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Daily and alternate day supplementation of urea or soybean meal to ruminants consuming low-quality cool-season forage: II. Effects on ruminal fermentation ☆, ☆☆, ★

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ABSTRACT

Five ruminally cannulated steers (initial BW = 464 ± 26 kg) consuming low-quality forage (5% CP; 78% NDF; DM basis) were used in an incomplete 5 × 4 Latin square with four 18-d periods to determine the influence of supplemental N source and supplementation frequency (**SF**) on ruminal fermentation dynamics. Treatments, arranged as a 2 × 2 factorial with a negative control, consisted of urea or soybean meal (**SBM**) supplements offered daily (**D**) or alternate days (**2D**) plus an unsupplemented treatment (**CON**). Urea supplements were provided to meet 100% of the degradable intake protein requirement while SBM supplements were provided on an isonitrogenous basis. All supplemented treatments received an equal quantity of supplemental N over a 2-d period. Ruminal indigestible acid detergent fiber (IADF) passage rate was increased with supplementation ($P \leq 0.03$) on the days when D and 2D supplements were provided, as well as when only D supplements were provided. In contrast, ruminal liquid fill and dilution rate were not affected by supplementation, N source, or SF on the days when D and 2D supplements were provided ($P \geq 0.24$). However, when only D supplements were offered, ruminal liquid dilution rate was greater ($P = 0.03$) for SBM supplemented steers compared with cohorts receiving supplemental urea, whereas ruminal liquid fill was greater ($P = 0.03$) for steers fed urea supplements. Nitrogen supplementation increased ($P < 0.01$) ruminal NH_3–N by 122% and 70%, compared with the CON, on the days when both D and 2D supplements were provided and when only D supplements were provided, respectively. We noted a N source × SF interaction for ruminal NH_3–N on the days when D and 2D supplements were provided ($P = 0.02$), as well as when only D supplements were provided ($P < 0.01$). On the days when D and 2D supplements were provided, urea increased NH_3–N by 61% (2.93 vs. 4.73 mM for D and 2D, respectively), whereas the increase in NH_3–N with SBM was only 15% (2.23 vs. 2.58 mM for D and 2D, respectively). However, when only D supplements were provided, NH_3–N was almost 36% less for the 2D compared with the D urea treatment (2.76 vs. 1.81 mM, respectively), whereas an 11% increase was noted for SBM 2D

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compared with SBM D (1.99 vs. 1.79 mM, respectively). Total concentration of VFA was increased on the days when both D and 2D supplements were provided ($P=0.03$), but not influenced by treatments on the days when only D supplements were provided ($P\geq0.50$). In summary, providing a urea-based supplement, as infrequently as every-other-day, was an effective alternative to a SBM-based supplement in maintaining acceptable ruminal fermentation of steers consuming low-quality, cool-season forage.

1. Introduction

Low ruminal NH_3–N (<2.94 mM) often limits microbial growth and ruminal fermentation (Satter and Slyter, 1974; Slyter et al., 1979) in ruminants consuming low-quality forage ($<7\%$ CP; DM basis). Consequently, provision of supplemental ruminally degradable protein (**RDP**) usually increases microbial CP production (Bohnert et al., 2002a; Hannah et al., 1991; Köster et al., 1996) and enhances ruminal fermentation (Bodine et al., 2000, 2001; Köster et al., 1996; Olson et al., 1999), thereby improving performance (Bohnert et al., 2002b; Clanton and Zimmerman, 1970; Mathis et al., 1999) and reproductive efficiency (Sasser et al., 1988; Wiley et al., 1991). However, protein supplementation is an expensive management practice, because of the costs of supplement, labor, and equipment associated with supplement delivery. Ruminant livestock producers can decrease these costs by purchasing supplements on a CP basis (cost/kg CP) and by decreasing the frequency of supplementation.

Non-protein N (**NPN**) sources are usually less expensive per unit of N than natural protein sources (i.e., soybean meal). Moreover, research has suggested that NPN can effectively be used as a source of supplemental N to ruminants consuming low-quality forage (Currier et al., 2004a,b; Köster et al., 1997, 2002). However, the use of NPN in N supplements can result in management concerns such as supplement palatability and refusal, urea toxicity, and decreased efficiency of N use compared with sources of natural protein (Chalupa, 1968; Clanton, 1978; Helmer and Bartley, 1971; Rush et al., 1976).

Previous research has indicated that providing CP supplements as infrequently as once every 7 d to ruminants consuming low-quality forage results in performance and nutrient utilization similar to daily supplementation (Bohnert et al., 2002a,b; Huston et al., 1999a,b). However, little data are available comparing infrequent supplementation of NPN and natural protein. Therefore, we hypothesized that offering a CP supplement in which NPN provided the primary source of supplemental N would maintain ruminal parameters comparable to a supplement in which natural protein provided the primary source of supplemental N, even when offered every-other-day. The objective of this study was to compare supplementation frequency (**SF**; daily and alternate day) of supplements in which urea or SBM provided the primary source of supplemental N on ruminal fermentation in steers consuming low-quality forage ($<7\%$ CP).

2. Materials and methods

The present experiment was conducted at the Oregon State University—Eastern Oregon Agricultural Research Center (EOARC), Burns. 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.

