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COMPARISON OF UREA AND BIURET AS NITROGEN SUPPLEMENTS TO LOW-QUALITY FORAGE: DAILY AND ALTERNATE DAY SUPPLEMENTATION EFFECTS ON DIGESTION AND RUMINAL FERMENTATION IN STEERS

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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 non-protein nitrogen (NPN) source and supplementation frequency (SF) on DMI and site of digestion in steers consuming low-quality forage (4% CP). Treatments (TRT) included an unsupplemented control (CON) and a urea or biuret supplement placed directly into the rumen daily (D) or every other day (2D) at 0700. Supplements were calculated to provide 90% of the DIP requirement. Urea and biuret supplements (29%CP) were provided on an isonitrogenous basis. Forage was provided at 120% of the previous 5 d average intake in two equal portions at 0715 and 1900. Ruminal fluid was collected 0, 3, 6, 9, 12, and 24 h after supplementation on a day of and a day before supplementation for all TRT. Forage DMI and DM digestibility were not affected ($P > 0.05$) by NPN supplementation, NPN source, or SF. However, total DMI was increased ($P < 0.01$) with supplementation. NH_3N increased ($P < 0.05$) the day of and the day before supplementation with supplemental CP. However, a NPN source × SF interaction ($P = 0.03$) on the day of supplementation indicated NH_3N increased at a greater rate for urea as SF decreased compared with biuret. The data suggest that ruminal degradation of biuret to NH_3N was more moderate and prolonged compared with urea, possibly improving use by ruminal microflora. Ruminal NH_3N on the day before supplementation was greater for D compared with 2D ($P = 0.02$). These results suggest that urea or biuret can be used effectively as a supplemental N source by steers consuming low-quality forage without adversely affecting DMI and DM digestibility, even when provided every other day. Also, biuret should be safer and more useful as a CP supplement when offered infrequently to ruminants because of its slower ruminal degradation to NH_3N compared with urea.

Key Words: Urea, Biuret, Forage, Non-protein Nitrogen, Supplementation, Frequency

Introduction

Many cattle in the western United States consume low-quality forage (< 6% CP) from late summer through winter. Supplementation with protein increases cow weight gain and body condition score (Clanton and Zimmerman, 1970; Rusche et al., 1993; Beaty et al., 1994), forage intake and digestibility (Kartchner, 1980; Köster et al., 1996), and can improve reproductive performance (Sasser et al., 1988; Wiley et al., 1991). Winter feed costs in the Intermountain West often total $100 to 200 per cow each year. In addition to the actual supplement costs, winter supplementation includes other expenses such as labor and equipment associated with supplement delivery.

Decreasing the frequency of supplementation is one management practice that decreases labor costs. Nolan and Leng (1972) suggested that recycling of absorbed N to the rumen may support fermentation between times of supplementation. In addition, research has shown that protein supplements can be fed at infrequent intervals and still maintain acceptable levels of performance (Hunt et al., 1989; Huston et al., 1997; Bohnert et al., 2001). Non-protein nitrogen (NPN) sources are an attractive protein replacement due to their low cost compared with natural proteins (per unit of nitrogen). Data has shown that hydrolysis of urea to ammonia and CO_2 occurs very rapidly, irrespective of dietary history (Helmer and Bartley, 1971). This can lead to ammonia toxicity if urea is consumed in large quantities within a short period of time (Raleigh and Wallace, 1963; Helmer and Bartley, 1971; Bartley et al., 1976). In contrast, biuret is less soluble in water and is degraded to ammonia at a slower rate compared with urea (Fonnesbeck et al., 1975). As a result, biuret is comparatively non-toxic (Hatfield et al., 1959) and does not elicit the negative effects on palatability and intake (Fonnesbeck et al., 1975; Clanton, 1978) often observed with urea; therefore, biuret can be incorporated into supplements at higher concentrations than urea. However, data is limited comparing the effects of urea and biuret supplemented at infrequent intervals on forage intake, site of digestion, and ruminal fermentation. The objective of this research is to compare daily and alternate day supplementation of urea or biuret on utilization of low-quality forage by steers. This knowledge will assist in developing management strategies that help reduce winter feed costs while maintaining acceptable levels of production.

