

## PROTEIN SUPPLEMENTATION OF LOW-QUALITY FORAGE: EFFECTS OF AMOUNT AND FREQUENCY ON INTAKE AND NUTRIENT DIGESTIBILITY BY LAMBS

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**ABSTRACT:** The objectives of this research were to determine the effects of protein supplementation frequency on intake and nutrient digestibility by lambs. Seven lambs were utilized in a 4 × 7 incomplete Latin square design. Dietary treatments were arranged as a 2 × 3 factorial (2 levels of CP and 3 supplementation frequencies); CON = unsupplemented control; D = supplemented daily 0.28% of BW of soybean meal (SBM); 5D = supplemented 1.4% of BW of SBM once every 5 d; 10D = supplemented 2.8% of BW of SBM once every 10 d; ½ D = supplemented at 50% of D; ½ 5D = supplemented at 50% of 5D; ½ 10D = supplemented at 50% of 10D. Full CP refers to D, 5D, and 10D and half CP refers to ½ D, ½ 5D, and ½ 10D dietary treatments. Lambs were supplemented with SBM immediately prior to feeding of low-quality forage (Chewings fescue grass seed straw). Each experimental period was 30 d, with intake measured d 19 to 28. Feces, urine, and blood were collected d 21 to 30. Straw and SBM DMI, total DMI, straw OM intake, OM intake, ADF intake, and NDF intake were not different ( $P \geq 0.26$ ) due to supplementation. Supplementation increased ( $P \leq 0.02$ ) DM, OM, and NDF digestibility compared with the CON. The CON lambs had reduced ( $P \leq 0.002$ ) N intake, urine N excretion, N balance, N digestibility, and digested N retained compared with supplemented lambs. Plasma urea N was increased in the supplemented lambs ( $P = 0.004$ ) compared with the CON lambs as well as for full CP compared with half CP lambs ( $P = 0.03$ ). Lambs supplemented with full CP had increased ( $P \leq 0.03$ ) urine N excretion and N digestibility compared with the half CP lambs; however, digested N retained was not different ( $P = 0.94$ ) due to supplementation amount. As supplementation frequency decreased, N digestibility was also reduced ( $P = 0.01$ ). Both DM and OM digestibility increased ( $P \leq 0.04$ ) as supplementation interval increased. These results suggest that increasing the supplementation interval may be utilized to maintain intake, digestibility, and reduce the labor costs associated with more frequent supplementation.

**Key words:** lambs, nitrogen balance, supplementation frequency

### INTRODUCTION

Grazing livestock in the western United States consume low-quality forage (< 6% CP) from late summer through winter (Bohnert et al., 2002b). Therefore, supplemental CP is

required to maintain livestock BW and BCS (Bohnert et al., 2002a; Schauer et al., 2005). Protein supplementation every day can be costly; therefore, decreasing supplementation frequency may reduce labor costs while maintaining livestock performance. Current research suggests that supplementation frequency can be reduced to once every 10 d while maintaining livestock performance (Schauer et al., 2010).

Previous research has indicated that supplementation frequency can be reduced to once every 6 or 10 d due to urea recycling and maintenance of nitrogen use efficiency (Bohnert et al., 2002a; Schauer et al., 2010). However, there is no research currently available to determine if the amount of CP can also be reduced while decreasing supplementation frequency. Therefore, we hypothesized that as supplementation frequency is reduced, ruminants will become more efficient in their N utilization and, with the increased efficiency, the amount of CP supplemented can be reduced. The objectives of the trial were to evaluate infrequent supplementation of differing amounts of CP on sheep nitrogen use efficiency.

### MATERIALS AND METHODS

All procedures were approved by the North Dakota State and Oregon State University Animal Care and Use Committees.

**Animals and Diets.** Seven wethers (western Whiteface; 31.6 ± 1.7 kg) were used in a 4 × 7 incomplete Latin square design to evaluate the efficiency of lambs fed low-quality forage supplemented CP at different amounts and frequencies. Treatments were arranged in a 2 × 3 factorial design (2 levels of CP and 3 supplementation frequencies); CON = unsupplemented control; D = supplemented daily 0.28% of BW of soybean meal (SBM); 5D = supplemented 1.4% of BW of SBM once every 5 d; 10D = supplemented 2.8% of BW of SBM once every 10 d; ½ D = supplemented at 50% of D; ½ 5D = supplemented at 50% of 5D; ½ 10D = supplemented at 50% of 10D. Full CP refers to D, 5D, and 10D and half CP refers to ½ D, ½ 5D, and ½ 10D dietary treatments. The full CP was estimated to meet the CP requirement of a 30 kg lamb gaining 0.20 kg per day; the half CP was 50% of the corresponding full CP. Trace mineralized salt was provided daily (16.0% Ca, 8.0% P, 21.0% Salt, 2.75% Mg, 3 ppm Co, 5 ppm Cu, 100 ppm I, 1400 ppm Mn, 20 ppm Se, 3000 ppm Zn, 113,500 IU/kg vitamin A, 11,350 IU/kg vitamin D, and 227 IU/kg vitamin E). In addition, an

