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Alfalfa Leaves as a Protein Source. Replacement Value of Alfalfa Leaves for Soybean Meal Protein in Forage-based Diets Fed to Mature Ewes

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Abstract

Four ruminally and duodenally cannulated mature ewes (BW 65.8 4.6 kg) were used in a 4 x 4 Latin square designed experiment to investigate the replacement value of alfalfa leaves (AL) for soybean meal (SBM) protein. Ewes received basal diets consisting of bromegrass hay, cracked corn, and one of four protein supplements. Diets were formulated to be isonitrogenous by replacing SBM with AL on a nitrogenous basis. Supplement treatments were: 100% SBM; 33.3% AL and 66.7% SBM; 66.7% AL and 33.3% SBM and 100% AL. Experimental periods were 14 days with 10 days of diet adaptation followed by 4 days of ruminal, duodenal, and fecal sample collection. Replacing SBM with AL decreased intake of OM and NDF (quadratic; $P < .02$). Intake of N increased as AL replaced SBM, which resulted in a cubic effect ($P = .06$). Organic matter flow and true ruminal OM digestibility were not affected (P .18) by replacement of SBM with AL. Lower and total tract OM digestibility increased from the 33.3% AL to the 66.7% AL diet and decreased from the 66.7% AL to the 100% AL dietary treatment resulting in cubic effects ($P = .01$ and .03, respectively). Nitrogen flow and true ruminal N digestibility were not influenced (P .18) by supplemental protein treatment. Greater lower tract N digestibility for 33 and 67% AL resulted in a quadratic effect ($P = .05$), whereas total tract N digestibility was not affected (P .13) by replacing SBM with AL. No treatment differences (P .20) were detected for NDF flow or ruminal NDF digestibility. Digestibility of NDF entering the small intestine tended to respond quadratically ($P = .09$) with a tendency for a cubic effect ($P = .08$) for total tract NDF digestibility as a result of greater digestibility of 66.7% AL diet. Ruminal pH increased quadratically ($P = .0004$), while ruminal NH_3 N tended to decrease linearly ($P = .10$) as AL replaced SBM. Ruminal particulate and fluid passage rates were not influenced (P .21) by replacing SBM with AL. Total VFA concentrations within the rumen decreased in a quadratic (P = .05) fashion. Acetate:propionate ratio responded quadratically (P = .0001) due to higher acetate:propionate ratios for diets containing a combination of supplementary protein. Alfalfa leaves can replace soybean meal as a protein supplement; however, greater nutritional value was apparent when the two protein sources constituted the supplement.

Key Words: Alfalfa, Protein, Replacement value, Digestion

Introduction

Protein supplementation is a common practice in the ruminant livestock industry, particularly with ruminants fed low-quality forages. Digestion of low-quality forages by ruminants usually increases when supplemental degradable intake protein (DIP) is fed. Studies indicate that alfalfa and high-protein concentrates, when fed to provide similar amounts of CP, elicit similar effects on forage digestion and utilization (Cochran et al., 1986; DelCurto et al., 1990a; Hannah et al., 1991).

Soybean meal (SBM) is a common source of supplemental protein for ruminants. However, the use of supplemental SBM in many areas of the United States may be limited due to low availability. Alternatively, alfalfa (*Medicago sativa* L.) is readily available because it is a widely grown forage crop (Barnes et al., 1988). Leaves in alfalfa hay are diluted by the stem fraction which is mainly composed of highly fibrous cell wall constituents of low CP content and high lignin content. Thus, alfalfa hay may not be comparable to SBM as a protein supplement. Results of two companion papers (Tsopito et al., 1999a,b) indicated that protein content and quality of alfalfa leaves (AL) was greater than that of alfalfa hay. The objective of this study was to investigate the replacement value of AL for SBM as a protein supplement.

