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[1,2]

T. A. Currier*, D. W. Bohnert*[3], S. J. Falck†, C. S. Schauer*[4], and S. J. Bartle‡

*Eastern Oregon Agricultural Research Center, Oregon State University, Burns, OR 97720; †Eastern Oregon Agricultural Research Center, USDA-ARS, Burns; and ‡ADM Alliance Nutrition, Inc., Quincy, IL 62305

ABSTRACT: Five steers (491 ± 21 kg BW) were used in an incomplete 5 × 4 Latin square with four 24-d periods to determine the influence of supplemental nonprotein N (NPN) source and supplementation frequency (SF) on nutrient intake and site of digestion in steers consuming low-quality grass straw (4% CP). Treatments (TRT) included an unsupplemented control and a urea- or biuret-containing supplement placed directly into the rumen daily (D) or every other day (2D) at 0700. The NPN treatments were formulated to provide 90% of the estimated degradable intake protein requirement. Daily TRT were supplemented CP at 0.04% of BW/d, whereas the 2D TRT were supplemented at 0.08% of BW every other day. Therefore, all supplemented TRT received the same quantity of supplemental CP over a 2-d period. Forage OM intake was not affected ($P > 0.05$) by NPN supplementation, NPN source, or SF; however, total OM and N intake were increased ($P < 0.01$) with CP supplementation. Duodenal flow of N was greater ($P = 0.04$) with CP supplementation compared with the control. In addition, duodenal bacterial N flow was increased with CP supplementation ($P = 0.04$) and for biuret compared with urea ($P < 0.01$). Bacterial efficiency (g bacterial N/kg OM truly digested in the rumen) was greater ($P = 0.05$) for biuret than for urea. Apparent total-tract N digestibility was increased with NPN supplementation ($P < 0.01$) but not affected by NPN source or SF. These results suggest that urea or biuret can be used effectively as a supplemental N source by steers consuming low-quality forage.

Key Words: Biuret, Forage, Frequency, Nonprotein Nitrogen, Supplementation, Urea

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Introduction

Many cattle in the Western United States consume low-quality forage (<6% CP) from late summer through winter. Crude protein supplementation of low-quality forage can increase cow weight gain and body condition score (Bohnert et al., 2002b) and forage intake and digestibility (Köster et al., 1996), and can improve reproductive performance (Wiley et al., 1991). However, annual winter feed and labor costs in the Intermountain West often total $100 to $200 per cow. Infrequent supplementation can decrease labor costs while maintaining acceptable levels of performance (Huston et al., 1999b; Bohnert et al., 2002b). In addition, nonprotein N (**NPN**), primarily urea, is an attractive protein replacement because of its low cost per unit of N compared with most sources of natural protein.

Urea and biuret are two sources of NPN commonly used in ruminant diets. Data have shown that hydrolysis of urea to ammonia and CO_2 occurs rapidly, irrespective of dietary history (Helmer and Bartley, 1971). This can result in ammonia toxicity if urea is consumed in large quantities within a short period of time (Helmer and Bartley, 1971). In contrast, biuret is less soluble in water and is degraded to ammonia at a slower rate compared with urea (Fonnesbeck et al., 1975). Biuret is also comparatively nontoxic (Hatfield et al., 1959) and does not decrease supplement palatability as does urea (Fonnesbeck et al., 1975). Consequently, biuret can be incorporated into supplements at higher concentrations compared with urea. Data

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[3]Correspondence: 67826-A Hwy 205 (phone: 541-573-8910; fax: 541-573-3042; e-mail: dave.bohnert@oregonstate.edu).

[4]Present address: Hettinger Res. and Ext. Center, Hettinger, ND 58639.

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Table 1. Supplement composition and feedstuff nutrient content

Item	Hard fescue straw	Urea supplement[a]	Biuret supplement[a]
Urea, %	—	5.3	—
Biuret, %	—	—	6.1
Soybean hulls, %	—	91.0	90.2
Dried molasses, %	—	3.7	3.7
Nutrient composition			
CP, % DM	4.0	28.9	29.0
DIP, % CP[b]	76.0	83.0	84.2
OM, % DM	94.3	90.8	92.7
NDF, % DM	77.4	60.1	56.3
ADF, % DM	41.2	39.7	39.1

[a]Pelleted supplements were provided by ADM Alliance Nutrition, Inc., Quincy, IL.
[b]Degradable intake protein. Estimates are based on Dacron bag degradabilities. Techniques were similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for straw and supplements, respectively.

are limited comparing the effects of urea or biuret supplemented infrequently on forage intake and nutrient digestibility in ruminants. Therefore, the objective of this research was to compare daily and alternate-day supplementation of urea or biuret on the utilization of low-quality forage by steers.

