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# Influence of amount and frequency of protein supplementation to steers consuming low-quality, cool-season forage: intake, nutrient digestibility, and ruminal fermentation

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## Abstract

This experiment evaluated the influence of protein supplementation frequency (**SF**) and amount offered on intake, nutrient digestibility, and ruminal fermentation by rumen-fistulated beef steers consuming low-quality [2.9% crude protein (**CP**); dry matter (**DM**) basis], cool-season forage. Seven Angus × Hereford steers (300 ± 27 kg) fitted with ruminal cannulas were randomly assigned to 1 of 7 treatments in an incomplete 7 × 4 Latin square. Treatments, in a 2 × 3 factorial design plus a non-supplemented control (**CON**), consisted of 2 levels of supplemental soybean meal, 100% (**F**) or 50% (**H**) of the estimated rumen-degradable protein requirement, provided daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**). Experimental periods were 30 d and dry matter intake (**DMI**) was measured from days 19 to 28. On days 21 (all supplements provided) and 30 (only daily supplements provided; day immediately prior to supplementation for 5D and 10D treatments) ruminal fluid was collected for ruminal pH, ammonia-N ($NH_3$), volatile fatty acids (**VFA**), and determination of ruminal fermentation variables. Forage and total DM, organic matter (**OM**), and nitrogen (**N**) intake increased with supplementation ($P \leq 0.04$). However, a linear effect of SF × amount of supplement interaction was observed for forage and total DM, OM, and N intake ($P \leq 0.04$), with each variable decreasing as SF decreased, but the decrease being greater with F vs. H. Apparent total tract DM, OM, and neutral detergent fiber digestibility was not affected by supplementation or amount of supplement provided ($P \geq 0.10$). In contrast, N digestibility increased with supplementation and for F vs. H ($P < 0.01$). Digestibility of DM, OM, and N increased linearly as SF decreased ($P \leq 0.03$). When all supplements were provided, ruminal $NH_3$, total VFA, and molar proportions of all individual VFA increased with supplementation ($P \leq 0.04$), whereas acetate:propionate ratio decreased ($P < 0.01$). When only daily supplements were provided, none of the aforementioned fermentation parameters were affected ($P \geq 0.09$). In summary, reducing the amount of supplemental CP provided to ruminants consuming low-quality forages, when supplementation intervals are >5 d, can be a management tool to maintain acceptable levels of DMI, nutrient digestibility, and ruminal fermentation while reducing supplementation cost.

**Key words:** beef steers, forage intake, low-quality cool-season forage, soybean meal, supplement amount, supplementation frequency

**Abbreviations**

| | |
|---|---|
| ADF | acid detergent fiber |
| CP | crude protein |
| DM | dry matter |
| DMI | dry matter intake |
| IADF | indigestible-ADF |
| N | nitrogen |
| NDF | neutral detergent fiber |
| $NH_3$ | ammonia-N |
| OM | organic matter |
| SF | supplementation frequency |
| VFA | volatile fatty acids |

## Introduction

Livestock operations using low-quality forages often require a supplementation strategy to address nutrient deficiencies (Schillo et al., 1992). Protein is traditionally considered the most limiting nutrient in Western U.S. cow-calf operations (DelCurto et al., 2000), although the costs associated with labor, feed, and fuel may offset the beneficial effects of supplementation (Miller et al., 2001). Ruminants have the ability to recycle N back to the rumen; hence, recycling of absorbed N may be one of the underlying mechanisms supporting ruminal fermentation between times of infrequent supplementation (Farmer et al., 2004). Decreasing the supplementation frequency (**SF**) of protein to once every 6 d has been shown to reduce operation costs without adversely affecting dry matter intake (**DMI**), nutrient digestibility, or bacterial crude protein (**CP**) synthesis in ruminants consuming low-quality forage (Bohnert et al., 2002a, 2002b, 2002c).

Schauer et al. (2010) reported that protein supplements can be fed as infrequently as once every 10 d without negative impacts on efficiency of nutrient utilization and performance of sheep consuming low-quality forage. Furthermore, Wickersham et al. (2008) suggested that ruminants might be able to adapt to, and utilize more efficiently, smaller amounts of supplemental N offered less frequently compared with a greater quantity offered at the same SF or with greater frequency. Based on this rationale, we hypothesized that steers consuming low-quality, cool-season forage and supplemented at extended intervals, would be able to adapt to reduced amounts of supplemental N by improving efficiency of N use and minimizing potential negative effects on nutrient utilization and ruminal fermentation. Hence, the objective of this experiment was to evaluate the effects of amount and SF of a CP supplement on intake, digestibility, and ruminal fermentation parameters of beef steers consuming low-quality, cool-season forage.

## Materials and Methods

This experiment was conducted at the Oregon State University—Eastern Oregon Agricultural Research Center (Burns Station). All animals utilized 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 (# 4061).

### Animals and treatments

Seven Angus × Hereford steers [initial body weight (**BW**) = 300 ± 27 kg] fitted with ruminal cannulas were randomly allotted to 1 of 7 treatments in an incomplete 7 × 4 Latin square (Cochran and Cox, 1957) and housed in individual pens (4 × 8 m) within an enclosed barn with continuous lighting throughout the study. Treatments, arranged as a 2 × 3 factorial design plus a nonsupplemented control (**CON**), consisted of 2 levels of supplemental soybean meal (**SBM**; Table 1) provided at 100% (**F**) or 50% (**H**) of the daily amount estimated to meet rumen degradable protein (**RDP**) requirements assuming a microbial efficiency of 10% (NRC, 2000; Model 1). The second factor was the frequency at which SBM was offered to the animals: daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**).

Within each level of supplementation, the amount of SBM provided was the same over a 10-d period. To minimize bias because of potential BW changes resulting from treatment regimens during each period, the quantity of supplement provided during each period was based on the initial BW at the beginning of the experiment. The SBM was added directly into the rumen via the ruminal cannula of each steer (0700 hours) according to the specific supplementation level and SF. Also, all steers received 57 g/d of a trace mineralized salt mix (7.3% Ca, 7.2% P, 27.8% Na, 23.1% Cl, 1.5% K, 1.7% Mg, 0.5% S, 2,307 ppm Mn, 3,034 ppm Fe, 1,340 ppm Cu, 3,202 ppm Zn, 32 ppm Co, 78 ppm I, 90 ppm Se, 79 IU/kg vitamin E, and 397 kIU/kg vitamin A; Cattleman's Choice, Performix Nutrition Systems, Nampa, ID) directly through the rumen cannula. Steers were provided continuous access to fresh water and chopped (4 to 8 cm; BC-900; Newhouse Manufacturing, Redmond, OR) grass seed straw (Chewings Fescue; Table 1). Grass seed straw was provided daily (0710 hours) at 120% of the average intake for the previous 5 d, with feed refusals from the previous day determined before the supplement feeding. An intramuscular injection of vitamins A, D, and E (500,000, 50,000, and 1500 IU of vitamins A, D, and E, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each steer at the onset of the trial to safeguard against deficiency.

