Ruminal Microbial Protein Synthesis in Sheep Fed Forages of Varying Nutritive Values

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Summary

Six wethers, fitted with ruminal and duodenal cannulae, were utilized in a 6 x 6 Latin Square metabolism trial to determine efficiency of microbial protein synthesis in the rumen of sheep fed forages with varying nutritional quality. Ground alfalfa hay, oat-berseem clover hay, and baled corn crop residues were fed at an *ad libitum* or limited intake level. Chromium-mordanted fiber, cobalt-EDTA, and purines were used to determine digesta flow and solid passage rate, dilution rate, and microbial protein production, respectively. Sheep fed alfalfa hay had greater organic matter (OM) intakes, and amounts of OM apparently and truly ruminally digested (g/d; $P < .05$) than sheep fed either oat-berseem clover or corn crop residues at the *ad libitum* intake level. Rates of slow solid and liquid passage, and postfeeding ruminal ammonia-nitrogen (N) and volatile fatty acids (VFA) concentrations were lower ($P < .05$) in sheep fed corn crop residues than those fed alfalfa or oat-berseem clover hay. Total duodenal flows (g/d) and efficiencies of ruminal synthesis (g crude protein/100 g of OM truly digested; $P < .05$) of microbial protein were less in sheep fed corn crop residues than in sheep fed alfalfa, and oat-berseem clover *ad libitum*. Whereas total duodenal microbial-N flow was related to organic matter intake (OMI; $r^2 = .97$) and OM truly digested in the rumen (OMTDR; $r^2 = .97$), microbial efficiency was related to g of nitroge truly digested in the rumen (NTDR)/100 g of OMTDR ($r^2 = .82$) and slow solid passage rate ($r^2 = .91$).

Introduction

Amino acids entering the duodenum of ruminant animals primarily come from either dietary, bacterial or endogenous origin. Because microbial protein synthesis in the rumen supplies 40 to 80% of the amino acids entering the duodenum of forage-fed cattle, the quantification of microbial nitrogen flow into the duodenum of animals is critical for diet calculations. One way to express the amount of microbial protein flow to the duodenum is by the use of efficiency of microbial protein synthesis expressed as g of microbial CP/ 100 g of TDN. It has been proposed that efficiency of microbial protein synthesis is 13.05 g of bacterial CP produced in the rumen per 100 g of TDN consumed, but the efficiency of bacterial CP synthesis has been reported to vary greatly among animals fed different forages. Although the efficiency of bacterial CP synthesis in the rumen of cattle fed different forages is known to vary, there has been little attempt to quantify the relationships between the efficiency of bacterial CP synthesis and other dietary factors. Understanding these relationships is necessary to maximize the efficiency of beef cow nutrition by optimizing protein supplementation.

Therefore, the objectives of this experiment were to determine the amount and efficiency of microbial protein synthesis in ruminants fed forages of differing nutritional value at two intake levels using sheep as a model. Furthermore, we wished to quantify the relationship between microbial protein synthesis and other dietary factors.

Materials and Methods

A digestibility trial was conducted using six wethers, surgically fitted with a rubber cannula in the rumen and a closed t-type cannula in the proximal duodenum, in a 6 x 6 Latin Square design. First harvest forage from a field containing a mixture of oats (Avena sativa L.) and berseem clover (Trifolium alexandrium cv. BigBee) was mowed when oats were at the boot stage. Third harvest alfalfa (Medicago sativa L.) forage was mowed at the first flower stage. The two hays and crop residues from corn (Zea mays L.) were baled as large round bales and stored outside on tires until initiation of the experiment on 3 December 1996. Prior to feeding, all forages were ground through the 5-cm screen of a tub-grinder and stored in a barn.

Diets consisted of corn crop residues, alfalfa hay, or oat-berseem clover hay fed at *ad libitum* and limited levels of intake. The *ad libitum* intake level was determined during the first 7 days of each adjustment period, and 115% of *ad libitum* intake was offered to those fed *ad libitum*. The limited intake level of corn crop residues was equal to 75% of the *ad libitum* intake level of corn crop residues, based on the percentage of metabolic body weight (BW). The limited intake levels of alfalfa and oat-berseem clover hays were equal to the *ad libitum* intake level of corn crop residues. Diets were fed twice daily at 0800 and 2000 h. Sheep had continuous access to clean water and a trace mineralized salt block during the experiment. Sheep were housed in pens within a temperature controlled barn.

