EFFECTS OF CHEMICAL TREATMENTS OF BARLEY STRAW ON LEACHING, AND DIGESTIBILITY BY RUMEN FLUID AND CELLULOLYTIC BACTERIA

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Summary

Effects of chemical treatments on *in sacco* and *in vitro* digestibility of barley straw by rumen fluid and pure cultures of cellulolytic bacteria were studied to evaluate the pretreatment and to improve the poor quality feed. Chemicals were applied by dissolving them in water equivalent to 40% of the weight of the straw (dry matter basis). Pretreatment with 5% NaOH yielded the largest increase *in sacco* digestion followed by pretreatment with 2% $(NH_4)_2SO_3$, 2.6% NH_4OH, 1.6% $NaHSO_3$ and untreated straw (control). *In sacco* dry matter digestibility of straw treated with NaOH and $(NH_4)_2SO_3$ continued to increase as the concentration of chemical increased (1 to 7.5%), as it was the *in vitro* dry matter loss by leaching. Treatment of barley straw with 5% NaOH enhanced significantly ($p < 0.01$) *in vitro* digestibility by rumen fluid, *Fibrobacter succinogenes* and *Ruminococcus albus* though the fermentation products by cellulolytic bacteria were low, whereas the treatment with 5% $(NH_4)_2SO_3$ inhibited *in vitro* digestibility by *F. succinogenes* and *R. albus* together with lower fermentation products. Dry matter loss by leaching and bacterial digestion from barley straw treated with NaOH and $(NH_4)_2SO_3$ suggested the effect of pretreatment with these chemicals were based on leaching, and the cellulolytic bacteria had little to do with digestion.

(**Key Words**: Barley Straw, Nylon Bag, *Ruminococcus albus*, *Fibrobacter succinogenes*, Chemical Treatments, Cellulose Digestion)

Introduction

The world has vast supplies of rice, barley and wheat straws which are composed of a lignin-cellulose complex. Because of this, energy in straw is not readily available to rumen microorganisms or the host animal. Untreated straw lacks sufficient nitrogen necessary to balance the large amounts of available carbon. Thus, untreated straw, by itself, can not support maintenance or growth requirements for energy or protein (Møller and Hvelplund, 1982) because energy is made available very slowly and contains insufficient nitrogen.

Various physical (Fernández and Greenhalgh, 1972; Kaufman, 1976) and chemical methods (Bergner et al., 1974; Dolberg et al., 1981; Fodgaard, 1982; Smith et al., 1983; Sundstøl, 1984) have been used to enhance the nutritive value of straw. Kennedy and Siebert (1972) and Bird (1974) have reported substantial increases in digestion, voluntary intake and nitrogen balance, when low-quality roughage diets containing urea were supplemented with sulphur. Elliot and Armstrong (1982) reported the addition of urea and urea plus sulphate supplements induced a stepwise reduction in digestibility of organic matter and resulted in increased microbial protein synthesis in the rumen and increased quantities of amino acid nitrogen entering the small intestine. Efficiency of microbial protein production was significantly increased by the addition of urea plus sulfate. The chemical reaction of the cell wall components with NH_3 makes the β-linked polysaccharide of the straw more accessible for microbial growth in the rumen. The ammonia, which bound to the straw during the reaction can be used as a source of nitrogen for microbial protein synthesis in the forestomachs (Cann et al., 1993). The present work was conducted to evaluate and develop chemical pretreatments to enhance the digestion of straw.

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Materials and Methods

Preparation of straw

Barley straw for the *in vitro* digestion experiment was cut with a pair of scissors into 2.5 cm segments. Samples of straw (approximately 800 g dry matter for each treatment) were placed in plastic bags and 0, 1.0, 2.5, 5.0 and 7.5% of NaOH and $(NH_4)_2SO_3$, 2.6% NH_4OH, 2.0% $(NH_4)_2SO_3$ and 1.6% of $NaHSO_3$ on dry matter (DM) basis were sprayed on the straw samples using a chromatography sprayer. Chemicals were dissolved in distilled water equivalent to 40% of the DM weight of straw. DM weight of straw was calculated just prior to chemical application by oven drying for 24 h at 80℃. Straw samples were then sealed in double plastic bags and stored for at least 10 days at room temperature (approximately 20℃).