Materials and Methods

Five cannulated (ruminal and duodenal) beef steers (491 ± 21 kg) were allotted randomly to one of five treatments in an incomplete 5 × 4 Latin square design and housed in individual pens (4 × 8 m) within an enclosed barn

with continuous lighting. Treatments consisted of an unsupplemented control and urea or biuret supplemented daily or every other day (**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). Supplemented treatments were formulated to provide 90% of the estimated degradable intake protein requirement assuming a microbial efficiency of 11% (NRC, 1996). 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 .069, .138, .085, and .170 g/kg BW on each supplementation day for UD, U2D, BD, and B2D, respectively. The amount of CP supplied by each supplement was approximately 0.04% of BW/d (averaged over a 2 d period). Protein supplements were placed directly into the rumen via the ruminal cannula at 0700 for the daily and alternate day treatments. Steers had continuous access to fresh water and low-quality grass seed straw. Nutrient content of the grass seed straw and protein supplements is listed in Table 1. 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 feeding. 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, 2307 ppm Mn, 3034 ppm Fe, 1340 ppm Cu, 3202 ppm Zn, 32 ppm Co, 78 ppm I, 90 ppm Se, 79 IU/kg vitamin E, and 397 kIU/kg vitamin A). In addition, an intramuscular injection of vitamins A, D, and E (500,000, 50,000, and 1500 IU of Vitamins A, D, and E, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each steer at the onset of the trial to safeguard against deficiency.

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 DM and fluid contents were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on a day of supplementation and a day preceding supplementation for all treatments. Total ruminal contents were weighed, mixed by hand, and sub-sampled 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 for DM; ground to pass a 1-mm screen in a Wiley mill; and composited within period and d by steer.

Gelatin capsules containing 9 g of chromic oxide was dosed intra-ruminally at 0700 and 1900 on d 14 to 24 for use as an indigestible marker of digesta flow. Samples of grass seed straw and protein 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 were collected at 0800, 1200, 1600, and 2000. Sub-samples (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 (0730). Fecal bags were weighed and emptied twice daily at 0730 and 1630. The feces collected at 1630 were stored in a sealed 50-gallon polyethelene bag for mixing with the 0730 collection the following morning (24 h fecal collection). Feces were manually mixed, a 2.5% sub-sample (wet weight) obtained, weighed, dried for 96 h at 55°C, re-weighed for DM, and composited by steer. Dried samples of hay, orts, and feces were ground as described previously. Duodenal samples were ground through a 1-mm screen using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) due to limited sample size.

On d 19 and 24 (day of and a day before protein supplementation for all treatments, respectively), ruminal fluid (approximately 100 mL) was collected from each steer by suction strainer immediately prior to feeding and at 3, 6, 9, 12, and 24 h post feeding. The 12 h collection was taken prior to the 1900 grass seed straw feeding. Ruminal fluid pH was measured immediately after collection. Five mL were acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for subsequent analysis of NH_3N by a modification (sodium salicylate substituted for phenol) of the procedure described by Broderick and Kang (1980) using a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

Frozen (-20°C) ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × *g*, 10 min), and collecting the supernatant. Ground samples of grass seed straw and protein 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). Duodenal and fecal samples were analyzed for Cr using atomic absorption spectroscopy (air/acetylene flame; Model 351 AA/AE Spectrophotometer, 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%.

Data were analyzed as an incomplete 5 × 4 Latin square using the GLM procedure of SAS (1996). 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) Control vs CP supplementation; 2) Urea vs Biuret; 3) D vs 2D; 4) NPN source × SF. Response variables included: DM and OM intake; ruminal, intestinal, and total tract digestibility of DM, OM, and N; rumen fluid volume; and rumen DM volume.

Ruminal pH and NH_3N collected at fixed times after feeding on d 19 and 24 were analyzed using the REPEATED statement with the MIXED procedure of SAS (1996). The model included steer, period, treatment, time,

and treatment × time. In addition, steer × period × treatment was used to specify variation between animals (using the RANDOM statement). Steer × period × treatment was used as the SUBJECT and autoregression used as the covariance structure. The same contrasts noted above were used to partition the treatment sums of squares.

Results and Discussion

Intake of hay DM and OM was not affected ($P > 0.10$) by CP supplementation or degradability (Table 2). However, hay DM and OM intake tended to be greater ($P = 0.08$) on daily versus alternate day supplementation. Total intake of DM, OM, N, and NDF increased ($P < 0.01$) with supplementation. In addition, N and NDF intake decreased ($P = 0.03$) and tended to decrease ($P = 0.09$), respectively, for alternate day compared with daily supplementation.

No differences ($P > 0.05$) were observed because of CP supplementation or SF for apparent ruminal OM and NDF digestibility (Table 2). Apparent ruminal N digestibility was negative for all treatments and was more negative ($P < 0.01$) for the CON compared with supplemented treatments, indicating that N recycling played an important role in ruminal N dynamics.

