Fungal Suppression and Its Effects on Some Ruminal Parameters

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Abstract The effect of feeding of sodium chlorite-treated rice straw (SCT) or polyoxin D (PD: a chitin synthesis inhibitor) on rumen microbes and metabolites was investigated with rumen cannulated sheep. The experiment consisted of three periods of pre-treatment (25 days), treatment (10 days) and post-treatment (10 days). During the pre- and post-treatment periods, the animals were offered untreated rice straw and concentrate. In the course of the treatment period, sheep on SCT received sodium chlorite-treated rice straw and concentrate, whereas those on PD were offered untreated rice straw, concentrate and polyoxin D. Both SCT and PD treatments suppressed fungal growth with concomitant increases in protozoal counts. During the period of treatments, concentration of acetate decreased while that of propionate and butyrate increased. Ruminal ammonia-N was not significantly altered at any stage of the experiment.

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The anaerobic fungi, which are members of the microbial population in the gut of herbivora, have been shown to colonize plant fragments in the rumen¹⁾. The isolates of these organisms produce extracellular enzymes important in the degradation of plant polymers^{8,9)} and thus making them important members of the ruminal ecology.

The availability of procedures for the elimination of protozoa from the rumen has shed light on their contribution to this complex ecosystem. On the other hand, studies concerning the elimination or suppression of the rumen anaerobic fungi, with the view of elucidating their role in the rumen are rare.

The objective of the present experiment was to investigate two possible ways of eliminating or suppressing the anaerobic fungi and their effects on the ruminal environment.

Materials and Methods

Polyoxin D, an antibiotic which suppresses fungal growth by inhibiting the conversion of uridine 5'-diphosphate N-acetylglucosamine to chitin, is a product of Kaken Pharmaceutical Co. Rice straw was treated with a sodium chlorite acetic acid mixture as previously described⁵⁾.

The experiment was divided into 3 periods: pre-treatment, treatment and post-treatment. During the treatment period two fungal growth-suppressing diets, namely a sodium chlorite-treated rice straw (SCT) diet (300 g SCT +100 g concentrate) and a polyoxin D (PD)-included diet (300 g untreated rice straw +100 g concentrate +8.8 g PD) were offered twice daily to two crossbreed sheep (Suffolk × Corriedale), each equipped with a permanent ruminal cannula. During pre- and post-treat-

Table 1. Chemical composition of untreated (UT)- and sodium chlorite-treated (SCT) rice straw (% dry matter basis)

Item	UT-rice straw	SCT-rice straw
DM	88.1	89.2
NDF	74.9	77.5
ADF	48.9	49.2
Hemicellulose	26.0	28.2
Cellulose	34.0	39.6
Lignin	6.8	0.7
Ash	8.1	9.0

The commercial formula feed for beef cattle contains the following (%): milo, corn, barley and rye, 65: wheat bran, corn gluten meal, rice bran, 19: soybean meal, rapeseed meal and cotton seed oil, 7: molasses, alfalfa meal, CaCO₃, NaCl and Ca₃(PO₄)₂, 9.

ment period, sheep received the same diet consisting of 300 g untreated rice straw and 100 g concentrate in a manner similar to the treatment period (Table 1).

There was an adaptation period of 21 days. The pre-treatment period ruminal samples were collected just before (0 hr) and 3 hr after the morning feed on the 2nd and 4th day after adaptation. During the treatment period, one

sheep was offered PD diet while the other received SCT diet over a period of 10 days within which ruminal samples were collected on 4 different days (2nd, 4th, 7th and 10th day) in the same manner as mentioned above. Ruminal samples also were collected on the 7th and 10th days of the post-treatment period. The experiment was repeated with a cross-over design.

On each day of sampling, aliquots of rumen samples taken just before the morning feed were used for total viable counts, total protozoal counts and percentage composition of species and fungal counts as described elsewhere^{3,6,11}. Ruminal fluid pH, ammonia-N, volatile fatty acids (VFA) and their molar composition of all rumen samples were determined as described earlier³.

Data were pooled and analyzed by analysis of variance and Duncan's multiple range test¹⁰, whenever appropriate.

Results

Fungal counts determined by the agar strip method indicated a significant decrease ($p < 0.01$) during the treatment period (Tables 2 and 3). Results from the roll tube method also

Table 2. Rumen microbial changes before, during and after the feeding of sodium chlorite-treated (SCT) rice straw diet (Mean $\pm$ S.E.)

