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INFLUENCE OF RUMEN PROTEIN DEGRADABILITY AND SUPPLEMENTATION FREQUENCY ON PERFORMANCE AND NITROGEN USE IN RUMINANTS CONSUMING LOW-QUALITY FORAGE: COW PERFORMANCE AND EFFICIENCY OF NITROGEN USE IN WETHERS

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ABSTRACT: Two experiments (Exp) were conducted to determine the influence of CP degradability (CPD) and supplementation frequency (SF) on ruminant performance and N efficiency. Treatments (TRT) included an unsupplemented control (CON) and degradable intake protein (DIP; 18% UIP) or undegradable intake protein (UIP; 60% UIP) provided daily, every 3 d, or every 6 d. Exp 1 was a N balance study using 7 wethers (36 ± 1 kg BW) with DIP and UIP TRT formulated to meet CP requirements. Exp 2 was a performance study using 84 cows (512 ± 42 kg BW) during the last third of gestation. DIP TRT were calculated to provide 100% of DIP requirement while UIP TRT were provided on an isonitrogenous basis compared with DIP TRT. Basal diets consisted of low-quality (5% CP) meadow hay. In Exp 1 forage DMI and N intake decreased ($P < 0.05$) linearly as SF decreased. DMI, OM intake, N retention, N digestibility, and digested N retained were greater ($P < 0.01$) for supplemented wethers compared with CON with no difference ($P > 0.10$) due to CPD. Nitrogen balance, DMI, and OM intake decreased linearly ($P < 0.05$) as SF decreased. Plasma urea (PU; mM) was measured over a 6 d period with supplemented lambs having increased ($P < 0.01$) PU compared with CON. PU linearly decreased ($P < 0.01$) as SF decreased. In Exp 2 cow pre- and post-calving (within 14 d and 24 hr of calving, respectively) weight and BCS change were more positive ($P < 0.05$) for supplemented groups compared with CON. No differences ($P > 0.10$) were observed for CPD or SF. Results suggest CP supplements consisting of 20 to 60% UIP can be effectively used by ruminants consuming low-quality forage without adversely affecting N efficiency and animal performance, even when provided as infrequently as once every 6 d.

Key Words: Protein, Nitrogen, Degradability, Supplementation, Frequency

Introduction

Protein can be divided into degradable intake protein (**DIP**) and undegradable intake protein (**UIP**). Degradable intake protein is broken down within the rumen by ruminal microorganisms while UIP "escapes" ruminal degradation and is presented to the small intestine for enzymatic digestion and potential absorption (NRC, 1985b; NRC, 1996). Degradable intake protein is used by the ruminal microflora to maintain fermentation and promote microbial growth. The resulting microbial protein is the main source of amino acids and peptides flowing to the small intestine of ruminants consuming low-quality forage. Undegradable intake protein that is absorbed (as amino acids and/or peptides) and not used by the animal for maintenance, growth, and/or production can be deaminated by the liver and the N used to produce urea N (Maynard et al., 1979). This endogenous urea N can enter the rumen by diffusion across the rumen wall and from saliva (Tillman and Sidhu, 1969; Helmer and Bartley, 1971) for use as a source of DIP.

The use of low-quality forage by ruminants is dependent on the cellulolytic activity of the microorganisms within the rumen. Many of the nutrients required for growth of these microorganisms, such as ammonia, peptides, amino acids, branch-chain VFA, and sulfur, are derived from the ruminal degradation of DIP (Allison, 1969; Russell and Hespell, 1981). Consequently, DIP is generally considered to be the most beneficial supplement to low-quality forages. Also, ammonia is probably the most important source of N for growth of ruminal bacteria (Allison, 1969; Tillman and Sidhu, 1969). However, ammonia not used for microbial protein synthesis is either absorbed through the rumen wall or flows out of the rumen for absorption from the abomasum and/or small intestine (Leng and Nolan, 1984). As a result, ammonia N is transported to the liver where it is converted to urea N via the urea cycle and released into the peripheral circulation (Maynard et al., 1979). Urea N not transferred to the gut increases blood urea N and urinary N excretion, thereby decreasing efficiency of N use. Infrequent supplementation with DIP will supply nitrogen in excess of the immediate demands of the rumen microbial population, especially on the day of supplementation, due to rapid degradation of DIP. Undegradable intake protein may be better suited to less frequent supplementation compared with DIP because of a potential to decrease urinary N excretion and increase N recycling to the gut. This study was designed to determine if infrequent supplementation of low-quality forage with UIP will allow for acceptable performance and more efficient use of dietary N by ruminants compared with DIP.