Materials and Methods

General

Four ruminally and duodenally cannulated mature ewes (avg. initial BW 65.8 4.6 kg) were used in accordance with a protocol approved by the University of Wyoming Animal Care and Use Committee. Ewes were housed in a temperature-controlled room with continuous lighting. Sheep were placed in individual metabolism crates and had ad libitum access to fresh water and trace mineralized salt (Trace Mineralized Salt, Akzo Salt, Inc.; guaranteed analysis [percentage of DM]: NaCl, 95 to 99; Co, Cu, I, Mn, Zn, and Fe, <1) throughout the study. Basal diets were fed at 95% of maintenance and consisted of bromegrass hay and cracked corn and one of four protein supplements. Diets were fed in equal allotments at 0700 and 1900 hours. Ewes were assigned to one of the four supplemental protein treatments according to the randomization table of Neter et al. (1990) for a 4 x 4 Latin square design experiment. Alfalfa leaves replaced supplemental protein from SBM to provide supplemental protein treatments of: 100% SBM; 33.3% AL plus 66.7% SBM; 66.7% AL plus 33.3% SBM; and 100% AL. Alfalfa leaves replaced increasing amounts of SBM and bromegrass hay to ensure isonitrogenous diets (Table 1).

Sampling

As an indigestible marker of digesta flow, Cr_2O_3 (2.5 g) was delivered intraruminally at each feeding. Experimental periods were 14 days with 10 days of diet adaptation and 4 days of sample collection. Representative samples of each feed ingredient were collected at each feeding, composited within each experimental period, and stored for analysis.

Beginning at 0500 hours on day 1 of each sampling period, duodenal and fresh fecal grab samples were collected at 4-hour intervals. Collection times were advanced 6 hours on day 2 of sampling to obtain samples that represented every 2 hours of a 24-hour period. Duodenal and fecal samples, respectively, were composited on an equal volume basis for each ewe within each sampling period.

Table 1. Ingredients and chemical composition of the diets fed to mature ewes

	Treatment[a]			
Item	**100% SBM**	**33.3% AL**	**66.7% AL**	**100% AL**
Ingredients, %				
Bromegrass hay	61.1	55.5	48.3	39.1
Cracked corn	25.2	25.2	25.1	25.2
Soybean meal	13.8	10.0	5.1	-
Alfalfa leaves	-	9.3	21.1	35.6
Chemical composition				
DM, %	95.2	95.1	94.8	95.1
OM, % of DM	88.2	88.0	87.8	87.5
	---------- % of OM ----------			
CP	20.0	20.1	20.4	20.9
NDF	58.7	57.4	55.5	53.0
ADF	30.5	30.1	29.2	28.2

[a]Treatment: supplemental protein = 100% soybean meal (SBM), supplemental protein = 33.3% alfalfa leaves (AL) and 66.7% SBM, supplemental protein = 66.7% AL and 33.3% SBM, and supplemental protein = 100% AL.

Duodenal samples were stored at -20 C. Fecal samples were refrigerated (4 C) until the end of the collection period and then dried in a forced-air oven at 55 C.

On day 2 of each sampling period, approximately 100 mL of whole ruminal contents were collected from each ewe immediately before feeding at 0700 (0-h sampling time). Ruminal contents were strained through four layers of cheesecloth and pH measured immediately. Strained samples were acidified with 0.1 mL of 7.2 *N* H_2SO_4/10 mL ruminal fluid, and frozen (-20 C). The resulting boluses from strained ruminal contents were dried in a forced-air oven (55 C for 48 h) and ground in a Wiley mill to pass a 2-mm screen. The remainder of ruminal contents (unstrained) were then placed into 3.8 L plastic jugs containing saline solution (9 g NaCl /1L H_2O) and stored at –20 C for isolation of ruminal bacteria. Following initial collection of ruminal contents, each ewe was dosed intraruminally with wetted 50 g Yb-labeled bromegrass hay (Teeter et al., 1984) and 50 mL of Co-EDTA (Uden et al., 1980) for determination of particulate and fluid passage rates, respectively. Additional ruminal samples were taken at 4, 8, 12, 16, and 20 h after feeding and processed in the same manner as the 0-h sample.

