1967

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A. S. Series 67-21

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It is well known that the ruminant forestomach (ruminoreticulum) harbors large populations of bacteria and protozoa. These organisms engage in a variety of degradative as well as synthetic activities beneficial to the host animal. A considerable amount of research has been devoted to the study of the fermentative capabilities of the rumen bacteria. However, the significance of the rumen protozoa in the nutrition of the ruminant is less well understood. Protozoa have been thought to perform most of the biochemical activities common to the bacteria. It has been reported that animals with protozoa are thriftier and make faster weight gains than animals without protozoa. The presence of protozoa in the rumen is also known to improve ration digestibility and nitrogen retention and to alter the nature of fermentation in the rumen.

Certain feeding and management conditions influence the size of protozoal populations in the rumen. Fine grinding or pelleting the ration and full feeding as opposed to restricting feed intake appear to reduce protozoal numbers. Increasing the level of concentrates in the ration has lowered protozoal numbers in some instances. However, recent results from this station show that sizable populations of protozoa were maintained in the rumen of cattle fed diets composed entirely of concentrates.

This experiment was conducted to determine the effect of energy level of the diet and the presence or absence of the rumen protozoa on the digestibility of ration components, nitrogen retention and rumen fermentation.

Procedure

Twenty-four crossbred wether lambs, averaging about 68 lbs., were made free of protozoa by starvation followed by treatment with copper sulfate. About 2 weeks after treatment 12 of the lambs were given a concentrated suspension of washed protozoa by means of a rubber tube introduced into the rumen via the esophagus. Six lambs in each protozoa treatment group were fed a low-energy and 6 lambs a high-energy ration. The low-energy ration was composed of 75% bromegrass hay and 25% concentrates. The high-energy ration contained 25% bromegrass hay and 75% concentrates. The ingredient composition and proximate analysis of the rations is shown in table 1. The roughage was passed through a field chopper and then through a hammermill equipped with a 1-inch screen. The ground hay was mixed with

Prepared for Area Sheep Field Days, November 27-30, December 1, 1967.

the concentrate portion at the time of feeding. The lambs were kept in individual metabolism cages during the 48-day trial. Each lamb was fed 1000 grams of feed daily in 2 equal portions. Water cups were emptied and filled with fresh water twice daily. All lambs were kept in a large room of the nutrition laboratory with a tarpauline suspended between lambs with protozoa and those without protozoa.

TABLE 1. Ingredient Composition and Proximate Analysis of Rations

	High-energy ration	Low-energy ration
Ingredient Composition, %		
Bromegrass hay	25.0	75.0
Ground corn	70.9	21.5
Soybean meal	1.5	1.5
Urea[a]	1.0	1.0
Trace mineral salt	0.5	0.5
Ground limestone	1.0	0.4
Dicalcium phosphate	0.1	0.1
Nopcay "30"[b]	1.67 gm.	1.67 gm.
Proximate Analysis (dry matter basis), %		
Crude protein	15.0	13.4
Crude fiber	10.7	27.0
Ether extract	2.2	1.7
Ash	5.2	7.2
Nitrogen-free extract	66.8	50.5

[a] 281% protein

[b] Contains 30,000 I.U. vitamin A per gram

Digestion and Nitrogen Balance Study

A 7-day total collection digestion and nitrogen balance trial was conducted following a 3-week adaptation period. Fecal collections were made each morning and evening and dried in a forced air oven at 75° C. for 48 hours. Urine was collected once daily in polyethylene vessels containing 10 ml. of 50% hydrochloric acid solution. A 10% aliquot of each day's urine output was stored under refrigeration for nitrogen analysis. Samples of feed, refused feed and dried feces were analyzed for proximate principles.

Rumen Fermentation and Blood Studies

At the completion of the digestion and balance trial, rumen fluid was collected using a stomach tube-suction strainer apparatus before and at 3 hours after the morning feeding. The procedure was to sample 2 lambs on each treatment per sampling day and to sample 3 days in each of 2 weeks for a total of 12 samples per treatment. Total volatile fatty acids (VFA), the proportions of VFA and the concentration of ammonia nitrogen were determined on each sample. Rumen contents of all lambs were examined periodically for the presence or absence of protozoa and counts of protozoa were made on lambs with protozoa at the end of the experiment. Jugular blood was collected before and at 6 hours after feeding and immediately analyzed for urea nitrogen. The sampling times for blood were the same as outlined for the rumen studies.

Results

The weight gains ranged from 0.25 to 0.27, with the high-energy ration and 0.14 to 0.18, for the low-energy ration with essentially no difference between lambs with or without protozoa. The gains were not typical of those expected under feedlot conditions. Nevertheless, all lambs appeared healthy and no digestive disturbances were encountered.

Digestion and Nitrogen Balance Study

The results of these studies are shown in table 2. The digestibility of nutrients in the high-energy ration was greater than for the low-energy ration in all instances except for crude fiber. These results are in agreement with what has been reported for mixed rations containing roughages and concentrates.

Digestibility of nutrients was higher for lambs with protozoa than for protozoa-free lambs except for ether extract in the high-energy ration. Any difference in ether extract digestibility would not be of great importance in view of its low concentration in the ration. Generally, the depression in digestibility in the absence of protozoa was slightly greater for lambs fed the higher energy ration.

