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Original article

Effects of 3 chemical treatments on *in vitro* fermentation of rice straw by mixed rumen microbes in the presence or absence of anaerobic rumen fungi

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Summary — Rice straw (Rs) was treated by a sodium chlorite/acetic acid mixture (Sct), ammoniation (At) and alkaline hydrogen peroxide (Athp) methods. The objective was to compare their degradation and fermentation products in the presence or suppression of anaerobic fungi. Significant differences ($P < 0.01$) in degradation of straws were observed during all periods of incubation with Sct having the highest digestibilities during the 48-h and 72-h incubations. The degradation of Sct straw was highest in both of the incubations with whole rumen fluid (WRF) and WRF plus cycloheximide. *In sacco* digestion followed the order of Sct > At > Rs > Athp. Suppressing fungal activity with cycloheximide resulted in a decrease in dry matter degradation, with concomitant decrease in total volatile fatty-acid concentration. While the suppression of fungal activity with cycloheximide depressed acetate and butyrate production, it favored an increase in propionate production.

rice straw / rumen microbe / cycloheximide / fermentation

Résumé — Effet de 3 traitements chimiques sur la fermentation *in vitro* de la paille de riz par les microbes du rumen, en présence ou après suppression des champignons anaérobies. *L'objectif de cette étude était de comparer la dégradation et les produits de fermentation, en présence ou après suppression des champignons anérobies, de la paille de riz (Rs) traitée par un mélange de chlorite de sodium et d'acide acétique (Sct), par ammoniation (At) ou par traitement alcalin au peroxyde d'hydrogène (Athp). Des différences significatives (P < 0,01) de dégradation des pailles ont été observées pendant toutes les périodes d'incubation, le produit Sct ayant les digestibilités les plus élevées pendant les incubations de 48 et 72 h. La dégradation de la paille Sct était la plus forte dans les 2 incubations, avec du fluide total du rumen sans (WRF) ou avec cycloheximide. La digestion* in sacco *suivait l'ordre suivant : Sct > At > Rs > Athp. La suppression de l'activité fongique par addition de cycloheximide conduisait à une diminution de la dégradation de la matière sèche, avec une baisse concomitante de la concentration en acides gras volatils totaux. Alors que la suppression de l'activité fongique diminuait la production d'acétate et de butyrate, elle favorisait l'augmentation de la production de propionate.*

paille de riz / microbe du rumen / cycloheximide / fermentation

INTRODUCTION

The utilization of the lignocellulosic materials abound in nature by ruminants is carried out by a host of microorganisms (bacteria, protozoa and fungi) present in the rumen that ferment these materials yielding mainly acetate, propionate and butyrate. These fermentation products are absorbed and serve as the main energy source for the ruminant. The degradation of these fibrous materials by the microorganisms is, however, constricted by their high lignin content. Chemical treatments, such as chlorite delignification, have been used as means of circumventing this limitation. In recent studies, Elliot *et al* (1987) and Cann *et al* (1993b) observed that treatment with sodium chloride/acetic acid mixture (Sct) decreased lignin content but also drastically depressed intake by sheep. This reduced intake of Sct-chaffed barley straw has been attributed to the fungicidal effect of the treated straw (Elliot *et al*, 1987). However, it has been reported by Windham and Akin (1984) that suppressing fungal activity *in vitro* did not decrease dry matter degradation of ground alfalfa and coastal bermuda grass.

To investigate the discrepancy, an experiment was conducted to assess the effect of sodium chlorite/acetic acid mixture treatment of rice straw on its degradability by mixed rumen bacteria. The degradability of this material was also compared with ammoniated rice straw and alkaline hydrogen peroxide treatment in the presence or absence of rumen fungi.

MATERIALS AND METHODS

Substrates

Rice straw (Rs) was treated chemically by 3 methods, namely, ammoniation at 3 g NH_3/100 g DM (At) (Cann *et al*, 1991), alkaline hydrogen peroxide treatment (Athp) (Lewis *et al*, 1988) and sodium chlorite/acetic acid mixture treatment (Elliot *et al*, 1987). During Athp treatment, NH_4OH was used as the alkali instead of NaOH. The treated straws were ground through a 1-mm screen in a Wiley mill and used as substrates.