Materials and Methods

Experiment 1. Seven wethers (36 ± 1 kg) were used in an incomplete 7 × 4 Latin square design to evaluate

the efficacy of N use in lambs supplemented with a DIP or UIP supplement (approximately 20 and 60% UIP as a percentage of CP, respectively; Table 1) every d, every third d, or every sixth d. Wethers were randomly allotted to treatments and housed in individual metabolism crates within an enclosed barn with continuous lighting.

Wethers had continuous access to fresh water and low-quality meadow hay (Table 1). Hay was provided daily in two equal portions (0730 and 1730) at 120% of the average intake for the previous 5 d, with feed refusals from the previous day determined before the 0730 feeding. A trace mineral salt mix was available free choice (2.4% Ca, 2.3% P, 20.4% Na, 31.65 Cl, 0.2% K, 0.4% mg, 0.1% S, 1309 ppm Mn, 2046 ppm Fe, 7 ppm Cu, 1930 ppm Zn, 42 ppm Co, 120 ppm I, 16 ppm Se, 1325 IU/kg Vitamin E, and 552 and 50 kIU/kg Vitamins A and D, respectively). In addition, an intramuscular injection of vitamins A, D, and E (200,000, 20,000, and 600 IU of Vitamins A, D, and E, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each lamb at the onset of the trial to safeguard against deficiency. Treatments were arranged in a 2 × 3 factorial design, two levels of ruminal protein degradability (**CPD**) and three supplementation frequencies (**SF**), with a negative control (**CON**; no supplementation). Protein supplements were offered every day (**1D**), every third day (**3D**), or every sixth day (**6D**). The DIP and UIP treatments received the same amount of total supplemental N over a 6 d period); therefore, the 3D and 6D treatments received threefold and sixfold the amount of supplement (N basis) on their respective supplementation d compared with 1D treatment. The amount of CP supplied by each supplement was approximately 0.19% of BW/d (averaged over a 6 d period) based on intake and protein requirements (NRC, 1985a).

Experimental periods were 24 d with at least 3 d between periods (to remove wethers from metabolism crates). Dry matter intake was determined on d 17 to 22. In addition, samples of meadow hay, protein supplements, and orts were collected on d 17 to 22 and dried at 55°C for 48 h. On d 19 to 24, total fecal and urine output was collected. Urine was composited daily by wether (10% of total; weight basis) and stored at 4°C. Sufficient 6 *N* HCl (150 mL) was added daily to urinals to maintain urine pH < 3. A sub-sample of each daily fecal sample (7.5%; weight basis) was dried at 55°C for 96 h for calculation of fecal DM. On d 19 to 24, 20 mL of blood was collected via jugular venipuncture 4 h after the 0730 feeding using a heparinized syringe. Blood samples were immediately transferred to vacutainers (Fisher Scientific, catalog no. 0268360), placed on ice for transport to the lab, centrifuged (5000 × *g*, 4°C, 15 min), and plasma harvested and stored (-20°C). Dried samples were ground through a Wiley mill (1-mm screen). Samples of ground meadow hay and protein supplements were composited by period and daily orts composited by lamb (within period) on an equal weight basis (20% as-fed). Feed, orts, and fecal samples were analyzed for DM and OM (AOAC, 1990) and NDF and ADF (Ankom 200 Fiber Analyzer, Ankom Co., Fairport, NY). Feed, orts, fecal, and urine samples were analyzed for N using a Kjeltec Auto 1030 Analyzer (Tecator AB, Höganäs, Sweden). Plasma samples were assayed for urea N using the Sigma Diagnostics Procedure 535 (Sigma Chemical Co., St. Louis, MO) and a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

Data were analyzed as an incomplete 7 × 4 Latin square using the GLM procedure of SAS (1996). The model included period, wether, and treatment. Because the treatment structure consisted of a 2 × 3 factorial plus a negative control, nonorthogonal contrasts were used to partition specific treatment effects. Contrast statements included: 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: 1) DM and OM intake; 2) total tract digestibility of DM, OM, and CP; 3) N balance; and 4) digested N retained. Plasma concentration of urea N was analyzed using the REPEATED statement with the MIXED procedure of SAS (1996). The model included lamb, period, treatment, day, and treatment × day. In addition, lamb × period × treatment was used to specify variation between animals (using the RANDOM statement). Lamb × period × treatment was used as the SUBJECT and autoregression used as the covariance structure. The same contrasts noted above were be used to partition treatment sums of squares.

