Effect of Sodium Hydroxide plus Hydrogen Peroxide Treated Mustard (*Brassica campestris*) Straw Based Diets on Rumen Degradation Kinetics (*In sacco*), Fermentation Pattern and Nutrient Utilization in Sheep

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ABSTRACT : Two experiments were conducted to determine the effect of alkaline hydrogen peroxide (AHP) treatment (1% NaOH+1.5% H_2O_2; 1 AHPMS, 2% NaOH+1.5% H_2O_2; 2AHPMS) on rate and extent of degradation of mustard straw (MS) *in sacco* in sheep, and its *in vivo* digestion and ruminal fermentation characteristics when fed to sheep with concentrate (200 g per sheep daily). The treatment of straw with 1 and 2% AHP increased its sodium content by 148 and 296% to that of untreated straw (UMS). There was significant decrease in NDF and hemicellulose contents of AHP treated straw and increase in cellulose and lignin contents. Phenolic acids like ferrulic, p-coumaric and o-coumaric significantly ($p<0.001$) reduced by AHP treatment of mustard straw. In first experiment the in sacco degradation of DM, OM and NDF was significantly ($p<0.01$) greater for 2 AHPMS than for UMS at all incubation periods. The disappearance of nutrient from 1 AHPMS and 2 AHPMS treated straws continue to increase up to 96 h whereas in UMS the peak disappearance was found at 48 h. By using the equation $\{(y=a+b)\ (1-e^{-ct})\}$ the degradation rates (c) for DM, OM, and NDF were significantly higher for UMS than AHP treated straws. Level of alkali (1 and 2%) had significant effect on degradation characteristics (a, b, c and $P_{0.05}$) of DM and NDF fraction of MS. However, the effect was not pronounced on OM fraction of MS. In feeding experiment, the intake of nutrients for DM, OM, cell wall constituents and energy was higher on 2 AHPMS, whereas no effect on the digestibility of these nutrients was observed. The apparent nitrogen retention was higher ($p<0.05$) both in 1 and 2 AHPMS groups. Water intake by animals was significantly increased due to AHP treated mustard straw feeding. Rumen liquor pH was higher in 2 AHPMS fed animals. The NH_3-N of rumen liquor was not affected by feeding of AHP treated MS based diets. Total VFA concentration was significantly ($p<0.01$) higher in UMS fed group. The fractional out flow rate of DM was higher ($p<0.05$) in animals fed on 2 AHPMS diets compared to UMS and lAHPMS fed groups. The population of large holotrichs was higher ($p<0.05$) on AHP treated MS fed diets compared to UMS. The study indicated that treatment of mustard straw with AHP changed its chemical composition towards a better feed. The nutritive value of 2% AHP treated mustard straw was better in terms of dry matter intake and apparent nitrogen retention. The higher in sacco DM, OM and NDF disappearance however, was not confirmed by *in vivo* data in this study. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 355-365)***

Key Words : Straw, Mustard Straw, Alkaline Hydrogen Peroxide, Sheep Feeding, *In sacco*, Digestibility, Rumen Fermentation

INTRODUCTION

Crop residues are important as livestock feed in South East Asian region, and interest in their use as livestock feed is increasing as the feeds are in short supply and availability of other better quality feeds is very low (Jackson, 1977). In India for example, conventional crop residues/dry fodders are deficit to the tune of Ca 40% (Mishra et al., 2000). With changing scenario of Indian agriculture, the adoption of dwarf varieties of wheat and paddy by farmers further reduced the availability of these traditional crop residues. Gradual mechanisation of harvesting and thrashing operations like use of automatic harvester also contributed to low recovery of straw in the field. Under a situation of inadequate availability of cereal straws for livestock feeding, there is a need to use other alternative crop residues in animal diet. In India the area under mustard is increasing (Chauhan et al., 1999) and at present Ca 20 million tones of mustard straw (MS) is being produced annually. The MS becomes available during the months of February to April in semi-arid region of the country. This period is considered most critical as the availability of surface vegetation and other roughage starts depleting due to harsh climatic conditions. However, MS remain unutilised, as ruminants in the raw form owing to its very low digestibility (Misra et al., 2000) do not consume it. If nutritional value of MS is improved through some process technology then enormous quantity of roughage will be made available for ruminant livestock. In our laboratory attempts were made to improve the MS through urea-NH_3 (Misra et al., 2000) and NaOH (Mishra et al., 2001) treatment. Recently Mishra et al. 2000 following the reports from Gould, 1984; Kerley et al., 1986 and Chaudhary, 1998 and 2000, confirmed the effectiveness of alkaline hydrogen peroxide ($NaOH+H_2O_2$; AHP) to modify cell wall composition and *in vitro* organic matter digestibility (82-112% higher than untreated MS) of MS. The increased digestion was perhaps due to increased rate and extent of MS degradation by rumen microbes in an *in vitro* system.

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Therefore, we conducted experiments to investigate the farm-scale application of the most effective treatments (1.0% NaOH+1.5% H_2O_2 and 2% NaOH+1.5% H_2O_2) found in our earlier experiment (Mishra et al., 2000) and to assess feeding value of AHP treated straw in in sacco (Ørskov, 1982).