Materials and Methods

Five Angus × Hereford steers (491 ± 21 kg) with ruminal and double L-shaped duodenal cannulas (Streeter et al., 1991) were allotted randomly to one of five treatments in an incomplete 5 × 4 Latin square (Cochran and Cox, 1957) and housed in individual pens (4 × 8 m) within an enclosed barn with continuous lighting. Treatments (**TRT**) consisted of an unsupplemented control and a urea or biuret supplement provided daily (**D**) or every other day (**2D**; **CON** = control, **UD** = urea supplement every day, **U2D** = urea supplement every other day, **BD** = biuret supplement every day, and **B2D** = biuret supplement every other day). The NPN treatments were formulated to provide 90% of the estimated degradable intake protein (**DIP**) requirement assuming a microbial efficiency of 11% (NRC, 1996). Protein supplements were placed directly into the rumen via the ruminal cannula at 0700 for supplemented treatments. The D TRT were supplemented CP at 0.04% of BW/d, whereas the 2D TRT were supplemented at 0.08% of BW every other day. Therefore, urea and biuret treatments received the same amount of total supplemental N over a 2-d period. To minimize potential bias because of different BW changes resulting from treatment regimes during each period, the quantity of supplement provided in each period was based on initial BW. Urea and biuret intake was approximately 0.069, 0.138, 0.085, and 0.170 g/kg BW on each supplementation day for UD, U2D, BD, and B2D, respectively. Estimates of DIP for hard fescue straw (*Festuca trachyphylla*) and supplements were based on in situ degradability using techniques similar to those described by Mass et al. (1999) and Bohnert et al. (1998), respectively. Steers had continuous access to fresh water and chopped (4 to 8 cm) hard fescue straw. Nutrient content of the hard fescue straw and protein supplements is listed in Table 1. Straw was provided daily at 120% of the previous 5-d average intake in two equal portions (at 0715 and 1900), with feed refusals from the previous day determined before feeding. A trace mineral salt mix was available free choice (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, DM basis). In addition, an intramuscular injection of vitamins A, D, and E (500,000, 50,000, and 1,500 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. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Oregon State University.

Experimental periods were 24 d, with 10 d of diet adaptation and 14 d of sampling. Intake was measured beginning d 11 and concluding d 22. On d 13 and 18, treatment effects on ruminal particulate fill were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on a day all supplements were provided and on a day on which only daily supplements were provided. Total ruminal contents were weighed, mixed by hand, and subsampled in triplicate (approximately 400 g). The remaining ruminal contents were replaced immediately into the animal. Ruminal samples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed in order to calculate DM, ground to pass a 1-mm screen in a Wiley mill, and composited within period and day by steer. A complete description of these procedures is provided in a companion paper (Currier et al., 2004b). Ruminal bacteria were isolated from ruminal contents on d 13. A 2-kg sample was weighed into a container and 1 L of cold (4°C) 0.9% (wt/vol) NaCl was added. This mixture was well mixed by hand and homogenized (Waring blender; Waring Products, New Hartford, CT) at high speed for 1 min and strained through

Table 2. Effects of nonprotein nitrogen (NPN) source and supplementation frequency on intake of dry matter, organic matter, and neutral detergent fiber and organic matter and neutral detergent fiber disappearance in steers

	Treatment[a]						*P*-value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	CON vs. Suppl.	Urea vs. Biuret	D vs. 2D	NPN source × SF
Daily DM intake, g/kg BW										
Straw	17.1	17.4	17.0	17.9	17.2	0.3	0.37	0.34	0.08	0.56
Supplement[d]	0.0	1.3	1.3	1.4	1.4					
Total	17.1	18.8	18.4	19.2	18.5	0.3	<0.001	0.32	0.08	0.57
Daily OM intake, g/kg BW										
Straw	16.1	16.5	16.1	16.9	16.2	0.3	0.37	0.34	0.08	0.56
Supplement[e]	0.0	1.2	1.2	1.3	1.3					
Total	16.1	17.7	17.3	18.1	17.4	0.3	0.009	0.29	0.08	0.57
Daily NDF intake, g/kg BW	13.2	14.3	14.0	14.6	14.0	0.2	0.004	0.55	0.09	0.56
Daily OM disappearance from stomach										
Apparent, % OM intake	38.5	41.5	38.4	39.2	37.3	2.3	0.80	0.48	0.31	0.80
True, % of OM intake[f]	55.1	56.7	55.2	57.7	54.8	1.7	0.62	0.86	0.23	0.69
Daily NDF disappearance from stomach, % of NDF intake	55.9	60.1	55.6	59.1	54.7	2.3	0.57	0.69	0.08	0.98
Daily duodenal OM flow, g/kg BW	9.9	10.4	10.6	11.0	10.9	0.4	0.08	0.23	0.84	0.59
Daily OM disappearance from intestines, g/kg BW	2.5	2.2	2.8	3.0	2.8	0.4	0.61	0.31	0.55	0.34
% of duodenal OM flow	24.7	20.9	26.2	25.8	25.2	2.4	0.94	0.46	0.36	0.26
% of OM intake	15.3	12.3	16.2	16.3	16.3	2.1	0.99	0.35	0.37	0.38
Apparent total tract OM disappearance, %	53.7	53.8	54.6	55.5	53.7	0.5	0.31	0.52	0.38	0.04

