Effects of supplemental protein type on intake, nitrogen balance, and site, and extent of digestion in whiteface wethers consuming low-quality grass hay[1]

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ABSTRACT: Two experiments were conducted to determine the effects of supplementing ruminally degradable intake protein (DIP) or ruminally undegradable intake protein (UIP) on N balance (Exp. 1; n = 6 wethers; initial BW = 48.7 ± 4.6 kg) and site and extent of digestion (Exp. 2; n = 5 wethers; initial BW = 36.9 ± 3.1 kg) in whiteface wethers consuming (as-fed basis) 69% blue grama and 31% love grass hay (mixture = 7.5% CP, 73.0% NDF, 36.0% ADF [DM basis]). Treatments were 1) no supplement (Control), 2) a supplement (219 g/d, as-fed basis) low in UIP (70 g/d of CP; 24.8 g/d of UIP), and 3) a supplement (219 g/d, as-fed basis) high in UIP (70 g/d of CP; 37.1 g/d of UIP). Both experiments were replicated 3 × 3 Latin square designs, with identical feeding and supplementation. Wethers had ad libitum access to the forage mixture and fresh water, and received supplement once daily. In Exp.1, forage intake (percentage of BW) was greatest (P = 0.04) for control, but total DMI (g/d) was greatest (P = 0.05) for lambs consuming supplement. Apparent total-tract OM digestibility was numerically greater (P = 0.11) for supplemented wethers than for controls, whereas total-tract ADF digestibility tended (P = 0.08) to be greater for control wethers. Lambs fed supplements consumed and retained more ($P \leq 0.01$) N (% of N intake) compared with controls, but no difference (P = 0.22) was observed between low and high UIP treatments. Similar to Exp. 1, forage intake (percentage of BW) tended (P = 0.06) to be greater for control than for supplemented wethers in Exp. 2. Ruminal NDF digestibility was 16.3% greater (P = 0.02) for supplemented wethers than for controls. Postruminal NDF and N digestibilities were greatest ($P \leq 0.03$) for controls, but apparent OM digestibility did not differ among treatments at all sites. Duodenal N flow was greatest (P = 0.05) for high UIP and least for control wethers. Nonmicrobial N flow was greater (P = 0.02) for high UIP compared with low UIP or controls. Control wethers had greater (P = 0.05) microbial efficiency. Ruminal ammonia concentration tended (P = 0.08) to be greatest for wethers fed low UIP and least for controls, with high-UIP wethers having intermediate ammonia concentrations. Results from these experiments suggest that in lambs fed low-quality forage there was no difference in apparent total-tract digestion or N balance (percentage of N intake) between lambs fed supplements that had the same CP but differed in the proportion of UIP and DIP; however, supplementing protein (regardless of UIP:DIP ratio) to wethers consuming low-quality forage increased N balance.

Key Words: Digestibility, Microbial Efficiency, Protein, Sheep

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Introduction

Protein-deficient forages for growing lambs (NRC, 1985) are commonly grazed in New Mexico and many other regions throughout the Great Plains. Grazed forages deficient in CP are generally supplemented with high-protein supplements to meet CP requirements (Knox, 1967) and improve intake and digestibility (Kartchner, 1981; Owens et al., 1991). More recent experiments have been conducted to determine the appropriate type (e.g., ruminally degradable intake protein **[DIP]**, ruminally undegradable intake protein **[UIP]**, or nonprotein N) of supplemental protein to use for

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ruminants fed low-quality forages. For example, Bohnert et al. (2002b) determined the influence of CP degradability on efficiency of N utilization in wethers fed low-quality (5% CP) meadow hay. Dry matter and OM intakes, N retention and digestibility, and digested N retained were greater for supplemented wethers than controls, although no difference was observed due to ruminal degradability of CP. Swanson et al. (2004) reported that infusing a portion of casein into the abomasum compared with infusing 100% of the casein into the rumen of wethers consuming low-quality forage increased N retention and the efficiency of protein utilization, with no effect on total-tract digestibility. Although several experiments have reported apparent digestibility of nutrients in the total-tract, few experiments have evaluated site and extent of digestion and microbial efficiency in ruminants supplemented with protein sources differing in ruminal degradability. Therefore, two experiments were conducted to investigate the effects of feeding two supplements with similar CP but differing in the proportion of UIP on intake, N balance, and site and extent of digestion in wethers consuming 69% blue grama grass (*Bouteloua gracilis*) and 31% love grass (*Eragrostis curvula*) hay.

Materials and Methods

All procedures contained in this manuscript were approved by and conducted in accordance with the Institutional Animal Care and Use Committee of New Mexico State University.

