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Potential Associative Effects of Increasing Dietary Forage in Limit-fed Ewes Fed a 6% Fat Diet

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Abstract

The objective of this study was to determine how site and extent of nutrient digestion are affected by dietary forage level in ewes when the diet contains 6% crude fat by soybean oil supplementation. Five mature ewes (66.5 ± 12.8 kg of initial BW) fitted with ruminal and duodenal cannulas were used in a 5 x 5 Latin square experiment. Diets (13.9% CP, DM basis) were fed at 1.3% of BW and included bromegrass hay, cracked corn, corn gluten meal, urea, and limestone. Dietary fat was adjusted to 6% (DM basis) with soybean oil and included one of five dietary forage levels (18.4%, 32.2%, 45.8%, 59.4%, and 72.9%). Chromic oxide was used as a digesta flow marker. Ruminal pH increased (linear, $P < 0.001$) from 5.7 to 6.5 and VFA concentration decreased (linear, $P < 0.001$) with increased dietary forage. Ruminal, post-ruminal, and total tract OM digestibility decreased (linear, $P \leq 0.06$) with increased dietary forage. Ruminal starch digestibility was unaffected ($P = 0.76$) by treatment. Post-ruminal and total tract starch digestibility decreased (linear, $P < 0.001$) with increased dietary forage. Ruminal NDF digestibility increased (linear, $P < 0.05$) as dietary forage increased, but post-ruminal and total tract NDF digestibilities did not differ ($P \geq 0.23$). True ruminal N digestibility was not affected ($P = 0.29$) by dietary forage level. In contrast, microbial efficiency increased from 34.3 to 47.3 g microbial N/kg of OM truly digested as dietary forage increased from 18.4 to 45.8% , then decreased to 37.1 g microbial N/kg of OM truly digested on the highest forage diet (quadratic, $P < 0.001$). Total tract N digestibility decreased (linear, $P < 0.05$) with increased dietary forage. We conclude that the general pattern of nutrient digestion reflects the quality of dietary ingredients when mature ewes are restricted-fed a 6% fat diet ranging from 18.4% to 72.9% forage. Total tract OM digestibility was greater than anticipated for the 59.4 and 72.9% forage diets, suggesting that positive associate effects are possible with high-forage diets containing 6% dietary fat.

Key words: Sheep, Ruminal digestion, Dietary fat

Introduction

Digestibility of nutrients by ruminants as affected by dietary forage level was thoroughly studied by Mould et al. (1983 a,b). Numerous studies have demonstrated associative effects between single sources of forage and concentrate (Ørskov and Ryle, 1990); however, little information exists on associative effects when diets contain supplemental fat in limit-fed rations for sheep. Although supplementing fat at up to 6% of diet DM did not cause problems with nutrient digestibility in cattle (Van der Honing et al., 1981) or sheep (Jenkins, 1997), adding greater than 2 to 3% dietary fat to the diet of ruminants can inhibit microbial activity and reduce diet digestibility (Palmquist, 1988). Feeding dairy cows an 8% soybean oil, high-fiber diet did not affect total tract digestibility of DM, OM, N, and NDF (Bateman and Jenkins, 1998). However, when wethers were fed an 8% tallow, high-fiber diet, digestibility of N and ash decreased, whereas, ADF digestibility increased (Olubobokun et al., 1985). In steers consuming a 5% fat diet composed of equal proportions of forage and concentrate, only ruminal and total tract OM digestibility decreased compared with diets that did not contain added fat (Elliott et al., 1997). When steers were fed a high-concentrate diet with 6% supplemental fat, ruminal digestibility of OM, NDF, starch, and feed N, and total tract digestibility of OM decreased compared with a high-concentrate diet without supplemental fat (Zinn et al., 2000). Thus, there are mixed results regarding nutrient digestibility of fat-supplemented diets when dietary forage is increased from low to high levels. Moreover, limit-fed sheep, which may occur during periods of drought, may benefit from supplemental fat if nutrient digestion is not adversely affected. Also, by providing soybean oil as the supplemental fat, the ewe may derive additional benefit from the polyunsaturated fatty acids. The purpose of the present study was to determine how site and extent of nutrient digestion are affected by forage level when a limit-fed diet contained 6% crude fat by soybean oil supplementation.