Sample Processing

Duodenal samples were freeze-dried (Model 10-MR-TR Freeze Dryer, Virtis Co., NY). Feed composites, and dried duodenal and fecal samples were ground to pass a 1 mm

screen using a Wiley mill. Frozen ruminal contents were thawed, saline (9 g NaCl /L H_2O) added and homogenized in a blender (Hamilton Beach/Proctor-Silex, Inc., Model 702R, Washington, NC) to release particulate-associated microbes from feed particles (Firkins et al., 1986). The mixture was strained through four layers of cheesecloth and the resulting fluid fraction was separated from the particulate fraction via centrifugation at 1,000 x *g* for 10 min. The supernantant was retained and re-centrifuged at 10,000 x *g* for 30 min. Following rinsing with saline (2 parts saline:1 part pellet) and recentrifugation (10,000 x g for 30 min), the resulting bacteria-rich pellet was isolated and stored in saline (9 g NaCl /L H_2O) on an equal volume basis for later analysis.

Acidified ruminal samples were thawed at room temperature and centrifuged at 10,000 x *g* for 10 min. The resulting supernatant was collected for VFA, Co, and NH_3 N concentration determination. To 2.5 mL of centrifuged ruminal fluid, .5 mL of 25% (wt/vol) metaphosphoric acid containing 2 g/L 2-ethylbutyrate (2-EB) were added, and the mixture centrifuged at 10,000 x *g* for 10 min. The supernatants were transferred into glass vials (2.2 mL), capped and stored (4 C).

Laboratory Analyses

Ground composites of feed, duodenal, and fecal samples were analyzed for DM, ash, Kjeldahl N (AOAC, 1990), ADF and NDF (Goering and Van Soest, 1970). Isolated ruminal bacteria were analyzed for purines (Zinn and Owens, 1986), DM, ash, and Kjeldahl N (AOAC, 1990). Chromium was extracted by perchloric acid from ground duodenal and fecal samples, and Cr concentrations determined by atomic absorption spectroscopy (Model 210 VDT AASpectr., E. Norwalk, CT) with an air-acetylene flame (Hill and Anderson, 1958; Williams et al., 1962). Ytterbium was extracted from ground ruminal boluses with .05 M EDTA containing 3.8 g of KCl/L as an ionization buffer (Teeter et al., 1984). Ytterbium concentrations were measured by atomic absorption spectroscopy with an air-acetylene and nitrous oxide flame (Hart and Polan, 1984). Ruminal fluid supernatant was analyzed for Co concentration by atomic absorption spectroscopy with an air-acetylene flame, NH_3 N by the phenol-hypochlorite assay (Broderick and Kang, 1980). Volatile fatty acid concentrations were determined by gas chromatography (Goetsch and Galyean, 1983; Whitney 1998) using a Hewlett Packard 5890 Series II GC equipped with a 15 m x .53 mm (i. d.) column (Nukol; Supelco, Bellefonte, PA) with oven temperature ramp of 110 C to 150 C at 8 C per min, and Helium as carrier gas (20 mL/min). Injector and detector temperatures were 250 C.

Calculations

Nutrient flows at the duodenum as well as fecal output were calculated by reference to Cr. Apparent ruminal, lower tract, and total tract digestion of nutrients were calculated as the difference between nutrient intake, duodenal flow, and fecal excretion of nutrients, respectively. The purine:N ratio of isolated ruminal bacteria was used in conjunction with the duodenal purine content to calculate microbial N flow to the duodenum. True ruminal OM and N digestibilities were determined by correcting apparent ruminal digestibilities for microbial OM and N flow to the duodenum. Bacterial protein synthesis was expressed as grams of microbial N per kilogram of OM truly fermented in the rumen.

Ruminal fluid and particulate passage rates were calculated by regressing the natural logarithm of Yb and Co concentration

against sampling time after dosing (Uden et al., 1980). Volatile fatty acids areas were adjusted for detector responses with a set of correction factors developed to determine the mg/g values and mmol values calculated from these values using formula weights for each VFA molecule.

