

EFFECT OF ALKALINE HYDROGEN PEROXIDE TREATMENT OF RICE STRAW ON IN SACCO RUMINAL DIGESTIBILITY

K. H. Myung¹ and J. J. Kennelly²

Chonnam National University
Kwangju 500-757, Korea

Summary

The objective of this experiment was to compare the effect of pH-regulated alkaline hydrogen peroxide (AHP) treatment of rice straw with those of sodium hydroxide (NaOH) and anhydrous ammonia (NH₃) treatments on in sacco digestibility. Three non-lactating ruminally cannulated Holstein cows were fed a diet containing 90% forage and 10% concentrate on a dry matter (DM) basis. The AHP treatment significantly ($p < 0.05$) reduced acid detergent lignin content of the straw, resulting in significant ($p < 0.05$) increase of neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose concentrations. Disappearance rates of DM and NDF of the straw significantly ($p < 0.05$) increased at the incubation time of 24 h. On the other hand, those of ADF and cellulose were significantly ($p < 0.05$) higher at the incubation time of 12 h than those of the others. The effective degradability of DM(EDDM), NDF(EDNDF), ADF(EDADF) and cellulose (EDCE) were determined using in sacco nylon bag technique on the basis of 0.05/h solid outflow rate. The greater differences ($p < 0.05$) of EDDM, EDNDF, EDADF and EDCE were found between AHP treated straw and the others. In general, AHP treatment of the straw recorded higher digestion coefficients than untreated straw as well as NaOH and NH₃ treated straws.

The results of this study demonstrate that AHP treatment can be used as a effective method for improving the nutritive value of rice straw for ruminants.

(Key Words: Alkaline Hydrogen Peroxide, Rice Straw, Disappearance Rate, Effective Degradability)

Introduction

The close physical and chemical association between lignin and plant cell-wall polysaccharides and the degree of crystallinity within the cellulose polymer (Cowling and Kirk, 1976) are major limitations to efficient utilization of straws for ruminant feeding.

A variety of chemical treatments have been applied to straw in an effort to increase the susceptibility of ligno-cellulosic substances to digestion by rumen microbes. Alkali (Klopfenstein, 1978; Lesoing et al., 1981) and the ammonia (NH₃) treatments (Garrett et al., 1979) have shown the most promising results. In general, NaOH treatment results in greater improvement in digestibility of straw and animal performance than NH₃ treatment (Males, 1987).

Recently, a new treatment method, which results in both partial delignification of cell wall and partial decrystallization of cellulose microfibrils, for enhancing the nutritive value of straw was developed by Gould (1984, 1985). Treatment of lignocellulosic materials such as wheat straw (Kerley et al., 1987) and aspen (Myung and Kennelly, 1989) with alkaline solutions of hydrogen peroxide (AHP) significantly increased degradability of plant cell wall constituents in the rumen. One disadvantage of AHP treatment was a loss of 50% of hemicelluloses when pH was not regulated during treatment. However, loss of hemicellulose was greatly reduced when the pH of the AHP reaction was maintained alkaline at pH 11.5.

This research was conducted to evaluate the efficacy of AHP, NaOH or NH₃ treatment of rice straw for enhancing in sacco ruminal digestibility of dry matter and fiber components.

Materials and Methods

Three non-lactating ruminally cannulated

¹Address reprint requests to Dr. K.H. Myung, Chonnam National University, Kwangju 500-757, Korea.

²University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

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TABLE 1. FORMULATION AND CHEMICAL COMPOSITION OF DIET FED TO COWS USED FOR IN SACCO STUDY

Items	%, DM basis
Ingredients	
Oat silage	90.00
Barley, rolled	7.51
Canola meal	0.57
Fish meal	0.36
Wheat short	0.55
Ground shelled corn	0.90
Molasses	0.55
Monophosphate	0.01
Trace mineral ^a	0.05
Chemical composition	
Crude protein	11.9
Acid detergent fiber	34.5
Neutral detergent fiber	50.3
Ash	7.5

^aContaining 25 mg/kg Se, 40 mg/kg Co, 0.35% Mg, 25% Cu, 0.01% I and 0.75% Zn.