Experiment 1

Six 8-mo old whiteface (Rambouillet × Columbia × Debouillet) wethers (initial BW = 48.7 ± 4.6 kg) were used to determine the effect of supplemental protein type on forage intake and N balance. Wethers were assigned randomly to one of three treatments in a replicated 3 × 3 Latin square design. Wethers were housed in metabolism crates (0.6 × 1.6 m) in the Nutrition and Physiology Building (21°C, 12 h light/d) on the main campus of New Mexico State University (Las Cruces). Treatments consisted of 1) forage only (control), 2) forage plus a supplement high in DIP and low in UIP (24.8 g/d of UIP), and 3) forage plus a supplement low in DIP and high in UIP (37.1 g/d of UIP). Supplements were the same in CP but differed in the amount of UIP and DIP (Table 1). The low-UIP supplement was formulated to represent supplements commonly used in range operations in the western United States (primarily oilseed meal). The high-UIP supplement was formulated to contain similar CP and energy, but the amount of UIP supplied by the supplement was increased by adding hydrolyzed feather meal and blood meal in place of soybean meal. Forage used in this experiment was a mixture (as-fed basis) of blue grama (69%; *B. gracilis*) and love grass (31%; *E. curvula*) hay. The mixture contained (DM basis) 7.5% CP, 73% NDF, and 2.07 Mcal/kg ME (NRC 1996), which is similar to forage grazed by sheep during the dormant (October through March) season in central New Mexico (Salisbury et al., 2000). Individual hays were kept separate until mixed for each wether before daily feeding. Forage was chopped (Bear Cat 5A, Western Bear Cat, Hastings, NE) and hand mixed for each wether daily to prevent sorting. Forage samples were taken from each bale after the hay was chopped. Forage was fed daily at 0800 at 115% of that consumed the previous 24 h so that each wether had ad libitum access, and refusals were weighed daily. Supplements were fed at 1700 daily at a rate of 219 g/d, which provided 70 g/d of CP and approximately 0.60 Mcal of ME. The recommended requirement for 50-kg medium mature weight lambs (similar weight to wethers used in the present experiment) to gain 100 g/d is 139 g of CP/d (NRC, 1985). Therefore, for supplemented wethers, 69 g/d of CP from forage would be required to meet the recommended NRC (1985) requirement.

Each period was 14 d long, which comprised a 7-d adaptation to treatments followed by 4 d of total fecal and urine collection. The wethers were removed from crates and provided forage only during the final 3 d of each period. Forage intake was measured from d 8 through 11 of each period. During a 4-d collection period (d 8 through 11), total fecal output was measured daily, and a 10% subsample was collected and frozen. Total

Table 1. Ingredient and nutrient composition of protein supplements used in both experiments

	Treatment[a]	
Item	Low UIP	High UIP
Ingredient, % of DM		
Corn (fine ground)	14.62	43.66
Hydrolyzed feather meal	0.0	31.08
Soybean meal, 49%	79.81	14.36
Blood meal (flash dry)	0.0	5.09
Potassium	0.0	1.1
Calcium carbonate	0.85	0.0
Ferrous sulfate, 30%	0.407	0.407
Cobalt carbonate, 46%	0.006	0.003
Manganese sulfate	0.10	0.10
Vitamin A	0.007	0.003
Carmel-molasses blend	4.2	4.2
Nutrient composition[b]		
ME, Mcal/kg	2.9	2.6
CP, %	32.0	32.0
DIP, % of CP	64.6	47.0
UIP, % of CP	35.4	53.0
NDF, %	40.21	36.63
ADF, %	5.28	11.14
Ca, %	0.55	0.31
P, %	0.55	0.33
K, %	2.13	1.20
Vitamin A, IU/kg	4,000	4,000

[a]Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP); High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP).
[b]Nutrient values (DM basis) represent calculated values (NRC, 1996) except CP, NDF, and ADF, which were analyzed.

urine was collected in a sealed-top container using a funnel and drainage hose. Each urine collection container contained 50 mL of 6 *N* HCl to bring the pH below 3.5 to stabilize urinary ammonia. Urine was quantified daily, and a 10% subsample was collected and frozen (–20°C). Urine and fecal samples were composited by animal within period, so that there was one urine and one fecal sample for each animal for every period.

Forage and orts samples were composited by animal within period, mixed by hand, and subsampled for analysis to yield one forage and one orts sample per wether and period. Fecal samples were lyophilized and ground in a Wiley mill to pass a 1-mm screen. Forage, orts, and fecal N was determined by the micro-Kjehldahl procedure (AOAC, 1990), and ADF was determined by the procedure described by Goering and Van Soest (1970). Urinary N was determined by the micro-Kjehldahl procedure using a 1-mL sample of urine.

Nitrogen retention was calculated as the total N intake (g/d) minus the sum (g/d) of fecal and urinary N excreted. Nitrogen retention was also expressed as a percentage of N consumed. Apparent total-tract digestibility was calculated using OM, ADF, and N intakes and OM, ADF, and N excreted in the feces.

Experiment 2

Six 8-mo-old whiteface (Rambouillet × Columbia × Debouillet) wethers (initial BW = 36.9 ± 3.1 kg) fitted with ruminal and duodenal cannulas were assigned randomly to one of the three treatments in a replicated 3 × 3 Latin square. One wether died and data for that wether were not included in the analyses; therefore, n = 5. Ruminal cannulas were inserted first followed by duodenal cannulas 14-d later. Duodenal cannulas were T-type cannulas, as recommended by Harmon and Richards (1997).