[a]CON = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.
[b]n = 4.
[c]CON vs. Suppl. = control vs. supplemented treatments; Urea vs. Biuret = urea vs biuret treatments; D vs. 2D = daily vs. alternate-day supplementation; NPN source × SF = interaction of NPN source vs. supplementation frequency.
[d]UD received 1.3 g/kg BW daily; U2D received 2.6 g/kg BW every other day; BD received 1.4 g/kg BW daily; B2D received 2.8 g/kg BW every other day.
[e]UD received 1.2 g/kg BW daily; U2D received 2.4 g/kg BW every other day; BD received 1.3 g/kg BW daily; B2D received 2.6 g/kg BW every other day.
[f]Corrected for bacterial OM.

four layers of cheesecloth. The bacteria were then separated from protozoa and feed particles by centrifugation (800 × *g* for 20 min). The resulting supernatant fluid was collected and stored (–20°C) for later isolation of ruminal bacteria. The supernatant fluid was thawed, transferred to 250-mL bottles, and centrifuged (10,000 × *g* for 15 min, 4°C) to pellet bacteria. The resulting supernatant fluid from this centrifugation was decanted and discarded. The pellet was resuspended using distilled water and centrifuged (10,000 × *g* for 15 min, 4°C). This step was repeated once and the bacteria were frozen (–20°C), lyophilized, ground with a mortar and pestle, and composited by treatment.

Gelatin capsules containing 9 g of chromic oxide were dosed intraruminally at 0700 and 1900 on d 14 to 24 for use as indigestible markers of digesta flow. Samples of hard fescue straw and CP supplements were collected on d 11 to 22 and orts were collected on d 12 to 23. Samples of feed and orts were dried at 55°C for 48 h. On d 19 to 24, approximately 200 g of duodenal digesta was collected at 0800, 1200, 1600, and 2000. Subsamples (75 g) were composited by steer and stored (–20°C). Composited duodenal samples were lyophilized. Feces were collected on d 19 to 24. Steers were fitted with harnesses and fecal bags on d 19 at 0730. Fecal bags were weighed and emptied twice daily at 0730 and 1630. Feces collected at 1630 were stored individually by steer in a sealed 189-L polyethylene bag for mixing with the 0730 collection the following morning (24-h fecal collection). Feces were manually mixed and a 2.5% subsample (wet weight) obtained, dried for 96 h at 55°C, reweighed for DM, and composited by steer within period. Dried samples of straw, orts, and feces were ground through a Wiley mill (1-mm screen). Duodenal samples were ground to pass a 1-mm screen using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) because of limited sample size.

Ground samples of hard fescue straw and CP supplements were composited by period and daily orts composited by steer (within period) on an equal-weight basis (5% as-fed). Feed, orts, duodenal digesta, and feces were analyzed for DM and OM (AOAC, 1990), N (Leco CN-2000, Leco Corporation, St. Joseph, MI), and 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). Samples of duodenal digesta and feces were prepared as described by Williams et al. (1962) for analysis of Cr using atomic absorption spectroscopy (air/acetylene flame; model 351 AA/AE Spectropho-

Table 3. Effects of nonprotein nitrogen (NPN) source and supplementation frequency on nitrogen intake and disappearance in steers

	Treatment[a]						*P*-value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	CON vs. Suppl.	Urea vs. Biuret	D vs. 2D	NPN source × SF
Daily N intake, g/kg BW	0.109	0.173	0.171	0.178	0.172	0.002	<0.001	0.06	0.03	0.28
Daily N flow at duodenum										
g/kg BW	0.282	0.295	0.310	0.331	0.310	0.010	0.04	0.12	0.79	0.13
Daily bacterial N at duodenum										
g/kg BW	0.213	0.208	0.229	0.271	0.251	0.010	0.04	0.002	0.92	0.07
% of total duodenal N	76.3	71.2	74.1	81.7	81.0	1.4	0.67	<0.001	0.43	0.22
Daily non-bacterial N at duodenum										
g/kg BW	0.069	0.087	0.081	0.060	0.059	0.004	0.53	<0.001	0.33	0.50
Bacterial N, % of DM	7.14	6.96	7.13	7.17	7.44					
Bacterial N:purine ratio	1.16	1.23	1.19	1.09	1.11					
Bacterial N synthesis										
g of N/kg of OMAD[d]	34.5	28.2	35.3	39.8	40.9	4.1	0.74	0.07	0.35	0.48
g of N/kg of OMTD[e]	24.1	20.8	24.2	26.2	26.9	1.8	0.84	0.05	0.28	0.46
Daily N disappearance from stomach										
Apparent, % of N intake	–160.4	–69.9	–80.8	–86.6	–80.6	8	<0.001	0.33	0.77	0.32
True, % of N intake[f]	37.5	50.9	53.0	65.0	66.1	4.1	0.002	0.01	0.71	0.90
True, g/kg BW[f]	0.040	0.086	0.090	0.118	0.113	0.005	<0.001	<0.001	0.97	0.39
Daily N disappearance from intestines										
g/kg BW	0.197	0.196	0.216	0.230	0.210	0.010	0.19	0.19	0.99	0.07
% of intake	181.9	112.5	126.0	130.2	121.9	7.4	<0.001	0.39	0.74	0.18
% of duodenal flow	69.7	66.3	69.7	69.2	67.3	0.9	0.17	0.77	0.39	0.02
ATTN disappearance, %[g]	21.6	42.6	45.2	43.5	41.3	1.2	<0.001	0.25	0.86	0.07