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Materials and Methods

Animals, diets, and sampling

Animals

Five mature, Western white-faced ewes (4 to 7 yr of age and initial BW = 66.5 ± 12.8 kg) were fitted with ruminal and duodenal cannulas to be used as an in vivo model of ruminal and post-ruminal digestion. Duodenal T-type cannulas were inserted proximal to the common bile and pancreatic duct. All surgical and experimental procedures were conducted under protocols approved by the University of Wyoming Animal Care and Use Committee. Ewes were housed in individual metabolism crates (1.4 m x 0.6 m) in a temperature-controlled room (22°C) under continuous lighting.

Diets

Ewes were assigned to one of five dietary treatments in a 5 x 5 Latin square experiment. Treatments consisted of five forage levels, with the remainder as concentrate mixture, as follows: 18.4, 32.2, 45.8, 59.4, and 72.9% forage (DM basis; Table 1). Dietary crude fat was adjusted to 6% (DM basis) by addition of soybean oil to each ration immediately before feeding. Isonitrogenous diets (13.9% CP) were formulated on a DM basis to meet the CP requirements of a 40-kg finishing lamb (NRC, 1985 a). In an effort to ensure complete consumption of all diets and to avoid confounding effects of DM intake, diets were fed at 1.3% of initial BW (DM basis) in two equal allotments at 0630 and 1830. Amount of feed offered daily was 92.9% of maintenance (TDN basis) recommended by the NRC (1985 a) for the highest forage diet. The TDN value used for the bromegrass hay was the value closest to our estimate of TDN determined by in vitro dry matter disappearance.

Sampling

Experimental periods lasted 25 d, with the first 14 d used for adaptation to the respective diet. No indications of ruminal disfunction were apparent during periods of diet changes. From d 15 to 21, Cr_2O_3 was dosed (5 g/d) via gelatin capsules into the rumen twice daily in two equal portions at each feeding for use as an indigestible marker of digesta flow. Sample collections were 4 d in duration. At the morning feeding on d 22, duodenal (50 mL) and fresh fecal grab samples were collected at 6-h intervals. Collection times were advanced 3 h on d 23 to provide one sample for every 3-h interval in a theoretical 24-h period. Samples of concentrate mix, hay, and soybean oil were collected daily for later analysis. Duodenal and fecal samples were composited on approximately equal volume bases. On d 24 of each sampling period ruminal samples were collected and processed for VFA analysis, ruminal microbe isolation, pH, and passage rate determination. Ytterbium-labeled corn and bromegrass hay were delivered in the same proportion as the forage to concentrate ratio of the respective diets. Ruminal contents were collected at 0630 and 3, 6, 9, 12, 15, 18, 21, 24, 36, and 48 h from the time of dosing with passage rate markers. Ruminal samples collected at 24, 36, and 48 h were only for Yb and Co determination.

Sample processing and analysis

Feedstuffs and duodenal and fecal samples were dried and ground. Frozen samples of whole ruminal contents were thawed and blended with 0.9% saline (one part saline to two parts ruminal contents) in a household blender and bacteria-rich fractions prepared by differential centrifugation (Firkins et al., 1986). Bacteria-rich preparations were freeze-dried and ground with mortar and pestle in preparation for analysis.

Feed, duodenal, and fecal samples were analyzed for DM, ash, Kjeldahl N (AOAC, 1990), starch (MacRae and Armstrong, 1968), and NDF by non-sequential methods (Goering and Van Soest, 1970; without decalin, $NaSO_3$, or ethoxyethanol). Isolated ruminal microorganisms were analyzed for DM, ash, and Kjeldahl N (AOAC, 1990). Chromium, Yb, and Co concentrations (digesta and passage rate markers) were determined by atomic absorption spectroscopy (Hill and Anderson, 1958; Teeter et al., 1984; and Hart and Polan, 1984, respectively). Ruminal fluid supernatant was analyzed for NH_3 by the phenol-hypochlorite procedure (Broderick and Kang, 1980). Volatile fatty acid concentrations were determined by GLC (Goetsch and Galyean, 1983) using a Hewlett Packard 5890 Series II gas chromotograph equipped with a 15 m x 0.53 mm (i.d.) column (Nukol; Supelco, Bellefonte, PA) with an initial oven temperature of 110°C to final temperature of 150°C at 8°C/min. Helium was used as carrier gas with column flow rate of 20 mL/min. Injector and detector temperatures were 250°C.