Daily duodenal OM flow (g/kg BW) tended to increase ($P = 0.08$) with CP supplementation, while duodenal flow of N (g/kg BW) increased ($P = 0.04$) with CP supplementation (Table 2). Daily intestinal disappearance of OM and N (g/kg BW; percentage of duodenal flow) were not affected ($P > 0.10$) by CP supplementation or SF. However, an NPN source by SF interaction ($P = 0.02$) was observed for apparent intestinal N digestion (% of duodenal flow) because N digestibility decreased as SF decreased with biuret compared with an increase in N digestibility as SF decreased with urea. In addition, intestinal disappearance of N, expressed as a percentage of N intake, was greater ($P < 0.01$) for CON compared with CP supplementation. This is most likely the result of greater N recycling with the CON compared with supplemented treatments. In other words, the greater N recycling with CON increased the duodenal flow of absorbable N presented to the small intestine when expressed as a percentage of N intake.

No differences ($P > 0.05$) were observed for apparent total tract digestion of DM and NDF (Table 2). An NPN source by SF interaction ($P = 0.04$) was observed for apparent total tract OM digestion. This indicates that, as with apparent intestinal N digestion, OM digestibility decreased as SF decreased with biuret compared with an increase in OM digestibility as SF decreased with urea. Apparent total tract N digestion was greater ($P < 0.01$) with CP supplementation.

Ruminal DM fill (g/kg BW) on the day of and day before supplementation (Table 3) was not affected by CP supplementation or SF ($P > 0.10$). No differences were observed ($P > 0.10$) for ruminal liquid volume (g/kg BW) because of CP supplementation or SF on the day of and day before supplementation.

Treatment × time interactions ($P < 0.01$) were observed for ruminal NH_3N on the day of and the day before CP supplementation. However, after considering the nature of the interactions, we concluded that discussing treatment means while providing the treatment × time figure would aid in interpretation and discussion of the data.

Ruminal NH_3N on the day of supplementation increased ($P < 0.05$) due to CP supplementation (Table 4; Figure 1). Also, an NPN source by SF interaction was observed ($P = 0.03$) indicating that ruminal NH_3N increased at a greater rate as SF decreased for urea compared with biuret. Figure 1 illustrates that ruminal NH_3N had a distinct peak on the U2D treatment compared with a prolonged and moderate increase on the B2D treatment. This is indicative of the decreased solubility and slower hydrolysis to NH_3N observed with biuret. On the day before supplementation, ruminal NH_3N was greater ($P < 0.05$) for CP supplemented steers and decreased ($P < 0.05$) as SF decreased.

There were no differences for ruminal pH ($P > 0.10$) because of CP supplementation or SF on the day of or the day before supplementation.

Implications

Daily or alternate day supplementation of urea or biuret to ruminants consuming low-quality forage does not adversely affect forage intake or nutrient digestibility. Biuret should be a safer and more useful crude protein supplement when offered infrequently to ruminants because of its slower ruminal degradation to ammonia nitrogen and lower probability of causing ammonia toxicity compared with urea. Alternate day supplementation of non-protein nitrogen sources may provide beef producers with a management alternative to decrease supplementation costs and improve economic returns.

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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
Soy Hulls	-	91.0	90.2
Dried Molasses	-	3.7	3.7
Nutrient Composition			
CP, % DM	4.0	28.9	29.0
DIP[b], %CP	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, respectivly.