Item	Before	During	After
No. of samples	4	8	4
Total viable count ¹	5.26 $\pm$ 0.49 ^{ab}	6.12 $\pm$ 0.23 ^b	3.82 $\pm$ 0.92 ^a
Fungal counts			
Agar strip method ²	1.67 $\pm$ 0.11 ^b	0.22 $\pm$ 0.05 ^a	1.97 $\pm$ 0.28 ^b
Roll tube method ³	3.68 $\pm$ 0.27 ^a	0.74 $\pm$ 0.26 ^a	9.83 $\pm$ 2.82 ^b
Total protozoal count ⁴	0.83 $\pm$ 0.05 ^a	1.48 $\pm$ 0.07 ^b	1.43 $\pm$ 0.09 ^b
% composition of major species			
<i>Entodinium nanellum</i>	23.53 $\pm$ 1.39	21.83 $\pm$ 1.74	26.60 $\pm$ 1.42
<i>Entodinium simplex</i>	42.86 $\pm$ 1.92	38.04 $\pm$ 2.25	33.30 $\pm$ 1.57
<i>Entodinium caudatum</i>	14.20 $\pm$ 1.83	14.97 $\pm$ 2.12	25.53 $\pm$ 5.43
<i>Entodinium longinucleatum</i>	7.75 $\pm$ 0.58 ^{ab}	11.55 $\pm$ 0.96 ^b	4.75 $\pm$ 2.12 ^a

Means within the same row with different letters differ (DMRT, $p < 0.01$)

¹; $\times 10^8$ /ml ruminal contents

²; $\times 10^3$ sporangia/cm²

³; $\times 10^3$ zoospores/ml ruminal contents

⁴; $\times 10^5$ /ml ruminal contents

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Table 3. Rumen microbial changes before, during and after the feeding of polyoxin D (PD) (17.6 g/d) diet (Mean±S.E.)

Item	Before	During	After
No. of samples	4	8	4
Total viable count ¹	5.01±1.16	4.80±1.60	4.11±1.61
Fungal counts			
Agar strip method ²	1.90±0.06 ^b	0.89±0.25 ^a	2.32±0.12 ^b
Roll tube method ³	7.43±2.54	2.78±1.31	9.60±3.20
Total protozoal count ⁴	1.73±0.11 ^a	2.11±0.11 ^{ab}	2.50±0.32 ^b
% composition of major species			
<i>Entodinium nanellum</i>	30.05±3.15	29.51±4.42	29.20±4.05
<i>Entodinium simplex</i>	27.25±2.88	31.70±3.27	27.08±4.35
<i>Entodinium caudatum</i>	19.98±5.55	22.73±6.04	24.43±6.24
<i>Entodinium longinucleatum</i>	6.32±0.89 ^b	2.46±0.60 ^a	3.28±0.81 ^a

Means within the same row with different letters differ (DMRT, p<0.01)

¹, ×10⁸/ml ruminal contents

², ×10³ sporangia/cm²

³, ×10³ zoospores/ml ruminal contents

⁴, ×10⁵/ml ruminal contents

Table 4. Ruminal fermentation patterns before, during and after the feeding of sodium chlorite-treated (SCT) rice straw diet (Mean±S.E.)

Item	Time(hr)	Before	During	After
No. of samples		4	8	4
Total VFA	0	80.1±2.1 ^a	76.2±3.7 ^a	113.1±14.1 ^b
(mmole/l)	3	101.2±8.0	98.3±1.0	99.5± 8.8
Molar % composition				
Acetate	0	62.4±3.6 ^a	40.9±0.6 ^b	57.4± 2.5 ^a
	3	60.9±2.5 ^a	42.0±0.6 ^b	58.2± 2.6 ^a
Propionate	0	20.2±0.2 ^a	43.1±1.1 ^c	28.2± 2.2 ^b
	3	24.6±0.6 ^a	42.0±0.6 ^b	27.4± 2.0 ^a
Iso-Butyrate	0	1.4±1.0	0.7±0.3	0
	3	0	0.9±0.3	0
n-Butyrate	0	8.2±1.0 ^a	11.3±0.6 ^b	10.5± 0.3 ^b
	3	9.1±0.5 ^a	12.0±0.0 ^c	10.7± 0.5 ^b
Iso-Valerate	0	4.8±0.8 ^b	2.1±0.5 ^a	3.8± 1.0 ^{ab}
	3	3.9±1.0	1.5±0.6	2.2± 0.4
n-Valerate	0	1.9±1.1	2.0±0.4	1.2± 0.6
	3	1.6±1.2	2.7±0.5	1.6± 0.5
NH ₃ -N (mg/dl)	0	14.3±2.7	15.8±2.3	20.0± 4.7
	3	13.4±2.1	16.6±2.5	14.1± 4.8
pH	0	7.0±0 ^b	6.9±0 ^a	6.8± 0.1 ^a
	3	6.9±0 ^b	6.8±0 ^c	6.7± 0.1 ^a

