

# Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: I. Site of digestion and microbial efficiency<sup>1</sup>

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**ABSTRACT:** Seven cannulated (rumen and duodenal) Angus × Hereford steers (264 ± 8 kg BW) consuming low-quality forage (5% CP; 61% NDF; 31% ADF) were used to determine the influence of CP degradability and supplementation frequency (SF) on DMI and nutrient digestion. Treatments included an unsupplemented control and degradable intake protein (DIP) or undegradable intake protein (UIP) provided daily, every 3 d, or every 6 d. The DIP treatments (18% UIP) were calculated to provide 100% of the DIP requirement, while the UIP treatments (60% UIP) were provided on an isonitrogenous basis compared with DIP. Forage DMI was not affected by treatment. Total DM and N intake, duodenal N flow, and intestinal N disappearance increased ( $P < 0.01$ ) with supplementation. Dry matter intake and duodenal N flow responded quadratically ( $P < 0.04$ ; greatest values on the every-third-day

treatments) as SF decreased. However, no differences in N intake or intestinal N disappearance were observed because of CP degradability or SF. Duodenal bacterial N flow and true bacterial N synthesis (g bacterial N/kg of OM truly digested in the rumen) were increased ( $P < 0.05$ ) with supplementation. Also, duodenal bacterial N flow was greater ( $P < 0.05$ ) for DIP compared with UIP. Duodenal nonbacterial N flow was increased ( $P = 0.02$ ) with CP supplementation and for UIP compared with DIP ( $P < 0.01$ ). Supplemental CP increased ( $P < 0.01$ ) total tract DM and N digestibility with no difference due to CP degradability or SF. Results suggest CP supplements consisting of 20 to 60% UIP can be effectively used by steers consuming low-quality forage without adversely affecting DMI, nutrient digestibility, or bacterial CP synthesis, even when provided as infrequently as once every 6 d.

Key Words: Degradation, Digestion, Forage, Frequency, Protein, Feed Supplementations

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## Introduction

Many cattle in the western United States consume low-quality forage (< 6% CP) from late summer through winter. Supplementation with protein has been shown to increase cow weight gain and body con-

dition score (Clanton and Zimmerman, 1970; Bohnert et al., 2002a) and forage intake and digestibility (Kartchner, 1980; Köster et al., 1996), and can improve reproductive performance (Sasser et al., 1988; Wiley et al., 1991). However, supplementation can be very expensive. Yearly feed costs in the Intermountain West often total 100 to \$200 per cow. In addition to actual supplement costs, supplementation includes other expenses, such as the labor and equipment associated with supplement delivery.

Decreasing the frequency of protein supplementation is one management practice that lowers labor costs. In addition, research has shown that ruminants can be fed protein supplements at infrequent intervals and still maintain acceptable levels of performance (Huston et al., 1999b; Farmer et al., 2001; Bohnert et al., 2002a). Also, infrequent supplementation (as infrequently as once every 6 d) of degradable intake protein (**DIP**) or undegradable intake protein (**UIP**) to wethers consuming low-quality forage resulted in

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efficiencies of N use that were similar to daily-supplemented individuals (Bohnert et al., 2002a). However, we are aware of no data comparing the effects of DIP and UIP supplementation at infrequent intervals on forage intake, nutrient digestibility, and rumen microbial efficiency in beef steers consuming low-quality forage. Therefore, the objective of this study was to determine the influence of rumen protein degradability and supplementation frequency (**SF**) on intake, nutrient digestion, and rumen microbial efficiency in steers consuming low-quality forage. This knowledge will assist in developing and understanding management strategies that help reduce protein supplementation costs while maintaining acceptable levels of production.

## Materials and Methods

Seven Angus × Hereford steers ( $264 \pm 8$  kg) with ruminal and double L-shaped duodenal cannulas (Streeter et al., 1991) were allotted randomly to one of seven treatments in an incomplete  $7 \times 4$  Latin square design (Cochran and Cox, 1957), and housed in individual pens ( $2 \times 4$  m) within an enclosed barn with continuous lighting. Treatments consisted of an unsupplemented control and DIP or UIP supplemented daily, every third day, or every sixth day (**CON**, **DIPD**, **DIP3D**, **DIP6D**, **UIPD**, **UIP3D**, and **UIP6D** for control, DIP daily, DIP every third day, DIP every sixth day, UIP daily, UIP every third day, and UIP every sixth day, respectively). The DIP treatments were formulated to provide 100% of the estimated DIP requirement, assuming a microbial efficiency of 11% (NRC, 1996). The DIP3D and DIP6D treatments received threefold and sixfold the amount of supplement (N basis) on their respective supplementation day compared with DIPD. An equal amount (N basis) of UIP supplement was provided; therefore, all supplemented treatments received the same amount of supplemental N over a 6-d period. The sources of CP used in formulating the DIP and UIP supplements were chosen based on their estimated CP degradability (ruminal and intestinal). Soybean meal has been demonstrated to be a satisfactory DIP source for beef cattle consuming low-quality forage (Church and Santos, 1981; Beaty et al., 1994; Mathis et al., 1999), while expeller-processed soybean meal (SoyPLUS; West Central Soy, Ralston, Iowa; Coenen and Trenkle, 1989; Harouna et al., 1996) and blood meal (Stock et al., 1981; Titgemeyer et al., 1989) have been reported to be excellent sources of UIP. Initially, soybean meal and expeller-processed soybean meal were chosen as sources of DIP and UIP, respectively. However, preliminary analysis indicated the UIP content of the expeller-processed soybean meal was not sufficient to meet the specifications for the UIP supplement. Consequently, blood meal was mixed with the expeller-processed soybean meal to formulate a supplement containing approximately 60% UIP (N basis). Estimates of UIP and DIP were based