Periods lasted for 22 days and consisted of 12 days of diet adaptation followed by 10 days of sample collection. To determine duodenal and fecal DM flow, 1.5 g chromium-mordanted fiber containing approximately 2% Cr was inserted through the ruminal cannula of each sheep at 0800 and 2000 h from d 5 to 16 of each period.

Feed and uneaten feed samples were collected on days 12 through 16 of each period. Duodenal digesta and rectal fecal samples were collected four times daily on days 14 through 16 to determine forage digestibility and bacterial protein synthesis. For determination of ruminal NH_3-N concentrations and to isolate ruminal bacteria, ruminal fluid samples were collected at 0, 2, 4, 6, 8, and 10 hours post-feeding on day 17 of each period. Rectal fecal samples were collected on days 16 through 20 to determine the solid and liquid passage rates using Cr-mordanted fiber and Co-EDTA, respectively.

Treatment differences are significant at the .05 level of probability.

Results and Discussion

As expected, both hays had lower neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations and higher crude protein (CP) concentrations than corn crop residues (Table 1). Furthermore, alfalfa hay had lower concentrations of NDF and ADF and a higher CP concentration than oat-berseem clover forage, reflecting the high proportion of oats in the first harvest forage.

Organic matter intakes of sheep fed alfalfa hay were greater than those fed oat-berseem clover hay or corn crop residues *ad libitum*, expressed either as grams per day or as a percentage of body weight (Table 2). As designed, limit-fed sheep had lower organic matter intakes than sheep fed *ad libitum*. Although there were large differences in organic matter intake in sheep fed alfalfa or oat-berseem clover hay at the *ad libitum* and limited intake levels, there was little difference in organic matter intake by sheep fed corn crop residues at the two intake levels.

Amounts of organic matter apparently or truly digested in the rumen and total tract were less in sheep fed corn crop residues than the two hays and were less in sheep fed oat-berseem clover hay than in those fed alfalfa hay because of differences in organic matter intake. As designed, amounts of organic matter apparently or truly digested in the rumen and total tract were greater in sheep fed *ad libitum* than those limit-fed. Apparent digestibility of organic matter in the rumen was not affected by forage species or intake level. However, the true digestibility of organic matter was greater in sheep fed hay than in those fed corn crop residues because the hays had lower NDF and ADF concentrations than corn crop residues. Apparent total tract digestibilities of organic matter were higher in sheep fed oat-berseem clover or alfalfa hay than in those fed corn crop residues. Forage intake level did not affect apparent total tract organic matter digestibility of corn crop residues. However, apparent total tract organic matter digestibilities of the two hays were higher at the *ad libitum* intake level than in sheep that were limit-fed.

Crude protein intake, represented by nitrogen intake, was higher in sheep fed the two hays than in those fed corn crop residues, was higher in sheep fed alfalfa hay than in those fed oat-berseem clover hay and was higher in sheep fed forages at the *ad libitum* intake level than in those limit-fed (Table 3). Because of differences in CP intake, amounts of CP apparently and truly digested in the rumen and total tract were lower in sheep fed corn crop residues than in those fed two hays, were lower in sheep fed oat-berseem clover hay than in those fed alfalfa hay, and were lower in limit-fed than in those fed *ad libitum*. Apparent and true ruminal and total tract CP digestibilities were higher in sheep fed the two hays than in those fed corn crop residues. Furthermore, apparent and true ruminal and total tract CP digestibilities of sheep fed alfalfa hay were greater than those fed oat-berseem clover hay.

Sheep fed oat-berseem clover or alfalfa hay had greater amounts of crude protein in the form of nitrogen flowing into the duodenum compared with sheep fed corn crop residues (Table 4). The greater nitrogen flow in sheep fed the hays than in those fed corn crop residues resulted from greater duodenal flows of microbial-, undegraded dietary-, and ammonia-nitrogen in sheep fed the two hays. The proportions of consumed crude protein in corn crop residues, oat-berseem clover hay, and alfalfa hay that escaped ruminal degradation were 60, 34 and 17% when limit-fed and 43, 31, and 15% when fed at the *ad libitum* intake level. Because sheep fed oat-berseem clover or alfalfa hay at the *ad libitum* intake level had greater microbial protein synthesis in the rumen and escape dietary protein entering the duodenum compared with sheep that were limit-fed, sheep fed oat-berseem clover or alfalfa hay at the *ad libitum* intake level had greater amounts of protein-nitrogen flowing into the duodenum than limit-fed sheep. Duodenal nitrogen flow in sheep fed corn crop residues was not affected by intake level. Efficiencies of microbial protein synthesis in the rumen, expressed as grams of microbial CP per 100 grams of organic matter truly digested in the rumen were greater in sheep fed oat-berseem clover or alfalfa hay than in those fed corn crop residues, but did not differ between sheep fed the two hays.