Substrate characteristics

Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, lignin and ash were determined as described elsewhere (Cann *et al*, 1991). X-ray diffraction was performed with a Mini Flex X-ray diffractometer (Rigaku–denki Co), operated under the following conditions: X-ray tube 30 kV, 10 mA; scanning speed 2°/min; divergence slit 1°; receiving slit 0.3 mm; time constant 2 s; irradiation CuKα, eliminating Kβ with an Ni-filter.

Microbial inoculum

Two sheep cannulated in the rumen and maintained daily on 600 g of alfalfa hay cubes and 200 g of a commercial formula feed (a grain and mineral supplement, crude protein 12%, total digestible nutrients (TDN) 72%) for beef cattle were used as donors of rumen fluid. Rumen fluid 200 ml/sheep was removed just before offering the morning feed (9.00 am), mixed together and squeezed through 4 layers of surgical gauze. The strained fluid was mixed with a pre-heated (39°C) McDougall's buffer (McDougall, 1948) in a ratio of 1:2 and used as inoculum (WRF). The microbial characteristics (table I) of the rumen fluid were determined as described previously (Cann *et al*, 1993c).

In vitro *studies*

Substrates of weight 0.5 g from the respective straws (Rs, At, Athp and Sct) were weighed into 70 ml bottles with 4 replicates per sample. The bottles were flushed with an oxygen-free CO_2, prepared by passage through a column of heated copper filings, for 2 min and 25 ml of inoculum (WRF) dispensed into each of the bottles and sealed under anaerobic conditions. To sup-

press fungal growth, 3 ml of cycloheximide (C-6255, Sigma) solution (5 mg/ml of distilled H_2O) was dispensed together with inoculum (4 replicates per sample) to study the effect of fungal suppression on the degradability and fermentation pattern of the treated and untreated straws The tubes receiving only whole rumen fluid (WRF) received additional 3 ml distilled water to bring their volume to that of the experiment with WRF plus cycloheximide (B + P: the inoculum consists essentially of bacteria (B) and protozoa (P)). Inoculum blanks were prepared to correct for the indigestible dry matter from inoculum residue. In addition, substrates were incubated with only McDougall's buffer (solubility in buffer: At > Rs > Athp > Sct) to correct for solubilization due to the buffer. The incubation was carried out in a water bath at 39°C for 12, 24, 48 and 72 h. After incubation, samples were filtered (Soseni teiryoroshi C-7000, Sanshin Kogyo, Japan) and the pH of the fluid determined immediately. The undigested material was washed with 100 ml boiling water and dried at 100°C overnight in an oven. The fluid content was stored on mercuric chloride in a freezer at –4°C until analyzed for ammonia nitrogen and volatile fatty acids by colorimetry and gas chromatography, respectively, as described elsewhere (Cann *et al*, 1991).

In sacco *study*

In this investigation, 0.5 g of each sample was weighed into a nylon bag with pore size 5 μm. Samples were then incubated in the rumen of the sheep donating the rumen digesta for the *in vitro* experiment, in a random fashion, over the periods stated above. After withdrawal, the nylon bags were washed gently with distilled water and the digestibility determined as described in the *in vitro* study.

Statistical analysis

The results were analyzed by analysis of variance and Duncan's multiple range test (DMRT) used to separate the means (Duncan, 1955).

RESULTS

The treatments involving washing (Athp and Sct) in the present experiment significantly ($P < 0.01$) increased NDF with a further increase being observed in ADF content of Athp (table II). An increase in cellulose was observed for Athp and Sct. Lignin content decreased in At and Sct with the most significant ($P < 0.01$) decrease occurring in the latter. The ash content of Athp was significantly ($P < 0.01$) higher compared to the other straws. The X-ray diffraction patterns (fig 1) showed a relative increase in the broad peak be-

Table I. Microbial characteristics of rumen fluid from sheep used for the present experiment.