on in situ degradability using techniques similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for meadow hay and supplements, respectively. The amount of CP supplied by each supplement was 0.10% of BW/d (averaged over a 6-d period). Protein supplements were placed directly into the rumen via the ruminal cannula at 0745 every day, every third day, or every sixth day for the daily, every third day, and every sixth day treatments, respectively. Steers had continuous access to fresh water and low-quality meadow hay. Low-quality meadow hay was harvested from native flood meadows consisting of approximately 82% meadow foxtail (*Alopecurus pratensis* L.), with the majority of the remaining vegetation consisting of rushes (*Juncus* spp.), sedges (*Carex* spp.), and blue wild rye (*Elymus triticoides* Buckl.; Wenick, 2000). Nutrient content of meadow hay and protein supplements is listed in Table 1. Hay was provided daily (0800) at 120% of the average intake for the previous 5 d, with feed refusals from the previous day determined before feeding. A trace mineralized salt mix was freely available (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, 85 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 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 experiment 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 DM and indigestible ADF fill were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on the day all supplements were offered and the day only daily supplements were offered (2 and 5 d after supplementation for the every-third- and sixth-day treatments, respectively). A more complete description of these procedures is provided in a companion paper (Bohnert et al., 2002b). Ruminal bacteria were isolated from ruminal contents on d 13. Briefly, 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 then homogenized (Waring blender; Waring Products, New Hartford, CT) at high speed for 1 min and strained through four layers of cheesecloth. The bacteria were then separated from protozoa and feed particles by centrifugation ( $800 \times g$  for 20 min). The resulting supernate was collected and stored ( $-20^\circ\text{C}$ ) for later isolation of ruminal bacteria. The supernate was thawed, put into 250-mL bottles, and centrifuged ( $10,000 \times g$  for 15 min, 4°C). The resulting supernate was decanted and discarded. The pellet was resuspended using distilled water and cen-

**Table 1.** Ingredient and nutrient content of meadow hay and supplements

Item	Meadow hay	DIP Supplement	UIP Supplement
Soybean meal	—	97.5	—
SoyPLUS <sup>b</sup>	—	—	67.7
Blood meal	—	—	29.8
Molasses	—	2.5	2.5
Nutrient composition			
CP, % DM	5.3	52.8	59.7
UIP, % CP <sup>c</sup>	19.0	17.6	59.9
OM, % DM	91.4	92.6	94.4
NDF, % DM	60.6	11.9	28.2
ADF, % DM	30.8	5.2	6.6

<sup>a</sup>DIP = degradable intake protein.

<sup>b</sup>SoyPLUS is an expeller-processed soybean meal from West Central Soy (Ralston, Iowa).

<sup>c</sup>Undegradable intake protein. Estimates are based on in situ degradabilities. Techniques were similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for meadow hay and supplements, respectively.

trifuged as before. This step was repeated once and the bacteria frozen ( $-20^{\circ}\text{C}$ ), lyophilized, ground with a mortar and pestle, and composited by treatment.

Gelatin capsules containing 9 g of chromic oxide were dosed intraruminally at 0600 and 1700 on d 14 to 24 for use as an indigestible marker of digesta flow. Samples of meadow hay, protein supplements, and orts were collected on d 13 to 22 and dried at  $55^{\circ}\text{C}$  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 ( $-20^{\circ}\text{C}$ ). Duodenal samples were lyophilized. Feces were collected on d 19 to 24. Steers were fitted with harnesses and fecal bags on d 19 (0700). Bags were emptied once daily, feces manually mixed, and a 2.5% subsample (wet weight) obtained, weighed, dried for 96 h at  $55^{\circ}\text{C}$ , reweighed for DM, and composited by steer. Dried samples of hay, orts, and feces were ground through a Wiley mill (1-mm screen). Duodenal samples were ground through a 1-mm screen using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) because of limited sample size.

Ground samples of meadow hay 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 (Kjeltec Auto 1030 Analyzer, Tecator AB, Höganäs, Sweden), 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 prepared as described by Williams et al. (1962) for analysis of 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  $100.1 \pm 1.7\%$ .

The purine content of duodenal digesta and ruminal bacteria was determined using the technique described by Zinn and Owens (1986) as modified by Makkar and Becker (1999). 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  $7 \times 4$  Latin square 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  $2 \times 3$  factorial plus a negative control, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were: 1) CON vs CP supplementation; 2) DIP vs UIP; 3) linear effect of SF; 4) quadratic effect of SF; 5) contrast 2  $\times$  contrast 3; 6) contrast 2  $\times$  contrast 4. Daily hay and total DM intake over the 6-d supplementation period were analyzed using the REPEATED statement with the MIXED procedure of SAS (SAS Inst. Inc.). The model included steer, period, treatment, day, and treatment  $\times$  day. In addition, steer  $\times$  period  $\times$  treatment was used to specify variation between steers (using the RANDOM statement). Steer  $\times$  period  $\times$  treatment was used as the SUBJECT, and autoregression was used as the covariance structure. The same contrasts noted above were used to partition treatment sums of squares.