2.1. Animals and diets

A full description of experimental procedures (excluding ruminal fermentation measurement and analysis) and diet composition is given in a companion paper (McGuire et al., this issue). Briefly, 5 mature Angus × Hereford steers (initial BW = 464 ± 26 kg) with ruminal cannulas were allotted randomly to 1 of 5 treatments in an incomplete 5 × 4 Latin square design (Cochran and Cox, 1957) and housed in individual pens (4 × 8 m) within an enclosed barn with continuous lighting. Treatments, arranged as a 2 × 2 factorial plus a negative control, consisted of a hard fescue (*Festuca trachyphylla*) straw diet (CON) and the CON diet plus urea (U) or soybean meal (SBM) supplements provided daily (D) or on alternate days (2D). The U treatments were formulated to provide 100% of the estimated RDP requirement assuming a microbial efficiency of 11 g bacterial CP/100 g TDN (NRC, 2000; Model 1) and the SBM treatments were provided on an isonitrogenous basis, at 0.4 g CP/kg BW and 0.8 g CP/kg BW for D and 2D, respectively. Protein supplements were placed directly into the rumen via the ruminal cannula at 0700 h for supplemented treatments on each respective supplementation day (1.3 and 2.6 g DM/kg BW for D and 2D, respectively). The basal diet consisted of hard fescue grass seed straw (4.7% CP; DM basis; McGuire et al., this issue).

2.2. Sampling

Experimental periods were 18 d, with 9 d of diet adaptation and 9 d of sampling. On d 11 and 12, treatment effects on ruminal DM and indigestible ADF (IADF) fill were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on a day when both D and 2D supplements were provided and a day when only D supplements were provided, respectively. Total ruminal contents were weighed, mixed by hand, and sub-sampled in triplicate (approximately 400 g per triplicate). The remaining ruminal contents were replaced immediately into the animal. Ruminal samples were weighed, dried in a force-air oven (55 °C) for 96 h, reweighed for DM, ground to pass a 1-mm screen in a Wiley mill, and composited within period and day by steer.

On d 13 and 18, each steer was intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-ml aqueous solution

(Udén et al., 1980) at 0700 h (the time supplements were provided). As described above for ruminal evacuations, this allowed sampling on a day when both D and 2D supplements were provided and a day when only D supplements were provided. The Co marker was administered throughout the rumen by injecting through a stainless steel probe with a perforated tip. Ruminal fluid (approximately 100 mL) was collected by suction strainer (Raun and Burroughs, 1962) immediately prior to dosing (0 h) and at 3, 6, 9, 12, and 24 h after dosing. Ruminal pH was measured immediately after collection (Orion SA 520, American Instrument Exchange Inc., Haverhill, MA). Twenty milliliters of the ruminal fluid was stored (−20 °C) for later analysis of Co-concentration and 5 mL was acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (−20 °C) for subsequent analysis of VFA and NH_3–N. Frozen (−20 °C) ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × *g* for 10 min at room temperature for VFA and NH_3–N, and 2000 × *g* for 20 min at room temperature for Co), and collecting the supernatant. Cobalt concentration in ruminal fluid was 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 described by Warner and Stacy (1968). Volatile fatty acids were analyzed as described by Harmon et al. (1985) and NH_3–N was analyzed by a modification (sodium salicylate substituted for phenol) of the procedure described by Broderick and Kang (1980) using an UV–visible spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

Feed, orts, and ruminal particulate samples (McGuire et al.,this issue) were analyzed for IADF using procedures described by Bohnert et al. (2002c). Procedures described by Van Soest (1982) were used to determine IADF passage rate by dividing IADF intake by the quantity of IADF in the rumen 4 h after feeding.

2.3. Statistical analysis

All data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation was used to determine the denominator degrees of freedom for the test of fixed effects. Ruminal liquid volume, liquid dilution rate, DM fill, IADF fill, and IADF passage rate were analyzed as an incomplete 5 × 4 Latin square. The model statement contained the effects of treatment and period as independent variables. Steer was used as the random variable. Because the treatment structure consisted of a 2 × 2 factorial plus a control, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were: (1) CON vs. Supplementation; (2) U vs. SBM; (3) D vs. 2D supplementation; (4) N source × SF.

Ruminal pH, NH_3–N, and VFA data, collected at the fixed times after feeding on a day that D and 2D supplements and a day only D supplements were provided (d 13 and 18, respectively), were analyzed using the repeated statement with the PROC MIXED procedure of SAS. The model statement contained the effects of treatment, time, treatment × time interaction and period as the independent variable. Steer was used as the random variable. The specified term for the repeated statement was hour, the subject was steer(period × trt), and the covariance structure utilized was autoregressive by it providing the lowest Akaike information criterion for all variables analyzed. The same contrasts described above were used to partition specific treatment effects.

Results are reported as least square means and separated using LSD. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant or according to highest-order interaction detected.

3. Results

For all intake and digestibility data, please refer to a companion paper (McGuire et al., this issue).

3.1. Ruminal fill and passage

Ruminal fill (DM and IADF), liquid fill, and dilution rate were not affected by supplementation, N source, or SF ($P \geq 0.24$) on the day when both D and 2D supplements were provided (Table 1). However, IADF passage rate was greater ($P = 0.03$) in supplemented steers compared with CON cohorts, but it was not affected by N source and SF ($P \geq 0.42$).

On the day when only D supplements were offered, ruminal liquid fill and liquid dilution rate were affected by N source ($P = 0.03$; Table 1). Ruminal liquid dilution rate was greater ($P = 0.03$) for steers supplemented with SBM than cohorts receiving supplemental U (10.9 vs. 10.1%/h, respectively), whereas ruminal liquid fill was greater ($P = 0.03$) for steers fed U treatments (159 vs. 146 ml/kg of BW for U and SBM, respectively). In addition, a N source × SF interaction was observed ($P = 0.05$) for IADF fill, whereas the same interaction tended ($P = 0.06$) to affect ruminal DM fill (Table 1), indicating that as SF decreased from D to 2D, ruminal DM and IADF fill increased for urea-supplemented steers, whereas it decreased for SBM-supplemented cohorts. Similarly to the aforementioned results, IADF passage rate was greater ($P < 0.01$) for supplemented compared with unsupplemented steers.