Calculations and statistical analysis

Calculations

Duodenal and fecal OM flows were calculated as Cr dosed per d (3.4 g) divided by Cr concentration (OM basis) in the respective samples. Nutrient flows were calculated by multiplying OM flow by the percentage of the particular nutrient in the OM. Duodenal microbial N percentage was calculated as duodenal purine:N ratio divided by the ratio of purine:N in isolated bacteria (Zinn and Owens, 1986). Duodenal flow of microbial N was calculated by multiplying duodenal N flow by the microbial N:total N ratio. Duodenal flow of microbial OM was calculated as flow of duodenal microbial N divided by ruminal bacterial N:OM ratio.

Apparent ruminal, post-ruminal, and total tract nutrient digestibilities were calculated as the difference between nutrient intake, duodenal flow, and fecal excretion of nutrients, respectively. True ruminal OM and N digestibilities were determined by correcting apparent ruminal digestibilities for microbial OM and N flows to the duodenum. Microbial efficiency was expressed as grams of microbial N per kilogram of OM truly digested 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).

Statistical analysis

All data were analyzed by ANOVA using the GLM procedure of SAS (SAS Inst., Cary, NC) for a Latin square design. Ruminal data (pH, NH_3, and VFA) were analyzed as a split-plot to examine effects of time. The main plot was dietary forage level tested against the animal x dietary forage level interaction. Subplots included sampling time and forage level x time interaction tested against residual error. There were no treatment x time interactions ($P > 0.10$) detected for pH, NH_3 N, or VFA; therefore, only least-squares treatment means are presented. When *F*-tests were

significant, single degree of freedom orthogonal contrasts for unequally spaced treatments (Carmer and Seif, 1963) were used to determine linear, quadratic, cubic, and quartic effects of increasing forage level. Few cubic and quartic effects were observed; therefore, these effects will only be indicated in the text.

Results and Discussion

Ruminal measurements

pH

Ruminal pH was lowest ($P < 0.01$) with the highest concentrate diet (pH = 5.7) and increased (linear, $P < 0.01$) to 6.5 with the highest forage diet (Table 2). This response was expected because of the greater rate of digestion of processed, high-concentrate diets compared with that of high-forage diets (Mertens, 1993).

Ammonia concentrations

Ruminal NH_3 concentrations decreased (linear, $P < 0.05$) as dietary forage level increased. Ruminal NH_3 concentrations approached or were within ranges reported for maximal microbial growth (3 to 5 mg/dL; Satter and Roffler, 1975). Nonetheless, ruminal NH_3 values in the present study, per se, seemed low, which may have occurred because the diets were supplemented with soybean oil. Jenkins and Fotouhi (1990) also noted decreased ruminal NH_3 levels in lambs receiving diets containing 5.2% lecithin or 2.4% corn oil. Although ruminal pH is influenced by ammonia concentrations, changes in ruminal ammonia were not consistent with changes in pH. In the present study, changes in ruminal pH were more closely related to VFA concentrations.

Volatile fatty acids

Total VFA (Table 2) decreased (linear, $P < 0.001$; and quadratic, $P < 0.05$) as dietary forage increased. When dietary forage increased to 32.2%, total VFA concentration increased to 87.1 m*M*, then decreased to 65.8 m*M* with 72.9% forage. Although greater ruminal VFA was expected with increased levels of dietary concentrate, lower ruminal concentrations of VFA for ewes fed 18.4% forage than ewes fed 32.2% forage must be interpreted with caution because lower pH promotes VFA absorption from the rumen (Van Soest, 1994).