### Results and Discussion

Treatment  $\times$  day interactions ( $P < 0.07$ ) were observed for forage and total DM and OM intake by steers over the 6-d supplementation period; however, after considering the nature of the interactions, we concluded that discussing treatment means (Table 2) while providing the daily intake data for forage and

**Table 2.** Effect of protein degradability and supplementation frequency on DM, OM, and NDF intake and OM and NDF disappearance by steers

Item	Treatment <sup>a</sup>										P-value <sup>c</sup>					
	CON	DIPD	DIP3D	DIP6D	UIPD	UIP3D	UIP6D	SEM <sup>b</sup>	Con vs Supp	DIP vs UIP	L SF	Q SF	L SF vs CPD	Q SF vs CPD		
Daily DM intake, g/kg BW	22.7	24.6	26.2	23.0	23.7	25.2	23.3	0.9	0.14	0.51	0.31	0.03	0.56	0.67		
Hay	0.0	1.9	1.9	1.9	1.6	1.6	1.6									
Supplement <sup>d</sup>	22.7	26.5	28.1	24.9	25.3	26.8	24.9	0.9	0.006	0.31	0.31	0.03	0.56	0.67		
Total																
Daily OM intake, g/kg BW	20.7	22.4	23.8	21.0	21.6	22.9	21.2	0.9	0.15	0.51	0.34	0.03	0.56	0.66		
Hay	0.0	1.8	1.8	1.8	1.5	1.5	1.5									
Supplement <sup>e</sup>	20.7	24.1	25.6	22.7	23.1	24.4	22.8	0.9	0.007	0.33	0.34	0.03	0.56	0.66		
Total																
Daily NDF intake, g/kg BW	13.9	15.2	16.0	14.2	14.8	15.6	14.6	0.6	0.09	0.79	0.33	0.06	0.55	0.64		
Daily OM disappearance from stomach																
Apparent, % of OM intake	43.8	38.9	38.9	39.0	41.2	36.7	40.9	2.9	0.18	0.78	0.98	0.40	0.95	0.43		
True, % of OM intake <sup>f</sup>	59.5	59.4	59.0	59.8	57.7	58.3	59.6	2.4	0.83	0.68	0.65	0.83	0.76	0.94		
Daily NDF disappearance from stomach, % of NDF intake	50.5	45.8	45.8	45.1	48.9	46.9	49.4	2.7	0.25	0.22	0.97	0.68	0.83	0.58		
Daily duodenal OM flow, g/kg BW	11.5	14.6	15.6	13.7	13.6	15.4	13.4	0.5	<0.001	0.22	0.23	0.001	0.41	0.62		
Daily OM disappearance from intestines g/kg BW	1.81	5.32	4.89	4.41	4.22	5.42	4.04	0.61	0.001	0.66	0.29	0.19	0.43	0.31		
% of duodenal OM flow	16.5	36.1	31.3	29.6	30.8	35.7	30.2	3.9	0.003	0.94	0.40	0.58	0.44	0.32		
% of OM intake	9.5	22.4	19.2	18.9	18.1	22.2	17.8	3.0	0.01	0.75	0.55	0.60	0.62	0.30		
Apparent total-tract OM disappearance, %	53.3	61.3	58.1	58.0	59.3	59.0	58.7	1.1	0.001	0.87	0.11	0.46	0.26	0.44		

<sup>a</sup>CON = control; DIPD = degradable intake protein every day; DIP3D = DIP every third day; DIP6D = DIP every sixth day; UIPD = undegradable intake protein every day; UIP3D = UIP every third day; UIP6D = UIP every sixth day.