[a]CON = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.
[b]n = 4.
[c]CON vs. Suppl. = control vs. supplemented treatments; Urea vs. Biuret = urea vs. biuret treatments; D vs. 2D = daily vs. alternate-day supplementation; NPN source × SF = interaction of NPN source vs. supplementation frequency.
[d]OMAD = apparent OM disappearance from stomach.
[e]OMTD = true OM disappearance from stomach (corrected for bacterial OM).
[f]Corrected for bacterial N.
[g]ATTN = apparent total-tract N disappearance.

tometer; Instrumentation Laboratory, Inc., Lexington, MA). Duodenal Cr concentration was used in conjunction with nutrient concentration to determine duodenal nutrient flow (Merchen, 1988). Recovery of dosed Cr in the feces averaged 105 ± 1%.

The purine content of duodenal digesta and ruminal bacteria was determined using the technique of Zinn and Owens (1986) as modified by Makkar and Becker (1999) using torula yeast RNA (R-6625; Sigma Chemical Co., St. Louis, MO) as a purine standard. Total flow of bacterial N at the duodenum was estimated by dividing the average bacterial N:purine ratio of harvested bacteria by the N:purine ratio of the duodenal digesta and multiplying the quotient by the total N flow at the duodenum.

Statistical Analysis

Data were analyzed as an incomplete 5 × 4 Latin square (Cochran and Cox, 1957) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included period, steer, and treatment. Because the treatment structure consisted of a 2 × 2 factorial plus a negative control, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were 1) CON vs. CP supplementation, 2) urea vs. biuret, 3) D vs. 2D supplementation, 4) NPN source × supplementation frequency (**SF**).

Results

Intake of straw DM and OM was not affected ($P > 0.33$) by CP supplementation or NPN source (Table 2). However, total DM and OM intake was increased ($P < 0.01$) with CP supplementation compared with the control and tended ($P = 0.08$) to be greater for D compared with 2D treatments. Intake of NDF was increased ($P < 0.01$) with CP supplementation, whereas no difference was noted because of NPN source ($P = 0.55$); however, D treatments tended ($P = 0.09$) to have greater NDF intake compared with 2D treatments.

Apparent and true (corrected for bacterial OM) OM disappearance from the stomach was not affected by CP supplementation, NPN source, or SF ($P > 0.22$; Table 2). Similarly, NDF disappearance from the stomach was not altered by CP supplementation or for urea compared with biuret ($P > 0.56$). However, NDF disappearance tended ($P = 0.08$) to be greater for D compared with 2D treatments.

Daily duodenal OM flow (grams per kilogram of BW) tended to increase ($P = 0.08$) with CP supplementation, but was not affected by NPN source or SF ($P > 0.22$; Table 2). Daily intestinal disappearance of OM (grams per kilogram of BW; percentage of duodenal flow; percentage of OM intake) was not affected ($P > 0.25$) by CP supplementation, NPN source or SF. In addition, CP supplementation did not affect apparent total tract OM digestion ($P = 0.31$). However, we detected a NPN source × SF interaction ($P = 0.04$) for apparent total-tract OM digestion because OM disappearance decreased as SF decreased with biuret, whereas no difference was observed between urea D and 2D treatments.

Daily N intake was increased ($P < 0.01$) with CP supplementation and was greater ($P = 0.03$) for D compared with 2D supplementation (Table 3). Similarly, duodenal N flow increased ($P = 0.04$) with CP supplementation, with no difference because of NPN source or SF ($P > 0.11$). Bacterial N at the duodenum (grams per kilogram of BW) increased ($P = 0.04$) with CP supplementation and for biuret compared with urea ($P < 0.01$). However, duodenal bacterial N (expressed as a percentage of total duodenal N), and nonbacterial N (grams per kilogram of BW), were not affected by CP supplementation or SF ($P > 0.21$). Biuret had greater ($P < 0.01$) bacterial N flow at the duodenum (expressed as a percentage of total duodenal N) than urea, whereas urea had greater ($P < 0.01$) nonbacterial N flow at the duodenum compared with biuret.