Molar proportions of acetate increased (quadratic, $P < 0.001$) as dietary forage increased. Linear decreases ($P < 0.001$) in molar proportions of propionate and butyrate were observed as dietary forage level increased. For butyrate, as dietary forage level increased, the response also was quadratic ($P < 0.001$) because the decrease was most pronounced between 18.4% and 32.2% dietary forage. The change in acetate and propionate with increased dietary forage were in opposite directions; thus, acetate to propionate ratios increased (linear, $P < 0.001$) with increased dietary forage. Molar proportions of isobutyrate increased 3.5-fold (linear, $P < 0.001$) as dietary forage level increased. Valerate and isovalerate molar proportions decreased ($P < 0.001$) with increased dietary forage. Third order polynomial effects were observed for butyrate, valerate, and isobutyrate; however, the greatest change occurred between the 18.4 and 32.2% forage diet, suggesting that the quadratic effects were biologically most significant.

The VFA responses to dietary forage level observed in the present study were consistent with previous documentation (Owens and Goetsch, 1993). Corn contains proteins rich in the branched-chain amino acids, which upon digestion would give rise to the branched-and odd-chain VFA. Generally, as dietary forage level is increased, molar proportions of acetate should increase because cellulolytic bacteria favor production of acetate (Bergman, 1990). Furthermore, as the dietary proportion of concentrate is increased, production of propionate and butyrate should increase (Owens and Goetsch, 1993).

Intake and digestibility of OM, starch, NDF, and N

Organic matter

A linear decrease ($P = 0.06$) in true ruminal OM digestibility was observed as dietary forage increased (Table 3). However, Kalscheur et al. (1997a) reported lower true ruminal OM digestibility in lactating cows fed 75% concentrate compared with cows fed 40% concentrate (52 vs. 38%). The contrast between studies is peculiar considering our observation that the 59.4% forage diet had the second lowest value for ruminal digestibility. Differences in feed intake between our study and that of Kalscheur et al. (1997a) also may explain some of the contrast between the studies.

Post-ruminal and total tract OM digestibilities decreased (linear, $P < 0.001$) with increased dietary forage. Sloughed digestive tract cells and microbial cells may have confounded total tract OM digestibility because increased dietary fiber increases erosion of the digestive tract lining and microbial contributions to fecal output (NRC, 1985b). Changes in total tract OM digestibility associated with the various dietary forage levels were comparable to expected differences for these types of diets (Table 1).

Compared with estimates of TDN (Table 1), observed total tract OM digestibilities were approximately two percentage units higher than expected for the 18.4 and 32.2% forage diets. However, OM digestibilities were greater than expected for the two highest forage levels (59.4% forage = 4.3%; 72.9% forage = 4.9%) suggesting a positive associative effect may have occurred when dietary forage level increased in oil-supplemented diets.

Starch

As intended, starch intake (Table 4) decreased (linear, $P < 0.001$) with increased dietary forage. Ruminal starch digestibility was not affected ($P = 0.76$) by dietary forage level. Both post-ruminal and total tract starch digestibility decreased ($P < 0.001$) as forage level increased. Abomasal infusion of increasing amounts of starch, dextrin, or glucose resulted in increased non-structural carbohydrate disappearing from the small intestine of steers (Kreikemeier et al., 1991). Similary, Matejovsky and Sanson (1995) observed an increase in starch digestibility in lambs fed increasing levels of corn.

Neutral detergent fiber

Intake and ruminal digestibility of NDF increased (linear, $P < 0.05$) with increased dietary forage (Table 5). However, post-ruminal and total tract NDF digestibilities were not affected ($P = 0.18$) by forage level. Increased ruminal NDF digestibility with dietary forage greater than 45.8% may be attributed to associative effects. Addition of 13.2% corn to a 60% bromegrass hay and 26.4% soybean hull diet fed to steers at 90% of ad libitum intake increased ruminal

NDF digestibility (Grigsby et al., 1993). In the present study, 30 and 16% corn in 59.4 and 72.9% forage diets, respectively, may have provided energy to support fibrolytic bacterial growth. Thus, a positive associate effect seems to have occurred in ewes fed the 59.4 and 72.9% forage diets, further supporting the greater than expected OM digestibility for these two diets.

