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Relationship Between Rumen Ammonia Levels and the Microbial Population and Volatile Fatty Acid Proportions in Faunated and Defaunated Sheep[1]

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Cheviot wethers were defaunated by using dioctyl sodium sulfosuccinate and were constantly infused with urea to provide 2.87% of the daily N intake. Defaunation resulted in higher rumen dry matter and lower rumen *p*H. The molar per cent propionate was higher in defaunated sheep, whereas the molar per cent butyrate and acetate was lower. Apparent nitrogen digestibility, nitrogen utilization, and nitrogen balance were higher in defaunated sheep when compared with faunated animals. Urea infusion resulted in lower apparent nitrogen digestibility, nitrogen utilization, and nitrogen balance in faunated sheep, but did not affect nitrogen metabolism in defaunated sheep. Rumen ammonia-N levels in defaunated sheep were lower than those observed for faunated animals, and urea infusion into faunated sheep increased rumen ammonia-N levels to a greater extent than did the urea infusion into defaunated animals. Significant correlations were demonstrated between rumen ammonia-N levels and C_2/C_3, C_3/C_4 and C_2/C_4 volatile acid ratios. From this it was concluded that, as rumen ammonia-N levels increased, there was a shift from propionate to higher proportions of butyrate and acetate.

A decrease in rumen ammonia-N levels as a result of defaunation in animals fed identical rations is probably the most consistent effect recorded from studies with defaunated and faunated ruminants. The recorded values for rumen ammonia-N levels of defaunated animals as a per cent of those in control faunated animals are 47% by Abou Akkada and el-Shazly (2), 52% by Christiansen et al. (5), 44, 50, and 39% by Klopfenstein et al. (8) and 49 and 50% by Chalmers et al. (Proc. Nutr. Soc., p. 29A, 1968).

As a consequence of this change in rumen ammonia-N levels in defaunated ruminants, any attempt to relate the effect of the presence of protozoa per se to overall animal response is confounded with a marked change in the rumen environment, i.e., greatly increased rumen ammonia-N levels. The experiment reported in this paper was designed to investigate the effect of experimentally increased rumen ammonia-N levels in defaunated and faunated sheep.

MATERIALS AND METHODS

Twelve rumen-fistulated, 2-year-old Cheviot wethers, averaging 30 kg, were fed ration number 3, as previously described (8) at 6% body weight$^{0.75}$ throughout the entire experiment. Due to problems encountered with sheep refusing feed, the original 4 by 4 Latin square design had to be abandoned and unequal numbers per treatment combination resulted. The four treatments imposed were (i) urea infusion into the rumen of defaunated sheep (seven observations), (ii) urea infusion into the rumen of faunated sheep (seven observations), (iii) water infusion into the rumen of defaunated sheep (six observations), and (iv) water infusion into the rumen of faunated sheep (six observations). The water and urea infusions were continuous, a volume of 460 ml being infused in 24 hr. Urea was infused at a rate to provide 0.123% of the daily ration; this represented 2.87% of the daily N intake. Sodium sulfide was included in the infusate to provide a N to S ratio of 15:1. Although not yet experimentally proven, this was thought to be necessary to maintain optimal N to S ratios in the rumen at times when recycling differences could otherwise prevent effective N incorporation into microbial protein.

Defaunation was accomplished by using 12 ml of dioctyl sodium sulfosuccinate under the trade name Complemix (American Cyanamid Company) in a procedure only slightly modified from that used by Abou Akkada et al. (1). Volatile fatty acid concentrations were determined by using the procedure of Dehority et al. (6); all other analyses and sample collection procedures were as previously described (8).

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A 13-day adjustment period preceded 6 days of feed intake, urine production, and fecal output measurement. Two days later rumen samples and jugular blood samples were taken prefeeding and at 2 and 4 hr postfeeding, but the 2-hr postfeeding results are not presented for they were essentially identical to those obtained 4 hr postfeeding. Statistical analysis was by least squares and Duncan's multiple range test (7).

