

# Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: II. Ruminal fermentation characteristics<sup>1</sup>

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**ABSTRACT:** Seven ruminally and duodenally cannulated steers ( $264 \pm 8$  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 ruminal fermentation characteristics. 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. Ruminal  $\text{NH}_3\text{-N}$  was increased on the day all supplements were provided with supplemental CP ( $P = 0.04$ ) and for DIP compared with UIP ( $P < 0.01$ ). Also, because ruminal  $\text{NH}_3\text{-N}$  increased at a greater rate with DIP compared with UIP as SF decreased, a linear effect of SF  $\times$  CP degradability interaction ( $P = 0.02$ ) was observed. In addition,  $\text{NH}_3\text{-N}$  was greater on the day only daily supplements were provided for supplemented treatments ( $P = 0.04$ ), and decreased linearly ( $P < 0.01$ ) as SF decreased. Concentration of total VFA increased linearly ( $P = 0.02$ ) as SF decreased on the day all supplements were provided, whereas on the day only daily

supplements were provided, total VFA were greater for UIP compared with DIP ( $P = 0.01$ ), and decreased linearly ( $P < 0.01$ ) as SF decreased. An interaction concerning the linear effect of SF and CP degradability ( $P = 0.02$ ) was observed for ruminal liquid volume on the day all supplements were provided. This was the result of an increase in liquid volume with DIP as SF decreased compared with a minimal effect with UIP. In contrast, there was no influence of supplementation on liquid volume the day only daily supplements were provided. Ruminal liquid dilution rate was greater ( $P = 0.02$ ) with CP supplementation on the day all supplements were provided. We did observe a quadratic effect of SF  $\times$  CP degradability interaction ( $P = 0.01$ ) for dilution rate because of a quadratic response with DIP (greatest value with the every-third-day treatment) compared with a decrease as SF decreased for UIP. On the day only daily supplements were provided, ruminal liquid dilution rate decreased linearly ( $P = 0.02$ ) as SF decreased. These results suggest that DIP and UIP elicit different effects on ruminal fermentation when supplemented infrequently to ruminants consuming low-quality forage while not adversely affecting nutrient intake and digestibility.

Key Words: Degradation, Fermentation, Forage, Frequency, Protein, Supplements

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## Introduction

Ruminants consuming low-quality forage deficient in CP face the problem of inadequate ruminal N that hinders microbial growth and, consequently, decreases ruminal fermentation and the quantity of potentially absorbable N presented to the small intestine (Hannah et al., 1991; Köster et al., 1996; Bohnert et al., 2002a). Supplemental degradable intake protein (DIP) and/or recycled N (from digested and absorbed undegradable intake protein [UIP], mobilized tissue protein, or digested and absorbed microbial protein; Tillman and Sidhu, 1969; Harmeyer and Martens, 1980) provide the

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main source of N for the growth of ruminal microorganisms (Allison, 1969; Tillman and Sidhu, 1969).

Decreasing the frequency of protein supplementation to ruminants consuming low-quality forage has been shown to result in acceptable levels of performance (Huston et al., 1999a,b; Bohnert et al., 2002b), with only minimal impacts on nutrient intake and digestibility (Huston et al., 1999a,b; Bohnert et al., 2002a,b). In addition, research has shown that lambs (Brown et al., 1996; Bohnert et al., 2002b) and steers (Coleman and Wyatt, 1982) are able to maintain N efficiency when supplemented infrequently with protein compared with daily supplemented individuals. The capacity to maintain N efficiency is probably a consequence of the N recycling ability of ruminants, which agrees with the suggestion by Cocimano and Leng (1967) that recycling of absorbed N to the rumen might support fermentation between supplementation events. There are limited data available concerning the effects of infrequent supplementation on ruminal fermentation (Beaty et al., 1994; Farmer et al., 2001), and none comparing infrequent supplementation of DIP and UIP. Therefore, the objective of our study was to determine ruminal fermentation characteristics in response to infrequent supplementation of DIP and UIP to steers consuming low-quality forage.

## Materials and Methods

A full description of experimental procedures (excluding ruminal fermentation measurement and analysis) and diet composition is given in a companion paper (Bohnert et al., 2002a). Briefly, seven cannulated (ruminal and duodenal) beef steers ( $264 \pm 8$  kg) 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).

Experimental periods were 24 d, with 10 d of diet adaptation and 14 d of sampling. On d 13 and 18, treatment effects on ruminal DM and indigestible ADF (**IADF**) fill were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on the day all supplements were provided and the day only daily supplements were provided (2 and 5 d after supplementation for the every-third and sixth-day treatments), respectively. Total ruminal contents were weighed, mixed by hand, and subsampled in triplicate (approximately 400 g each). The remaining ruminal contents were replaced immediately into the ani-

mal. Ruminal samples were weighed; dried in a force-air oven ( $55^\circ\text{C}$ ; 96 h); reweighed for DM; ground to pass a 1-mm screen in a Wiley mill; and composited within period and day by steer.

