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Effects of feeding small amounts of ammonia treated straw on degradation rate and intake of untreated straw

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ABSTRACT

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Four sheep, each fitted with a rumen cannula, were fed barley straw either untreated plus urea (10 g kg^{-1}) (Control), treated with ammonia (AS) at 35 g kg^{-1}, or untreated plus urea supplemented with 200 g or 400 g AS (200 AS or 400 AS) in a Latin square design. The straws were supplemented with minerals and vitamin. Daily intake of untreated straw was 641, 816 and 667 g per animal on Control, 200 AS and 400 AS diets. Total straw dry matter intake was 641, 1003, 1019 and 1152 g day^{-1} per animal on Control, 200 AS, 400 AS and AS diets, respectively ($P<0.01$).

Studies on degradation rate in the rumen were carried out to provide some explanation for the changes in intake. Degradability studies using the nylon bag method showed that the rate of degradation of untreated straw was increased from 0.024 h^{-1} to 0.031 and 0.041 in the rumens of sheep fed 400 AS and AS diets, respectively ($P<0.05$). Rumen fluid from the sheep was also used as an inoculum to ferment untreated straw in vitro. Rate of gas production was observed to be significantly higher ($P< 0.05$) when rumen fluid from animals fed 200 AS, 400 AS and AS diets was used to ferment the straw. The extent of degradation or total gas production was not affected by diet. Differences in straw intake were attributed to differences in degradation rate caused by the stimulatory effect in the rumen of the increased supply of fermentable energy provided by the ammonia treated straw.

INTRODUCTION

There is a growing interest in the use of fibrous feeds with a high content of readily digestible cellulose and hemicellulose fractions as supplements to animals consuming poor quality roughage diets. Supplements of dried grass (Mbatya et al., 1983 a,b), corn fibre (Oliveros et al., 1989), unmolassed su-

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garbeet pulp (Highfill et al., 1987; Huhtanen, 1988; Silva and Ørskov, 1988) and legume hay (Ndlovu and Buchanan-Smith, 1985; Smith et al., 1990) have been shown to increase intake and utilization of poor quality roughage. Silva and Ørskov (1988) demonstrated that supplements of unmolassed sugar beet pulp and citrus pulp improved the rumen environment for the growth of cellulolytic microbes, thus facilitating a greater rate of fibre digestion and roughage intake.

Kolankaya et al. (1985) showed that ammonia treated straw supported a higher growth rate of selected cellulolytic bacteria in vitro. In the study by Silva and Ørskov (1988) attempts to increase the degradation rate of straw using unmolassed sugarbeet pulp were largely unsuccessful, suggesting that ammonia treated straw already provides optimum rumen conditions for cellulolysis. On the basis of this observation, the current study was undertaken to evaluate the potential for the use of ammonia treated straw as a supplement for sheep consuming untreated straw. Of interest was the assessment of intake, changes in rumen environment and fibre digestion.

MATERIALS AND METHODS

Animals

Four mature castrated male sheep weighing 50.5–69.6 kg were used. The sheep had been fitted with permanent rumen cannulae of 45 mm diameter.

Treatments

The experiment had a 4×4 Latin square design such that the four sheep were fed four diets and measurements were taken over four periods. The diets were: (1) untreated barley straw (Control); (2) ammonia treated barley straw (AS); (3) untreated straw together with 200 g air dried AS (200 AS); (4) untreated straw together with 400 g air dried AS (400 AS).

Straw was treated with ammonia (35 g kg^{-1} dry matter (DM)), and covered with polythene sheet for 3 weeks. The straws were supplemented with minerals (0.3 kg sodium chloride, 0.5 kg calcium dihydrogen phosphate, 0.15 kg sodium sulphate per 100 kg DM) and a trace mineral plus vitamin mixture (0.1 kg 100 kg^{-1} DM), and urea (10 g kg^{-1} DM) was added to untreated straw.

