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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,† A. A. Clark,‡ M. L. Merrill,§ S. J. Falck,# and D. L. Harmon‖

*Eastern Oregon Agricultural Research Center, Oregon State University, Burns 97720; †Eastern Oregon Agricultural Research Center, Oregon State University, Union 97883; ‡Bureau of Land Management, Department of Interior, Colorado River District, Kingman, AZ 86401; §University of California, Cooperative Extension, Alturas 96101; #Eastern Oregon Agricultural Research Center, ARS-USDA, Burns 97720; and ‖University of Kentucky, Lexington 40546

ABSTRACT: An in situ study (Exp. 1) using 4 ruminally cannulated steers (343 ± 11 kg of BW) in a completely randomized design was used to compare ruminal degradation characteristics of low-quality cool-season (C3; Kentucky bluegrass straw; *Poa pratensis*; 6.3% CP; DM basis) and warm-season (C4; tallgrass prairie; 5.7% CP; DM basis) forage. Four ruminally cannulated steers (252 ± 8 kg of BW; Exp. 2) and 4 wethers (38 ± 1 kg of BW; Exp. 3) were used in two 2 × 2 factorial arrangements of treatments to determine the influence of supplemental CP (CPSupp; soybean meal; 0.09 and 0.19% of BW, CP basis, for steers and lambs, respectively) on nutrient intake and digestion of C3 and C4 forages. Steers and wethers were allotted to separate 4 × 4 Latin squares that ran simultaneously with 20-d periods. In Exp. 1, C3 had a greater A fraction (fraction of total pool disappearing at a rate too rapid to measure) and effective degradability of DM and NDF compared with C4 ($P < 0.01$). In addition, C3 had a greater ($P < 0.01$) A fraction and effective degradability of N, whereas the C fraction (fraction of total pool unavailable in the rumen) was less ($P < 0.01$) than those for C4. Consequently, RDP accounted for 84.7% of total CP in C3 as compared with 66% for C4 ($P < 0.01$). In Exp. 2, a CPSupp × forage interaction ($P < 0.01$) was noted for forage and total DMI, with CPSupp increasing intake of C4 by 47% and intake of C3 forage by only 7%. Dry matter digestibility responded similarly, with a CPSupp × forage interaction ($P = 0.05$; CPSupp increased digestibility by 21% with C4 and by 9% with C3 forage). In addition, CPSupp × forage interactions were noted for ruminal liquid retention time ($P = 0.02$; CPSupp decreased retention by 3.6 h with C4 and by only 0.6 h with C3 forage) and particulate passage rate ($P = 0.02$; CPSupp increased passage by 46% with C4 and by 10% with C3 forage). As in Exp. 2, a CPSupp × forage interaction ($P = 0.01$; CPSupp increased digestibility by 18% with C4 and by 7% with C3 forage) was observed with DM digestibility in Exp. 3. In contrast, only N balance ($P < 0.01$) and N digestibility ($P < 0.01$) were affected by CPSupp. These data suggest that intake and digestion of low-quality C3 and C4 forages by ruminants are not similar and, more important, that the physiological response of ruminants to protein supplementation of low-quality forage is dependent on forage type.

Key words: cattle, forage, intake, protein, sheep, supplementation

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INTRODUCTION

Forages represent the predominant class of feed for cow-calf operations. Because of 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 consume low-quality forages (<7% CP) for extended periods during a year (Turner and DelCurto, 1991). To meet the nutritional needs of these animals, supplemental CP is often provided to increase forage intake (Lintzenich et al., 1995), DM digestibility (DelCurto et al., 1990), and BW gain (Bodine et al., 2001).

Forage types can be grouped into cool season (**C3**) and warm season (**C4**). Physiological and biochemical differences distinguish C3 (first organic product during C fixation is 3-C 3-phosphoglycerate) from C4 (first organic product is the 4-C oxaloacetate) grasses (Lambers et al., 1998). It is generally assumed that C3

[1]Corresponding author: dave.bohnert@oregonstate.edu
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grasses have greater nutritional quality than C4 grasses (Galyean and Goetsch, 1993; Barbehenn et al., 2004), which has been attributed to greater nonstructural carbohydrates and protein and less fiber (Wilson et al., 1983; Barbehenn and Bernays, 1992).

Despite agronomic research evaluating physiological differences between C4 and C3 grasses and nutritional research demonstrating the advantages of CP supplementation of ruminants consuming low-quality forage, data comparing the utilization of low-quality C3 vs. C4 grasses by ruminants is limited. Research does suggest that CP supplementation of ruminants consuming low-quality C3 forages does not increase forage DMI in a manner similar to that observed with C4 forages (Horney et al., 1996; Mathis et al., 2000; Bohnert et al., 2002a). Therefore, the objectives of this experiment were to compare in situ ruminal degradation of a C3 and C4 forage as well as intake and nutrient utilization of ruminants offered low-quality C4 and C3 hays with and without CP supplementation.

MATERIALS AND METHODS

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

Exp. 1. In Situ Degradation of a C3 and a C4 Low-Quality Forage

Four ruminally cannulated Angus × Hereford steers (343 ± 11 kg of BW) were used in a completely randomized design to evaluate the ruminal degradation characteristics of low-quality C3 (Kentucky bluegrass straw; *Poa pratensis*) and C4 (tallgrass prairie from Oklahoma) forages containing 6.3 and 5.7% CP (DM basis), respectively (Table 1). Steers had ad libitum access to low-quality meadow hay (6.5% CP; DM basis) consisting of approximately 82% meadow foxtail (*Alopecurus pratensis* L.), with the majority of the remaining vegetation being rushes (*Juncus* spp.), sedges (*Carex* spp.), and blue wild rye (*Elymus triticoides* Buckley; Wenick et al., 2008). The steers were offered the low-quality meadow hay diet for at least 90 d before the start of Exp. 1.