On d 19 and 24, each steer was intraruminally pulse-dosed with 5 g of Co-EDTA in a 150-mL aqueous solution (Uden et al., 1980) at 0745 (the time supplements were provided). As described above for ruminal evacuations, this allowed sampling on the day all supplements were provided and the day only daily supplements were provided (2 and 5 d after supplementation for the every-third and sixth-day treatments), respectively. The Co marker was administered throughout the rumen using a stainless-steel probe with a perforated tip. Ruminal fluid (approximately 100 mL) was collected by suction strainer (Raun and Burroughs, 1962; 19-mm diameter, 1.6-mm mesh) immediately prior to dosing and at 3, 6, 9, 12, and 24 h post dosing. Ruminal fluid pH was measured immediately after collection (Orion SA 520, Boston, MA). Twenty milliliters was stored ( $-20^\circ\text{C}$ ) for later analysis of Co concentration, and 5 mL was acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored ( $-20^\circ\text{C}$ ) for subsequent analysis of VFA and  $\text{NH}_3\text{-N}$ . Frozen ( $-20^\circ\text{C}$ ) ruminal samples were prepared for analysis by thawing, centrifuging ( $15,000 \times g$  for 10 min for VFA and  $\text{NH}_3\text{-N}$ , and  $2,000 \times g$  for 20 min for Co), and collecting the supernatant. Cobalt concentration in ruminal fluid was analyzed by atomic absorption using an air/acetylene flame (Model 351 AA/AE spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA). Ruminal liquid volume and liquid dilution rate were estimated by regressing the natural logarithm of Co concentration against sampling time as described by Warner and Stacy (1968). Volatile fatty acids were analyzed as described by Horney et al. (1996), and  $\text{NH}_3\text{-N}$  was analyzed 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 and Lomb, Inc., Rochester, NY).

Ground samples of meadow hay and protein supplements were composited by period and daily orts were composited by steer (within period) on an equal-weight basis (5% as-fed). Feed, orts, and ruminal particulate were analyzed for DM and OM (AOAC, 1990), and except for ruminal particulate, 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, ruminal particulate, and fecal samples (from Bohnert et al., 2002a) were analyzed for IADF using the following procedure. Duplicate samples (0.5 g) of hay, orts, ruminal particulate, and feces were weighed into Ankom filter bags (F57; Ankom Co., Fairport, NY) and, except for fecal samples, incubated for 16 h at  $39^\circ\text{C}$  in a solution containing 0.1% pepsin (Catalog # P-7012, Sigma, St. Louis, MO) and 10% 1 N HCl using a Daisy<sup>II</sup> incubator (24 sample bags and 2 L per incubation vessel; Ankom Co., Fairport, NY). Samples were then rinsed with

warm (39°C) tap water, placed into a lingerie bag along with the fecal samples, and incubated for 96 h in the rumen of a cannulated steer consuming low-quality forage ad libitum. The sample bags were then removed from the rumen, rinsed with warm (39°C) tap water until the rinse water was clear, and analyzed for ADF as described above. Fecal recovery of IADF was  $96.0 \pm 1.4\%$ . Digesta kinetics techniques described by Van Soest (1982) were used to determine IADF passage by dividing IADF intake by the quantity of IADF in the rumen 4 h after feeding.

### Statistical Analysis

Ruminal liquid volume, liquid dilution rate, DM fill, IADF fill, and IADF passage rate were analyzed as an incomplete  $7 \times 4$  Latin square using the GLM procedure of SAS (1996). The model included period, steer, and treatment. Because the treatment structure consisted of a  $2 \times 3$  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. Ruminal VFA, pH, and  $\text{NH}_3\text{-N}$  data collected at fixed times on the day all supplements were provided and the day only daily supplements were provided (2 and 5 d after supplementation for the every-third and sixth-day treatments, respectively) were analyzed using the REPEATED statement with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included steer, period, treatment, time, and treatment  $\times$  time. 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 used as the covariance structure. The same contrasts noted above were used to partition treatment sums of squares.

## Results

On the day all supplements were provided, ruminal DM fill tended ( $P = 0.07$ ) to increase for DIP compared with UIP and linearly increased ( $P = 0.005$ ) as SF decreased (Table 1). However, no difference was observed for ruminal IADF fill because of CP supplementation, CP degradability, or SF. Also, IADF passage rate was not influenced by our treatments on the day all supplements were provided.

A linear effect of SF  $\times$  CP degradability interaction ( $P = 0.02$ ) was observed for ruminal liquid volume on the day all supplements were provided (Table 1). This was because of a linear increase in ruminal liquid volume with DIP as SF decreased and minimal change was observed with UIP. Liquid volume increased by 6, 10, and 36% compared with the CON for DIPD, DIP3D, and DIP6D, respectively. In contrast, liquid volume was increased by 9, 9, and 3% compared with the CON for UIPD, UIP3D, and UIP6D, respectively. Ruminal liq-

uid dilution rate on the day all supplements were provided was increased with CP supplementation ( $P = 0.02$ ). In addition, a quadratic effect of SF  $\times$  CP degradability interaction ( $P = 0.01$ ) was noted for liquid dilution rate on the day all supplements were provided because of a quadratic response with DIP in which the greatest dilution rate was observed with the 3-d treatment. In contrast, liquid dilution rate with UIP was greatest for the daily treatment and decreased with the 3- and 6-d treatments.