Animals

Three fistulated Holstein steers were used. Animals were fed cubed alfalfa hay at 0.5% of body weight in two daily portions. Chopped barley straw was offered free choice. Animals were allowed free access to water and salt block.

Collection of *in sacco* samples

Straw (9.0 g DM/bag) was placed into 9 × 16 cm nylon mesh bags (Nytex No. 130, 10-N, 0.13 mm opening) and suspended in the rumen of fistulated steers. Weights were attached to each bag to keep the sample immersed in rumen fluid. Replication was obtained by incubating duplicate samples of each straw treatment in 3 steers on 3 different days at each of 6 time-points (18 bags/treatment-time). After 6, 12, 24, 36, 48 and 72 h in the rumen, the nylon bags were withdrawn and the exterior of the bags were washed under tap cold water and oven dried for 48 h at 80℃ to allow for a measure of DM disappearance. In the first experiment, comparisons were made for straw treated with 1.6% $NaHSO_3$, 5% NaOH, 2.6% NH_4OH, 2% $(NH_4)_2SO_3$ and untreated straw. In the second experiment, straws treated with 0, 1.0, 2.5, 5.0 and 7.5% NaOH were compared. The third experiment was similar except that $(NH_4)_2SO_3$ was used instead of NaOH.

Samples for the leaching experiment were placed in nylon bags (12 replicates/treatment) and incubated for 24 h at 38℃ in autoclaved rumen fluid. NaN_3 (0.5%, wt/vol) was added to prevent bacterial growth. All measurements of DM at 0 h were taken by freeze drying the straw since SO^{2-}_3 treatment will cause decomposition of straw if heat is applied.

Media and inoculum

The artificial medium of Scott and Dehority (1965) without soluble carbohydrate was used for *in vitro* bacterial digestion experiment. 4.2 g straw (DM basis) was incubated anaerobically (under CO_2 atmosphere) in 300 ml medium with 10 ml microbial inoculum at 38℃ for 4 days. Straw was added to flask just prior to autoclaving. Rumen fluid was filtered through two layers of cheesecloth. Two strains of cellulolytic bacteria, *Ruminococcus albus* 6A41 and *Fibrobacter succinogenes* BL2, were tested for the effects of 5% NaOH and $(NH_4)_2SO_3$ treatments of barley straw on digestibility. The isolation of *R. albus* 6A41 from vole (*Microtus montebelli*) stomach content is described previously (Kudo et al., 1979). *F. succinogenes* BL2 was from the Lethbridge culture collection, which was isolated by Stewart et al. (1981). These cellulolytic bacteria were maintained with Scott and Dehority's medium without soluble carbohydrate and 1 cm^2 of Whatman No. 1 filter paper was added as a carbon source. The anaerobic technique used throughout this investigation was that of Hungate (1950) as modified by Bryant and Burkey (1953).

Analysis of fermentation products

Samples were deproteinized overnight with 24% (wt/vol) m-phosphoric acid in 5 N sulfuric acid and then centrifuged at 7,700 g for 30 min. For volatile fatty acid analyses, the supernatant was mixed with the same volume of 30 mM crotonic acid as an internal standard. For succinic acid analyses, 0.5 ml of supernatant was mixed with 0.5 ml of 20 mM malonic acid as an internal standard, methylated overnight at 38℃ with 1 ml of 14% (wt/vol) boron trifluoride-methanol and extracted with 0.5 ml of chloroform. Samples were analyzed for VFAs (Column: 3% phosphoric acid, Porapak Q 80/100) and succinic acid (10% polyethylene adipate + 3% phosphoric acid, chrom W 60/80) in a Varian Model 3,700 gas chromatograph equipped with a Shimadzu chromatopac C-R 1B data processor.