Management

The sheep were housed in individual pens with slatted floors. Drinking water was given ad libitum. Straw was chopped prior to feeding using a bale grinder fitted with a 4 cm screen. Fresh straw was offered daily and uneaten food was

removed and weighed. The daily straw allowance was fed in two equal meals at 08:00 and 16:00 h. For treatments 200 AS and 400 AS, the treated and untreated straw was given in separate troughs. The basal straws were given ad libitum to ensure > 10% refusals.

Measurements

Measurements were taken after an 18 day adaptation period.

Nylon bag studies

Degradability of untreated straw was determined by incubating the straw in nylon bags in the rumen of each sheep. The straw was ground through a 2.5 mm screen and 3 g of straw were incubated in each bag. Incubation periods were 8, 16, 24, 48, 72 and 96 h with duplicate bags for each period. On removal from the rumen, the bags were washed in cold water for 20 min in a domestic washing machine (Indesit 2550). The bags were then dried for 48 h at 60°C in an oven. DM loss was calculated and expressed as percentage degradability of the original DM incubated.

Soluble DM loss was estimated by placing nylon bags with straw samples in warm water (39°C) for 1 h followed by washing and drying as described above. Soluble DM was expressed as percentage solubility of the original DM in the nylon bag.

Degradability data were fitted to the non-linear model $p=a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979), where p is degradability after time t, a is the intercept of the degradation curve at time zero, b is the fraction which degrades with time at a rate constant c, and $a+b$ represents potential degradability or the fraction that will be degraded in the rumen given sufficient time (Ørskov and McDonald, 1979).

Sampling rumen fluid

At the end of nylon bag incubations, samples of rumen fluid were collected just before morning feed at 08:00 h and at 09:00, 10:00, 12:00 and 14:00 h, to represent sampling 0, 1, 2, 4 and 6 h after feeding. At each sampling, 20 ml of rumen fluid were collected and strained through two layers of surgical gauze; the pH was determined immediately. The rumen liquor was then acidified and centrifuged at 48 $300\times g$ and volatile fatty acids and ammonia were analysed in the supernatant.

Particle bound carboxymethyl cellulase (CMCase) activity

The method for assaying for particle bound CMCase used in this study was originally developed by Nossal and Heppel (1966) and modified by Silva et al. (1987). Two grams of untreated straw were incubated in nylon bags in the rumen of each sheep for 24 h. The bags were washed as described above. One

gram of wet residue was incubated with 1 ml 0.4 mg ml^{-1} lysozyme in 19 ml of 10 mM phosphate buffer (pH 6.8) and 2.5 ml carbon tetrachloride at 39°C to release the enzymes. After 3 h the samples were centrifuged at 27 200×*g* for 15 min and 1 ml of supernatant was incubated with 1.5 ml 2% sodium carboxymethylcellulose (Sigma, Poole, Dorset) at 39°C for 2 h. The glucose released was estimated by reading absorbance at 560 nm after adding 3,5-dinitrosalicylic acid (DNS reagent) and placing the tubes in a boiling water-bath for 5 min (Miller et al., 1960). D-Glucose was used as a standard. Enzyme activity was expressed as μmol glucose released h^{-1} g^{-1} straw DM.

In vitro gas production

The in vitro gas method used was that described by Menke et al. (1979) and Menke and Steingass (1988). On the last day of each measurement period, rumen liquor was collected from each sheep, strained through two layers of surgical gauze, mixed with sodium and ammonium carbonate buffer (35 g $NaHCO_3$+4 g $(NH_4)_2CO_3$ per 1000 ml) (2:1, v/v), and used in the measurement of gas production in vitro using untreated straw substrate. Fermentation was carried out in calibrated glass syringes of 32 mm diameter and 150 ml volume incubated in a water bath at 39°C. Three replicates were used for the substrate and blank (rumen fluid+buffer without substrate). Gas volume was recorded after 8, 12, 24, 48, 72 and 96 h incubation. Net gas volume at each incubation period was calculated by subtracting the mean gas volume of the blanks from the mean volume of gas in tubes with substrate. The data were then fitted to the non-linear model of Ørskov and McDonald (1979) to obtain an estimation of potential and rate of gas production.