On the day only daily supplements were provided, ruminal DM fill was greater ( $P = 0.03$ ) for the CON compared with supplemented treatments (Table 1). In contrast, ruminal IADF fill was not affected by CP supplementation. However, a linear effect of SF  $\times$  CP degradability interaction ( $P = 0.01$ ) was observed for IADF fill, indicating that IADF fill increased linearly as SF of DIP decreased compared with a decrease in IADF fill as SF decreased with UIP. Consequently, this coincides with the results observed for IADF passage rate on the day only daily supplements were provided. Supplementation increased ( $P = 0.03$ ) IADF passage rate and a linear effect of SF  $\times$  CP degradability interaction ( $P = 0.02$ ) was observed for IADF passage rate. This was in response to a decrease in IADF passage rate as SF decreased with DIP compared with an increase as SF decreased with UIP.

CP supplementation, CP degradability, and SF did not affect ruminal liquid volume on the day only daily supplements were provided. However, liquid dilution rate tended ( $P = 0.08$ ) to be greater with CP supplementation and decreased linearly ( $P = 0.02$ ) as SF decreased.

Treatment  $\times$  time interactions ( $P < 0.01$ ) were noted for ruminal  $\text{NH}_3\text{-N}$  on the day all supplements were provided and the day only daily supplements were provided (2 and 5 d after supplementation for the every-third and sixth-day treatments, respectively). In addition, treatment  $\times$  time interactions ( $P < 0.05$ ) were observed for ruminal pH, total VFA, and molar proportions of acetate, propionate, butyrate, isovalerate, and valerate on the day all supplements were provided. However, after considering the nature of the interactions, we concluded that discussing treatment means while providing treatment  $\times$  time figures would aid in interpretation and discussion of the data.

On the day all supplements were provided, ruminal  $\text{NH}_3\text{-N}$  increased ( $P = 0.04$ ; Table 2; Figure 1) and pH decreased ( $P < 0.01$ ; Table 2; Figure 2) with CP supplementation. In addition, an interaction concerning the linear effect of CP degradability and SF ( $P = 0.02$ ) was observed for ruminal  $\text{NH}_3\text{-N}$ , indicating  $\text{NH}_3\text{-N}$  increased at a greater rate as SF decreased with DIP compared with UIP. A linear increase ( $P = 0.02$ ) in the concentration of total VFA was noted on the day all supplements were provided as SF decreased (Table 2; Figure 3). A linear effect of SF  $\times$  CP degradability interaction ( $P = 0.04$ ) was observed for the molar proportion of acetate on the day all supplements were provided, primarily because acetate decreased at a greater rate

**Table 1.** Effects of protein degradability and supplementation frequency on ruminal DM fill, indigestible acid detergent fiber (IADF) fill, liquid volume, and liquid and IADF passage rates in steers offered low-quality meadow hay

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	
Day all supplements provided															
DM fill, g/kg BW	41.9	40.3	43.3	46.2	41.3	39.7	43.1	1.1	0.73	0.07	0.005	0.25	0.09	0.22	
IADF fill, g/kg BW	9.6	9.3	9.8	8.7	10.2	9.1	9.7	0.6	0.92	0.42	0.42	0.96	1.00	0.15	
IADF passage, %/h	1.62	1.87	1.96	1.84	1.78	1.99	1.78	0.13	0.10	0.73	0.92	0.20	0.92	0.65	
Liquid volume, mL/kg BW	230	244	252	312	251	251	236	16	0.13	0.10	0.12	0.50	0.02	0.24	
Liquid dilution rate, %/h	9.0	10.3	12.0	8.7	11.2	10.4	10.8	0.5	0.02	0.26	0.08	0.06	0.29	0.01	
Day only daily supplements provided <sup>d</sup>															
DM fill, g/kg BW	42.1	39.0	39.4	38.7	39.3	39.1	35.7	1.4	0.03	0.40	0.19	0.40	0.26	0.67	
IADF fill, g/kg BW	9.9	9.3	10.0	10.1	10.2	10.3	8.8	0.4	0.78	0.96	0.44	0.15	0.01	0.46	
IADF passage, %/h	1.58	1.87	1.90	1.63	1.73	1.76	1.94	0.08	0.03	0.82	0.87	0.62	0.02	0.15	
Liquid volume, mL/kg BW	229	219	228	233	243	216	232	14	0.97	0.78	0.92	0.44	0.41	0.35	
Liquid dilution rate, %/h	9.5	12.3	11.6	9.6	10.8	10.6	10.2	0.6	0.08	0.23	0.02	0.49	0.12	0.61	

<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; 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>2 and 5 d after supplementation for the every third and sixth day treatments, respectively.