3.2. Rumen fermentation

Treatment × time interactions ($P < 0.01$) were noted for ruminal NH_3–N on the day when both D and 2D supplements were provided, as well as on the day when only D supplements were provided.

Ruminal NH_3–N increased ($P < 0.01$) 122% with supplementation on the day when both D and 2D supplements were provided (Table 2; Fig. 1). Also, we noted a N source × SF interaction ($P = 0.02$) in which ruminal NH_3–N increased with supplemental U (2.93 vs. 4.73 mM, respectively; SEM = 0.37), whereas it remained relatively

Table 1
Effects of supplemental N source and supplementation frequency daily (D) or every-other-day (2D) on ruminal liquid fill and liquid dilution rate in steers fed hard fescue straw.

Item	Treatment[a]					SEM[b]	*P*-value[c]			
	CON	UD	U2D	SBMD	SBM2D		Con vs Supp	Urea vs SBM	D vs 2D	N Source × SF
D and 2D supplements provided										
DM fill, g/kg BW	29.0	30.2	29.2	29.9	28.9	1.01	0.60	0.76	0.26	0.95
IADF fill, g/kg BW	8.22	8.50	8.55	8.64	8.24	0.37	0.41	0.76	0.53	0.41
IADF passage rate, %/h	1.92	2.08	2.15	2.10	2.14	0.15	0.03	0.97	0.42	0.83
Liquid fill, mL/kg BW	136	138	137	149	133	11.0	0.79	0.74	0.41	0.47
Liquid dilution rate, %/h	9.1	10.6	9.8	10.1	10.7	0.88	0.24	0.80	0.88	0.46
Only D supplements provided										
DM fill, g/kg BW	28.0	27.8	29.4	30.3	26.6	1.23	0.69	0.89	0.39	0.06
IADF fill, g/kg BW	7.76	7.66	8.03	8.81	7.54	0.48	0.54	0.39	0.25	0.05
IADF passage rate, %/h	2.02	2.32	2.34	2.10	2.32	0.19	< 0.01	0.09	0.09	0.13
Liquid fill, mL/kg BW	145	162	156	142	151	7.6	0.19	0.03	0.83	0.16
Liquid dilution rate, %/h	9.9	9.9	10.3	11.1	10.8	0.54	0.11	0.03	0.90	0.22

[a] CON=control; UD=urea supplement provided daily; U2D=urea supplement provided every-other-day; SBMD=soybean meal supplement provided daily; SBM2D=soybean meal supplement provided every-other-day.
[b] $n=4$.
[c] Con vs Supp=control vs supplemented treatments; Urea vs SBM=urea vs soybean meal treatments; D vs 2D=daily vs alternate day supplementation; N Source × SF=interaction of N source vs supplementation frequency.

Table 2
Effects of supplemental N source and supplementation frequency on steer ruminal fermentation characteristics when daily (D) and alternate day (2D) supplements were provided.

Item	Treatment[a]					SEM[b]	P-Value[c]			
	CON	UD	U2D	SBMD	SBM2D		Con vs Supp	Urea vs SBM	D vs 2D	N Source × SF
Ammonia N, mM	1.42	2.93	4.73	2.23	2.58	0.37	< 0.01	< 0.01	< 0.01	0.02
pH	6.57	6.59	6.58	6.55	6.58	0.04	0.80	0.47	0.66	0.34
Total VFA, mM	74.3	77.7	78.3	78.3	78.8	2.49	0.03	0.71	0.71	0.99
VFA, mol/100 mol										
Acetate	75.4	75.8	75.3	74.9	75.3	0.30	0.80	0.17	0.91	0.15
Propionate	17.3	16.7	17.3	16.9	16.9	0.31	0.18	0.61	0.18	0.14
Isobutyrate	0.45	0.41	0.47	0.51	0.47	0.03	0.66	0.07	0.72	0.09
Butyrate	6.2	6.4	6.3	6.8	6.5	0.18	0.06	0.04	0.16	0.56
Isovalerate	0.37	0.40	0.41	0.44	0.45	0.023	< 0.01	< 0.01	0.59	0.83
Valerate	0.31	0.29	0.35	0.43	0.40	0.033	0.10	< 0.01	0.63	0.14
Acetate:Propionate	4.4	4.6	4.4	4.4	4.5	0.09	0.46	0.96	0.36	0.15

[a] CON=control; UD=urea supplement provided daily; U2D=urea supplement provided every-other-day; SBMD=soybean meal supplement provided daily; SBM2D=soybean meal supplement provided every-other-day.
[b] $n=4$.
[c] Con vs Supp=control vs supplemented treatments; Urea vs SBM=urea vs soybean meal treatments; D vs 2D=daily vs alternate day supplementation; N Source x SF=interaction of N source vs supplementation frequency.

constant with SBM (2.23 vs. 2.58 mM, respectively; SEM=0.37) as SF decreased from D to 2D.

On the day when only D supplements were provided, supplementation increased ($P<0.01$) ruminal NH_3–N by 71% compared with the CON (Table 3; Fig. 1) and U-supplemented steers had greater ($P<0.01$) ruminal NH_3–N compared with SBM cohorts (2.29 vs. 1.89 mM, respectively). Moreover, infrequent supplementation reduced ($P<0.01$) ruminal NH_3–N for steers receiving U supplements (2.76 vs. 1.81 mM, respectively; SEM=0.15), whereas no further changes were observed for SBM-supplemented cohorts (1.79 vs. 1.99 mM, respectively; SEM=0.15), which resulted in a N source × SF interaction ($P<0.01$).