TABLE 2. Apparent Digestibility and Nitrogen Retention Data

	Protozoa		Protozoa-free	
	High Energy	Low Energy	High Energy	Low Energy
No. of lambs	5[a]	6	6	6
Dry matter intake, gm.	866	823	862	861
	Digestibility, %			
Dry matter	80.8	68.0	76.3	64.9
Crude protein	80.0	76.6	75.0	73.8
Crude fiber	51.6	56.8	40.2	53.4
Ether extract	70.5	55.3	71.3	51.7
Nitrogen-free extract	88.2	73.4	80.4	71.9
	Nitrogen Balance			
Nitrogen intake, gm.	22.5	19.4	22.4	20.1
Fecal nitrogen, gm.	4.5	4.6	5.6	5.3
Urinary nitrogen, gm.	10.0	9.5	7.6	9.5
Nitrogen retained, gm.	8.1	5.3	9.2	5.4
As % of intake	36.0	27.3	41.1	26.9
As % of absorbed	44.8	35.7	54.5	34.8

[a]Data are not included for one lamb whose feed consumption was low.

Nitrogen retention expressed as percent of intake or absorbed was esstentially the same for lambs with protozoa as for lambs free of protozoa with the low-energy ration. In the case of the high-energy ration, nitrogen retained as a percentage of absorbed was 45% for lambs with protozoa as compared to 55% for protozoa-free lambs. The higher nitrogen retention was the result of a marked reduction in urinary nitrogen even though fecal nitrogen was somewhat greater than in lambs with protozoa.

Rumen Fermentation and Blood Studies

The results of these studies are presented in table 3. Ruminal pH ranged from 6.2 to 6.5 with only small differences between the different treatments. Rumen fluid of lambs with protozoa had about 10 micromoles per milliliter or about 12% more total volatile fatty acids (VFA) than rumen fluid from lambs without protozoa with either level of dietary energy. Differences in VFA levels between before feeding and 3 hours after feeding suggest a faster rate of fermentation for the lambs with protozoa. Molar proportions of individual VFA were essentially the same for lambs with protozoa as for those without protozoa with the low-energy ration. However, important differences in the propertions of VFA were found with the high-energy ration. Production of acetic acid was lower and that of propionic acid was higher for lambs with protozoa as compared to protozoa-free lambs. This is illustrated in a narrower ratio of acetic to propionic acid. These findings are of particular significance since an increase in total VFA or a narrowing of the acetic to propionic ratio would contribute materially to the energy economy of the host. Propionic acid, with a lower heat increment, is utilized more efficiently than acetic acid in the fattening ruminant.

Ruminal levels of ammonia nitrogen were higher in lambs with protozoa than in those without protozoa both before and at 3 hours after feeding. However, the differences were larger with the high-energy ration. The higher levels of ammonia nitrogen in the presence of protozoa in the rumen is indicative as a more rapid protein degradation. A more rapid rate of fermentation in the presence of protozoa is also indicated by the higher levels of VFA in these lambs. The effect of energy content of ration on ruminal ammonia levels observed by other workers was apparent in this study with ammonia levels being lower with the higher energy ration.

Blood urea nitrogen values were within the normal range of 15 to 20 mg.% with principle differences occuring between levels of dietary energy. Levels of blood urea obtained with the high-energy ration were 16 mg.% and with the low-energy ration, about 20 mg.%. Some workers have suggested that lower levels of blood urea nitrogen are indicative of a more efficient utilization of dietary nitrogen. Nitrogen retention data from this experiment would lend support to this statement. The effect of dietary energy on protein utilization was apparent in this study when either nitrogen retention or blood urea values are used as a measure of nitrogen utilization.

Summary

A digestion and nitrogen balance trial and a rumen fermentation and blood study was utilized to determine the utilization of a high-energy or a low-energy ration by normal and protozoa-free lambs. Digestibility of nutrients, except for crude fiber, and nitrogen retention were higher for the high-energy ration than for the low-energy ration. Likewise, ruminal volatile fatty acid (VFA) and ammonia nitrogen concentrations were higher with the high-energy ration. Blood urea nitrogen levels followed an inverse trend with 16 mg.% for the high-energy ration and 20 mg.% for the low-energy ration.

The absence of protozoa in the rumen resulted in a lowering of the digestibility of nutrients except in 1 instance with either level of dietary energy. Nitrogen retention expressed as percent of nitrogen absorbed was 45 and 55% for lambs with or without protozoa, respectively, with the high-energy ration and about 35% for both groups of lambs fed the low-energy ration. Ruminal VFA and ammonia concentrations were markedly higher in lambs with protozoa than in those without protozoa with either level of energy. Differences in the molar percentage of VFA were found between protozoa treatments with the high-energy ration. In this instance there was a decrease in acetic acid and an increase in propionic acid in lambs with protozoa as compared to protozoa-free lambs. Blood urea nitrogen levels were essentially the same between protozoa treatments.

TABLE 3. Rumen Fermentation and Blood Data

	Protozoa		Protozoa-free	
	High Energy	Low Energy	High Energy	Low Energy
No. lambs	6	6	6	6
No. observations	12	12	12	12
Rumen Fermentation				
pH	6.2	6.5	6.4	6.5
Total VFA, micromoles/milliliter				
Before feeding	66.4	74.7	59.8	67.2
3 hours after feeding	83.4	88.3	73.6	77.9
Molar % VFA[a]				
Acetic	39.0	49.6	45.8	48.9
Propionic	31.5	23.8	24.9	24.1
Butyric	23.1	22.3	23.6	24.3
Valeric	2.1	1.1	2.6	0.6
Branched-chain[b]	4.3	3.2	3.1	2.1
Acetic: propionic[a]	1.3	2.1	2.1	2.1
Ammonia nitrogen, mg. %				
Before feeding	6.1	8.6	2.6	5.5
3 hours after feeding	10.3	11.5	5.3	8.3
Blood Urea Nitrogen, Mg.%				
Before feeding	13.6	19.2	13.3	16.4
6 hours after feeding	16.1	20.1	15.6	19.2

[a] 3 hours after feeding

[b] Includes iso butyric and iso valeric acids