**Table 2.** Effects of protein degradability and supplementation frequency on steer ruminal fermentation characteristics on the day all supplements were provided

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
Ammonia N, mM	0.89	1.68	2.06	5.77	1.15	1.68	1.92	0.60	0.04	0.008	0.002	0.17	0.02	0.11
pH	6.60	6.40	6.42	6.32	6.51	6.43	6.41	0.05	0.004	0.10	0.10	0.78	0.86	0.34
Total VFA, mM	81.4	83.7	84.3	95.1	82.3	85.9	84.3	2.5	0.12	0.11	0.02	0.58	0.09	0.10
VFA, mol/100 mol														
Acetate	71.2	71.8	70.0	68.5	71.4	71.6	69.9	0.4	0.14	0.01	<0.001	0.28	0.04	0.16
Propionate	17.4	17.5	18.3	19.2	17.8	17.2	18.8	0.3	0.04	0.11	<0.001	0.04	0.24	0.07
Isobutyrate	0.61	0.44	0.36	0.47	0.42	0.39	0.45	0.05	0.006	0.96	0.60	0.15	0.93	0.55
Butyrate	9.7	9.2	10.2	10.5	9.4	9.8	9.7	0.2	0.78	0.08	0.005	0.12	0.04	0.81
Isovalerate	0.44	0.36	0.33	0.46	0.28	0.34	0.38	0.05	0.18	0.26	0.08	0.50	0.96	0.36
Valerate	0.62	0.77	0.81	0.83	0.70	0.74	0.77	0.06	0.04	0.15	0.26	0.90	0.92	0.96

<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; 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.

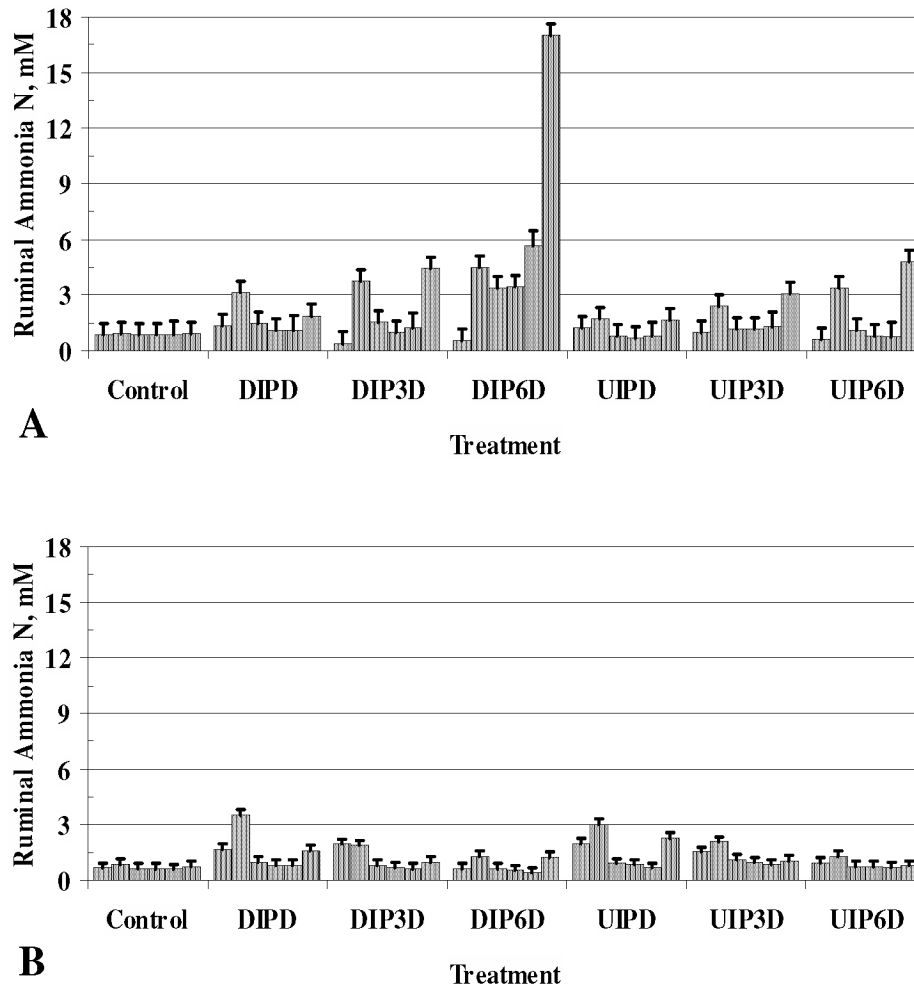
as SF decreased with DIP compared with UIP. The molar proportion of propionate on the day all supplements were provided was increased ( $P = 0.04$ ) with CP supplementation. In addition, propionate increased linearly ( $P < 0.01$ ) and quadratically ( $P = 0.04$ ) as SF decreased, and the molar proportion of isobutyrate was decreased ( $P < 0.01$ ) with CP supplementation on the day all supplements were provided. As with acetate, a linear effect of SF  $\times$  CP degradability interaction ( $P = 0.04$ ) was noted for the molar proportion of butyrate on the day all supplements were provided; however, this interaction was primarily because butyrate increased at a greater rate as SF decreased with DIP compared with UIP. The molar proportion of valerate was increased ( $P = 0.04$ ) with supplemental CP on the day all supplements were provided.