Sheep fed corn crop residues had lower ruminal NH_3-N concentrations at all sampling times than sheep fed either of the hays (Figure 1). Furthermore sheep fed alfalfa hay had greater NH_3-N concentrations at 2 and 4 hours postfeeding than sheep fed oat-berseem clover hay. Intake level did not affect NH_3-N concentrations.

The slow rate of particulate passage, reflecting the time required for particle size to be reduced, and the rate of liquid passage from the rumen were greater in sheep fed the two hays than in those fed corn crop residues (Table 5). Feed intake level had no effect on digesta passage rates. The slow rate of particulate passage and the rate of liquid passage, however, tended to be lower in sheep limit-fed oat-berseem clover or alfalfa hay than in those fed *ad libitum*, but did not differ in sheep fed corn crop residues at the two intake levels.

In linear regressions predicting daily microbial protein synthesis, organic matter intake as a percentage of body weight (r^2=.97), the amount of organic matter truly digested in the rumen expressed as a percentage of body weight (r^2=.98), the slow rate of particulate passage (r^2=.91), and the rate of liquid passage (r^2=.90) were significant variables (Table 6 and Figure 2). The efficiency of microbial protein synthesis was predicted by quadratic equations for the slow rate of particulate passage (r^2=.92), the rate of liquid passage

(r^2=.78), and the ratio of the amount of nitrogen truly digested in the rumen to the amount of organic matter truly digested in the rumen (r^2=.82).

In stepwise multiple regressions predicting daily microbial CP synthesis in the rumen, the amounts of organic matter truly digested in the rumen as a percentage of body weight (OMTDR) and the ratio of the amount of nitrogen truly digested in the rumen to the amount of organic matter truly digested in the rumen (NTDR:100 g OMTDR) were significant variables {y = -1.50 + 5.77 (OMTDR) + .51 (NTDR/100 g OMTDR); r^2=.99}. The only variable chosen to predict efficiency of microbial CP synthesis in the rumen was the ratio of the amount of nitrogen truly digested in the rumen to the amount of organic matter truly digested in the rumen {y = 10.55 + .83 (NTDR/100 g OMTDR); r^2=.75}. These results indicate that daily microbial protein synthesis is dependent on the amount of organic matter digested in the rumen as affected by forage intake and digestibility and the ratio of the amounts of nitrogen and organic matter digested in the rumen, implying a need for a balance in available nitrogen and energy. However, the efficiency of microbial protein synthesis is primarily a function of the ratio of the amounts of nitrogen and organic matter digested in the rumen.

Implications

Microbial protein synthesis and efficiency were lower in animals fed corn crop residues compared with animals fed either alfalfa hay or oat-berseem clover hay. Microbial protein synthesis could be predicted from organic matter intake, the amount of organic matter truly digested in the rumen, and the rate of passage. But the efficiency of microbial protein synthesis was, however, related to the rate of passage, the amount of organic matter truly digested in the rumen and the ratio of the amounts of nitrogen and organic matter truly digested in the rumen. The significance of these values is that if an efficiency of microbial protein synthesis of 10.9 grams of microbial CP is synthesized per 100 grams of organic matter truly digested in the rumen is assumed for a cow consuming corn crop residues, this cow should be supplemented with 2.3 lb. of a protein supplement containing 40% CP to yield a dietary CP concentration of 7.5%. However, if the default efficiency in the NRC requirements of 13.05 grams of microbial protein synthesized per 100 grams of organic matter truly digested in the rumen is assumed for a cow consuming corn crop residues, this cow would need to be supplemented with 3.1 lb. of the 40% CP supplement to yield a dietary CP concentration of 8.5%.

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Table 1. Chemical composition of forage species fed to sheep.

Item	Forage species: Corn crop residue	Oat\berseem clover	Alfalfa	SE[a]
DM, %	90.8	91.5	90.9	.29
% of DM				
OM	93.3[b]	89.6[c]	91.9[d]	.35
CP	4.1[b]	13.9[c]	18.1[d]	.51
NDF	75.7[b]	62.2[c]	42.0[d]	1.05
ADF	43.3[b]	37.4[c]	28.4[d]	1.05

[a] Standard error of means
[bcd] Row means without a common superscript differ from each other, P<.05.