Statistical Analyses

Data were analyzed by ANOVA using GLM procedure of SAS (1989). Intakes, fluid and particulate passage rates, digesta flows, and digestibilities were analyzed by ANOVA in a 4 x 4 Latin square design. Ruminal pH, NH_3 N, and VFA data were analyzed as a split-plot to examine the effects of time. Treatment x sampling time interactions were not detected ($P > .10$); therefore, treatment sums of squares for all data were partitioned into linear, quadratic, and cubic effects of AL replacement. One ewe died on day 4 of the third experimental period due to unknown causes. Hence, treatment means were calculated using the LSMEANS option of SAS (1989).

Results and Discussion

Intakes

Although intakes were controlled at 95% of maintenance requirements, OM intake decreased quadratically ($P = .02$) and N intake increased linearly ($P = .0001$) as AL replaced supplemental SBM (Table 2 and 3). The response noted for OM intake corresponded with lower levels of OM in AL (Table 1). Due to the lower CP content of AL compared to SBM, AL also replaced bromegrass hay as AL dietary percentage increased. Therefore, NDF intake decreased quadratically ($P = .0003$) as AL replaced supplemental SBM (Table 4).

Table 2. Intake, flow and digestibility of organic matter by mature ewes fed alfalfa leaves as a replacement for soybean meal protein

	Treatment[a]				Contrast[b]		
Item	100% SBM	33.3% AL	66.7% AL	100% AL	Linear	Quadratic	Cubic
OM intake, g/d	1048.9 ± .8	1046.7 ± .6	1044.4 ± .6	1037.1 ± .8	.0003	.02	.13
Total duodenal OM flow, g/d	642.8 ± 32.0	647.9 ± 24.2	654.6 ± 24.2	602.3 ± 32.0			
	.43	.40	.61				
Microbial OM flow, g/d	223.7 ± 11.3	224.2 ± 8.5	214.6 ± 8.5	202.4 ± 11.3	.18	.57	.86
Apparent ruminal OM digestion, g/d	406.0 ± 32.6	398.9 ± 24.6	389.9 ± 24.6	434.8 ± 32.6	.60	.44	.65
Apparent ruminal OM digestibility, % of intake	38.7 ± 3.1	38.1 ± 2.4	37.3 ± 2.4	41.9 ± 3.1	.60	.41	.53
OM truly fermented, g/d	629.7 ± 39.9	623.0 ± 30.2	604.5 ± 30.2	637.1 ± 39.9	.98	.62	.67
True ruminal OM digestibility, % of intake	60.0 ± 3.9	59.5 ± 2.9	57.8 ± 2.9	61.4 ± 3.9	.91	.61	.60
Fecal OM output, g/d	274.4 ± 11.4	276.8 ± 8.6	236.7 ± 8.6	265.6 ± 11.4	.22	.72	.27
Lower tract OM digestion, g/d	368.5 ± 24.7	371.1 ± 18.6	417.9 ± 18.6	336.7 ± 24.7	.65	.14	.11
Lower tract OM digestibility, % entering	57.3 ± 1.3	57.3 ± 1.0	63.8 ± 1.0	55.9 ± 1.3	.80	.05	.01
Total tract OM digestion, g/d	774.5 ± 11.8	769.9 ± 8.9	807.8 ± 8.9	771.5 ± 11.8	.58	.22	.05
Total tract OM digestibility, % of intake	73.8 ± .9	73.5 ±.7	77.4 ± .7	74.4 ± .9	.25	.25	.03

[a]Treatment: supplemental protein = 100% soybean meal (SBM), supplemental meal = 33.3% alfalfa leaves (AL) and 66.7% SBM, supplemental protein = 66.7% AL and 33.3% SBM, and supplemental protein = 100% AL.

[b]Observed *P*-values for polynomial contrasts.