Dacron bags (10 × 20 cm; Ankom Technology Corp., Fairport, NY) were labeled with a waterproof permanent marker and weighed, 4 g (air equilibrated) of ground (2 mm; Model 4 Wiley mill, Arthur H. Thomas, Philadelphia, PA) C3 or C4 forage was added, and the bags were sealed with an impulse sealer (TISH-200, TEW Electric Heating Equipment Co. Ltd., Taipei, Taiwan). Triplicate bags for each forage source were placed in a bucket containing warm water (39°C), and introduced into the rumen within 5 min. Bags were placed in a weighted polyester mesh bag within the rumen of each steer (0, 2, 8, 12, 24, 48, and 96 h) in reverse order, allowing all bags to be removed simultaneously. Three blank Dacron bags were incubated for 96 h and used to correct for microbial and feed contamination. Upon removal, the Dacron bags were rinsed under tap water until the effluent was clear and then dried at 55°C for 24 h. The dried triplicates were allowed to air equilibrate for 24 h at room temperature; weighed for residual DM; composited by steer, time, and forage type; and analyzed for NDF (Robertson and Van Soest, 1981) by using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). The NDF residue was then weighed and analyzed for N (Leco CN-2000, Leco Corp., St. Joseph, MI). Effective degradability values of DM, NDF, and N were determined as described by Hoffman et al. (1993), using a ruminal passage rate of 2%/h (Mass et al., 1999). Rumen-degradable protein was calculated as described by Ørskov and McDonald (1979), with RUP calculated as 1 − RDP.

Exp. 2. Influence of CP Supplementation of C3 vs. C4 Forage on Intake, Digestibility, and Ruminal Fermentation by Steers

Four ruminally cannulated Angus × Hereford steers (252 ± 8 kg of BW) were used in a 4 × 4 Latin square design and housed in individual pens (2 × 4 m) within an enclosed barn with continuous lighting. Treatments were arranged as a 2 × 2 factorial (C3 or C4 with or without supplemental protein). The supplemented treatments were formulated to provide 100% of the estimated degradable intake protein requirement, assuming a microbial efficiency of 11% and an estimated forage intake of 2.5% BW (level 1; NRC, 2000). To minimize potential bias attributable to BW changes resulting from treatment regimens, the quantity of supplement provided in each period was based on initial BW at the beginning of the experiment. The amount of CP supplied by soybean meal (**SBM**) was 0.09% of BW/d. Soybean meal was supplemented by placement directly into the rumen via the ruminal cannula at 0700

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

Nutrient,[2] %	C4	C3	SBM
Exp. 1 and 2			
CP	5.7	6.3	52.6
OM	93.8	90.5	92.6
NDF	69.8	66.4	13.0
ADF	36.6	36.2	5.3
IADF	19.1	19.0	2.5
NFC	9.3	17.0	26.4
WSC	8.8	14.1	16.3
Exp. 3			
CP	5.7	6.3	51.8
OM	93.2	90.0	92.6
NDF	69.7	68.1	14.8
ADF	35.5	35.8	5.2

[1]C4 = warm-season forage (tallgrass prairie hay); C3 = cool-season forage (bluegrass straw); SBM = dehulled soybean meal.

[2]IADF = indigestible ADF; NFC = nonfibrous carbohydrates; WSC = water-soluble carbohydrates.

h daily (**CPSupp**). Steers were provided continuous access to fresh water and chopped (4 to 8 cm; BC-900, Newhouse Manufacturing, Redmond, OR) C3 or C4 hay from Exp. 1. Forage was provided daily (0710 h) at 120% of the average intake for the previous 5 d, with feed refusals from the previous day determined before the 0700 h supplement feeding. A trace mineralized salt mix (22 g/d; ≥96% NaCl, ≥0.20% Mn, ≥0.10% Fe, ≥0.10% Mg, ≥0.05% S, ≥0.025% Cu, ≥0.01% Co, ≥0.008% Zn, and ≥0.007% I) was placed directly into the rumen 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 (500,000, 50,000, and 1,500 IU of vitamins A, D, and E, respectively; Vitamin E-AD 300, AgriLabs, St. Joseph, MO).

Experimental periods were 20 d, with at least 3 d allowed between periods, when steers were removed from individual pens and placed in a common outdoor pen (22 × 34 m). Between periods, steers were provided ad libitum access to the low-quality meadow hay referenced in Exp. 1 with continuous access to water. Intake measurement began on d 14 and concluded on d 18. On d 15, treatment effects on ruminal DM and indigestible ADF (**IADF**) were determined by manually removing the contents from the reticulorumen from each steer 4 h after feeding. The total ruminal contents were weighed, mixed by hand, and subsampled (approximately 400 g) in triplicate. 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 (Arthur H. Thomas), and composited within period and steer.

Samples of forages and SBM were collected on d 14 through 18, whereas orts were collected on d 15 through 19. Feed and orts samples were 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 h). Bags were emptied once daily; feces were manually mixed; and a 2.5% subsample (wet weight) was obtained, weighed, dried for 96 h at 55°C, reweighed for DM, and composited by steer. Dried samples of hay, orts, and feces were ground as described above. Ground samples of forages and SBM were composited by period and daily orts were composited by steer (within period) on an equal-weight basis (5%, as fed). Feed, orts, and feces were analyzed for DM and OM (AOAC, 1990), N (Leco CN-2000, Leco Corp.), and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) by using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). Feed, orts, feces, and ruminal particulate samples were analyzed for IADF as described by Bohnert et al. (2002c; fecal recovery of IADF was 102 ± 4%). Digesta kinetics techniques, as 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.

