1.73	0.97	0.45	0.02	0.60	0.08	0.52
Supplement	0.0	2.8	2.8	2.8	1.4	1.4	1.4							
Total	18.8	24.4	22.3	16.8	21.6	20.7	20.1	1.73	0.26	0.80	0.02	0.60	0.08	0.54
OM intake, g/kg BW														
Forage	17.4	20.0	18.0	12.9	18.6	17.8	17.3	1.60	0.97	0.46	0.02	0.60	0.08	0.52
Supplement	0.0	2.5	2.5	2.5	1.3	1.3	1.3							
Total	17.4	22.5	20.5	15.4	19.9	19.1	18.6	1.60	0.26	0.79	0.02	0.61	0.08	0.54
NDF intake, g/kg BW	14.9	17.8	15.9	11.6	16.0	15.4	15.0	1.40	0.78	0.73	0.02	0.65	0.07	0.57
IADF intake, g/kg BW	3.9	4.5	4.0	2.9	4.1	4.0	3.8	0.38	0.96	0.51	0.02	0.60	0.09	0.67
Digestibility, %														
DM	37.4	40.6	45.7	49.6	43.5	43.5	43.5	1.98	<0.01	0.28	0.04	0.87	0.04	0.87
OM	39.6	43.6	48.0	52.0	45.7	45.4	45.5	1.85	<0.01	0.14	0.04	0.99	0.03	0.89
NDF	42.2	43.6	46.9	48.6	46.0	45.7	44.7	1.39	0.02	0.43	0.19	0.64	0.04	0.86

[1]CON, control; D, daily; 5D, once every 5 d; 10D, once every 10 d; F, amount provided to meet CP requirements; H, 50% of F.
[2]CON vs. Supp, non-supplemented treatment vs. protein supplemented treatments; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. Amt, linear effect of SF × supplementation amount; Q vs. Amt, quadratic effect of SF × supplementation amount.