Palmquist (1988) suggested that there is a negative impact of dietary fat on digestibility of fiber, which is attenuated when dietary forage level increases. Moreover, Mould et al. (1983a) showed that ruminal pH less than 6.2 depressed fiber digestion. Cellulolytic bacteria are inhibited when ruminal pH is less than 6.0 (Chen and Russell, 1989). Thus, lower ruminal NDF digestibility, when on the higher concentrate diets, was probably related to the lower ruminal pH associated with these diets as well as the potential negative impact that the supplemental oil may have had on fiber digestibility at the lower forage levels.

Negative associative effects were likely not related to ruminal pH with the diet containing 45.8% forage because ruminal pH was not low enough (pH = 6.2) to inhibit cellulolytic bacteria. Ørskov and Ryle (1990) noted that when rolled barley was added to ground hay the observed digestibility of DM was substantially less than would have been expected. In summarizing data of Mould et al. (1983b), Ørskov and Ryle (1990) calculated the digestibility of the hay and found it was reduced by 37.2% when the diet contained 57% rolled barley and 43% ground hay. Ørskov and Ryle (1990) also indicated that rapid utilization of nutrients by fast-growing bacteria may deprive slower growing cellulolytic organisms, which could result in reduced fiber digestion. Alternatively, Ørskov and Ryle (1990) suggested that the ruminal protozoa may be involved in determining associative effects. Ruminal protozoa are known to produce butyrate during fermentation (Wolin et al., 1997). Ruminal molar proportions of butyrate were lower when ewes were fed 45.8% forage than when fed the 18.4 and 32.2% forage diets, suggesting that the ruminal protozoa were less prevalent when fed the 45.8% forage diet. Because the protozoa remove starch from the ruminal mileu, the possibility of negative associative effects associated with substrate competition would be greater in ewes fed the 45.8% forage diet.

A shift in fiber digestibility from the rumen to the post-ruminal digestive tract was reported by Elliott et al. (1997), who indicated no change in total tract digestibility of fiber with diets containing supplemental fat. Microbial digestion of fiber in the lower gut may compensate for the reduction in ruminal fiber digestion caused by dietary fat (Demeyer, 1991). The same response appeared to have occurred in the present study because total tract NDF digestibility was not different across treatments despite detection of differences among treatments for ruminal NDF digestibility. Numerical trends for post-ruminal NDF digestibility across dietary forage levels would also support this supposition.

Nitrogen

Duodenal flow of total N increased from 23.5 to 26.1 g/d when dietary forage increased from 18.4 to 32.2%, but then duodenal N flow decreased thereafter, resulting in an overall quadratic effect ($P < 0.05$; Table 6). Duodenal flow of feed N and NH_3 N were not affected by diet ($P = 0.39$), but duodenal flow of microbial N decreased (linear, $P < 0.05$) with dietary forage level. Therefore, true ruminal N digestibility was not affected ($P = 0.29$) by dietary forage level. Generally, values for duodenal flow of N appear to be greater than N intake. We attribute this difference to N recycling that probably occurred in response to the low level of ruminal degradable protein consumed (5.6%) as determined by the relationship between CP and true ruminal digestibility.

Microbial efficiency was highest for the 45.8% forage diet (quadratic, $P < 0.001$). Values for microbial efficiencies in the present study were in the range of those reported by Kalscheur et al. (1997a,b). Our results for microbial efficiency support the possibility that the ruminal protozoa were less prevalent in ewes fed 45.8% forage. Ruminal protozoa often prey upon bacteria, which would decrease microbial N flow and efficiency (Ushida et al., 1991). Alternatively, if the faster growing bacteria were more prevalent than the slower growing fibrolytic bacteria in ewes fed the 45.8% forage diet, greater microbial efficiency would be expected because faster growing bacteria expend proportionally less energy to support maintenance functions (Russell et al., 1992). In that regard, Kalscheur et al. (1997a) reported higher microbial efficiencies for cows fed a low-forage diet compared with a high-forage diet. Furthermore, in sheep fed at maintenance, high-concentrate diets supported less efficient microbial protein synthesis which could be attributed to lower ruminal dilution rates characteristic of this type of diet (Harrison and McAllen, 1980).