### Sampling

Experimental periods lasted 30 d and a minimum of 3 d, but no more than 5 d, were allowed between periods where steers were removed from individual pens and placed in a common outdoor pen (22 × 34 m). Between periods, steers were provided ad libitum access to the same grass seed straw used during experimental periods. Intake was measured from days 19 to 28 of each experimental period with subsamples of straw and SBM (~150 g/d as-fed) obtained daily during this time. Orts were measured and subsampled (5% of total daily refusal; as-fed basis) from days 20 to 29. Samples of grass seed straw, orts, and SBM were dried at 55 °C for 48 hr, reweighed for calculation of DM, ground in a Wiley mill (1-mm screen), and composited by source for grass seed straw and SBM and by steer for orts. In addition, fecal grab samples (100 g) were collected at 0800, 1600, and 2400 hours from days 21 through 30, composited by steer and stored (–20 °C) until further analysis. Fecal samples were

**Table 1.** Nutrient content of the feedstuffs used in the present experiment

| Item | Grass seed straw[1] | Soybean meal |
|---|---|---|
| *Nutrient composition, %DM* | | |
| OM | 92.6 | 92.2 |
| CP | 2.9 | 51.4 |
| NDF | 79.5 | 15.0 |
| ADF | 46.7 | 4.8 |
| IADF | 24.7 | 1.6 |

[1]Chewings fescue.

dried in a forced-air oven at 55 °C for 96 hr and ground in a Wiley mill (1-mm screen).

On days 11 (day when all animals were supplemented, except for CON) and 20 (day when only D supplements were provided; day prior to supplementation for the 5D and 10D treatments), treatment effects on ruminal DM and indigestible-ADF (**IADF**) fill were determined by manually removing reticulorumen contents 4 hr after feeding. Total rumen contents were weighed, manually mixed, and subsampled in triplicate (~400 g/triplicate). The remaining rumen contents were replaced immediately into the animal. Ruminal samples were weighed, dried in a forced-air oven at 55 °C for 96 hr, reweighed in order to calculate DM, ground to pass a 1-mm screen in Wiley mill, and composited within period and day by steer.

Ground samples were analyzed for DM and organic matter (OM; AOAC, 1996), N (Leco TruMac CN Leco Corp., St. Joseph, MI), NDF (Robertson and Van Soest, 1981), and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 fiber analyzer (Ankom Co., Fairport, NY). Moreover, samples were analyzed for IADF as described by Bohnert et al. (2002c). Digesta kinetics techniques described by Van Soest (1982) were used to determine IADF passage by dividing IADF intake by the quantity of IADF in the rumen 4 hr after feeding. Diet digestibility was determined using IADF fecal concentration in conjunction with nutrient concentration of grass seed straw, orts, and SBM (Merchen, 1988).

On days 21 (day when all supplemented treatments received supplement) and 30 (day when only D supplements were offered), each steer was intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 200-mL aqueous solution at 0700 hours (Uden et al., 1980). The Co marker was administered throughout the rumen by injecting through a stainless-steel probe with a perforated tip. Ruminal fluid (~100 mL) was collected by suction strainer (Raun and Burroughs, 1962) immediately prior to dosing (0 hr) and at 3, 6, 9, 12, 18, and 24 hr after dosing. Ruminal pH was measured immediately after each collection (Orion SA 520, American Instrument Exchange Inc., Haverhill, MA). Following the collection, 20 mL of the ruminal fluid were immediately stored (–20 °C) for later analysis of Co concentration and an additional 5 mL acidified with 1 mL of 25% (w/v) meta-phosphoric acid and stored (–20 °C) for subsequent analysis of ruminal ammonia N (**$NH_3$**). Frozen (–20 °C) ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × *g* for 10 min at room temperature for volatile fatty acids (VFA) and $NH_3$; 2,000 × *g* for 30 min at 4 °C for Co), and collecting the supernatant. Ruminal $NH_3$ was analyzed by a modification (sodium salicylate substituted for phenol) of the procedure described by Broderick and Kang (1980), while VFA were analyzed as previously described by Harmon et al. (1985). Ruminal Co concentrations were analyzed by atomic absorption using an air-acetylene flame (Model 351 AA/AE Spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA). Ruminal liquid fill and liquid dilution rate were estimated by regression of the natural logarithm of Co concentration against sampling time as previously described by Warner and Stacy (1968).

Blood samples were collected daily, 4 hr after feeding, on days 21 through 30 for determination of plasma-urea N (**PUN**). Samples were obtained via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10-mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 USP units of freeze-dried sodium heparin. All blood samples were placed immediately on ice for transport to the lab, subsequently centrifuged (2,500 × *g* for 30 min; 4 °C) for plasma harvest and stored at –80 °C on the same day of collection. Plasma urea-N concentrations were determined using a quantitative colorimetric kit (#B7551; Pointe Scientific, Inc., Canton, MI). The intra- and interassay CV were, respectively, 5.27% and 9.61%.

### Statistical analysis

Data were analyzed as an incomplete 7 × 4 Latin square using the PROC MIXED procedure of SAS (version 9.4; SAS Inst., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model included treatment and period as independent variables. Steer was used as a random variable. 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 included were: (1) CON vs. protein supplementation, (2) F vs. H of estimated RDP requirement, (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. Daily DMI, ruminal pH, $NH_3$, VFA, and PUN data, collected at the fixed times, were analyzed using the repeated statement with the PROC MIXED procedure of SAS (version 9.4; SAS Inst.). The model statement contained the effects of treatment, hour (pH, $NH_3$, and VFA) or day (DMI and PUN), as well as the resultant interaction, and period as an independent variable. Steer was used as the random variable. The specified term for the repeated statement was hour or day, whereas steer(period × treatment) was included as the subject. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion, and hence the best fit for the variables analyzed. The same contrasts denoted above were used to partition treatment sums of squares. All results are reported as least square means, whereas significance was set at $P \leq 0.05$ and tendencies were denoted if $0.05 < P \leq 0.10$.

## Results

### Nutrient intake and digestibility

Treatment × day interactions ($P \leq 0.09$) were observed for forage and total 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 add in interpretation and discussion of the observed response. Supplemental SBM increased forage and total DM and OM intake, as well as intake of NDF, IADF, and N compared to nonsupplemented cohorts ($P \leq 0.04$; Table 2). Interestingly, we noted, for each of the aforementioned variables, an interaction between the linear effect of SF and the amount of supplement provided ($P \leq 0.04$; Table 2). For each variable, as SF decreased from daily to once every 10 d, intake decreased roughly by 15% for the F treatments, whereas little to no change was observed for H treatments. This response is primarily attributed to the observed reduction in forage DMI 2 d following supplementation for the F10D treatment (Figure 1).