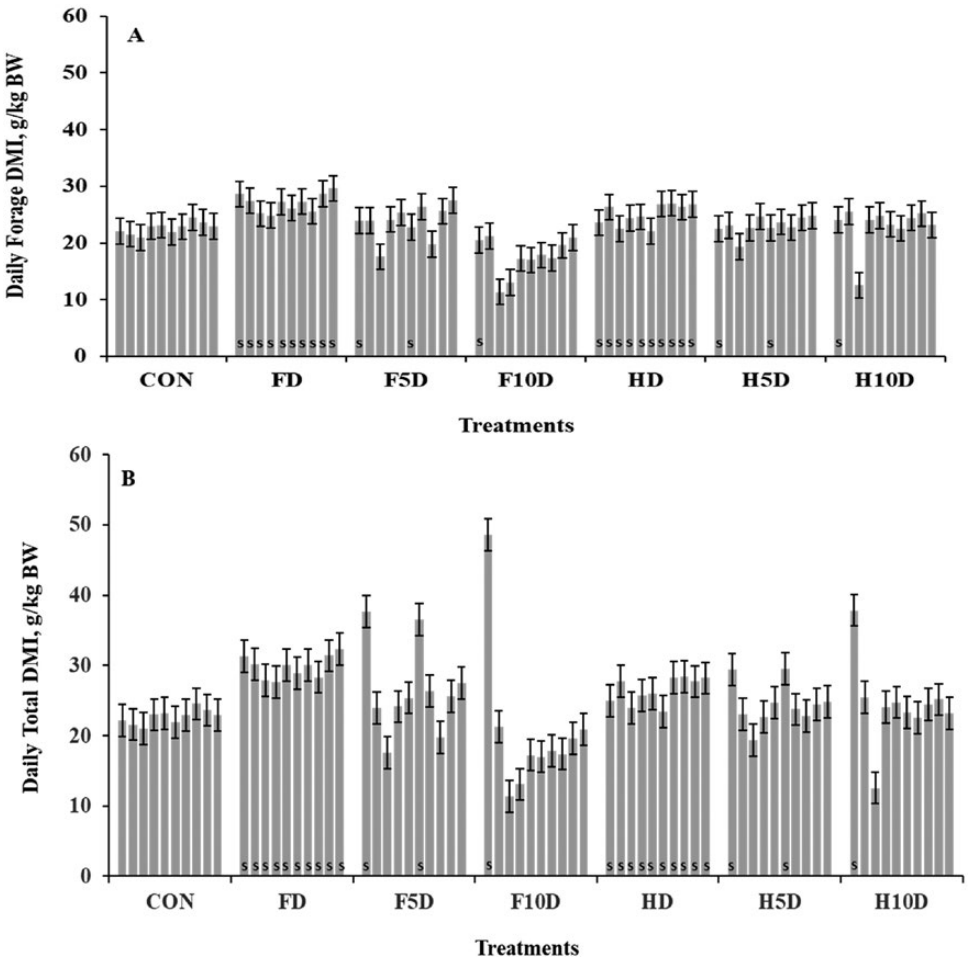

Figure 1. Daily forage (A) and total (B) DMI in lambs consuming low-quality, cool-season forage and receiving SBM or CON D, 5D, or 10D in differing amounts (F and H). Columns for each treatment represent, left to right, DMI from day 1 through 10 of the DMI measurement period. S, supplementation. Treatment × day interactions were observed for daily and total hay DMI ($P < 0.01$). CON, control; D, daily; 5D, once every 5 d; 10D, once every 10 d; F, amount provided to meet CP requirements; H, 50% of F.

supplementation for the 5D and 10D SF and returned to nadir approximately 48 and 96 h post-supplementation, respectively (Figure 2). A quadratic effect of SF by supplement quantity interaction ($P = 0.04$; Table 3) was noted, with PUN being greatest for FD, decreasing slightly for F5D, and increasing for F10D, while the greatest PUN concentration for H-supplemented wethers was H5D with HD and H10D being similar.

Experiment 2

As expected, no treatment effects were detected on initial cow BW ($P = 0.16$; Table 4) and BCS ($P = 0.99$; Table 4). Supplementation improved ($P \leq 0.03$) pre- and post-calving BW and BCS change compared with non-supplemented cows (Table 4). Furthermore, cows fed F had greater ($P = 0.05$) pre-calving and post-calving BW and BCS changes compared with cohorts fed H (Table 4). Post-calving BW change linearly increased ($P < 0.01$) in a negative fashion as SF decreased for both F- and H-supplemented cows. Linear effects of SF × supplementation amount were detected on pre-calving BW and BCS change ($P \leq 0.05$; Table 4). As SF decreased, pre-calving BW and BCS change linearly decreased for cows receiving both the F and H amount of supplement; however, the magnitude of decrease as SF decreased from D to 10D was greater for F compared with H. Moreover, no treatment effects ($P \geq 0.19$) were observed on calf birth weight (Table 4).

Discussion

The data reported in this manuscript comprise a series of experiments to evaluate the performance, digestibility, ruminal fermentation, and N use in ruminants consuming low-quality, cool-season forage and offered two levels of supplement provided at three SF. The rationale underlying these experiments is that reducing the amount and frequency of supplement offered to ruminants consuming a low-quality forage with a concomitant maintenance of performance would benefit the profitability of the operation, as feed and labor costs play a key role in the success of beef production systems (Miller et al., 2001). A companion paper (Cappellozza et al., 2021) reports nutrient intake and digestibility as well as ruminal fermentation parameters in rumen-fistulated beef steers provided the same treatments utilized herein.

Experiment 1

The lack of supplementation effects on forage and total DMI corroborates data from Bohnert et al. (2011), who demonstrated that CP supplementation did not increase DMI in cattle consuming low-quality, cool-season forage. Moore et al. (1999) suggested that forage intake is not impacted by CP supplementation when OM intake is ≥17.5 g/kg BW, whereas OM intake for the CON group in the present experiment was

Table 3. Nitrogen utilization in lambs consuming low-quality, cool-season forage and receiving SBM or CON D, 5D, or 10D in differing amounts (F and H)