Item	*Count*
Total viable bacterial count (x 10^8 cells/ml of rumen fluid)	3.7 ± 0.7
Amylolytic bacterial count (x 10^8 cells/ml of rumen fluid)	3.2 ± 0.8
Proteolytic bacterial count (x 10^8 cells/ml of rumen fluid)	3.8 ± 0.3
Lactate-fermenting bacterial count (x 10^7 cells/ml of rumen fluid)	1.3 ± 0.3
Cellulolytic bacterial count (x 10^6 cells/ml of rumen fluid)	2.6 ± 0.5
Fungal count (agar strip) (x10^3 sporangia/cm^2)	1.2 ± 0.1
Fungal count (roll tube) (x 10^3 zoospores/ml of rumen fluid)	1.4 ± 0.5
Total protozoal count (x 10^5 cells/ml of rumen fluid)	2.6 ± 0.3
% Composition	
Entodinium sp	90.7 ± 2.5
Isotricha prostoma	2.3 ± 0.3
Polyplastron multivesiculatum	5.0 ± 0.5

tween 19–26° for Athp and Sct. The microbial characteristics (table I) indicated a total viable count, fungal count and total protozoal counts of 3.7 x 10^8, 1.4 x 10^3 and 2.6 x 10^5, respectively. The dominant protozoal species belonged to the genus *Entodinum*. Digesting the straws with WRF for 12 h did not show any difference for Sct and Athp digestibilities, but resulted in significantly higher values for At and Rs. The digestibilities during the 24, 48 and 72 h periods indicated higher ($P < 0.01$) values for the treated straws (Sct, At and Athp) compared with Rs (fig 2a), with Sct showing the highest digestibility followed by At. Compared with WRF, suppressing fungal activity with cycloheximide (fig 2b) did not result in changes in digestibility values during the 12-h and 24-h incubations. The digestibilities during the 48-h and 72-h periods, however, showed a decreased trend. The digestibility patterns among treatments were, however, similar to that with WRF. The results from *in sacco* digestion showed an identical pattern to that of

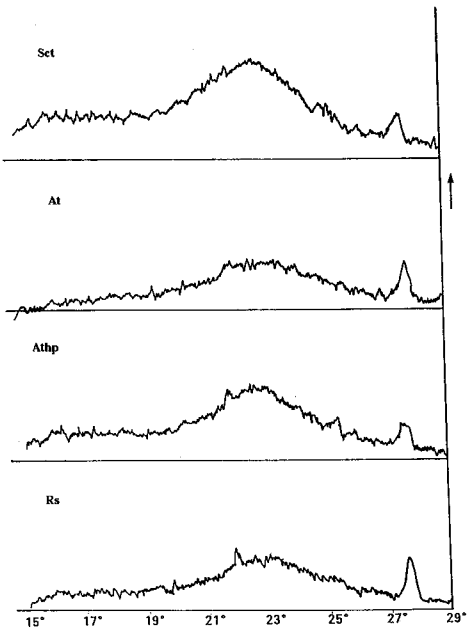

Fig 1. X-ray diffraction patterns of rice straw and treated rice straw. The abscissa is intensity and the ordinate is the angle of diffraction 2ϑ.

Table II. Chemical analyses of treated and untreated straws (%).

Item	*Rs*	*At*	*Athp*	*Sct*
DM	88.1 ± 0.2 [a]	88.1 ± 0.2 [a]	90.2 ± 0.3 [c]	89.2 ± 0.2 [b]
NDF	74.9 ± 0.4 [a]	76.2 ± 0.9 [ab]	82.1 ± 0.2 [c]	77.5 ± 0.1 [b]
ADF	48.9 ± 0.3 [a]	48.9 ± 0.2 [a]	53.1 ± 0.5 [b]	49.2 ± 0.3 [a]
Hemicellulose	26.0 ± 0.9	27.3 ± 1.7	29.0 ± 0.8	28.3 ± 0.5
Cellulose	34.0 ± 0.1 [a]	34.1 ± 0.1 [a]	37.7 ± 0.3 [b]	38.6 ± 0.2 [c]
Lignin	6.8 ± 0.3 [b]	6.1 ± 0.2 [c]	6.6 ± 0.2 [b]	2.7 ± 0.3 [a]
Ash	8.1 ± 0.1 [a]	8.6 ± 0.2 [ab]	8.8 ± 0.3 [b]	8.0 ± 0.1 [a]

