EFFECTS OF AMMONIATED RICE STRAW FEEDING ON MICROBES AND THEIR FERMENTATION END-PRODUCTS IN THE RUMEN AND CAECUM OF SHEEP

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Summary

Three sheep fitted with cannulas in the rumen and the caecum were used in a 3 × 3 Latin square design to study the changes in ruminal and caecal microbial populations and their metabolite composition with ammoniated rice straw feeding. The 3 diets contained either 80% untreated rice straw (UTS) or ammoniated rice straw (ATS) and 20% formula feed. These were a control diet (C), a urea supplemented diet (U) containing urea at 1.1% and an ammoniated rice straw diet (AT). Data were analyzed by analysis of variance and means separated by the Student Neumann Keul's multiple comparison. AT feeding increased ruminal bacterial counts, in particular cellulolytic bacterial counts ($p < 0.05$) which were 1.8, 2.4 and 7.0 ($\times 10^6$/ml ruminal fluid) for C, U and AT, respectively. There was an increasing tendency ($p < 0.10$) in ruminal fungal population with U; values were 2.0, 5.2, 3.1 ($\times 10^3$/ml ruminal fluid) for C, U and AT, respectively. Ruminal protozoal counts were not significantly ($p > 0.05$) altered with diets. Caecal total viable bacterial count with AT was about thrice the value with C. Total VFA concentration in the rumen was significantly increased ($p < 0.025$) (7.7 mmol/dl for C and 8.2 mmol/dl for AT) and correspondingly, pH lowered when AT was fed. Sheep on AT tended to produce less acetate and more butyrate in the rumen without significance ($p > 0.05$). Similar to the rumen, total VFA concentrations of 4.4, 3.8 and 5.2 mmol/dl were detected, respectively, for C, U and AT. Caecal ammonia-nitrogen concentrations were about six-fold of that in the rumen, though there were no differences ($p > 0.05$) among treatments.

(**Key Words**: Ammoniated Rice Straw, Microbes, End-products, Rumen, Caecum)

Introduction

The utilization of straws, which have an annual production of more than 2000 million tonnes (Jackson, 1977), is limited by their low digestibilities and inadequate nitrogen content. Chemical procedures to improve the nutritive value of cereal straws have been tried since the beginning of this century (Lehman, 1905). Of these procedures, ammonia and sodium hydroxide treatments have received the greatest attention. The effects of alkali treatment on straws have been summarized (Theander, 1981) as the cleavage of linkages between lignin and other cell wall constituents, saponification of bound acetic acid and phenolic acids, swelling of cell walls and partial solubilization of hemicelluloses, lignin, protein and silica. Ammonia treatment, however, has an advantage over sodium hydroxide, because when the potential digestibility is increased by structural changes with ammoniation, ammonia absorbed into straws will fulfill increased microbial requirement for nitrogen (Ørskov et al., 1983).

Over the last two decades, many experiments have been carried out on treated straws, but these have concentrated on intake, digestibility and animal performance (Garrett et al., 1979; Brown et al., 1987). There have been a few studies (Kolankaya et al., 1985 and Minato et al., 1989) on the influence of ammoniation on the microbial flora in the digestive tract.

The objectives of the present experiment were to investigate the changes in bacterial, protozoal and fungal populations and the fermentation (volatile fatty acids, % molar composition of acids, ammonia-nitrogen and pH) pattern in the rumen and caecum of sheep fed ammoniated rice straw diet.

Materials and Methods

Ammoniation of rice straw

Rice straw in bales was ammoniated (2 g NH_3

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/100 g DM) by the stack method (Sundstøl, 1978) under environmental conditions (average temperature of 25℃).

Formulation of diets

Three diets each containing 80% of straw (chopped to an average size of 5 cm) and 20% formula feed, were formulated. These were a control diet (C) containing 80% untreated rice straw and 20% commercial formula feed (table 1), an ammoniated rice straw diet (AT) containing 80% ammoniated rice straw and 20% formula feed and an urea supplemented diet (U) containing urea at 1.1%. Diet U was formulated to make it isonitrogenous with AT. The composition of feeds is shown in table 1.

TABLE 1. INGREDIENTS AND CHEMICAL COMPOSITION OF DIETS (%)

Diet	Ingredients				Chemical composition		
	ATS	UTS	Formula feed	Urea	DM	CP	NDF
C	–	80.0	20.0	–	93.9	6.7	57.9
U	–	80.0	18.9	1.1	94.0	9.4	57.6
AT	80.0		20.0		94.0	9.9	57.4

ATS: Ammoniated rice straw (2 g NH_3/100 g DM).
UTS: Untreated rice straw.
C: Control.
U: Urea supplemented diet.
AT: Ammoniated rice straw diet.