RESULTS

The mean rumen protozoal concentration for the faunated sheep was 313.2 ± 179.4 × 10^3/ml, at the prefeeding sample time. Rumen dry matter was higher in defaunated sheep at all sampling times, when compared to faunated animals (Table 1), and the difference was significant ($P < 0.01$) at the prefeeding sampling time. Sheep receiving the water infusion also had higher rumen dry matter percentages than did sheep which received the urea infusion. Rumen *p*H was slightly higher in faunated sheep and in sheep that received the urea infusion (Table 1); however, these differences were not significant.

The effect of the absence of protozoa from the rumen on rumen volatile fatty acids (VFA) is outlined in Tables 2 and 3. There were no significant differences observed in total VFA concentrations at any of the sampling times between faunated and defaunated sheep. The molar proportion of acetate was slightly higher in faunated wethers at prefeeding and 4 hr postfeeding (Table 2 and 3) and was significantly ($P < .05$) higher at 2 hr postfeeding. The molar proportion of propionate was consistently higher when sheep had been defaunated and at prefeeding this was significant ($P < .05$). Butyrate was lower in defaunated sheep at all sampling times ($P < 0.05$). No significant differences were observed in the molar proportions of valerate or the combined branched-chain VFA. These differences in the molar proportions of acetate, propionate, and butyrate were also evident in the ratios of the volatile fatty acids. The C_2 to C_3 ratio was consistently lower when sheep had been defaunated, a difference that was significant ($P < 0.01$) at prefeeding and at 2 and 4 hr postfeeding ($P < 0.05$). Although there were no significant differences recorded for the C_2 to C_4 and C_3 to C_4 ratios, some interesting trends were observed. The acetate to butyrate ratio was consistently higher in defaunated sheep. Thus, when the sheep were defaunated, there was a shift from acetate and butyrate to higher levels of propionate, and these results closely correspond to those of Klopfenstein et al. (8).

Dry matter digestion was not significantly affected when the treatment effects of faunation and defaunation or of urea infusion were analyzed (Table 4).

Apparent nitrogen digestibility was similar for all defaunated animals. The urea infusion lowered

TABLE 1. *Influence of faunation and urea infusion upon pH and dry matter*

Treatment	*p*H		Dry matter	
	Zero time	4 hr Postfeeding	Zero time	4 hr Postfeeding
			%	%
Faunated				
Water infused	6.07[a]	5.51	12.75	16.10
Urea infused	6.30[b]	5.92	11.70	16.96
Mean	6.19	5.72	12.22A[d]	16.53
Defaunated				
Water infused	5.78[a]	5.43	17.37	18.54
Urea infused	5.83[b]	5.48	15.67	17.55
Mean	5.80	5.46	16.52B	18.05
Water mean	5.92	5.47	15.06	17.32
Urea mean	6.06	5.70	13.68	17.26
SE[c]	0.12	0.08	0.55	1.30

[a] Mean of six values.
[b] Mean of seven values.
[c] Standard error of the means.
[d] Values having different letters are significantly different ($P < 0.01$).

TABLE 2. *Influence of faunation and urea infusion upon volatile fatty acid distribution, prefeeding*[a]

Treatment	Acetate	Propionate	Butyrate	Valerate	Branch chain	Total[b]
Faunated						
Water	60.71[c]	22.15	11.70	1.22	4.22	53.88
Urea	62.81[d]	16.21	14.78	1.16	5.23	60.47
Mean	61.66	19.18a[f]	13.24a	1.19	4.72	57.15
Defaunated						
Water	56.79[c]	31.19	6.20	1.48	4.33	67.31
Urea	57.73[d]	28.69	7.83	1.42	4.31	65.97
Mean	57.26	29.94b	7.02b	1.45	4.32	66.64
Water mean	58.75	26.67	8.95	1.35	4.27	60.57
Urea mean	59.45	22.45	11.31	1.29	4.77	63.22
SE[e]	0.01	0.02	0.01	0.00	0.00	2.48

[a] Unless otherwise indicated, values are expressed as molar per cent.
[b] Values in total column are expressed in micrometers per milliliter.
[c] Mean of six values.
[d] Mean of seven values.
[e] Standard error of means.
[f] Values having different letters (a, b) are significantly different ($P < 0.05$).