Table 2. Organic matter intake and OM digested in the rumen and total tract of sheep fed different forages.

	Forage species (sp) and intake level (i)						Significance			
	Corn crop residue		Oat/berseem clover hay		Alfalfa hay					
Item	Low	High	Low	High	Low	High	SE[a]	sp	i	sp x i
OM intake,										
g/d	446	452	539	883	519	1189	37.0	.01	.01	.01
% of BW	1.36	1.59	1.63	2.86	1.71	4.15	.122	.01	.01	.01
OM digested, g/d										
Apparent ruminal	202	205	245	368	245	589	24.8	.01	.01	.01
True ruminal	267	268	335	528	335	836	29.8	.01	.01	.01
Apparent total tract	237	240	288	515	306	769	34.3	.01	.01	.01
Digestion coefficient, % of intake,										
Apparent ruminal	45.1	44.3	45.7	42.5	47.4	49.6	1.56	.57	.61	08
True ruminal	58.6	58.2	62.5	61.2	64.9	70.4	2.42	.07	.64	.18
Apparent total tract	53.2	52.5	55.0	58.4	59.2	64.5	2.53	.01	.04	.06

[a] Standard error of means

Table 3. Nitrogen intake and crude protein digested in the rumen and total tract of sheep fed different forages.

	Forage species (sp) and intake level (i)						Significance			
	Corn crop residue		Oat/berseem clover hay		Alfalfa hay					
Item	Low	High	Low	High	Low	High	SE[a]	sp	i	sp x i
N intake,										
g/d	3.32	3.92	13.5	21.7	16.4	38.5	1.51	.01	.01	.01
CP digested, g/d										
Apparent ruminal	-15.5	-10.2	16.6	23.7	38.8	97.7	7.5	.01	.01	.01
True ruminal	6.7	12.5	52.3	89.1	80.7	195.6	10.6	.01	.01	.01
Apparent total tract	3.5	6.5	51.9	87.8	74.9	182.2	9.2	.01	.01	.01
Digestion coefficient, % of intake,										
Apparent ruminal	-74.6	-45.5	18.9	16.9	36.5	39.8	4.1	.01	.03	.04
True ruminal	35.7	43.9	62.8	65.5	77.7	82.2	9.6	.01	.53	.93
Apparent total tract	15.0	25.8	61.3	64.2	72.2	75.1	3.1	.01	.14	.52

[a] Standard error of means

Table 4. Duodenal N flow and efficiency of microbial protein synthesis in sheep fed different forages.

	Forage species (sp) and intake level (i)									
	Corn crop residue		Oat/berseem clover hay		Alfalfa hay		Significance			
Item	Low	High	Low	High	Low	High	SE[a]	sp	i	sp x i
N flow to duodenum, g/d										
Total-N	5.81	5.55	10.86	17.92	10.23	22.82	.78	.01	.01	.01
Microbial-N	3.56	3.62	5.76	10.47	6.71	15.67	.97	.01	.01	.01
NH_3-N	0.24	0.23	0.55	0.76	0.71	1.22	.10	.01	.02	.13
Dietary and Endogenous-N	2.01	1.69	4.55	6.70	2.82	5.94	1.08	.01	.16	.50
Efficiency of microbial protein synthesis										
MOEEF, G CP/100 g OMTDR	10.8	10.9	12.7	14.2	14.4	12.9	1.15	.01	.93	.45

[a]Standard error of means

Table 5. The rates of fast solid (k_{pf}), slow solid (k_{ps}), and liquid (k_d) passage of sheep fed different forages.

	Forage species (sp) and intake level (i)									
	Corn crop residue		Oat/berseem clover hay		Alfalfa hay		Significance			
Item	Low	High	Low	High	Low	High	SE[a]	sp	i	sp x i
	h^{-1}									
k_{pf},	.268	.280	.264	.231	.201	.263	.025	.63	.59	.27
k_{ps},	.015	.013	.018	.025	.022	.058	.006	.01	.23	.10
k_d,	.075	.077	.088	.089	.084	.103	.005	.01	.41	.10

[a]Standard error of means

Table 6. Regressions predicting the amount and efficiency of microbial protein synthesis from intake and digestion values in sheep fed different forages.