	Treatments[1]								Contrasts[2]					
Item	CON	FD	F5D	F10D	HD	H5D	H10D	SEM	CON vs. Supp	F vs. H	SF L	SF Q	L vs. Amt	Q vs. Amt
N, g/kg BW														
Intake	0.148	0.384	0.368	0.328	0.264	0.264	0.256	0.01	<0.01	<0.01	0.04	0.54	0.12	0.75
Fecal	0.129	0.194	0.155	0.112	0.146	0.138	0.132	0.02	0.36	0.27	0.01	0.99	0.05	0.94
Urine	0.052	0.148	0.176	0.203	0.103	0.120	0.112	0.01	<0.01	<0.01	0.05	0.61	0.15	0.63
Retention	−0.033	0.042	0.038	0.012	0.016	0.006	0.012	0.01	<0.01	0.09	0.22	0.92	0.36	0.43
N digestibility, %	12.2	49.5	58.4	65.8	45.5	48.9	48.4	3.4	<0.01	<0.01	0.01	0.67	0.07	0.85
Digested N retained[3], %	−460.6	21.8	17.9	3.9	12.4	2.4	7.3	120.0	<0.01	0.94	0.92	0.99	0.96	0.95
PUN, mg/dL	9.0	18.9	15.4	16.5	11.7	16.4	12.4	1.73	<0.01	0.03	0.63	0.51	0.39	0.04

[1]CON, control; D, daily; 5D, once every 5 d; 10D, once every 10 d; F, amount provided to meet CP requirements; H, 50% of F.
[2]CON vs. Supp, non-supplemented treatment vs. protein supplemented treatments; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. Amt, linear effect of SF × supplementation amount; Q vs. Amt, quadratic effect of SF × supplementation amount.
[3]Calculated as (Daily N Retention, g/kg BW/Daily N digested, g/kg BW) × 100.

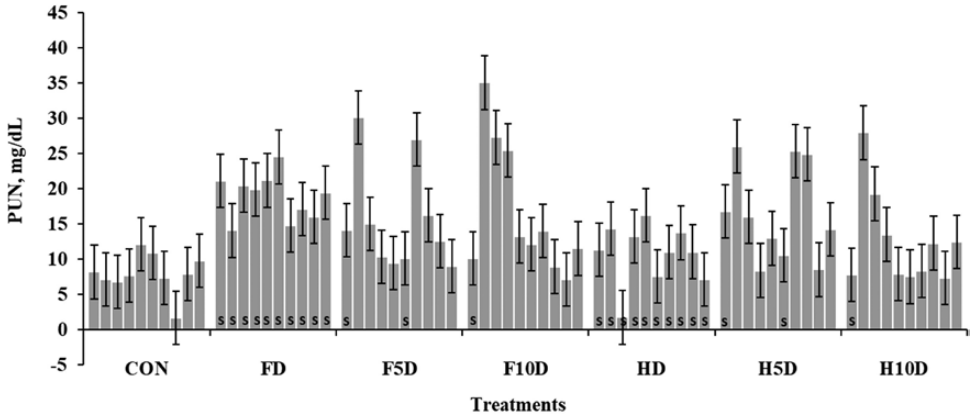

Figure 2. Daily PUN concentration in lambs consuming low-quality, cool-season forage and receiving SBM or CON D, 5D, or 10D in differing amounts (F and H). Columns for each treatment represent, left to right, PUN 4 h after feeding from day 1 through 10 of the DMI measurement period; S, supplementation. A treatment × day ($P < 0.01$) interaction was observed for PUN (SEM = 3.79). CON, control; D, daily; 5D, once every 5 d; 10D, once every 10 d; F, amount provided to meet CP requirements; H, 50% of F.

17.4 g/kg BW. Likewise, Mertens (1985, 1994) suggested that DMI is maximized when NDF intake is approximately 12.5 g · kg BW^{-1} · d^{-1}. In the current study, NDF intake of the non-supplemented animals was roughly 14.9 g · kg BW^{-1} · d^{-1}, likely explaining the lack of effects on DMI observed herein and by others (Mathis et al., 2000; Currier et al., 2004).