Formula feed contains the following: Milo, corn, barley, and rye (65%), wheat bran, corn gluten meal, rice bran, corn and barley (distillers grain soluble) (19%), soybean meal, rapeseed meal and cotton seed oil (7%), molasses, alfalfa meal, $CaCO_3$, NaCl and $Ca_3(PO_4)_2$ (9%).

Animals and their management

Three crossbred (Corriedale × Suffolk) sheep with an average weight of 42.8 kg (range 34-50 kg) and cannulated in the rumen and caecum were randomly assigned to the diets. The diets were fed in two equal portions of 450 g, at 09:00 A.M. and 05:00 P.M., to these animals in a 3 × 3 Latin square. The sheep were kept in spacious cages throughout the experimental period, with free access to water and mineral salt licks.

Experimental procedure

Each experimental period consisted of 15 days. The first 12 days were for an adaptation to diets, and on the 13th day, just before the morning feed, ruminal and caecal samples were collected. Soon after sampling, agar strips were introduced into the rumen of sheep to estimate fungal population size in the rumen by a slight modification of the in situ procedure (Ushida et al., 1989).

Sample preparations and analytical methods

Ruminal and caecal samples were strained through two layers of surgical gauze, and pH of the strained fluid determined immediately with a glass electrode (Hitachi-Horiba D5). Portions of both the strained ruminal and caecal fluids were diluted serially, and appropriate dilutions used to enumerate total viable, cellulolytic, lactate fermenting (Olumeyan et al., 1986), amylolytic (Kistner, 1960), proteolytic (Holdeman, 1977) bacterial and fungal (Joblin, 1981) counts. Ruminal samples used for the enumeration of protozoa were blended with equal volumes of MFS solution (containing 0.05% methylgreen in 10% formalin). The counting and generic classification were carried out according to the method used elsewhere (Cann et al., 1991).

Volatile fatty acids (VFAs) and ammonia-nitrogen were determined by gas chromatography and colorimetry, respectively (Cann et al., 1991).

After withdrawal, agar strips were gently washed with distilled water, and stained with a few drops of lactophenol blue (Merck, Rahway, N. J., USA). Stained fungal sporangia were then observed and counted under a light microscope equipped with an eyepiece grid.

Statistical procedure

Results were subjected to analysis of variance as a 3 × 3 Latin square, and where significant differences were detected, means were separated by the Student Neumann-Keul's Test (Snedecor and Cochran, 1967).

Results

Total viable, amylolytic, proteolytic and cellulolytic bacterial counts all increased when animals were fed AT (table 2). However, statistical significance ($p < 0.05$) was only detected in the case of cellulolytic bacteria. The increase in cellulolytic bacteria with AT feeding was about four-fold of that of C. Lactate fermenting bacterial count in sheep on C was three times that of animals on AT. Fungal population was generally increased when urea supplemented diet was fed. The increase showed statistical significance ($p < 0.10$) when the roll tube method was used for the estimation of the population size. On the other hand, with the agar strip method the difference did not achieve statistical significance ($p > 0.05$). Total protozoal count increased with AT feeding, but the increase was not significant ($p > 0.05$) (table 2). The predominant species were *Entodinium nanellum*, *Entodinium simplex* and *Entodinium caudatum*. These three species together formed 92%, 92.3% and 90% of the total protozoal counts in C, U and AT, respectively. The percentage proportions were not significantly

TABLE 2. VIABLE COUNTS OF BACTERIA, FUNGI, PROTOZOA AND THEIR PERCENTAGE COMPOSITION IN THE RUMEN OF SHEEP FED DIFFERENT DIETS[j]

Type of microorganism	Diets		
	C	U	AT
Total viable counts[d]	1.3 ± 0.1	1.1 ± 0.1	1.8 ± 0.4
Amylolytic bacteria[d]	1.1 ± 0.5	0.7 ± 0.3	1.3 ± 0.6
Proteolytic bacteria[d]	0.6 ± 0.3	0.4 ± 0.3	0.7 ± 0.3
Lactate fermenters[d]	0.6 ± 0.3	0.1 ± 0	0.2 ± 0
Cellulolytic bacteria[e]*	1.8 ± 0.4[a]	2.4 ± 0.7[b]	7.0 ± 0.3[c]
Fungi			
Roll tube method[f]#	2.0 ± 0.6[a]	5.2 ± 2.6[b]	3.1 ± 1.5[a]
Agar strip method[g]	0.5 ± 0.2	0.8 ± 0.1	0.7 ± 0.1
Total protozoal counts[h]	1.4 ± 0.4	1.8 ± 0.6	2.4 ± 0.5
Composition (%)			
Entodinium nanellum	49.0 ± 0.8	37.7 ± 1.6	52.1 ± 9.9
Entodinium simplex	31.2 ± 3.7	42.0 + 4.4	29.9 ± 7.9
Entodinium caudatum	11.8 ± 6.3	12.6 ± 3.2	8.5 ± 2.8
Entodinium bursa	1.4 + 1.2	0.7 ± 0.7	2.0 ± 0.7
Entodinium exiguum	2.3 ± 1.8	1.7 ± 1.0	2.3 ± 0.1
Entodinium minimum	1.6 ± 1.2	0	0.8 ± 0.8
Entodinium longinucleatum	3.8 + 1.9	3.1 ± 1.5	3.6 ± 1.2
Entodinium ovinum	0	0	0.5 ± 0.5
Isotricha prostoma	0.9 ± 0.9	0.7 ± 0.7	0.7 ± 0.5
Dasytricha ruminantium	0.6 ± 0.6	0.1 ± 0.1	0
Polyplastron multivesiculatum	0.5 ± 0.3	0.8 ± 0.5	0.7 ± 0.4