Experiment 2. Eighty-four pregnant (approximately 180 d) beef cows (512 ± 42 kg BW) were stratified by age, body condition score (**BCS**; 1 = emaciated, 9 = obese; Herd and Sprott, 1996), and expected calving date, and assigned randomly within stratification to one of seven treatments (as described in Exp. 1 above) in a 2 × 3 factorial arrangement (two levels of CPD and three SF) with a negative control (no supplementation). They were then sorted by treatment and allotted randomly to 1 of 21 pens (4 cows/pen; 3 pens/treatment). 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). Cows were provided ad libitum access to low-quality meadow hay (Table 2).

The DIP treatments were formulated to provide 100% of the estimated DIP requirement assuming a microbial efficiency of 11% (NRC, 1996). An equal amount (N basis) of the UIP supplement was provided, thereby ensuring that the DIP and UIP treatments were providing the same amount of total supplemental N over a 6 d period. Protein supplements were offered D, 3D, or 6D to provide approximately 0.08% of BW/d of CP (averaged over a 6 d period) until calving. The experiment began on January 19, 2000, with experimental diets fed from start date to calving (78 ± 4 d). After calving all cows and calves were managed and treated equally until weaning.

Cow BW and BCS were measured every 14 d until calving and within 24 h of calving. All weights were obtained following an overnight shrink (16-h). Cow BCS was judged independently by at least two observers. The same technicians measured BCS throughout the experiment. In addition to cow weights, calf weights were

obtained within 24 h of birth. Hay and supplement samples (approximately 200 g) were collected weekly, dried at 55°C for 48 h, ground through a Wiley mill (1-mm screen), and composited by period for analysis of ADF and NDF (Ankom 200 Fiber Analyzer, Ankom Co., Fairport, NY), N (Kjeltec Auto 1030 Analyzer, Tecator AB, Höganäs, Sweden), and OM (AOAC, 1990).

Cow performance data were analyzed as a randomized complete block design. The model included block and treatment. The same nonorthogonal contrasts described in Exp.1 were used to partition specific treatment effects. Response variables included: 1) cow weight change; 2) cow BCS change; 3) calf birth weight.

Results and Discussion

Experiment 1. Intake of hay DM and OM by lambs was not affected ($P > 0.10$) by CP supplementation or degradability, while total DM, OM, and N intake increased ($P < 0.01$) with supplementation (Table 3). Also, Hay DM and OM, total DM and OM, N, and NDF intake decreased linearly ($P < 0.05$) as SF decreased. Therefore, total nutrient intake decreased as SF decreased.

Total tract digestibility of DM, OM, N, and NDF was increased ($P < 0.01$) with CP supplementation, while UIP increased ($P < 0.05$) total tract digestibility of NDF compared with DIP (Table 3). Also, an interaction was noted with OM digestibility ($P < 0.05$) concerning the linear effect of SF × CPD. This interaction was a result of a decrease in OM digestibility with DIP as SF decreased compared with an increase in digestibility with UIP.

Daily fecal and urinary excretion of N (g/kg BW) was increased ($P < 0.01$) with CP supplementation (Table 3). However, no differences were noted due to CPD or SF. Daily N balance and digested N retained (g/kg BW) were greater with CP supplementation. In addition, daily N balance decreased linearly ($P < 0.05$) as SF decreased. This is most likely due to the linear decrease in N intake that occurred as SF decreased. This is supported by the fact that there was no difference ($P > 0.10$) in digested N retained by supplemented lambs.

Treatment × time interactions ($P < 0.01$) were observed for plasma urea N (mM). However, after considering the nature of the interactions, we concluded that discussing treatment means while providing the treatment × time figure would aid in interpretation and discussion of the data.

Lamb plasma urea N (mM) was greater ($P < 0.01$) in CP supplemented lambs compared with CON (Table 3). No difference was observed due to CPD; however, plasma urea N decreased ($P < 0.01$) as SF decreased. This can be at least be partially explained by the decrease in N intake observed as SF decreased. Figure 1 provides an illustration of plasma urea N (mM) over the six d supplementation period. It is of interest to note the peaks in urea N on the d following supplementation for the 3D and 6D treatments. Plasma urea N concentration demonstrated a bimodal pattern in the 3D supplemented lambs (a moderate peak following each supplementation d), while a large, single peak in urea N was observed on the d following supplementation with the 6D treatment.