Chemical analysis

Dry matter was determined by drying samples at 60°C for 48 h in a forced draught oven. Ash content was determined by incinerating samples at 450°C for 4 h. Total nitrogen in samples was determined by the automated Kjeldahl method with mercuric oxide catalyst (Davidson et al., 1970). Acid detergent fibre (ADF) was determined according to the method of Goering and Van Soest (1970). Ammonia nitrogen concentration in rumen fluid was determined using a Technicon analyser and the calorimetric method described by Whitehead et al. (1967). Volatile fatty acid (C_2–C_5) concentrations in the rumen fluid were determined by the gas–liquid chromatography method described by Ottenstein and Baertley (1971).

Statistical analysis

Data were analysed as for a balanced Latin square design using analysis of variance with the aid of the GENSTAT package; differences between treatment means were tested using the *t*-test (Steel and Torrie, 1980).

RESULTS

Composition of the straws

The composition of the untreated straw plus 1% urea and of the ammonia treated straw are shown in Table 1. It can be observed that the straws had a similar nitrogen content although the treated straw had slightly less ADF. The soluble DM content of the untreated and treated straw was 158 g kg^{-1} and 147 g kg^{-1}, respectively.

TABLE 1

Composition of straws used in the experiment

	Dry matter (g kg^{-1})	DM (g kg^{-1})		
		Ash	Nitrogen	ADF[1]
Untreated straw (plus 1% urea)	882	56.5	10.7	509
Ammonia treated straw	886	53.7	12.6	466

[1]ADF, acid detergent fibre.

TABLE 2

Daily intake of DM measured in sheep receiving Control, AS or 200 AS and 400 AS diets

	Diet				
	Control	200 AS	400 AS	AS	SED
DM intake (g)					
Untreated straw	641	816	667	-	-
Treated straw	-	187	352	1152	-
Total	641[a]	1003[b]	1019[b]	1152[b]	103.2
g $W^{-0.75}$	30.9[a]	47.5[b]	47.8[b]	53.2[b]	4.47
Degradability of straw (%) after 48 h incubation					
Untreated straw	50.5	47.7	52.1	-	-
Treated straw	-	55.2	61.2	58.4	-
Estimated digestible DM intake (g d^{-1})	324	492	563	673	

[a,b]Means in the same row with different superscripts are significantly different ($P<0.01$), each value being the mean of four observations.
[1]DM digestibility assumed to be equal to 48 h degradability of the straws incubated in nylon bags in the rumen of sheep on the different diets.

TABLE 3

Dry matter degradability (%) of untreated straw incubated in the rumen of sheep fed Control, AS or 200 AS and 400 AS diets

	Diet				
	Control	200 AS	400 AS	AS	SED
Incubation time (h)					
8	23.8	25.9	25.9	26.0	0.93
16	33.8	33.7	36.2	35.9	1.59
24	38.8	39.9	42.4	45.9	1.52
48	50.5	47.7	52.1	53.3	1.76
72	57.5	58.1	59.0	58.2	1.93
96	60.6	60.2	62.2	60.7	1.59
$a+b$[1]	63.4	63.8	63.8	60.7	1.35
c[1]	0.024[a]	0.023[a]	0.031[b]	0.041[c]	0.0024

[a,b]Means in the same row with different superscripts are significantly different ($P<0.05$), each value being the mean of four observations.
[1]$a+b$ is potential degradability, c is rate constant of degradation (h^{-1}) in the model $p=a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979).

Feed intake

Feed intake is summarized in Table 2. Dry matter intake was lowest on the control diet ($P<0.01$). Supplements of ammonia treated straw increased total feed intake to a level comparable to the treated straw diet (AS). Compared with the control diet the supplement of 200 g AS and 400 g AS increased intake of untreated straw by 27% and 4%, respectively.