Statistical analysis

Data from *in sacco* experiments were subjected to ANOVA with variation due to animals and days removed. Data from in the *in vitro* and leaching

experiments were subjected to ANOVA with inoculum and type of chemical (*in vitro*) or type and level of chemical (leaching) as main effects. Differences between main effect means in all experiments were tested using the Tukey-Kramer method (Kramer 1956).

Results and Discussion

The effects of various chemical treatment of barley straw on *in sacco* DM disappearance are shown in table 1. Treatment with 5% NaOH yielded the largest DM disappearance followed by treatments with 2% $(NH_4)_2SO_3$, 2.6% NH_4OH and 1.6% $NaHSO_3$. This difference could be observed, even at the early stage of incubation (6 h). It may have been due to leaching (solubility) rather than bacterial digestion, since bacterial degradation would not be expected to occur to this extent at such an early stage. Loss of micro-particulate matter from nylon bags may also have contributed. Graham and Åman (1984) noted that ammonia treatment appeared to cause a reduction in DM disappearance relative to DM in the initial stage of digestion and caused the release of hemicellulose that were soluble in buffer and it was possible that a small fraction of the lignin was also solubiliz ed. The rate of DM disappearance was faster at 12 to 24 h, especially in straw treated with 5% NaOH.

TABLE 1. EFFECTS OF CHEMICAL TREATMENTS ON THE DRY MATTER DISAPPEARANCE (%) OF STRAW *IN SACCO*

Incubation time (Hours)	Treatments of straw					SEM
	Control	1.6%$NaHSO_3$	5%NaOH	2.6%NH_4OH	2%$(NH_4)_2SO_3$	
6	9.4[a]	10.0[a]	16.2[b]	10.5[a]	11.2[a]	0.67
12	12.5[a]	13.4[a]	18.8[b]	13.7[a]	14.2[a]	0.66
24	20.1[a]	19.4[a]	37.4[b]	20.8[a]	21.4[a]	0.79
36	23.3[d]	24.6[cd]	43.2[a]	26.9[bc]	27.8[b]	0.97
48	29.9[c]	30.2[c]	51.8[a]	32.3[bc]	34.7[b]	0.90
72	33.5[c]	34.2[c]	57.4[a]	37.4[b]	40.4[b]	0.93

[a,b,c,d] Means with different superscripts in the same row are different ($p < 0.05$).
SEM: Standard error of the mean.

The enhancement of DM disappearance by increasing the concentrations (2.5 to 7.5%) of NaOH or $(NH_4)_2SO_3$ treatment is evident in table 2 and table 3. The higher the concentration of chemicals, the larger the percentage of DM disappearance was observed. Extents of DM disappearances *in sacco* after 72 h incubation were 35, 44.8 and 55.5% for the untreated, 5% $(NH_4)_2SO_3$ and 5% NaOH treated straw respectively. Again, the differences in DM disappearance were observed at early stages of incubation (6 h) and vigorous extents of DM disappearance were noted at 12 to 24 h, especially in straw treated with NaOH. This high rates was similar to results obtained by Graham and Åman (1984), with straw treated with 3% anhydrous ammonia at 95℃ for 15 h.

As the higher rate of DM disappearance at the early stage of incubation can not be explained by bacterial degradation alone, an experiment was conducted to determine the total percent DM leached by NaOH and $(NH_4)_2SO_3$ treatments. The soluble fraction of NaOH and $(NH_4)_2SO_3$ treated straw was determined separately by incubating for 24 h at 38℃ in sterilized rumen fluid with added NaN_3 and the results are presented in table 4. As chemical concentration increased for 1 to 7.5% the effect of concentration on leaching was linear with NaOH, whereas little additional effects were observed with $(NH_4)_2SO_3$ levels above 5%. The effect of NaOH and $(NH_4)_2SO_3$ treatment on DM disappearance occurred even without bacterial degradation. Morrison and Brice (1984) noted that ammonia treatment caused the hydrolysis of covalent bonds in the cell wall with the solubilization of the original cell wall components. Our results indicate the importance of solubility in chemical treatments of straw.

