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INFLUENCE OF RUMEN PROTEIN DEGRADABILITY AND SUPPLEMENTATION FREQUENCY ON STEERS CONSUMING LOW-QUALITY FORAGE: RUMINAL FERMENTATION AND SITE OF DIGESTION

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ABSTRACT: Seven cannulated (rumen and duodenal) steers (264 ± 8 kg BW) consuming low-quality forage (5% CP) were used to determine the influence of CP degradability (CPD) and supplementation frequency (SF) on DMI, ruminal fermentation, and nutrient digestion. Treatments (TRT) 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 TRT (18% UIP) were calculated to provide 100% of the DIP requirement while the UIP TRT (60% UIP) were provided on an isonitrogenous basis compared with DIP TRT. Forage DMI was not affected ($P > 0.10$) by TRT. Total DM and N intake, duodenal N flow, and intestinal N disappearance increased ($P < 0.01$) with supplementation. No differences in DMI, N intake, duodenal N flow, or intestinal N disappearance were observed due to CPD or SF ($P > 0.10$). Supplemental CP increased ($P < 0.01$) total tract DM and N digestibility with no difference ($P > 0.10$) due to CPD or SF. Rumen fluid was collected 0, 3, 6, 9, 12, and 24 h after feeding on a d of and a d before supplementation for all TRT. Ammonia N (mM) increased ($P < 0.05$) the d of and the d before supplementation for all protein TRT. However, a CPD × SF interaction ($P < 0.05$) on the d of supplementation indicated that NH_3 N increased at a greater rate for DIP as SF decreased compared with UIP. Ammonia N linearly decreased ($P < 0.01$) as SF decreased the d before supplementation. Results suggest CP supplements consisting of 20 to 60% UIP can be effectively used by steers consuming low-quality forage without adversely affecting DMI and nutrient digestibility, even when provided as infrequently as once every 6 d.

Key Words: Protein, Degradability, 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 (**BCS**; Clanton and Zimmerman, 1970; 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). However, winter supplementation can be very expensive. Winter feed costs in the intermountain west often total $100 to 200 per cow per year. In addition to actual supplement costs, winter supplementation includes other expenses such as the labor, time, and equipment associated with supplement delivery.

Decreasing frequency of protein supplementation is one management practice that decreases labor and time 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 (Huston et al., 1997); however, data are limited comparing the effects of degradable intake protein (**DIP**) and undegradable intake protein (**UIP**) supplemented at infrequent intervals on forage intake, forage digestibility, and efficiency of N use. The objective of this research is to determine the influence of ruminal protein degradability (**CPD**) and supplementation frequency (**SF**) on utilization of low-quality forage by ruminants. This knowledge will assist in developing management strategies that help reduce winter feed costs while maintaining acceptable levels of production.

Materials and Methods

Seven cannulated (ruminal and duodenal) beef steers (264 ± 8 kg) were allotted randomly to one of seven treatments in a incomplete Latin square design and housed in individual pens (2 × 4 m) within an enclosed barn with continuous lighting. Treatments consisted of an unsupplemented control and DIP or UIP supplemented daily, every third d, or every sixth d (**CON**, **DIPD**, **DIP3D**, **DIP6D**, **UIPD**, **UIP3D**, and **UIP6D** for control, DIP daily, DIP every third d, DIP every sixth d, UIP daily, UIP every third d, and UIP every sixth d, 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 d 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 amount of CP supplied by each supplement was approximately 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 d, every third d, or every sixth d for the daily, every third d, and every

sixth d treatments, respectively. Steers had continuous access to fresh water and low-quality meadow hay. 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 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, 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 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 day by steer.

Gelatin capsules containing 9 g of chromic oxide were dosed intra-ruminally 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°C for 48 h. On d 19 to 24, approximately 200 g of duodenal digesta was collected at 0800, 1200, 1600, and 2000. Sub-samples (75 g) were composited by steer and stored (-20°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% 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 above. 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. 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_3 N 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 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 and ADF (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 100.1 ± 1.7 %.

Data were analyzed as an incomplete 7 × 4 Latin square using the GLM procedure of SAS (1996). The model included period, steer, and treatment. Because treatment structure consisted of a 2 × 3 factorial plus a negative control, nonorthogonal contrasts were used to partition specific treatment effects. Contrast statements were: 1) Control vs protein supplementation; 2) DIP vs UIP; 3) linear effect of SF; 4) quadratic effect of SF; 5) contrast 2 × contrast 3; 6) contrast 2 × contrast 4. Response variables included: DM and OM intake; ruminal, intestinal, and total tract digestibility of DM, OM, and CP; rumen fluid volume; and rumen DM volume.