Forage and supplements were the same and were fed in the same manner as described for Exp. 1. Each period was 21 d and consisted of a 13 d adaptation to treatments followed by 2 d of intensive ruminal and duodenal sampling. Wethers were then allowed 1 d of rest, followed by 3 d of in situ sampling. Wethers were allowed 2 d of rest between periods, during which they received only forage. Wethers were housed outside from October to November in individual pens (1.4 × 3.6 m) with free access to clean fresh water and shade. At 0700 and 1700, a gelatin capsule containing 3.5 g of chromic oxide was placed directly in the rumen of each wether to facilitate estimating rate of duodenal flow and fecal output (Merchen, 1988). Chromic oxide dosing began 3 d before the initiation of the first period and continued every day, even during days between periods, until the end of the experiment, in an attempt to maintain equilibrium concentrations throughout the digestive tract.

Ruminal and duodenal contents were collected at 0, 4, 8, 12, 16, and 20 h during d 1 and at 2, 6, 10, 14, 18, and 22 h during d 2 to represent every 2 h of a 24-h period. Zero-hour sampling occurred immediately before feeding. At each collection time, approximately 100 g of whole ruminal contents was collected from the base of the fiber mat of each wether. The sample was placed in a plastic bag and immediately frozen (–20°C) until later analysis. Additionally, ruminal contents were strained through four layers of cheesecloth to obtain ruminal fluid. Ruminal fluid pH was immediately measured (HI 9024, Hanna Instruments SRL, Italy). Ten milliliters of ruminal fluid was placed in a 15-mL plastic conical centrifuge tube and frozen (–20°C) for VFA analysis. Another 10 mL was placed in a 15-mL plastic conical centrifuge tube containing 1 mL of 6 *N* HCl and frozen (–20°C) for subsequent ammonia analysis. At the same time, duodenal contents were collected by inserting a plastic tube (1.2 cm diameter × 16 cm long), with a 45° angle cut at the end, into the opening of the cannula to divert all contents to the outside. A 100-mL sterile plastic bag was attached to the end of the diverting tube. Duodenal contents were diverted to the bag until 100 mL was collected or for 20 min. Following collection, samples were immediately frozen (–20°C) until analyses. At the 4-, 12-, and 20-h collections for d 1, and the 2-, 10-, and 18-h collections for d 2, fecal grab samples were collected.

After all collections, fecal and duodenal samples were lyophilized, ground with a mortar and pestle, and composited by animal (equal weight basis) within period. Neutral detergent fiber (Goering and Van Soest, 1970), N (AOAC, 1990), and Cr (Galyean, 1997) concentrations were determined in fecal and duodenal samples. Acidified ruminal fluid samples were analyzed for ammonia concentrations using the phenol-hypochlorite method (Broderick and Kang-Meznarich, 1980). This procedure was adapted for a microtiter plate reader (Richards, 1999), in which the reaction steps of the assay were conducted at normal volumes and 200 μL of the product was pipetted into each well of a 96-well microtiter plate and read (630 nm, ELX808 ultra microplate reader; Bio-Tek Instruments, Inc., Winooski, VT). Ruminal fluid samples were analyzed for VFA concentrations using the procedures outlined by Galyean (1997). The 100-mL samples of whole ruminal contents were thawed and homogenized (model 1120 blender, Waring, New Hartford, CT) with an equal weight of physiological saline and strained through eight layers of cheesecloth. Samples were then pooled on an equal-volume basis to obtain a composite for each animal/period combination. Procedures for bacterial isolation and purine analysis were conducted according to Zinn and Owens (1986), with modifications (Klopfenstein et al., 2001). The bacterial pellet was also analyzed for N by the micro-Kjeldahl method (AOAC, 1990). Additionally, purine content was determined in the composited duodenal samples following the same procedures described above.

On d 17 of each experimental period, 5 g of the forage mixture (ground to pass a 1-mm screen and mixed following grinding) was placed in Dacron bags (5 × 10 cm, average pore size was 53 μm) for determination of in situ DM disappearance. Incubation times were 0, 2, 6,

Table 2. Intake and digestibility by whiteface wethers consuming low-quality forage and receiving no supplement or supplement with two different proportions of undegradable intake protein and degradable intake protein, Exp. 1[a]

	Treatments[a]					
Item	Control	Low UIP	High UIP	SEM[b]	C vs. S[c]	L vs. H[d]
Forage DMI, g/d	1,124	985	987	15.1	0.001	0.83
Forage DMI, % of BW	2.33	2.03	2.03	0.03	0.04	0.45
Total DMI, g/d	1,124	1,196	1,195	15.1	0.05	0.63
Total OMI, g/d	1,027	1,091	1,096	13.8	0.03	0.61
ADF intake, g/d	405	366	379	5.4	0.03	0.67
ME intake, Mcal/d	2.33	2.68	2.55	0.26	0.19	0.13
Total-tract digestibility, %						
DM	47.60	50.95	49.05	1.89	0.20	0.72
OM	50.95	54.27	54.44	2.26	0.11	0.77
ADF	47.12	37.49	40.21	3.65	0.08	0.27

[a]Treatments were Control = low-quality forage only; Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP); and High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP).
[b]Standard error of the least squares means, n = 6.
[c]*P*-value for Control vs. supplemented wethers.
[d]*P*-value for Low UIP vs. High UIP wethers.