No treatment × time interactions ($P\geq0.15$) were observed for ruminal pH or VFA; therefore, only overall treatment means are presented. On the day when both D and 2D supplements were provided, no treatment effects ($P>0.51$) were detected on ruminal pH (Table 2). However, total VFA concentrations were increased due to supplementation ($P=0.03$; Table 2). Also, SBM-supplemented steers had greater ($P\leq0.04$) butyrate, isovalerate, and valerate concentrations (mol/100 mol) compared with steers receiving U supplements. In addition, the molar proportion of isovalerate was greater ($P<0.01$) for supplemented steers compared with CON cohorts.

On the day when only D supplements were provided, ruminal pH was greater ($P<0.01$) for steers offered U than cohorts offered SBM supplements (6.59 vs. 6.51, respectively; Table 3). No further effects were observed on ruminal pH due to supplementation and SF ($P\geq0.25$). Concentration of total VFA was not affected by

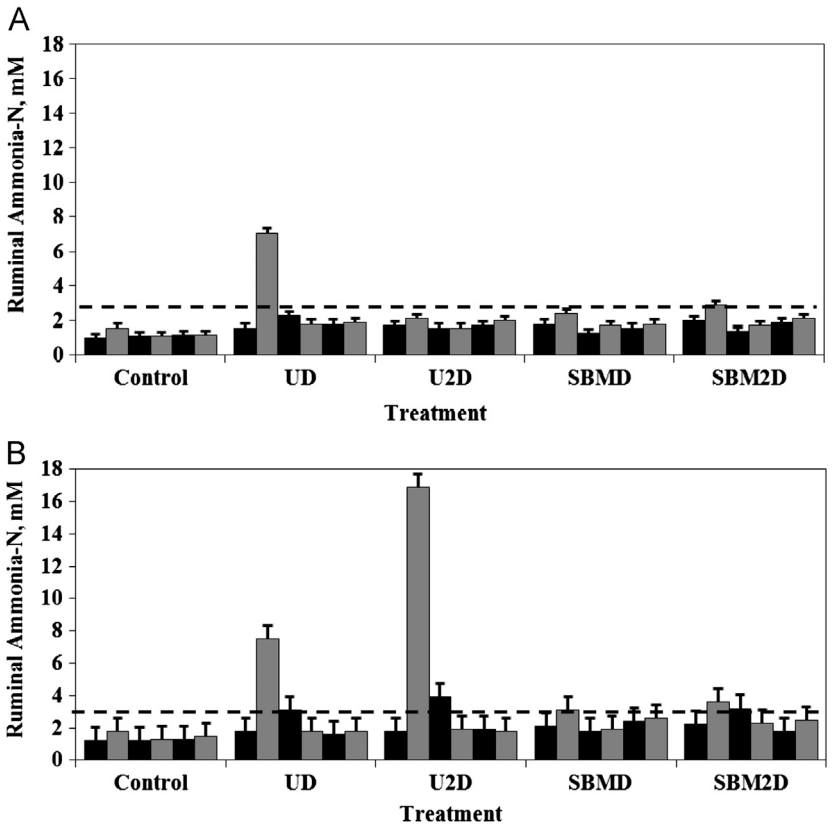

Fig. 1. Effects of supplemental N source and supplementation frequency on steer ruminal ammonia-N on the day when only daily supplements were provided (A) and on the day when all supplements were provided (B). Dashed lines in the graphs represent the suggested minimum ruminal ammonia levels required for optimal rumen microbial growth (2.94 mM; Slyter et al., 1979). Bars from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 h post-feeding, respectively. Treatments were: Control; UD=urea supplement provided every day; U2D=urea supplement provided every-other-day; SBMD=soybean meal supplement provided every day; and SBM2D=soybean meal supplement provided every-other-day. Treatment × time interactions for A and B are $P < 0.01$. The SEM for A and B are 0.24 and 0.82, respectively.

Table 3
Effects of supplemental N source and supplementation frequency on steer ruminal fermentation characteristics when only daily (D) supplements were provided.

Item	Treatment[a]					SEM[b]	*P*-value[c]			
	CON	UD	U2D	SBMD	SBM2D		Con vs Supp	Urea vs SBM	D vs 2D	Source × SF
Ammonia N, mM	1.22	2.76	1.81	1.79	1.99	0.15	< 0.01	< 0.01	< 0.01	< 0.01
pH	6.51	6.59	6.59	6.49	6.52	0.04	0.25	< 0.01	0.73	0.60
Total VFA, mM	78.5	80.5	79.2	81.0	78.5	2.65	0.60	0.93	0.50	0.90
VFA, mol/100 mol										
Acetate	75.9	76.2	75.7	74.9	75.3	0.23	0.11	< 0.01	0.98	0.02
Propionate	16.6	16.2	16.4	16.9	16.6	0.26	0.64	< 0.01	0.47	0.04
Isobutyrate	0.46	0.41	0.49	0.50	0.53	0.019	0.27	< 0.01	< 0.01	0.13
Butyrate	6.3	6.5	6.6	6.8	6.7	0.14	< 0.01	0.04	0.62	0.28
Isovalerate	0.38	0.38	0.40	0.43	0.40	0.024	0.19	0.08	0.63	0.15
Valerate	0.35	0.32	0.37	0.45	0.43	0.022	0.02	< 0.01	0.30	0.06
Acetate:Propionate	4.6	4.7	4.6	4.4	4.6	0.08	0.97	< 0.01	0.64	0.05

[a] CON=control; UD=urea supplement provided daily; U2D=urea supplement provided every-other-day; SBMD=soybean meal supplement provided daily; SBM2D=soybean meal supplement provided every-other-day.
[b] $n=4$.
[c] Con vs Supp=control vs supplemented treatments; Urea vs SBM=urea vs soybean meal treatments; D vs 2D=daily vs alternate day supplementation; N Source x SF=interaction of N source vs supplementation frequency.

supplementation, N source, or SF ($P \geq 0.50$; Table 3). However, molar proportions of acetate, propionate, butyrate, isobutyrate, valerate, and acetate:propionate ratio were affected by N source. Steers supplemented with U had a greater ($P < 0.01$) molar proportion of acetate, acetate:propionate ratio, and reduced ($P \leq 0.04$) propionate,

butyrate, isobutyrate, and valerate than steers fed SBM. Valerate and butyrate concentrations were also greater ($P \leq 0.02$) for supplemented vs. CON cohorts.