[a,b,c] Means in the same row with different letters differ (S.N.K.).
[d] × 10^6/ml ruminal fluid.
[e] × 10^6/ml ruminal fluid.
[f] × 10^3/ml ruminal fluid.
[g] × 10^3/ml sporangia/cm^2.
[h] × 10^5/ml ruminal fluid.
[j] Means ± S.E. from 3 observations.
*; $p < 0.05$ #; $p < 0.10$.

altered ($p > 0.05$) with diets.

Total viable and amylolytic bacterial counts were increased three fold above C in the caecum of animals fed AT, though the increases were not statistically significant (table 3). Unlike the rumen, proteolytic, cellulolytic and lactate fermenting bacteria all insignificantly decreased ($p > 0.05$) in U and AT feeding. In the caecum, fungal population sizes estimated with the roll tube method tended to increase in animals on AT, but the incidence of the organisms was highly variable.

TABLE 3. VIABLE COUNTS OF BACTERIA IN THE CAECUM OF SHEEP FED DIFFERENT DIETS[a]

Type of microorganism	Diets		
	C	U	AT
Total viable counts[b]	1.9 ± 0.5	3.6 ± 2.4	6.0 ± 3.4
Amylolytic bacteria[b]	0.5 ± 0.3	0.4 ± 0.1	1.9 ± 0.6
Proteolytic bacteria[b]	10.7 ± 9.4	3.7 ± 1.5	7.6 ± 6.1
Lactate fermenters[b]	0.9 ± 0.8	0.7 ± 0.6	0.7 ± 0.6
Cellulolytic bacteria[c]	0.8 ± 0.6	0.4 ± 0.1	0.6 ± 0.2
Fungi			
Roll tube method[d]	2.0 ± 0.7	9.9 ± 9.1	11.7 ± 10.4

[a] Means ± S.E. from 3 observations.
[b] × 10^6/ml caecal fluid.
[c] × 10^9/ml caecal fluid.
[d] × 10^2/ml caecal fluid.

The total VFA concentrations in the ruminal fluid of sheep were significantly different among diets ($p < 0.025$) (table 4) and the value was the highest in sheep on AT. Acetate proportion was reduced and propionate and butyrate proportions were slightly increased without significant differences ($p > 0.05$) in AT feeding. Corresponding to the VFA concentrations, pH values were significantly different ($p < 0.025$) among diets: the pH in AT fed animals was lower than those in C and U.

Similar to the rumen, the VFA concentration in the caecum was the highest in AT fed animals (table 4). There were no significant differences

TABLE 4. TOTAL VFA CONCENTRATIONS, MOLAR PERCENTAGES OF ACIDS AND pH VALUES IN THE RUMINAL AND CAECAL FLUIDS OF SHEEP FED DIFFERENT DIETS[d]

Diet	Total VFA**	Percentages					pH**
	(mmol/dl)	Acetate	Propionate	n-Butyrate	i-Val.	n-Val.	
Ruminal fluid							
C	7.7 ± 1.0[b]	69.1 ± 1.5	22.0 ± 1.0	8.3 ± 0.8	0.6 ± 0.6	–	6.8 ± 0.1[a]
U	6.2 ± 0.9[a]	68.7 ± 1.1	21.1 ± 1.0	10.2 ± 0.5	0	–	6.8 ± 0.1[a]
AT	8.2 ± 0.6[c]	66.0 ± 1.7	23.9 ± 0.8	10.1 ± 1.0	0	–	6.6 ± 0.0[b]
Caecal fluid #							
C	4.4 ± 0.8[a]	59.7 ± 1.4	29.0 ± 3.0	6.7 ± 0.8	2.0 ± 1.1	2.6 ± 1.3	7.5 ± 0.2
U	3.8 ± 1.3[a]	60.7 ± 1.0	31.8 ± 3.1	4.1 ± 2.0	2.3 ± 1.2	1.1 ± 1.1	7.6 ± 0.1
AT	5.8 ± 0.9[b]	61.5 ± 2.2	27.7 ± 0.6	7.6 ± 0.8	2.1 ± 1.1	1.2 ± 0.9	7.3 ± 0.1

[a,b,c] Means with different letters in the same column differ (S.N.K.).
[d] Mean ± S.E. from 3 observations.
**; $p < 0.025$.
#; $p < 0.1$.
Val; Valerate.