On d 20, each steer was intraruminally pulse-dosed with 5 g of Co-EDTA in a 150-mL aqueous solution (Udén et al., 1980). 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 (Raun and Burroughs, 1962) immediately before dosing and at 3, 6, 9, 12, 18, and 24 h after dosing. Ruminal fluid pH was measured immediately after collection (Orion SA 520, American Instrument Exchange Inc., Haverhill, MA). Twenty milliliters of ruminal fluid 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 NH_3-N. Frozen (−20°C) ruminal samples were prepared for analysis by thawing, centrifuging (15,000 × *g* for 10 min at room temperature for VFA and NH_3-N, and 2,000 × *g* for 20 min at room temperature for Co), and collecting the supernatant. Cobalt 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 Harmon et al. (1985), and NH_3-N was analyzed by a modification (sodium salicylate substituted for phenol) of the procedure described by Broderick and Kang (1980) using a UV-visible spectrophotometer (UV Mini 1240, Shimadzu Scientific Instruments, Columbia, MD).

Exp. 3. Influence of CP Supplementation of C3 vs. C4 Forage on Efficiency of N Use by Lambs

Four wethers (38 ± 1 kg of BW) 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 and 2 (Table 1). Treatments were the same as described in Exp. 2; wethers were randomly allotted to treatments and housed in individual metabolism crates within an enclosed barn with continuous lighting. The quantity of supplemental CP provided (at 0700 h) was 0.19% of BW (CP basis). To minimize potential bias attributable to BW changes resulting from treatment regimens during each period, the quantity of supplement provided in each period was based on initial BW. Forage was provided at 120% of the previous 5-d average intake in 2 equal portions (0710 and 1700 h), with feed refusals from the previous day determined before supplement feeding at 0700 h. In addition, 35 g of a trace mineral salt mix (2.4% Ca, 2.3% P, 20.4% Na, 31.65% Cl, 0.2% K, 0.4% Mg, 0.1% S, 1,309 mg/kg of Mn, 2,046 mg/kg of Fe, 7 mg/kg of Cu, 1,930 mg/kg of Zn, 42 mg/kg of Co, 120 mg/kg of I, 16 mg/kg of Se, 1,325 IU/kg of vitamin E, and 552 and 50 kIU/kg of vitamins A and D, respec-

tively) was provided daily to each lamb at 0700 h. 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) was administered to each lamb at the onset of the trial to safeguard against deficiency.

Experimental periods were 20 d, with at least 3 d allowed between periods to remove wethers from metabolism crates. Intake was determined on d 14 through 18. In addition, samples of forages and SBM were collected on d 14 to 18, whereas orts were collected on d 15 to 19. Samples of feed (100 g) and orts (10% wet weight) were dried at 55°C for 48 h. On d 16 to 20, total fecal and urine output was collected. Urine was composited daily by wether (50% of total; weight basis) and stored (4°C). Sufficient 6 *N* HCl (approximately 25 mL) was added to urinals daily to maintain urine pH <5 to minimize bacterial growth and N loss. A subsample 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 a jugular vein 4 h after forage feeding by using a heparinized syringe (2 mL of 10,000 USP units/mL of heparin solution was drawn into a 10-mL syringe and then dispensed back into the vial; the remaining residue within the syringe resulted in a heparinized syringe). Blood samples were immediately transferred to Vacutainer tubes (catalog number 0268360, Fisher Scientific, Waltham, MA), placed on ice for transport to the laboratory, and centrifuged (5,000 × *g* for 15 min; 4°C), and plasma was harvested and stored (−20°C).

Dried samples were ground as described in Exp. 2. Samples of ground forages and SBM were composited by period, and daily orts were composited by lamb within period. Feed, orts, and fecal samples were analyzed for DM, OM (AOAC, 1990), and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) by using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). Feed, orts, fecal, and urine samples were analyzed for N using a Leco CN-2000 instrument (Leco Corp.). Plasma samples were assayed for urea-N following the manual procedure described by Marsh et al. (1965) by using a UV-visible spectrophotometer.

Statistical Analyses

Exp. 1. Kinetic variables for DM, NDF, and N digestibility were estimated with SAS software (SAS Inst., Inc., Cary NC) by using the modified nonlinear regression procedure described by Fadel (2004). Data were analyzed with steer as the experimental unit with the GLM procedure of SAS. The model included steer and forage type. Means were separated using LSD protected by a significant *F*-test ($P \leq 0.05$).

Exp. 2. Intake and digestibility data were analyzed as a 4 × 4 Latin square with 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 included the main effects comparing forage type (C3 vs. C4 forage) and supplementation (CPSupp vs. not supplemented). In addition, the interaction of main effects was evaluated (forage type × supplementation). 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 period, treatment, time, and treatment × time. In addition, steer was used to specify variation (using the RANDOM statement). Steer (period × treatment) was used as the SUBJECT and autoregression (i.e., AR1) was determined to be the most appropriate covariance structure based on the Akaike information criterion. The same contrasts noted above were used to partition the treatment sums of squares. If no treatment × time interactions were detected ($P > 0.10$), measurements were averaged and the treatment means were compared as described above.

Exp. 3. Data were analyzed as described in Exp. 2. Plasma urea-N was analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included period, treatment, day, and treatment × day. In addition, lamb was used to specify variation (using the RANDOM statement). Lamb (period × treatment) was used as the SUBJECT and autoregression (i.e., AR1) was used as the covariance structure based on the Akaike information criterion. The same contrasts noted above were used to partition treatment sums of squares.