intramuscular injection of vitamins A, D, and E (100,000, 10,000, and 300 IU of vitamins A, D, and E, respectively; Vitamin E-AD; VetOne, Neogen Corp., Lexington, KY). Lambs had continuous access to fresh water and low-quality cool season hay (Chewings fescue grass seed straw; 4.9% CP).

**Sampling and Laboratory Analysis.** Wethers were weighed on d 0 and 1 of each 30 d experimental period, with a total of 4 periods. Wethers were housed in an enclosed room with a 13 h light and 11 h dark cycle. Lambs were adapted to diets from d 1 to 18. Soybean meal (SBM; 49.9% CP) was used as the CP supplement and offered to lambs immediately prior to hay feeding along with the trace mineralized salt. Hay was provided daily at 0830 h at 120% of the average daily intake for the previous 5 d. Feed refusals from the previous day were determined prior to feeding.

Dry matter intake was determined on d 19 to 28. Additionally, samples of hay and SBM were collected on d 19 to 28 and dried at 55°C for 48 h to determine DM. Orts were collected on d 20 to 29 and dried at 55°C for 48 h. Total fecal and urine output were collected on d 21 to 30. A subsample of each daily fecal sample (7.5% of total, wet basis) was dried at 55°C for 96 h for calculation of fecal DM. Urine was composited daily by wether (10% of total; wet basis) and stored at 4°C. Sufficient 6 N HCL (100 mL) was added daily to urinals to maintain urine pH < 3. On d 21 to 30, 10 mL of blood were collected via jugular venipuncture 4 h after feeding using Vacutainers (VWR, catalogue no. VT6480). Blood was cooled at 4°C for 2 h, centrifuged (3,640 × g; 20 min), and plasma harvested and stored (-20°C).

Dried fecal samples were ground through a Wiley mill (2-mm screen) and composited by lamb. Daily samples of hay and SBM were composited for the collection period, and Orts were composited by lamb on an equal weight basis (20%; as fed basis). Feed, Orts, and fecal samples were analyzed for DM, OM, NDF, and ADF. Feed, Orts, fecal, and urine samples were analyzed for N. Plasma samples were analyzed for urea-N.

**Statistical Analysis.** Data were analyzed as an incomplete 4 × 7 Latin square (Cochran and Cox, 1957) using the Mixed procedure (SAS Inst. Inc., Cary, NC) with Satterwaite approximation. Period, wether, and dietary treatment were included in the model and lamb used as the random variable. Orthogonal contrasts were used to determine dietary treatment effects because of the treatment structure. Orthogonal contrasts were 1) CON vs. CP supplementation; 2) full vs. half CP; 3) linear effect of supplementation frequency (SF); 4) quadratic effect of SF; 5) contrast 2 × contrast 3; and 6) contrast 2 × contrast 4. Plasma urea-N concentrations were analyzed using the MIXED procedure of SAS. Lamb, period, treatment, day, and treatment × day were used in the model. The slice option of the MIXED procedure was used to determine treatment and time differences. The random statement included lamb × period × day. The contrasts above were used to determine treatment sums of squares. Quadratic contrasts were not significant ( $P > 0.05$ ) and will not be presented.