The relationship between DM disappearance by leaching *in vitro* and DM disappearance *in sacco* (bacterial degradation + leaching) of straw

TABLE 2. EFFECTS OF NaOH CONCENTRATIONS ON DRY MATTER DISAPPEARENCE (%) OF STRAW *IN SACCO*

Incubation time (Hours)	Concentrations of NaOH (%)					SEM
	0	1.0	2.5	5.0	7.5	
6	9.0[a]	9.7[a]	11.7[b]	16.0[c]	24.4[d]	0.30
12	11.3[a]	11.8[a]	12.2[b]	18.4[c]	27.3[d]	0.20
24	21.0[a]	21.6[a]	25.4[b]	36.5[c]	44.3[d]	0.77
36	25.6[a]	24.7[a]	29.7[b]	42.6[c]	51.7[d]	0.31
48	29.5[a]	28.5[a]	35.8[b]	50.1[c]	59.5[d]	0.99
72	36.1[a]	35.5[a]	45.3[b]	55.5[c]	68.9[d]	0.81

[a,b,c,d] Means with different superscripts in the same row are different ($p < 0.05$).
SEM: Standard error of the mean.

TABLE 3. EFFECTS OF $(NH_4)_2SO_3$ CONCENTRATIONS ON DRY MATTER DISAPPEARANCE (%) OF STRAW *IN SACCO*

Incubation time (Hours)	Concentrations of $(NH_4)_2SO_3$					SEM
	0	1.0	2.5	5.0	7.5	
6	8.4[a]	8.9[a]	12.5[b]	15.9[c]	17.5[c]	0.58
12	11.0[a]	11.0[a]	15.6[b]	18.1[bc]	20.5[c]	0.78
24	19.4[a]	19.2[a]	23.6[b]	26.3[b]	30.8[c]	0.95
36	25.7[ab]	22.3[a]	27.5[b]	34.4[c]	39.2[d]	1.00
48	32.3[a]	32.9[a]	36.3[a,b]	41.1[c]	45.1[d]	1.03
72	33.8[a]	34.6[a]	40.9[b]	44.8[c]	52.1[d]	0.81

[a,b,c,d] Means with different superscripts in the same row are different ($p < 0.05$).
SEM: Standard error of the mean.

TABLE 4. LEACHING OF DRY MATTER FROM STRAW TREATED WITH NaOH OR $(NH_4)_2SO_3$

Concentration (%)	NaOH	$(NH_4)_2SO_3$
0	11.3	
1.0	11.8[a]	14.7[a]
2.5	17.7[b]	15.6[a]
5.0	24.2[c]	20.6[b]
7.5	31.7[d]	21.6[b]
S_x	0.42	

[a,b,c,d] Means with different superscripts in the same row are different ($p < 0.05$).

Samples were incubated for 24 h at 38°C in sterlized rumen fluid added NaN_3 (0.5%, wt/vol) with nylon bag. With each sample, duplicates six experiments were conducted.

treated with NaOH or $(NH_4)_2SO_3$ after 24 h incubation is shown in figure 1 and figure 2 respectively. Increasing NaOH concentration from 2.5 to 7.5% gave a marked increase in DM loss with each distinguishable concentration group. 1% NaOH resulted in a small improvement. The effects of concentrations of NaOH or $(NH_4)_2SO_3$ were linear, particularly with NaOH treatment. The relatively high rate of DM disappearance in the initial stage *in sacco* digestion could be explained by its solubilization. Our results suggest that the differences observed in digestibility patterns *in sacco* by treatment with NaOH and $(NH_4)_2SO_3$ were mainly due to solubilization as the difference between the DM digestibility *in sacco* and solubility *in vitro* was not large enough to account for bacterial degradation. Latham et al. (1979) postulated from bacterial adherence that alkali treatment of straw should produce a dual effect of decrease in the undegradable fraction and stimulation of the rate of DM disappearance.

