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PROTEIN SUPPLEMENTATION OF RUMINANTS CONSUMING LOW-QUALITY COOL- OR WARM-SEASON FORAGE: DIFFERENCES IN INTAKE AND DIGESTIBILITY

D. W. Bohnert[1], T. DelCurto[2], A. A. Clark[2], M. L. Merrill[1], S. J. Falck[3], and D. L. Harmon[4]
Eastern Oregon Agricultural Research Center, Oregon State University, Burns[1] and Union[2], OR
[3]Eastern Oregon Agricultural Research Center, ARS-USDA, Burns, OR
[4]University of Kentucky, Lexington, KY

ABSTRACT: Four steers (252 ± 8 kg BW; Exp. 1) and four wethers (38 ± 1 kg BW; Exp. 2) were used in two 2 × 2 factorial design experiments to determine the influence of protein supplementation of low-quality cool- (C3; bluegrass straw) and warm-season (C4; tall grass-prairie hay) forage (6.3 and 5.7% CP, respectively) on intake and nutrient digestion. Steers and wethers were allotted to 4 × 4 Latin squares with 20-d periods. Animals were provided forage at 120% of the previous 5 d average intake. Soybean meal (SBM; 52% CP) was used as the CP supplement. In Exp. 1, feed and digesta were collected on d 14 through 18 for estimation of nutrient digestibility and ruminal fluid was sampled on d 20. In Exp. 2, feed, feces, and urine were collected on d 16 to 20 for calculation of N balance. Contrasts were: 1) supp. vs unsupp.; 2) C3 vs C4; 3) supp. × forage type. A supp. × forage type interaction ($P < 0.01$) was noted for forage and total DMI in Exp. 1, with supplementation increasing intake of C4 and C3 forage by 47 and 7%, respectively. DM digestibility responded similarly with a supp. × forage type interaction ($P = 0.05$; supp. increased digestibility 12% with C4 and 9% with C3 forage). Also, supp. × forage type interactions were noted for ruminal liquid retention time ($P = 0.02$; supp. decreased retention time from 15.3 to 11.7 h with C4 and from 9.7 to 9.1 h with C3 forage) and particulate passage rate ($P = 0.02$; supp. increased particulate passage 46% with C4 and 10% with C3 forage). As in Exp. 1, a supp. × forage type interaction ($P = 0.01$; supp. increased digestibility 18% with C4 and 7% with C3 forage) was observed with DM digestibility in Exp. 2. In contrast, only supplementation effects were noted for N balance ($P = 0.002$) and N digestibility ($P < 0.001$), which increased with supplementation. These data suggest that intake and digestion of low-quality C3 and C4 forages by ruminants are not similar and, more importantly, the physiological response of ruminants differs with protein supplementation of C3 versus C4 forages.

Keywords: Cattle, Metabolism, Sheep

Introduction

Forages represent the predominant class of feed within most ruminant livestock operations. Due to differences in plant variety, stage of maturity, and management practices, forages vary significantly with respect to quality parameters such as DM digestibility, CP, and palatability. In addition, many ruminants, especially in the Intermountain West, consume low-quality forages (<7% CP) for extended periods during the annual production cycle (Turner and DelCurto, 1992). In an effort to meet the nutritional needs of these animals, supplemental CP is often provided because it has been shown to increase forage OM intake (Lintzenich et al., 1995), forage DMD (DelCurto, 1990), and animal performance (Bodine et al., 2001).

The forage types available to ruminants can be broadly grouped into cool-season (**C3**) and warm-season (**C4**). Physiological and biochemical differences distinguish C3 (first organic product during carbon fixation is three-carbon 3-phosphoglycerate) from C4 (first organic product is the four-carbon oxaloacetate) grasses (Lambers et al., 1998). It is generally considered that C3 grasses have a higher nutritional quality than C4 grasses (Barbehenn et al., 2004), which has been attributed to higher levels of nonstructural carbohydrates, protein, and water and lower levels of fiber (Wilson et al., 1983; Barbehenn and Bernays, 1992).