4. Discussion

The main goal of the present study was to evaluate the effects of different N sources (natural [SBM] or NPN [urea]) and supplementation frequency (daily or alternate day) on ruminal fermentation dynamics of beef steers consuming low-quality hard fescue straw (4.7% CP; DM basis). Infrequent CP supplementation helps to reduce the labor and fuel costs associated with supplementation, while maintaining performance comparable to daily supplementation (Bohnert et al., 2002a; Huston et al., 1999a,b; Schauer et al., 2005). Farmer et al. (2004a) suggested a few mechanisms that may play a role in buffering the impact of infrequent supplementation and minimize differences in performance of cattle. These likely include N recycling, a lag in peak ruminal ammonia concentration, and prolonged elevation of NH_3–N.

In the present study, independently of the collection day (D and/or 2D), IADF passage rates were greater for supplemented steers, which is in agreement with the intake data reported by McGuire et al. (this issue). Supplementation, independently of N source, increased straw DM and OM intake compared with unsupplemented cohorts, likely due in part to our noted increase in IADF passage rate as well as increased ruminal NH_3–N. Infrequent supplementation did not affect DM or OM intake by beef steers consuming low-quality forages (McGuire et al., this issue). Several researchers have demonstrated that CP supplementation increases DM and OM intake (Bandyk et al., 2001; DelCurto et al., 1990; Huston et al., 1999a) and decreased SF does not negatively affect total DMI by ruminants consuming low-quality forage (Farmer et al., 2004b; Huston et al., 1999a; Krehbiel et al., 1998; Wickersham et al., 2008b). The reason for the N source × SF interactions on ruminal variables (DM fill and IADF fill) on the day when only D supplements were provided is not clear and deserves further investigation.

Crude protein supplementation has been shown to increase total tract digestibility in beef steers consuming low-quality forage (Bohnert et al., 2002a; Currier et al., 2004c). However, in a companion paper (McGuire et al., this issue), neither supplementation nor N source increased DM, OM, NDF, and ADF digestibilities. Similarly, Wickersham et al. (2008b) reported no difference in total tract digestibility of OM and NDF by beef steers offered increasing amounts of RDP (casein). Nevertheless, another study from the same group (Wickersham et al., 2008a) demonstrated that increasing the RDP content of the diet increased total tract digestibility of OM and NDF. One likely explanation for the lack of effects on digestibility by steers receiving CP supplements (Wickersham et al., 2008b; McGuire et al., this issue) may be in part to the greater ruminally available N supply (Guthrie and Wagner, 1988; Olson et al., 1999), which increased ruminal particulate passage rate and, thereby, decreased the time ruminal microbes had access to the substrate (Wickersham et al., 2008b).

On the day when both D and 2D supplements were offered, ruminal liquid fill and dilution rate were not affected by supplementation, N source, or SF. Supporting our results, Currier et al. (2004c) fed different sources of NPN (urea or biuret) to beef steers consuming low-quality forage and reported minimal effects on ruminal fluid dynamics due to decreased SF. Conversely, Bohnert et al. (2002c) reported a linear increase in ruminal liquid fill of beef steers consuming low-quality forage and supplemented with RDP (soybean meal) as SF decreased from daily to once every 3 d or once every 6 d. The same authors reported increased ruminal liquid dilution rate when all supplements were provided. Nevertheless, the reasons why no differences were observed in these parameters due to N source on the days when all supplements were offered remain unknown and deserve further investigation.

In contrast to the aforementioned results, greater ruminal liquid fill for U-supplemented steers and increased liquid dilution rate for steers supplemented with SBM on the day when only D supplements were offered suggests that rumen function may be altered by the N source used, even if equal amounts of CP (as % of BW) are provided. Olson et al. (1999) argued that differences in the type of CP supplements used (such as CP quantity and degradability) may help to explain different results associated with these ruminal parameters. Urea is extremely soluble in water and is rapidly hydrolyzed to NH_3–N within the rumen (Bartley et al., 1976) and one can speculate that there was an osmotic imbalance within the rumen, allowing more water to enter the rumen and, subsequently affecting ruminal liquid fill. Another possible explanation for the observed response herein is that U supplementation stimulated water intake to a greater extent compared with the SBM supplement. However, none of these parameters were analyzed in the current study.

Previous research has shown that CP supplementation increases ruminal NH_3–N in ruminants consuming low-quality forage (Köster et al., 1996; Weder et al., 1999). This is in agreement with our observation that supplementation increased ruminal NH_3–N by 122% on the day when both D and 2D supplements were provided and by 71% on the day when only D supplements were provided compared with CON. Similarly, Currier et al. (2004c) observed an increase in ruminal NH_3–N of approximately 240 and 152% on the day when both D and 2D supplements were provided and on the day when only D supplements were provided, respectively, for beef steers consuming low-quality grass seed straw (4% CP). Also, Bohnert et al. (2002b) reported that steers fed 5% CP meadow foxtail hay and offered low- or high-RDP supplements daily, once every 3 d, or once every 6 d had, on average, 267 and 173% increased ruminal NH_3–N on the day that all supplements and the day only daily supplements were provided, respectively, compared with an unsupplemented control. We should note that the unsupplemented treatment (CON) had ruminal NH_3–N concentrations close to the lower threshold known to maximize the growth of ruminal microbes (1.18 to 2.94 mM; Slyter et al., 1979). Therefore, ruminal N availability may have been limiting for the CON steers.