RESULTS AND DISCUSSION

Exp. 1

The C3 and C4 forages had similar percentages (DM basis) of CP, NDF, ADF, and IADF (Table 1). However, the cool-season forage had greater concentrations of nonfibrous carbohydrates (17.0% vs. 9.3%) and water-soluble carbohydrates (14.1 vs. 8.8%) compared with the warm-season forage.

It is generally assumed that C3 forages are of greater nutritional quality than C4 forages (Caswell et al., 1973; Galyean and Goetsch, 1993; Barbehenn et al., 2004) at a similar phenological stage because of differences in the proportions and arrangements of tissues resulting from the different photosynthetic pathways used by C3 and C4 plants (Akin, 1989; Lambers et al., 1998). There have been studies comparing the ruminal degradation characteristics of moderate- to high-quality C3 and C4 forages (Mertens and Loften, 1980; Mitchell et al., 1997; Coblentz et al., 2004), but to our knowledge, this is the first study that directly compared the ruminal degradation of low-quality (≤6.5% CP) cool- and warm-season forages.

The A fraction (soluble fraction; total pool disappearing at a rate too rapid to measure) of DM and NDF was greater for C3 compared with C4 ($P < 0.01$),

with no differences in B (degradable fraction; total pool disappearing at a measurable rate) and C (undegradable fraction; total pool unavailable in the rumen) fractions or lag time ($P \geq 0.17$; Table 2). The greater A fraction for C3 is most likely a result of the greater nonfibrous carbohydrate and water-soluble carbohydrate concentrations (Table 1). Furthermore, the lack of differences in the B and C fractions as well as the lag time of DM and NDF support this assumption. Our lack of a difference in lag time for C3 and C4 contrasts with the work of Mertens and Loften (1980), in which the authors noted a decreased lag time with fescue and orchardgrass (C3 forages) compared with coastal bermudagrass (C4 forage). However, the forages used by Mertens and Loften (1980) ranged from 14 to 16% CP compared with the 6% CP forages used in our study. It is possible that differences in the species, maturity, and quality of forages used in the 2 studies may have resulted in different effects on lag time.

Both DM ($P = 0.09$) and NDF ($P = 0.12$) fractional rates of degradation tended to be greater for C3 than C4. In addition, the effective degradability values of DM (54 vs. 48%) and NDF (47 vs. 41%) were greater ($P < 0.01$) for C3 than C4. This agrees with numerous studies demonstrating that C3 forages have a greater extent of digestion than do C4 forages (Mertens and Loften, 1980; Reid et al., 1988; Galloway et al., 1991; Coblentz et al., 2004). However, to our knowledge, our study is the first that has directly compared ruminal degradation characteristics of low-quality C3 and C4 forages with similar concentrations of CP, NDF, and ADF.

All ruminal N degradation variables were influenced ($P < 0.01$), or tended to be influenced ($P \leq 0.11$), by forage type (Table 2). Similar to what was observed with DM and NDF, the A fraction for N was 36% greater ($P < 0.01$) for C3 than C4. However, the degradable N pool (B fraction) tended to be approximately 6% less ($P = 0.11$) for C3 than C4, whereas the C fraction was 79% greater ($P < 0.01$) for C4 than C3. These data, along with the tendency for the rate of N degradation to be greater for C3 than C4 ($P = 0.07$), resulted in an effective N degradability for C3 that was 10% greater than that observed for C4 ($P < 0.01$; 89.4 vs. 80.9%). These results are in contrast to those reported by Mathis et al. (2001), in which the authors noted no difference in the A, B, or C fractions or the rate of degradation in N pools across 3 warm-season forages (Bermuda grass, forage sorghum, tallgrass prairie) and 1 cool-season forage (brome). It is not clear why there

Table 2. Degradation parameters of a low-quality cool-season (C3) and a low-quality warm-season (C4) forage (Exp. 1)

Degradation parameter	C4	C3	SEM[1]	*P*-value
DM				
Fraction,[2] %				
A	20.89	24.20	0.277	<0.01
B	55.48	53.95	1.255	0.45
C	23.62	21.85	1.121	0.34
Lag time, h	3.80	3.27	0.440	0.47
K_d,[3] %/h	1.92	2.48	0.159	0.09
Effective degradability,[4] %	47.94	53.95	0.547	<0.01
NDF				
Fraction,[2] %				
A	6.98	11.55	0.358	<0.01
B	69.46	65.99	1.347	0.17
C	23.56	22.46	1.067	0.52
Lag time, h	4.76	4.20	0.458	0.46
K_d, %/h	1.88	2.42	0.175	0.12
Effective degradability,[4] %	41.04	47.03	0.665	<0.01
N				
Fraction,[2] % of total N				
A	31.36	42.70	1.144	<0.01
B	49.59	46.74	0.902	0.11
C	19.05	10.56	0.400	<0.01
K_d, %/h	4.07	6.86	6.47	0.07
Effective degradability,[4] %	80.93	89.43	0.401	<0.01
RDP,[5] % of CP	65.96	84.72	0.304	<0.01
RUP[6]	34.04	15.28	0.314	<0.01

[1]n = 4.

[2]A = soluble fraction (total pool disappearing at a rate too rapid to measure); B = degradable fraction (total pool disappearing at a measurable rate); C = undegradable fraction (total pool unavailable in the rumen).

[3]Fractional rate constant.

[4]Calculated as A + {B × [(K_d/(K_d + Kp)]}, where Kp was the ruminal passage rate, which was set at 2%/h (Hoffman et al., 1993). The units used for K_d in the equation were per hour.