10, 16, 24, 48, and 72 h. In situ bags were placed in small mesh bags (31 × 31 cm) and inserted into the rumen of each wether. Duplicate bags with a blank at each time were placed into the rumen in reverse order, so that all bags were removed at the same time. At removal time, the 0-h bags were introduced to the mesh bag and were rinsed with the others. For washing, mesh bags containing the in situ bags were placed in a plastic 19-L bucket of tap water. The bag was gently agitated for several minutes and then transferred to another bucket of clean water. This procedure was repeated until the bags went through three buckets of water in which the color of the water did not change. Individual in situ bags were then rinsed with low-pressure, low-volume tap water at a sink to work all the contents to the bottom of the bag. Bags were then placed on a plastic tray and dried in a forced-air oven at 50°C for 48 h. Residue was weighed, and lag time, rate, and extent of DM digestibility were calculated according to the procedures outlined by Wilkerson (1992).

Statistical Analyses

Experiment 1 was analyzed as a replicated 3 × 3 Latin square design. Animal was the experimental unit. All data collected were single-point collections, and data were analyzed using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). The model included treatment and period. Lamb was considered a random effect. Experiment 2 was analyzed as a replicated 3 × 3 Latin square design with one missing cell in each period. All intake, digestibility, and flow rate data were single-point samples with one missing animal (i.e., cell) in each period; data were analyzed using the Mixed procedure of SAS. The model included treatment and period. Lamb was considered a random effect. Data repeated over time (ruminal pH, ammonia, and VFA concentrations) were analyzed using the Mixed procedure of SAS. The model included treatment, period, time, and time × treatment interactions (Little et al., 1998). Lamb was considered a random effect and the lamb × period interaction was the subject. The covariance structure used was autoregressive 1. Contrast statements were used to separate treatment means with contrasts comparing control vs. supplements and low UIP vs. high UIP. Results were considered significant at $P < 0.05$, with tendencies considered at $P \leq 0.10$.

Results and Discussion

Feed Intake and Apparent Digestibility

In Exp. 1, forage intake (g/d and % of BW) was approximately 14% greater ($P \leq 0.04$) for control wethers than for supplemented wethers (Table 2). In contrast, total DMI (g/d) by supplemented wethers was 6.4% greater ($P = 0.05$) than for control wethers. In Exp. 2, numeric trends were similar to Exp. 1; forage intake (percentage of BW) tended ($P = 0.06$) to be greater for control wethers than for supplemented wethers (Table 3). In addition, wethers consuming high UIP tended ($P = 0.09$) to have greater forage intake (percentage of BW) than wethers fed low UIP. Similar to forage intake, intake of ADF was 8.6% greater ($P = 0.03$) for control wethers than for supplemented wethers in Exp. 1 (Table 2). Intake of NDF did not differ ($P = 0.16$) between supplemented and control wethers, or between supplements ($P = 0.14$) in Exp. 2 (Table 3).

Intake of low-quality forage often increases with protein supplementation (McCollum and Galyean, 1985; Beaty et al., 1994; Krehbiel et al., 1998). However, others (Ferrell et al., 1999; Swanson et al., 2000; Bohnert et al., 2002a) have reported no difference in forage intake when ruminants fed low-quality forages were supple-

Table 3. Feed intake, duodenal flow, fecal output, and digestibility in ruminally and duodenally cannulated wethers consuming low-quality forage and receiving no supplement or supplement with two different proportions of undegradable intake protein (UIP) and degradable intake protein, Exp. 2[a]

	Treatments[a]					
Item	Control	Low UIP	High UIP	SEM[b]	C vs. S[c]	L vs. H[d]
Forage DMI, g/d	816	653	753	51.9	0.12	0.19
Forage DMI, % of BW	2.23	1.77	2.05	0.12	0.06	0.09
Total DMI, g/d	816	883	983	51.9	0.08	0.19
Total OM intake, g/d	751	813	905	47.80	0.08	0.14
NDF intake, g/d	548	531	589	33	0.16	0.14
Duodenal flow, g/d						
OM	493.7	519.2	538.2	48.20	0.36	0.57
NDF	335.9	335.1	343.7	26.30	0.47	0.52
Ruminal digestibility, %						
OM	39.8	44.4	49.3	4.07	0.26	0.43
NDF	47.9	54.3	57.1	2.15	0.02	0.41
Fecal output, g/d						
OM	374.3	380.8	402.2	21.90	0.18	0.27
NDF	184.8	217.5	217.7	15.30	0.11	0.65
Postruminal digestibility, %						
OM	13.3	15.5	11.8	5.30	0.66	0.31
NDF	23.5	15.7	15.6	2.40	0.03	0.70
Postruminal digestibility, % of entering						
OM	20.4	28.0	21.3	8.96	0.60	0.46
NDF	45.4	34.2	35.1	5.12	0.10	0.32
Total-tract digestibility, %						
OM	50.2	53.2	55.6	3.82	0.35	0.24
NDF	66.3	59.0	63.0	1.98	0.12	0.21