Rs: untreated rice straw; At: ammoniated rice straw; Athp: alkaline hydrogen peroxide-treated rice straw; Sct: sodium chlorite/acetic acid mixture-treated rice straw; values are means ($n = 4$) ± SE; means within the same row with different letters differ (Duncan's multiple range test, $P < 0.01$).

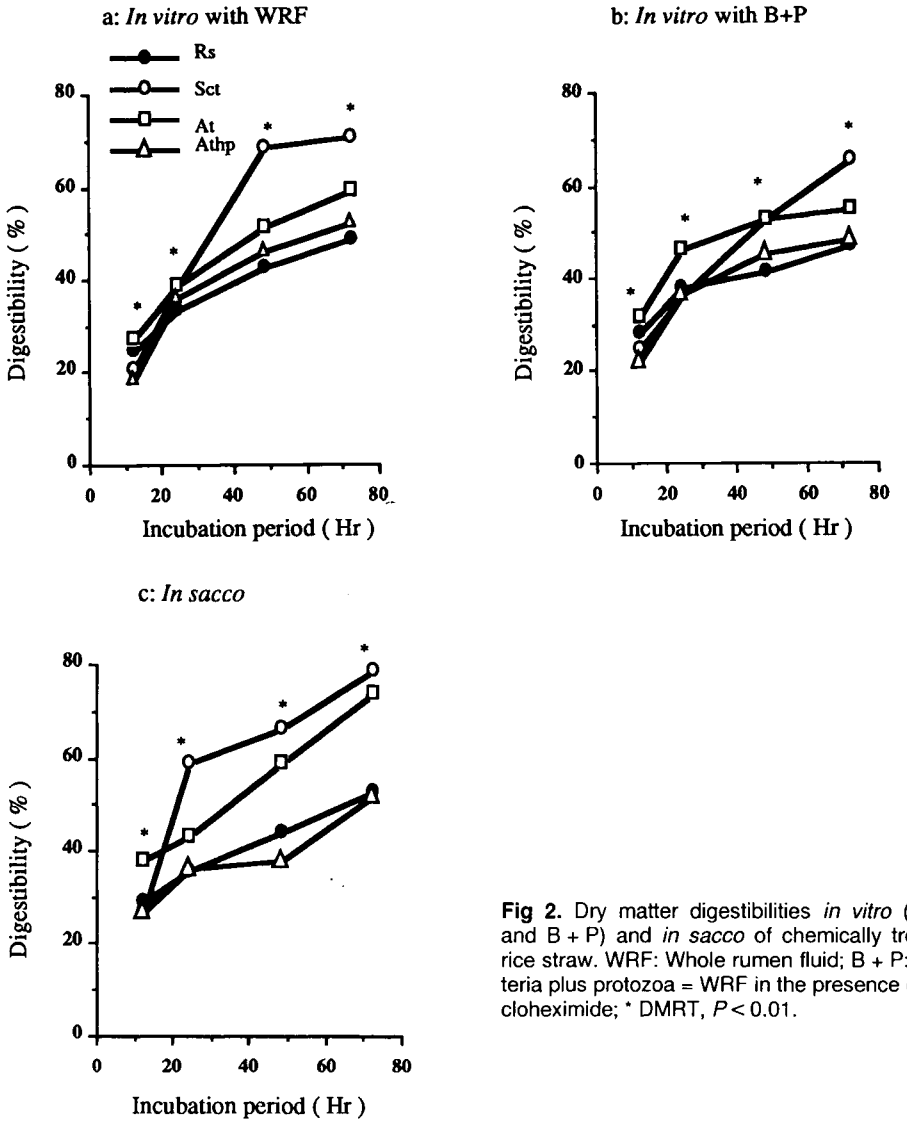

Fig 2. Dry matter digestibilities *in vitro* (WRF and B + P) and *in sacco* of chemically treated rice straw. WRF: Whole rumen fluid; B + P: bacteria plus protozoa = WRF in the presence of cycloheximide; * DMRT, $P < 0.01$.