MATERIALS AND METHODS

The study was conducted at Central Sheep and Wool Research Institute, Avikanagar. Experimental animals were housed in asbestos roofed open sided shed in individual pens. During the experimental period the mean (±SD) maximum and the minimum temperature was 37.3±3.5°C and 17.5±3.8°C, respectively. Mean relative humidity was 37.3±3.5% at 14:30 h. The feeding trial was initiated in the month of March and continued for 40 days.

Straw, chemicals and treatment

The MS used for study was obtained from a nearby farm and contained upper half portion of the plant. MS was pulverized in a hammer mill to 1 to 2 cm, particle sizes before treatment. NaOH as flakes and H_2O_2 as 50% solution (W/V) were procured from CDH, India and used to treat the MS. Untreated mustard straw (UMS) served as control.

1% AHP (1.0% NaOH–1.5% H_2O_2) treatment : Alkaline hydrogen peroxide (AHP) treatment of MS was done in cemented tank (0.36 m^3). To, 100 L solution containing 1.0 kg NaOH and 3.0 l H_2O_2, 33.3 kg straw dry matter (DM) was dipped and left for 6h for treatment. The initial pH of solution was 13.1 and bath ratio of liquid: straw was 3:1. After draining the left over solution the treated straw (1 AHPMS) was removed and sun dried.

2% AHP (2.0% NaOH–1.5% H_2O_2) treatment : A 2% AHP treated MS (2 AHPMS) was prepared as described earlier. The amounts of NaOH and H_2O_2 were 2.0 kg and 3.0 L, respectively in 100 L solution used to treat straw. During the treatment, pH of solution was around 13.8.

Experiment 1

In sacco degradability : Three Malpura rams (35±1.4 kg BW and 34 to 37 months old), fitted with rumen cannulae, were used in this experiment. Rams were maintained on Cenchrus straw based diet (roughage to concentrate ratio, 65:35). The untreated (UMS) and AHP treated (1 AHPMS and 2 AHPMS) straws were ground through 1 mm sieves before in sacco rumen incubation. Triplicate samples 2.5 g DM of each test straw was weighed in to separate nylon bags. Nylon bags were suspended in the rumen as per the method described by Ørskov (1982) for 6, 12, 24, 48, 72 and 96 h. After incubation, the bags with residues were taken out from rumen, dipped immediately into cold water to stop the microbial activity and then rinsed with cold water to remove the particles from outside the bags. There after the bags of different incubation periods were washed in a washing machine. The bags were dried at 45°C till the constant weights were achieved. To determine the content of water-soluble fraction (a), bags with each test straw underwent the same washing procedures as the incubated bags. The samples of test straw together with residues of incubated samples were analysed for DM and organic matter (OM) as per AOAC (1990) and neutral detergent fibre (NDF, Van Soest et al., 1991). Disappearance of each nutrient was assumed to be due to degradation in rumen and calculated by difference between nutrient composition of unwashed and non-incubated samples of that test straw and residue after each incubation period in the rumen. The data obtained were fitted into the exponential model [$(y=a+b)(1-e^{-ct})$] of Ørskov and McDonald (1979) to obtain estimates of a, b and c for each straw in each sheep. Here, a represented water-soluble (or quickly degradable) and b insoluble (or slowly degradable) fractions, where as c is degradation rate of b and t the hours of incubation. While apparent degradability represented by a+b, predicted rumen degradability ($P_{0.05}$) for each straw was also calculated from the equation, P=a+(bc/c +k), where the k was the rumen out flow rate (0.05 per h). These estimates were statistically analysed by using one-way analysis of variance in SPSS Base 10.0 (SPSS Soft ware Products, USA) to test the effect of AHP treatment on rate and extent of in sacco degradability of MS.

Experiment 2

Metabolism and rumen fermentation experiment : Eighteen adult Bharat Merino (Rambouillet/Russian Merino X Chokla, stabilized at 65% level of exotic inheritance) rams, (range, 21.1 to 23.1 kg live weight) were used. Rams were adopted from a flock previously maintained on Cenchrus *(Cenchrus ciliaris)* predominant mixed pasture. The animals were randomly allocated to three equal groups on the basis of their live weight. These groups either received UMS, 1 AHPMS or 2 AHPMS straw *ad libitum*. In addition, each animal received 200 g concentrate mixture (barley, 370; mustard cake, 240; wheat bran, 370; mineral mixture, 10 and common salt, 10 g kg^{-1}) in individual feeding troughs, daily. Weighed quantity of concentrate mixture and straws were offered daily at 8.00 h after discarding the residue of previous day. Animals were offered clean drinking water free of choice.