The DM and OM intake results observed in the current study as SF decreased agree with those of Schauer et al. (2010). They used a similar experimental design and observed that forage and total DMI linearly decreased as SF also decreased from daily to once every 5 or 10 d. Additionally, Cappellozza et al. (2021) reported that forage DMI of steers receiving the same forage and treatments used in the current study was decreased as SF decreased from D to 10D. However, in contrast to Cappellozza et al. (2021), it is worth noting that we observed a lag in daily DMI reduction as SF decreased for both F and H supplementation amounts, with the greatest reduction occurring 3 d following supplementation. The differences observed between the studies likely relate to the method by which supplements were delivered, given that supplements in Cappellozza et al. (2021) were administered directly into the rumen via ruminal cannula, whereas in the current study supplements were offered in the feed bunk and consumed over a 24-h period. Consequently, feeding behavior and the resulting potential differences in ruminal fermentation may explain part of the lag in forage DMI reduction observed in the current study. Furthermore, Bohnert et al. (2011) suggested that forage intake in response to protein supplementation of ruminants consuming low-quality forages is highly dependent on forage type, with forage intake often increasing when CP supplements are offered to animals consuming low-quality (<7% CP), warm-season forages, whereas little to no increase is often observed for ruminants consuming low-quality, cool-season forages. Additionally, especially for the 10D treatments, offering the supplements via the rumen cannula likely resulted in an acute abundance of nutrients within the reticulorumen (i.e., N) that could have resulted in alterations of ruminal microflora, ruminal fermentation, and site of nutrient utilization. Also, Cappellozza et al. (2015) offered beef cows 7 kg of SBM at a supplementation event (once weekly) and reported significant alterations in PUN and hepatic enzymes involved in the urea cycle compared with daily (1 kg) or 3× per week (2.3 kg) supplementation, suggesting that the rumen microflora and liver can adapt to this type of acute protein supplementation.

Past work from our group suggests that not only the type of protein (RDP vs. rumen undegadable protein) but also the amount and SF of supplemental protein provided impact nutrient digestibility (Bohnert et al., 2002b). In the current study and in our companion paper (Cappellozza et al., 2021), nutrient digestibility increased due to CP supplementation; however, Cappellozza et al. (2021) noted a linear increase in

Table 4. Performance of late-gestating beef cows consuming low-quality, cool-season forage and receiving SBM or CON D, 5D, or 10D in differing amounts (F and H)[1]

	Treatments[2]								Contrasts[3]				
Item	CON	FD	F5D	F10D	HD	H5D	H10D	SEM	Con vs. Supp	F vs. H	SF L	SF Q	L vs. Amt	Q vs. Amt
Initial BW[4], kg	563	563	553	559	533	572	567	11.1	0.66	0.93	0.17	0.47	0.08	0.10
Initial BCS[4]	4.78	4.81	4.82	4.81	4.84	4.78	4.89	0.22	0.84	0.89	0.91	0.85	0.91	0.82
BW change, kg														
Pre-calving	−11	32	24	−2	11	3	7	5.8	<0.01	0.01	<0.01	0.76	0.01	0.11
Post-calving	−70	−28	−47	−52	−43	−62	−65	6.9	<0.01	0.01	<0.01	0.18	0.87	0.90
BCS change[4]														
Pre-calving	−0.35	0.09	−0.03	−0.26	−0.24	−0.23	−0.28	0.09	0.03	0.01	0.02	0.53	0.05	0.90
Post-calving	−0.64	−0.21	−0.25	−0.46	−0.39	−0.35	−0.46	0.11	0.01	0.26	0.13	0.38	0.40	0.96
Calf birth weight[5], kg	37	40	37	37	39	37	36	1.9	0.92	0.77	0.19	0.48	0.98	0.76

[1]F supplements were offered at 1.5 g/kg BW for D, 7.5 g/kg BW for 5D, and 15.0 g/kg BW for 10D. Half (H) supplements were 50% of the F amount. Pen average cow weight was used for calculating the quantity of supplement provided.
[2]CON, control; D, daily; 5D, once every 5 d; 10D, once every 10 d; F, amount provided to meet CP requirements; H, 50% of F.
[3]CON vs. Supp, non-supplemented treatment vs. protein supplemented treatments; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. Amt, linear effect of SF × supplementation amount; Q vs. Amt, quadratic effect of SF × supplementation amount.
[4]Changes were calculated by subtracting the pre- (≤14 d of calving) or post- (within 24 h after calving) calving weight/BCS from the initial weight/BCS.
[5]Calf weights were obtained within 24 h of birth.