Holstein cows (average weight 769 kg) were fed a diet (table 1) with a forage to concentrate ratio (DM basis) of 90:10. Animals were fed twice daily (07:30 and 17:00 h) in equal portions.

Rice straw was chopped (5 cm length) and chemically treated with NaOH, NH₃ or AHP while untreated rice straw was used as a control. For NaOH treatment the straw was soaked in a 4% NaOH solution (w/w, as-fed basis) for 30 min at 100°C and then air-dried at 60°C for 48 h without washing. For NH₃ treatment, the straw was moistened with distilled water and treated with anhydrous ammonia gas at 4% level (w/w, as-fed basis) for 72 h at 60°C in a sealed glass bottle. For AHP treatment, the straw was soaked in a solution of H₂O₂ (1% w/v), adjusted to pH 11.5 with 50% NaOH solution (w/w), for 5.5 h with stirring at ambient temperature. While the reaction was running, the reaction pH was kept at 11.5±3.0 by adding concentrated HCl (Kerley et al., 1987). After the reaction, the insoluble residue was neutralized with concentrated HCl, filtered and washed with distilled water until the filtrate was pH < 8, and air-dried at 60°C for 48 h. All samples were ground through a 2 mm screen and air-equilibrated for 48 h prior to analysis or placement in nylon bags for ruminal incubation.

Treated and untreated straws were analyzed for dry matter(DM), crude protein, crude fat and crude ash (AOAC, 1985) and fiber (Georing and Van Soest, 1970).

The nylon bag procedure was as described by de Boer et al. (1987). Approximately 5 g (air dry) of test samples were placed in nylon bags. A total of 28 bags were placed into a polyester mesh bag and incubated in the rumen for 0, 6, 12, 24, 48 and 72 h. At the end of each incubation time, one bag per treatment per cow was removed from the rumen and mechanically washed and dried at 60°C in a forced air oven for 48 h prior to determination of DM, neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose.

Percent disappearance of DM, NDF, ADF and cellulose at each incubation time was calculated from the proportion remaining after incubation in the rumen. The disappearance rate was fitted to the following equation (Robinson and Kennelly, 1988):

$$\text{Disappearance} = a + b(1 - e^{-k/100(t-1)})$$

Where a = soluble fraction (% of total), b = degradable fraction (% of total), k = rate of degradation of the degradable fraction (h⁻¹), t = time of rumen incubation (h) and 1 = discrete time lag before degradation began in the rumen (h). Nonlinear parameters a, b and k were estimated by an iterative least-square procedure and best-fit values were chosen using the smallest sum of squares after 10 iterations. In addition effective degradability of DM(EDDM), NDF(EDNDF), ADF(EDADF) and cellulose (EDCE) were calculated according to the equation (Ørskov and McDonald, 1979):

$$\text{EDDM, EDNDF, EDADF or EDCE} = a + [(b \times k) / (k + r)]$$

Where r = estimated rate of outflow from the rumen.

Data obtained from the experiment were evaluated statistically by analysis of variance, and means were compared using the Student-Neuman-Keul's procedure (Steel and Torrie, 1960).

Results and Discussion

The chemical composition of the untreated and treated rice straw is in table 2. Dry matter content of treated straws were significantly (p < 0.05)

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TABLE 2. CHEMICAL ANALYSES OF TREATED AND UNTREATED RICE STRAW (%)