## RESULTS AND DISCUSSION

Supplementation of CP did not affect ( $P \geq 0.26$ ; Table 1) straw DMI, total DMI, straw OM intake, total OM intake, NDF intake, ADF intake, or indigestible ADF (IADF) intake. However, DM digestibility, OM digestibility, and NDF digestibility were increased ( $P \leq 0.03$ ) and ADF digestibility tended ( $P = 0.08$ ) to increase due to CP supplementation. Similar results were observed by Schauer et al. (2010), who observed that as supplementation of CP was reduced to once every 10 d but, total DMI and OM intake were not influenced. However, Bohnert et al. (2002a) observed that total DMI was increased with CP supplementation compared with unsupplemented control wethers. In contrast to the current results, Beaty et al. (1994) observed that steers consuming wheat straw and supplemented three times weekly had reduced straw and total DMI compared with steers supplemented daily. Similar to the current trial, daily supplementation of CP has been demonstrated to increase DM digestibility in steers (DelCurto et al., 1990). Supplementation of CP increased ( $P \leq 0.002$ ) N intake, urinary N excretion, N balance, N digestibility, and daily digested N retained compared with the unsupplemented control. However, fecal N excretion was not affected ( $P = 36$ ) by CP supplementation. These results were expected due to the increase in N available for digestion in the supplemented wethers. In a similar trial to ours, daily NDF intake was 13.1 g/kg BW for unsupplemented controls compared with 13.1, 11.3, and 10.8 for daily, every 5<sup>th</sup> day, and every 10<sup>th</sup> day CP supplementation, respectively (Schauer et al., 2010). Supplemental CP has caused an increase in forage intake in other studies. Bandyk et al (2001), DelCurto et al. (1990), and Köster et al. (1996) all observed that forage intake increased when NDF intake of the unsupplemented controls was 8.2, 6.4, and 5.1 g/kg BW per d (respectively). These studies suggest that lambs must consume less than 12.5 g/kg BW per d of NDF for protein supplementation to elicit an increase in forage intake. All lambs in the current trial were consuming more than 12.5 g/kg BW per d of NDF, which may explain the lack of a treatment effect on forage intake. Also, Bohnert et al. (2011) demonstrated that the forage intake response to CP supplementation of low-quality forage may be dependent on forage type, with intake of warm season forages increasing substantially while little to no increase in intake of cool-season forages, like that used in the current study, is commonly noted.

The amount of CP fed (full vs. half) did not affect ( $P \geq 0.14$ ) DM, OM, or fiber intake or digestibility parameters. Similarly, the amount of supplemental CP did not affect ( $P = 0.27$ ) fecal N excretion or daily digested N retained ( $P = 0.94$ ). As was expected, N intake was increased ( $P < 0.001$ ) in full CP wethers compared with half CP wethers. Half CP wethers had reduced ( $P \leq 0.002$ ) urinary N excretion and N digestibility and tended ( $P = 0.09$ ) to have reduced N balance compared with full CP wethers. Full CP wethers had a greater reduction ( $P = 0.05$ ) in fecal N excretion as SF decreased than the half CP wethers. Full CP wethers tended ( $P = 0.07$ ) to have a greater increase in N digestibility as SF reduced compared with half CP wethers. Nitrogen

**Table 1.** Effect of CP amount and supplementation frequency on intake and diet digestibility of wethers

Item	Dietary Treatment <sup>1</sup>										P-value <sup>3</sup>	
	CON	D	5D	10D	½ D	½ 5D	½ 10D	SEM <sup>2</sup>	CON vs. Supp	Full vs. Half	L SF	L SF vs. Amt
Intake												
Straw DMI, g/kg BW	18.8	21.6	19.5	14.0	20.2	19.3	18.7	1.73	0.97	0.45	0.02	0.08
Supplement DMI, g/kg BW	0.0	2.8	2.8	2.8	1.4	1.4	1.4					
Total DMI, g/kg BW	18.8	24.4	22.3	16.8	21.6	20.7	20.1	1.73	0.26	0.80	0.02	0.09
Straw OM intake, g/kg BW	17.4	20.0	18.0	12.9	18.6	17.8	17.3	1.60	0.97	0.46	0.02	0.08
Supplement OMI, g/kg BW	0.0	2.5	2.5	2.5	1.3	1.3	1.3					
Total OM intake, g/kg BW	17.4	22.5	20.5	15.4	19.9	19.1	18.6	1.60	0.26	0.79	0.02	0.09
NDF intake, g/kg BW	14.9	17.8	15.9	11.6	16.0	15.4	15.0	1.40	0.78	0.73	0.02	0.07
ADF intake, g/kg BW	8.6	10.2	9.0	6.5	9.1	8.8	8.5	0.84	0.88	0.73	0.02	0.07
Indigestible ADF intake, g/kg BW	3.9	4.5	4.0	2.9	4.1	4.0	3.8	0.38	0.96	0.51	0.02	0.10
Digestibility, %												
DM	37.4	40.6	45.7	49.6	43.5	43.5	43.5	1.98	0.004	0.28	0.04	0.04
OM	39.6	43.6	48.0	52.0	45.7	45.4	45.5	1.85	0.002	0.14	0.04	0.03
NDF	42.2	43.6	46.9	48.6	46.0	45.7	44.7	1.39	0.03	0.43	0.19	0.04
ADF	43.0	43.4	46.0	48.4	46.5	45.2	44.8	1.38	0.08	0.69	0.25	0.03
N balance												
N intake, g/kg BW	0.15	0.38	0.37	0.33	0.26	0.26	0.26	0.015	<0.001	<0.001	0.04	0.12
Fecal N excretion, g/kg BW	0.13	0.19	0.16	0.11	0.15	0.14	0.13	0.017	0.36	0.27	0.01	0.05
Urine N excretion, g/kg BW	0.05	0.15	0.18	0.20	0.10	0.12	0.11	0.015	<0.001	<0.001	0.05	0.15
N balance, g/kg BW	-0.03	0.04	0.04	0.01	0.02	0.01	0.01	0.013	0.002	0.09	0.22	0.36
N digestibility, %	12.2	49.5	58.4	65.8	45.5	48.9	48.4	3.40	<0.001	0.002	0.01	0.07
Daily digested N retained, g/kg BW <sup>4</sup>	-460.6	21.8	17.9	3.9	12.4	2.4	7.3	119.95	0.002	0.94	0.93	0.96