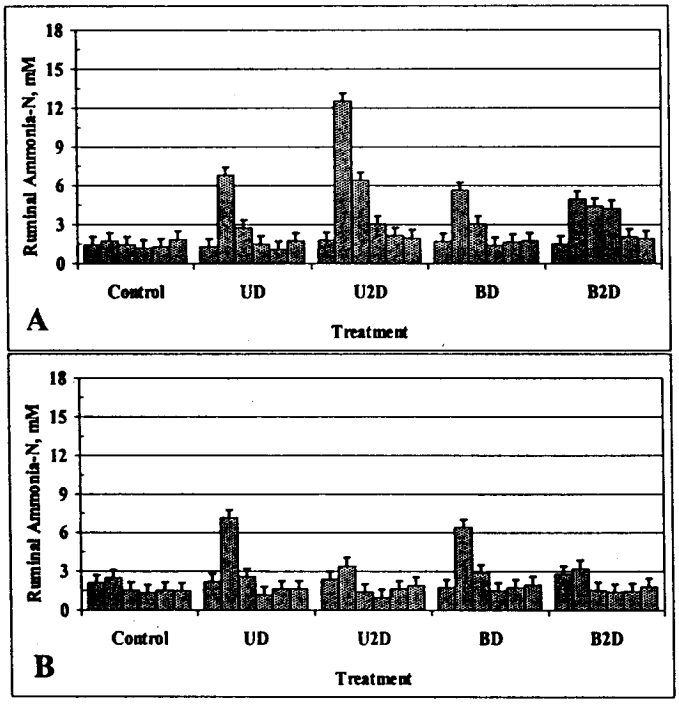

Figure 1. Effect of non-protein nitrogen source and supplementation frequency on steer ruminal ammonia-N the day of (A) and the day before (B) supplementation. Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 h post-feeding, respectively. Treatments were: Control; UD = urea supplement every day; U2D = urea every other day; BD = biuret every day; B2D = biuret every other day.

Table 2. Effect of protein degradability and supplementation frequency on steer dry matter intake and diet digestibility

Item	Treatment[a]					SEM[b]	P-Value[c]			
	CON	UD	U2D	BD	B2D		Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source × SF
Daily DM Intake, g/kg BW										
Hay	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										
Hay	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 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 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
Apparent Ruminal Digestion, %										
OM	38.5	41.5	38.4	39.2	37.3	2.3	0.80	0.48	0.31	0.80
N	-160.4	-69.9	-80.8	-86.6	-80.6	8	<0.001	0.33	0.77	0.32
NDF	55.9	60.1	55.6	59.1	54.7	2.3	0.57	0.69	0.08	0.98
Daily Duodenal Flow, g/kg BW										
OM	9.9	10.4	10.6	11.0	10.9	0.4	0.08	0.23	0.84	0.59
N	0.282	0.295	0.310	0.331	0.310	0.010	0.04	0.12	0.79	0.13
Apparent Intestinal Digestion, %										
OM disappearance, g/kg BW	2.5	2.2	2.8	3.0	2.8	0.4	0.61	0.31	0.55	0.34
OM, % of duodenal flow	24.7	20.9	26.2	25.8	25.2	2.4	0.94	0.46	0.36	0.26
OM, % of intake	15.3	12.3	16.2	16.3	16.3	2.1	0.99	0.35	0.37	0.38
N disappearance, g/kg BW	0.197	0.196	0.216	0.23	0.21	0.01	0.19	0.19	0.99	0.07
N, % of duodenal flow	69.7	66.3	69.7	69.2	67.3	0.9	0.17	0.77	0.39	0.02
N, % of intake	181.9	112.5	126	130.2	121.9	7.4	<0.001	0.39	0.74	0.18
Total Tract Digestibility, %										
DM	50.6	50.7	51.6	52.5	50.7	0.6	0.28	0.48	0.45	0.06
OM	53.7	53.8	54.6	55.5	53.7	0.5	0.31	0.52	0.38	0.04
N	21.6	42.6	45.2	43.5	41.3	1.2	<0.001	0.25	0.86	0.07
NDF	55.8	54.9	56.0	56.4	55.2	0.7	0.82	0.66	0.92	0.12

[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 Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = 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.

Table 3. Effect of protein degradability and supplementation frequency on steer ruminal evacuation parameters

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source × SF
Day of Supplementation										
Ruminal DM, g/kg BW	33.9	35.3	35.3	34.0	36.4	0.8	0.14	0.87	0.16	0.16
Ruminal Liquid, g/kg BW	198	198	196	199	205	5	0.81	0.38	0.73	0.43
Day before Supplementation										
Ruminal DM, g/kg BW	35.7	37.2	35.1	37.6	35.6	1.0	0.59	0.67	0.09	0.93
Ruminal Liquid, g/kg BW	206	203	196	206	201	4	0.33	0.30	0.15	0.77

[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 Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source × SF = interaction of NPN source vs supplementation frequency.

Table 4. Effect of protein degradability and supplementation frequency on steer ruminal ammonia-N and pH

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source × SF
Day of Supplementation										
Ruminal NH_3N, mM	1.36	2.57	4.47	2.72	3.30	0.24	<0.001	0.07	0.001	0.03
Ruminal pH	6.45	6.49	6.50	6.54	6.50	0.04	0.22	0.49	0.75	0.56
Day before Supplementation										
Ruminal NH_3N, mM	1.59	2.78	1.80	2.88	2.18	0.27	0.03	0.41	0.02	0.61
Ruminal pH	6.48	6.53	6.54	6.47	6.53	0.05	0.50	0.52	0.48	0.68

[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 Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source × SF = interaction of NPN source vs supplementation frequency.