Means within the same row with different letters differ (DMRT, p<0.01)

gave decreases in fungal counts during this period. However, the changes were not significant probably due to high variations during the period of sampling. The effect of SCT diet on rumen fungal counts was more drastic than that of PD diet. Fungal counts after the cessation of the feeding of SCT and PD diet tended to be restored to previous counts.

The period of fungal suppression coincided with that of an increasing tendency of total viable and protozoal counts (Table 2). Reversing to the pre-treatment feed resulted in a decreased total viable count (Table 2). With the exception of *Entodinium longinucleatum* which increased ($p<0.01$) during SCT feeding, percentage composition of protozoal species was not altered. PD feeding did not alter total viable counts, though it showed a tendency toward increases in total protozoal counts (Table 3). Unlike the SCT diet, the percentage of *E. longinucleatum* decreased ($p<0.01$) during and after PD treatment (Table 2 and 3).

When the SCT diet was fed, total VFA concentration at 0 hr showed no alteration, whereas the concentration during post-treatment period increased significantly (Table 4). The concentration at 3 hr after feeding was not altered. On the other hand, even though total VFA concentrations increased during and after cessation of PD diet feeding, the increases were not statistically significant (Table 5) probably due to the high variations observed during these periods. In both treatment periods, acetate proportion decreased significantly ($p<0.01$) while propionate ($p<0.01$) and butyrate proportions increased (Table 4 and 5). The increase in butyrate proportions was maintained after cessation of fungal growth-suppressing diet administration. The changes in butyrate was, however, not statistically significant in the results with PD diet. The depressed iso-valerate proportion ($p<0.01$) observed in the feeding of SCT was not observed in PD administration. Ruminal ammonia-N

Table 5. Ruminal fermentation patterns before, during and after the feeding of polyoxin D (PD) (17.6 g/d) diet (Mean $\pm$ S.E.)

Item	Time (Hr)	Before	During	After
No. of samples		4	8	4
Total VFA	0	90.5 $\pm$ 3.6	110.0 $\pm$ 10.3	120.5 $\pm$ 12.9
(mmole/l)	3	105.9 $\pm$ 5.0	122.5 $\pm$ 7.9	116.9 $\pm$ 6.5
Molar % composition				
Acetate	0	64.0 $\pm$ 0.2 ^b	57.4 $\pm$ 1.1 ^a	60.2 $\pm$ 1.5 ^{ab}
	3	60.3 $\pm$ 0.1 ^b	56.8 $\pm$ 0.4 ^a	61.2 $\pm$ 0.1 ^b
Propionate	0	24.3 $\pm$ 0.2 ^a	28.2 $\pm$ 0.9 ^b	26.6 $\pm$ 1.4 ^{ab}
	3	27.0 $\pm$ 0.6 ^a	29.1 $\pm$ 0.9 ^b	26.2 $\pm$ 0.9 ^a
n-Butyrate	0	8.2 $\pm$ 0.3	9.7 $\pm$ 0.8	9.8 $\pm$ 0.7
	3	9.7 $\pm$ 0.2	10.2 $\pm$ 0.5	9.8 $\pm$ 0.5
Iso-Valerate	0	2.5 $\pm$ 0.2	3.6 $\pm$ 0.6	3.9 $\pm$ 0.7
	3	2.0 $\pm$ 0.1	2.5 $\pm$ 0.4	2.1 $\pm$ 0.1
n-Valerate	0	0.9 $\pm$ 0.5	1.1 $\pm$ 0.6	1.4 $\pm$ 0.9
	3	1.3 $\pm$ 0.6	1.3 $\pm$ 0.4	0.7 $\pm$ 0.4
NH ₃ -N (mg/dl)	0	22.1 $\pm$ 1.2	25.0 $\pm$ 1.0	28.9 $\pm$ 5.5
	3	17.1 $\pm$ 0.9	27.0 $\pm$ 2.3	23.9 $\pm$ 5.5
pH	0	6.9 $\pm$ 0.1	6.8 $\pm$ 0	6.7 $\pm$ 0.1
	3	6.7 $\pm$ 0.1	6.7 $\pm$ 0	6.6 $\pm$ 0.1

Means within the same row with different letters differ (DMRT, $p<0.01$)

concentrations were not statistically altered in both SCT and PD feeding. The values of ruminal pH significantly ($p < 0.01$) dropped during and after SCT feeding.