After 21 days of adaptation, the digestibility and balance measurements were made. These occurred between days 23 to 30. Faeces and urine from all sheep were collected quantitatively. Sub-samples of faeces were taken for DM determination and further samples were kept for subsequent freezing and chemical analysis. The urine was mixed with sulphuric acid during collection and sub-

Table 1. Chemical composition (g kg^{-1} DM) of concentrate mixture (CM), untreated (UMS) and AHP treated mustard straw used in metabolism and rumen fermentation experiment (means of 3 determinations)

Item	CM	UMS	1 AHPMS	2 AHPMS	SEM	Regression analysis	
						Linear	Quadratic
DM	948	962	962	955	2.41	NS	NS
OM	936	935^a	932^a	906^b	2.82	**	**
CP	219	44	44	41	1.70	**	NS
NDF	472	782^a	766^b	756^b	1.00	**	NS
ADF	151	664	662	657	2.70	NS	NS
Hemicellulose[1]	321	118^a	105^b	98^b	2.00	**	NS
Cellulose	104	490^a	513^b	509^b	2.12	**	**
ADL	34	137^a	143^b	143^b	1.00	**	**
GE kcal	50	38	40	41	1.23	NS	NS
Sodium	6.71	3.79^a	9.41^b	14.99	0.31	***	NS
Phenolic acids (mg g^{-1} cell wall)							
Ferrulic	ND	2.66^a	1.21^b	0.98^b	0.05	***	***
p-coumaric	ND	0.60^a	0.03^b	0.03^b	0.01	***	***
o-coumaric	ND	0.20^a	0.01^b	0.01^b	0.00	***	***

1 AHPMS-Mustard straw treated with a solution containing 1% NaOH and 1.5% H_2O_2.
2 AHPMS-Mustard straw treated with a solution containing 2% NaOH and 1.5% H_2O_2.
Linear: Significance of linear effects of NaOH levels used in AHP treatment.
Quadratic: Significance of quadratic effects of NaOH levels used in AHP treatment.
[1] NDF-ADF. [a,b] Statistical significance was tested among UMS, 1 AHPMS and 2 AHPMS only.
** $p<0.001$. *** $p<0.001$: ND=Not determined, means values bearing different letters in row differ significantly.

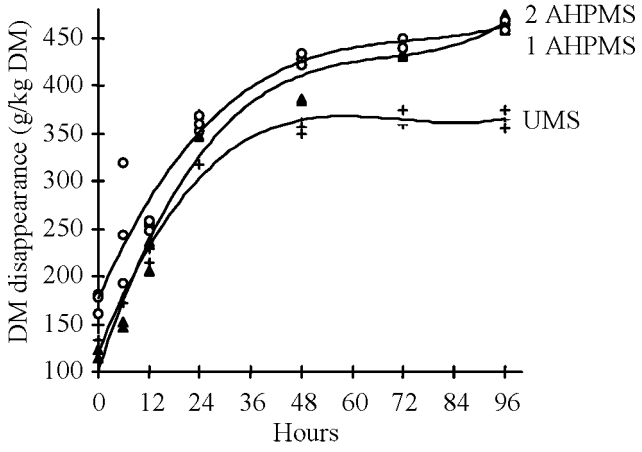

Figure 1. Patterns of dry matter (DM) disappearance from untreated (UMS) and AHP treated 1AHPMS (1% NaOH+1.5% H_2O_2) and 2 AHPMS (2% NaOH+1.5% H_2O_2), mustard straw during in sacco incubation.

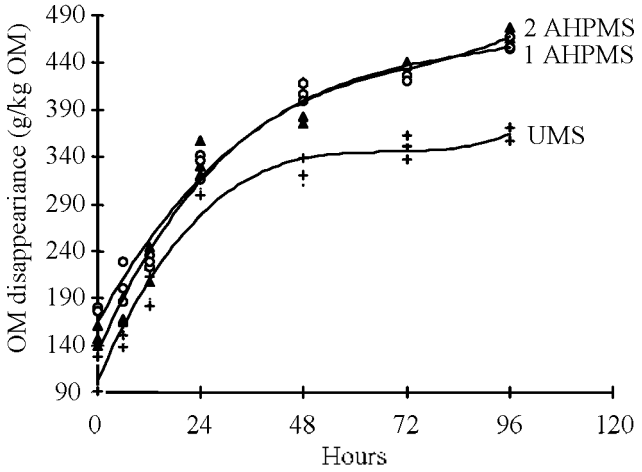

Figure 2. Patterns of organic matter (OM) disappearance from untreated (UMS) and AHP treated 1 AHPMS (1% NaOH+1.5% H_2O_2) and 2 AHPMS (2% NaOH+1.5% H_2O_2), mustard straw during in sacco incubation.

samples were taken for analysis. Rumen fermentation and rate of passage study was carried out between days 34 to 36 and 38 to 40, respectively, involving 4 sheep from each group. Rumen fluid samples (20 ml) were taken by a stomach tube at 0, 3 and 6 h post feeding for three consecutive days. Rumen fluid was filtered through four layers of cheesecloth and the pH was measured in the strained rumen liquor (SRL). These samples were acidified with 0.2 ml of 10 N H_2SO_4 and stored for later analysis. The rate of passage of solid digesta was determined by feeding Cr-mordanted fibre, prepared as per the method suggested by Uden et al. (1980) and modifications adopted by Chaudhry (1998) for bulk preparation.