significantly ($P < 0.05$) the apparent nitogen digestibility of the faunated wethers, when compared with the water-infused faunated wethers (Table 4). The nitrogen utilization and nitrogen balance figures that were observed in this experiment were extremely high for sheep on a maintenance ration; however, significant differences were observed (Table 4). Defaunation resulted in a significantly ($P < 0.01$) higher nitrogen balance, and the infusion of urea into defaunated sheep did not affect this. However, urea infusion into faunated sheep resulted in lower nitrogen utilization and nitrogen balance, and, although not significant, these differences approached significance. When faunated sheep received the urea infusion, fecal nitrogen as a per cent of nitrogen intake was significantly ($P < 0.05$) higher (Table 4).

TABLE 3. *Influence of faunation and urea infusion upon volatile fatty acid distribution 4 hr postfeeding*[a]

Treatment	Acetate	Propionate	Butyrate	Valerate	Branch chain	Total[b]
Faunated						
Water	60.01[c]	24.86	11.19	1.20	2.72	74.33
Urea	62.83	21.74	13.07	0.96	2.66	74.47
Mean	61.42	23.30	12.13a[f]	1.08	2.69	74.40
Defaunated						
Water	57.53[c]	31.16	6.27	1.48	3.08	81.61
Urea	57.47[d]	29.44	8.79	1.64	2.54	81.99
Mean	57.50	30.30	7.53b	1.56	2.81	81.80
Water mean	58.77	28.01	8.73	1.34	2.90	77.97
Urea mean	60.15	25.59	10.93	1.30	2.60	78.23
SE[e]	0.01	0.02	0.01	0.00	0.00	4.07

[a] Unless otherwise indicated, values are expressed as molar per cent.
[b] Values in total column are expressed in micrometers per milliliter.
[c] Mean of six values.
[d] Mean of seven values.
[e] Standard error of means.
[f] Values having different letters (a, b) are significantly different ($P < 0.05$).

The rumen ammonia-N levels that were obtained in this study were consistent with those previously reported in the literature from defaunation studies. Defaunation of the wethers in this study resulted in significantly lower rumen ammonia-N levels at 2 ($P < 0.01$) and at 4-hr ($P < 0.05$) postfeeding (Fig. 1). The infusion of urea raised rumen ammonia-N levels in faunated sheep an average of 3.44 mg per 100 ml and in defaunated sheep an average of only 1.88 mg per 100 ml.

Regression analysis, using ammonia and VFA data obtained 4 hr postfeeding from the results of Klopfenstein et al. (8) and this investigation, showed a linear relationship between rumen ammonia concentration and the C_2 to C_3 VFA ratio ($r = 0.91$; $P < 0.01$; Fig. 2), a curvilinear relationship between ammonia concentrations and the C_2 to C_4 VFA ratio ($r = 0.89$; $P < 0.01$; Fig. 3), and a curvilinear relationship between ammonia concentration and the C_3 to C_4 VFA ratio ($r = 0.81$; $P < 0.05$; Fig. 4). Similar relationships were demonstrated for the data taken prefeeding, but the correlation values were less.

TABLE 4. *Influence of faunation and urea infusion upon dry matter digestibility and nitrogen metabolism*

Treatment	Dry matter digestibility	Nitrogen intake	Apparent nitrogen digestibility	Nitrogen utilization[a]	Fecal nitrogen/ nitrogen intake	Nitrogen balance
	%	g/day	%	%	%	g/day
Faunated						
Water	78.58[b]	13.78	71.75a[e]	53.28	27.61a	5.200
Urea	76.79[c]	13.84	68.40b	33.86	33.31b	3.084
Mean	77.68	13.81	69.90	43.57A	29.46	4.142a
Defaunated						
Water	77.61[b]	11.83	70.63	71.69	30.19	6.396
Urea	76.64[c]	12.74	70.63	75.12	30.20	6.789
Mean	77.12	12.28	70.63	73.54B	30.19	6.593b
Water mean	78.09	12.80	71.19	62.61	28.90	5.798
Urea mean	76.71	13.29	69.34	54.49	30.75	4.937
SE[d]	0.60		0.87	4.68	0.87	0.449

[a] Nitrogen retained/nitrogen absorbed × 100.
[b] Mean of six values.
[c] Mean of seven values.
[d] Standard error of means.
[e] Values with different capital letters (A, B) are significantly different ($P < 0.01$); values with different small letters (a, b) are significantly different ($P < 0.05$).