Ruminal pH and NH_3 N concentrations 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, day, and treatment × day. 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 were not affected ($P > 0.10$) by CP supplementation or degradability, while total intake of DM, OM, and N increased ($P < 0.01$) with supplementation (Table 2). Also, intake of hay and total DM and OM increased quadratically ($P < 0.05$) as SF decreased.

No differences ($P > 0.05$) were observed due to CP supplementation, CPD, or SF for apparent ruminal OM, N, or NDF digestibility (Table 2). Apparent ruminal N digestibility was negative for all treatments, suggesting that N recycling played an important role in ruminal N dynamics.

Daily duodenal flow of OM and N (g/kg BW) increased ($P < 0.01$) with CP supplementation and increased quadratically ($P < 0.01$) due to SF. Given the tendency ($P = 0.11$) for N intake to increase quadratically as SF decreased, duodenal flow of OM and N corresponded

directly to the observed intake of OM and N noted with CP supplementation.

Daily intestinal disappearance of OM and N (g/kg BW) was greater ($P < 0.01$) with CP supplementation. In addition, intestinal disappearance of N increased quadratically as SF decreased. The lack of a difference in N disappearance from the intestine for DIP and UIP treatments suggests that intestinal digestibility of N flowing to the duodenum for all supplemented treatments was similar.

Apparent total tract digestion of DM, OM, N, NDF, and ADF was greater ($P < 0.01$) with CP supplementation. This agrees with other studies that have reported increased digestibility when N was supplemented to beef cattle consuming low-quality forage (DelCurto et al., 1990; Scott and Hibberd, 1990).

Ruminal DM fill (g/kg BW) on the d of supplementation (Table 3) was not affected by CP supplementation ($P > 0.10$); however, it increased as SF decreased. This may be due in part to the large amount of supplement DM dosed into the rumen on the d of supplementation for the every third and sixth d treatments (the amount supplement DM dosed on the d of supplementation for DIP3D, DIP6D, UIP3D, and UIP6D was 5.7, 11.4, 4.8, and 9.6 g/kg BW, respectively). In contrast, CP supplementation decreased ($P < 0.05$) ruminal DM fill on the d before supplementation compared with CON. Ruminal liquid volume (g/kg BW) was decreased ($P < 0.05$) with CP supplementation on the d of and before supplementation. Also, an interaction involving the linear effect of SF × CPD ($P < 0.05$) was noted for ruminal liquid volume on the d of supplementation. This interaction suggests that liquid volume increased for DIP as SF decreased compared with little to no change for UIP.

Treatment × time interactions ($P < 0.01$) were observed for ruminal NH_3 N on the d of and the d before CP supplementation. In addition, time × treatment interactions ($P < 0.01$) were observed for ruminal pH on the d of supplementation. However, after considering the nature of the interactions, we concluded that discussing treatment means while providing treatment × time figures would aid in interpretation and discussion of the data.

Ruminal NH_3 N on the d of supplementation increased ($P < 0.05$) due to CP supplementation (Table 4; Figure 1). In addition, an interaction concerning the linear effect of SF × CPD ($P < 0.01$) was observed indicating that ruminal NH_3 N increased at a greater rate for DIP compared with UIP as SF decreased. It is of interest to note that the greatest ruminal NH_3 N concentrations on the third d and sixth d treatments for DIP and UIP occurred 24 h after feeding (Figure 1). This delayed NH_3 N peak agrees with work by Beaty et al. (1994) in which infrequently supplemented steers (three times per week) had later peaks in NH_3 N compared with daily supplemented animals. On the d before supplementation, ruminal NH_3 N was greater ($P < 0.05$) for CP supplemented steers and decreased linearly ($P < 0.01$) as SF decreased (Table 4; Figure 1).

Ruminal pH decreased ($P < 0.01$) due to supplemental CP on the d of supplementation (Table 4; Figure 2). However, it was not influenced ($P > 0.05$) by CP supplementation on the d before supplementation (Table 4). Ruminal pH was lower ($P < 0.05$) for UIP compared with DIP and increased ($P < 0.01$) as SF decreased on the d before supplementation.