Table 3. Intake, flow and digestion of nitrogen by mature ewes fed alfalfa leaves as a replacement for soybean meal protein

	Treatmenta				Contrastb		
Item	100% SBM	33.3% AL	66.7% AL	100% AL	Linear	Quadratic	Cubic
N intake, g/d	33.6 ± .04	33.7 ± .03	34.1 ± .03	34.6 ± .04	.0001	.01	.06
Total duodenal N flow, g/d	20.8 ± 2.2	24.1 ± 1.7	23.8 ± 1.7	21.7 ± 2.2	.81	.26	.85
Duodenal NH_3 N, g/d	2.3 ± .8	3.1 ± .6	4.0 ± .6	2.4 ± .6	.70	.18	.36
Feed N flow, g/d	6.9 ± 2.1	9.2 ± 1.6	8.3 ± 1.6	6.9 ± 2.1	.93	.38	.74
Microbial N flow, g/d	11.7 ± .6	11.8 ± .4	11.5 ± .4	12.4 ± .6	.49	.53	.40
Microbial efficiency, g microbial N flow/kg OM truly fermented	18.4 ± .9	19.2 ± .7	20.0 ± .7	19.7 ± .9	.24	.56	.74
Apparent ruminal N digestion, g/d	12.8 ± 2.3	9.6 ± 1.8	10.3 ± 1.8	12.7 ± 2.3	.81	.34	.71
Apparent ruminal N digestibility, % of intake	38.1 ± 6.7	28.5 ± 5.1	30.2 ± 5.1	36.8 ± 6.7	.71	.33	.83
True ruminal N digestibility, % of intake	60.6 ± 3.6	63.5 ± 2.7	62.6 ± 2.7	62.2 ± 3.6	.91	.71	.92
Fecal N output, g/d	5.9 ± .3	5.7 ± .2	4.9 ± .2	6.0 ± .3	.64	.07	.09
Lower tract N digestion, g/d	14.9 ± 2.2	18.4 ± 1.6	18.9 ± 1.6	15.9 ± 2.2	.75	.17	.93
Lower tract N digestibility, % of intake	44.4 ± 6.4	54.6 ± 4.8	55.4 ± 4.8	46.0 ± 6.4	.50	.05	.18
Total tract N digestion, g/d	27.7 ± .34	28.0 ± .26	29.2 ± .26	28.6 ± .34	.15	.08	.05
Total tract N digestibility, % of intake	82.5 ± 1.1	83.1 ± .8	85.6 ± .8	82.7 ± 1.1	.95	.13	.84

aTreatment: supplemental protein = 100% soybean meal (SBM), supplemental protein = 33.3% alfalfa leaves (AL) and 66.7% SBM, supplemental protein = 66.7% AL and 33.3% SBM, and supplemental protein = 100% AL.

bObserved *P*-values for polynomial contrasts.

Table 4. Intake, flow and digestibility of neutral detergent fiber by mature ewes fed alfalfa leaves as a replacement for soybean meal protein

	Treatmenta				Contrastb		
Item	100% SBM	33.3% AL	66.7% AL	100% AL	Linear	Quadratic	Cubic
NDF intake, g/d	614.3 ± 1.7	600.3 ± 1.0	579.3 ± 1.0	549.7 ± 1.7	.0001	.003	.73
Total duodenal NDF flow, g/d	264.9 ± 19.5	290.0 ± 14.7	260.4 ± 14.7	244.9 ± 19.5	.32	.32	.37
Apparent ruminal NDF digestion, g/d	349.3 ± 19.8	310.8 ± 15.0	319.2 ± 15.0	305.1 ± 19.8	.20	.54	.37
Apparent ruminal NDF digestibility, % of intake	56.8 ± 3.3	51.8 ± 2.5	55.1 ± 2.5	55.5 ± 3.3	.94	.43	.42
Fecal NDF output, g/d	185.9 ± 11.5	183.3 ± 8.7	149.9 ± 8.7	170.0 ± 11.5	.15	.35	.10
Lower tract NDF digestion, g/d	79.0 ± 16.6	106.7 ± 12.6	110.4 ± 12.6	74.9 ± 16.6	.91	.11	.80
Lower tract NDF digestibility, % entering	29.8 ± 4.1	36.8 ± 3.1	42.4 ± 3.1	30.6 ± 4.1	.85	.09	.21
Total tract NDF digestion, g/d	428.3 ± 10.7	417.5 ± 8.1	429.6 ± 8.1	380.0 ± 10.7	.04	.12	.08
Total tract NDF digestibility, % of intake	69.7 ± 1.8	69.6 ± 1.4	74.2 ± 1.4	69.1 ± 1.8	.93	.21	.08