($p > 0.05$) in VFA proportions among treatments, although acetate and n butyrate proportions tended to be higher when AT was fed. The pH values were not influenced by diet although there were significant differences ($p < 0.1$) in total VFA concentrations.

Ruminal ammonia concentrations in animals on U and AT were not significantly different ($p > 0.05$) from C (table 5). Ammonia concentrations of caecal contents were six times higher than those of ruminal contents, and were not affected by diets.

TABLE 5. AMMONIA-NITROGEN CONCENTRATIONS (mg/dl) IN THE RUMEN AND CAECUM OF SHEEP FED DIFFERENT DIETS[a]

Source	Diets		
	C	U	AT
Rumen	10.8 ± 1.5	12.2 ± 2.5	12.0 ± 0.5
Caecum	66.8 ± 11.5	80.2 ± 16.4	75.5 ± 9.6

[a] Means ± S.E. from 3 observations.

Discussion

Treating rice straw with ammonia increased ruminal cellulolytic bacteria in the present experiment, a result that was in agreement with a previous study with sheep (Cann et al., 1991) and of others with steers (Minato et al., 1989) who isolated the cellulolytic organisms *Ruminococcus albus* and *Fibrobacter succinogenes* at high dilutions. The general increase in bacterial counts with ammoniation was also consistent with the previous study (Cann et al., 1991). These findings suggest that the breakdown of partial linkages between lignin and other carbohydrates (Jackson, 1977) caused the bacterial proliferation in the rumen.

The significant increase in fungal population size (roll tube method) in the rumen of sheep on U in the present experiment provided information only on the "free" phase of these organisms. It has been suggested that the presence of these organisms can be seen as being in two phases, namely the "free" phase and the "fixed" (hence active) phase, and if environmental conditions render it easy for attachment, the number of free spores decreases without the action of fungi diminishing (Grenet et al., 1989). Rice straw ammoniation increases fragility (Zorrilla-Rios et al., 1985), and sites for fungal attachment are mainly damaged plant surfaces, cut ends and the stomata (Ho et al., 1990). The fragility and the other physical properties of ammoniated rice straw may enhance fungal attachment, and accordingly a procedure for comparing fungal populations between treated and untreated straw must consider both the "free" and "fixed" phases. Further investigations and improved methods are needed to quantify fungal activities in ammoniated straw feeding.

Similarity of total protozoal counts among diets agreed with the previous reports (Minato et al., 1989) where feeding of ammoniated rice straw did not alter total protozoal counts, except *Dasytricha sp.* which was increased in steers on untreated rice straw diet. In the present experiment, even though not in large numbers, *Dasytricha ruminantium* was observed only in the rumen of sheep on untreated diets (U and C). The major protozoal species in the rumen of sheep on rice straw were the *Entodinium sp.* in agreement with previous results (Cann et al., 1991).

The results of the present experiment demonstrated that the feeding of AT increased ruminal production of volatile fatty acids and thereby lowered ruminal fluid pH. This is uniform with previous papers (Minato et al., 1989).

The significantly high VFA concentration observed in the caecal contents of sheep on AT was in constrast to the low VFA concentration in the rectal contents of sheep on a diet similar to the present study (Cann et al., 1991). Ammoniation of straws has been found to increase the digestibility leading to an increased supply of

digestible nutrients to the lower tract, bacterial activity in the lower tract and the observed higher production of VFAs compared with other diets.

In the previous report (Minato et al., 1989), increased butyrate concentration in the rumen was suspected to be due to the significant presence of *Eubacterium sp.*, butyrate producers, in animals on AT. Accordingly, it is possible in the present experiment that when AT was fed, the butyrate producing *Eubacterium sp.* was increased not only in the rumen, but also in the caecum.

The high ammonia-nitrogen concentrations in the caecum may, in part, explain the high pH values. Ammonia-nitrogen concentration range required for optimal activity of ruminal cellulolytic bacteria has been reported as 5-28 mg/dl (Durand et al., 1989). The concentrations at the period of sampling indicated that ammonia-nitrogen was not a limiting factor and that the improvements observed in this experiment are rather attributable to changes in the cell wall structure due to ammoniation.

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