Composition	Untreated	Treated			SE ^d
		NaOH ^a	NH ₃ ^b	AHP ^c	
Dry matter	93.5 ^e	92.5 ^f	91.2 ^g	90.8 ^h	0.03
Percent of dry matter					
Crude protein	6.0 ^e	5.7 ^f	15.1 ^g	5.2 ^h	0.04
Crude fat	1.7 ^e	1.3 ^f	1.4 ^f	1.4 ^f	0.05
Crude ash	11.7 ^e	16.6 ^f	10.8 ^e	5.6 ^g	0.58
Neutral detergent fiber	72.1 ^e	79.2 ^f	74.8 ^g	83.1 ^h	0.31
Acid detergent fiber	40.0 ^e	48.0 ^f	42.5 ^e	52.2 ^g	0.38
Hemicellulose	32.1 ^e	31.2 ^f	32.2 ^e	30.9 ^f	0.16
Cellulose	36.7 ^e	41.4 ^f	37.8 ^g	46.5 ^g	0.18
Acid detergent lignin	5.5 ^e	5.3 ^{ef}	5.1 ^f	4.7 ^g	0.07
Acid insoluble ash	3.5 ^e	3.6 ^e	3.4 ^e	2.4 ^f	0.18

^aSoaked in a 100°C solution of 4% NaOH (w/w, as-fed basis) for 30 min and artificially dried without washing.

^bAmmoniated with NH₃ at 4% (w/w, as-fed basis) for 72 h at 60°C.

^cDelignified with 1% alkaline hydrogen peroxide (AHP) solution at ambient temperature for 5.5 h by regulating pH at 11.5.

^dStandard error of the mean.

^{e,f,g,h}Means in the same row without a common letter in their superscripts differ ($p < 0.05$).

lower than that of untreated material, suggesting an increase in the water absorbancy. This increase in absorbancy may have resulted from the increase in the proportion of total cellulose found in low crystalline structures (Gould, 1985). Both crude protein and fat contents were decreased with treatment except for the higher ($p < 0.05$) crude protein content of the ammoniated straw. The higher ($p < 0.05$) ash content of the NaOH treated straw was expected because 2.3% sodium is added when 4% NaOH is used (Garrett et al., 1979). In contrast, the decrease ($p < 0.05$) in ash content of the AHP treated straw was in agreement with results of Gould and Freer (1984). The NDF and ADF contents of AHP treated straw increased ($p < 0.05$) suggesting that washing the substrate with water removed reactant chemicals and solubilized components of the straw. Cellulose contents followed trends similar to those for NDF and ADF, suggesting that most of the cellulose initially present in the straw remained after the AHP treatment (Gould and Freer, 1984). However, losses of hemicellulose and lignin with treatment supports reported preferential losses of hemicellulose and lignin associated with AHP treatment (Gould, 1984).

Disappearance of DM and NDF from nylon

bags as a function of ruminal incubation time are summarized in table 3. DM disappearance of treated straws was higher ($p < 0.05$) than that of the untreated straws after 12 h. However, disappearance at 0 h (soluble fraction) was lower for AHP and NaOH treatments than for control samples. Ammonia treatment resulted in highest soluble DM values. At 72 h, the DM disappearance for AHP was higher ($p < 0.05$) than that for NaOH or NH₃ treatments. Disappearance of NDF was greatest for AHP treatment with intermediate values being observed for NaOH and NH₃ treatment. Acid detergent fiber and cellulose disappearance as a function of ruminal incubation time are shown in table 4. Disappearance rates for ADF and cellulose followed similar trends to those observed for DM and NDF. This may result from chemical delignification as well as a physical change (Gould and Freer, 1984) in properties of the lignocellulosic material. During the course of AHP treatment, the integrity of the individual residue particles is completely lost as the residue disintegrates into small, highly water-absorbent fibers with a pulp-like consistency. This reduced the degree of crystallinity within the cellulose fibers, yielding a more hydrate, open structure (Gould and Freer, 1984). Although the magnitude

TABLE 3. DRY MATTER AND NEUTRAL DETERGENT FIBER DISAPPEARANCE TREATED AND UNTREATED RICE STRAW FROM NYLON BAGS (n=3) AS A FUNCTION OF INCUBATION TIME (%)