Table 5 shows the effect of 5% NaOH and 5% $(NH_4)_2SO_3$ treatments of barley straw on digestibility and organic acids production by rumen fluid and cellulolytic bacteria *in vitro*. With rumen fluid inoculum, 5% NaOH-treated straw showed the highest rate of DM disappearance and VFA

produced. Although the extent of DM disappearance was slightly higher in 5% $(NH_4)_2SO_3$-treated straw than in control, acetic acid production was much less. In experiments with fistulated sheep, Borhami et al. (1983) found higher ammonia and lower VFA concentration in the rumen with NH_3-treated straw than an isonitrogenous diet of NaOH-treated straw plus urea. The values of DM disappearance *in vitro* were lower than those *in sacco* despite longer incubation time. This discrepancy between digestibility values measured *in vitro* and *in vivo* has been noted previously (Van Soest et al., 1984). If the values of DM disappearance with incubation of rumen fluid were subtracted from those of without incubation, values were not as notable. "True bacterial degradation" was less in the straw treated with 5% $(NH_4)_2SO_3$ than in untreated straw. Incubations with cellulolytic bacteria (*F. succinogenes* and *R. albus*) also showed an inhibitory effect of 5% $(NH_4)_2SO_3$-treated straw on DM disappearance and fermentation products. While the straw treated with 5% NaOH showed greater DM disappearance but less fermentation products than untreated straw.

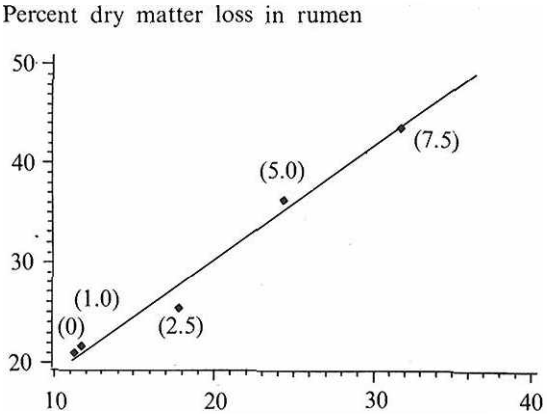

Figure 1. Effects of concentrations of NaOH on dry matter loss by eaching and dry matter loss by bacterial degradation. The horizontal values shows percent dry matter loss after incubation for 24 h at 38℃ in autoclaved rumen fluid added NaN_3 (0.5% wt / vol) with nylon bag (leaching), whereas the vertical shows percent dry matter loss after incubation for 24 h in the rumen with nylon bag (bacterial degracation + leaching). n = 12, (•): Concentration of NaOH.

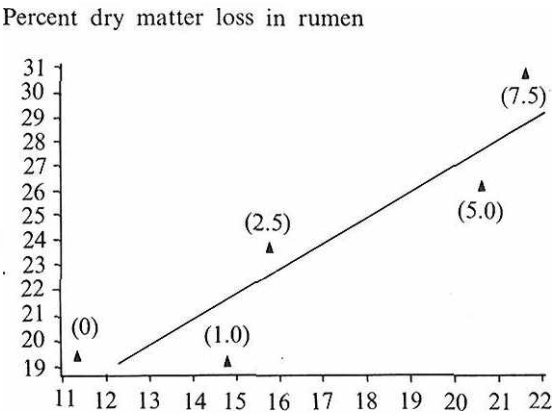

Figure 2. Effects of concentration of $(NH_4)_2SO_3$ on dry matter loss by leaching and dry matter loss by bacterial degradation. The horizontal value shows percent dry matter loss after incubation for 24 h at 38℃ in autoclaved rumen fluid added NaN_3 (0.5% wt / vol) with nylong bag (leaching), whereas the vertica shows percent dry matter loss after incubation for 24 h in the rumen with nylon bag (bacterial degradation + leaching). n = 12, (▲): Concentration of $(NH_4)_2SO_3$.