Degradability studies

Table 3 summarizes the DM degradability of untreated straw in the rumen of sheep receiving the different straw diets. It is apparent that, although the potential degradability ($a+b$) of untreated straw was not altered, degradability up to 48 h was consistently higher on the 400 AS and AS diets and approached significance ($P<0.10$) at 24 h and 48 h. The rate of degradation (c) of untreated straw was significantly higher on 200 AS, 400 AS and AS diets ($P<0.05$) than in the rumens of animals receiving untreated straw.

Gas production in vitro

Gas production in vitro from untreated straw incubated with rumen fluid from sheep on the different straw diets is shown in Table 4. Significant treatment differences were observed for the rate of gas production ($P<0.05$). The

TABLE 4

Gas production (ml) in vitro when untreated straw was incubated with rumen fluid from sheep fed Control, AS or 200 AS and 400 AS diets

	Diet				
	Control	200 AS	400 AS	AS	SED
Incubation time (h)					
8	4.4	4.8	4.8	4.6	1.07
12	7.1	8.0	7.4	8.0	1.77
24	16.5	17.0	16.9	19.8	2.56
48	25.6	27.3	26.6	27.5	2.54
72	30.1	33.1	31.5	32.3	2.61
96	33.1	35.6	33.8	35.5	2.35
$a+b$[1]	36.3	39.6	39.0	37.9	1.96
c[1]	0.029[a]	0.033[b]	0.035[b]	0.041[b]	0.0026

[a,b]Means in the same row with different superscripts are significantly different ($P<0.05$), each value being the mean of four observations.
[1]$a+b$ is potential gas, c is rate constant of gas production (h^{-1}) in the model $p=a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979).

TABLE 5

Rumen pH, ammonia, volatile fatty acids (VFA) and particle bound CMCase activity measured in sheep fed Control, AS or 200 AS and 400 AS diets

	Diet				
	Control	200 AS	400 AS	AS	SED
Rumen pH	7.12	6.88	6.94	7.02	0.158
Ammonia-nitrogen (mg 100 ml^{-1})	44.6	46.2	40.2	31.7	10.72
VFA (mg 100 ml^{-1})					
C_2	54.4 (730)[1]	60.1 (724)	67.2 (752)	55.1 (732)	9.24
C_3	13.1 (176)	15.4 (186)	15.6 (186)	13.9 (184)	1.79
C_4	6.1 (82)	6.5 (78)	5.7 (78)	5.4 (72)	0.83
C_5	0.9 (12)	0.9 (12)	0.9 (12)	0.8 (10)	0.16
total	74.5	82.9	89.4	75.2	12.4
CMCase (μmol glucose g^{-1} DM h^{-1})					
Untreated straw	114	152	147	146	69.4
Treated straw	188	246	288	305	98.6

[1]Values in parentheses are the respective molar proportions expressed as mmol/mol.

200 AS, 400 AS and AS diets gave higher rates of gas production than the Control diets. It is also of interest to note that the potential gas production was the same across treatments as was observed for potential degradability using the nylon bag method.

Rumen parameters

There was no significant difference in rumen pH, ammonia, volatile fatty acids and CMCase activity between diets (Table 5). The CMCase values tended to be higher on the 200 AS, 400 AS and AS diets.

DISCUSSION

Food intake

In the current experiment, treatment of straw with ammonia or supplementing straw with 200 g or 400 g of ammonia treated straw resulted in an 80%, 56% and 59% increase in intake, respectively. The increase in straw intake following treatment with ammonia was expected, as had been observed in other studies elsewhere (e.g. Sundstol and Coxworth, 1984). It is of particular interest that supplements of ammonia treated straw actually increased untreated straw intake by 27% and 4% on 200 AS and 400 AS diets, respectively. Compared with the control, the increase in intake was sufficient to increase estimated metabolizable energy intake of the sheep (50–70 kg liveweight) from 0.5 maintenance to near maintenance. In the study by Silva and Ørskov (1988), a supplement of unmolassed sugarbeet pulp (15% of total DM intake) increased total DM intake by 37% and untreated straw intake by 22%. The results of the current experiment are also similar to some reported work on the supplementation of cereal straws using legume forage; for example, Minson and Milford (1967) using lucerne, Mosi and Butterworth (1985) using trifolium hay, and Smith et al. (1990) using cow pea, lab lab and pigeon pea hay, showed that maximum increase in intake of the basal roughage is achieved between a 10 and 20% level of supplementation. Higher levels of supplementation generally cause a depression in intake of the basal roughage. Mbatya et al. (1983a,b) observed similar responses in intake using a supplement of dried grass on straw diets.