Samples of feed offered, ort and faeces and urine voided were analysed for DM, nitrogen (N) and ash contents according to AOAC (1990), while NDF, ADF, cellulose and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991). Gross energy (GE) content was determined by ballistic bomb calorimeter (Gallenkamp, UK). Phenolic acids were determined on HPLC system (Shimadzu 10AD, Japan) as per the procedure described by Shakil et al., (1999). Rumen liquor samples were analysed for total-N (AOAC, 1990), NH_3-N (Conway, 1962), total volatile fatty acids (TVFA; Bernett and Reid, 1957).

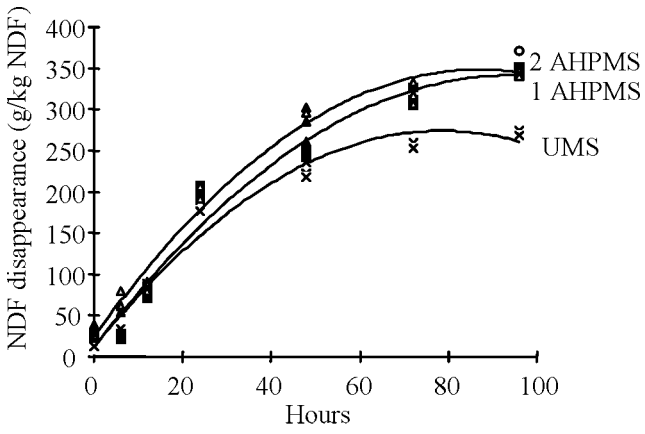

Figure 3. Patterns of neutral detergent fibre (NDF) disappearance from untreated (UMS) and AHP treated 1 AHPMS (1% NaOH+1.5% H_2O_2) and 2 AHPMS (2% NaOH+1.5% H_2O_2), mustard straw during in sacco incubation.

Sodium was analysed on atomic absorption spectrophotometer (Perkin-Elmer, 2380, USA) following the standard procedure (Perkin-Elmer, 1982). Cr in mordanted fibre and faeces was analysed calorimetrically at 440 nm as dichromate after wet digestion (Owen et al., 1967).

Total and differential counts of protozoa were made in 30 microscopic fields in a haemocytometer counting chamber at a magnification of 100×. Ciliates were identified according to the method of Hungate (1966). Spirotrichs not identified to a generic level, were classified in to small spirotrichs (mainly entodinia with an average size of 42×23 μm) and large spirotrichs (largely diplodinia with an average size of 132×66 μm).

Statistical analysis

Chemical composition, intake and digestibility data were analysed by using one way analysis of variance procedure of SPSS Base 10 (SPSS software products. USA) and group differences were compared by using Duncan's Multiple Range Test (Duncan, 1955). Chemical constituents of straw were also analysed for linear and quadratic effects of two levels of NaOH used in AHP treatment. Rumen fermentation variables were analysed for treatment (T) and sampling time (H) as main effects and T×H interaction using the following mathematical model in two-way analysis of variance procedure of SPSS Base 10:

$$Y_{ijk}=\mu+T_i+P_j+(TP)i_j+e_{ijk}$$

Where, μ=general mean. Ti=effect of i^{th} treatment, P_j=effect of j^{th} period, (TP) i_j=interaction effect of i^{th} treatment with j^{th} period. e_{ijk}=random error.

RESULTS

Effect of AHP treatment on cell wall composition, phenolic acids and sodium content

The cell wall composition. phenolic acids and sodium content along with other chemical constituents of untreated

Table 2. Degradable fractions (g kg^{-1}) of dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF) in untreated (UMS) and AHP treated mustard straw after in sacco rumen incubation in sheep

Parameters	UMS (1)	1 AHPMS (2)	2 AHPMS (3)	SEM	P		
					1-2	1-3	2-3
DM							
a	117.37	129.30	163.47	4.70	NS	**	**
b	254.6	367.2	310.7	5.36	**	**	**
c (per h)	0.050	0.04	0.04	0.003	*	*	NS
a+b	372.00	470.50	474.10	5.20	**	**	NS
$P_{0.05}$	247.00	260.33	294.00	2.32	**	**	**
OM							
a	104.73	135.30	164.50	7.24	*	**	NS
b	256.7	349.1	320.9	8.05	**	**	NS
c (per h)	0.042	0.029	0.027	0.003	*	**	NS
a+b	361.40	484.40	485.40	9.44	**	**	NS
$P_{0.05}$	222.00	264.00	276.30	3.39	**	**	*
NDF							
a	-5.25	4.06	13.37	1.78	**	**	*
b	248.0	401.1	335.5	8.49	**	**	**
c (per h)	0.05	0.02	0.03	0.002	**	**	**
a+b	242.80	405.13	348.87	9.12	**	**	*
$P_{0.05}$	113.00	123.67	141.67	0.72	**	**	*

1 AHPMS-Mustard straw treated with a solution containing 1% NaOH and 1.5% H_2O_2.

2 AHPMS-Mustard straw treated with a solution containing 2% NaOH and 1.5% H_2O_2.

a and b represent quickly and slowly degradable fraction: c. degradable rate of b; a−b and $P_{0.05}$ represent apparent (asymptote) and predicted extent of degradation. respectively.

