

# Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: I. Effects on cow performance and the efficiency of nitrogen use in wethers<sup>1,2</sup>

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**ABSTRACT:** Two experiments were conducted to determine the influence of supplemental nonprotein N (NPN) provided daily (D) or every other day (2D) on ruminant performance and N efficiency. Treatments included an unsupplemented control (CON) and a urea (28.7% CP) or biuret (28.6% CP) supplement provided D or 2D at 0700. In Exp. 1, five wethers ( $39 \pm 1$  kg BW) were used in an incomplete  $5 \times 4$  Latin square with four 24-d periods to determine the influence of supplemental NPN source and supplementation frequency (SF) on the efficiency of N use in lambs consuming low-quality grass straw (4% CP). The amount of CP supplied by each supplement was approximately 0.10% of BW/d (averaged over a 2-d period). In Exp. 2, 80 Angus  $\times$  Hereford cows ( $540 \pm 8$  kg BW) in the last third of gestation were used to determine the effect of NPN source and SF on cow performance. The NPN treatments were formulated to provide 90% of the estimated degradable intake protein requirement. The supplemented treatments received the same amount of supplemental N over a 2-d

period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day than the D treatments. In Exp. 1, total DM, OM, and N intake; DM, OM, and N digestibility; N balance; and digested N retained were greater ( $P < 0.03$ ) for supplemented than for CON wethers, with no difference ( $P > 0.05$ ) between NPN sources or SF. Plasma urea-N (PUN) was increased with N supplementation compared with CON ( $P < 0.01$ ), and urea treatments had greater PUN than biuret ( $P < 0.01$ ). In addition, PUN was greater ( $P = 0.02$ ) for D than for 2D treatments. In Exp. 2, pre- and postcalving (within 14 d and 24 h after calving, respectively) cow weight and body condition score change were more positive ( $P < 0.05$ ) for supplemented groups than for CON. These results suggest that supplements containing urea or biuret as the primary source of supplemental N can be effectively used by lambs and cows consuming low-quality forage, even when provided every other day.

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

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## Introduction

It has been 38 yr since Virtanen (1966) demonstrated that ruminants can convert nonprotein N (NPN) to milk protein. Sources of NPN are an attractive protein replacement because of their low cost com-

pared with most natural proteins (N basis). Consequently, numerous studies have been conducted evaluating NPN as a source of supplemental N for ruminants. Urea, the most commonly used NPN source, is very soluble in water and is rapidly hydrolyzed to  $\text{NH}_3\text{-N}$  within the rumen. This can lead to  $\text{NH}_3\text{-N}$  toxicity if urea is consumed in large quantities within a short period of time (Bartley et al., 1976). In contrast, biuret is not very soluble in water and is degraded to  $\text{NH}_3\text{-N}$  at a slower rate than urea (Fonnesbeck et al., 1975). Thus, biuret is comparatively non-toxic compared with urea and, therefore, can be incorporated into supplements at higher concentrations than urea (Hatfield et al., 1959). Also, biuret does not elicit the negative effects on supplement palatability and intake often observed with urea (Fonnesbeck et al., 1975).

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**Table 1.** Ingredient and nutrient content of hard fescue straw and supplements

Item	Hard fescue straw	Urea supplement <sup>a</sup>	Biuret supplement <sup>a</sup>	Hard fescue straw	Urea supplement <sup>a</sup>	Biuret supplement <sup>a</sup>
	Lamb study (Exp. 1)			Cow study (Exp. 2)		
Supplement composition, % of DM						
Urea	—	5.3	—	—	5.3	—
Biuret	—	—	6.1	—	—	6.1
Soybean hulls	—	91.0	90.2	—	91.0	90.2
Dried molasses	—	3.7	3.7	—	3.7	3.7
Nutrient composition						
CP, % DM	4.3	28.7	28.6	4.0	29.1	28.6
DIP, % CP <sup>b</sup>	76.0	83.0	84.2	76.0	83.0	84.2
OM, % DM	93.6	90.2	92.4	93.8	93.1	94.0
NDF, % DM	73.8	57.9	55.4	75.9	58.6	55.6
ADF % DM	32.0	38.1	38.2	42.0	40.8	40.0

<sup>a</sup>Pelleted supplements were provided by ADM Alliance Nutrition, Inc., Quincy, IL.

<sup>b</sup>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.

There are few data available concerning the effects of infrequent supplementation of NPN on the efficiency of N use and performance by ruminants consuming low-quality forage. In addition, certain self-fed supplements, such as liquid-molasses mixes, molasses tubs, and molasses blocks, are becoming increasingly popular with beef producers because of the decreased labor associated with their use. However, these types of supplements are generally consumed at infrequent intervals and often contain a significant portion of their supplemental CP in the form of NPN. Consequently, this study was designed to determine whether daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage (<6% CP) would allow for acceptable levels of performance and nutrient utilization compared with unsupplemented controls.