the *in vitro* experiment, but the digestibilities were higher *in sacco* than *in vitro*, especially for Sct and At (fig 2c). The yield of total volatile fatty acids during the fermentation followed the pattern observed for digestion (fig 3). The total volatile fatty-acid concentration was highest ($P < 0.01$) during the 48-h and 72-h incubation for Sct. The values for Athp and Rs were statistically similar. In all experiments with WRF, acetate proportions due to Sct were significantly ($P < 0.01$) lower compared to the other treatments (At and Athp) and Rs (table III). As the period of incubation increased, acetate proportion decreased, a tendency that was also observed during

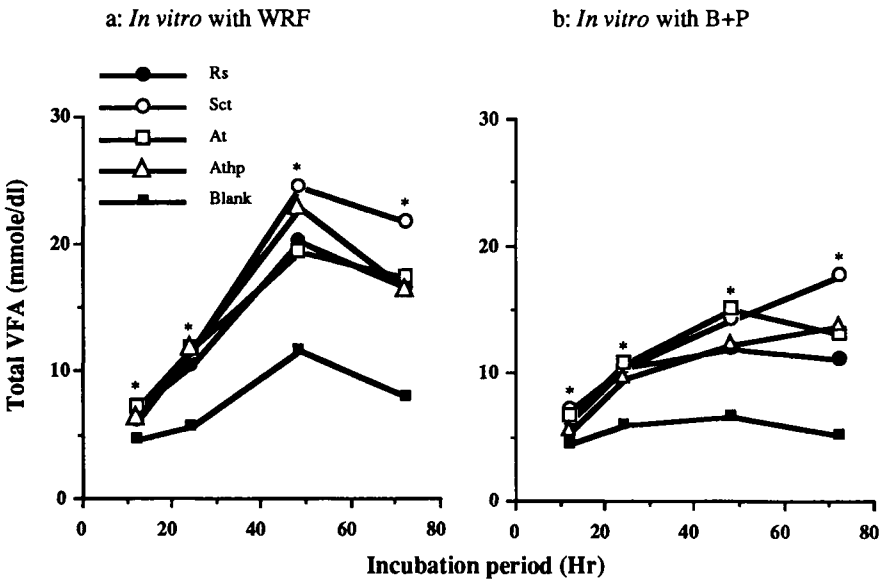

Fig 3. Total volatile fatty-acid concentrations from treated and untreated rice straws incubated with WRF or B + P. WRF: whole rumen fluid; B + P: bacteria plus protozoa = WRF in the presence of cycloheximide; * DMRT, $P < 0.01$.

cycloheximide inhibition (B + P) (table IV). The reverse of this trend was noted for propionate proportions. However, the propionate proportions during fungal suppression (B + P) were higher than those of WRF, resulting in decreased acetate/propionate ratios in all incubations with (B + P). In Sct, the ratio decreased below 1.0 during the 48-h and 72-h incubations for both WRF and (B + P) (table III and IV). The proportion of *n*-butyrate and *i*-valerate were higher in WRF than (B + P), with the values being highest ($P < 0.01$) in Rs. The values for *n*-valerate in the present experiment were not altered ($P > 0.05$) due to treatment. Ammonia N concentrations were higher during digestion with WRF than (B + P). The values in both cases were highest in At with Sct having the lowest concentration. The pH values observed for Rs, At and Athp were slightly higher during (B + P) incubation than for WRF. The values for Sct decreased sharply from the 24-h incubation and continued to the 72-h incubation. This observation was made in both WRF and (B + P) incubations.