Mean bacterial N for all treatments was 7.17% (DM basis) and average bacterial N:purine ratio was 1.16 (Table 3). Apparent bacterial N synthesis (grams of bacterial N per kilogram of OM apparently digested in the rumen) tended ($P = 0.07$) to be greater for biuret compared with urea and was not affected by CP supplementation or SF ($P > 0.34$). Likewise, true bacterial N synthesis (grams of bacterial N per kilogram of OM truly digested in the rumen; corrected for bacterial OM) was not affected by CP supplementation or SF ($P > 0.27$) but was greater ($P = 0.05$) for biuret compared with urea.

Apparent N disappearance from the stomach (as a percentage of N intake) was more negative for the CON compared with NPN treatments ($P < 0.01$; Table 3). Also, true N disappearance from the stomach (corrected for bacterial N) was greater with CP supplementation ($P < 0.01$) and for biuret compared with urea ($P < 0.01$).

Intestinal N disappearance (grams per kilogram of BW) was not affected ($P > 0.06$) by CP supplementation, NPN source, or SF. Intestinal N disappearance was greater ($P < 0.01$) for the CON compared with CP supplemented treatments when expressed as a percentage of N intake. Crude protein supplementation did not influence disappearance of N from the intestines compared with the CON when expressed as a percentage of duodenal N flow ($P > 0.16$). However, we did note an NPN source × SF interaction ($P = 0.02$). This was because N disappearance increased as SF decreased with urea, whereas no change in disappearance was noted with biuret as SF decreased. In addition, apparent total-tract N disappearance increased ($P < 0.01$) twofold with CP supplementation (Table 3).

Discussion

This is the first research of which we are aware that compares the effects of supplemental urea or biuret on microbial protein production and duodenal flow and intestinal disappearance of nutrients in ruminants consuming low-quality forage (<6% CP). Additionally, we provided NPN supplements on a D or 2D basis to better represent the infrequent consumption often observed with commercial, NPN-based, self-fed supplements. These data should add to our understanding of N metabolism in ruminants consuming N-deficient, forage-based diets. In addition, it provides ruminant nutritionists with information useful in evaluating NPN supplements for use by livestock operations that rely on low-quality forage as a primary feed source.

Intake of low-quality forage by ruminants has been reported to increase with CP supplementation (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001). However, this response was not observed in the current study or in a companion paper in which wethers consumed similar hard fescue straw and identical NPN treatments (Currier et al., 2004a). A possible explanation for our lack of a forage intake response with CP supplementation is NDF intake. Mertens (1985, 1994) suggested that forage DM intake is maximized when NDF intake is approximately 12.5 g·kg BW^{-1}·d^{-1}. Therefore, based on the high-NDF intake observed in the current study (13.2 g·kg BW^{-1}·d^{-1} without supplementation), we did not anticipate an increase in forage intake with CP supplementation. Moreover, the increase in NDF intake with CP supplementation in the current study was most likely a result of the high-NDF concentration in the supplements (approximately 57%; DM basis). This is supported by the lack of a difference in straw intake with CP supplementation.

In contrast to forage intake, total DM and OM intake increased with CP supplementation, indicating that total nutrient intake was increased. This agrees with numerous studies that have noted that CP supplementation of ruminants consuming low-quality forage increases total DMI (Ferrell et al., 1999; Bohnert et al., 2002a,b). No difference in straw and/or total DM and OM intake because of NPN source is comparable to results from other studies comparing urea and biuret as CP supplements given to ruminants consuming forage-based diets (Oltjen et al., 1969; Chicco et al., 1971; Currier et al., 2004a).

The tendency for daily supplementation to increase forage and total DM and OM intake compared with supplementation every other day is comparable to results reported in a companion paper (Currier et al., 2004a) and by Bohnert et al. (2002b). In contrast,

Krehbiel et al. (1998) and Huston et al. (1999a) noted no affect of protein SF on DMI by ewes consuming low-quality forage.

It is not readily apparent why 2D supplementation tended to decrease intake compared with D supplementation in the current study. It is possible that the greater quantity of supplement provided on the 2D treatments may have substituted for forage intake on the day of supplementation, thereby decreasing total forage and DMI. Furthermore, Bohnert et al. (2002b,c) suggested that infrequent supplementation may disrupt rumen function for a period of time because of the larger quantity of supplement provided during a supplementation event. This is supported in the current study by the tendency for ruminal NDF digestibility to be greater for D compared with 2D treatments. However, ruminal fermentation characteristics (rumen liquid fill, liquid dilution rate, pH) reported in a companion paper are not consistent with this suggestion (Currier et al., 2004b). The lack of a SF effect on apparent and true (corrected for bacterial OM) ruminal OM digestion suggests that ruminal fermentation was not negatively affected by 2D supplementation.