## Materials and Methods

### Experiment 1: N Balance Study

Five wethers ( $39 \pm 1$  kg) were used in an incomplete  $5 \times 4$  Latin square design (Cochran and Cox, 1957) to evaluate the efficiency of N use in lambs supplemented with a urea or biuret supplement (Table 1) every day or every other day. Estimates of degradable intake protein (DIP) were determined based on in situ degradability using techniques similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for hard fescue straw and supplements, respectively. Wethers were allotted randomly to treatments and housed in individual metabolism crates within an enclosed barn with continuous lighting. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Oregon State University.

Wethers had continuous access to fresh water and chopped (4 to 8 cm in length) hard fescue (*Festuca trachyphylla*) straw (Table 1). Treatments were arranged as a  $2 \times 2$  factorial, two sources of supplemental

NPN and two supplementation frequencies (**SF**), with a negative control (**CON**; no supplementation). Crude protein supplements were offered every day (**D**) or every other day (**2D**) at 0700. The urea and biuret treatments received the same amount of total supplemental N over a 2-d period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day compared with D treatments. Urea and biuret intake was approximately 0.175, 0.350, 0.207, and 0.416 g/kg BW on each supplementation day for urea D, urea 2D, biuret D, and biuret 2D, respectively. The amount of CP supplied by each supplement was approximately 0.10% of BW/d (averaged 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. Forage was provided daily at 120% of the average intake for the previous 5 d in two equal portions (0715 and 1900), with feed refusals from the previous day determined before the 0700 feeding. Also, 35 g of a trace mineral salt mix (2.4% Ca, 2.3% P, 20.4% Na, 31.65 Cl, 0.2% K, 0.4% Mg, 0.1% S, 1,309 ppm Mn, 2,046 ppm Fe, 7 ppm Cu, 1,930 ppm Zn, 42 ppm Co, 120 ppm I, 16 ppm Se, 1,325 IU/kg Vitamin E, and 552 and 50 kIU/kg vitamins A and D, respectively, DM basis) was provided daily to each lamb at 0700. In addition, an intramuscular injection of vitamins A, D, and E (200,000, 20,000, and 600 IU, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each lamb at the onset of the trial to safeguard against deficiency.

Experimental periods were 24 d with at least 3 d between periods (to remove wethers from metabolism crates). Dry matter intake was determined on d 17 to 22. Samples of hard fescue straw and CP supplements were collected on d 17 to 22, whereas orts were collected on d 18 to 23. Samples of feed and orts were dried at 55°C for 48 h. On d 19 to 24, total fecal and urine output was collected. Sufficient 6 N HCl (100

mL) was added daily to urinals to maintain urine pH < 3 (verified with pH paper during the urine collection period) to minimize bacterial growth and N loss. Urine was composited daily by wether (50% of total; weight basis) and stored at 4°C. A subsample of each daily fecal sample (7.5%; wet weight basis) was dried at 55°C for 96 h for calculation of fecal DM. On d 19 to 24, 12 mL of blood was collected via jugular venipuncture 2, 4, and 6 h after the 0715 straw feeding using a heparinized syringe. Blood samples were immediately transferred to Vacutainers (Fisher Scientific, Pittsburgh, PA, Catalog No. 0268360), placed on ice for transport to the lab, centrifuged (5,000 × g for 15 min; 4°C), and plasma harvested and stored (-20°C).

Dried samples were ground through a Wiley mill (1-mm screen). Samples of ground hard fescue straw and CP supplements were composited by period and daily orts composited by lamb (within period) on an equal-weight basis (20% as-fed). Ground fecal samples were composited by lamb within period. Feed, orts, and fecal samples were analyzed for DM, OM (AOAC, 1990), 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). Feed, orts, fecal, and urine samples were analyzed for N using a Leco CN-2000 (Leco Corp., St. Joseph, MI). Plasma samples were assayed for urea-N using the Sigma Diagnostics Procedure 535 (Sigma Chemical Co., St. Louis, MO) and a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

### *Experiment 2: Performance Study*

Eighty pregnant (approximately 210 d) Angus × Hereford cows (540 ± 8 kg BW) were stratified by age, BCS (1 = emaciated, 9 = obese; Herd and Sprott, 1996), and weight and were assigned randomly within stratification to one of five treatments (as described in Exp. 1) in a 2 × 2 factorial arrangement (two types of NPN and two SF) with a negative CON (no supplementation). Cows were then sorted by treatment and allotted randomly to 1 of 20 pens (four cows/pen; four pens/treatment). A trace-mineralized 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). Cows were provided ad libitum access to hard fescue straw (Table 1).