In the present study, steers supplemented with U had greater ruminal NH_3–N compared with SBM-supplemented cohorts on the day when both D and 2D supplements were provided, as well as on the day when only D supplements were provided. Bohnert et al. (2002c) reported that high-RDP supplements increased ruminal NH_3–N compared with rumen undegradable protein supplements. As previously mentioned, U is rapidly hydrolyzed into NH_3–N when compared with other RDP sources (i.e., soybean meal), which are more slowly digested to NH_3–N. In addition, U 2D increased ruminal NH_3–N on the day when both D and 2D supplements were provided, whereas on the day when only D supplements were offered U D steers had greater ruminal NH_3–N levels. In agreement with our results, Currier et al. (2004c) demonstrated that U supplementation increased NH_3–N levels when compared with biuret as SF decreased. Farmer et al. (2004a) suggested that one of the mechanisms by which infrequent CP supplementation is effective is by prolonging NH_3–N elevation (up to 24 h after supplementation), reflecting both recycling as well as time-series differences in the prevalence of ruminal bacteria that can ferment peptides and free AA and produce NH_3–N (Yang and Russell, 1993). An attenuated peak and prolonged maintenance of elevated ruminal NH_3–N would facilitate the maintenance of fibrolytic activity and the conservation of N, thus reducing potential negative effects as a result of the infrequent supplementation.

Ruminal pH averaged approximately 6.6 for all treatments on the day when both supplements were provided and on the day when only D supplements were provided. In agreement, Farmer et al. (2004b) reported that inclusion of urea (0 or 30% of RDP) and/or supplementation frequency (daily or alternate day) did not affect rumen pH. These results suggest that ruminal pH values were within the range (6.3 to 6.8) normally sufficient to support adequate fiber digestion of beef steers across all treatments (Yokoyama and Johnson, 1988).

Total VFA have been shown to increase with CP supplementation in ruminants consuming low-quality forage (Bohnert et al., 2002c, 2011; Hannah et al., 1991; Köster et al., 1996), which supports our data, on the day when both D and 2D supplements were provided. However, in agreement with Collins and Pritchard (1992), we did not observe increased concentration of total VFA on the day when only D supplements were provided. Leng (1973) reported that branched-chain VFA arise from fermentation of the branched chain amino acids present in SBM and, accordingly, steers supplemented with SBM had greater molar proportions of the branched-chain VFA isovalerate and isobutyrate compared with U-supplemented cohorts. Also, Wickersham et al. (2008a) reported that steers supplemented with increasing amounts of RDP had increased molar proportions of propionate, isobutyrate, valerate, isovalerate, and decreased molar proportion of acetate. Likewise, we noted that the molar proportion of butyrate increased for SBM-supplemented steers on the day when all supplements were provided and on the day when only D supplements were provided. Additionally, acetate decreased on the day when only D supplements were offered. The increased acetate:propionate ratio for U-supplemented steers observed on the day when only D supplements were offered, agrees with our observation that acetate increased and propionate decreased for animals receiving U supplements. However, we should point out that this difference (less than 5%) may not be of physiological importance.

Nevertheless differences were observed for ruminal parameters of beef steers supplemented with U or SBM. Results from the companion paper (McGuire et al., this issue) and the literature available demonstrate that NPN sources (i.e., urea) may be used as an acceptable alternative to sources of natural protein for mature beef cattle consuming low-quality forage. Supporting our results, Cooke and Arthington (2008) reported that mature beef cows grazing winter range and receiving molasses-based supplements containing NPN (urea) or a blend of natural protein (cottonseed meal and feather meal) had similar performance (BW and BCS) and pregnancy rates during the annual breeding season. Farmer et al. (2004b) demonstrated that feeding urea as 0 or 30% of RDP to cows during the last trimester of gestation did not affect calf birth weight and calf ADG. Nonetheless, replacing natural protein sources with NPN for growing cattle (yearling heifers and steers) negatively affected ADG and reproductive performance (Karges et al., 1992; Pate et al., 1995). These differences can be explained by the fact that a greater supply of amino acids, and subsequently MP, are available in cattle supplemented with natural protein sources compared with NPN (Cooke and Arthington, 2008), and growing cattle have greater requirements for MP in order to sustain tissue growth (NRC, 2000) and acceptable ADG.

5. Conclusions

Supplemental U and SBM resulted in acceptable ruminal parameters for beef cattle consuming hard fescue straw. Therefore, our data suggest that with mature beef cattle consuming low-quality, cool-season forages, U can be used to replace SBM when designing protein supplements for ruminants deficient in RDP, even if consumed on alternate days.

References

Bandyk, C.A., Cochran, R.C., Wickersham, T.A., Titgemeyer, E.C., Farmer, C. G., Higgins, J.J., 2001. Effects of ruminal vs. postruminal administration of degradable protein on utilization of low-quality forage by beef steers. J. Anim. Sci. 79, 225–231.

Bartley, E.E., Davidovich, A.D., Barr, G.W., Griffel, G.W., Dayton, A.D., Deyoe, C.W., Bechtle, R.M., 1976. Ammonia toxicity in cattle: I. Rumen and blood changes associated with toxicity and treatment models. J. Anim. Sci. 43, 835–841.