Such data indicate that, as ammonia concentrations increased, acetate and butyrate proportions increased, with butyrate increasing more than acetate. At the same time, propionate proportions decreased.

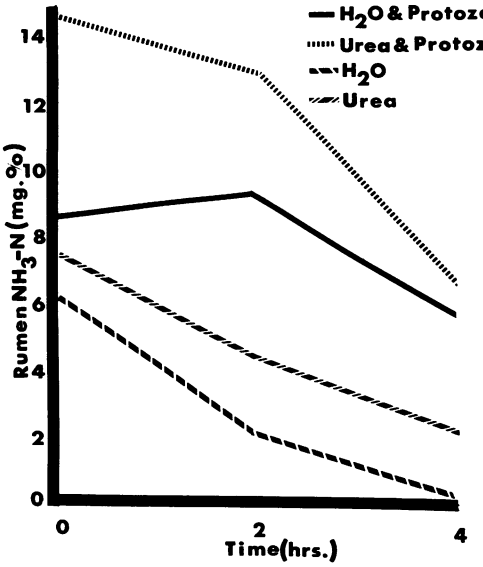

FIG. 1. *Rumen ammonia-N concentration in faunated and defaunated sheep receiving water or urea infusions.*

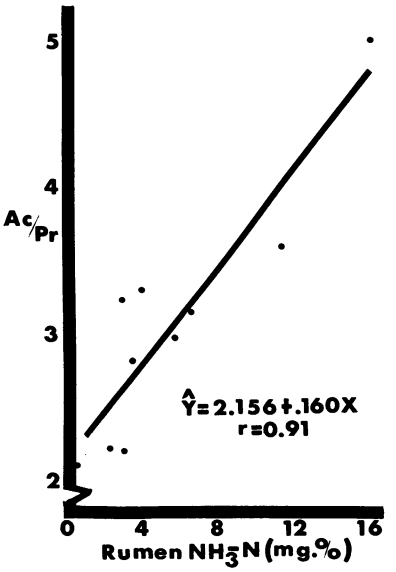

FIG. 2. *Relationship between acetate to propionate ratios and ammonia-N concentrations in the rumen. Samples taken 4 hr postfeeding from faunated and defaunated animals receiving three different rations and from faunated and defaunated animals receiving urea or water infusion into the rumen.*

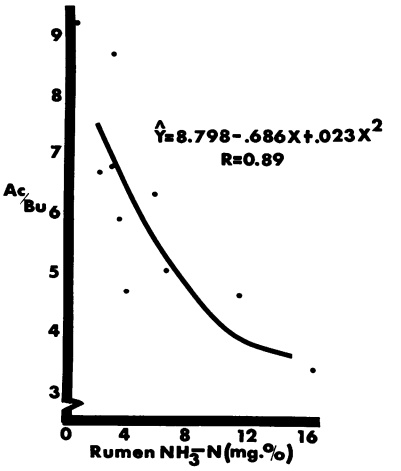

FIG. 3. *Relationship between acetate to butyrate ratios and ammonia-N concentrations in the rumen. Conditions as described in Fig. 2.*

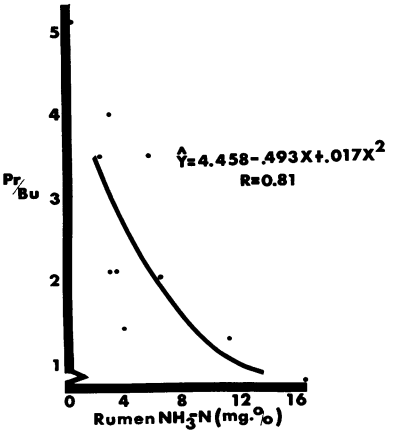

FIG. 4. *Relationship between propionate to butyrate ratios and ammonia-N concentrations in the rumen. Conditions as described in Fig. 2.*