Apparent gastrointestinal tract DM digestibility tended to be greater ($P = 0.10$) and N digestibility increased ($P < 0.01$) for supplemented steers, whereas OM and NDF digestibility were not altered by supplemental CP ($P \geq 0.64$; Table 2). Offering the F amount of supplement improved N digestibility 29% compared with the H treatments (Table 2); however, no difference ($P \geq 0.52$)

**Table 2.** Intake, apparent digestibility, and plasma urea-N in steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in differing amounts [**F** = 100% of estimated RDP requirement and **H** = 50% of F][1]

| | Treatments | | | | | | | | 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 | 16.1 | 19.6 | 19.2 | 16.2 | 17.6 | 17.0 | 17.5 | 0.75 | 0.03 | 0.08 | 0.02 | 0.52 | 0.02 | 0.14 |
| Supplement | 0.00 | 1.33 | 1.33 | 1.33 | 0.67 | 0.67 | 0.67 | | | | | | | |
| Total | 16.1 | 21.0 | 20.5 | 17.6 | 18.3 | 17.7 | 18.1 | 0.75 | <0.01 | 0.01 | 0.02 | 0.52 | 0.02 | 0.14 |
| *OM intake, g/kg BW* | | | | | | | | | | | | | | |
| Forage | 15.1 | 18.2 | 17.8 | 15.1 | 16.3 | 15.8 | 16.2 | 0.67 | 0.04 | 0.07 | 0.01 | 0.47 | 0.02 | 0.13 |
| Supplement | 0.00 | 1.23 | 1.23 | 1.23 | 0.62 | 0.62 | 0.62 | | | | | | | |
| Total | 15.1 | 19.4 | 19.0 | 16.3 | 16.9 | 16.4 | 16.8 | 0.67 | <0.01 | 0.01 | 0.01 | 0.47 | 0.02 | 0.13 |
| *NDF intake, g/kg BW* | 12.7 | 15.8 | 15.4 | 13.0 | 14.0 | 13.5 | 13.9 | 0.61 | 0.02 | 0.05 | 0.01 | 0.54 | 0.03 | 0.13 |
| *IADF intake, g/kg BW* | 4.0 | 4.9 | 4.8 | 4.1 | 4.4 | 4.2 | 4.4 | 0.18 | 0.01 | 0.04 | 0.01 | 0.38 | 0.02 | 0.10 |
| *N intake, g/kg BW* | 0.078 | 0.204 | 0.202 | 0.188 | 0.140 | 0.138 | 0.139 | 0.004 | <0.01 | <0.01 | 0.02 | 0.41 | 0.04 | 0.22 |
| *Digestibility, %* | | | | | | | | | | | | | | |
| DM | 45.0 | 45.8 | 44.7 | 48.6 | 45.7 | 45.1 | 47.3 | 0.86 | 0.10 | 0.57 | 0.01 | <0.01 | 0.39 | 0.34 |
| OM | 49.3 | 48.9 | 48.1 | 51.9 | 48.8 | 48.6 | 50.1 | 0.95 | 0.87 | 0.52 | 0.03 | 0.05 | 0.33 | 0.36 |
| NDF | 48.2 | 47.5 | 46.2 | 49.0 | 47.8 | 47.4 | 48.5 | 1.03 | 0.64 | 0.68 | 0.26 | 0.12 | 0.69 | 0.48 |
| N | 3.6 | 49.4 | 49.5 | 55.1 | 37.5 | 39.2 | 43.0 | 1.88 | <0.01 | <0.01 | 0.01 | 0.27 | 0.97 | 0.62 |
| *Plasma urea-N, mg/dL* | 6.0 | 11.8 | 10.7 | 13.6 | 9.7 | 9.7 | 10.2 | 0.72 | <0.01 | <0.01 | 0.10 | 0.07 | 0.38 | 0.16 |

[1]This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control.
[2]CON vs. Supp, non-supplemented treatment vs. protein supplemented treatments; F vs. H, 100% of estimated RDP requirement vs. 50% of estimated RDP requirement; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. amount, linear effect of SF × supplementation amount; Q vs. amount, quadratic effect of SF × supplementation amount.

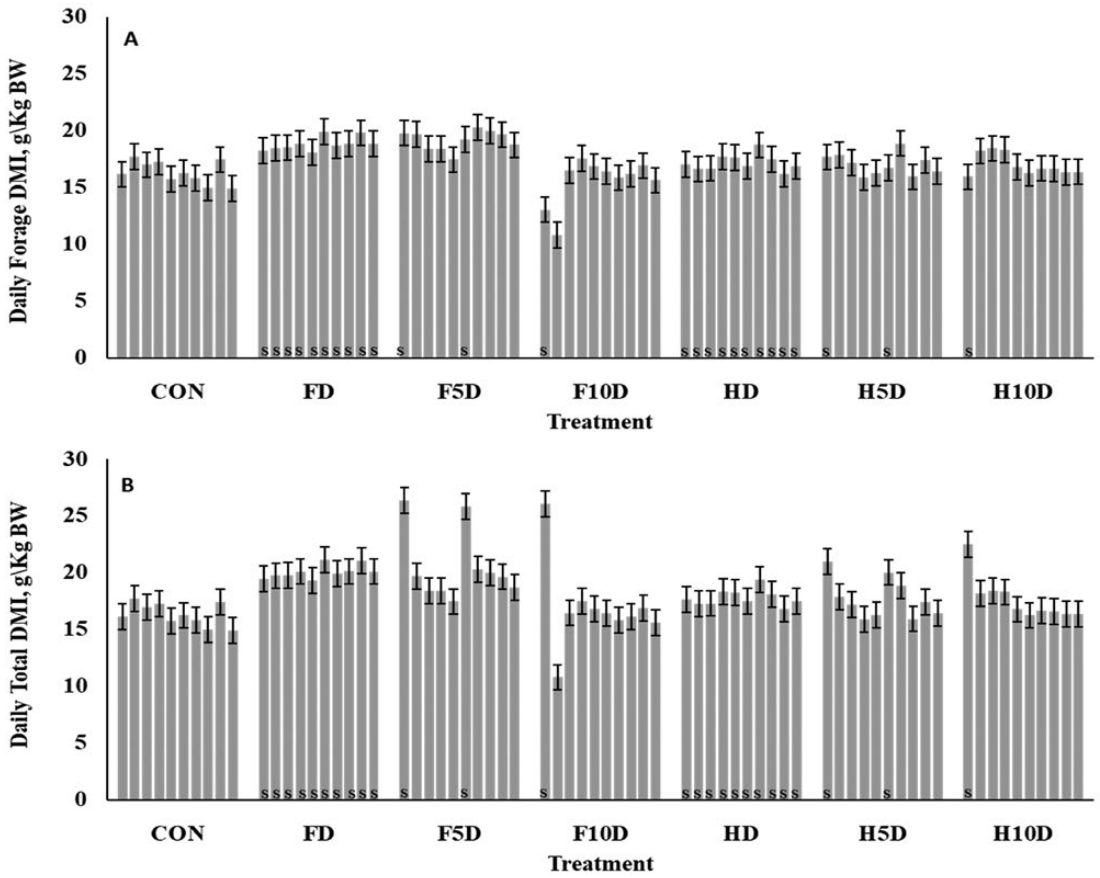


**Figure 1.** Daily forage (**A**) and total (**B**) DMI variation in steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in differing amounts [**F** = 100% of estimated rumen degradable protein (RDP) requirement and **H** = 50% of F]. This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control. Columns for each treatment represent, left to right, forage DMI from days 1 through 10 of the supplementation period; S = supplementation. A treatment × day interaction for daily forage DMI ($P = 0.09$; SEM = 1.12) and total DMI ($P < 0.01$; SEM = 1.13).