P: Statistical significance, NS; Non significant, * $p<0.05$, ** $p<0.001$. SEM: standard error of means.

Table 3. Digestible nutrients intake (g day^{-1}) and digestibility coefficients (%) in sheep fed untreated (UMS) or AHP treated mustard straw based diets[1] (values for 6 sheep per treatment)

Digestible nutrients intake	UMS (1)	1AHPMS (2)	2AHPMS (3)	SEM	P		
					1-2	1-3	2-3
DM							
Intake	253.6	272.9	330.0	19.56	NS	*	NS
Digestibility	52.3	51.5	50.5	1.74	NS	NS	NS
OM							
Intake	251.5	269.8	310.9	18.88	NS	*	*
Digestibility	56.4	54.6	52.3	1.70	NS	NS	NS
CP							
Intake	49.3	51.9	55.9	3.51	NS	NS	NS
Digestibility	53.1	51.9	45.8	3.19	NS	NS	NS
NDF							
Intake	124.2	157.6	198.2	15.52	NS	*	NS
Digestibility	40.9	44.4	44.9	1.99	NS	NS	NS
ADF							
Intake	84.3	106.3	145.9	14.71	NS	*	NS
Digestibility	38.9	41.5	43.4	2.20	NS	NS	NS
Hemicellulose							
Intake	53.1	56.8	56.1	3.49	NS	NS	NS
Digestibility	57.9	55.6	52.3	3.50	NS	NS	NS
Cellulose							
Intake	76.8	107.2	141.7	12.94	NS	*	NS
Digestibility	48.6	54.0	54.0	2.12	NS	NS	NS

[1] Concentrate supplement at 200 g per sheep daily.

1 AHPMS-Mustard straw treated with a solution containing 1% NaOH and 1.5% H_2O_2.

2 AHPMS-Mustard straw treated with a solution containing 2% NaOH and 1.5% H_2O_2.

P: Statistical significance, NS; Non significant. * $p<0.05$, SEM; standard error of means.

and AHP treated MS are presented in Table 1. Both type of AHP treatment reduced ($p<0.01$) NDF and hemicellulose and increased ($p<0.05$) the cellulose and lignin content of MS. The efficacy of two levels of alkali in AHP treatment was similar. Content of acid detergent fibre was not affected by AHP treatment with either level of alkali. The sodium (Na) content of UMS was 3.79 g kg^{-1} of DM and it increased to 9.41 and 15.0 in 1 AHPMS and 2 AHPMS, respectively. Content of phenolic acid decreased linearly ($p<0.001$) with increasing level of alkali in AHP solution.

In sacco disappearance and degradation characteristics of nutrients

Patterns of DM, OM and NDF disappearance from UMS, 1 AHPMS and 2 AHPMS during rumen incubation are illustrated in Figure 1, 2 and 3 where as the estimates of degradation characteristics (a, b, a+b, c and $P_{0.05}$) of these nutrients are presented in Table 2. The disappearance of DM, OM and NDF was significantly ($p<0.01$) greater for 2 AHPMS, than for UMS at almost all incubation times. Whereas, in 1 AHPMS, the disappearance of these nutrients was not greater ($p<0.01$) than that of UMS up to 12 h of incubation and thereafter the observed differences were significantly higher ($p<0.01$) for 1 AHPMS but similar to 2 AHPMS. The losses of DM and OM from 2 AHPMS on washing with water (0 h) were higher ($p<0.01$) than that from 1 AHPMS and UMS. The disappearance of nutrients from 1 AHPMS and 2AHPMS treated straws continued to increase to 96 h, whereas in UMS the peak disappearance of nutrients was observed at 48 h. The AHP treatment with 2% alkali (2 AHPMS) caused greatest disappearance of nutrients from MS followed by 1 AHPMS ($p<0.01$).

Quickly degradable (a) fraction of MS DM was significantly higher ($p<0.01$) in 2 AHPMS than UMS and 1 AHPMS. However, slowly degradable fraction (b) was significantly ($p<0.01$) affected by AHP treatment. The AHP treatment significantly ($p<0.01$) affected the degradation of OM of MS. The a and b fractions of MS for NDF were significantly ($p<0.01$) affected by AHP treatment. The effect of two levels (1 and 2%) of alkali for AHP treatment on degradation characteristics (a, b) of DM and NDF was significant, whereas the effect was not pronounced on OM fraction of MS. Unexpectedly, the degradation rates (c) of DM, OM and NDF were significantly ($p<0.01$) faster for untreated straw (UMS) compared with AHP treated straws (1 AHPMS and 2 AHPMS). However, predicted degradability ($P_{0.05}$) of DM, OM and NDF was greatest for AHP treated straw ($p<0.01$) compared with UMS.