The NPN treatments were formulated to provide 90% of the estimated DIP requirement assuming a microbial efficiency of 11% (NRC, 1996; level 1). The urea and biuret treatments received the same amount of total supplemental N over a 2-d period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day compared with D treatments. Crude protein supplements (Table 1) were offered D or 2D at 0700 to provide

approximately 0.04% of BW/d (averaged over a 2-d period) of CP until calving. The experiment began on January 11, 2002, with experimental diets fed from start date until calving (70 ± 1 d).

Cow BW and BCS were measured every 14 d to calving and within 24 h after calving. All weights were obtained following an overnight shrink (16 h). Cow BCS was judged independently by four observers. The same technicians measured BCS throughout the experiment. In addition, calf weights were obtained within 24 h of birth. Hard fescue straw and supplement samples (approximately 200 g) were collected weekly, dried at 55°C for a minimum of 48 h, ground through a Wiley mill (1-mm screen), and composited by period for analysis of ADF, NDF, N, and OM as described in Exp. 1.

### *Statistical Analysis*

*Experiment 1: N Balance Study.* 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, wether, and treatment. Because the treatment structure consisted of a 2 × 2 factorial plus a negative CON, orthogonal contrasts were used to partition specific treatment effects. Contrast statements included 1) CON vs. CP supplementation, 2) urea vs. biuret, 3) D vs. 2D supplementation, and 4) NPN source × SF. Response variables included 1) DM and OM intake; 2) total-tract digestibility of DM, OM, and N; 3) N balance; and 4) digested N retained. Plasma urea-N (**PUN**) was analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included lamb, period, treatment, hour, frequency (day all supplements provided or day only daily supplements provided), treatment × frequency, treatment × hour, and treatment × hour × frequency. In addition, lamb × period × treatment was used to specify variation between animals (using the RANDOM statement). Autoregression was used as the covariance structure. The same contrasts noted above were used to partition treatment sums of squares.

*Experiment 2: Performance Study.* Cow performance data were analyzed as a randomized complete block design using the GLM procedure of SAS. The model included block and treatment. The same orthogonal contrasts described in Exp. 1 were used to partition specific treatment effects. Response variables included 1) cow weight change, 2) cow BCS change, and 3) calf birth weight.

## **Results**

### *Experiment 1: N Balance Study*

Intake of straw DM and OM by lambs was not affected ( $P = 0.56$ ) by CP supplementation; however, there was a tendency ( $P = 0.08$ ) for straw DM and OM

**Table 2.** Effects of nonprotein nitrogen (NPN) source and supplementation frequency on intake, diet digestibility, and nitrogen balance in lambs consuming low-quality forage

Item	Treatment <sup>a</sup>					SEM <sup>b</sup>	P-value <sup>c</sup>			
	CON	UD	U2D	BD	B2D		CON vs. Suppl.	Urea vs. Biuret	D vs. 2D	NPN source × SF
Daily DMI, g/kg BW										
Straw	26.4	28.2	26.1	28.2	26.1	1.1	0.56	0.99	0.08	0.99
Supplement <sup>d</sup>	0.0	3.3	3.3	3.4	3.4					
Total	26.4	31.5	29.4	31.6	29.4	1.1	0.009	0.97	0.08	0.99
Daily OM intake, g/kg BW										
Straw	24.8	26.5	24.5	26.4	24.4	1.0	0.56	0.97	0.08	0.97
Supplement <sup>e</sup>	0.0	3.0	3.0	3.1	3.1					
Total	24.8	29.5	27.5	29.5	27.5	1.0	0.01	0.96	0.08	0.97
Daily NDF intake, g/kg BW	19.6	22.9	21.3	22.8	20.8	0.7	0.02	0.68	0.04	0.75
Daily N intake, g/kg BW	0.183	0.347	0.331	0.343	0.347	0.012	<0.001	0.58	0.63	0.42
Total-tract digestibility, %										
DM	39.2	48.0	47.9	47.8	45.3	1.9	0.006	0.50	0.51	0.55
OM	42.8	51.4	51.2	51.0	48.7	2	0.009	0.49	0.56	0.63
NDF	42.2	50.8	51.1	49.5	46.1	2.3	0.02	0.20	0.51	0.44
ADF	42.9	52.5	52.0	51.8	46.9	2.4	0.02	0.27	0.31	0.40
N	24.3	53.0	48.5	51.9	52.3	2.9	<0.001	0.66	0.51	0.43
Daily N excretion, g/kg BW										
Fecal	0.136	0.160	0.170	0.167	0.164	0.008	0.01	0.92	0.71	0.46
Urinary	0.059	0.144	0.148	0.135	0.142	0.007	<0.001	0.27	0.45	0.79
Daily N balance, g/kg BW	-0.012	0.042	0.013	0.041	0.041	0.014	0.02	0.36	0.34	0.35
Daily digested N retained, % <sup>f</sup>	-54.4	16.3	6.5	28.6	19.4	15.6	0.003	0.45	0.56	0.98
Plasma urea N, mM	2.40	5.86	5.36	4.46	4.05	0.16	<0.001	<0.001	0.02	0.78

<sup>a</sup>CON = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.