was observed in DM, OM, or NDF digestibility due to amount of supplement provided. Changing SF altered digestibility of DM, OM, and N, so that DM and OM increased quadratically, and N linearly increased, as the time between supplementation events increased ($P \leq 0.05$; Table 2). No SF × amount interactions were observed for DM, OM, NDF, and N digestibility ($P \geq 0.33$; Table 2).

Treatment × day interactions ($P \leq 0.01$) were observed for PUN 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 PUN data (Figure 2) would add in interpretation of the observed response. In agreement with N intake and digestibility data, PUN increased with supplementation (6.0 vs. 11.0 mg/dL; $P < 0.01$), being 21% greater for F vs. H ($P < 0.01$), and tended to increase quadratically as SF decreased ($P = 0.07$). No interaction was observed for SF × supplement amount ($P \geq 0.16$).

## Rumen fill and passage rate

Ruminal IADF fill was not altered for supplemented vs. CON steers ($P \geq 0.20$), but we did note that IADF fill was greater for F vs. H-supplemented animals on the day all supplements were provided and when only daily supplements were provided ($P \leq 0.02$; Table 3). Similarly, ruminal IADF passage rate was not affected by treatments on the day all supplemented steers were provided supplement ($P \geq 0.31$), but increased as a result of CP supplementation on the day that only daily supplements were provided ($P = 0.03$).

Compared with nonsupplemented controls, ruminal liquid fill increased with supplementation ($P < 0.01$) on the day all supplements were provided (Table 3). We also noted a linear effect of SF × supplement amount interaction ($P = 0.03$), in which liquid fill increased by 37% as supplementation interval increased from daily to 10 d for F treatments but only 14% for H treatments. Conversely, no supplementation or SF effects were observed for liquid fill on the day that only daily supplements were provided ($P \geq 0.10$).

In contrast to fill, ruminal liquid dilution rate was similar ($P \geq 0.62$) between supplemented and nonsupplemented steers when all supplements were provided, but dilution rate was greater for supplemented steers on the day when only daily supplements were provided ($P = 0.03$; Table 3). However, on the day all supplements were provided, ruminal dilution rate linearly decreased by 56% for the steers receiving F supplement as SF decreased from D to 10D, while H treatments decreased <6% ($P < 0.01$; SF × amount interaction) for the same time frame.

## Rumen fermentation characteristics

On the day when all supplements were provided, no time by treatment interaction was noted for ruminal pH ($P > 0.05$);

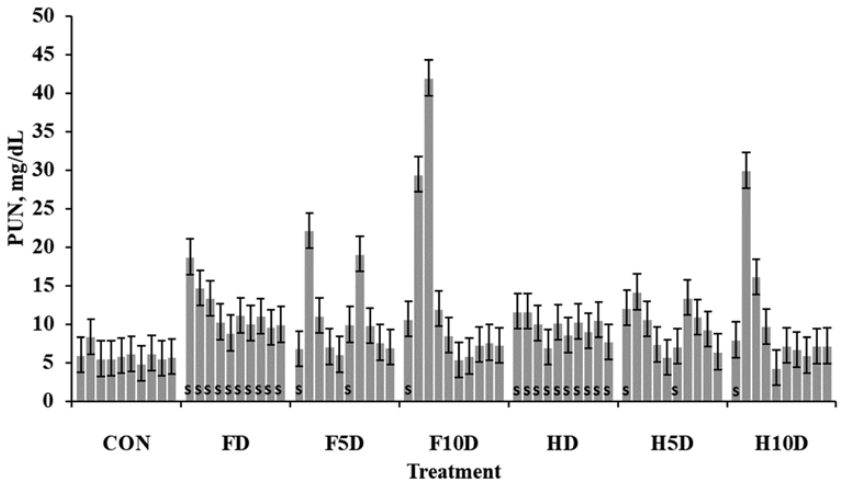


**Figure 2.** Daily plasma urea-N (**PUN**) concentration in beef steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in differing amounts [**F** = 100% of estimated rumen degradable protein (RDP) requirement and **H** = 50% of F]. This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control. Columns for each treatment represent, from left to right, PUN 4 hr after feeding from days 1 through 10 of the DMI measurement period; S = supplementation. A treatment × day interaction was detected ($P < 0.01$; SEM = 2.29).

therefore, only treatment means will be discussed. Ruminal pH tended to decrease ($P = 0.06$; Table 4) with supplementation vs. CON cohorts, while F steers also had a reduced ruminal pH vs. H ($P = 0.03$). Moreover, as the supplementation interval increased from daily to once every 10 d, ruminal pH decreased ($P < 0.01$).

On the day when all supplements were provided, a treatment × time interaction was detected for ruminal $NH_3$ ($P > 0.01$); however, after considering the nature of the interaction we believe discussing treatment means while providing the time × treatment figure would add in interpretation of the data. Ruminal ammonia (mM) was greater ($P < 0.01$) for supplemented vs. nonsupplemented cohorts on the day all supplements were provided (Table 4; Figure 3). Moreover, as SF decreased from D to 10D, ruminal ammonia increased 9.0 mM for F but only 4.9 mM for H treatments ($P = 0.02$).

With the exception of isobutyrate ($P = 0.03$), no treatment × hour interactions were observed ($P > 0.05$) for any of the VFA evaluated in the current study on the day all supplements were provided. As with ruminal $NH_3$, we concluded that discussing isobutyrate treatment means would facilitate interpretation and discussion of the data while still providing an effective understanding of the overall treatment effects. Total VFA and molar proportions of propionate, butyrate, isobutyrate, and isovalerate were greater ($P \leq 0.04$) for supplemented animals, while acetate and acetate:propionate ratio decreased ($P < 0.01$; Table 4). In addition, total VFA and molar proportions of propionate and valerate linearly increased ($P \leq 0.02$), whereas acetate:propionate ratio linearly decreased ($P < 0.01$) as SF decreased. As the amount of supplement fed increased, the molar proportion of propionate also increased ($P = 0.01$), decreasing the acetate:propionate ratio ($P = 0.01$). We also noted a tendency for an interaction for acetate linearly decreasing as SF decreased, the reduction being greater for F vs. H (7 vs. 4%, respectively; $P = 0.06$). Conversely, butyrate linearly increased as SF decreased, resulting in a 25% and 9% increase for F and H, respectively ($P < 0.01$).