On the day only daily supplements were provided, ruminal  $\text{NH}_3\text{-N}$  was greater ( $P = 0.04$ ) with CP supplementation but decreased linearly ( $P < 0.01$ ) as SF decreased (Table 3; Figure 1). Ruminal pH was greater ( $P = 0.03$ ) for UIP compared with DIP and increased as SF decreased ( $P < 0.01$ ). Also, a quadratic effect of SF  $\times$  CP degradability interaction ( $P = 0.04$ ) was observed for the concentration of total VFA. This was because total VFA decreased linearly as SF decreased with DIP, compared with a quadratic response observed with UIP (greatest total VFA concentration with the 3 d treatment). The molar proportion of acetate increased linearly ( $P = 0.04$ ) as SF decreased on the day only daily supplements were provided. Also, the molar proportion of propionate tended ( $P = 0.06$ ) to decrease as SF decreased. A linear effect of SF  $\times$  CP degradability interaction ( $P = 0.03$ ) was observed with butyrate on the day only daily supplements were provided. This was a consequence of the molar proportion of butyrate decreasing linearly as SF decreased with DIP, and butyrate increased from 9.1 mol/100 mol total VFA with UIP daily to 9.5 mol/100 mol total VFA for UIP every 3 and 6 d.

## Discussion

This is the first study of which we are aware that has attempted to evaluate the influence of supplemental CP degradability and SF on ruminal fermentation characteristics. These data will add information to our understanding of the N metabolism of ruminants consuming low-quality forage, specifically as to how SF affects ruminal N metabolism, liquid volume and dilution rate, and particulate passage (rate and fill). As a result, this research should improve our ability to design supplementation strategies that help improve the sustainability of ruminant operations that rely on the use of low-quality forage.

The increase in ruminal DM fill as SF decreased on the day all supplements were provided is probably a consequence of the quantity (DM basis) of supplement provided. This assumption is supported—given the low concentration of IADF in the DIP and UIP supplements (1.5 and 3.8% of DM, respectively; data not shown) and

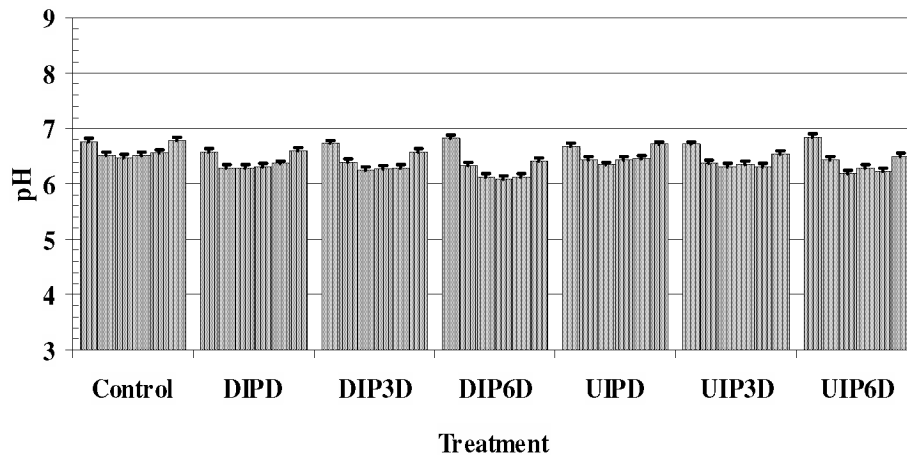


**Figure 1.** Effect of protein degradability and supplementation frequency on steer ruminal ammonia N the day all supplements were provided (A) and the day only daily supplements were provided; 2 and 5 d after supplementation for the every-third- and every-sixth-day treatments, respectively (B). Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 h postfeeding, 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. Treatment  $\times$  time interactions for A and B are ( $P < 0.0001$ ). SEM for A and B are 0.78 and 0.29, respectively.

the short period of time after supplementation (4 h) that ruminal samples were obtained—by the lack of a treatment effect on ruminal IADF fill. These results agree with other studies in which protein supplementation of low-quality forage has little to no effect on ruminal particulate fill or passage rate (Krysl et al., 1989; Olson et al., 1999; Weder et al., 1999). In addition, Beaty et al. (1994) reported no effect of SF (7 d/wk vs 3 d/wk) on IADF passage rate in steers consuming low-quality forage. However, this contrasts with what we observed on the day only daily supplements were provided. Ruminal DM fill decreased and IADF passage rate increased with CP supplementation. Also, we noted interactions concerning the linear effect of SF  $\times$  CP degradability for IADF fill and passage rate. It is not readily apparent why, on the day only daily supplements were provided, ruminal IADF fill increased with DIP and decreased with UIP as SF decreased. As a

result, ruminal IADF passage rate decreased linearly with DIP and increased linearly with UIP. The lack of a consistent response in the quantity and passage rate of particulate in the rumen has been observed in other studies involving protein supplementation of low-quality forage (DelCurto et al., 1990; Köster et al., 1996). Olson et al. (1999) suggested that differences in the type of CP supplements used (such as varying CP quantity, degradability, and/or concentration) could possibly explain the lack of a consistent response in the outflow of digesta from the rumen as well as the quantity of digesta held in the rumen.