Implications

Infrequent supplementation of rumen degradable and undegradable intake protein is a valid alternative to daily supplementation. It appears that ruminants consuming low-quality forage are able to effectively utilize supplemental nitrogen, even with supplementation once every six days, independent of ruminal degradability. Therefore, infrequent supplementation of nitrogen provides 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	Meadow Hay	DIP Supplement	UIP Supplement
Soybean meal	-	97.5	-
SoyPLUS[a]	-	-	67.7
Blood meal	-	-	29.8
Molasses	-	2.5	2.5
Nutrient Composition			
CP, % DM	5.3	52.8	59.7
UIP, %CP	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

[a] SoyPLUS is an expeller-processed soybean meal from West Central Soy, Ralston, Iowa

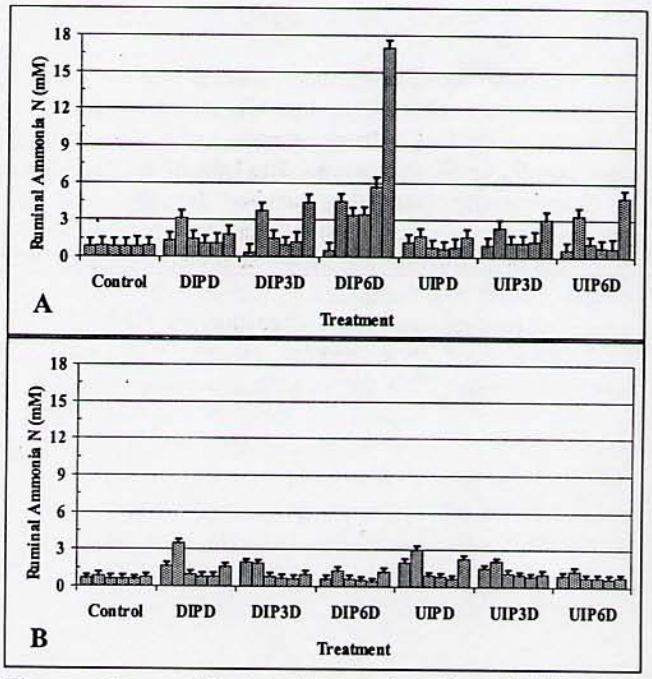

Figure 1. Effect of protein degradability and supplementation frequency on steer ruminal ammonia the day of (A) and the day before (B) supplementation. Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 hours post-feeding, respectively. Treatments were: Control; DIPD = degradable 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.

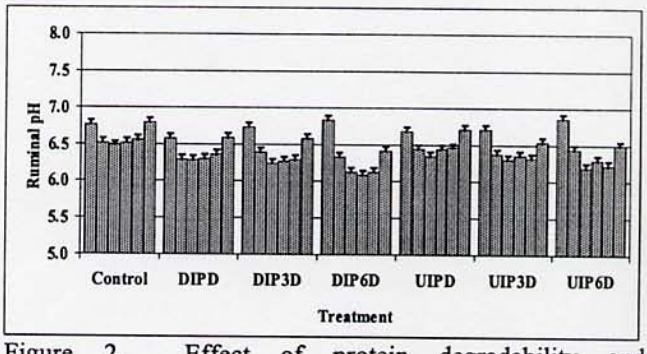

Figure 2. Effect of protein degradability and supplementation frequency on steer ruminal pH the day of supplementation. Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 hours post-feeding, respectively. Treatments were: Control; DIPD = degradable intake protein every day; 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.