Incubation time h	Untreated	Treated			SE ^d
		NaOH ^a	NH ₃ ^b	AHP ^c	
Dry matter					
0	19.7 ^e	15.8 ^f	25.4 ^g	9.3 ^h	0.30
6	21.8 ^e	20.2 ^f	29.8 ^g	13.4 ^h	0.37
12	25.6 ^e	29.9 ^f	39.2 ^g	28.4 ^f	0.59
24	38.5 ^e	45.2 ^f	49.3 ^g	45.0 ^f	0.79
48	45.2 ^e	64.1 ^f	62.9 ^f	61.4 ^f	1.05
72	48.4 ^e	68.5 ^f	72.8 ^g	81.4 ^h	1.02
Neutral detergent fiber					
0	3.1 ^e	1.8 ^f	0.9 ^g	2.5 ^h	0.15
6	7.1 ^e	9.4 ^f	10.8 ^g	7.2 ^h	0.27
12	10.7 ^e	20.8 ^f	22.7 ^{fg}	23.6 ^g	0.60
24	25.8 ^e	37.1 ^f	37.5 ^f	42.0 ^g	0.99
48	34.9 ^e	60.8 ^g	54.6 ^f	59.2 ^g	0.74
72	43.0 ^e	64.7 ^f	66.7 ^f	80.5 ^g	1.56

^{a,b,c}See table 2 for details of treatments.

^dStandard error of the mean.

^{e,f,g,h}Means in the same row without a common letter in their superscripts differ ($p < 0.05$).

TABLE 4. ACID DETERGENT FIBER AND CELLULOSE DISAPPEARANCE FOR TREATED AND UNTREATED RICE STRAW FROM NYLON BAGS (n=3) AS A FUNCTION OF INCUBATION TIME (%)

Incubation time h	Untreated	Treated			SE ^d
		NaOH ^a	NH ₃ ^b	AHP ^c	
Acid detergent fiber					
0	1.2 ^e	1.6 ^e	0.5 ^f	1.6 ^e	0.17
6	5.6 ^e	1.6 ^f	4.6 ^e	2.6 ^f	0.40
12	7.3 ^e	12.3 ^f	15.6 ^g	18.1 ^h	0.51
24	23.4 ^e	29.0 ^f	30.7 ^f	35.4 ^g	0.90
48	25.9 ^e	61.1 ^f	49.4 ^g	54.5 ^h	0.72
72	35.3 ^e	65.3 ^f	64.7 ^f	78.9 ^g	0.82
Cellulose					
0	2.3 ^e	0.5 ^h	0.9 ^f	0.7 ^g	0.05
6	5.7 ^e	1.6 ^f	5.7 ^e	1.3 ^f	0.16
12	10.0 ^e	12.8 ^f	18.0 ^g	20.3 ^h	0.59
24	26.1 ^e	30.2 ^{ef}	31.8 ^{ef}	36.7 ^f	1.78
48	29.1 ^e	63.0 ^f	50.9 ^g	57.3 ^h	0.88
72	35.4 ^e	66.5 ^f	67.3 ^f	80.5 ^g	1.44

^{a,b,c}See table 2 for details of treatments.

^dStandard error of the mean.

^{e,f,g,h}Means in the same row without a common letter in their superscripts differ ($p < 0.05$).

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of the differences observed in the present study was slightly lower, the above results agree with those of Myung and Kennelly (1989). In general, the disappearance of DM and major structural carbohydrates in rice straw was greater for AHP than for NaOH or NH₃ treatments.

The non-linear parameters of effective degrad-

abilities of DM, NDF, ADF and cellulose are summarized in table 5. As discussed for disappearance rate, soluble fractions of DM and cellulose were lower ($p < 0.05$) than those of untreated and/or NaOH and NH₃ treated straw probably due to washing with water after AHP treatment. However, the degradable fractions were

TABLE 5. NONLINEAR PARAMETERS AND EFFECTIVE DEGRADABILITY OF DRY MATTER (EDDM), NEUTRAL DETERGENT FIBER (EDNDF), ACID DETERGENT FIBER (EDADF) AND CELLULOSE (EDCE) FOR TREATED AND UNTREATED RICE STRAW