Post-ruminal N digestibility decreased (linear, $P < 0.05$) with increased dietary forage, perhaps reflecting the lower quality protein of the forage. Although not a great decrease (6% of the 18.4% forage diet value), total tract N digestibility decreased (linear, $P < 0.01$) with increased dietary forage. Kalscheur et al. (1997a) reported no differences in total tract N digestibility between cows fed low- or high-forage diets. As mentioned previously for OM digestibility, however, results of total tract N digestibility may be confounded by greater erosion of the digestive tract lining and microbial contributions to fecal output as dietary fiber increased with increased dietary forage.

Overall, incrementally increasing forage level in a diet that contained soybean oil and fed at a restricted level to mature ewes resulted in slightly higher than expected total tract OM digestibility for the lower fiber diets, and approximately 5.5% greater than expected OM digestibility for the higher fiber diets. Combined with a high post-ruminal OM digestibility, total tract OM digestibility appeared normal. As forage level increased, ruminal NDF digestibility increased indicating inhibitory effects of the dietary oil were overcome by increasing dietary fiber. Post-ruminal digestion of protein decreased as dietary forage level increased, and total tract protein digestibility coefficients were comparable to expected values.

Implications

Much of what has been learned regarding digestion in ruminants as the level of dietary forage is increased or decreased would not be altered by feeding mature ewes restricted diets that contain soybean oil at about 3 to 4% of dietary DM.

However, additional research evaluating associative effects in high-forage, low-concentrate diets with inclusion of dietary fat is warranted.

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Table 1. Ingredient and nutrient composition of diets fed to ewes

	Dietary forage, %[a]				
Item	18.4	32.2	45.8	59.4	72.9
Ingredient[b]					
Bromegrass hay, chopped (2.54 cm)	18.4	32.2	45.8	59.4	72.9
Cracked corn	72.7	58.7	44.5	30.3	16.5
Soybean oil	3.2	3.4	3.9	4.4	4.7
Corn gluten meal	2.8	3.0	3.3	3.6	3.9
Urea	0.7	0.7	0.7	0.7	0.7
Limestone	2.3	2.0	1.8	1.6	1.3
Nutrient composition[c]					
DM, %	92.0	92.9	93.2	94.1	94.7
	---------- % of DM ----------				
OM	86.3	86.8	86.6	86.5	86.6
ADF	13.2	18.5	23.9	28.9	34.3
NDF	28.1	36.5	44.1	51.7	59.3
CP	14.2	14.0	13.9	13.8	13.8
Crude fat	6.5	6.4	6.4	6.4	6.3
Starch	52.5	44.1	32.6	25.3	15.2
TDN[c]	81.8	78.0	74.7	70.2	66.6

[a]All diets included 33 mg of Rumensin per kg of diet.
[b]Ingredient values are expressed as a percentage of DM.
[c]Calculated from tabular values (NRC, 1985a).

Table 2. Ruminal pH, ammonia, passage rates, and volatile fatty acids (VFA) of ewes fed diets with different forage levels containing 6% crude fat

	Dietary forage, %						
Item	18.4	32.2	45.8	59.4	72.9	SEM	Contrast[a]
PH	5.7	5.9	6.2	6.2	6.5	0.08	L^{***}
NH_3, mg/dL	4.1	3.6	3.4	4.0	2.8	0.04	L*
Particulate passage rate, %/h	4.7	3.4	3.4	3.6	3.0	0.93	
Fluid passage rate, %/h	6.0	5.8	5.8	5.9	5.6	0.31	
VFA :							
Total, m*M*	80.5	87.1	77.2	78.3	65.8	3.3	L^{***} Q^{*}
	----------mol/ 100 mol----------						
Acetate	46.3	54.5	58.5	61.3	64.3	0.8	L^{***} Q^{***}
Propionate	34.7	33.2	30.9	27.6	25.6	0.7	L^{***}
Butyrate	14.4	9.9	7.6	7.9	7.2	0.4	L^{***} Q^{***} C^{*}
Isobutyrate	0.2	0.3	0.3	0.6	0.7	0.1	L^{***}
Valerate	2.2	1.0	1.3	1.2	1.0	0.1	L^{***} Q^{***} C^{***}
Isovalerate	2.2	1.0	1.3	1.2	1.0	0.1	L^{***} Q^{***} C^{***}
A:P[b]	1.4	1.7	1.9	2.3	2.6	0.1	L^{***}

[a]Contrasts: L = linear, Q = quadratic, C = cubic, and Qr = quartic. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.
[b]Acetate:propionate ratio.