[a]Treatments were Control = low-quality forage only; Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP); and High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP).
[b]Standard error of the least squares means, n = 5.
[c]*P*-value for Control vs. supplemented wethers.
[d]*P*-value for Low UIP vs. High UIP wethers.

mented with protein. Swanson et al. (2000) reported that forage intake by mature ewes fed a forage containing 6.7% CP did not increase in response to increasing supplemental UIP (5.2, 22.1, and 41.3% of supplement DM). They suggested that DIP from forage (49 g/d for control ewes) might have been adequate for maintaining ruminal fermentation, and therefore, no forage intake response resulted from additional DIP. This suggestion agrees with the present experiments with lambs fed 7.5% CP from forage; no increase in forage DMI was observed when wethers were supplemented with supplements equal in CP but differing in UIP levels. In addition, NDF intake by wethers consuming forage only in Exp. 2 was 14.9 g·kg BW^{-1}·d^{-1}. This is greater than the level of 12.5 g·kg BW^{-1}·d^{-1} (approximately 1.2% of BW) suggested as the maximal level of NDF intake, above which DMI will not be increased (Mertens, 1987; Ferrell et al., 1999; Bohnert et al., 2002a). Based on the summary by Mertens (1987) and data with ruminants consuming low-quality forages, Ferrell et al. (1999) suggested that when forage NDF intake without supplementation is relatively low (approximately 1.2% of BW or less), an intake response to supplementation may be expected, whereas when forage NDF intake without supplementation is relatively high (>1.2% of BW), then an intake response to supplementation is not likely to occur. In addition, Moore et al. (1999) suggested that when forage OM intake is greater than 1.75% of BW, forage OM intake should not be expected to increase with supplementation. Forage DM intake by control wethers was 2.33 and 2.23% of BW in the present Exp. 1 and 2, respectively, which is greater than intakes by ruminants grazing low-quality forages reported by others (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001), where protein supplementation increased forage DMI. The greater forage intake by control wethers and the greater total DMI for supplemented wethers suggest that DMI of forage was substituted by supplements in the present experiment.

In Exp. 1, apparent total-tract OM digestibility was numerically greater ($P = 0.11$) for supplemented wethers, whereas total-tract ADF ($P = 0.08$; Exp. 1) and NDF ($P = 0.12$; Exp. 2) digestibility were numerically greater for control wethers (Tables 2 and 3). Apparent total-tract digestibility of OM did not differ ($P = 0.35$ for control vs. supplemented wethers) among treatments in Exp. 2 (Table 3). Swanson et al. (2000) fed wethers low-quality forage (6.7% CP) supplemented with increasing UIP and reported no difference in apparent digestibility

Table 4. Nitrogen intake, duodenal flow and digestibility in ruminally and duodenally cannulated wethers consuming low-quality forage and receiving no supplement or supplement with two different proportions of undegradable intake protein and degradable intake protein, Exp. 2

	Treatments[a]					
Item	Control	Low UIP	High UIP	SEM[b]	C vs. S[c]	L vs. H[d]
N intake, g/d	9.6	22.1	23.2	0.61	0.0001	0.42
Duodenal N flow, g/d						
Total	15.8	18.1	20.6	1.4	0.05	0.36
Microbial	13.9	16.2	13.4	1.3	0.45	0.04
Non-microbial	1.9	1.9	7.2	1.1	0.05	0.02
Fecal N output, g/d	7.2	8.8	10.0	0.6	0.04	0.06
N digesitibility						
Ruminal, %	–48.0	23.6	17.2	6.61	0.02	0.52
Postruminal, %	79.8[d]	39.5[e]	41.3[e]	8.36	0.002	0.48
Postruminal, % of entering	52.5	50.3	50.1	3.49	0.64	0.62
Total-tract, %	25.0	63.2	58.5	3.35	0.001	0.32
Microbial efficiency, g/kg of OM apparently fermented	11.7	10.4	10.2	0.6	0.05	0.29

[a]Treatments were Control = low-quality forage only; Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP); and High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP).
[b]Standard error of the least squares means, n = 5.
[c]*P*-value for Control vs. supplemented wethers.
[d]*P*-value for Low UIP vs. High UIP wethers.

of DM, NDF, and ADF in one trial, but increasing digestibility of DM and OM in a second trial; NDF and ADF digestibility were not affected in the second trial. Others (Bandyk et al., 2001; Swanson et al., 2004) have reported increased apparent OM digestibility with casein infusion compared with no supplemental protein, but no difference between ruminal or abomasal infusion, suggesting that apparent total-tract digestion is not affected by site (or source) of protein digestion. However, Bohnert et al. (2002b) reported increased total-tract digestibility of NDF when UIP rather than DIP was supplemented.