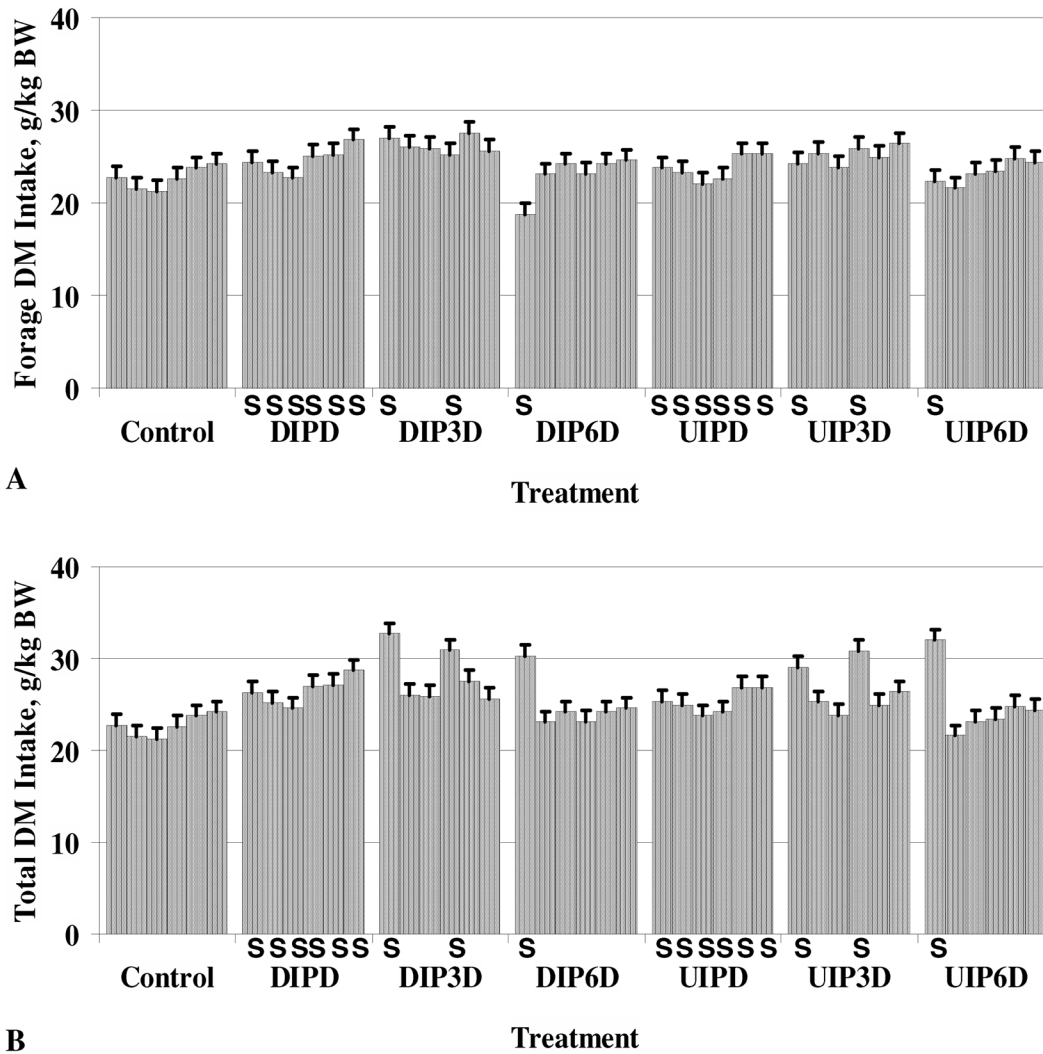
<sup>b</sup>n = 4.

<sup>c</sup>CON vs Supp = control vs supplemented treatments; DIP vs UIP = DIP vs UIP treatments; L SF = linear effect of supplementation frequency; Q SF = quadratic effect of supplementation frequency; L SF vs CPD = interaction of the linear effect of supplementation frequency and ruminal protein degradability; Q SF vs CPD = interaction of the quadratic effect of supplementation frequency and ruminal protein degradability.

<sup>d</sup>DIPD received 1.9 g/kg BW daily; DIP3D received 5.7 g/kg BW every third day; DIP6D received 11.4 g/kg BW every sixth day; UIPD received 1.6 g/kg BW daily; UIP3D received 4.8 g/kg BW every third day; UIP6D received 9.6 g/kg BW every sixth day.

<sup>e</sup>DIPD received 1.8 g/kg BW daily; DIP3D received 5.4 g/kg BW every third day; DIP6D received 10.8 g/kg BW every sixth day; UIPD received 1.5 g/kg BW daily; UIP3D received 4.5 g/kg BW every third day; UIP6D received 9.0 g/kg BW every sixth day.

<sup>f</sup>Corrected for bacterial OM.



**Figure 1.** Effect of protein degradability and supplementation frequency on (A) daily forage and (B) total DM intake by steers. Columns from left to right for each treatment represent d 1, 2, 3, 4, 5, and 6 of a 6-d supplementation period, respectively. Treatments were: Control; DIPD = degradable intake protein every day; DIP3D = DIP every third day; DIP6D = DIP every sixth day; UIPD = undegradable intake protein every day; UIP3D = UIP every third day; UIP6D = UIP every sixth day. Each column with an S below it represents a supplementation day. Treatment  $\times$  day interactions are ( $P = 0.06$ ) and ( $P < 0.01$ ) for A and B, respectively. SEM = 1.2 for A and B.

total DM intake (Figure 1) would aid in interpretation of the data. Intake of hay DM and OM were not affected by CP supplementation or degradability, while total intake of DM and OM increased ( $P < 0.01$ ) with supplementation (Table 2); therefore, CP supplementation increased total nutrient intake compared with unsupplemented controls. This contrasts with other data that has demonstrated protein supplementation increases intake of low-quality forage (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001). The most probable explanation for this apparent discrepancy lies in differences in NDF intake. Mertens (1985, 1994) suggested that DMI is maximized when NDF intake is approximately  $12.5 \text{ g}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{d}^{-1}$ . In the current study, NDF intake of the unsupplemented CON was  $13.9 \text{ g}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{d}^{-1}$ , and ranged from 14.2 to  $16.0 \text{ g}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{d}^{-1}$  in supplemented steers. This is