aTreatment: supplemental protein = 100% soybean meal (SBM), supplemental prote in = 33.3%alfalfa leaves (AL) and 66.7% SBM, supplemental protein = 66.7% AL and 33.3% SBM, and supplemental = 100% AL.

bObserved *P*- values for polynomial contrasts.

Organic Matter Digestion

An increase from 33.3% AL to 66.7% AL and a decrease from 66.7% AL to 100% AL caused quadratic and cubic effects (P = .05 and .01, respectively) for lower tract OM digestibility while causing cubic (P = .03) effects for total tract OM digestibility as SBM was replaced with AL. Galyean and Owens (1991) suggested that source of supplemental N generally seemed unimportant in altering site of OM digestion. Results of this study were similar to those of Stafford et al. (1996) who observed increased total tract OM digestibility when supplementing tallgrass-prairie forage with high-quality alfalfa hay (17.5 % CP). However, Stafford et al. (1996) noted decreased total tract OM digestibility with supplemental pelleted alfalfa dehy (17.5 % CP) compared to higher CP soybean meal-based supplements. Alfalfa leaves are normally of higher quality than alfalfa hay. Therefore, AL is a likely replacement for supplemental SBM.

Nitrogen Digestion

An increase from 100% SBM to 66.7% AL and a decrease from 66.7% AL to 100% AL induced a quadratic (P = .05) effect for lower tract N digestibility. Cotta and Hespell (1986) noted that the amount of N apparently digested in the small intestine was a good indication of the protein available to the host animal. Therefore, 33.3 and 66.7% AL treatments provided greater metabolizable protein than each protein source fed alone.

Neutral Detergent Fiber Digestion

Digestibility of NDF in the lower tract tended to increase from 100% SBM to 66.7% AL, and to decrease from 66.7% AL to 100% AL causing a quadratic (P = .09) effect as supplemental SBM was replaced with AL; similar fluctuations also induced a cubic (P = .08) effect on total tract NDF digestibility. Stefanon et al. (1996) showed that alfalfa hay had less structural fiber than bromegrass hay, but this fiber appeared to contain a relatively slower digesting component. A portion of this slower digesting fiber component of the AL treatments may have escaped ruminal digestion, then was digested in the lower tract. Greater lower tract NDF digestibility may not have occurred for the 100% AL treatment because of the greater proportion of fiber from bromegrass hay reaching the lower tract for this treatment. Particulate passage rate for the 100% AL treatment was 10% greater than that of the other treatments suggesting greater duodenal flow of bromegrass hay because bromegrass hay was labeled with Yb for this experiment.

Ruminal pH, Ammonia, and Passage Rates

Replacing supplemental SBM with AL increased ruminal pH from the 100% SBM to the 33.3% AL diet and decreased pH from the 33.3% AL to the 100% AL diet such that linear (P = .03) and quadratic (P = .0004) effects occurred (Table 5). Ruminal NH_3 N concentration tended to decrease linearly (P = .10) as AL replaced supplemental SBM (Table 5). Broderick et al. (1993) observed a similar trend in lactating cows fed SBM, unprocessed and processed alfalfa hay. Erdman et al. (1986) indicated that the concentration of ruminal NH_3 N needed for maximum digestion is a function of the fermentability of the diet. Although OM truly fermented in the rumen was not statistically different across treatments, it was worth noting numerical differences of OM truly fermented that may have a biological effect on some ruminal fermentation parameters such as ruminal NH_3 N concentrations.