DISCUSSION

Higher ammonia levels in faunated animals have been consistently observed, and it has been suggested that this is due to the fact that the protozoa contribute significantly to protein degradation and deamination (8, 10). Such an interpretation now appears to be entirely misleading, and, in fact, it seems that ammonia levels in the rumen of defaunated animals are lower than in faunated animals as a result of greater bacterial concentrations, thus causing greater ammonia utilization. This interpretation of the results suggests that ammonia levels are higher in faunated animals as a result of an inability to effectively utilize ammonia rather than

as a result of excessive ammonia production, a conclusion also arrived at by Smith (11). The reasons for suggesting this interpretation are given below.

Firstly, greater rumen bacterial protein quantities at the expense of rumen protozoan protein should give rise to greater excretion of nitrogen in the feces, for protozoan protein is more digestible than bacterial protein (3, 9). Such a relationship is shown by the results with the water-infused animals in this experiment (27.61 versus 30.19) and by the results of Klopfenstein et al. (8). No explanation for the effect of urea infusion upon apparent nitrogen digestibility in faunated animals can be given at this time.

Secondly, the quantity of urea infused in this experiment had been calculated to provide an increase of 4 mg per 100 ml of rumen fluid. This value was approached in the faunated animals (3.44 mg/100 ml), but an increase of only 1.88 mg per 100 ml was realized in defaunated animals, thus suggesting a more rapid uptake of ammonia in these animals. The slight possibility of a deficiency of urease in these animals was eliminated when it was shown that in fact urease activity (per milliliter of rumen contents) in defaunated animals was double that of faunated animals (Purser and Dehority, *unpublished data*).

A second, but variable, effect of defaunation is a lowered dry matter digestibility as compared to faunated animals. This was not true in this experiment, but see, for example, Klopfenstein et al. (8). A large portion of any such change (3% units) may be attributed to the decreased digestibility of bacteria, as compared with protozoa. The above value is arrived at by assuming a 20% cell yield (cell yield per 100 g of digestible substrate), equal distribution of cell matter in protozoa and bacteria in faunated animals, and digestibilities of 90 and 60% for protozoa and bacteria, respectively. A difference of 3% units would account for a large proportion of the changes recorded in the literature, but differences due to possible changes in the bacterial population composition cannot be ignored (4).

The ammonia-VFA proportion relationships demonstrated in this work are potentially of very great importance to ruminant metabolism in general. For example, if a causal relationship does exist between ammonia levels and VFA proportions, the relationship is of potential value as a guide for the manipulation of rations causing milk fat depression problems. Rations markedly different in nitrogen content and energy availability were included and additional treatments in which urea infusions were the only variables were also included and all fitted the relationships. However, it remains to be seen whether the phenomenon is in fact as general as these results would suggest.

One of the major aims of this work was to determine whether changes in animal response upon defaunation were the result of the presence of protozoa per se or were due to indirect effects mediated by some other factors. At this point, it seems likely that any changes in the VFA proportions as a result of defaunation are, in fact, a result of a change in rumen ammonia levels, either directly or indirectly. The explanation for this relationship cannot be given at this time, but both metabolic changes within individual organisms and population composition changes must be considered. An ammonia limitation rather than an excess would appear to be the causal factor.

Two major relationships have been discussed in this paper, and a number of quite important implications follow from the acceptance of these data. Not the least is the selection of appropriate control animals to be used in defaunation work, for the results of the present experiment show that, when isonitrogenous rations are used, different rumen ammonia levels occur which apparently cause different VFA proportions. On the other hand, this secondary effect may be overcome by supplementary nitrogen to raise rumen ammonia levels, but this then means that isonitrogenous comparisons are no longer being made. It seems that careful experimentation is required to eliminate these conflicting factors.

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