[5]Calculated as described by Ørskov and McDonald (1979).

[6]Calculated as 1 − RDP.

Table 3. Nutrient intake and digestibility by steers consuming low-quality cool-season (C3) and warm-season (C4) grass hay with or without soybean meal (CP) supplementation (Exp. 2)

	Treatment					*P*-value[2]		
Item	C4	C4 + CP	C3	C3 + CP	SEM[1]	CP vs. no CP	C4 vs. C3	CPSupp × type
DMI, g/kg of 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
OM intake, g/kg of BW								
Forage	14.7	21.4	21.6	23.1	0.6	<0.01	<0.01	<0.01
Soybean meal	0.0	1.6	0.0	1.6				
Total	14.7	23.0	21.6	24.7	0.61	<0.01	<0.01	<0.01
N intake, g/kg of BW	0.147	0.356	0.228	0.385	0.007	<0.01	<0.01	<0.01
NDF intake, g/kg of BW	10.8	16.0	15.6	16.9	0.5	<0.01	<0.01	<0.01
Apparent digestibility, %								
DM	42.8	51.8	49.7	54.2	0.9	<0.01	<0.01	0.05
OM	45.6	54.6	53.6	58.5	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

[1]n = 4.
[2]CPSupp = CP supplementation; type = forage type.

was a differential response between our study and that of Mathis et al. (2001); however, differences in the species of forages compared, or the samples obtained, may be partially responsible. For example, it is worth noting that our results for the A, B, and C fractions of the N pool for the only forage common to both studies, tallgrass prairie, yielded results comparable with those of Mathis et al. (2001; 31.4 vs. 32.2, 49.6 vs. 50.4, and 19.0 vs. 17.5, respectively). Consequently, ruminal degradation characteristics may not be consistent across all C3 and C4 forages.

The RDP content of C3 was 28% greater ($P < 0.01$) and the RUP was 55% less ($P < 0.01$), as a percentage of total CP, than the RDP content of C4 (Table 2). This agrees with other data indicating that cool-season forages have a greater proportion of CP as RDP and less as RUP than do warm-season forages (Mullahey et al., 1992; Mitchell et al., 1997; Coblentz et al., 2004). These data increase the body of work suggesting that cool-season forages provide more rumen-available N for fermentation and microbial protein production than do warm-season forages.

Exp. 2

Intake and Digestibility. We noted CPSupp × forage interactions ($P < 0.01$) for forage and total DM and OM intake, N intake, and NDF intake by steers (Table 3). In each instance, the C4 forage had decreased overall intake, and intake increased more with CPSupp than with the C3 forage (Table 3). As an example, overall forage DMI was 19.2 g/kg of BW for steers consuming unsupplemented C4 compared with 24.5 g/kg of BW for steers consuming unsupplemented C3. In addition, the difference in forage DMI between unsupplemented and CPSupp C3 was 23.7 and 25.3 g/kg of BW, respectively (7% increase). In contrast, forage DMI was 15.6 and 22.9 g/kg of BW for unsupplemented and CPSupp C4, respectively, a 47% increase.

It is generally believed that CPSupp of low-quality forage (<7% CP) increases forage intake (Paterson et al., 1994; Moore and Kunkle, 1995; Mathis, 2003). This assumption is 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 studies with CPSupp of low-quality C3 forages (Mathis et al., 2000; Bohnert et al., 2002a,b; Currier et al., 2004). Our data suggest the forage intake response to CPSupp in ruminants consuming low-quality forage is dependent on forage type. A probable explanation for this is that forage intake with low-quality C3 forages is often maximized without CPSupp. For example, greater forage intake is routinely observed (forage DMI >1.7% of BW) in almost all studies with low-quality C3 forages, resulting in no increase in forage intake attributable to CPSupp (Mathis et al., 2000; Bohnert et al., 2002a,b; Currier et al., 2004). In contrast, intake of low-quality C4 forages is almost never maximized without CPSupp (routinely less than 1% of BW) and has been shown to increase with CPSupp from 30 to 100% compared with unsupplemented controls (DelCurto et al., 1990; Köster et al., 1996; Mathis et al., 1999).

A potential reason for the differences noted above in forage intake with CPSupp of C3 and C4 forages may be related to NDF intake. Mertens (1985, 1994) suggested that DMI is maximized when NDF intake is approximately 12.5 g·kg of $BW^{-1}\cdot d^{-1}$. Our data and other research seem to support this hypothesis. In the current study, NDF intake of the unsupplemented C4 was below 12.5 g·kg of $BW^{-1}\cdot d^{-1}$ (10.8 g·kg of $BW^{-1}\cdot d^{-1}$) and increased to 16.0 g·kg of $BW^{-1}\cdot d^{-1}$ with CPSupp (result-

ing in an almost 50% increase in forage intake), whereas NDF intake was already above 12.5 g·kg of $BW^{-1}·d^{-1}$, and we noted a minimal (less than 7%) change in forage intake with CPSupp for the C3 forage. Our results with the C3 forage are comparable with those noted by Bohnert et al. (2002a,b), in which NDF intake by unsupplemented steers and lambs consuming C3 forage was above 12.5 g·kg of $BW^{-1}·d^{-1}$ (13.9 and 13.0, respectively) and averaged 15.1 and 13.8 g·kg of $BW^{-1}·d^{-1}$ in steers and lambs provided CPSupp, respectively, with no statistical difference in forage intake attributable to CPSupp. Likewise, other studies (Galloway et al., 1991; Mathis et al., 2000) have noted that NDF intake was above 12.5 g·kg of $BW^{-1}·d^{-1}$ for unsupplemented ruminants consuming C3 forage and did not increase forage intake with CPSupp. In contrast to these results, NDF intake in unsupplemented ruminants consuming C4 forages was less than 12.5 g·kg of $BW^{-1}·d^{-1}$ in the studies by DelCurto et al. (1990), Köster et al. (1996), and Bandyk et al. (2001; 6.4, 5.1, and 8.2 g·kg of $BW^{-1}·d^{-1}$, respectively) and increased to 14.3, 11.3, and 13.3 g·kg of $BW^{-1}·d^{-1}$, respectively, with supplementation. Each of these studies reported a statistical increase in intake of C4 forages attributable to CPSupp.