Table 3. Intake and digestibility of OM in ewes fed diets with different forage levels containing 6% crude fat

	Dietary forage, %						
Item	18.4	32.2	45.8	59.4	72.9	SEM[a]	Contrast[b]
Intake, g/d	713.9	728.0	728.6	735.0	726.9	7.1	
True digestibility, % of intake	49.7	49.3	29.1	33.8	34.7	7.4	L†
Post-ruminal digestibility, % entering duodenum	77.1	74.3	70.3	68.5	63.4	2.2	L***
Total tract digestibility, % of intake	84.0	79.5	74.3	74.5	70.5	1.6	L***

[a]N = 5.
[b]Contrasts: L = linear, Q = quadratic, and C = cubic. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\dagger}P < 0.10$.

Table 4. Intake and digestibility of starch in ewes fed diets with different forage levels containing 6% crude fat

	Dietary forage, %						
Item	18.4	32.2	45.8	59.4	72.9	SEM[a]	Contrast[b]
Intake, g/d	429.5	370.1	274.5	214.6	127.9	11.3	L***
Apparent ruminal Digestibility, % of intake	84.4	79.9	78.4	85.6	79.3	4.9	
Post-ruminal digestibility, % entering duodenum	97.0	96.0	89.7	81.2	79.1	3.0	L***
Total tract digestibility , % of intake	99.6	99.3	98.1	97.5	96.2	0.5	L***

[a]N = 5.
[b]Contrasts: L = linear. $^{***}P < 0.001$, $^{**}P < 0.01$.

Table 5. Intake and digestibility of NDF in ewes fed diets with different forage levels containing 6% crude fat

	Dietary forage, %						
Item	18.4	32.2	45.8	59.4	72.9	SEM[a]	Contrast[b]
Intake, g/d	236.8	305.7	370.8	439.5	497.9	10.0	L***
Apparent ruminal Digestibility, % of intake	26.1	33.1	22.8	39.6	42.9	5.1	L*
Post-ruminal digestibility, % entering duodenum	60.4	56.3	61.8	58.3	51.4	3.7	
Total tract digestibility, % of intake	70.7	70.5	70.7	75.0	72.9	2.1	

[a]N = 5.
[b]Contrasts: L = linear and Q = quadratic. *** $P < 0.001$, * $P < 0.05$.

Table 6. Intake, digestibility, and duodenal flow of N in ewes fed diets with different forage levels containing 6% crude fat

	Dietary forage, %						
Item	18.4	32.2	45.8	59.4	72.9	SEM[a]	Contrast[b]
Intake, g/d	18.7	18.7	18.7	18.8	18.5	0.2	
Duodenal flow, g/d:							
Total N	23.5	26.1	25.3	24.6	22.1	1.3	Q*
Feed N	9.5	11.7	11.3	11.8	10.9	1.3	
NH_3 N	2.1	3.2	1.7	2.9	1.4	0.6	
Microbial N	11.3	10.3	11.0	9.8	9.8	0.5	L*
True ruminal digestibility, % of intake	47.3	36.6	37.7	37.4	42.0	7.3	
Microbial efficiency[c]	34.3	39.4	47.3	40.1	37.1	2.6	Q***
Post-ruminal digestibility, % entering duodenum	82.4	83.6	79.4	79.9	78.1	1.9	L*
Total tract Digestibility, % of intake	78.7	76.9	72.1	73.9	73.7	1.8	L*

[a]N = 5.
[b]Contrasts: L = linear and Q = quadratic. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and † $P < 0.1$.
[c]Microbial efficiency was calculated as duodenal flow of microbial N, grams, divided by the kilograms of OM truly fermented.