Bodine, T.N., Purvis II, H.T., Ackerman, C.J., Goade, C.L., 2000. Effects of supplementing prairie hay with corn and soybean meal on intake, digestion, and ruminal measurements by beef steers. J. Anim. Sci. 78, 3144–3154.

Bodine, T.N., Purvis II, H.T., Lalman, D.L., 2001. Effects of supplement type on animal performance, forage intake, digestion, and ruminal measurements of growing beef cattle. J. Anim. Sci. 79, 1041–1051.

Bohnert, D.W., Schauer, C.S., Bauer, M.L., DelCurto, T., 2002a. Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: I. Site of digestion and microbial efficiency. J. Anim. Sci. 80, 2967–2977.

Bohnert, D.W., Schauer, C.S., DelCurto, T., 2002b. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: cow performance and efficiency of nitrogen use in wethers. J. Anim. Sci. 80, 1629–1637.

Bohnert, D.W., Schauer, C.S., Falck, S.J., DelCurto, T., 2002c. Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: II. Ruminal fermentation characteristics. J. Anim. Sci., 80; 2978–2988.

Bohnert, D.W., DelCurto, T., Clark, A.A., Merrill, M.L., Falck, S.J., Harmon, D. L., 2011. Protein supplementation of ruminants consuming low-quality cool- or warm-season forage: differences in intake and digestibility. J. Anim. Sci. 89, 3707–3717.

Broderick, G.A., Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63, 64–75.

Chalupa, W., 1968. Problems in feeding urea to ruminants. J. Anim. Sci. 27, 207–219.

Clanton, D.C., 1978. Non-protein nitrogen in range supplements. J. Anim. Sci. 47, 765–779.

Clanton, D.C., Zimmerman, D.R., 1970. Symposium on pasture methods for maximum production in beef cattle: Protein and energy requirements for female beef cattle. J. Anim. Sci. 30, 122–132.

Cochran, W.G., Cox, G.M., 1957. Experimental Designs. John Wiley & Sons, New York, NY.

Collins, R.M., Pritchard, R.H., 1992. Alternate day supplementation of corn stalk diets with soybean meal or corn gluten meal fed to ruminants. J. Anim. Sci 70, 3899–3908.

Cooke, R.F., Arthington, J.D.., 2008. Case study: effects of protein source added to molasses-based supplements on performance of range cows. Prof. Anim. Sci. 24, 264–268.

Currier, T.A., Bohnert, D.W., Falck, S.J., Bartle, S.J., 2004a. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: I. Effects on cow performance and efficiency of nitrogen use in wethers. J. Anim. Sci. 82, 1508–1517.

Currier, T.A., Bohnert, D.W., Falck, S.J., Schauer, C.S., Bartle, S.J., 2004b. Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: II. Effects on site of digestion and microbial efficiency in steers. J. Anim. Sci., 82; 1518–1527.

Currier, T.A., Bohnert, D.W., Falck, S.J., Schauer, C.S., Bartle, S.J., 2004c. Daily and alternate-day supplementation of urea or biuret to ruminants consuming low-quality forage: III. Effects on ruminal fermentation characteristics in steers. J. Anim. Sci. 82, 1528–1535.

DelCurto, T., Cochran, R.C., Harmon, D.L., Beharka, A.A., Jacques, K.A., Towne, G., Vanzant, E.S., 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of varying supplemental protein and(or) energy levels on forage utilization characteristics of beef steers in confinement. J. Anim. Sci. 68, 515–531.

Farmer, C.G., Cochran, R.C., Nagaraja, T.G., Titgemeyer, E.C., Johnson, D.E., Wickersham, T.A., 2004a. Ruminal and host adaptations to changes in frequency of protein supplementation. J. Anim. Sci. 82, 895–903.

Farmer, C.G., Woods, B.C., Cochran, R.C., Heldt, J.S., Mathis, C.P., Olson, K.C., Titgemeyer, E.C., Wickersham, T.A., 2004b. Effect of supplementation frequency and supplemental urea level on dormant tallgrass-prairie hay intake and digestion by beef steers and prepartum performance of beef cows grazing dormant tallgrass-prairie. J. Anim. Sci. 82, 884–894.

Guthrie, M.J., Wagner, D.G., 1988. Influence of protein or grain supplementation and increasing levels of soybean meal on intake, utilization, and passage rate of prairie hay in beef steers and heifers. J. Anim. Sci 66, 1529–1537.

Hannah, S.M., Cochran, R.C., Vanzant, E.S., Harmon, D.L., 1991. Influence of protein supplementation on site and extent of digestion, forage intake, and nutrient flow characteristics in steers consuming dormant bluestem-range forage. J. Anim. Sci. 69, 2624–2633.

Harmon, D.L., Britton, R.A., Prior, R.L., Stock, R.A., 1985. Net portal absorption of lactate and volatile fatty acids in steers experiencing glucose-induced acidosis or fed a 70% concentrate diet ad libitum. J. Anim. Sci. 60, 560–569.

Helmer, L.G., Bartley, E.E., 1971. Progress in the utilization of urea as a protein replacer for ruminants. A review. J. Dairy Sci. 54, 25–51.

Huston, J.E., Engdahl, B.S., Bales, K.W., 1999a. Supplemental feeding interval for adult ewes. Sheep Goat Res. J. 15, 87–93.

Huston, J.E., Lippke, H., Forbes, T.D.A., Holloway, J.W., Machen, R.V., 1999b. Effects of supplemental feeding interval on adult cows in western Texas. J. Anim. Sci. 77, 3057–3067.

Karges, K.K, Klopfenstein, T.J., Wilkerson, V.A., Clanton, D.C.., 1992. Effects of ruminally degradable and escape protein supplements on steers grazing summer native range. J. Anim. Sci. 70, 1957–1964.