Another possible explanation for the difference in forage intake response with CPSupp of C3 and C4 forages is OM intake. Moore et al. (1999), in a thorough review of the effects of supplementation on voluntary forage intake, suggested that when forage OM intake is greater than 1.75% of BW, it should not be expected to increase further with supplementation. In the current study and in the studies by Mathis et al. (2000) and Bohnert et al. (2002a,b), forage OM intake was greater than 17.5 g·kg of $BW^{-1}·d^{-1}$ for unsupplemented ruminants consuming C3 with no increase in forage intake noted with CPSupp. In addition, Mathis et al. (2000) reported that when forage OM intake was greater than 17.5 g·kg of $BW^{-1}·d^{-1}$ for steers consuming bermudagrass hay (C4), no increase in forage intake was observed with CPSupp. Unsupplemented C4 forage OM intake in the current study was 14.7 g·kg of $BW^{-1}·d^{-1}$ and increased to 21.4 g·kg of $BW^{-1}·d^{-1}$ with CPSupp. This agrees with most other studies using low-quality C4 forages, in which forage OM intake was less than 17.5 g·kg of $BW^{-1}·d^{-1}$ without supplementation and increased with CPSupp (Lintzenich et al., 1995; Köster et al., 1996; Mathis et al., 2000). It should be noted that studies have been conducted with ruminants in which forage OM intake less than 1.75% resulted in no increase with CPSupp (Horney et al., 1996; Currier et al., 2004). Nevertheless, based on the forage NDF and OM intakes observed in the current experiment, it is not surprising that differences in forage intake were noted between C4 and C3. The available data suggest that low-quality C3 forages are consumed in greater amounts (BW basis) than are low-quality C4 forages. As a result, CPSupp of low-quality C3 forages often results in little or no increase in forage intake, whereas CPSupp of C4 forages routinely results in a 30 to 100% increase in forage intake.

Apparent digestibility of DM and OM responded similarly to intake, with CPSupp × forage type interactions ($P = 0.05$; Table 3). Dry matter digestibility averaged approximately 47 and 52% for C4 and C3, respectively, and increased by 21 and 9%, respectively, with CPSupp. Likewise, OM digestibility averaged 50 and 56% and increased 20 and 9% with CPSupp for C4 and C3, respectively. Neutral detergent fiber digestibility tended ($P = 0.07$) to be greater for C3 compared with C4 forage, whereas N and NDF apparent digestibility increased with CPSupp ($P < 0.03$).

Apparent diet digestibility values of low-quality C3 (Horney et al., 1996; Weder et al., 1999; Bohnert et al., 2002a,b) and C4 (DelCurto et al., 1990; Köster et al., 1996; Mathis et al., 1999) forages have been reported to increase with CPSupp. In addition, most studies have noted that C3 forages are more digestible than C4 forages (Mertens and Loften, 1980; Reid et al., 1988; Galloway et al., 1991; Coblentz et al., 2004). Our data support this observation, with DM and NDF apparent digestibility averaging almost 6 percentage points greater for C3 than C4. In addition, this agrees with the increase in effective DM and NDF degradability values noted for C3 compared with C4 in Exp. 1. We are aware of no additional data that has directly compared the in vivo digestibility of low-quality C3 and C4 forages; nevertheless, Foster et al. (1996) noted that NDF and ADF in vitro digestibility values of C3 forages were greater than those for C4 forages sampled at the same time throughout the year. The greater degradability of C3 compared with C4 has been suggested to be related to differences in proportions and arrangements of tissues, such as the degradability of mesophyll cells and parenchyma bundle sheath cells (Akin, 1989; Galyean and Goetsch, 1993). In addition, diet digestibility is positively correlated with voluntary DMI (Minson and Wilson, 1994), which corroborates our data. Consequently, the increased diet digestibility frequently noted with C3 forages most likely contributes to the greater forage intake often observed with C3 compared with C4 forages.

Ruminal Fermentation. Treatment × time interactions ($P \leq 0.02$) were noted for ruminal NH_3-N and molar proportions of acetate, propionate, and acetate:propionate. However, after considering the nature of the interactions, we concluded that discussing treatment means would facilitate interpretation and discussion of the data while still providing an effective understanding of the overall treatment effects. Ruminal NH_3-N for the unsupplemented treatments averaged 0.64 and 0.52 m*M* for C4 and C3, respectively. Previous research has suggested that the ruminal NH_3-N concentration required for maximal growth of rumen microbes ranges from 1.18 to 2.94 m*M* for in vivo fermentation (Slyter et al., 1979) and 2.94 m*M* for in vitro fermentation (Satter and Slyter, 1974). We can assume, based

on these data, that ruminal NH_3-N was limiting for maximal ruminal fermentation in the unsupplemented treatments. In response to supplementation, we noted a CPSupp × forage interaction ($P = 0.02$; Table 4), in which supplemental SBM increased average ruminal NH_3-N 134% with the C4 forage and 335% with the C3 forage. This supports the majority of past research with low-quality forages, which has consistently demonstrated increased ruminal NH_3-N with CPSupp (Köster et al., 1996; Mathis et al., 1999; Bohnert et al., 2002c). In addition, given the greater proportion of forage CP as RDP for C3 (Table 2), it is probable that ruminal NH_3-N production was greater with C3 than C4, especially given the observed differences in DMI, which should potentially yield greater ruminal NH_3-N concentrations with C3.