Our observation that apparent and true (corrected for bacterial OM) OM and NDF disappearance from the stomach were not affected by CP supplementation or NPN source agrees with the statement of Galyean and Owens (1991) that supplemental CP source (NPN, natural protein, DIP, or UIP) has little to no effect on site of digestion of low-quality forage. Other studies support our results that CP supplementation did not increase ruminal OM disappearance (Spragg et al., 1986; Bohnert et al., 2002a). However, these results contrast with those of Lintzenich et al. (1995) and Köster et al. (1996). The hays used by Lintzenich et al. (1995) and Köster et al. (1996) were of very poor quality (1.9 and 2.8% CP, respectively) compared with the hard fescue straw in the current study (4% CP) and the forages used in the aforementioned studies of Spragg et al. (1986) and Bohnert et al. (2002a), which contained 11 and 5% CP, respectively). Therefore, ruminal N was probably more deficient and could have been more limiting to ruminal digestion. Ruminal NH_3-N concentrations in unsupplemented cattle support this hypothesis. They were 0.16, 0.24, 6.90, and 0.80 m*M* in the studies of Lintzenich et al. (1995), Köster et al. (1996), Spragg et al. (1986), and Bohnert et al. (2002a), respectively. Ruminal NH_3-N in the current study was 1.48 m*M* (Currier et al., 2004b). Consequently, CP supplementation may have had a more positive effect on ruminal digestion in the studies of Lintzenich et al. (1995) and Köster et al. (1996) than in the present study.

The tendency for duodenal OM flow to increase with CP supplementation coincides with increased total OM intake. In addition, other research has reported similar results with CP supplementation of forage-based diets (Spragg et al., 1986; Bohnert et al., 2002a). However, the lack of a CP supplementation effect on intestinal OM disappearance contrasts with results reported by Bohnert et al. (2002a). They supplemented steers fed low-quality forage with low- or high-DIP supplements daily, once every 3 d, or once every 6 d and reported that CP supplementation increased intestinal OM disappearance compared with an unsupplemented control. A possible explanation for the conflicting results cited above for intestinal OM disappearance is that we noted a tendency ($P = 0.08$) to increase duodenal OM flow with CP supplementation whereas Bohnert et al. (2002a) significantly ($P < 0.001$) increased duodenal OM flow. Duodenal OM flow increased with CP supplementation in the current study by approximately 8%, whereas Bohnert et al. (2002a) reported an increase of 25% compared with an unsupplemented control. Therefore, Bohnert et al. (2002a) may have had a greater chance of measuring an increase in intestinal OM disappearance. Bohnert et al. (2002a) noted, as in the current study, no effect of CP source or SF on intestinal OM disappearance.

In contrast to our results, Bohnert et al. (2002a) and Currier et al. (2004a) reported increased apparent total-tract OM disappearance with CP supplementation of ruminants consuming low-quality forage. Both studies noted that OM disappearance was not affected by CP source or SF, whereas we observed an NPN source × SF interaction. However, other researchers have reported results similar to ours for total-tract OM digestibility (Romero et al., 1976; Köster et al., 1996; Mathis et al., 2000).

It is not clear why, as SF decreased, that apparent total-tract OM disappearance decreased with biuret but was not different for urea D and 2D. Bohnert et al. (2002b) reported a similar response with lambs consuming low-quality forage (5% CP). They provided a low- or high-DIP supplement daily, once every 3 d, or once every 6 d and noted a CP degradability × SF interaction in which apparent total-tract OM digestibility increased as SF decreased with the low-DIP supplement and decreased as SF decreased with the high-DIP supplement. In another study, Farmer et al. (2001) supplemented steers fed dormant tallgrass-prairie hay with a 43% CP supplement 7, 5, 3, or 2 d/wk and reported that total-tract OM digestibility decreased as SF decreased. Romero et al. (1976) provided urea once a day, twice a day, or once every other day to steers fed low-quality forage and did not affect total-tract OM digestibility. The reason for inconsistent results for total-tract OM digestibility with infrequent supplementation is not clear.

The approximately 60% increase in N intake with CP supplementation was expected because of treatment design. However, the decrease in N intake as SF decreased contradicts results reported in a companion paper (Currier et al., 2004a). The reason for greater N intake for D compared with 2D supplementation is because of differences in forage N intake, which agrees with results reported by Bohnert et al. (2002b) for

ruminants consuming low-quality forage and provided supplemental CP infrequently.