DISCUSSION

The treatments involving oxidation and washing (Athp and Sct) in the present experiment with a higher NDF indicated that the treatments removed substantial components of straw normally solubilized during NDF solution extraction. A similar explanation can be offered for the increase in ADF in Athp. The 2 treatments, however, increased cellulose fractions. The increase in the intensity of the broad peak observed in Athp and Sct (fig 1) may reflect the relative increase in the cellulose content due to the 2 treatments. The increases in NDF and ADF proportions observed for Athp were also obtained by other workers using

Table III. Fermentation products, pH and NH_3-N concentrations of treated and untreated rice straw with whole rumen fluid (WRF) *in vitro**.

Item	*Incubation period (h)*	*Molar percentages of acids*					*A:P*	*pH*	*NH_3-N (mg/dl)*
		Acetate	*Prop*	n-*Buty*	i-*Valerate*	n-*Valerate*			
Rs	12	52.6^{b}	19.4^{b}	15.4	5.9	3.0^{a}	2.9	6.6^{c}	32.3^{a}
	24	56.7^{c}	23.1^{b}	12.6^{b}	5.8^{c}	1.7	2.5	6.5^{b}	29.2^{a}
	48	51.8^{d}	24.2^{b}	13.1^{b}	6.5^{d}	–	2.1	6.5^{c}	29.8^{a}
	72	51.6^{c}	28.9^{b}	12.4^{b}	4.6^{c}	2.6^{a}	1.8	6.5^{c}	24.2^{a}
Sct	12	48.8^{a}	26.7^{c}	14.8	5.3	3.0^{a}	1.8	6.4^{a}	35.2^{a}
	24	48.8^{a}	35.6^{d}	11.2^{b}	3.4^{a}	1.6	1.4	6.3^{a}	26.3^{a}
	48	40.7^{a}	41.7^{d}	12.1^{b}	2.1^{a}	–	1.0	5.8^{a}	32.5^{a}
	72	39.8^{a}	44.6^{d}	9.8^{a}	2.3^{a}	3.4^{b}	0.9	5.5^{a}	29.8^{a}
At	12	53.7^{b}	20.8^{b}	14.0	5.5	3.5^{a}	2.6	6.5^{b}	50.4^{b}
	24	58.0^{c}	24.2^{b}	11.3^{b}	4.5^{b}	1.6	2.4	6.4^{b}	48.9^{b}
	48	53.3^{e}	26.3^{bc}	12.8^{b}	5.5^{b}	–	2.0	6.3^{b}	69.2^{c}
	72	51.9^{c}	31.2^{c}	10.7^{ab}	3.9^{b}	2.3^{a}	1.7	6.2^{b}	62.3^{c}
Athp	12	51.5^{ab}	19.5^{b}	15.3	6.3	3.7^{a}	2.6	6.5^{b}	34.3^{a}
	24	53.3^{b}	30.2^{c}	10.7^{a}	4.1^{ab}	1.3	1.8	6.5^{b}	32.5^{a}
	48	50.9^{c}	28.3^{c}	11.3^{a}	6.0^{c}	–	1.8	6.4^{b}	43.2^{b}
	72	51.5^{c}	31.3^{c}	10.9^{ab}	3.9^{b}	2.5^{a}	1.6	6.3^{b}	46.1^{b}
Blank	12	49.1^{a}	16.0^{a}	13.7	8.9	6.8^{b}	3.1	6.5^{b}	58.0^{c}
	24	68.4^{d}	13.3^{a}	11.3^{b}	9.7^{d}	–	5.1	6.5^{b}	53.0^{c}
	48	47.5^{b}	17.4^{a}	14.6^{c}	11.2^{e}	–	2.7	6.6^{d}	85.2^{d}
	72	50.6^{b}	17.3^{a}	16.9^{c}	8.3^{d}	3.8^{c}	2.9	6.7^{c}	83.0^{d}

* The values are means of 4 observations; means within the same column and period with different letters differ (DMRT, $P < 0.01$); A:P: acetate/propionate ratio; Prop: propionate; *n*-Buty: *n*-butyrate.

wheat straw (Lewis *et al,* 1988). On the other hand, using NaOH as the alkali in the alkaline hydrogen peroxide treatment decreased lignin content about 5 percentage units (Lewis *et al,* 1988). However, in the present experiment, this response was not observed probably due to NH_4OH being a weaker base than NaOH.