Ruminal pH was not affected by CPSupp ($P = 0.23$; Table 4); however, C3 did have lower average pH compared with C4 ($P = 0.04$; 6.52 vs. 6.65). Ruminal pH never declined below 6.3 (data not shown) for all treatments and sampling times. This is within the range considered adequate to maintain fiber digestion and support the growth of cellulolytic bacteria provided other nutrients are present in adequate amounts (Yokoyama and Johnson, 1988). No CPSupp × forage type interaction was noted for ruminal pH ($P = 0.46$).

Total VFA were greater with CPSupp ($P = 0.03$; 79.4 vs. 71.1 m*M*; Table 4), which was expected because the quantity of supplement provided increased the fermentable substrate available to the ruminal microflora. Molar proportions of the branched-chain VFA isovalerate ($P = 0.01$) and valerate ($P = 0.01$) increased and isobutyrate tended to increase ($P = 0.10$) with CPSupp. This was anticipated because branch-chain VFA arise from fermentation of the branched-chain amino acids present in SBM (Leng, 1973). The molar proportion of acetate was less with C3 than C4 ($P < 0.01$), whereas proportions of propionate, butyrate, isovalerate, and valerate were greater ($P < 0.01$). In addition, with the lesser acetate and greater propionate, the acetate:propionate was less with C3 than C4 ($P < 0.01$; 3.9 vs. 5.4), suggesting greater energetic efficiency with the C3 forage.

Ruminal fluid and particulate dynamics were affected by forage type and supplemental CP (Table 4). Ruminal liquid volume was less ($P < 0.01$) for C4 than C3 (234 and 311 mL/kg of BW, respectively; Table 4) and was not affected by CPSupp ($P = 0.28$), whereas liquid dilution rate increased with CPSupp ($P = 0.03$) and for C3 compared with C4 ($P < 0.01$). A CPSupp × forage interaction ($P = 0.02$) was noted for liquid retention time, with CPSupp decreasing retention time by 24% (15.3 to 11.7 h) with the C4 forage, whereas little change was noted with C3.

A CPSupp × forage interaction was also noted for IADF intake ($P < 0.01$; Table 4), with intake increasing by 45% (2.9 to 4.2 g/kg of BW) with CPSupp of C4 forage, whereas intake of C3 increased by only 7% (4.4 to 4.7 g/kg of BW). In addition, we observed a CPSupp × forage interaction for IADF passage rate ($P = 0.02$); C4 averaged 1.6%/h and C3 averaged 2.0%/h with CPSupp, increasing passage rates by 46 and 10% for C4 and C3, respectively. Likewise, ruminal outflow

Table 4. Ruminal fermentation dynamics of steers consuming low-quality cool-season (C3) and warm-season (C4) grass hay with or without soybean meal (CP) supplementation (Exp. 2)

	Treatment					*P*-value[2]		
Item	C4	C4 + CP	C3	C3 + CP	SEM[1]	CPSupp vs. no CP	C4 vs. C3	CPSupp × type
NH_3-N, m*M*	0.64	1.50	0.52	2.26	0.13	<0.01	0.05	0.02
pH	6.70	6.60	6.54	6.51	0.05	0.23	0.04	0.46
Total VFA, m*M*	66.8	78.0	75.4	80.7	3.0	0.03	0.11	0.37
VFA, mol/100 mol								
Acetate	76.9	76.4	71.2	70.0	0.5	0.11	<0.01	0.54
Propionate	14.2	14.5	18.0	18.4	0.3	0.22	<0.01	0.82
Isobutyrate	0.49	0.55	0.55	0.61	0.03	0.10	0.13	0.97
Butyrate	7.8	7.7	9.3	9.7	0.31	0.56	<0.01	0.48
Isovalerate	0.34	0.48	0.42	0.56	0.04	0.01	0.08	0.99
Valerate	0.30	0.40	0.61	0.72	0.03	0.01	<0.01	0.88
Acetate:propionate ratio	5.5	5.3	4.0	3.8	0.1	0.16	<0.01	0.95
Ruminal liquid								
Volume, mL/kg of 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[3]								
IADF intake, g/kg of BW	2.9	4.2	4.4	4.7	0.1	<0.01	<0.01	<0.01
Fill, g/kg of 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
Outflow, g·kg of BW^{-1}·h^{-1}	0.119	0.176	0.184	0.196	0.006	<0.01	<0.01	<0.01

[1]n = 4.

[2]CPSupp = CP supplementation; type = forage type.

[3]IADF = indigestible ADF.

of IADF increased by 48% with CPSupp of C4, whereas IADF outflow increased by 6% with C3 (CPSupp × forage interaction; $P < 0.01$). Nevertheless, ruminal IADF fill was not affected by CPSupp or forage type ($P > 0.54$). This supports the theory that, with low-quality diets, ruminants consume feed to an amount that matches the capacity of the gastrointestinal tract to accommodate digesta (Mertens, 1994).