Despite agronomic research evaluating physiological differences between C4 and C3 grasses and the nutritional evaluation of low-quality forage CP supplementation, information on the comparative utilization of low-quality C3 vs. C4 grasses by ruminants is limited. Therefore, the objective of this experiment was to compare intake, digestibility, and N balance of ruminants offered low-quality C4 (tall grass-prairie hay) and C3 (bluegrass straw) grasses with and without protein supplementation.

Materials and Methods

All experimental procedures used in this study were approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP# 3372).

Experiment 1: Influence of CP Supplementation of C3 versus C4 Forage on Intake, Digestibility, and Ruminal Fermentation by Steers

Four ruminally cannulated Angus x Hereford steers (252 ± 8 kg BW) were used in a 4 × 4 Latin square design and housed in individual pens (2 x 4 m) within an enclosed barn with continuous lighting. Steers were provided continuous access to fresh water and low-quality C3 (bluegrass straw) or C4 (tall grass-prairie hay) forage (6.3 and 5.7% CP, respectively; Table 1). Forage was provided daily (0700) 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 provided daily. In addition, an intramuscular injection of vitamins A, D, and E was administered to each steer at the onset of the trial to safeguard against deficiency. Treatments were arranged in a 2 × 2 factorial design (two forage types with or without supplemental protein). Soybean meal (**SBM**) was placed directly into the rumen via the ruminal cannula for supplemented treatments. The amount of CP supplied by SBM was 0.09% of BW/d. The supplemented treatments were formulated to provide 100% of the estimated DIP requirement assuming a microbial efficiency of 11%.

Experimental periods were 20 d, with intake measured beginning d 14 and concluding d 18. On d 15, treatment effects on ruminal DM, indigestible ADF (**IADF**), and fluid contents were determined by manually removing the contents from each steer's reticulo-rumen 4 h after feeding. The total ruminal contents were weighed, mixed by hand, and sub-sampled in triplicate (approximately 400 g). The remaining ruminal contents were immediately replaced 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 steer.

Samples of forages, SBM, and orts were collected on d 14 through 18 and dried at 55°C for 48 h. Total fecal collection was conducted on d 16 to 20. Steers were fitted with harnesses and fecal bags on d 16 (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.

On d 20, each steer was intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-ml aqueous solution. The Co marker was administered throughout the rumen by injecting through a stainless steel probe with a perforated tip. Ruminal fluid (approximately 100 mL) was collected by suction strainer immediately prior to dosing and at 3, 6, 9, 12, 18, and 24 h post-dosing. Ruminal fluid pH was measured immediately after collection. Twenty milliliters was stored (-20°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°C) for subsequent analysis of VFA and NH3-N. Frozen (-20°C) ruminal samples were prepared for analysis by thawing, centrifuging, and collecting the supernatant. Cobalt was analyzed by atomic absorption using an air/acetylene flame.

Ground samples of forages and SBM were composited by period and daily orts composited by steer (within period) on an equal weight basis (5% as-fed). Feed, orts, and feces were analyzed for DM and OM, N, and NDF and ADF. Feed, orts, feces, and ruminal particulate samples were analyzed for IADF (IADF recovery was 102 ± 4%).

Data were analyzed as a 4 × 4 Latin square using the GLM procedure of SAS. The model included period, steer, and treatment. Because the treatment structure consisted of a 2 × 2 factorial, orthogonal contrasts were used to partition specific treatment effects. Contrast statements include: 1) C3 vs C4 forage; 2) supplemented vs unsupplemented; 3) contrast 1 × contrast 2. Ruminal pH, NH_3-N, and VFA data, collected at the fixed times after feeding, were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included steer, period, treatment, time, and treatment × time. In addition, steer × period × treatment were used to specify variation between steers (using the RANDOM statement). Steer × period × treatment were used as the SUBJECT and autoregression (AR1) used as the covariance structure. The same contrasts noted above were used to partition the treatment sums of squares. If no treatment × day interaction is detected ($P > .10$) measurements were averaged and the treatment means compared as described above.