Digestible nutrients intake and digestibility

The mean values of digestibility of DM, OM, crude protein (CP) and fibre fractions and intake of their digestible portion are presented in Table 3. One way analysis of variance indicated significant effects ($p<0.05$)

Table 4. Intake and excretion pattern of nitrogen (N), sodium (Na) and water in sheep fed untreated (UMS) or AHP treated mustard straw based diets[1] (values for 6 sheep per treatment)

Attributes	UMS (1)	1AHPMS (2)	2AHPMS (3)	SEM	P		
					1-2	1-3	2-3
Nitrogen (g day^{-1})							
Intake	7.88	8.31	8.95	0.31	NS	NS	NS
Faecal excretion	3.68	4.07	4.95	0.40	NS	NS	NS
Urinary excretion	3.05	2.07	1.69	0.22	**	**	NS
N retained	1.15	2.18	2.31	0.29	*	*	NS
Sodium (mg/day)							
Intake	2,331	4,476	8,522	604.70	***	***	***
Faecal excretion	973	1526	2157	324.00	*	*	*
Urinary excretion	18	30	53	12.79	NS	*	NS
Water intake (l day^{-1})	2.03	2.61	3.01	0.11	*	*	*
Water excretion (ml day^{-1})							
Faeces	164.20	323.00	502.20	74.14	*	*	*
Urine	217.10	266.10	292.80	79.93	NS	NS	NS

[1] Concentrate supplement at 200 g per sheep daily.
1 AHPMS-Mustard straw treated with a solution containing 1% NaOH and 1.5% H_2O_2.
2 AHPMS-Mustard straw treated with a solution containing 2% NaOH and 1.5% H_2O_2.
P: Statistical significance, NS; Non significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, SEM; standard error of means.

Table 5. Energy intake and excretion (day^{-1}) in sheep fed untreated (UMS) or AHP treated mustard straw based diets[1] (values for 6 sheep per treatment)

Attributes	UMS (1)	1AHPMS (2)	2AHPMS (3)	SEM	P		
					1-2	1-3	2-3
Gross energy (GE) intake							
Mcal	3.20	3.74	5.03	0.41	NS	*	*
kcal/kg $W^{0.75}$	321.78	362.02	474.69	34.80	NS	*	NS
Faecal energy				3.12			
Mcal	0.99	1.14	1.67	0.132	NS	**	NS
kcal/kg $W^{0.75}$	100.67	110.34	157.02	10.76	NS	**	**
% of GE	31.43	31.27	33.0	3.12	NS	NS	NS
Digestible energy intake							
Mcal	2.20	2.60	3.36	0.298	NS	*	NS
kcal/kg $W^{0.75}$	221.10	251.68	317.67	25.87	NS	*	NS
% of GE	68.57	68.73	67.00	1.55	NS	NS	NS
Urinary energy							
kcal	38.11	31.96	30.99	1.89	*	*	NS
kcal/kg $W^{0.75}$	3.90	3.14	2.93	0.42	NS	NS	NS
% of GE	1.20	0.92	0.66	0.11	NS	**	NS

[1] Concentrate supplement at 200 g per sheep daily.
1 AHPMS-Mustard straw treated with a solution containing 1% NaOH and 1.5% H_2O_2.
2 AHPMS-Mustard straw treated with a solution containing 2% NaOH and 1.5% H_2O_2.
P: Statistical significance, NS; Non significant, * $p<0.05$, ** $p<0.01$, SEM; standard error of means.

due to AHP treatment applied with 2% NaOH (2 AHPMS) except for hemicellulose and CP. Digestible intakes of DM, OM and cell wall constituents were higher in 2 AHPMS compared to U MS. Whereas the differences in intakes of these nutrients between UMS and 1 AHPMS and 1 AHPMS and 2 AHPMS were non-significant. Either level of NaOH used for AHP treatment did not affect the digestibility of nutrients.

The mean values of intake of N and Na and their excretion through faeces and urine are presented in Table 4. The N intake and N excreted through faeces was similar in all groups. However, losses of urinary N were greater ($p<0.01$) for the sheep fed UMS diet than those fed AHP treated MS diets (1 AHPMS and 2 AHPMS). Sheep given AHP treated MS diets retained more nitrogen compared to UMS ($p<0.05$), however, the difference between AHP treated MS diets were non-significant.

Sodium intake and its excretion in faeces ($p<0.01$) and urine in sheep fed treated MS diets were higher than those given untreated MS diet. Similarly due to feeding of AHP treated straw based diets total water intake increased significantly ($p<0.01$). The daily water intake increased linearly with the amount of NaOH used in AHP treatment (Figure 5). The faecal moisture contents were also affected ($p<0.05$) by treatment of straw and water losses through faeces were higher in animals consuming 1 AHPMS and 2

Table 6. Influence of AHP treatment on ruminal metabolites, flow rate of dry matter and average count ciliate protozoa ($\times 10^4$/ml) in the strained rumen liquor[2] of sheep fed untreated (UMS) or AHP treated mustard straw based diets[1] (4 sheep per treatment)