comparable to the results observed by Bohnert et al. (2002a) with lambs in a similarly designed study. However, NDF intake in unsupplemented controls was lower than  $12.5 \text{ g}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{d}^{-1}$  in the studies of DelCurto et al. (1990), Köster et al. (1996), and Bandyk et al. (2001; 6.4, 5.1, and  $8.2 \text{ g}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{d}^{-1}$ , respectively), and increased to 14.3, 11.3, and  $13.3 \text{ g}\cdot\text{kg}^{-1} \text{ BW}\cdot\text{d}^{-1}$  with supplementation, respectively. Another possible reason we observed no increase in forage intake with supplemental protein was the high level of forage intake observed for all treatments, including the CON. Moore et al. (1999), in a thorough review of the effects of supplementation on voluntary forage intake, suggested that when forage OM intake is greater than 1.75% of BW, forage OM intake should not be expected to increase with supplementation. In the current study, forage OM intake was 2.07, 2.24, 2.38, 2.10,

2.16, 2.29, and 2.12% of BW for CON, DIPD, DIP3D, DIP6D, UIPD, UIP3D, and UIP6D, respectively. Therefore, based on the NDF and forage OM intakes noted in the current study, it is not surprising that no differences in forage intake were observed with CP supplementation.

Intake of hay and total DM and OM responded quadratically ( $P = 0.03$ ) as SF decreased, with the greatest response occurring in the every-third-day treatments. In a similar study, Bohnert et al. (2002a) reported that forage and total DM intake by lambs decreased linearly as SF decreased. The reason for the quadratic response in the current study is not readily apparent. Forage intake was not substantially depressed on the day of supplementation with the every-third-day treatments; however, as noted with lambs by Bohnert et al. (2002a), forage intake was decreased on the day of supplementation with the every-sixth-day treatments and subsequently increased over the next 5 d (Figure 1). The depression in forage intake because of supplementation (substitution of supplement for forage) in the every-sixth-day treatments was not overcome later in the 6-d supplementation period and may have contributed to the quadratic effect observed for forage and total DM intake.

Farmer et al. (2001) supplemented steers with a 43% CP supplement 7 d/wk, 5 d/wk, 3 d/wk, or 2 d/wk. They reported that forage and total OM intake decreased linearly as SF decreased. However, they did observe a tendency for forage and total OM intake to respond cubically ( $P = 0.07$ ) as SF decreased, with the greatest intake occurring on the 7 d/wk treatment, decreasing with 5 d/wk, increasing with 3 d/wk, and decreasing again for the 2 d/wk treatment. As with the current study, the reason for the oscillating intake is not readily apparent. Farmer et al. (2001) did suggest that particulate passage rate might have been related to the response observed for liquid dilution rate (linear, quadratic, and cubic response to decreasing SF), which may have affected intake in a similar manner. However, when compared with forage and total DM intake in the current study, we did not observe a similar response for indigestible ADF passage rate or rumen liquid dilution rate (Bohnert et al., 2002b). Likewise, Beaty et al. (1994) reported no difference between 7 and 3 d/wk supplemented steers for ruminal indigestible ADF passage rate or liquid dilution rate, even though forage and total DM intake were different (greater with 7 d/wk supplementation). In contrast to the aforementioned studies, Huston et al. (1999a) and Krehbiel et al. (1998) reported that SF had no effect on forage and total intake by mature ewes. Huston et al. (1999a) supplemented ewes consuming wheat straw with cottonseed meal daily or once every 7 d and noted no difference in forage or total intake because of SF. Also, Krehbiel et al. (1998) supplemented ewes consuming bromegrass hay with soybean meal every 24 or 72 h and reported no difference in forage or total DM intake.

No differences ( $P > 0.05$ ) were observed due to CP supplementation, CP degradability, or SF for apparent and true OM and NDF disappearance from the stomach (Table 2). This agrees with other studies where ruminal digestion did not improve with protein supplementation (Spragg et al., 1986; Lintzenich et al., 1995). Also, Galyean and Owens (1991) reported that source of supplemental N (nonprotein N, natural protein, DIP, or UIP) has little to no effect on site of digestion of low-quality forage. Spragg et al. (1986) supplemented heifers consuming alkali-treated oat straw with cottonseed meal and actually decreased the proportion of digestible OM apparently digested in the stomach from 75% with unsupplemented steers to 67% for those receiving cottonseed meal once a day. In addition, Lintzenich et al. (1995) reported that steers consuming harvested, dormant bluestem-range forage, supplemented with various forms of alfalfa, did not have increased apparent ruminal OM or NDF digestibility compared with unsupplemented controls. However, they did observe that true ruminal OM digestibility (corrected for bacterial OM) increased with supplementation. This concurs with other research that has demonstrated improved ruminal digestion with protein supplementation of low-quality forage (Pritchard and Males, 1985; Köster et al., 1996). Pritchard and Males (1985) noted that increasing dietary CP from 8 to 10% increased ruminal DM digestion by mature beef cows when the supplement was fed twice daily. Similarly, Köster et al. (1996) supplemented mature beef cows consuming low-quality forage with increasing amounts of casein twice a day and observed that true ruminal OM and NDF digestion were increased compared with an unsupplemented control.