Experiment 2: Influence of CP Supplementation of C3 versus C4 Forage on Efficiency of Nitrogen Use by Lambs

Four wethers (38 ± 1 kg BW kg) were used in a 4 × 4 Latin square design. Wethers were provided continuous access to fresh water and the same low-quality C3 or C4 forage used in Exp. 1 (Table 1). Forage was provided at 120% of the previous 5-d average intake in two equal portions (0700 and 1700), with feed refusals from the previous day determined before the 0700 feeding. A trace mineral salt mix was provided daily. In addition, an intramuscular injection of vitamins A, D, and E was administered to each lamb at the onset of the trial to safeguard against deficiency. Treatments were the same as described in Experiment 1. The quantity of supplemental CP provided was 0.19% of BW/d (CP basis). Wethers were randomly allotted to treatments and housed in individual metabolism crates within an enclosed barn with continuous lighting.

Experimental periods were 20 d, with DMI determined on d 14 through 18. In addition, samples of forages, SBM, and orts were collected on d 14 to 18 and dried at 55°C for 48 h. On d 16 to 20, total fecal and urine output were collected. Urine was composited daily by wether (50% of total; weight basis) and stored at 4°C. Sufficient 6 *N* HCl (approximately 25 mL) was added to urinals daily to maintain urine pH < 5. 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 16 to 20, 10 mL of blood was collected from the jugular vein 4 h after feeding using vacutainers containing EDTA. Blood samples were centrifuged and plasma harvested and stored (-20°C). Dried samples were ground through a Wiley mill (1-mm screen). Samples of ground forages and SBM were composited by period and daily orts composited by lamb (within period) on an equal weight basis (10% as-fed). Feed, orts, and fecal samples were analyzed for DM and OM and NDF and ADF. Feed, orts, fecal, and urine samples were analyzed for N. Plasma samples were assayed for urea-nitrogen using a UV/VIS spectrophotometer.

Data were analyzed as described above. Plasma urea-N was analyzed using the REPEATED statement with the MIXED procedure of SAS. 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 was used as the covariance structure. The same contrasts noted above were used to partition treatment sums of squares.

Results and Discussion

Experiment 1

We noted CP supplementation × forage type interactions ($P < 0.01$) for forage and total DMI, N intake, and NDF intake by steers (Table 2). In each instance, the C4 forage had decreased overall intake and intake increased more with CP supplementation compared with the C3 forage. For example, forage DMI averaged 19.2 and 24.5 g/kg BW for steers consuming C4 and C3, respectively. Also CP supplementation increased C4 forage intake by 47% compared with 7% with C3. This may help explain some of the apparent inconsistencies reported in the literature for forage intake in response to CP supplementation. It is generally believed that CP supplementation of low-quality forage (< 7% CP) will increase forage intake up to 100%. This assumption has been based almost exclusively on research with C4 forages (McCollum and Galyean, 1985; DelCurto et al., 1990; Köster et al., 1996). However, forage intake has not been reported to increase in most, if not all, of the studies with CP supplementation of low-quality C3 forages (Horney, et al., 1996; Mathis et al., 2000; Bohnert et al., 2002).

Digestibility of DM responded similarly to intake, with a CP supplementation × forage type interaction ($P = 0.05$; Table 2) in which DM digestibility averaged approximately 47 and 52% and increased 12 and 9% with CP supplementation for C4 and C3, respectively. Neutral detergent fiber digestibility tended ($P = 0.07$) to be greater for C3 compared with C4 forage, while N and NDF digestibility increased with CP supplementation ($P < 0.03$). Diet digestibility has been reported to increase with CP supplementation of low-quality forage (Horney et al., 1996; Bohnert et al., 2002). We are aware of no data that has compared the in vivo digestibility of low-quality C3 and C4 forage; however, Foster et al. (1996) noted that NDF and ADF in vitro digestibility of C3 forages was greater than C4 forages sampled at the same time throughout the year.