<sup>b</sup>n = 4.

<sup>c</sup>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.

<sup>d</sup>UD received 3.3 g/kg BW daily; U2D received 6.6 g/kg BW every other day; BD received 3.4 g/kg BW daily; B2D received 6.8 g/kg BW every other day.

<sup>e</sup>UD received 3.0 g/kg BW daily; U2D received 6.0 g/kg BW every other day; BD received 3.1 g/kg BW daily; B2D received 6.2 g/kg BW every other day.

<sup>f</sup>Calculated as (daily N retention, g/kg BW / daily N digested, g/kg BW) × 100.

intake to decrease as SF decreased (Table 2). Total DM, OM, NDF, and N intake increased ( $P < 0.03$ ) with supplementation. Also, total DM and OM intake tended to decrease ( $P = 0.08$ ) as SF decreased. Similarly, NDF intake decreased ( $P = 0.04$ ) as SF decreased.

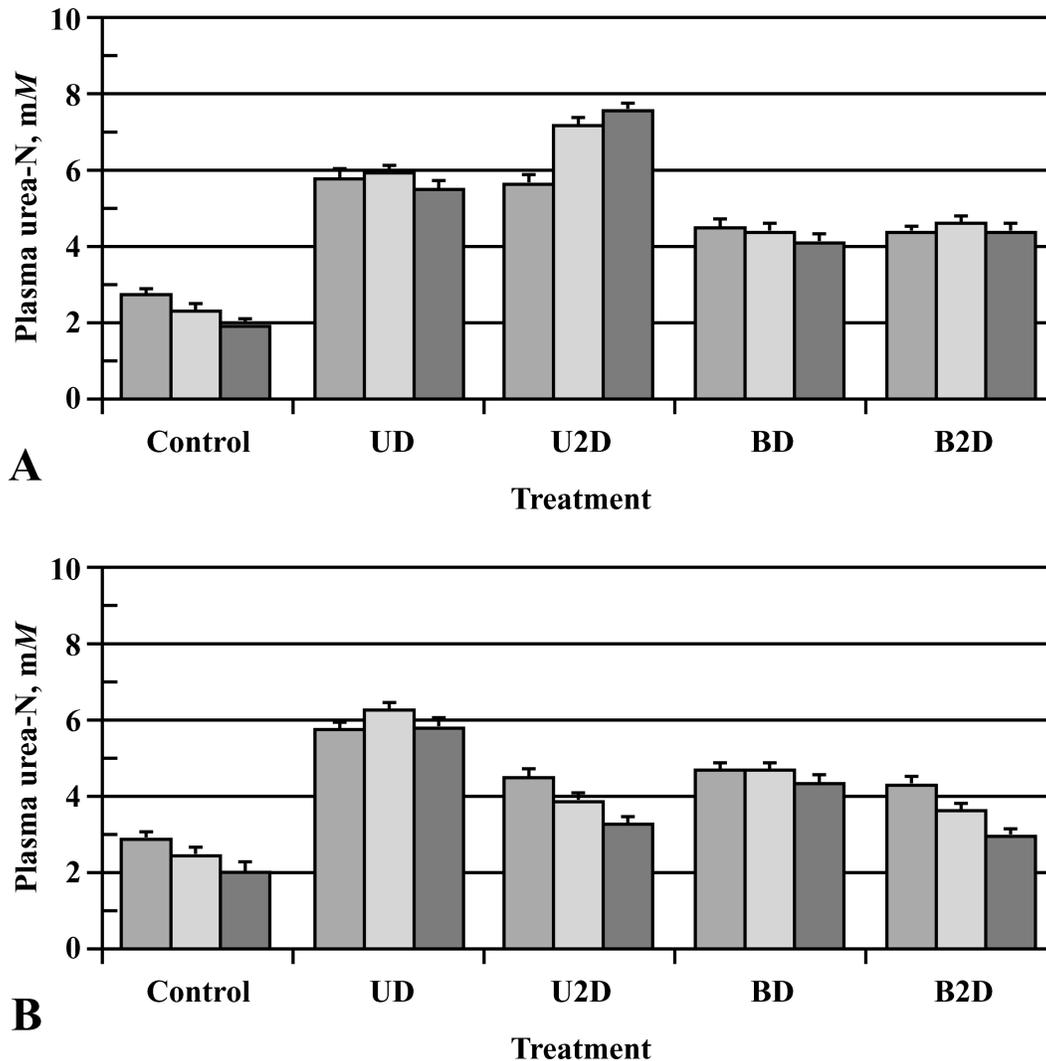
Total-tract digestibility of DM, OM, NDF, ADF, and N increased ( $P < 0.03$ ) with CP supplementation (Table 2). Daily fecal and urinary N excretion (g/kg BW) were increased ( $P < 0.02$ ) with CP supplementation; however, no differences were noted because of NPN source or SF. Daily N balance and digested N retained were greater ( $P < 0.03$ ) with CP supplementation with no difference because of NPN source or SF.