Attributes	Diets			P			Hours post feeding			P			Interaction	SEM
	UMS	1 AHPMS	2 AHPMS				0 h	3 h	6 h					
	(1)	(2)	(3)	1-2	1-3	2-3	1	2	3	1-2	1-3	2-3	TxH[3]	
pH	6.85	6.93	7.00	NS	**	NS	7.18	6.81	6.80	**	*	NS	NS	0.05
NH_3-N (mg dl^{-1})	8.30	8.35	8.29	NS	NS	NS	7.12	9.67	8.16	**	**	**	**	0.07
Total VFA (mML^{-1})	8.69	8.42	8.24	NS	**	NS	6.94	9.92	8.49	**	**	**	NS	0.09
Large holotrichs	0.16	0.23	0.23	*	*	NS	0.10	0.28	0.25	**	**	NS	NS	0.01
Small holotrichs	0.52	0.63	0.67	NS	NS	NS	0.43	0.75	0.66	**	**	NS	NS	0.08
Total holotrichs	0.68	0.87	0.90	NS	NS	NS	0.53	1.02	0.91	**	**	NS	NS	0.11
Large spirotrichs	4.18	4.35	4.66	NS	NS	NS	8.86	17.10	16.14	**	**	NS	NS	0.37
Small spirotrichs	13.40	14.13	14.56	NS	NS	NS	2.68	5.51	5.11	**	**	NS	NS	1.23
Total spirotrichs	17.59	18.49	19.21	NS	NS	NS	11.54	22.51	21.26	**	**	NS	NS	1.44
Total protozoea	18.29	19.36	20.12	NS	NS	NS	12.07	23.53	22.16	**	**	NS	NS	1.40
Flow rate of DM (g day^{-1})	691.88	710.33	867.93	NS	*	*	-	-	-	-	-	-	-	34.82

[1] Concentrate supplement at 200 g per sheep daily.

[2] Each value is an average of 12 observations (4 sheep: 3 sampling times i.e. 0, 3 and 6 h post feeding). [3] Treatment×hour interaction.

1 AHPMS-Mustard straw treated with a solution containing 1% NaOH and 1.5% H_2O_2.

2 AHPMS-Mustard straw treated with a solution containing 2% NaOH and 1.5% H_2O_2.

P; Statistical significance, NS; Non significant, * $p<0.05$, ** $p<0.01$, SEM, standard error of means.

AHPMS diets compared to control whereas, urinary excretion of water remain unaffected.

GE intake was more ($p<0.05$) in sheep given 2 AHPMS diet than animals given UMS diet (Table 5). They also excreted more energy in faeces compared to UMS. The GE digestibility did not differ among the groups. Urinary energy (UE) losses (kcal day^{-1}) were more ($p<0.01$) in sheep given UMS diet compared to those fed on treated straw diets, whereas the variation between these two groups was non-significant.

Ruminal fermentation and protozoa population

The values averaged across the sampling times of 0, 3 and 6 h post feeding for pH, NH_3-N and total VFA are presented in Table 6. Feeding of 2 AHPMS increased ($p<0.01$) ruminal pH of sheep. However, variations in UMS vs. 1 AHPMS and 1 AHPMS vs. 2 AHPMS were non-significant. The ammonia N fraction of rumen liquor was not affected by feeding of AHP treated MS based diets. Total VFA concentration was significantly ($p<0.01$) higher in UMS fed group compared to 2 AHPMS diets, whereas the differences between UMS and 1 AHPMS were non-significant. Ruminal out flow rate of DM was greater ($p<0.05$) in animal consuming 2 AHPMS diet compared to UMS and 1 AHPMS diets but the later two did not differ with each other. Ruminal counts of large holotrichs were significantly increased ($p<0.05$) on feeding of AHP treated MS diets compared to UMS (Table 6). However, the distribution of other type protozoal population in rumen was unaffected due to feeding of AHP treated MS diets. The pH of rumen fluid was significantly ($p<0.01$) lower and concentration of NH_3-N, total VFA and rumen protozoa population were greater ($p<0.01$) at 3 h post feeding. The interaction between treatment and hour is non significant in all the parameters except in NH_3-N. Over all on an average the lowest rumen pH was observed at 3 h post feeding while total VFA and NH_3-N production at similar time intervals exhibited reversal trend. The values for rumen pH at 3 and 6 h post feeding were almost similar. However, ruminal concentration of total VFA and NH_3-N were lower ($p<0.01$) at 6 than 3 h post feeding.

Ruminal counts of large holotrichs were higher ($p<0.01$) of AHP treated MS diets compared to UMS diet (Table 6). However, the distribution of other type protozoa population in rumen was unaffected due to feeding of AHP treated MS diets. Lowest number of ciliated protozoa count was observed just before feeding with increased in total, holotrichs and spirotrich protozoa count at 3 h post feeding followed by decreased at 6 h post feeding.