<sup>1</sup>CON = unsupplemented control; D = 0.28 % of BW/d of SBM; 5D = 1.4 % of BW of SBM once every 5 d; 10D = 2.8 % of BW of SBM once every 10 d; ½ D = 50 % of D; ½ 5D = 50 % of 5D; ½ 10D = 50 % of 10D.

<sup>2</sup>n = 4.

<sup>3</sup>CON vs. Supp = control vs. supplemented treatments; Full vs. Half = full vs. half amount of CP; L SF = linear effect of supplementation frequency; L SF vs. Amt = interaction of the linear effect of supplementation frequency and amount of CP.

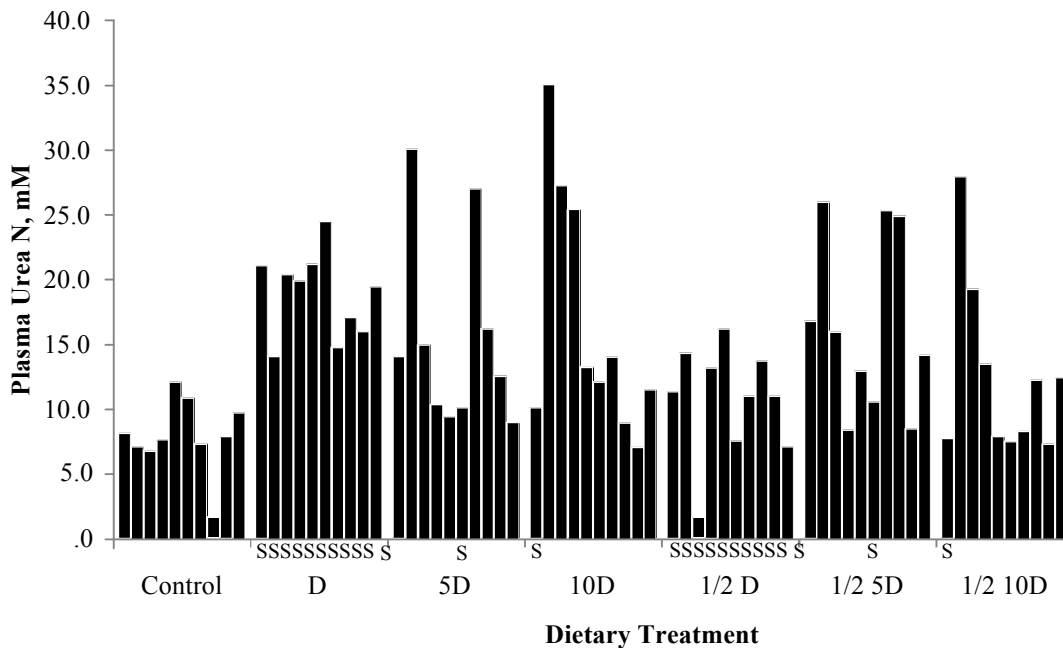
<sup>4</sup>Calculated as (Daily N retention, g/kg BW/Daily N digested, g/kg BW) x 100.

digestibility of the half CP lambs was similar to that of the full CP daily supplemented lambs. These results suggest that the amount of CP fed may be reduced and still elicit similar results to the full CP fed wethers.