Parameter ^a	Untreated	Treated			SE ^e
		NaOH ^b	NH ₃ ^c	AHP ^d	
Dry matter					
soluble (a), % of total	19.9 ^f	15.9 ^g	25.4 ^h	9.3 ⁱ	0.34
degradable (b), % of total	29.8 ^f	58.5 ^g	57.7 ^g	92.5 ^h	3.32
degradation rate of the degradable fraction (k), (h ⁻¹)	0.05 ^f	0.04 ^g	0.02 ^h	0.02 ^h	0.001
lag (l), (h)	6.1 ^f	4.1 ^{fg}	1.9 ^g	2.4 ^g	0.81
EDDM	34.6 ^f	40.5 ^g	44.1 ^g	36.4 ^f	0.73
Neutral detergent fiber					
soluble (a), % of total	3.1 ^f	1.8 ^f	0.7 ^h	2.5 ^{fg}	0.21
degradable (b), % of total	46.8 ^f	70.7 ^g	75.6 ^g	95.9 ^h	3.40
degradation rate of the degradable fraction (k), (h ⁻¹)	0.03 ^g	0.04 ^g	0.03 ^f	0.02 ^h	0.001
lag (l), (h)	3.7 ^f	3.1 ^f	0.4 ^g	2.5 ^f	0.20
EDNDF	20.1 ^f	31.1 ^h	27.7 ^g	32.4 ⁱ	0.36
Acid detergent fiber					
soluble (a), % of total	1.2 ^f	1.6 ^f	0.5 ^g	1.6 ^f	0.14
degradable (b), % of total	36.6 ^f	74.3 ^g	84.4 ^h	114.8 ⁱ	2.80
degradation rate of the degradable fraction (k), (h ⁻¹)	0.03 ^f	0.03 ^f	0.02 ^g	0.02 ^g	0.002
lag (l), (h)	3.0 ^f	8.1 ^g	3.0 ^f	4.0 ^f	0.31
EDADF	15.6 ^f	31.7 ⁱ	24.9 ^g	29.1 ^h	0.35
Cellulose					
soluble (a), % of total	2.3 ^f	1.0 ^g	0.9 ^g	0.7 ^h	0.04
degradable (b), % of total	33.6 ^f	75.6 ^g	100.1 ^g	106.4 ^g	11.10
degradation rate of the degradable fraction (k), (h ⁻¹)	0.05 ^f	0.04 ^f	0.02 ^g	0.02 ^g	0.001
lag (k), (h)	4.5 ^f	7.9 ^g	1.6 ^h	4.4 ^f	0.34
EDCE	18.8 ^f	32.4 ^g	25.6 ^h	30.5 ^g	0.90

^aa, b and k are non-linear parameters. l is discrete lag before degradation began. EDDM, EDNDF, EDADF, EDADF and EDCE are calculated on the basis of 0.05/h solid outflow rate.

^{b,c,d}See table 2 for details of treatments.

^eStandard error of the mean.

^{f,g,h,i}Means in the same row without a common letter in their superscripts differ ($p < 0.05$).

greatly increased ($p < 0.05$) with AHP treatment. The rate of degradation of the degradable fraction for treated straw of all items studied showed almost the same trend as that for untreated straw.

But, a direct comparison is not very meaningful because of the marked differences in the fraction. Although discrete time lag before degradation began in the rumen was not always significant ($p < 0.05$), there was a trend to decreased lag values for AHP treated straw, suggesting a greater susceptibility to microbial attack in the rumen (Kerley et al., 1985).

Effective degradabilities of DM, NDF, ADF and cellulose calculated on the basis of 0.05/h solid outflow rate were significantly ($p < 0.05$) greater for AHP treated straw compared to untreated samples.

The pH-regulated AHP treatment of rice straw could be a potent methods for delignification. The increased in sacco disappearance of AHP treated rice straw compared to NaOH and NH_3 treatment suggests that AHP treatment can effectively improve the nutritive value of the straw for use as a ruminant feedstuff.

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