Discussion

The two methods used in the present experiment substantially suppressed fungal growth *in vivo*. However, unlike SCT barley straw which was reported to have eliminated anaerobic fungi completely from the rumen⁴⁾, SCT rice straw feeding failed in complete elimination. This is not clear at present, because the exact mechanism by which SCT straw inhibits fungal growth is still unknown. Polyoxin D fed at 17.6 g/d was effective as a fungal inhibitor, suggesting that chitin synthesis is essential for fungal proliferation in the rumen.

The increase in protozoal counts observed during the period of fungal suppression was in agreement with the previous report⁴⁾. The decrease in fungal population in the presence of similar quantities of nutrients is likely to stimulate growth in the two major rumen microbes, namely bacteria and protozoa. In the experiment with SCT, straw refusals of 112 ± 39.9 and 71.5 ± 25.4 grams at each feeding time during the period of treatment and post-treatment, respectively, were observed. The total VFA concentrations during these periods were, however, statistically equal to the pre-treatment period characterized by high fungal activity and high feed intake. This observation was contrary to that of others⁴⁾ who reported a fall of approximately 13 mmole/l of total VFA during the period of fungal absence and reduced feed intake. This discrepancy may either be due to the inability, in the present experiment, to eliminate the anaerobic fungi completely or structural differences between rice straw and barley straw. The decrease in intake without a fall in the volatile fatty acid concentration suggests that rumen digestibility is improved when rice straw is treated with the sodium chlorite acetic acid mixture.

Rumen anaerobic fungi are known to produce acetate as a major fermentation product during cellulose degradation^{7,9)}. The contribution of these organisms to the ruminal acetate pool is, therefore, certain and it is highly likely that a decreased fungal activity results in the decrease in acetate production in the rumen as observed in the present study and by others⁴⁾. The decrease of acetate was accompanied by an increase in propionate⁴⁾. However, in the present experiment, decreasing acetate was not only compensated with propionate increase, but also butyrate. Whereas the decrease in straw intake due to SCT feeding may result in an increased concentrate : fibre intake with subsequent changes in rumen fermentation pattern, in an experiment using strained rumen fluid to digest SCT rice straw (without concentrate) results similar to those reported in the present experiment were observed (CANN *et al.* unpublished results). In addition, incubation of untreated rice straw with sodium chlorite in the presence of rumen fluid failed to yield results as observed in that with SCT rice straw (CANN *et al.* unpublished results). It may, therefore, be assumed that the changes observed in the rumen fermentation pattern in sheep on SCT were mostly due to changes in fibre characteristics and suppression of the anaerobic fungi.

In conclusion, the present study confirmed that (the absence or) a decreased fungal population in the rumen will lead to an increase in protozoal and bacterial activity. The fermentation pattern is likely to shift to that of a decreased acetate production concomitant with a higher propionate synthesis and material (fiber source) dependently, a higher butyrate production.

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ルーメン藻菌数の抑制とそれが各種ルーメン 指標に及ぼす影響

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過塩素酸ナトリウム処理した稲わらまたはキチン合成の阻害剤であるポリオキシシン D をルーメンカニューレを装着したヒツジに給与して、ルーメン微生物数や代謝産物への影響を観察した。実験には 2 頭の雑種（サフォーク×コリデール）ヒツジを供試し、上記処理稲わらまたはポリオキシシン D 給与前期（25 日間）、給与期（10 日間）、給与後期（10 日間）を設けた。給与期には 1 頭のヒツジには過塩素酸処理わらと濃厚飼料を、他の 1 頭には無処理稲わらと濃厚飼料およびポリオキシシン D を給与し、この給与をヒツジを代えて繰り返した。給与前、後期にはいずれも無処理の稲わらと濃厚飼料を与えた。処理稲わらおよびポリオキシシン D 給与は藻菌数を減少させ、プロトゾア数を増加させた。またこれらの飼料給与期には、ルーメン液中の酢酸割合が減少し、プロピオン酸、酪酸割合の増加がみられた。しかしアンモニア態窒素濃度に影響はみられなかった。これらの結果から藻菌数の抑制は他の微生物数に影響を与え、ルーメンの VFA 組成にも影響が現れることを確認した。

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