Our data suggest that infrequent supplementation of protein to steers consuming low-quality forage does not depress ruminal digestion. However, this is the first study of which we are aware that evaluates the effects of SF on ruminal digestion. Therefore, we are aware of no studies with which to compare our ruminal digestion data resulting from altering SF. However, research has shown that infrequently supplemented sheep are able to maintain elevated plasma urea-N compared with unsupplemented controls, even when supplemented as infrequently as once every 6 d (Huston et al., 1999a; Krehbiel et al., 1998; Bohnert et al., 2002a). This suggests that ruminal  $\text{NH}_3\text{-N}$  may be increased on those days between supplementation events because of N recycling, which could help maintain ruminal fiber digestion similar to daily supplemented individuals. This is supported by data in a companion paper (Bohnert et al., 2002b) and by Farmer et al. (2001) that indicate ruminal  $\text{NH}_3\text{-N}$  is increased on the days between supplementation events compared with unsupplemented controls. Consequently, infrequent supplementation (as infrequently as once every 6 d) of digestible CP (ruminal or postruminally) to ruminants consuming low-quality

forage appears to allow for ruminal digestion that is similar to daily-supplemented individuals.

Duodenal flow of OM increased ( $P < 0.01$ ) with CP supplementation and responded quadratically ( $P = 0.01$ ) in response to SF, with the greatest duodenal OM flow occurring with the every-third-day treatments (Table 2). This corresponds directly with what we observed for total OM intake. However, daily OM disappearance from the intestines and total tract was greater ( $P < 0.01$ ) for supplemented treatments compared with the CON, with no difference because of CP degradability or SF (Table 2). This supports other research that has demonstrated increased diet digestibility with protein supplementation of low-quality forages (Church and Santos, 1981; DeCurto et al., 1990; Bandyk et al., 2001). The increased disappearance of OM from the intestines (as a percentage of duodenal flow) for the supplemented treatments suggests that the digestibility of OM flowing to the small intestine was increased with supplementation, thereby improving nutrient utilization. Also, the observation that total-tract OM disappearance increased with supplementation strengthens the assumption that overall nutrient utilization was improved with supplemental protein, regardless of CP degradability or SF. This is similar to results reported by Bohnert et al. (2002a). They provided a DIP or UIP supplement daily, every third day, or every sixth day to lambs consuming low-quality meadow hay and reported that total tract DM and OM digestibility were increased with CP supplementation. However, a linear effect of SF  $\times$  CP degradability interaction was observed (DM and OM digestibility decreased with DIP and increased with UIP as SF decreased). The response observed with the DIP treatments by Bohnert et al. (2002a) is similar to that reported by Farmer et al. (2001). They supplemented steers consuming low quality forage with a 58% DIP (as a percentage of CP) supplement 7 d/wk, 5 d/wk, 3 d/wk, or 2 d/wk, and noted that total tract OM digestibility decreased linearly as SF decreased. Bohnert et al. (2002a) and Farmer et al. (2001) suggested that the decrease in total tract OM digestibility may be attributed to altered ruminal fermentation on less frequently supplemented treatments. Although we noted a tendency ( $P = 0.11$ ) for total tract OM digestibility to decrease as SF decreased in the current study, OM disappearance from the stomach was not altered by CP degradability or SF.

Daily N intake was increased ( $P < 0.001$ ) by approximately 80% for supplemented treatments compared with the CON, with no difference observed for CP degradability or SF (Table 3). Similarly, daily duodenal N flow was increased ( $P < 0.001$ ) by approximately 77% for supplemented steers compared with those not receiving supplement. However, we observed a quadratic effect because of SF ( $P = 0.002$ ) for duodenal N flow, with the every-third-day treatments having the greatest flow. This is consistent with what we observed for OM intake and duodenal OM flow. Increased duode-

nal N flow has been demonstrated in numerous studies in which supplemental CP was provided to ruminants consuming forage-based diets (Donaldson et al., 1991; Hannah et al., 1991; Köster et al., 1996).

Daily bacterial N flow at the duodenum was increased ( $P < 0.01$ ) with CP supplementation and was greater ( $P = 0.04$ ) for DIP compared with UIP (Table 3). On average, DIP and UIP treatments increased bacterial N flow at the duodenum by 79 and 48%, respectively, compared with the CON. Also, the proportion of total duodenal N that was comprised of bacterial N was greater ( $P = 0.02$ ) for DIP compared with UIP, with no difference noted between supplemented treatments and the CON or because of SF. Approximately 70% of duodenal N in the current study was of bacterial origin, emphasizing the importance of bacterial protein to the N metabolism of ruminants consuming low-quality forage. The bacterial N:total duodenal N ratio observed for the treatments in the current study is within the range reported by Merchen and Bourquin (1994) for animals offered forage-based diets.

Duodenal nonbacterial N flow was increased ( $P = 0.02$ ) with CP supplementation and for UIP compared with DIP ( $P < 0.01$ ; Table 3). No influence of SF was observed for nonbacterial N flow. The increase in nonbacterial N flow with UIP compared with DIP was expected based on supplement in situ CP degradabilities—60% and 18% UIP for UIP and DIP treatments, respectively (Table 1). If we assume that in situ and in vivo CP degradabilities are comparable, we would expect the UIP treatments to have approximately three times the quantity of nonbacterial N at the duodenum compared with the DIP treatments. This is very close to what we actually observed. If we subtract the CON duodenal nonbacterial N from the supplemented treatments, we find that the average nonbacterial N flow was approximately 280% greater for UIP compared with DIP. This data agrees with the majority of studies that have demonstrated increased duodenal nonbacterial N flow with UIP supplementation (Titgemeyer et al., 1989; Cecava and Parker, 1993; Bohnert et al., 1998).