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

	Treatment[a]								P-Value[c]					
Item	CON	DIPD	DIP3D	DIP6D	UIPD	UIP3D	UIP6D	SEM[b]	Con vs Supp	DIP vs UIP	L SF	Q SF	L SF vs CPD	Q SF vs CPD
Daily DM Intake, g/kg BW														
Hay	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
Supplement[d]	0.0	1.9	1.9	1.9	1.6	1.6	1.6							
Total	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
Daily OM Intake, g/kg BW														
Hay	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
Supplement[e]	.0	1.8	1.8	1.8	1.5	1.5	1.5							
Total	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
Daily N Intake, g/kg BW	.209	.383	.396	.369	.371	.375	.364	0.009	<0.001	0.11	0.27	0.11	0.72	0.47
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
Apparent Ruminal Digestion, %														
OM	43.4	36.2	38.5	39.4	40.8	37.1	41.3	3.6	0.28	0.58	0.62	0.62	0.71	0.48
N	-51.4	-47.1	-43.9	-34.5	-39.1	-61.4	-41.3	6.6	0.36	0.33	0.45	0.05	0.29	0.14
NDF	50.1	43.3	45.4	45.5	48.6	47.3	49.8	3.3	0.36	0.19	0.63	0.88	0.89	0.62
Daily Duodenal Flow, g/kg BW														
OM	11.6	15.2	15.7	13.6	13.7	15.3	13.4	0.6	<0.001	0.14	0.10	0.008	0.27	0.62
N	0.309	0.565	0.569	0.497	0.517	0.604	0.512	0.022	<0.001	0.98	0.12	0.006	0.18	0.20
Apparent Intestinal Digestion, %														
OM	1.89	5.89	4.98	4.06	4.30	5.34	3.96	0.78	0.005	0.51	0.19	0.39	0.36	0.39
N	0.159	0.376	0.365	0.322	0.332	0.403	0.338	0.021	<0.001	0.84	0.28	0.04	0.18	0.18
Total Tract Digestibility, %														
DM	49.6	57.2	54.3	54.5	55.6	55.2	55.0	1.1	<0.001	0.94	0.15	0.42	0.34	0.44
OM	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
N	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
NDF	43.0	52.6	48.5	46.8	51.7	48.8	52.6	1.8	0.003	0.27	0.21	0.17	0.09	0.49
ADF	36.9	53.6	45.0	40.2	46.4	40.6	47.8	2.8	0.02	0.58	0.06	0.12	0.02	0.37

[a] CON = control; DIPD = degradable intake protein every d; DIP3D = DIP every third d; DIP6D = DIP every sixth d; UIPD = undegradable intake protein every d; UIP3D = UIP every third d; UIP6D = UIP every sixth d.

[b] n = 4.

[c] 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.

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

[e] DIPD received 1.8 g/kg BW daily; DIP3D received 5.4 g/kg BW every third d; DIP6D received 10.8 g/kg BW every sixth d; UIPD received 1.5 g/kg BW daily; UIP3D received 4.5 g/kg BW every third d; UIP6D received 9.0 g/kg BW every sixth d.

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

	Treatment[a]								P-Value[c]					
Item	CON	DIPD	DIP3D	DIP6D	UIPD	UIP3D	UIP6D	SEM[b]	Con vs Supp	DIP vs UIP	L SF	Q SF	L SF vs CPD	Q SF vs CPD
Day of Supplementation														
Ruminal DM, 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
Ruminal Liquid, g/kg BW	243	226	237	249	226	224	222	5	0.04	0.005	0.08	0.99	0.02	0.92
Day before Supplementation														
Ruminal DM, 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
Ruminal Liquid, g/kg BW	242	207	212	217	212	214	210	6	<0.001	0.97	0.50	0.73	0.37	0.78

[a] CON = control; DIPD = degradable intake protein every d; DIP3D = DIP every third d; DIP6D = DIP every sixth d; UIPD = undegradable intake protein every d; UIP3D = UIP every third d; UIP6D = UIP every sixth d.
[b] n = 4.
[c] 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.

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

	Treatment[a]								P-Value[c]					
Item	CON	DIPD	DIP3D	DIP6D	UIPD	UIP3D	UIP6D	SEM[b]	Con vs Supp	DIP vs UIP	L SF	Q SF	L SF vs CPD	Q SF vs CPD
Day of Supplementation														
Ruminal NH_3 N, mM	0.89	1.68	2.06	5.77	1.15	1.68	1.92	0.60	0.0428	0.0075	0.0017	0.1732	0.0179	0.1098
Ruminal pH	6.60	6.40	6.42	6.32	6.51	6.43	6.41	0.05	0.0043	0.1054	0.1053	0.7854	0.8600	0.3374
Day before Supplementation														
Ruminal NH_3 N, mM	0.70	1.57	1.17	.80	1.62	1.27	0.87	0.20	0.0378	0.6581	0.0031	0.9742	0.9529	0.9094
Ruminal pH	6.64	6.55	6.73	6.72	6.52	6.56	6.65	0.04	0.7534	0.0323	0.0056	0.3893	0.6092	0.1757

[a] CON = control; DIPD = degradable intake protein every d; DIP3D = DIP every third d; DIP6D = DIP every sixth d; UIPD = undegradable intake protein every d; UIP3D = UIP every third d; UIP6D = UIP every sixth d.
[b] n = 4.
[c] 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