Treatment × hour × SF interactions ( $P < 0.01$ ) were observed for PUN. However, after considering the nature of the interactions, we concluded that discussing treatment means, while providing the treatment × hour × SF figure, would aid in interpretation and discussion of the data (Figure 1). Lamb PUN was greater ( $P < 0.01$ ) for CP supplemented lambs and urea had greater ( $P < 0.01$ ) PUN than biuret (Table 2). In addition, PUN decreased ( $P = 0.02$ ) as SF decreased. Figure

1 provides an illustration of average PUN means for 2, 4, and 6 h after feeding on the day all supplements were offered and the day on which only daily supplements were offered over the 6-d collection period. Plasma urea-N was similar at 2, 4, and 6 h after feeding on the day all supplements were offered for urea D but increased from 2 to 6 h after feeding for urea 2D. In contrast, PUN was similar over the collection period on the day all supplements were offered for biuret D and biuret 2D. On the day only daily supplements were offered, PUN responded in a like manner for the urea 2D and biuret 2D treatments (decreasing over the collection period). However, the average difference between D and 2D treatments was less for biuret D and biuret 2D (0.93 mM) compared with urea D and urea 2D (2.05 mM).

#### Experiment 2: Performance Study

Precalving (within 14 d of calving) and postcalving (within 24 h after calving) weight and BCS change were greater ( $P < 0.01$ ) with CP supplementation (Table 3). In addition, calf birth weight was not affected by NPN supplementation, NPN source, or SF ( $P > 0.10$ ).



**Figure 1.** Effects of nonprotein nitrogen source and supplementation frequency (SF) on lamb plasma urea-N (mM) on the day all supplements were offered (A) and the day on which only daily supplements were offered (B). Columns from left to right for each treatment represent lamb plasma urea-N at 2, 4, and 6 h after feeding. Treatments were as follows: Control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day. Treatment  $\times$  hour  $\times$  SF interaction is ( $P < 0.001$ ). SEM for treatment  $\times$  hour  $\times$  SF = 0.20.

## Discussion

### Experiment 1: N Balance Study

The lack of a CP supplementation effect on straw DM and OM intake contrasts with other studies in which protein supplementation increased forage intake (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001). However, Bohnert et al. (2002a,b) reported that CP supplementation did not increase forage intake of steers and lambs consuming low-quality forage. This coincides with the results observed in the current study. Bohnert et al. (2002a,b) suggested that the lack of an increase in forage intake could be related to NDF intake. They based this on the concept pro-

posed by Mertens (1985, 1994) that DMI is maximized when NDF intake is approximately  $12.5 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ . Bohnert et al. (2002a,b) noted that NDF intake by steers and lambs ranged from  $13.9$  to  $16.0 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$  and  $12.7$  to  $15.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ , respectively. Similarly, forage intake was not expected to increase with CP supplementation in the current study because NDF intake ranged from  $19.6$  to  $22.9 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ . The increase in NDF intake with CP supplementation in the current study is probably a consequence of supplement NDF intake. This is supported by the lack of a CP supplementation effect on straw intake and the high NDF concentration in the NPN supplements (approximately 57%; Table 1).

**Table 3.** Effects of nonprotein nitrogen (NPN) source and supplementation frequency on cow performance and calf birth weight

Item	Treatment <sup>a</sup>					SEM <sup>b</sup>	P-value <sup>c</sup>			
	CON	UD	U2D	BD	B2D		CON vs. Suppl.	Urea vs. Biuret	D vs. 2D	NPN source × SF
Supplement DMI, g/d <sup>d</sup>	0.0	689	689	738	738					
Initial BW, kg	538	556	541	536	531					
Initial BCS	4.85	4.85	4.86	4.87	4.89					
Weight change, kg										
Pregalving <sup>e</sup>	10	31	33	35	33	5	0.002	0.70	0.92	0.65
Postcalving <sup>f</sup>	-40	-13	-19	-6	-14	5	<0.001	0.27	0.19	0.88
BCS change										
Pregalving <sup>e</sup>	-0.08	0.29	0.20	0.18	0.18	0.08	0.006	0.44	0.56	0.56
Postcalving <sup>f</sup>	-0.55	0.20	0.21	0.18	0.02	0.11	<0.001	0.40	0.51	0.48
Calf birth date, ordinal day of year	75	69	66	67	73	3	0.08	0.41	0.58	0.10
Calf birth weight, kg <sup>f</sup>	37	38	39	37	38	1	0.59	0.19	0.33	0.86

<sup>a</sup>CON = control; UD = urea supplement every day; U2D = urea supplement every-other-day; BD = biuret supplement every day; B2D = biuret supplement every other day.