Decreased ruminal NH_3 N concentrations for the 100% AL diet may also have been due to the quality of protein presented to ruminal microbes for degradation. Van Soest (1994) noted that leaf proteins were of higher quality than storage proteins found in plant seeds (SBM) because of their extent of solubility. Storage proteins tend to be rapidly soluble compared with leaf proteins that contain extensin proteins of the plant cell wall. The extensin proteins account for a small portion in the leaves, but tend to reduce rapid degradability of the leaves (Van Soest, 1994). Thus, soybean meal proteins (which are storage proteins) tend to be more rapidly degraded in the rumen, producing higher levels of ruminal NH_3 N. Nonetheless, ruminal NH_3 N concentrations observed in this study were within the 2.2 to 23.5 mg/dL range suggested by Clark and Davis (1983) to be optimal for OM digestion. Moreover, NH_3 N concentrations were above the 3.3 to 8.5 mg/dL range considered optimal for ruminal fermentation (Kang-Meznarich and Broderick, 1981), and above the 6.2 mg/dL required when dietary CP is over 6% (Hoover, 1986).

Ruminal Volatile Fatty Acids

Total ruminal VFA concentrations (Table 6) decreased from the 100% SBM to the 66.7% AL diet, then increased from the 66.7% AL to the 100% AL diet resulting in a quadratic ($P = .05$) effect which was similar to the trends noted for OM truly fermented in the rumen. However, trends for ruminal pH resembled those of total ruminal VFA concentrations. Higher total VFA concentrations are known to lower ruminal pH (Stokes et al., 1988; DelCurto et al., 1990b; Sunvold et al., 1991).

Ruminal molar proportions of acetate were influenced (quadratic, cubic and linear; $P = .0003$, .02, and .07, respectively) as supplemental SBM was replaced by AL.

Dietary treatment effects (quadratic and cubic; $P = .0001$ and .01, respectively) were detected for ruminal molar proportions of propionate as AL replaced supplemental SBM. Ruminal acetate:propionate (A:P) ratio had a quadratic ($P = .0001$) and cubic ($P = .003$) response due to greater A:P ratios for the 33.3 and 66.7% AL treatments. The A:P ratio is an indicator of the metabolic pathway used by ruminal bacteria in converting monosaccharides to VFA (Stefanon et al., 1996). Changes in the A:P ratio associated with the various diets may also be due to the fiber component being degraded differently within the rumen. Greater A: P ratios for the 33.3 and 67.7% AL treatments indicate the predominance of acetate producing bacteria. Conversely, lower A:P ratios for the 100% SBM and 100% AL diets indicate a bacterial species predominately synthesizing propionate. Ruminal pH for these treatments (pH 5.9) also was supportive of changes in ruminal A:P ratios. Ruminal pH less than 6.0 restricts the activity of cellulolytic bacteria (Orskov,1992), which are responsible for acetate synthesis.

Ruminal molar proportions of butyrate were influenced linearly ($P = .02$), quadratically ($P = .05$), and cubically ($P = .01$). Butyrate increased when the diet was changed from 100% SBM to 33.3% AL, and then decreased when the diet was changed from 33.3% AL to 100% AL. The higher ruminal molar proportions of butyrate for the 33.3% AL treatment compared to other dietary treatments induced the quadratic response since if this treatment was not considered, the response would have been a linear decrease in butyrate concentrations as replaced SBM. Increased digestion of NSC in SBM (Schadt et al., 1999) may have contributed to the response noted for ruminal molar proportions of butyrate. Additionally, the relatively high ruminal molar

Table 5. Ruminal pH, ammonia and passage rate mature ewes fed alfalfa leaves as a replacement for soybean meal protein