The increased degradation of At was expected as has been reported in previous studies (Graham and Aman, 1984; Cann *et al,* 1991). The higher digestibilities of At and Sct compared with Athp and Rs can be explained by the decrease in lignin content, which is one of the major limiting factors of digestion, in At and Sct. The decreases in lignin in At and Sct agree with findings reported elsewhere (Brown *et al,* 1987; Cann *et al,* 1993b). The fact that the degradation of Sct during the 48-h and 72-h incubations was 17% and 12% units, respectively, above that of At could be explained by the further decrease in lignin content of Sct, and supports the previous assumption that the decrease in the intake of Sct by sheep may not be due to micro-

Table IV. Fermentation products, pH and NH_3-N concentrations of treated and untreated rice straw with whole rumen fluid (WRF) in the presence of cycloheximide (bacteria + protozoa: B+P) *in vitro**.

Item	*Incubation period (h)*	*Molar percentages of acids*					*A:P*	*pH*	*NH_3-N (mg/dl)*
		Acetate	*Prop*	n-*Buty*	i-*Valerate*	n-*Valerate*			
Rs	12	57.6^{b}	26.4^{a}	10.2	3.6	2.2	2.2	6.6	13.7^{a}
	24	53.6^{b}	30.3^{b}	9.4^{b}	4.5^{b}	2.2	1.8	6.4^{b}	18.0^{a}
	48	53.1^{b}	30.0^{b}	10.5^{b}	4.0^{c}	2.4	1.8	6.1^{b}	32.6^{b}
	72	50.8^{b}	32.8^{b}	9.5^{b}	4.7^{c}	2.3^{a}	1.6	6.3^{b}	41.7^{b}
Sct	12	50.3^{a}	32.7^{b}	10.4	3.8	1.9	1.5	6.6	10.2^{a}
	24	45.9^{a}	41.3^{d}	8.1^{a}	2.8^{a}	1.8	1.1	6.2^{a}	12.2^{a}
	48	42.3^{a}	43.7^{d}	9.9^{a}	2.1^{a}	1.9	1.0	5.8^{a}	26.3^{a}
	72	37.7^{a}	48.0^{c}	9.8^{c}	2.4^{a}	2.1^{a}	0.8	5.7^{a}	33.2^{a}
At	12	58.3^{b}	27.1^{a}	9.5	3.4	1.6	2.2	6.6	27.8^{b}
	24	58.2^{c}	29.4^{b}	8.0^{a}	2.8^{a}	1.6	2.0	6.3^{b}	26.5^{b}
	48	55.1^{c}	29.7^{b}	9.9^{a}	3.2^{b}	2.2	1.9	6.1^{b}	45.0^{d}
	72	51.4^{b}	33.2^{b}	8.9^{a}	4.5^{c}	2.1^{a}	1.6	6.4^{b}	54.1^{c}
Athp	12	57.5^{b}	26.7^{a}	10.3	3.6	2.0	2.2	6.5	12.9^{a}
	24	54.4^{b}	32.7^{c}	8.0^{a}	3.1^{a}	1.7	1.7	6.3^{b}	15.5^{a}
	48	53.7^{b}	31.4^{c}	9.7^{a}	3.1^{b}	2.1	1.7	6.2^{b}	32.1^{b}
	72	50.7^{b}	34.5^{b}	9.0^{ab}	3.9^{b}	1.8^{a}	1.5	6.2^{b}	41.5^{b}
Blank	12	56.7^{b}	26.2^{a}	10.4	4.6	1.9	2.2	6.6	26.2^{b}
	24	57.1^{c}	18.9^{a}	9.8^{b}	7.0^{b}	1.8	3.0	6.7^{c}	33.3^{c}
	48	52.7^{b}	24.1^{a}	13.6^{c}	6.6^{d}	2.7	2.2	6.5^{c}	38.7^{c}
	72	52.0^{b}	21.7^{a}	11.6^{d}	8.7^{d}	3.1^{b}	2.4	6.7^{c}	39.9^{b}