On the day that only daily supplements were provided, no treatment × hour interactions ($P \geq 0.16$) were noted for pH, $NH_3$, total VFA, or molar proportions of the individual VFA (Table 5). Ruminal pH was not altered by supplementation amount or frequency ($P \geq 0.34$), whereas ruminal $NH_3$ concentration tended ($P = 0.09$) to decrease less as SF decreased from D to 10D for H compared with F (27 and 60%, respectively; Table 5).

Total VFA concentration did not differ among CON and supplemented cohorts on the day that only D supplements were provided ($P = 0.80$; Table 5); however, it did decrease quadratically as SF decreased, with D and 10D treatments presenting the greatest total VFA concentrations ($P = 0.02$). Molar proportions of acetate, propionate butyrate, valerate, isovalerate, and acetate:propionate ratio were not affected by supplementation ($P \geq 0.11$; Table 5), while isobutyrate tended to increase with CP supplementation ($P = 0.09$). Interactions (linear effect of SF and quantity of supplement) were noted for acetate ($P = 0.01$) and butyrate ($P = 0.05$). Briefly, acetate increased and butyrate decreased as SF decreased, but to a greater extent with F vs. H. The branch chain VFA isobutyrate, valerate, and isovalerate linearly decreased ($P < 0.01$) as SF decreased for supplemented treatments.

## Discussion

### Nutrient intake and digestibility

Economical nutritional strategies that promote or maintain nutrient intake and ruminal function of ruminants consuming low-quality forages are warranted. Such strategies might include the reduced frequency by which protein supplements are offered (Farmer et al., 2004) and/or providing reduced amounts of these supplements to the animals. Based on this rationale, we conducted a series of experiments to evaluate nutrient intake and ruminal response of steers consuming low-quality, cool-season forage while receiving differing amounts of a supplement as infrequently as once every 10 d. The present experiment compared intake, digestibility, and ruminal fermentation parameters in rumen-fistulated steers receiving SBM supplements at 3 SF and 2 amounts, while an experiment reported in a companion paper (Cappellozza et al., 2021) evaluated performance of pregnant beef cows in the last

**Table 3.** Ruminal liquid and particulate dynamics in beef steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in different amounts [**F** = 100% of estimated rumen degradable protein (RDP) requirement and **H** = 50% of F)[1]

| Item | Treatments | | | | | | | | Contrasts[2] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CON | FD | F5D | F10D | HD | H5D | H10D | SEM | CON vs. Supp | F vs. H | SF L | SF Q | L vs. Amount | Q vs. Amount |
| *All supplements provided*[3] | | | | | | | | | | | | | | |
| IADF fill, g/kg BW | 9.0 | 10.2 | 10.7 | 9.9 | 9.5 | 8.9 | 8.9 | 0.45 | 0.20 | <0.01 | 0.30 | 0.63 | 0.77 | 0.25 |
| IADF passage rate, % | 1.92 | 2.03 | 1.97 | 1.87 | 2.02 | 2.18 | 1.99 | 0.126 | 0.50 | 0.31 | 0.44 | 0.38 | 0.58 | 0.48 |
| Liquid fill, mL/kg BW | 255 | 262 | 274 | 360 | 276 | 289 | 314 | 12.5 | <0.01 | 0.60 | <0.01 | 0.06 | 0.03 | 0.17 |
| Liquid dilution rate, %/h | 7.5 | 9.2 | 8.7 | 4.0 | 7.2 | 7.0 | 6.8 | 0.65 | 0.62 | 0.50 | <0.01 | 0.05 | <0.01 | 0.04 |
| *Only daily supplements provided*[3] | | | | | | | | | | | | | | |
| IADF fill, g/kg BW | 9.7 | 10.9 | 10.1 | 10.2 | 9.5 | 9.1 | 9.0 | 0.58 | 0.85 | 0.02 | 0.34 | 0.55 | 0.88 | 0.75 |
| IADF passage rate, % | 1.54 | 1.92 | 2.01 | 1.77 | 1.86 | 1.91 | 1.97 | 0.144 | 0.03 | 0.90 | 0.92 | 0.52 | 0.38 | 0.52 |
| Liquid fill, mL/kg BW | 256 | 264 | 244 | 284 | 249 | 278 | 247 | 20.4 | 0.82 | 0.71 | 0.64 | 0.98 | 0.58 | 0.10 |
| Liquid dilution rate, %/h | 7.9 | 10.8 | 10.3 | 9.0 | 9.6 | 8.6 | 9.2 | 0.65 | 0.03 | 0.11 | 0.12 | 0.71 | 0.26 | 0.27 |

[1]This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control.
[2]CON vs. Supp, nonsupplemented treatment vs. protein supplemented treatments; F vs. H, 100% of estimated RDP requirement vs. 50% of estimated RDP requirement; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. amount, linear effect of SF × supplementation amount; Q vs. amount, quadratic effect of SF × supplementation amount.
[3]Rumen samplings were performed on a day all supplements were provided and on the day when only D supplements were provided (day immediately prior to supplementation for the 5D and 10D treatments).

trimester of gestation, as well as intake and efficiency of N use in wethers utilizing the same treatments described herein.

The observed improvement on intake of DM and other nutrients is comparable to other work demonstrating that increasing supplemental CP to ruminants consuming low-quality forage often increases forage and overall DMI (DelCurto et al., 1990; Köster et al. 1996; Wickersham et al., 2008). Furthermore, research from our group (Bohnert et al., 2002a; Bohnert et al., 2002b) and others (Beaty et al., 1994; Schauer et al., 2010) reported that forage DMI is decreased as SF is decreased. Bohnert et al. (2002a) and Schauer et al. (2010) noted that as the amount of supplement offered at each supplementation event increased, a corresponding reduction in forage DMI was observed on the day(s) following supplementation. Others have reported no influence of decreasing SF on forage DMI (Huston et al., 1999; Krehbiel et al., 1998; Wickersham et al., 2008). DMI was not affected when ewes consuming wheat straw were supplemented with cottonseed meal daily or once every 7 d (Huston et al., 1999). Krehbiel et al. (1998) and Wickersham et al. (2008) also reported no change in forage DMI in ruminants offered supplemental CP daily or once every 3 d. It is not clear the reason for these conflicting results. A possible explanation not evaluated in the current study is timing of supplementation. Work with grazing dairy cows has suggested that when a supplement is provided during the day (a.m. vs. p.m.), it can alter grazing behavior and marginal milk production (Sheahan et al., 2013); nevertheless, Barton et al. (1992) noted no effect of timing of supplementation on DMI or nutrient digestibility by steers grazing dormant intermediate wheatgrass. Interestingly, when we calculated the supplemental N intake per supplementation event for each of the studies, as well as the current one and our companion paper (Cappellozza et al., 2021), the data suggest that when supplemental N was offered at a supplementation rate ≥ 0.6 g/kg BW in cattle and ≥ 1.0 g/kg BW in sheep, forage DMI is depressed. As an example, in the current study, N intake at each supplementation event for D, 5D, and 10D was 0.06, 0.30, and 0.60 g/kg BW for H treatments and 0.11, 0.55, and 1.10 g/kg for F treatments, respectively. Moreover, these data corroborate with the linear SF × supplement amount interaction observed for forage and total DMI.