<sup>b</sup>n = 4.

<sup>c</sup>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.

<sup>d</sup>UD received 689 g daily; U2D received 1,378 g every other day; BD received 738 g daily; B2D received 1,476 g every other day.

<sup>e</sup>Within 14 d of calving.

<sup>f</sup>Within 24 h after calving.

Straw and total DMI for urea and biuret treatments are similar to results from other studies comparing urea and biuret as N supplements given to ruminants consuming forage-based diets (Oltjen et al., 1969; Chicco et al., 1971; Bond and Rumsey, 1973). The tendency for forage and total DM and OM intake to be greater for D compared with 2D treatments agrees with the results of Bohnert et al. (2002b). They supplemented lambs with a high- or low-DIP supplement every day, once every 3 d, or once every 6 d. They reported that as SF decreased from daily to once every 6 d, lambs receiving the high- and low-DIP supplements had 8 and 19% decreases in forage and 7 and 17% decreases in total DMI, respectively. This was partially attributed to the substitution of supplement intake for forage intake on the day of supplementation for the once-every-6-d treatments (the large quantity of supplement consumed on the day of supplementation resulted in a depression in forage intake on that day, which affected total forage intake for the 6-d period). Bohnert et al. (2002b,c) suggested that infrequent supplementation may have disrupted rumen function for a period of time because of the larger quantity of supplement provided during a supplementation event as SF decreased. This could explain the tendency for depressed total DMI for 2D compared with D treatments in the current study. However, other research has reported no effect of SF on total DMI by ruminants consuming low-quality forage (Krehbiel et al., 1998; Huston et al., 1999a).

Increased DM, OM, and NDF digestibility with CP supplementation of low-quality forage has been reported in numerous studies (DelCurto et al., 1990; Hor-

ney et al., 1996; Bohnert et al., 2002b). This has been attributed to improved N availability by the ruminal microflora, which increases ruminal fiber digestion (Campling et al., 1962; Petersen, 1987).

As observed in the current study, Chicco et al. (1971) and Ammerman et al. (1972) indicated that DM and OM digestibility, respectively, were not affected by NPN source when urea or biuret supplied the majority of supplemental N provided to ruminants consuming low-quality forage. Studies by Coleman and Wyatt (1982) and Bohnert et al. (2002a) demonstrated that infrequent supplementation of CP to ruminants consuming low-quality forage does not negatively affect DM and/or OM digestibility compared with daily and/or no supplementation. This coincides with our results, which suggest that supplementing urea or biuret daily or every other day to ruminants consuming low-quality forage can increase nutrient digestibility compared with not supplementing.

Apparent total-tract N digestibility for supplemented wethers was approximately 110% greater than the CON. This is comparable to other results observed with ruminants consuming low-quality forage and provided supplemental CP (Ferrell et al., 1999; Bohnert et al., 2002a,b). Similarly, Bohnert et al. (2002a,b) supplemented steers and lambs, respectively, with a high- or low-DIP supplement daily, once every 3 d, or once every 6 d and noted that CP supplementation increased apparent total-tract N digestibility by approximately 81% with the steers and 170% with the lambs compared with unsupplemented controls. The greater N digestibility with CP supplementation is most likely because CP supplements are generally more digestible

than low-quality forage (N basis) and/or metabolic fecal N can be a significant proportion of total fecal N in unsupplemented ruminants (Ferrell et al., 1999). Metabolic fecal N constitutes a greater proportion of total fecal N of unsupplemented ruminants consuming low-quality forage because of their low N intake and relatively constant quantity of metabolic fecal N (5.35 g N/kg DMI; NRC, 1985); therefore, the combination of greater N digestibility in CP supplements and increased metabolic fecal N (as a percentage of N intake) in unsupplemented ruminants results in increased apparent total-tract N digestibility with CP supplementation.

Increased urinary and/or fecal N excretion has been observed with CP supplementation of ruminants consuming low-quality forage compared with unsupplemented individuals (Ammerman et al., 1972; Bohnert et al., 2002b). Increased urinary N excretion with increased N intake has been demonstrated with ruminants (Huntington et al., 2001) primarily because of greater excretion of urea-N (Waterlow, 1999; Huntington et al., 2001). Therefore, the increased excretion of urinary N with CP supplementation in the current study is most likely because of increased N intake and greater urea-N excretion compared with CON.