nutrient digestibility as SF decreased for both F and H groups with no interactions. Herein, we noted linear effects of SF by the quantity of supplement interactions for nutrient digestibility, with digestibility increasing as SF decreased for F and little to no change for the H treatments. As noted for DMI, differences between results from the present study and the companion paper may be in response to the method by which supplements were offered to the animals (consumption by the animal vs. ruminal dosing, respectively). Nevertheless, Atkinson et al. (2010) also observed an increase in OM digestibility as SF decreased from daily to once every other day. The observed increases in apparent total tract digestibility of nutrients agree with previous work from our group (Bohnert et al., 2011).

As expected, we noted greater N intake as the amount of CP supplement provided increased, which agrees with other studies in which supplemental CP was provided to ruminants consuming low-quality forage (Sawyer et al., 2012). In another study, as SF of casein decreased and the amount provided per supplementation event increased, urinary urea-N tended to increase (Wickersham et al., 2008). Coleman and Wyatt (1982) observed no differences in daily fecal N excretion of steers consuming, or not, CP supplements daily, every other day, or once every 3 d, whereas fecal N excretion linearly decreased as SF of CP supplements also decreased from D to 5D or 10D (Schauer et al., 2010). Atkinson et al. (2010) also observed greater N digestibility as SF decreased in lambs supplemented with protein and consuming low-quality forages.

PUN is positively correlated with N intake (Harmeyer and Martens, 1980) and the type of protein consumed (RDP vs. RUP; Sawyer et al., 2012). Furthermore, Cappellozza et al. (2015) reported that beef cows consuming low-quality, cool-season forage and offered SBM as infrequent as once every 7 d had PUN peaks at 28 h after supplements were offered. In the companion paper (Cappellozza et al., 2021), PUN peaked 2 d after supplementation for F10D and H10D. Both gastrointestinal tract permeability to urea and regulation of renal urea excretion can be altered by low-protein diets and/or restricted feeding (Harmeyer and Martens, 1980; Kennedy and Milligan, 1980). Also, Krehbiel et al. (1998) reported that infrequently supplementing ewes consuming low-quality forage with protein resulted in greater efficiency of N use between supplementation events. They reported that the net removal of urea N by the portal-drained viscera was over 600% greater on the days following a supplementation event compared with the day of supplementation. However, Bohnert et al. (2002b) noted that as SF of CP supplements decreased from daily to once every 6 d, N balance linearly decreased, indicating that N retention was also being reduced. Yet, in the present experiment, as SF became less frequent, the percentage of digested N retained was not affected by the amount or SF of CP. This provides further evidence that ruminants consuming low-quality forage and supplemented infrequently have the ability to remove urea N from the blood between supplementation events and, thereby, help sustain efficient utilization of dietary N.

The increased circulating PUN observed on days between supplementation events for 5D and 10D supports increased ruminal ammonia concentrations and N recycling, which should help support adequate rumen fermentation (Bohnert et al., 2002a; Atkinson et al., 2010), nutrient utilization, and animal performance.

The greater PUN concentration for supplemented vs. CON and for F vs. H supports the statement that PUN reflects the amount of CP consumed by ruminants (Broderick and Clayton, 1997; Hammond, 1997; Cappellozza et al., 2014a, 2014b).

The greater circulating concentration of PUN in animals fed increased supplemental CP also supports the results observed herein for urine N, given that after conversion from ammonia in the liver, urea may be excreted via urine or recycled back to the gut through either direct transfer from blood across the epithelial tissue or via saliva (Van Soest, 1982; Reynolds and Kristensen, 2008).

To the best of our knowledge, this is the first series of research studies evaluating the effects of different supplementation amounts along with different SF schedules on efficiency of N utilization and nutrient intake parameters of ruminants consuming low-quality, cool-season forages. Excessive supplement DMI for SF ≥ 5 d likely affects forage intake by enhancing the substitution effect of the supplement for forage (Bohnert et al., 2002a). Furthermore, excessive protein intake might affect ruminal pH due to the greater release of ammonia, stimulating ruminal absorption of this molecule into the portal blood. Conversely, the results from our companion paper (Cappellozza et al., 2021) demonstrated that ruminal pH linearly decreased as SF decreased but remained at the adequate level for proper rumen function and forage utilization (6.2 to 6.8; Yokoyama and Johnson, 1988), suggesting that the reduced forage DMI as SF decreased is most likely due to the aforementioned substitution effect or some other fermentation metabolite(s).