Our results indicate that treatment of barley straw with NaOH or $(NH_4)_2SO_3$ increased DM disappearance, but DM disappearance *in sacco* must not necessarily be interpreted solely as bacterial degradation. It may simply be a physico-chemical phenomenon of solubilization within the rumen due to chemical treatment with minor assistance of bacteria. If so, this could help to explain the discrepancy between *in vitro* and *in vivo* digestibility. NaOH treatment showed better results in DM disappearance than $(NH_4)_2SO_3$ treatment. But $(NH_4)_2SO_3$ treatment has the advantage over NaOH that it is much less corrosive, while ammonia bound to the straw during the reaction can serve as source of non-protein nitrogen for microbial protein synthesis in the rumen. In this paper only cellulolytic bacteria are described although protozoa (Kudo et al., 1990), fungi (Akin and Rigsby, 1987; Ho et al., 1988; Kudo et al., 1990) and even noncellulolytic bacteria (Kudo et al., 1987a) are

TABLE 5. EFFECTS OF NaOH AND $(NH_4)_2SO_3$ TREATMENTS OF BARLEY STRAW ON DRY MATTER DISAPPEARANCE AND NET FERMENTATION END-PRODUCTS BY RUMEN FLUID AND CELLULOLYTIC BACTERIA *IN VITRO*

Inoculum	Straw treatment	Dry matter disappearance (%)	Net fermentation products (mmoles)									
			Acet.	Prop.	Iso-buty.	buty.	Iso-valer.	valer.	Total VFAs	Lact.	Fumar.	Succ
Main effects												
Inoculum												
F. succinogenes		35.2[c]	23.9[a]	0.4[a]	0.4[a]	1.4[a]	0[a]	0[a]	26.1[a]	3.5[b]	0.1	37.0[c]
R. albus		28.2[b]	44.4[b]	0[a]	0[a]	0[a]	0[a]	0[a]	44.4[a]	0[a]	0	0[a]
Rumen fluid		40.9[d]	126.2[c]	52.8[b]	4.3[c]	17.8[c]	6.2[b]	16.8[b]	224.1[b]	0[a]	0	0[a]
No. inoculum		16.5[a]	–	–	–	–	–	–	–	–	–	–
Treatment												
	Untreated	36.3[c]	75.4[b]	16.3[b]	1.4[a]	7.0[b]	2.2[b]	5.6	102.3	1.3[b]	0.1	25.8[c]
	5% NaOH	22.8[b]	83.2[c]	23.1[c]	1.9[b]	6.9[b]	2.3[b]	5.6	123.0	2.2[c]	0	19.9[b]
	5% $(NH_4)_2SO_3$	20.9[a]	35.9[a]	13.8[a]	1.4[a]	5.2[a]	1.7[a]	5.7	63.7	0[a]	0	1.7[a]
	S_x	0.30	0.89	0.52	0.06	0.12	0.13	0.17	8.14	0.09	0.03	0.42
F. succinogenes	Untreated	37.5	60.5	0	0	4.2	0	0	64.7	3.8	0.17	51.7
	5% NaOH	48.8	59.7	0	0	0	0	0	59.7	6.7	0	56.0
	5% $(NH_4)_2SO_3$	19.2	13.0	1.2	1.2	0	0	0	15.4	0	0	3.3
R. albus	Untreated	25.8	39.0	0	0	0	0	0	39.0	0	0	25.8
	5% NaOH	40.2	25.8	0	0	0	0	0	25.8	0	0	3.8
	5% $(NH_4)_2SO_3$	18.5	7.0	0	0	0	0	0	7.0	0	0	1.5
Rumen fluid	Untreated	33.3	126.7	49.0	4.2	16.8	6.7	16.6	220.0	0	0	0
	5% NaOH	53.5	164.2	69.2	4.2	20.9	5.0	17.2	280.7	0	0	0
	5% $(NH_4)_2SO_3$	36.2	87.8	40.2	4.7	15.7	6.8	16.6	171.8	0	0	0
S_e		0.51	1.53	0.90	0.09	0.21	0.23	0.29	14.1	0.15	0.06	0.73

[a,b,c,d] Means within main effects with different superscripts are different ($p < 0.05$).

involved in the digestion of fastidious cellulosic feed. The mechanism of cellulose digestion by bacteria was demonstrated (Kudo et al., 1987b). A full understanding of these consortia may allow us to manipulate digestion and improve animal performance on a wide range of diets. Although further investigations are required, an entire particle of barley straw for conventional feed seems to be possible for feeding animals, such treatments would probably enhance the possibility of an economically beneficial utilization of straw as a feed for ruminants.

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