The lack of an effect of NPN source on urinary and fecal N excretion agrees with other research comparing urea and biuret as CP supplements to low-quality forage (Oltjen et al., 1969; Ammerman et al., 1972). However, Chicco et al. (1971) noted that urinary N excretion was greater for urea-supplemented compared with biuret-supplemented bulls consuming mature, green-chopped elephant grass. They reported no difference in fecal N excretion. Also, infrequent supplementation of CP has had little effect on fecal or urinary N excretion, even when CP was provided once every 6 d (Tudor and Morris, 1971; Coleman and Wyatt, 1982; Bohnert et al., 2002b).

The increase in N balance and digested N retained observed with CP supplementation in the current study agrees with other research in which supplemental CP was provided to ruminants consuming low-quality forage (Egan, 1965; Ammerman et al., 1972; Bohnert et al., 2002b). In his classical work, Egan (1965) supplemented mature wethers consuming wheat straw with casein (6 g/d of N) and noted that supplementation increased N balance from 0.70 g/d without supplementation to 5.47 g/d with supplemental casein. In addition, Ammerman et al. (1972) increased N balance and digested N retained (expressed as a percentage of N intake) in wethers consuming low-quality forage (2.6% CP) and supplemented with soybean meal, urea and soybean meal (50:50 N basis), or biuret and soybean meal (50:50 N basis) compared with wethers receiving just forage. In a supplementation frequency study, Bohnert et al. (2002b) provided a low- or high-DIP supplement to lambs consuming 5.2% CP hay and increased average N balance by 0.103 and 0.096 g·kg BW<sup>-1</sup>·d<sup>-1</sup> and average digested N retained

by 47 and 43%, respectively, compared with an unsupplemented control. Our observation that NPN source did not affect N balance or digested N retained agrees with the work of Oltjen et al. (1969) and Ammerman et al. (1972). These results suggest that urea or biuret can be effectively used as a source of supplemental N by ruminants consuming low-quality forage (<7% CP).

Infrequent supplementation of CP to ruminants consuming low-quality forage has resulted in similar N balance and digested N retained compared with daily supplementation (Romero et al., 1976; Coleman and Wyatt, 1982; Bohnert et al., 2002b). Romero et al. (1976) provided steers consuming 2% CP spear grass hay with urea as an oral drench twice daily, once daily, or once every 2 d. Nitrogen balance was -5.0 g/d for unsupplemented steers and increased to 7.0, 5.7, and 5.7 g/d for steers supplemented twice daily, once daily, and once every 2 d, respectively. Supplementation frequency did not affect N balance. Similarly, Coleman and Wyatt (1982) supplemented steers consuming range hay with cottonseed meal daily, once every 2 d, or once every 4 d and noted that N balance and digested N retained were not affected by SF. Bohnert et al. (2002b) supplemented wethers consuming 5.2% CP meadow hay with a low- or high-DIP supplement daily, once every 3 d, or once every 6 d and increased N balance compared with an unsupplemented control; however, they reported a linear decrease in N balance as SF decreased. They attributed this to a similar decrease in N intake as SF decreased. This assumption is supported by digested N retained, which averaged approximately 29% for supplemented treatments (-16% for the control) and was not influenced by SF. Therefore, based on the results in the current and aforementioned studies, infrequent supplementation of CP to ruminants consuming low-quality forage has only minimal effects on N balance and digested N retained compared with daily supplementation.

Plasma urea concentration is positively correlated with N intake (Harmeyer and Martens, 1980). This coincides with the 87 and 105% increase in average N intake and PUN, respectively, that we observed with CP supplementation compared with the CON. Other research with ruminants has demonstrated increased PUN with CP supplementation of low-quality forage (Krehbiel et al., 1998; Ferrell et al., 1999; Bohnert et al., 2002b). However, limited research, with conflicting results, has compared the affect of biuret or urea supplementation on PUN (Oltjen et al., 1969; Chicco et al., 1971). Oltjen et al. (1969) supplemented steers consuming timothy hay with urea or biuret and reported that PUN was greater with biuret compared with urea (6.11 vs. 5.66 mM). Chicco et al. (1971) supplemented young bulls consuming green-chopped elephant grass with urea or biuret and noted that NPN supplementation increased PUN compared with an unsupplemented control, with no difference because of NPN source. In the current study, urea-supplemented lambs had 32% greater PUN compared with those re-