Experiment 2. Precalving (within 14 d of calving) and postcalving (within 24 hr of calving) weight and BCS change were more positive ($P < 0.01$) with CP supplementation (Table 4). All weight and BCS changes were positive except for postcalving weight change on the CON treatment. These cows lost 39 kg compared with gains of 8, 17, 3, 4, 6, and 9 kg for the DIPD, DIP3D, DIP6D, UIPD, UIP3D, and UIP6D cows, respectively. In addition, an interaction concerning the linear effect of SF × CPD was observed ($P < 0.05$) for precalving weight change. This was due to the decrease in weight change with DIP as SF decreased compared with essentially no change due to SF with UIP.

Crude protein supplementation, CPD, and SF had no effect ($P > 0.05$) on calf birth date or calf birth weight.

Implications

Occasional supplementation (as infrequently as once every six days) of rumen degradable and undegradable intake protein to ruminants consuming low-quality forage (< 6% crude protein) results in nitrogen efficiency and animal performance that is similar to daily supplemented individuals. Ruminants appear to have the ability to conserve nitrogen over extended periods, thereby storing it for use between periods of supplementation. Infrequent supplementation of protein with ruminal degradability ranging from 80 to 40% is a management alternative that can help lower costs associated with supplementation without being detrimental to animal performance.

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Table 1. Supplement composition and feedstuff nutrient content in Experiment 1 (lamb study)

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	5.2	52.8	59.8
UIP, %CP	19.0	17.6	59.9
OM, % DM	91.6	92.6	94.4
NDF, % DM	60.1	11.5	28.6
ADF, %DM	32.0	5.1	6.6

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

Table 2. Supplement composition and feedstuff nutrient content in Experiment 2 (cow study)

Item	Meadow Hay	DIP Supplement	UIP Supplement
Soybean meal	-	100.0	-
SoyPLUS[a]	-	-	69.4
Blood meal	-	-	30.6
Nutrient Composition			
CP, % DM	5.0	54.8	62.8
UIP, %CP	19.0	17.6	59.9
OM, % DM	91.8	92.5	94.8
NDF, % DM	57.7	8.5	25.7
ADF, %DM	32.1	3.6	5.1

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

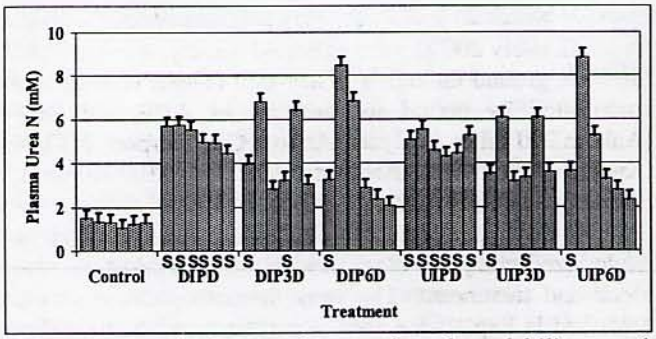

Figure 1. Effect of protein degradability and supplementation frequency on lamb plasma urea N (mM). Columns from left to right for each treatment represent d 1, 2, 3, 4, 5, and 6 of a 6 d supplementation period, respectively. Treatments were: Control; DIPD = degradable intake protein every day; DIP3D = 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 d.

Table 3. Effect of protein degradability and supplementation frequency on lamb intake, diet digestibility, and nitrogen balance