Few experiments have reported the effect of supplemental protein type on site and extent of digestion in ruminants fed low- to moderate-quality forage. In Exp. 2, supplemental protein or protein type did not affect lag time (average = 4.58 h, $P = 0.36$), rate (average = 2.97%/h, $P = 0.27$), or extent (average = 63.1%, $P = 0.19$) of in situ ruminal forage DM digestibility. In addition, apparent ruminal ($P = 0.43$) and postruminal ($P = 0.31$) digestibility of OM did not differ among treatments. However, digestibility of NDF in the rumen was 16.3% greater ($P = 0.02$), and postruminal NDF digestibility was 49.7% less ($P = 0.03$) in supplemented than in control wethers (Table 3). In contrast with the present results, Krysl et al. (1989) reported no effect of SBM on ruminal NDF digestion by steers grazing blue grama rangeland, and Donaldson et al. (1991) reported no effect of low (125 g/d) or high (250 g/d) UIP on apparent ruminal NDF digestion by steers grazing ryegrass. Similarly, Bohnert et al. (2002a) observed no differences in apparent and true OM and NDF disappearance from the stomach due to CP supplementation or CP ruminal degradability. In contrast, when increasing levels of casein were supplemented to mature beef cows consuming low-quality forage, true ruminal OM and NDF digestion were increased (Köster et al., 1996). In Exp. 2, ruminal and postruminal NDF digestibility were not different between low and high UIP supplemented wethers, suggesting that DIP was not limiting forage digestion. Galyean and Owens (1991) suggested that source of supplemental N has little effect on site of digestion of low-quality forage, possibly due to increased N recycling when supplements high in UIP are fed (Bandyk et al., 2001; Bohnert et al., 2002; Swanson et al., 2004). Our results confirm that supplemental protein can increase ruminal NDF digestion, whereas source of protein seems less influential when low-quality forages are consumed. However, because wethers substituted intake of protein supplement for forage, it should be noted that increased apparent ruminal NDF digestibility might have resulted from digestibility of the supplement, whereas digestibility of forage NDF might have been similar or decreased compared with control wethers. Interestingly, lower NDF digestion in the rumen resulted in more NDF being digested in the postruminally (most likely cecum and large intestine) of control wethers.

Nitrogen Digestibility and Retention

As designed, N intake was greater ($P = 0.001$) for supplemented vs. control wethers, whereas N intake did not differ ($P = 0.42$) among wethers fed low vs. high UIP (Exp. 2; Table 4). Supplemented wethers had greater ($P = 0.05$) total duodenal N flow (g/d) than control wethers. In addition, fecal N output was greater ($P = 0.04$) for supplemented than for control wethers,

Table 5. Nitrogen balance and utilization in whiteface wethers consuming low-quality forage and receiving no supplement or supplement with two different proportions of undegradable intake protein and degradable intake protein, Exp. 1

	Treatments[a]					
Item	Control	Low UIP	High UIP	SEM[b]	C vs. S[c]	L vs. H[d]
Total N intake, g/d	14.60	25.10	25.20	0.19	0.001	0.31
Fecal N, g/d	7.15	8.95	8.40	0.38	0.04	0.24
Urinary N, g/d	7.40	13.21	14.91	0.52	0.01	0.08
Total N excreted, g/d	14.55	22.16	23.31	0.62	0.01	0.13
Retained N, g/d	0.05	2.97	1.84	0.53	0.01	0.11
N retained, % intake	0.30	11.70	7.17	3.09	0.001	0.22

[a]Treatments were Control = low-quality forage only; Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP); and High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP).
[b]Standard error of the least squares means, n = 5.
[c]*P*-value for Control vs. supplemented wethers.
[d]*P*-value for Low UIP vs. High UIP wethers.