	Treatmenta				Contrastb		
Item	100% SBM	33.3% AL	66.7% AL	100% AL	Linear	Quadratic	Cubic
pH	5.7 ± .07	6.1 ± .06	6.0 ± .06	5.9 ± .07	.03	.0004	.10
NH_3 N, mg/dL	16.3 ± 1.2	14.7 ± 0.9	14.8 ± 0.9	13.6 ± 1.2	.10	.90	.47
Particulate passage rate, %/h	4.1 ± 0.3	3.9 ± 0.2	4.1 ± 0.2	4,5 ± 0.3	.55	.30	.21
Fluid passage rate, %/h	6.2 ± 0.9	6.1 ± 0.7	5.4 ± 0.7	7.4 ± 0.9	.50	.30	.34

aTreatment: supplemental protein = 100% soybean meal (SBM), supplemental protein = 33.3% alfalfa leaves (AL) and 66.7% SBM, supplemental protein = 66.7% AL and 33.3% SBM, and supplemental protein = 100% AL.

[b]Observed *P*-values for polynomial contrasts.

Table 6. Ruminal v olatile fatty acids of mature ewes fed alfalfa leaves as a replacement for soybean meal protein

	Treatmenta				Contrastb		
Item	100% SBM	33.3% AL	66.7% AL	100% AL	Linear	Quadratic	Cubic
Total VFA, m*M*	108.6 ± 4.7	102.2 ± 3.5	98.6 ± 3.5	109.7 ± 4.7	.99	.05	.47
	---------- mol/100 mol ----------						
Acetate	61.5 ± .4	63.9 ± .3	63.2 ± .3	62.7 ± .4	.07	.0003	.02
Propionate	21.5 ± .6	18.2 ± .5	20.0 ± .5	21.4 ± .6	.55	.0001	.01
Acetate:Propionate	2.9 ± .1	3.6 ± .1	3.2 ± .1	3.0 ± .1	.68	.0001	.003
Butyrate	13.0 ± .4	14.2 ± .3	12.6 ± .3	12.2 ± .4	.02	.05	.01
Isobutyrate	.9 ± .2	.9 ± .1	.9 ± .1	.7 ± .2	.57	.66	.79
Valerate	1.3 ± .1	1.3 ± .1	1.6 ± .1	1.5 ± .1	.0003	.32	.001
Isovalerate	1.8 ± .7	1.7 ± .1	1.8 ± .1	1.5 ± .7	.002	.15	.002

aTreatment: supplemental protein = 100% soybean meal (SBM), supplemental protein = 33.3% alfalfa leaves (AL) and 66.7% SBM, supplemental protein = 66.7% AL and 33.3% SBM, andsupplemental protein = 100% AL.

[b]Observed *P*-values for polynomial contrasts.

proportions of butyrate reported in the present study may have been due to inclusion of cracked corn in the basal diets of the ewes to synchronize energy with N provided by dietary treatments. Ruminal molar proportions of butyrate have been shown to increase with increasing starch intake (Stern et al., 1978; Grisby et al., 1991; Hess et al., 1996).

Ruminal molar proportions of valerate increased when dietary AL was increased from 33.3% to 66.7% and then decreased when dietary AL was increased from 66.7% to 100% (cubic effect, $P = .001$) and a decrease from 66.7% AL to 100% AL. Increasing dietary AL from 33.3% to 100% resulted in a positive cubic ($P = .002$) response in ruminal molar proportions of isovalerate. These minor ruminal VFA responses were considered unimportant (Gorosito et al., 1985; Gunter et al., 1990) in terms of enhanced ruminal digestion.

Conclusions

Alfalfa leaves can replace soybean meal as a protein supplement; however, greater nutritional value was apparent when the two protein sources constituted the supplement. Lower and total tract digestion of OM, N and NDF were all greater for diets containing a combination of supplemental alfalfa leaves and soybean meal, suggesting complementary effects of combining alfalfa leaf and soybean meal protein. Although alfalfa leaves can be used to replace SBM as a protein supplement, the availability, separation process, and cost of AL will determine the benefits of replacing traditional protein sources such as SBM.

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