	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	22.1	22.5	21.1	20.7	24.7	23.6	20.1	0.9	0.97	0.10	0.005	0.65	0.16	0.31
Supplement[d]	0.0	3.6	3.6	3.6	3.1	3.1	3.1							
Total	22.1	26.2	24.8	24.3	27.8	26.7	23.1	0.9	0.006	0.32	0.005	0.65	0.16	0.31
Daily OM Intake, g/kg BW														
Hay	20.2	20.6	19.3	19.0	22.6	21.6	18.4	0.8	0.95	0.10	0.004	0.69	0.16	0.32
Supplement[e]	0.0	3.4	3.4	3.4	2.9	2.9	2.9	0.0						
Total	20.2	24.0	22.7	22.3	25.6	24.5	21.3	0.8	0.005	0.29	0.004	0.69	0.16	0.32
Daily N Intake, g/kg BW	0.185	0.486	0.487	0.477	0.494	0.487	0.455	0.010	<0.001	0.60	0.04	0.33	0.16	0.69
Daily NDF Intake, g/kg BW	13.0	13.9	13.1	12.7	15.6	14.9	12.9	0.6	0.24	0.03	0.008	0.71	0.24	0.42
Total Tract Digestibility, %														
DM	50.4	58.7	57.8	56.6	57.3	59.1	59.4	0.9	<0.001	0.27	0.98	0.60	0.04	0.72
OM	54.0	62.4	61.9	60.4	60.9	63.2	63.2	0.9	<0.001	0.28	0.87	0.34	0.04	0.68
N	23.7	66.2	64.5	65.7	62.2	63.8	65.4	1.7	<0.001	0.27	0.44	0.63	0.30	0.63
NDF	45.9	53.1	53.9	49.7	53.2	57.8	57.4	1.8	<0.001	0.02	0.83	0.13	0.05	0.97
Daily N excretion, g/kg BW														
Fecal	0.140	0.167	0.172	0.164	0.189	0.174	0.158	0.009	0.007	0.44	0.07	0.65	0.13	0.66
Urinary	0.054	0.217	0.227	0.219	0.208	0.220	0.228	0.008	<0.001	0.68	0.18	0.48	0.26	0.64
Daily N balance, g/kg BW	-0.009	0.101	0.088	0.094	0.098	0.094	0.068	0.008	<0.001	0.25	0.04	0.94	0.18	0.15
Daily digested N retained[f], %	-16.3	35.3	27.7	29.6	31.2	25.8	24.0	7.6	<0.001	0.54	0.41	0.63	0.92	0.82
Plasma urea N, mM	1.30	5.22	4.39	4.32	4.88	4.31	4.36	0.22	<0.001	0.50	0.008	0.10	0.41	0.85

[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 3.6 g/kg BW daily; DIP3D received 10.8 g/kg BW every third d; DIP6D received 21.6 g/kg BW every sixth d; UIPD received 3.1 g/kg BW daily; UIP3D received 9.3 g/kg BW every third d; UIP6D received 18.6 g/kg BW every sixth d.

[e] DIPD received 3.4 g/kg BW daily; DIP3D received 10.2 g/kg BW every third d; DIP6D received 20.4 g/kg BW every sixth d; UIPD received 2.9 g/kg BW daily; UIP3D received 8.7 g/kg BW every third d; UIP6D received 17.4 g/kg BW every sixth d.

[f] Calculated as (Daily N retention, g/kg BW / Daily N digested, g/kg BW) × 100.

Table 4. Effect of protein degradability and supplementation frequency on cow performance and calf birth weight

	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
Supplement DMI, g/d[d]	0.0	740	740	740	627	627	627							
Initial weight, kg	521	514	514	511	519	516	490							
Initial body condition score	5.06	5.00	4.98	4.96	4.91	4.91	4.90							
Weight change, kg														
Precalving[e]	2	57	50	38	42	47	41	4	<0.001	0.17	0.04	0.30	0.04	0.68
Postcalving[f]	-39	8	17	3	4	6	9	6	<0.001	0.57	0.99	0.32	0.45	0.31
Body condition score change														
Precalving[e]	0.21	0.79	0.77	0.73	0.69	0.72	0.73	0.08	<0.001	0.44	0.90	0.89	0.52	0.98
Postcalving[f]	0.12	0.65	0.56	0.50	0.63	0.59	0.65	0.09	<0.001	0.48	0.50	0.68	0.40	0.80
Calf birth date, Gregorian d	83	78	74	73	78	71	85	4	0.16	0.40	0.79	0.13	0.22	0.22
Calf birth weight, kg	39	38	40	37	41	38	39	1	0.60	0.26	0.14	0.65	0.64	0.05

[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 = 3.

[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 740 g daily; DIP3D received 2,220 g every third d; DIP6D received 4440 g every sixth d; UIPD received 627 g daily; UIP3D received 1881 g every third d; UIP6D received 3762 g every sixth d.

[e] Within 14 d of calving.

[f] Within 24 hr after calving.