Exp. 3

Forage and total DMI by lambs tended ($P = 0.06$) to be greater for C3 than C4 forage (Table 5), with total DMI increasing with CPSupp ($P < 0.01$). In addition, total OM intake was greater with CPSupp ($P < 0.01$). It is worth noting that there tended to be CPSupp × forage type interactions for both forage and total DMI ($P = 0.11$) and forage and total OM intake ($P = 0.09$), similar to differences observed in Exp. 2. However, 2 principal differences between forage intake in Exp. 2 and that observed in Exp. 3 are worth mentioning. All treatments in the current experiment resulted in NDF intakes greater than 12.5 g/kg of BW (ranging from 17.8 to 20.0 g/kg) and OM intakes greater than 1.75% of BW (ranging from 2.40 to 2.67%). Consequently, as noted in Exp. 2, we did not anticipate a forage intake response to CPSupp on the basis of observed NDF (Mertens, 1985, 1994) and OM (Moore et al., 1999) intakes. Our statistical design did not allow for the direct comparison of forage intake between steers and lambs (Exp. 2 and 3); nevertheless, it is not clear what caused the lambs consuming unsupplemented C4 to have proportionally greater intakes of NDF and OM and, consequently, no response to CPSupp compared with steers on the same treatment in Exp. 2. It is interesting that cattle are normally expected to have greater intakes, as a proportion of BW, than sheep (Rees and Little, 1980; Reid et al., 1988, 1990); however, this did not appear to occur in the present study. Nitrogen intake increased with CPSupp ($P < 0.01$; Table 5) and was greater for C3 than C4 forage ($P = 0.01$) because of a greater intake and greater CP concentration in the C3 forage (6.3 vs. 5.7%; Table 1).

Apparent total tract DM and OM digestibility had CPSupp × forage interactions ($P \leq 0.01$; Table 5). Supplementation increased DM and OM digestibility values for both forages; however, both DM and OM digestibility values increased by 18% with CPSupp of C4 forage compared with approximately 7% for C3. This is comparable with the results reported in Exp. 2. Apparent NDF digestibility tended ($P = 0.09$) to be greater with C3 than C4, which, again, is comparable with the results of Exp. 2. Similarly, apparent total tract N digestibility increased with CPSupp ($P < 0.01$) and was

Table 5. Nutrient intake, diet digestibility, and N balance of lambs consuming low-quality cool-season (C3) and warm-season (C4) grass hay with or without soybean meal (CP) supplementation (Exp. 3)

	Treatment					*P*-value[2]		
Item	C4	C4 + CP	C3	C3 + CP	SEM[1]	CPSupp vs. no CP	C4 vs. C3	CPSupp × type
DMI, g/kg of 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	0.89	<0.01	0.06	0.11
OM intake, g/kg of BW								
Forage	24.0	25.9	26.7	25.4	0.79	0.70	0.22	0.09
Soybean meal	0.0	3.4	0.0	3.4				
Total	24.0	29.3	26.7	28.7	0.79	<0.01	0.22	0.09
NDF intake, g/kg of BW	17.8	19.7	20.0	19.6	0.59	0.25	0.13	0.09
N intake, g/kg of BW	0.246	0.558	0.288	0.577	0.008	<0.01	0.01	0.21
Apparent digestibility,[3] %								
DM	44.7	52.8	48.9	52.4	0.54	<0.01	0.01	0.01
OM	46.7	55.0	52.4	56.4	0.40	<0.01	<0.01	<0.01
NDF	46.7	50.0	50.4	51.2	1.2	0.14	0.09	0.33
ADF	41.7	44.6	46.0	46.5	1.7	0.36	0.12	0.51
N	35.3	65.2	36.5	63.0	1.2	<0.01	0.68	0.20
N excretion, g/kg of BW								
Fecal	0.159	0.195	0.183	0.214	0.007	<0.01	0.02	0.72
Urinary	0.065	0.221	0.080	0.261	0.017	<0.01	0.15	0.50
N balance, g/kg of BW	0.022	0.143	0.025	0.102	0.019	<0.01	0.35	0.30
Digested N retained,[4] %	23.4	39.2	23.2	27.9	9.6	0.33	0.57	0.59
Plasma urea-N, m*M*	2.2	4.9	3.0	6.6	0.28	<0.01	<0.01	0.14

[1]n = 4.

[2]CPSupp = CP supplementation; type = forage type.

[3]Apparent total tract digestibility.

[4]Calculated as [daily N retention (g/kg of BW)/daily N digested (g/kg of BW)] × 100.

not affected by forage type ($P = 0.68$). No differences were noted for apparent total tract ADF digestibility ($P \geq 0.12$).

Fecal and urinary N excretion, as well as N balance, increased ($P < 0.01$; Table 5) with CPSupp, whereas fecal N excretion was greater for C3 than C4 ($P = 0.02$). No difference was noted for digested N retained in response to CPSupp or forage type ($P \geq 0.32$). Plasma urea-N was greater with CPSupp ($P < 0.01$; 5.8 vs. 2.6 m*M*) and for C3 compared with C4 ($P < 0.01$; 4.8 vs. 3.6 m*M*). Plasma urea-N responded in the same manner as N intake, which supports the contention that plasma urea concentration is positively correlated with N intake (Harmeyer and Martens, 1980).

Conclusions

The results of these experiments indicate that intake and digestibility of C3 and C4 forages in the current study were not similar and, more important, that the physiological response of ruminants to supplemental protein may depend, in part, on the cell wall structure of the basal diet, with intake and digestibility of C4 forages increasing to a greater extent with supplementation compared with C3 forages of similar nutritional quality. In addition, on the basis of our work and other published data, the intake and digestibility of C3 forages appear to be greater than the intake and digestibility of C4 forages with comparable nutritional indices (e.g., CP, ADF, NDF). Therefore, further research comparing additional species of low-quality C3 and C4 forages is warranted to confirm that the observed digestive responses in our study are reflective of a broad range of low-quality C3 and C4 forages.

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