As SF was reduced, a linear reduction ( $P = 0.02$ ) in straw DMI, total DMI, straw OM intake, total OM intake, NDF intake, ADF intake, and IADF intake were observed. However, we noted a linear increase ( $P = 0.04$ ) in DM and OM digestibility as supplementation became less frequent, with NDF and ADF digestibility not affected ( $P \geq 0.19$ ). Frequency of supplementation did not affect N balance ( $P = 0.22$ ) or daily digested N retained ( $P = 0.93$ ). Similar results were observed by Bohnert et al. (2002b), as supplementation frequency did not affect the apparent total tract N disappearance of steers. Nitrogen intake and fecal N excretion decreased ( $P \leq 0.04$ ) as SF was reduced. Urinary N excretion and N digestibility increased ( $P \leq 0.05$ ) as SF reduced. In similar trials, as the frequency of supplementation was reduced to once every 10 or 6 d, urinary N excretion and N digestibility was increased (Schauer et al., 2010 and Bohnert et al., 2002a, respectively). As with Bohnert et al. (2002a) and Schauer et al. (2010), the reduction in straw and total DMI can partially be explained by the reduction in forage intake on the days following supplementation (data not shown).

A tendency existed ( $P \leq 0.10$ ) for a CP amount  $\times$  SF interaction on all intake variables. Lambs offered full CP had a greater reduction in straw intake, total DMI, straw OM intake, total OM intake, NDF intake, ADF intake, and IADF intake as SF decreased compared with the lambs offered half CP. Dry matter, OM, NDF, and ADF digestibility increased as SF decreased for the full CP wethers compared with the half CP wethers, which remained relatively constant ( $P \leq 0.04$ ). These results suggest that with a scenario similar to the current study, the amount of CP supplemented can be reduced to 50 % of that required while maintaining N efficiency similar to full CP supplemented wethers.

There was a dietary treatment  $\times$  day effect ( $P < 0.001$ ; Figure 1) for plasma urea N concentration. Plasma urea N concentration was increased on the day post-supplementation for the 5D, 10D,  $\frac{1}{2}$  5D, and  $\frac{1}{2}$  10D wethers ( $P \leq 0.05$ ). Similar results were observed by Bohnert et al. (2002a), with increased plasma urea N concentration on the first day post-supplementation. Also, Bohnert et al. (2002c) observed a similar increase in steer ruminal ammonia N concentration, with concentration greater 24 h post-supplementation compared with the time of supplementation. Both gastrointestinal tract permeability to urea and regulation of renal urea excretion can be altered by low-protein diets and/or restricted feeding (Harmeyer and Martens, 1980 and



**Figure 1.** Influence of supplementation frequency and amount of CP supplemented on plasma urea N concentrations of wethers. Columns from left to right for each dietary treatment represent d 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of a 10 d supplementation window, respectively. Treatments were CON = unsupplemented control; D = supplemented daily 0.28% of BW of soybean meal (SBM); 5D = supplemented 1.4% of BW of SBM once every 5 d; 10D = supplemented 2.8% of BW of SBM once every 10 d;  $\frac{1}{2}$  D = supplemented at 50% of D;  $\frac{1}{2}$  5D = supplemented at 50% of 5D;  $\frac{1}{2}$  10D = supplemented at 50% of 10D. Full CP refers to D, 5D, and 10D and half CP refers to  $\frac{1}{2}$  D,  $\frac{1}{2}$  5D, and  $\frac{1}{2}$  10D dietary treatments. Treatment  $\times$  day interaction ( $P < 0.001$ ). SEM = 3.80.

Kennedy and Milligan, 1980). Three factors influence the excretion of urea from the kidneys: 1) changes in filtered urea loads correspond with changes in plasma urea concentrations, 2) changes in glomerular filtration rate, and 3) changes in tubular resorption of urea (Harmeyer and Martens, 1980). This would suggest that the lambs in the current trial were more efficient in conserving and recycling urea N as SF and amount of CP decreased.

### IMPLICATIONS

Reducing the amount of CP supplemented and decreasing SF did not negatively impact N retention in wethers. Therefore, our data suggests that reducing the amount of CP while reducing SF is a potential strategic supplementation practice that can maintain N use efficiency. The reduction in SF and amount of CP fed will minimize labor and feed costs during times when only low-quality forage is available.

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