Köster, H.H., Cochran, R.C., Titgemeyer, E.C., Vanzant, E.S., Abdelgadir, I., St. Jean, G., 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass–prairie forage by beef cows. J. Anim. Sci. 74, 2473–2481.

Köster, H.H., Cochran, R.C., Titgemeyer, E.C., Vanzant, E.S., Nagaraja, T.G., Kreikemeier, K.K., St. Jean, G., 1997. Effect of increasing proportion of supplemental nitrogen from urea on intake and utilization of low-quality, tallgrass–prairie forage by beef steers. J. Anim. Sci. 75, 1393–1399.

Köster, H.H., Woods, B.C., Cochran, R.C., Vanzant, E.S., Titgemeyer, E.C., Grieger, D.M., Olson, K.C., Stokka, G., 2002. Effect of increasing proportion of supplemental N from urea in prepartum supplements on range beef cow performance and on forage intake and digestibility by steers fed low-quality forage. J. Anim. Sci. 80, 1652–1662.

Krehbiel, C.R., Ferrell, C.L., Freetly, H.C., 1998. Effects of frequency of supplementation on dry matter intake and net portal and hepatic flux of nutrients in mature ewes that consume low-quality forage. J. Anim. Sci. 76, 2464–2473.

Leng, R.A., 1973. Salient features of the digestion of pastures by ruminants and other herbivores. In: Butler, G.W., Bailey, R.W. (Eds.), Chemistry and Biochemistry of Herbage, Academic Press, New York, NY, pp. 82–129.

Mathis, C.P., Cochran, R.C., Stokka, G.L., Heldt, J.S., Woods, B.C., Olson, K.C., 1999. Impacts of increasing amounts of supplemental soybean meal on intake and digestion by beef steers and performance by beef cows consuming low-quality tallgrass–prairie forage. J. Anim. Sci. 77, 3156–3162.

McGuire D.L., Bohnert D.W., Schauer C.S. and Falck S.J., Daily and alternate day supplementation of urea or soybean meal to ruminant consuming low-quality cool-season forage: I. Effects on efficiency of nitrogen use and nutrient digestion, Liv. Sci. 'this issue' http://dx.doi.org/10.1016/j.livsci.2013.05.015.

NRC, 2000. Nutrient Requirements of Beef Cattle—Update. National Academy Press, Washington, DC2000.

Olson, K.C., Cochran, R.C., Jones, T.J., Vanzant, E.S., Titgemeyer, E.C., Johnson., D.E., 1999. Effects of ruminal administration of supplemental degradable intake protein and starch on utilization of low-quality warm-season grass hay by beef steers. J. Anim. Sci. 77, 1016–1025.

Pate, F.M., Brown, W.F., Hammond, A.C., 1995. Value of feather meal in a molasses-based liquid supplement fed to yearling cattle consuming a forage diet. J. Anim. Sci. 73, 2865–2872.

Raun, N.S., Burroughs, W., 1962. Suction strainer technique in obtaining rumen fluid samples from intact lambs. J. Anim. Sci. 21, 454–457.

Rush, I.G., Johnson, R.R., Totusek, R., 1976. Evaluation of beef cattle range supplements containing urea and biuret. J. Anim. Sci. 42, 1297–1308.

Sasser, R.G., Williams, R.J., Bull, R.C., Ruder, C.A., Falk, D.G., 1988. Postpartum reproductive performance in crude protein-restricted beef cows: return to estrus and conception. J. Anim. Sci. 66, 3033–3039.

Satter, L.D., Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32, 199–208.

Schauer, C.S., Bohnert, D.W., Ganskopp, D.C., Richards, C.J., Falck, S.J., 2005. Influence of protein supplementation frequency on cows consuming low-quality forage: performance, grazing behavior, and variation in supplement intake. J. Anim. Sci. 83, 1715–1725.

Slyter, L.L., Satter, L.D., Dinius, D.A., 1979. Effect of ruminal ammonia concentration on nitrogen utilization by steers. J. Anim. Sci. 48, 906–912.

Udén, P., Colucci, P.E., Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31, 625–632.

Van Soest, P.J., 1982. The kinetics of digestion, Nutritional Ecology of the RuminantCornell University Press, Ithaca, NY211–229.

Warner, A.C.I., Stacy, B.D., 1968. The fate of water in the rumen: 1. A critical appraisal of the use of soluble markers. Br. J. Nutr. 22, 369–387.

Weder, C.E., DelCurto, R., Svejcar, T., Jaeger, J.R., Bailey, R.K., 1999. Influence of supplemental alfalfa quality on the intake, use and subsequent performance of beef cattle consuming low-quality roughages. J. Anim. Sci. 77, 1266–1276.

Wickersham, T.A., Titgemeyer, E.C., Cochran, R.C., Wickersham, E.E., Gnad, D.P., 2008a. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86, 3079–3088.

Wickersham, T.A., Titgemeyer, E.C., Cochran, R.C., Wickersham, E.E., Moore, E.S., 2008b. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86, 3089–3099.

Wiley, J.S., Petersen, M.K., Ansotegui, R.P., Bellows, R.A., 1991. Production from first-calf beef heifers fed a maintenance or low level of prepartum nutrition and ruminally undegradable or degradable protein postpartum. J. Anim. Sci. 69, 4279–4293.

Yang, C.J., Russell, J.B., 1993. The effect of monensin supplementation on ruminal ammonia accumulation in vivo and the numbers of amino acid-fermenting bacteria. J. Anim. Sci. 71, 3470–3476.

Yokoyama, M.T., Johnson, K.A., 1988. Microbiology of the rumen and intestine. In: Church, D.C. (Ed.), The Ruminant Animal, Simon and Shuster, New York, pp. 125–144.