The responses observed for ruminal liquid volume and liquid dilution rate with DIP as SF decreased on the day all supplements were provided suggests that infrequent supplementation of DIP may have disrupted rumen function for a period of time, especially with the every-sixth-day treatment. This supports the findings



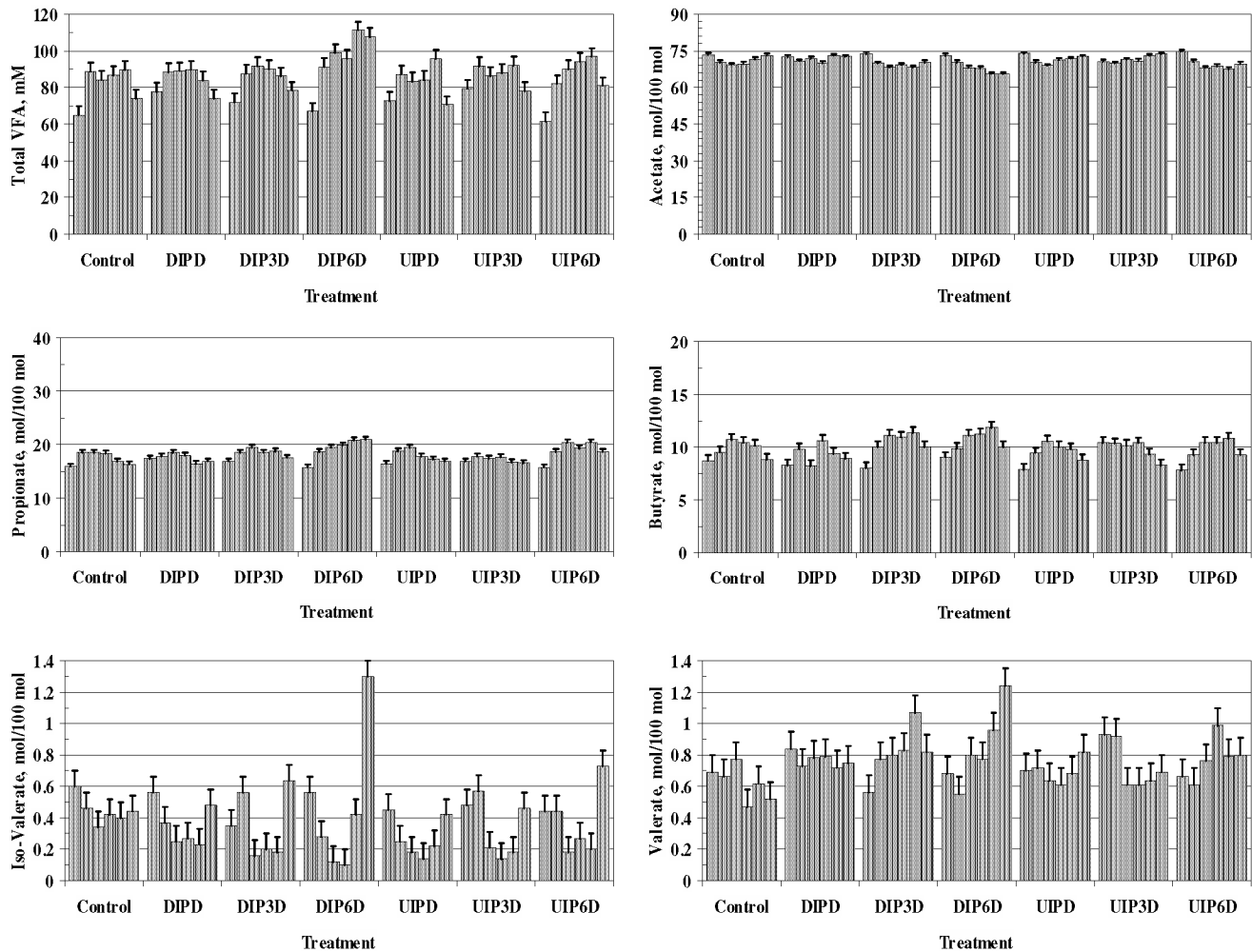
**Figure 2.** Effect of protein degradability and supplementation frequency on ruminal pH the day all supplements were provided. Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 h postfeeding, respectively. Treatments were: Control; DIPD = degradable intake protein every 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. Treatment  $\times$  time interaction ( $P < 0.01$ ). SEM = 0.06.

by Farmer et al. (2001), in which they provided a 58% DIP (as a percentage of CP) supplement 7 d/wk, 5 d/wk, 3 d/wk, or 2 d/wk, and reported that liquid passage rate responded quadratically on the day all supplements were provided with the lowest rate occurring on the 2 d/wk treatment. In contrast, SF of UIP had essentially no effect on ruminal liquid volume or ruminal liquid dilution rate on the day all supplements were provided in the current study, suggesting that the lower CP degradability of the UIP supplement may have allowed for more consistent ruminal fermentation. This assumption is further supported by the lack of a CP effect on ruminal liquid volume the day only daily supplements were provided. The decrease in liquid dilution rate as SF decreased was expected based on other studies in which protein supplementation increased rumen fluid dilution rate (Köster et al., 1996; Olson et al., 1999; Bodine et al., 2000). The every-third-day and every-sixth-day treatments had not been supplemented for 2 and 5 d, respectively, and could be considered “unsupplemented” treatments on the day only daily supplements were provided.