Apparent bacterial N synthesis tended to be greater with CP supplementation ( $P = 0.08$ ) and for DIP compared with UIP ( $P = 0.10$ ; Table 3). However, true bacterial N synthesis was increased with CP supplementation ( $P = 0.04$ ), and tended ( $P = 0.09$ ) to increase for DIP compared with UIP. No effects of SF were observed for apparent or true bacterial N synthesis. The increase in bacterial N synthesis with CP supplementation agrees with the work of Köster et al. (1996), in which bacterial N synthesis of steers consuming low-quality forage was increased with protein supplementation compared with an unsupplemented control. However, the values obtained for bacterial N synthesis in the current study are approximately 200% of those reported by numerous researchers for beef cattle consuming low-quality forage (Stokes et al., 1988; Krysl et al., 1989; Olson et al., 1994; Lintzenich et al., 1995;

Table 3. Effect of protein degradability and supplementation frequency on N intake and digestibility by steers

Item	Treatment <sup>a</sup>										P-value <sup>c</sup>					
	CON	DIPD	DIP3D	DIP6D	UIPD	UIP3D	UIP6D	SEM <sup>b</sup>	CON vs Supp	DIP vs UIP	L SF	Q SF	L SF vs CPD	Q SF vs CPD		
Daily N intake, g/kg BW	0.209	0.383	0.396	0.369	0.371	0.375	0.364	0.009	<0.001	0.11	0.27	0.11	0.72	0.47		
Daily N flow at duodenum, g/kg BW	0.306	0.546	0.566	0.500	0.514	0.607	0.514	0.019	<0.001	0.63	0.26	0.002	0.26	0.16		
Daily bacterial N at duodenum, g/kg BW	0.237	0.410	0.441	0.419	0.309	0.413	0.328	0.038	0.004	0.04	0.73	0.09	0.90	0.33		
Daily bacterial N at duodenum, % of total duodenal N	78.7	74.8	77.8	84.8	61.4	67.7	63.9	6.4	0.34	0.02	0.35	0.78	0.57	0.54		
Daily nonbacterial N at duodenum, g/kg BW	0.069	0.136	0.125	0.081	0.205	0.194	0.186	0.030	0.02	0.006	0.25	0.78	0.56	0.73		
Bacterial N, % DM	6.29	7.18	7.39	8.06	7.02	6.68	6.77									
Bacterial N:purine ratio	0.71	0.86	0.93	0.98	0.84	0.81	0.88									
Bacterial N synthesis g of N/kg of OMAD <sup>d</sup>	28.2	47.5	44.1	53.1	32.4	45.6	35.2	7.1	0.08	0.10	0.56	0.66	0.84	0.17		
g of N/kg of OMTD <sup>e</sup>	20.0	28.9	29.0	32.0	23.2	28.8	24.0	3.0	0.04	0.09	0.52	0.48	0.72	0.22		
Daily N disappearance from Stomach																
Apparent, % of N intake	-51.0	-41.7	-43.1	-35.2	-38.4	-62.2	-42.0	5.3	0.26	0.11	0.80	0.01	0.36	0.08		
True, % of N intake <sup>f</sup>	67.7	65.4	67.0	78.1	45.9	47.0	49.8	9.1	0.39	0.01	0.39	0.73	0.64	0.81		
True, g/kg BW <sup>g</sup>	0.140	0.247	0.270	0.288	0.166	0.181	0.177	0.031	0.03	0.003	0.42	0.82	0.65	0.90		
Intestines																
g/kg BW	0.156	0.356	0.362	0.325	0.329	0.406	0.341	0.017	<0.001	0.44	0.56	0.008	0.23	0.12		
% of intake	78.3	92.4	91.5	88.1	88.5	108.9	94.4	5.4	0.02	0.17	0.88	0.07	0.37	0.11		
% of duodenal flow	52.1	65.1	63.9	64.7	63.7	67.6	66.0	2.0	<0.001	0.48	0.64	0.64	0.52	0.32		
Apparent total-tract N disappearance, %	27.7	50.6	48.4	52.9	50.1	46.7	52.3	2.3	<0.001	0.64	0.36	0.08	0.99	0.79		

<sup>a</sup>CON = control; DIPD = degradable intake protein every day; DIP3D = DIP every third day; DIP6D = DIP every sixth day; UIPD = undegradable intake protein every day; UIP3D = UIP every third day; UIP6D = UIP every sixth day.

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

<sup>c</sup>CON vs Supp = control vs supplemented treatments; DIP vs UIP = DIP vs UIP treatments; L SF = linear effect of supplementation frequency; Q SF = quadratic effect of supplementation frequency; L SF vs CPD = interaction of the linear effect of supplementation frequency and ruminal protein degradability; Q SF vs CPD = interaction of the quadratic effect of supplementation frequency and ruminal protein degradability.