The low N digestibility for the nonsupplemented treatment (3.6%) is indicative of the high fiber and low CP of the forage used in the current study (Table 1). This should result in a significant proportion of N in the feces being metabolic fecal N. Supporting this statement, Ferrell et al. (1999) calculated metabolic fecal N from lambs consuming low-quality forage (4.3% CP, 74% NDF) and estimated that 90% to 105% of observed fecal N loss was attributed to metabolic fecal N. Based on these results, the authors suggested that caution should be used when trying to interpret apparent N digestibility when ruminants are consuming low-quality forage (Ferrell et al., 1999). The lack of a more pronounced supplementation effect on DM, OM, and NDF digestibility, although comparable results have been noted by other researchers (Schauer et al., 2005; Sawyer et al., 2012), was unexpected given that greater nutrient digestibility following a CP supplementation has been demonstrated in numerous studies with ruminants consuming low-quality forage (Bohnert et al., 2002a; Bohnert et al., 2002b; Currier et al., 2004a; Wickersham et al., 2008). We used a low-quality, cool-season forage in the current study and it should be noted that Bohnert et al. (2011) compared protein supplementation of cool-season and warm-season forages and reported that supplementation allowed for a greater increase in apparent digestibility of DM and OM for the warm-season forage compared with the cool-season forage,

**Table 4.** Ruminal fermentation characteristics in beef steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in different amounts [**F** = 100% of estimated RDP requirement and **H** = 50% of F] on a day when all supplements were offered[1]

| | Treatments | | | | | | | Contrasts[2] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON | FD | F5D | F10D | HD | H5D | H10D | SEM | CON vs.Supp | F vs.H | SF L | SF Q | L vs. amount | Q vs. amount |
| pH | 6.74 | 6.66 | 6.61 | 6.39 | 6.81 | 6.65 | 6.55 | 0.075 | 0.06 | 0.03 | <0.01 | 0.61 | 0.96 | 0.26 |
| Ammonia, mM | 1.4 | 2.2 | 7.6 | 11.2 | 2.4 | 3.1 | 7.3 | 0.78 | <0.01 | <0.01 | <0.01 | 0.52 | 0.02 | 0.07 |
| Total VFA, mM | 90.0 | 86.7 | 111.4 | 121.8 | 87.2 | 103.3 | 106.6 | 5.34 | 0.04 | 0.10 | <0.01 | 0.16 | 0.16 | 0.92 |
| *VFA, mol/100 mol* | | | | | | | | | | | | | | |
| Acetate | 88.0 | 86.4 | 82.6 | 80.3 | 86.2 | 84.8 | 82.8 | 0.67 | <0.01 | 0.01 | <0.01 | 0.70 | 0.06 | 0.34 |
| Propionate | 20.8 | 21.2 | 22.5 | 24.5 | 20.5 | 21.6 | 22.7 | 0.48 | 0.02 | 0.01 | <0.01 | 0.72 | 0.27 | 0.70 |
| Butyrate | 8.0 | 8.0 | 9.0 | 10.0 | 8.1 | 8.6 | 8.8 | 0.20 | <0.01 | <0.01 | <0.01 | 0.71 | <0.01 | 0.65 |
| Iso-butyrate | 1.20 | 1.44 | 1.77 | 1.47 | 1.65 | 1.48 | 1.66 | 0.135 | 0.02 | 0.73 | 0.89 | 0.56 | 0.96 | 0.05 |
| Valerate | 0.97 | 1.35 | 2.02 | 1.89 | 1.66 | 1.75 | 1.9 | 0.152 | <0.01 | 0.89 | 0.02 | 0.18 | 0.32 | 0.12 |
| Iso-valerate | 1.03 | 1.64 | 2.17 | 1.85 | 1.92 | 1.70 | 2.08 | 0.266 | 0.01 | 0.96 | 0.49 | 0.78 | 0.94 | 0.13 |
| Acetate:propionate | 4.3 | 4.1 | 3.7 | 3.3 | 4.2 | 4.0 | 3.8 | 0.12 | <0.01 | 0.01 | <0.01 | 0.76 | 0.23 | 0.92 |

[1]This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control.
[2]CON vs. Supp, nonsupplemented treatment vs. protein supplemented treatments; F vs. H, 100% of estimated RDP requirement vs. 50% of estimated RDP requirement; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. amount, linear effect of SF × supplementation amount; Q vs. amount, quadratic effect of SF × supplementation amount.

suggesting forage type can alter the response in digestibility associated with protein supplementation.

Beaty et al. (1994) supplemented beef cows consuming low-quality forage daily or 3 times per week and reported that as the time between supplementation events increased, DM digestibility increased from 50% to 54%. Nevertheless, Schauer et al. (2010) noted no effect on DM, OM, NDF, or N digestibility as SF decreased over 3 supplementation intervals (daily, 5 d, and 10 d). Moreover, total tract OM and NDF digestibility was not affected in ruminants offered different levels of supplemental CP at different frequencies (Wickersham et al., 2008). In the companion paper (Cappellozza et al., 2021), a linear effect of SF × supplementation amount was detected for DM, OM, and NDF digestibility in lambs receiving the same treatment design described herein, with nutrient digestibility increasing as SF decreased for the F treatments only. Therefore, it may be speculated that the animals receiving infrequent CP supplementation herein and in Cappellozza et al. (2021), experienced a substitution effect as SF decreased resulting in a decrease in forage DMI and a subsequent improvement in overall nutrient digestibility directly related to intake of the CP supplement.