Increased ruminal  $\text{NH}_3\text{-N}$  with CP supplementation of low-quality forage has been demonstrated in numerous studies (Caton et al., 1988; Köster et al., 1996; Weder et al., 1999). However, we are aware of limited ruminal fermentation data comparing the effects of DIP and UIP supplementation of ruminants consuming low-quality forage (Bandyk et al., 2001). Also, there is limited data available evaluating the effects of SF on ruminal fermentation (Hunt et al., 1989; Beaty et al., 1994; Farmer et al., 2001), with none comparing DIP and UIP supplemented infrequently. Bandyk et al. (2001) infused casein at approximately 0.10% of BW (CP basis; the same as used in the current study) directly into the rumen or abomasum of steers consuming low-quality

forage, and increased ruminal  $\text{NH}_3\text{-N}$  compared with an unsupplemented control. They attributed the increase in ruminal  $\text{NH}_3\text{-N}$  with abomasal infusion of casein to N recycling. In addition, they noted ruminal infusion of casein increased ruminal  $\text{NH}_3\text{-N}$  by approximately 200% compared with abomasal infusion. A similar response was observed in the current study on the day all supplements were provided. Ruminal  $\text{NH}_3\text{-N}$  was increased with CP supplementation and was 100% greater for DIP compared with UIP.

We noted an approximately 24-h delay in the peak ruminal  $\text{NH}_3\text{-N}$  concentration with DIP and, to a lesser extent, with the UIP treatments as SF decreased. This agrees with the results reported by Beaty et al. (1994) and Farmer et al. (2001), in which infrequent supplementation of CP to steers consuming low-quality forage resulted in delayed peaks in ruminal  $\text{NH}_3\text{-N}$  as SF decreased. In addition, this coincides with the response in plasma urea-N reported by Bohnert et al. (2002b) with lambs allotted to the same treatments used in the current study. They reported that plasma urea-N was greatest 24 h after supplementation for lambs supplemented infrequently. Also, they noted lambs were able to maintain elevated plasma urea-N for an extended period of time between supplementation events. This suggests that infrequently supplemented ruminants may be able to maintain greater ruminal  $\text{NH}_3\text{-N}$  between periods of supplementation. Consequently, that was what we observed on the day only daily supplements were provided in the current study. Supplemented steers had greater ruminal  $\text{NH}_3\text{-N}$  compared with the CON. However, ruminal  $\text{NH}_3\text{-N}$  did decrease linearly as SF decreased for DIP and UIP treatments. This corresponds to the linear decrease in lamb plasma urea-N as SF decreased, as reported by Bohnert et al. (2002b). These results suggest that ruminants consum-



**Figure 3.** Effect of protein degradability and supplementation frequency on ruminal total VFA, acetate, propionate, butyrate, isovalerate, and valerate the day all supplements were provided. Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 h postfeeding, 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. Treatment  $\times$  time interactions are  $P = 0.005$ ;  $P < 0.0001$ ;  $P < 0.0001$ ;  $P < 0.05$ ;  $P < 0.0001$ ; and  $P < 0.03$ ; and SEM are 4.8, 0.80, 0.55, 0.54, 0.10, and 0.11 for total VFA, acetate, propionate, butyrate, isovalerate, and valerate, respectively.

ing low-quality forage are able to efficiently use digestible CP (either ruminally or postruminally) when supplemented infrequently. This is validated further by the results of Bohnert et al. (2002b), in which lambs supplemented infrequently with DIP or UIP were able to maintain N efficiency (digested N retained) comparable to daily supplemented individuals. Also, Bohnert et al. (2002a) reported that CP degradability and SF did not affect rumen bacterial N synthesis (g bacterial N/kg OM truly digested in the rumen), N disappearance from the intestines (as a proportion of intake and duodenal flow), or total tract N disappearance in steers.

Ruminal pH never fell below 6.0 on the day all supplements were provided or the day only daily supplements were provided. This is within the range considered adequate to maintain fiber digestion and support cellulolytic bacteria (Yokoyama and Johnson, 1988). Our re-

sults support those of Beaty et al. (1994) and Farmer et al. (2001), in which infrequent supplementation resulted in lower ruminal pH compared with daily supplementation when all treatments received supplement but higher ruminal pH on the days between supplementation events. It is of interest to note that providing supplement as infrequently as once every 6 d appears to sustain a ruminal environment sufficient to maintain normal fiber digestion. This agrees with the results reported in the companion article (Bohnert et al., 2002a), in which ruminal OM and NDF digestion were not influenced by SF.