Increased duodenal flow of total and bacterial N with CP supplementation of ruminants consuming low-quality forage has been reported in numerous studies (Hannah et al., 1991; Köster et al., 1996; Bohnert et al., 2002a). Likewise, research involving urea supplementation of ruminants consuming forage-based diets has been reported to increase bacterial N production, or bacterial numbers, compared with no supplementation (Hsu et al., 1991; Bowen et al., 1998). Additionally, increasing the proportion of supplemental DIP provided by urea, at the expense of casein DIP, did not affect microbial N production in ruminants fed dormant tallgrass-prairie forage (Köster et al., 1997). Kropp et al. (1977) noted that the production of microbial N in steers consuming low-quality roughage (cottonseed hulls) was not altered when urea provided 75, 100, or 115% of the estimated CP requirement. Biuret supplementation in the current study increased duodenal bacterial N flow, expressed as grams per kilogram of BW and as a percentage of total duodenal N, by almost 20 and 12%, respectively, compared with urea. Therefore, because NPN source did not have an affect on the total flow of duodenal N whereas biuret increased duodenal bacterial N flow compared with urea, duodenal nonbacterial N flow was greater with urea-supplemented steers compared with those receiving biuret. This could possibly be the result of increased flow of NH_3-N to the small intestine with the urea treatments. This would coincide with the increased ruminal NH_3-N noted for urea compared with biuret treatments in a companion paper (Currier et al., 2004b). It is of interest to note that bacterial N flow at the duodenum averaged almost 150% of N intake for all steers, indicating that N recycling played a large role in steer N metabolism.

We are aware of no research that has compared the effects of supplemental urea and biuret on bacterial N production. However, Oltjen et al. (1969) reported that the average number of total bacteria in rumen fluid was 422 and 439 $\times$ 10^8 cells/mL for steers fed timothy hay and supplemented with urea or biuret, respectively. This was not a significant difference but agrees numerically with our observation of increased bacterial N production for biuret compared with urea. It is possible that the slower and more sustained release of ruminal NH_3-N often observed with biuret compared with urea (NRC, 1976; Bartle et al., 1998) may have allowed for a ruminal environment that supported increased bacterial growth. The slower and more prolonged increase in ruminal NH_3-N concentration with biuret compared with urea is especially evident with 2D supplementation (companion paper; Currier et al., 2004b). Currier et al. (2004b) reported that ruminal NH_3-N at 3, 6, and 9 h after supplementation on the day all supplements were provided was approximately 12, 6, and 3 m*M* and 5, 5, and 5 m*M* for urea and biuret 2D treatments, respectively. It should be noted that there were numerical differences in N:purine ratio and N concentration of ruminal bacteria in the current study that may have affected our results; however, we were not able to analyze for statistical differences because we composited bacteria by treatment. However, differences in N:purine ratio and N concentration between urea and biuret were small and averaged approximately 9% (1.21 vs. 1.10) and 4% (7.0 vs. 7.3), respectively. It is possible that type of supplemental NPN may have altered the bacterial population, thereby potentially changing the N percentage and N:purine ratio. This would agree with the review by Clark et al. (1992) that the N:purine ratio of ruminal bacteria can be altered by the source of CP, amount of CP, and time after feeding.

Bacterial N averaged approximately 76% of total duodenal N for all treatments, emphasizing the importance of bacterial protein to the N status of ruminants consuming low-quality forage. Merchen and Bourquin (1994) noted in their review that bacterial N has ranged from 47 to 81% of total duodenal N in ruminants consuming low-quality forage; therefore, our values are at the high end of this range. This was not unexpected considering that the majority of the supplemental N was in the form of NPN and the hard fescue straw had a low-N and high-DIP content (Table 1). Therefore, bacterial N should be a significant proportion of total duodenal N flow. Clark et al. (1992) noted in their comprehensive review that microbial protein averaged 7.7% N (DM basis), ranging from 4.8 to 10.6%, whereas the average N:purine ratio was 1.06 and ranged from 0.61 to 2.13. Our results for average bacterial N (7.2% N; range of 7.0 to 7.4%) and N:purine ratio (1.16; range of 1.09 to 1.23) are well within these ranges.

Our observation that CP supplementation did not affect apparent or true (corrected for bacterial OM) bacterial efficiency, expressed as grams of bacterial N per kilogram of OM ruminally digested, agrees with other studies in which ruminants consumed low-quality forage (Krysl et al., 1989; Lintzenich et al., 1995; Bowen et al., 1998). However, these results contradict other studies in which CP supplementation increased rumen microbial efficiency in ruminants consuming low-quality forage (Köster et al., 1996; Bohnert et al., 2002a). Possible reasons for the increased bacterial efficiency with CP supplementation in the studies of Köster et al. (1996) and Bohnert et al. (2002a) would be the increased rumen liquid dilution rate and/or greater DIP deficiency. Rumen liquid dilution rate increased linearly as the quantity of supplemental CP increased in the study of Köster et al. (1996) and was approximately 16% greater for supplemented treatments compared with an unsupplemented control in the study of Bohnert et al. (2002c). In contrast, CP supplementation did not increase rumen liquid dilution rate in the current study (Currier et al., 2004b) or in the studies of Krysl et al. (1989) and Lintzenich et al. (1995). The estimated DIP content of the diet without supplemen-

tation in the current study was approximately 72% of requirement and increased to 92% with CP supplementation (assuming a microbial efficiency of 11%; NRC, 1996). In contrast, the estimated DIP content of diets reported by Köster et al. (1996) increased from approximately 21% of requirement without supplementation to 45, 62, 76, and 98% with increasing DIP supplementation (11% microbial efficiency; NRC, 1996). The estimated DIP levels in diets reported by Bohnert et al. (2002a) were 64 and 100% of requirement for unsupplemented and supplemented treatments, respectively. Consequently, the greater DIP deficiency in the studies of Köster et al. (1996) and Bohnert et al. (2002a) may have allowed for a greater affect on microbial efficiency with CP supplementation compared with the current study.