Ruminal fluid and particulate dynamics were affected by forage type and supplemental CP. Ruminal liquid fill was greater ($P < 0.01$) for C3 than C4 (311 and 234 mL/kg BW, respectively; Table 2) and was not affected by CP supplementation ($P = 0.28$), whereas liquid dilution rate increased with CP supplementation ($P = 0.03$) and for C3 compared with C4 ($P < 0.01$). A CP supplementation × forage type interaction ($P = 0.02$) was noted for liquid retention time, with CP supplementation decreasing retention time from 15.3 to 11.7 h (24%) with the C4 and from 9.7 to 9.1 h (6%) with the C3 forage. Ruminal IADF fill was not affected by CP supplementation or forage type ($P > 0.54$); however, we did observe a CP supplementation × forage type interaction ($P = 0.02$) for IADF passage rate; C4 averaged 1.6 and C3 averaged 2.0 %/h with CP supplementation increasing passage rate by 46 and 10% for C4 and C3, respectively.

Ruminal NH_3-N responded with a CP supplementation × forage type interaction ($P = 0.02$; data not shown). Ammonia-N averaged 1.1 and 1.4 mM for C4 and C3, respectively, while providing supplemental SBM increased ruminal NH_3-N from 0.64 to 1.5mM with C4 forage and from 0.52 to 2.26 mM with C3 forage. Total VFA were greater with CP supplementation ($P = 0.03$; 79.4 vs 71.1 mM; data not shown) and tended to be greater for C3 vs C4 ($P = 0.11$; 78.0 vs 72.4 mM). Interestingly, the molar proportion of Ac was lower with C3 compared with C4 ($P < 0.01$) and Pr was greater ($P < 0.01$; data not shown). Consequently, The Ac:Pr was lower with C3 than C4 ($P < 0.01$; 3.9 vs 5.4), suggesting greater energetic efficiency with the C3 forage.

Experiment 2

Forage and total DMI by lambs showed a tendency ($P = 0.06$) to be greater with C3 compared with C4 forage (Table 2), with total DMI increasing with CP supplementation ($P < 0.01$). It is worth noting that there tended to be a CP supplementation × forage type interaction ($P = 0.11$) for both forage and total DMI, similar to that observed in Exp. 1 (C3 forage intake decreased 5% with CP supplementation and C4 intake increased 8%). Likewise, DM digestibility had a CP supplementation × forage type interaction in which digestibility averaged approximately 49% for C4 and 51% for C3, with CP supplementation increasing digestibility by 18 and 7%, respectively.

Nitrogen intake was increased with CP supplementation ($P < 0.01$; Table 2). Also, N intake was greater for C3 compared with C4 forage ($P = 0.01$) because of greater forage intake and greater forage CP concentration with C3 (6.3 vs 5.7%; Table 1). Similarly, plasma urea-N was greater with CP supplementation ($P < 0.01$; 5.8 vs 2.6 mM) and for C3 compared with C4 ($P < 0.01$; 4.8 vs 3.6 mM; data not shown). Fecal and urinary N excretion was increased ($P < 0.01$) with CP supplementation, and fecal N increased for C3 compared to C4 ($P = 0.02$). Nevertheless, efficacy of N use (N balance, N digestibility, and digested N retained) by lambs was not effected by forage type ($P > 0.34$), while N balance and N digestibility were greater with CP supplementation ($P < 0.01$).