## Rumen fill and passage rate

Rumen fermentation dynamics are greatly influenced by supplement type and amount (Olson et al., 1999). Our work agrees with past research showing that CP supplementation has little to no effect on ruminal particulate fill compared with non-supplemented controls (Krysl et al., 1989; Olson et al., 1999; Weder et al., 1999). Additionally, the observed decrease in ruminal IADF fill on both measurement days for F vs. H is most likely due to a combination of IADF intake (Table 2) and the provision of supplement. Besides the fact that IADF concentration of the supplement is extremely low (Table 1), supplement IADF likely did not have a suitable opportunity for significant digestion when ruminal evacuations were performed at 4 hr postsupplementation. Regarding IADF passage rate, our results favorably agree with past work from our research group that compared CP supplements with different ruminal degradability and 3 supplementation intervals (daily and once every 3 or 6 d; no differences noted when all supplements were provided but a supplementation effect when only daily supplements provided) to steers consuming low-quality forage (Bohnert et al., 2002b). Moreover, Beaty et al. (1994) supplemented steers either daily or 3 times per week and reported no effect of SF on IADF passage rate. It is worth noting that varying CP quantity, degradability, and/or concentration has been suggested as a likely rationale for altering digesta outflow from the rumen, as well as the quantity of digesta retained in the rumen (Olson et al., 1999).

The response for ruminal liquid fill concurs with a previous study conducted by our research group (Bohnert et al., 2002b) reporting that steers receiving a highly rumen degradable supplement (SBM) had 28% greater ruminal liquid fill as SF decreased from daily to once every 6 d on the day when all supplements were provided. Bohnert et al. (2002b) also evaluated a ruminal undegradable protein (**RUP**) supplement and reported that ruminal liquid fill was not affected over the same supplementation interval and time period, indicating that the utilization of an RUP supplement may have allowed for more consistent ruminal fermentation when the greater quantities of supplement were provided on each supplementation event. On the other hand, previous research reported the lack of a supplementation effect on ruminal liquid volume when only

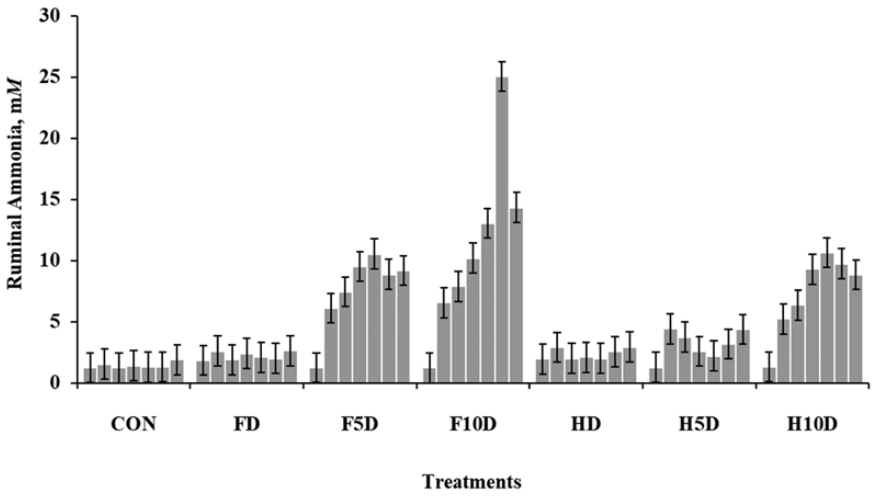


**Figure 3.** Ruminal ammonia in beef steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in differing amounts [**F** = 100% of estimated rumen degradable protein (RDP) requirement and **H** = 50% of F] on a day when all supplements were offered. This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control. Columns for each treatment represent, from left to right, ruminal fluid samples collected immediately before SBM was provided (0 hr) and at 3, 6, 9, 12, 18, and 24 hr after SBM was provided. A treatment × hour interaction was detected ($P < 0.01$; SEM = 1.222).

daily supplements were fed (Bohnert et al., 2002b; Currier et al., 2004b; Klein et al., 2015).

CP supplementation often increases rumen fluid dilution rate (Köster et al., 1996; Olson et al., 1999; Bodine et al., 2000). More specifically, Bohnert et al. (2002c) offered a CP supplement daily, once every 3, or once every 6 d, and reported that supplementation increased dilution rate compared with nonsupplemented controls, in agreement with our data on the day only daily supplements were provided. A likely explanation for the lack of consistent results when all supplements were provided in the current study might include the amount of supplement offered for 5D and 10D, which, as previously noted, may have disrupted normal ruminal fermentation for a period of time and, in turn, increased ruminal liquid fill and decreased ruminal liquid dilution rate. Other studies from our research group seem to support this statement, as no differences were observed on ruminal liquid fill and dilution rate on the day urea or SBM supplements were offered to beef animals consuming a low-quality, cool-season forage (Cappellozza et al., 2013). Additionally, previous studies demonstrated that the source of the N supplement might play a role by impacting rumen function (Olson et al., 1999), even when supplements were offered in equal amounts (as % of BW; Cappellozza et al., 2013).

## Rumen fermentation characteristics

Although supplementation and SF effects were observed on rumen pH, at no sampling point during our measurement did ruminal pH drop below 6.0 (data not shown), therefore staying within the range suggested by Yokoyama and Johnson (1988) as adequate to maintain ruminal fiber digestion and support cellulose digestion. The results for ruminal pH on the day all supplements were provided is consistent with other research evaluating ruminal fermentation with supplementation intervals greater than 3 d (linear reduction in pH as SF increased; Bohnert et al., 2002c; Farmer et al., 2001), likely due to the quantity of supplement provided at each supplementation event and the consequent effects on microbial fermentation.

As expected, CP supplementation increased ruminal $NH_3$ concentrations and is supported by the PUN data herein and with many other studies demonstrating an improved ruminal N status when supplemental protein was fed to ruminants consuming a low-quality forage (DelCurto et al., 1990; Bohnert et al., 2002c; Cappellozza et al., 2013). Moreover, the different $NH_3$ concentrations among F and H might be attributed to a direct result of the supplement amount provided at each supplementation event, as verified by the similarity between ammonia concentrations for F5D and H10D (Figure 3), which had the same CP intake, but at different SF. The mechanisms by which infrequent supplementation of CP is a feasible alternative for ruminants consuming low-quality forages may be due to increased N recycling, a lag in peak ruminal ammonia, and prolonged elevation of ammonia (Bohnert et al., 2002c; Currier et al., 2004b; Farmer et al., 2004). Likewise, infrequent CP supplementation has not resulted in a negative effect on N utilization, which may be attributed to the recycling of PUN into the rumen, thereby helping to maintain ruminal N availability for microbial protein synthesis on the days between supplementation events (Krehbiel et al., 1998; Archibeque et al., 2007).

An observed slower drop in $NH_3$ for H vs. F as SF decreased was most likely due, as noted above in the discussion on the day all supplements were provided, to the different amount of supplement provided for the F and H treatments. To the best of our knowledge, the current study (SF of daily, 5 d, and 10 d) and Bohnert et al. (2002c; SF of daily, 3 d, and 6 d) are the only CP supplementation studies to evaluate ruminal ammonia concentrations with SF of greater than 5 d. It may be argued that ruminal ammonia concentrations of infrequently supplemented treatments appear to decrease to a level comparable to non-supplemented treatments ~4 to 5 d following a supplementation event, suggesting that an SF of 5 to 6 d may be the maximal interval to maintain elevated ruminal ammonia concentrations in ruminants consuming low-quality forage.