Ammonia circulating freely in the blood is toxic to ruminants; thus, ammonia absorbed through the rumen wall must be removed from the circulation and converted to urea in the liver (Van Soest, 1982; Bach et al., 2005). This mechanism allows for N recycling and helps sustain rumen microbial metabolism and is useful when the dietary supply of protein is scarce (Ludden et al., 2009). Moreover, the amount of CP, more specifically RDP, offered may modulate the number and expression of urea transporters-B in the rumen, increasing the transport of urea into the blood (Marini and Van Amburgh, 2003; Ludden et al., 2009). One strategy to avoid excessive hepatic conversion of ammonia into urea, consequently reducing energy waste, may include strategic supplementation with RUP sources (i.e., corn gluten meal), which may improve the efficiency of N utilization by slowing the deamination of amino acids and contributing to the ruminal N supply, thereby reducing potential ruminal N losses, enhancing N recycling (Coomer et al., 1993), and providing a more consistent supply of available N (Atkinson et al., 2010). Sawyer et al. (2012) reported that feeding smaller amounts of RUP sources to beef cattle allows for maintenance of adequate rumen function, improves CP utilization efficiency by minimizing ruminal and metabolic N losses, and maintains NDF fermentation when compared with feeding large amounts of RDP to beef cattle.

Experiment 2

In agreement with others, CP supplementation positively benefited BW (Cappellozza et al., 2014b) and BCS (Bohnert et al., 2013) change of ruminants consuming low-quality, cool-season forages. The negative impacts of reducing CP SF on cow BW and BCS change are corroborated by the decrease in nutrient intake of F10D compared with FD and F5D noted in exp. 1 and in our companion paper (Cappellozza et al., 2021). Beaty et al. (1994) also reported reduced BW and BCS losses as cows were fed protein supplements daily instead of three times per week.

Several studies have demonstrated that decreasing SF is a feasible alternative to reduce costs associated with supplementation, while improving or maintaining desired performance of ruminants consuming low-quality, cool-season forages (Bohnert et al., 2002a; Schauer et al., 2005). Also, using the same SF as that of the current study, Schauer et al. (2010) reported that as SF decreased, pre-lambing weight change tended to increase in a linear fashion for gestating ewes, with no effects on pre-lambing BCS or post-lambing weight and BCS change. These authors concluded that SF of protein supplements can be decreased to once every 10 d for ewes consuming low-quality forages. However, Farmer et al. (2001) observed an increase in BW and BCS loss when beef cows were supplemented with protein supplements three times a week vs. daily. Our data suggest that the quantity of a CP supplement provided infrequently could yield differing impacts on nutrient utilization and performance of ruminants, likely by altering the overall nutrient intake and digestibility (Cappellozza et al., 2021). Our data also agree with prior studies in which SF did not affect calf birth weight (Beaty et al., 1994; Farmer et al., 2001; Schauer et al., 2010).

Overall conclusions

In summary, when evaluating supplementation frequencies greater than 5 d, reducing the amount of a CP supplement provided by half did not negatively affect forage DMI and maintained adequate N status of wethers, likely being a feasible management strategy to maintain acceptable levels of intake and digestibility of nutrients while reducing supplementation costs. Therefore, our data from the current study and the companion paper suggest that reducing the overall quantity of supplemental N provided at each supplementation event to ≤0.6 g/kg BW (Cappellozza et al., 2021) should be considered to maintain acceptable levels of DMI, nutrient digestibility, and ruminal fermentation while reducing supplementation costs. Protein supplementation improved pre- and post-calving weight and BCS change of cows. However, pre-calving weight and BCS change for H treatments were similar across the range of SF evaluated compared with a linear decrease in performance for F. Further research evaluating the reproductive and gestational programming consequences of the amount and type of protein supplementation at extended supplementation frequencies will provide information to help develop nutritional management strategies that improve the profitability of ruminant production systems reliant on low-quality forages.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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