A consistent response to supplementation was an increase in the rate of degradation of untreated straw estimated using the nylon bag and the in vitro gas method (Tables 3 and 4). Silva and Ørskov (1988) showed an increase in the rate and extent of straw degradation when supplements of unmolassed sugar beet pulp and citrus pulp were used. In the current study, rumen ammonia concentrations on all the diets were above the level known to limit microbial growth (see Satter and Slyter, 1974). Rumen pH was also not a

limiting factor for microbial growth (e.g. Mould and Ørskov, 1983), nor were minerals and vitamins, since these were added to the straws. The response was also not associated with a change in the level of volatile fatty acids in the rumen. An obvious difference between the straw diets was the supply of fermentable energy. This would have promoted a higher growth of cellulolytic microbes on the 200 AS, 400 AS and AS diets as can be predicted from the findings of Kolankaya et al. (1985) and Silva and Ørskov (1988).

In the current study, both nylon bag and gas methods showed that the potential degradation of straw was not altered by diet. Potential degradation is influenced to a large extent by roughage characteristics such as lignification and as such can not be expected to be altered easily by manipulating microbial populations. Within limits of rumen pool size, an increase in the rate of degradation will cause an increase in feed intake. However, it can be calculated from Table 2 that the increase in intake from both supplementation and feeding ammonia treated straw on its own resulted in an increase in the intake of undigested DM. This suggests that the change in intake was either associated with an increase in rumen volume, or rate of passage of digesta, or both. These factors were not measured in this trial. Treatment differences in rate of digestion might well have been greater than the in sacco and in vitro values because of the contribution of chewing during eating and rumination to rate of fibre digestion in the rumen (Poppi et al., 1981).

CMCase activity

The rate of degradation of fibre depends on the extent to which the rumen environment allows an adherent cellulolytic microbial population to develop (Silva et al., 1987). Using hay and concentrate diets, Silva et al. (1987) obtained high correlations between CMCase and fibre degradation. In the current experiment, diet differences in CMCase activity were not significant but showed the predicted trend. It is worth noting that roughage also imposes limitations to microbial attachment because of the wax cuticle and lignification (Chesson and Forsberg, 1988), and differences in rumen microbial populations may need to be sufficiently large (e.g. on roughage vs. concentrate diets) to show differences in microbial colonization of straw particles. Differences in CMCase activity between treated and untreated straw were large (Table 5), confirming that ammoniation facilitates more rapid colonization and degradation of straw by microbes.

Relationship between nylon bag and in vitro gas method

In the current experiment, a close relationship was observed between the asymptote for nylon bag and the in vitro gas methods ($r=0.71$). The gas method may be a more accurate method for estimating rumen degradation of

roughage because it overcomes the possible errors in nylon bag measurements resulting from the loss of materials through the pores of the bags by non-fermentative processes. Some of the material lost from nylon bags may consist of non-fermentable phenolic and lignin–carbohydrate complexes released during the course of cell wall degradation. The deviation of in vitro gas values from nylon bag values may be a useful indicator of the presence of antinutritional factors in feed.

CONCLUSION

The results of the current study show that it is possible to increase the intake and degradation of untreated straw in the rumen by supplementation with small amounts of ammonia treated straw in situations where all other factors necessary for optimum cellulolysis such as pH, rumen ammonia, mineral and vitamin nutrition are kept optimal. The practical implications of these results are of particular relevance to small-holder farming systems in developing countries where lack of financial and capital resources prohibit large scale treatment of straw by the urea-ammonia process. These results, however, need to be substantiated under practical on-farm feeding conditions. Further work is also needed to elucidate factors regulating feed intake in ruminants in situations where roughage supplements are used.

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