The PUN data we reported and discussed earlier, along with Schauer et al. (2010), provides further evidence for this rationale. Additionally, the large quantity of CP provided at each supplementation event with infrequent supplementation

**Table 5.** Ruminal fermentation characteristics in beef steers consuming low-quality, cool-season forage and receiving or not (**CON**) SBM daily (**D**), once every 5 d (**5D**), or once every 10 d (**10D**) in different amounts [**F** = 100% of estimated rumen degradable protein (RDP) requirement and **H** = 50% of F] on a day when only D supplements were offered[1]

| | Treatments | | | | | | | Contrasts[2] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON | FD | F5D | F10D | HD | H5D | H10D | SEM | CON vs. Supp | F vs. H | SF L | SF Q | L vs. amount | Q vs. smount |
| pH | 6.75 | 6.72 | 6.87 | 6.80 | 6.80 | 6.82 | 6.86 | 0.072 | 0.39 | 0.63 | 0.34 | 0.43 | 0.87 | 0.36 |
| Ammonia, mM | 1.1 | 2.0 | 1.2 | 0.8 | 1.5 | 1.2 | 1.1 | 0.24 | 0.43 | 0.64 | <0.01 | 0.38 | 0.09 | 0.81 |
| Total VFA, mM | 98.4 | 107.3 | 88.2 | 99.5 | 101.1 | 92.8 | 93.9 | 4.44 | 0.80 | 0.51 | 0.11 | 0.02 | 0.94 | 0.19 |
| *VFA, mol/100 mol* | | | | | | | | | | | | | | |
| Acetate | 86.2 | 83.4 | 86.4 | 87.3 | 85.4 | 86.5 | 86.6 | 0.53 | 0.60 | 0.24 | <0.01 | 0.08 | 0.01 | 0.53 |
| Propionate | 20.9 | 21.4 | 20.2 | 20.5 | 20.5 | 20.2 | 20.8 | 0.46 | 0.52 | 0.59 | 0.61 | 0.15 | 0.21 | 0.74 |
| Butyrate | 8.7 | 9.4 | 9.1 | 8.4 | 8.9 | 8.9 | 8.6 | 0.20 | 0.44 | 0.22 | <0.01 | 0.40 | 0.06 | 0.91 |
| Iso-butyrate | 1.43 | 1.85 | 1.44 | 1.42 | 1.67 | 1.44 | 1.44 | 0.062 | 0.09 | 0.31 | <0.01 | 0.01 | 0.11 | 0.54 |
| Valerate | 1.35 | 1.97 | 1.43 | 1.00 | 1.70 | 1.43 | 1.18 | 0.143 | 0.50 | 0.78 | <0.01 | 0.78 | 0.12 | 0.83 |
| Iso-valerate | 1.34 | 1.98 | 1.41 | 1.31 | 1.85 | 1.51 | 1.29 | 0.124 | 0.11 | 0.90 | <0.01 | 0.19 | 0.65 | 0.42 |
| Acetate:propionate | 4.1 | 3.9 | 4.3 | 4.3 | 4.2 | 4.3 | 4.2 | 0.12 | 0.60 | 0.47 | 0.13 | 0.07 | 0.13 | 0.69 |

[1]This experiment was designed as a 2 × 3 + 1 factorial design, composed by 2 supplementation amounts (F or H), 3 supplementation frequencies (D, 5D, or 10D), and a nonsupplemented control.
[2]CON vs. Supp, nonsupplemented treatment vs. protein supplemented treatments; F vs. H, 100% of estimated RDP requirement vs. 50% of estimated RDP requirement; SF L, linear effect of SF; SF Q, quadratic effect of SF; L vs. amount, linear effect of SF × supplementation amount; Q vs. amount, quadratic effect of SF × supplementation amount.

may result in deposition of nitrogenous compounds, other than urea and ammonia, into body pools that may be subsequently catabolized over time and converted into urea, thereby helping buffer the effects of infrequent CP supply (Reynolds and Kristensen, 2008). In fact, urea is the major metabolite resulting from rumen digestion of protein and is detected in the blood of ruminants (Bach et al., 2005). Therefore, the increase in PUN following CP supplementation, and for F compared with H, is consistent with work demonstrating that PUN is positively correlated with N intake (Harmeyer and Martens, 1980; Cappellozza et al., 2015). Previous research has shown that a reduction in CP SF leads to an increase in circulating concentrations of PUN when compared with daily-supplemented cohorts (Krehbiel et al., 1998; Bohnert et al., 2002b; Schauer et al., 2010), likely a result of elevated ruminal ammonia and N recycling on the days between supplementation events (Krehbiel et al., 1998; Wickersham et al., 2008).

In the present study, PUN peaked 2 d after supplements were provided for the F10D group and 1 d following supplementation events for F5D, H5D, and H10D groups, whereas all steers receiving D treatments had relatively stable circulating concentrations of PUN (Figure 2). These results are consistent with our lamb data reported in the companion paper (Cappellozza et al., 2021). Similarly, Cappellozza et al. (2015) reported that beef cows fed SBM as infrequently as once a week had a greater PUN peak on the day following supplementation compared with cohorts fed on a daily basis. The same authors reported a consistent and stable PUN concentration in cows supplemented daily. Wickersham et al. (2008) provided direct evidence that, with extended supplementation intervals, urea recycling enables relatively efficient utilization of N by ruminants consuming low-quality forage, especially when reduced quantities of supplemental N are offered.

The reduction in acetate with a concomitant increase in propionate and butyrate observed for CP-supplemented animals on the day all supplements were provided agrees with past studies where ruminants were fed a low-quality forage and provided supplemental protein (Köster et al., 1996; Mathis et al., 2000; Bohnert et al., 2002c). Wickersham et al. (2008) reported that steers supplemented with increasing amounts of RDP also had increased molar proportions of propionate and reduced acetate. The decrease in acetate and increase in butyrate is consistent with the fact that both VFA share acetyl-CoA as a precursor and a change in acetate molar proportion could be expected to coincide with an opposing change in butyrate.

In summary, providing 100% of the estimated RDP requirement to beef steers consuming low-quality forage at extended SF (i.e., 10 d) reduced forage DMI, likely due to a substantial substitution effect on the day of and on the day following a supplementation event. However, when the amount of supplement was reduced to 50% of the estimated RDP requirement, forage DMI was maintained or increased at extended SF. Consequently, when supplementation intervals are >5 d, our data suggest that reducing the overall quantity of supplemental N provided at each supplementation event to ≤ 0.6 g/kg BW is a management strategy that will maintain acceptable levels of DMI, nutrient digestibility, and ruminal fermentation while reducing supplementation costs.

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## Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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