* The values are means of 4 observations; means within the same column and period with different letters differ (DMRT, $P < 0.01$); A:P: acetate/propionate ratio; Prop: propionate; *n*-Buty: *n*-butyrate.

bial factors but rather to animal factors such as reduced palatability (Cann *et al*, 1993b). The rumen is a complex ecological system in which the numerous microorganisms present are inter-dependent. The removal of the microbes from their natural habitat is likely to have adverse effects on those organisms most susceptible to micro-environmental changes. That the degradation of the straws in the *in sacco* experiment is higher than that for the *in vitro* studies, may thus be attributed to a decrease in the efficiency of the microbial inter-relationship in the *in vitro* environment. The effect was more pronounced in the more easily digestible straw (At and Sct).

The use of cycloheximide in the present experiment to suppress fungal activity resulted in higher propionate proportions with decreasing butyrate proportions (tables III and IV). The significantly higher proportion of propionate resulting from Sct fermentation was shown to be due to the fungicidal effect of Sct (Cann *et al*, 1993b; Elliot *et al*, 1987). The A:P ratio of less than 1 is rarely encountered during the fer-

mentation of fibrous materials (Miron and Ben-Ghedalia, 1987; Cameron *et al,* 1991; Cann *et al,* 1991). The suppression of the anaerobic fungi leading to a higher propionate proportion at the expense of acetate proportion has been demonstrated by using other antifungal substances, namely polyoxin D (Cann *et al,* 1993a,b) and monensin (Elliot *et al,* 1987). The results of the present experiment, therefore, show that the suppression of rumen fungi may decrease the microbial degradation of fibrous materials and will surely shift the fermen-tation toward increased production of propionate while suppressing the acetate pathway. This view is consistent with the finding that rumen fungi produce acetate but not butyrate (Mountfort and Asher, 1985). Rumen methane is mainly produced by the reduction of CO_2 with hydrogen gas. The carbon dioxide originates from the conversion of pyruvic acid to acetate. Methane production is a by-product of high energy value. A decrease in the production of acetate will, therefore, minimize this waste in the fermentation process. In addition, propionate is more efficiently used by tissues with 17–18 mol ATP resulting from the oxidation of propionate while only 10 mol ATP are produced from the oxidation of acetate. Cycloheximide, which totally inhibited fungal growth, is a protein synthesis inhibitor (Pelczar *et al,* 1977). Windham and Akin (1984) indicated that some protozoa, especially the holotrichs, retain some activity in the presence of this antibiotic. The entodiniomorphid and holotrichs found in the rumen samples used in the present experiment produce butyrate as an end-product of metabolism (Williams and Coleman, 1988). The decrease in butyrate proportion during (B + P) fermentation may be due to the effect of cycloheximide on the rumen protozoa. In addition, this limited suppressive effect of the antibiotic on rumen protozoa may have influenced the extent of degradation of plant material by the rumen microorganisms (fig 2b). Whereas the decrease in the degradation during incubation with B + P cannot be entirely attributed to fungal suppression, the addition of this antibiotic (cycloheximide) resulted in a fermentation pattern simlar to that reported for other antifungal substances (Elliot *et al,* 1987; Cann *et al,* 1993a,b). The effect of cycloheximide inclusion resulting in lower digestibilities during the 48-h and 72-h incubations also throws light on the importance of rumen fungi during the later stages of fermentation when more refractory materials remain in the fermentation system.

The significantly low pH (table III and IV) during Sct fermentation was inconsistent with the results *in vivo* with sheep, where the ruminal pH of the animals being fed Sct was between 6.8 and 6.9 (Elliot *et al,* 1987; Cann *et al,* 1993b). This observation is explained by the buffering ability of the rumen ecosystem.

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