ceiving biuret (5.6 vs. 4.3 mM). It is not readily apparent why there are conflicting responses in PUN with urea and biuret supplementation. However, experimental diets used by Oltjen et al. (1969) were 85% timothy hay and approximately 13.5% CP. Consequently, protein may not have been the first-limiting nutrient. This could have masked any supplemental NPN effects on PUN. Also, Chicco et al. (1971) collected plasma samples from young bulls 2 h after feeding for measurement of urea-N. This may not have been sufficient time after feeding to determine supplemental NPN effects on PUN. For instance, they reported that ruminal  $\text{NH}_3\text{-N}$  was 60 and 24 mg/100 mL rumen fluid for urea and biuret treatments, respectively, 2 h after feeding. Plasma samples taken at a later time may have resulted in NPN effects on PUN because of the potential for increased  $\text{NH}_3\text{-N}$  absorption from the rumen with urea supplementation, which should increase urea-N production by the liver. Similarly, we sampled PUN at 2, 4, and 6 h after feeding on the day all supplements were provided and the day when only daily supplements were provided; therefore, we did not sample over a full 24-h period. This may have biased PUN results in the current study because of differences in ruminal  $\text{NH}_3\text{-N}$  profiles between urea and biuret treatments (Currier et al., 2004).

Decreased PUN with decreased SF has been reported in other studies with ruminants consuming forage-based diets (Huston et al. 1999a; Bohnert et al., 2002b). The general response observed with infrequent supplementation of CP to ruminants is a larger peak in PUN following a supplementation event compared with daily supplementation. This normally occurs within 24 h of supplementation and is proportional to the quantity of supplement provided. Plasma urea-N then decreases until the next supplementation event (Huston et al., 1999a; Bohnert et al., 2002b). The decrease in average PUN observed with the alternate day compared with daily treatments in the current study is because the decline in PUN on the day on which only daily supplements were provided was greater than the increase observed on the day all supplements were provided (Figure 1).

### *Experiment 2: Performance Study*

Supplementation of CP to beef cows consuming low-quality forage has routinely improved weight and BCS change compared with not providing a CP supplement (Horney et al., 1996; Bohnert et al., 2002b). This was also noted in the current study. Precalving (within 14 d of calving) weight gain was increased by approximately 23 kg and precalving BCS was approximately 0.30 unit greater with CP supplementation compared with the CON.

Research suggests that biuret supplementation of ruminants consuming low-quality forage results in similar, or slightly improved, performance compared with urea (Raleigh and Turner, 1968; Rush et al.,

1976). This agrees with the similar cow performance observed in the current study with urea and biuret supplementation.

Infrequent supplementation of CP to beef cows consuming low-quality forage has been shown to maintain acceptable levels of performance compared with daily supplementation (Huston et al., 1999b; Farmer et al., 2001; Bohnert et al., 2002b). The aforementioned studies used sources of natural protein as CP supplements; therefore, further discussion concerning infrequent supplementation of NPN is warranted given the use of urea and biuret in the current study. However, we are aware of only three studies that have evaluated the impact of infrequent supplementation of urea or biuret on ruminant performance (Thomas and Armitage, 1972; Oltjen et al., 1974; Farmer et al., 2002). Thomas and Armitage (1972) supplemented steer calves (fed approximately 6 kg/d grass hay) daily or every other day with 20% CP supplements in which soybean meal, urea, or biuret provided the source of supplemental CP. They noted, as in the current study, that weight gain was not affected by CP source or SF. Also, Oltjen et al. (1974) supplemented growing steers consuming pangola grass hay with urea, biuret, or cottonseed meal every day or three times a week. They reported that steer daily gain was not affected by CP source or SF, but was greater for CP supplementation compared with a control (mineral supplement only). This agrees with the data of Farmer et al. (2002). They supplemented pregnant cows (last third of gestation) consuming dormant tallgrass prairie forage (4% CP) daily or three times per week with 40% CP supplements in which urea provided 0, 15, 30, or 45% of the supplemental DIP (soybean meal provided the remainder of DIP). They reported no difference in cow BCS change because of SF when urea provided 0, 15, or 30% of the supplemental DIP. However, they reported a more negative BCS change when urea provided 45% of the supplemental DIP compared with the other DIP treatments. This was attributed to supplement refusal because of the high urea content. These results support our observation that daily and alternate-day supplementation of urea or biuret yielded similar effects on cow performance. In addition, this agrees with the N balance data obtained in Exp. 1 that suggests N retention was improved with CP supplementation and not influenced by SF. Therefore, the lambs and cows in the current study were able to use the supplemental N provided by urea or biuret, even when provided every other day, to maintain BW and improve N status.

### **Implications**

Ruminants consuming low-quality forage (<6% crude protein) can effectively use supplemental non-protein nitrogen to maintain nitrogen status and performance. This suggests that nonprotein nitrogen may be an economical alternative to natural protein for

use in hand-fed and self-fed supplements provided to ruminants consuming low-quality forage.

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