	Equations		r^2	
Independent Variables	Growth	Efficiency	Growth	Efficiency
DMI, %of BW	y= 3.83x - 1.49	$y=-1.22x^2 + 7.82x + 2.43$	.97	.62
OMI, %of BW	y = 4.23x – 1.72	$y= -1.48x^2 + 8.71x + 2.03$	.97	.60
OMTDR, % of BW	y = 6.52x –1.22	$y= - 3.66x^2 + 13.61x +2.43$	.98	.71
NDFI, % of BW	y= 7.56x – 2.53	$y= 9.15x^2 – 25.63 + 28.6$	.65	.71
ADFI, % of BW	y= 12.57x + 2.93	---[a].81--[a]		
k_p, h^{-1}	y = 267.87x +1.08	$y= -7948.5x^2 + 614.9x +4.13$	.91	.92
k_d, h^{-1}	y = 441.26x – 30.32	$y= -11725x^2 + 2171.9x – 86.455$	.90	.79
NTDR/OMTDR	y = 2.06x – 2.43	$y= -.306x^2 + 2.26x +9.53$	.50	.82

[a] Not significant, P>.05.

Figure 1. Ruminal NH_3-N concentrations in sheep fed different forages. CS=corn crop residue, Cs-L=corn crop residue at low level of intake, O\Br=oat-berseem clover, O\Br-L= oat-berseem clover at low level of intake, Alf=alfalfa hay, Alf-L=alfalfa hay low level of intake. SE=1.59, 2.64, 1.98, 1.52, .82, and .82 for from 0 to 10 h post-feeding, respectively.

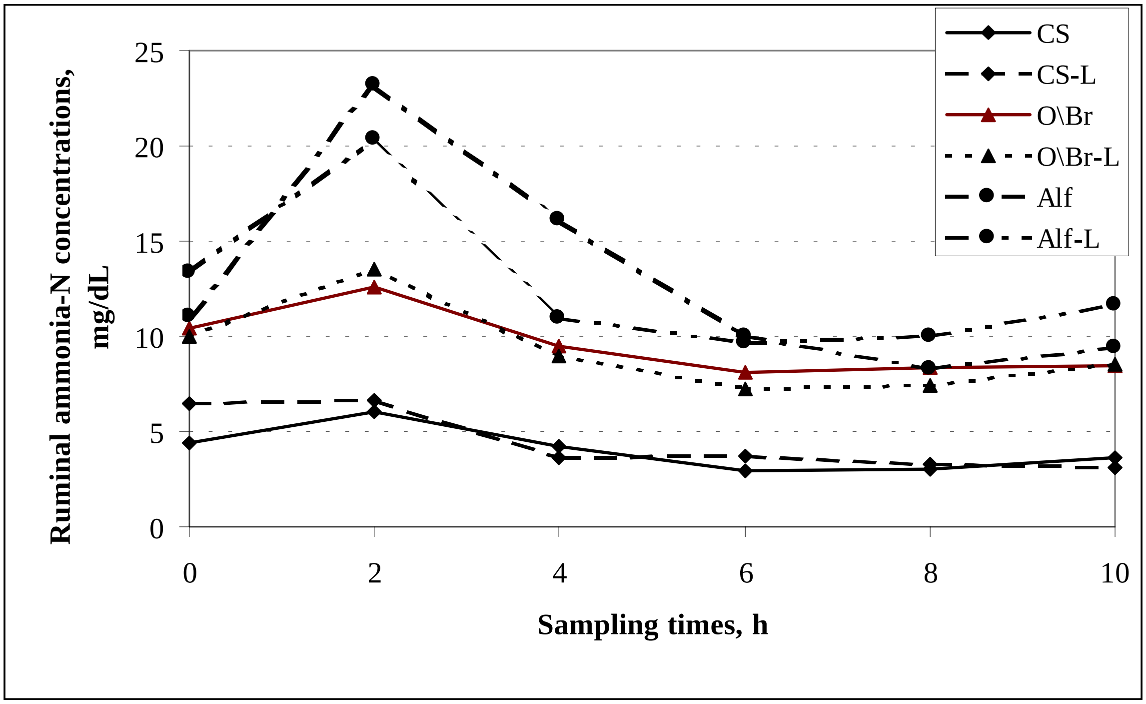

Figure 2. Regressions predicting relationship between microbial protein synthesis and efficiency. Mic=microbial nitrogen production (g/d), MOEFF=efficiency of microbial protein synthesis (g MCP/100 g OMTDR).