<sup>d</sup>OMAD = apparent OM disappearance from stomach.

<sup>e</sup>OMTD = true OM disappearance from stomach (corrected for bacterial OM).

<sup>f</sup>Corrected for bacterial N.



Köster et al., 1996). A possible explanation for this discrepancy is our use of the Makkar and Becker (1999) modification of the Zinn and Owens (1986) procedure to measure the purine content of ruminal bacteria and duodenal digesta. All of the aforementioned research used the original Zinn and Owens (1986) procedure. Briefly, Makkar and Becker (1999) reported that, even though purine recovery was sufficient with pure isolations of bacteria using the Zinn and Owens (1986) procedure, using milder hydrolysis conditions improved purine recovery from samples containing matrices of cellulose, starch, NDF, and/or undigested hay residue (common constituents of duodenal digesta). They found that purine recovery was approximately 50% when the Zinn and Owens (1986) procedure was used compared with essentially 100% when milder hydrolysis conditions were used. By increasing purine recovery in duodenal digesta, the quantity of bacterial N measured in the duodenal digesta should increase and, consequently, increase bacterial efficiency (bacterial N synthesis/kg OM digested in the rumen). A further discussion of the Makkar and Becker (1999) modification of the Zinn and Owens (1986) purine procedure is provided by Klopfenstein et al. (2001). In addition, they recommend great caution in interpreting results using the original procedure and suggest that the Makkar and Becker (1999) modification should be considered for future purine analyses.

Apparent N disappearance from the stomach was negative for all treatments and responded quadratically ( $P = 0.01$ ) as SF decreased, with the most negative values occurring on the every-third-day treatments (Table 3). The negative apparent ruminal N digestibilities reported here agree with other research in which beef cattle were consuming low-quality forage (Hannah, 1991; Lintzenich et al., 1995; Köster et al., 1996) and are most likely the result of N recycling (Bunting et al., 1989). True N disappearance (as a percentage of intake) from the stomach was greater ( $P = 0.01$ ) for DIP compared with UIP, indicative of the differences in supplement ruminal CP degradability, with no effect because of SF. The total quantity of true N disappearance from the stomach (g/kg BW) was lower for the CON compared with the supplemented treatments ( $P = 0.03$ ), and for UIP compared with DIP ( $P < 0.01$ ). No difference was noted because of SF.

Daily disappearance of N from the intestines was greater for supplemented treatments compared with the CON ( $P < 0.03$ ), with no difference because of CP degradability. Also, N disappearance from the intestines, when expressed as g/kg BW, responded quadratically with respect to SF ( $P < 0.01$ ). This coincides with the results we observed with duodenal N flow (greatest values occurring with the treatments receiving supplement once every 3 d). However, intestinal N disappearance was not affected by CP degradability or SF when expressed as a percentage of N intake or duodenal N flow. Therefore, digestibility of N flowing to the intestines was increased with CP supplementation and was

not affected by CP degradability or SF. This concurs with the results reported by Bohnert et al. (2002a). They measured N efficiency in lambs and cow performance during the last third of gestation using the same treatment structure as in the current study. They noted increased digested N retained in lambs and increased weight and body condition score gain in cows with no difference because of CP degradability or SF.

There is a lack of information concerning the effects of protein SF on intestinal N disappearance; nevertheless, we can make some assumptions based on the data of Krehbiel et al. (1998). They supplemented multi-catheterized ewes consuming bromegrass hay with soybean meal every day or every third day. If we assume that SF does not affect the digestibility of duodenal digesta and that flux of  $\alpha$ -amino N across the portal-drained viscera is related to disappearance of  $\alpha$ -amino N from the small intestine, we can assume that intestinal disappearance of  $\alpha$ -amino N and portal-drained viscera  $\alpha$ -amino N flux should have been similar over the 3-d supplementation period. As was the case, portal drained viscera  $\alpha$ -amino N flux was similar when averaged over the 3-d supplementation period for the daily and every-third-day treatments (48 and 52 mmol/h, respectively). This would agree with our lack of a SF effect on intestinal N disappearance. These results suggest that the quality of protein reaching the small intestine was similar for DIP and UIP treatments, even though differences existed in the quantity of bacterial and nonbacterial N flowing to the duodenum. This agrees with the hypothesis posed by Cecava and Parker (1993) that effects of protein source on the quality of intestinally absorbable protein are relatively small.

Apparent total tract N disappearance was greater for supplemented treatments compared with the CON ( $P < 0.01$ ), with no difference because of CP degradability or SF (Table 3). These results are comparable to those reported by Bohnert et al. (2002a) for lambs consuming low-quality forage. They reported that apparent total-tract N disappearance was approximately 170% greater for supplemented lambs compared with the CON. We found apparent total-tract N disappearance was approximately 80% greater for supplemented steers compared with the CON.

## Implications

Infrequent supplementation of rumen degradable and/or undegradable intake protein is a valid alternative to daily supplementation of ruminants consuming low-quality forage. It appears that ruminants consuming low-quality forage are able to effectively use supplemental nitrogen, even when supplemented once every six days, independent of ruminal crude protein degradability. Therefore, infrequent supplementation of protein provides beef producers with a management alternative to decrease supplementation costs and improve economic returns.

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