In summary, these data indicate that intake and digestibility of the C3 and C4 forages in the current study were not similar and, more importantly, the physiological response of ruminants to supplemental protein depends, in part, on the cell wall structure of the basal diet. Therefore, further research comparing other low-quality C3 and C4 forages is warranted to determine if the observed responses in the current study are indicative of differences in utilization of low-quality C3 and C4 forages by ruminants.

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Table 1. Feedstuff[a] nutrient content (DM basis)

Nutrient,%	C3	C4	SBM
Exp. 1			
CP	6.3	5.7	52.6
OM	90.5	93.8	92.6
NDF	66.4	69.8	13.0
ADF	36.2	36.6	5.3
IADF	19.0	19.1	2.5
Exp. 2			
CP	6.3	5.7	51.8
OM	90.0	93.2	92.6
NDF	68.1	69.7	14.8
ADF	35.8	35.5	5.2

[a] C3 = cool season forage (bluegrass straw); C4 = warm season forage (tall grass-prairie hay); SBM = soybean meal.

Table 2. Intake, digestibility, ruminal dynamics, and efficiency of N use by ruminants consuming low-quality cool-season (C3) and warm-season (C4) grass hay with or without soybean meal (CP) supplementation

	Treatment					P-Value[b]		
Item	C4	C4+CP	C3	C3+CP	SEM[a]	CP vs No CP	C4 vs C3	CP × Type
Exp. 1 – Steers								
DMI, g/kg BW								
Forage	15.6	22.9	23.7	25.3	0.6	<0.01	<0.01	<0.01
Soybean meal	0.0	1.7	0.0	1.7				
Total	15.6	24.6	23.7	27.0	0.6	<0.01	<0.01	<0.01
N Intake, g/kg BW	0.147	0.356	0.228	0.385	0.007	<0.01	<0.01	<0.01
NDF Intake, g/kg BW	10.8	16.0	15.6	16.9	0.5	<0.01	<0.01	<0.01
Digestibility, %								
DM	42.8	51.8	49.7	54.2	0.9	<0.01	<0.01	0.05
N	28.4	54.5	37.5	55.2	3.5	<0.01	0.21	0.27
NDF	43.5	50.0	48.0	52.7	1.7	0.02	0.07	0.61
Ruminal Liquid								
Fill, mL/kg BW	220	249	306	316	16	0.28	<0.01	0.56
Dilution Rate, %/h	6.5	8.7	10.5	11.0	0.5	0.03	<0.01	0.13
Retention Time, h	15.3	11.7	9.7	9.1	0.5	<0.01	<0.01	0.02
Ruminal IADF[c]								
Fill, g/kg BW	9.5	9.3	9.6	9.1	0.5	0.55	0.92	0.79
Passage Rate, %/h	1.3	1.9	1.9	2.1	0.06	<0.01	<0.01	0.02
Exp. 2 – Lambs								
DMI, g/kg BW								
Forage	25.8	27.8	29.5	28.2	0.9	0.69	0.06	0.11
Soybean meal	0.0	3.6	0.0	3.6				
Total	25.8	31.4	29.5	31.8	0.9	<0.01	0.06	0.11
DM Digestibility, %	44.7	52.8	48.9	52.4	0.5	<0.01	0.01	0.01
Daily N Intake, g/kg BW	0.246	0.558	0.288	0.577	0.008	<0.01	0.01	0.21
Fecal N, g/kg BW	0.159	0.195	0.183	0.214	0.007	<0.01	0.02	0.72
Urine N, g/kg BW	0.065	0.221	0.080	0.261	0.017	<0.01	0.15	0.50
N Balance, g/kg BW	0.022	0.143	0.025	0.102	0.019	<0.01	0.35	0.30
N Digestibility, %	35.3	65.2	36.5	63.0	1.15	<0.01	0.68	0.20
Digested N Retained, %	23.4	39.2	23.2	27.9	9.64	0.33	0.57	0.59

[a] n = 4.

[b] CP = CP supplementation; Type = forage type.

[c] IADF = indigestible ADF.