and wethers fed high UIP tended ($P = 0.06$) to have greater fecal N output than wethers fed low UIP. Supplementation did not affect ($P = 0.45$) duodenal microbial N flow, but wethers fed low UIP had 18.2% greater ($P = 0.04$) duodenal microbial N flow than wethers fed high UIP. Bohnert et al. (2002a) reported that bacterial N flow at the duodenum was increased with CP supplementation, and was greater for DIP than for UIP. In the present Exp. 2, wethers fed high UIP had greater nonmicrobial N flow at the duodenum than both control ($P = 0.05$) and low UIP ($P = 0.02$) treatments. Similar results were reported by Bohnert et al. (2002a). Although not measured in the present experiments, ammonia N averaged 8.0% of total N flow to the abomasum and did not differ among treatments when supplements varying in proportion of cracked corn, distiller's dried grains with solubles, and menhaden fish meal were fed to steers grazing ryegrass pasture (Donaldson et al., 1991). Assuming ammonia N averaged 8.0% of total duodenal N flow in the present Exp. 2, feed N flow to the duodenum was 4.1, 2.5, and 26.9% (4.1, 2.8, and 34.4 g/d of CP) of total duodenal N flow for control, low UIP, and high UIP, respectively. In a study with low-quality grass hay (6.2% CP) and increasing levels of crambe meal, Caton et al. (1994) reported ammonia N was 0.23% of duodenal flow in steers. Assuming the 0.23% value, duodenal flow of feed N was estimated to be 11.8, 10.3, and 34.7% (11.7, 11.7, and 44.7 g/d of CP) of total duodenal N flow for control, low UIP, and high UIP in the present experiment. Problems associated with using markers to estimate both duodenal (Cr) and microbial N (purines) flow most likely contribute to variation within and among experiments. However, duodenal flow of nonmicrobial N was 3.8-fold greater in wethers fed high UIP in the present Exp. 2, which agrees with previous experiments (Bohnert et al., 1998; 2002a). Microbial efficiency (g of N/kg of OM apparently fermented) was greater ($P = 0.05$) for control than for supplemented wethers. Numerical decreases in microbial efficiency with CP supplementation have been reported (Krysl et al., 1989; Caton et al., 1994).

Postruminal N digestibility (percentage) was nearly twofold greater ($P = 0.002$) for control wethers than for supplemented wethers (Table 4); however, when expressed as the percentage of N entering the duodenum, postruminal digestibility of N did not differ among treatments. Total-tract N digestibility was 2.4-fold greater ($P = 0.001$) for supplemented than for control wethers (Exp. 2; Table 4). Similar to our experiments, increased apparent total-tract N digestibility has been reported with increasing supplemental CP from soybean meal or blood and feather meal (Ferrell et al., 1999) or from ruminal and/or abomasal casein infusion (Swanson et al., 2004). Interestingly, source (Ferrell et al., 1999) or site (Swanson et al., 2004) of protein digested did not affect apparent total-tract N digestibility, which is similar to the results of the present experiment. Our data suggest that although site of digestion might be affected, total-tract digestion is not affected by altering DIP:UIP ratio when ruminants are fed low-quality forage.

Similar to results of Exp. 2, total N intake was greater ($P = 0.001$) for supplemented wethers than control wethers in Exp. 1 (Table 5). In addition, fecal (17.3%) and urinary (90%) N output was greater ($P = 0.04$ and $P = 0.01$, respectively) for supplemented than for control wethers. Retained N was greater ($P = 0.01$) for supplemented wethers (average = 2.41 g/d) than for control wethers (0.05 g/d). Similarly, when expressed as a percentage of N intake, supplemented wethers retained more ($P = 0.001$) N than control wethers. Swanson et al. (2004) recently conducted an experiment in lambs fed low-quality brome hay (6.2% CP) with ratios of infusions of casein into the rumen:abomasum of 100:0, 67:33, 33:67, or 0:100, respectively. As in the present experiment, total N excretion was greater in lambs receiving casein infusion compared with controls. However, retained N (g/d and percentage of N intake) increased as casein infusion was shifted from 100% ruminal:0% abomasal to 33% ruminal:67% abomasal. Based on regression analysis, the optimal proportion of casein infusion into the abomasum to maximize N retention

Table 6. Mean ruminal pH, ruminal volatile fatty acid concentrations, and acetate:propionate ratio of ruminally cannulated wethers consuming a low-quality forage and receiving no supplement or supplement with two different proportions of undegradable intake protein and degradable intake protein, Exp. 2

	Treatments[a]					
Item	Control	Low UIP	High UIP	SEM[b]	C vs. S[c]	L vs. H[d]
Ruminal pH	6.36	6.30	6.27	0.71	0.62	0.81
Total VFA, m*M*	163.9	228.6	142.1	37.4	0.11	0.04
Ruminal VFA[d]		mol/100 mol				
Acetate	72.70	70.94	71.31	0.85	0.11	0.36
Propionate	16.94	18.31	17.13	0.17	0.10	0.08
Butyrate	7.31	7.70	8.26	0.71	0.11	0.24
Isobutyrate	1.15	1.13	1.17	0.45	0.20	0.31
Valerate	0.82	0.90	0.92	0.34	0.18	0.23
Isovalerate	1.10	1.10	1.16	0.44	0.28	0.25
Acetate:propionate	4.30	4.65	4.33	0.37	0.75	0.84

[a]Treatments were Control = low-quality forage only; Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP); and High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP).
[b]Standard error of the least squares means, n = 5.
[c]*P*-value for Control vs. supplemented wethers.
[d]*P*-value for Low UIP vs. High UIP wethers.

was 68%. Lack of a response in N retention in the present experiment most likely resulted from similar total N flow to the duodenum with supplemental protein and similar apparent N digestibility (percentage of N entering the duodenum) in the post stomach. Our data support other work (Bohnert et al., 2002b), in which no differences (P = 0.22) in N retention were observed when natural protein supplements differing in ruminal degradability were compared.