The lack of a CP supplementation effect on total VFA concentration the day all supplements were provided and the day only daily supplements were provided, agrees with other authors who reported comparable results when the ruminal concentration of total VFA



**Table 3.** Effects of protein degradability and supplementation frequency on steer ruminal fermentation characteristics on the day only daily supplements were provided (2 and 5 d after supplementation for the every-third- and sixth-day treatments, respectively)

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
Ammonia N, mM	0.70	1.57	1.17	0.80	1.62	1.27	0.87	0.20	0.04	0.66	0.003	0.97	0.95	0.91
pH	6.64	6.55	6.73	6.72	6.52	6.56	6.65	0.04	0.75	0.03	0.006	0.39	0.61	0.18
Total VFA, mM	74.9	79.2	68.0	65.8	78.6	81.9	71.9	2.7	0.83	0.01	0.003	0.65	0.23	0.04
VFA, mol/100 mol														
Acetate	72.7	72.1	73.0	74.0	73.1	73.5	73.2	0.4	0.37	0.53	0.04	0.70	0.07	0.71
Propionate	16.2	16.8	16.1	16.1	16.4	15.6	15.9	0.3	0.99	0.15	0.06	0.12	0.79	0.64
Isobutyrate	0.57	0.38	0.52	0.52	0.43	0.52	0.46	0.06	0.17	0.64	0.19	0.17	0.36	0.92
Butyrate	9.6	9.7	9.4	8.6	9.1	9.5	9.5	0.3	0.38	0.56	0.24	0.40	0.03	0.90
Isovalerate	0.37	0.38	0.35	0.31	0.31	0.34	0.32	0.03	0.27	0.35	0.30	0.56	0.23	0.68
Valerate	0.63	0.69	0.60	0.52	0.62	0.59	0.62	0.05	0.63	0.87	0.10	0.69	0.11	0.85

<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; 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.

were similar to those observed in the current study (McCullum and Galyean, 1985; Caton et al., 1988; Bandyk et al., 2001). The increase in ruminal total VFA concentration as SF decreased on the day all supplements were provided was most likely a result of the greater quantity of supplement (DM basis) provided with the treatments receiving supplement once every 3 and 6 d. This should have resulted in a greater amount of fermentable substrate available to the ruminal microflora as SF decreased. In contrast, on the day only daily supplements were provided, ruminal total VFA concentration decreased for DIP and UIP treatments as SF decreased. However, the UIP treatments appeared to maintain a greater concentration of total VFA compared with the DIP treatments.

The decrease in the molar proportion of acetate (along with increased propionate) as SF decreased on the day all supplements were provided is supported by other research that has demonstrated decreased acetate and increased propionate molar proportions with DIP supplementation of low-quality forage (Köster et al., 1996; Heldt et al., 1999; Mathis et al., 2000). However, as observed with ruminal NH<sub>3</sub>-N, infrequent supplementation of UIP appeared to minimize changes in the molar proportions of acetate and propionate as SF decreased compared with DIP. In contrast, on the day only daily supplements were provided, the molar proportion of acetate increased and propionate tended to decrease as SF decreased. These responses for acetate and propionate on the day only daily supplements were provided were not surprising considering that it had been 2 or 5 d since a supplementation event for the treatments receiving supplement once every 3 or 6 d, respectively. Also, the molar proportion of butyrate increased as acetate decreased on the day all supplements were provided while decreasing as acetate increased on the day only daily supplements were provided. This is consistent with the fact that both acetate and butyrate share acetyl-CoA as a precursor. Therefore, a change in acetate molar proportion could be expected to cause an opposing change in butyrate.

We expected the branch chain VFA (isobutyrate, isovalerate, and valerate) to increase with CP supplementation, especially for the DIP treatments (Köster et al., 1996; Mathis et al., 2000; Bandyk et al., 2001), on the day all supplements were provided. However, except for valerate, this was not the case. A possible explanation for this response may involve the increased ruminal fluid dilution rate and the tendency for ruminal liquid volume to increase with CP supplementation. Also, some authors have reported results similar to those in the current study for branch-chain VFA (Krysl et al., 1989; Bodine et al., 2000). Krysl et al. (1989) supplemented steers grazing blue grama rangeland with soybean meal, and noted supplementation did not alter the molar proportions of isobutyrate or isovalerate but increased valerate. In addition, Bodine et al. (2000) supplemented beef steers consuming prairie hay with

increasing amounts of soybean meal and did not report an effect of DIP on any branch-chain VFA.

### Implications

Infrequent supplementation of protein with ruminal degradability ranging from 40 to 80% to ruminants consuming low-quality (< 6% CP) forage is a viable alternative to daily supplementation. Ruminants appear to be able to maintain a productive ruminal environment (adequate fiber digestion, fluid dynamics, and particulate passage) when supplemented infrequently with degradable intake protein or undegradable intake protein, even though degradable and undegradable intake protein elicit different effects on ruminal fermentation end products. This supports other research that has demonstrated ruminants consuming low-quality forage and supplemented infrequently (as infrequently as once every 6 d) are able to maintain performance, nitrogen efficiency, dry matter intake, nutrient utilization, and microbial efficiency comparable to daily supplemented individuals.

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