It is unclear why apparent bacterial efficiency tended to increase and true bacterial efficiency was increased with biuret compared with urea supplementation. Forage intake, supplement intake, and ruminal OM digestion were similar between NPN sources, as were rumen liquid and particulate passage rates (Currier et al., 2004b). It is possible that adaptation to biuret by ruminal bacteria allowed for a species composition shift that could have been more efficient in the use of NH_3-N and/or OM from low-quality forage. However, we have no data to support this proposition. As discussed above for bacterial N production, it is possible that the slower and more sustained release of ruminal NH_3-N often observed with biuret compared with urea may have allowed for a ruminal environment that increased bacterial efficiency.

The negative apparent ruminal N disappearance reported for all treatments indicates that N recycling played a major role in ruminal N metabolism (Bunting et al., 1989). This was especially evident with the CON in which apparent N disappearance was –160% of N intake (compared with an average of –79.5% with CP supplementation). Negative values for apparent ruminal N disappearance have been reported in many studies with ruminants consuming low-quality forage (Hannah et al., 1991; Lintzenich et al., 1995; Köster et al., 1996). In contrast to apparent ruminal N disappearance, true ruminal N disappearance (corrected for bacterial N) was approximately 57 and 154% greater with CP supplementation compared with the control when expressed as a percentage of N intake and grams per kilogram of BW, respectively. This is indicative of the high-DIP content of the NPN supplements. Biuret supplementation resulted in approximately 26% greater true ruminal N disappearance, expressed as a percentage of N intake, whereas the total quantity of N that truly disappeared from the rumen (grams per kilogram of BW) was 31% greater compared with urea.

We noted no difference in the quantity of total N (grams per kilogram of BW) that disappeared from the intestines of supplemented and unsupplemented steers. The greater intestinal N disappearance with the CON, as a percentage of N intake, suggests that N recycling played a larger role in the N metabolism of unsupplemented steers compared with those receiving supplemental N. This agrees with our observation that duodenal bacterial N flow (grams per kilogram of BW) was approximately 195% of N intake with the CON compared to an average of 138% with CP supplementation. The increase in intestinal N disappearance (as a percentage of duodenal N flow) with urea as SF decreased, compared with no affect with biuret, suggests that the digestibility of N flowing to the small intestine was increased with U2D compared with UD. However, a more probable explanation is that the U2D treatment had a greater quantity of NH_3-N flowing to the small intestine, specifically on the day of supplementation, compared with UD. This would be consistent with the greater ruminal NH_3-N concentration with U2D compared with UD on the day all supplements were provided (Currier et al., 2004b). A portion of this NH_3-N could have flowed to the small intestine where it may have been absorbed, resulting in increased intestinal N digestibility.

Bohnert et al. (2002a) reported that SF had no effect on intestinal N disappearance. They supplemented steers consuming low-quality forage (5% CP) with a low- or high-DIP supplement daily, once every 3 d, or once every 6 d and reported that intestinal N disappearance, reported as a percentage of N intake or duodenal N flow, was not affected by SF. These results are consistent with our observations that intestinal N disappearance (grams per kilogram of BW and as a percentage of intake) was not affected by NPN source or SF. This supports results noted with wethers and cows in a companion paper (Currier et al., 2004a). Currier et al. (2004a) reported that D or 2D supplementation of NPN to ruminants consuming low-quality forage resulted in similar N balance, N retention, and animal performance. Therefore, our results suggest that every-other-day supplementation of NPN to ruminants consuming low-quality forage has a minimal affect on intestinal N disappearance compared with daily supplementation.

Increased apparent total-tract N disappearance with CP supplementation has been reported in other studies with ruminants consuming low-quality forage (Ferrell et al., 1999; Bohnert et al., 2002a,b). These results concur with those reported for apparent total-tract N disappearance in a companion study (Currier et al., 2004a). This agrees with other research that has reported no effect of SF on total-tract N disappearance in ruminants consuming forage-based diets (Coleman and Wyatt, 1982; Brown et al., 1996; Bohnert et al., 2002a,b).

Implications

Daily and alternate-day supplementation of urea or biuret to ruminants consuming low-quality forage does not adversely affect forage intake, nutrient digestibility, site of digestion, or microbial efficiency compared

with unsupplemented animals. Ruminants consuming low-quality forage seem to efficiently use urea and biuret as sources of supplemental nitrogen. Consequently, alternate-day supplementation of nonprotein nitrogen may provide beef producers with a management alternative to decrease supplementation costs and improve economic returns.

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