Ruminal Fermentation. Time × treatment interactions were not observed for ruminal pH, and there were no differences among treatments (Table 6). A time × treatment interaction (P = 0.001) was observed for ruminal ammonia concentration (Figure 1). The underlying trend for all time periods was for control wethers to have the lowest ruminal ammonia concentration and low UIP wethers to have the greatest; concentrations in high UIP wethers were intermediate. These results confirm that differences in ruminal degradability of supplements occurred in the present experiment. Similarly, Bohnert et al. (2002c) reported that ruminal ammonia N was increased with CP supplementation, and that it was 100% greater for DIP than for UIP in steers consuming low-quality forage. In addition, Schloesser et al. (1993) reported that ewes supplemented with soybean meal had the greatest ruminal ammonia concentrations, ewes supplemented with blood meal had the least, and all combinations of soybean meal:blood meal were intermediate. Although ruminal ammonia concentration was increased by CP supplementation in Exp. 2, ruminal ammonia concentration most likely did not limit microbial growth, even for control wethers (>2.0 mg/100 mL of ruminal fluid; Satter and Slyter, 1974).

No time × treatment interactions occurred for total VFA concentration. Wethers fed low UIP had a greater (P = 0.04) ruminal concentration of total VFA than

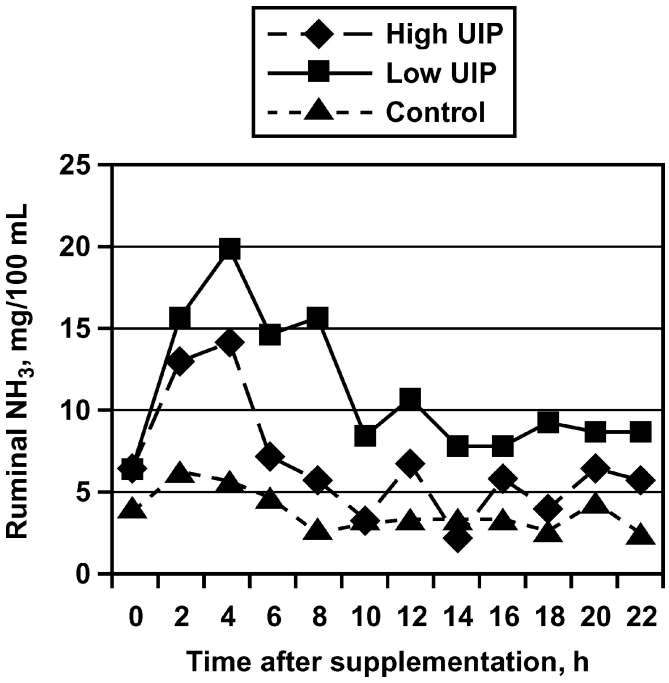

Figure 1. Ruminal ammonia concentrations of cannulated wethers consuming low-quality forage and receiving no supplement or supplement with two different proportions of undegradable intake protein and degradable intake protein, Experiment 2. Treatments were Control = low-quality forage only, Low UIP = low ruminally undegradable intake protein (24.8 g/d of UIP), and High UIP = high ruminally undegradable intake protein (37.1 g/d of UIP). There was a treatment × sampling time interaction (P = 0.001; SEM = 0.96).

wethers fed high UIP. Ruminal proportion of propionate tended ($P = 0.08$) to be greater in wethers fed low UIP compared with high UIP, whereas control wethers tended ($P = 0.10$) to have the lowest proportion of ruminal propionate (Table 6). Delcurto et al. (1990) reported no differences in VFA concentrations between supplemental protein types, but they found that propionate concentrations increased with level of supplement, regardless of type. Others (Köster et al., 1996; Bohnert et al., 2002c) reported decreased acetate and increased propionate molar proportions with DIP supplementation of low-quality forage. In addition to increased total N flow to the duodenum, increased molar proportion of ruminal propionate from CP (and energy) supplementation in ruminants consuming low-quality forage might increase total glucose precursors to the liver, providing for greater efficiency of acetate utilization by peripheral tissues, and resulting in increased N and energy balance (Blaxter, 1989). No effects of supplement ($P = 0.11$ to 0.18) or supplement type ($P = 0.23$ to 0.31) were observed for proportions of isobutyrate, butyrate, isovalerate, or valerate.

Implications

Providing a protein supplement to wether lambs consuming low-quality forage increased total dry matter intake and nitrogen balance. In addition, ruminally undegradable intake protein varied from at least 35 to 53% of supplemental crude protein, with no effect on total-tract nutrient digestion or nitrogen balance. Therefore, if ruminally degradable intake protein is adequate, it seems that true protein sources fed to growing ruminants grazing protein-deficient forages can vary in ruminal degradability with no expected difference in animal performance.

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