DISCUSSION

Chemical composition

The pH of residual solutions after soaking the MS was in range of 12.1 to 12.7, which would be considered optimal for effective AHP treatment (Chaudhary, 1998, 2000). The untreated MS had nearly neutral pH (7.1), whereas the pH of AHP treated straws ranged in between 10.5 to 11.1. An obvious effect of AHP treatment was on Na content of straw. The level of Na retention in straw was to the tune of about 33% of sodium present in AHP solution. This caused an increase of 148 and 296% in sodium content of straw due to 1 AHP and 2 AHP treatment respectively. The phenolic acids are able to form bridges between cell wall constituents (cellulose, hemicellulose and lignin) through formation of ester linkages (Hartley and Jones, 1977; Hartley, 1981). These ester linkages are not hydrolysed by rumen microbes but are alkali labile and a direct relationship exists between

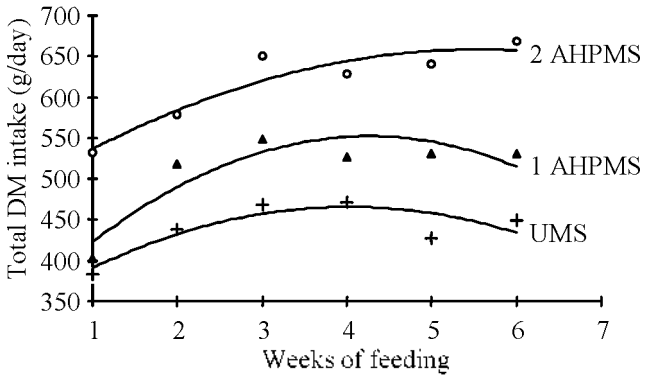

Figure 4. Effect of alkaline hydrogen peroxide treatment of mustard straw (MS) with 1 and 2 percent NaOH (1 AHPMS and 2 AHPMS) on total DM intake in sheep fed with concentrate (190 g DM per sheep daily).

loss of phenolic acids and the improvement in digestibility, which occurred when alkali was applied on cereal straw (Hartley and Jones, 1978; Chesson, 1981). The decrease in NDF content by AHP treatment agrees with our earlier findings on MS (Mishra et al., 2000) and other straws (Rexen and Thomsen, 1976). However ADF (cellulose+lignin) did not decreased ($p>0.05$) with AHP treatments, which indicated that the reduction in NDF was produced by solublization of hemicellulose in neutral detergent solution. The increase in lignin content by the AHP treatment is consistent (Chaudhary and Miller, 1996; Chaudhary, 1998; 2000, Mishra et al., 2000). However, it was contrary to that reported by Gould (1985); Kerley et al. (1987); Brand et al. (1989) where substantial reduction in lignin content resulted due to AHP treatment. AHP treatment of wheat straw at pH 11.5 removed about 15-50 per cent of the lignin as water-soluble products (Gould, 1984; Chaudhary and Millar, 1994). On the other hand during the process some of the phenolic acids get mobilized (Chaturvedi et al., 2001).

In sacco degradation

The DM and OM disappearance from straws was relatively higher than that of NDF during all incubation periods. The absolute disappearance as well as rate and extent of degradation of these nutrients from untreated and AHP treated MS was smaller when compared with similarly treated wheat straw (Miller and Oddoye, 1989; Chaudhary, 2000). The variation in response may largely be attributed to straw type, as the responses of alkali and AHP treatments are species specific (Jackson, 1977; Huntingdon and Givens, 1995). During initial 24 h of degradation the AHP treatment done with 1% NaOH did not show any statistically significant improvement in in sacco degradation, as the nutrient disappearance from 1 AHPMS was not much different from UMS. Whereas, in case of AHP treatment applied with 2% NaOH i.e. 2 AHPMS, the disappearance of nutrient was significantly ($p<0.05$) larger compared to UMS during initial 24 h incubation times. This could be due to the smaller amount of NaOH (3 g kg^{-1} DM) used in 1 AHPMS. The optimum level of alkali has varied from experiment to experiment and it might well be different for different straws. However, a uniform finding of various experiments has been an approximately linear increase in digestibility with increasing amounts of alkali up to a level of 10 g NaOH 100 g^{-1} straw and a levelling off thereafter (Jackson, 1977). The higher disappearance of nutrients from 2 AHPMS was a consequence of higher level of alkali (2% NaOH) used in AHP treatment, which causes swelling and changes in crystalline structure of cellulose (Guggolz et al., 1971; Klopfenstein, 1978) and renders the fibre more susceptible to ruminal microbial degradation. In sacco degradability of OM observed in AHP treated straws (396 and 407 g kg^{-1} OM in 1 AHPMS and 2 AHPMS) at 48 h of incubation is reasonably higher to that of our previous *in vitro* study (Mishra et al., 2000) conducted with similarly treated MS (291 and 360 g kg^{-1} OM). However the efficacy of AHP treatment was of lesser magnitude and only 29 and 33 per cent improvement in in sacco degradability could be observed with 1 AHPMS and 2 AHPMS as against 82-112 per cent improvement in its *in vitro* degradation at 48 h (Mishra et al., 2000). It appeared that straw used in this study would have contained more of these digestible fractions, thus showing higher initial digestibility. In general the AHP treatment of MS with 1 and 2% NaOH increased the extent of degradation of MS in in sacco, whereas the rate of in sacco degradation (c) of treated MS was slower than UMS. The effect of AHP treatment of MS on in sacco nutrients disappearance was more pronounced during later 48 h of incubations. The added advantage of higher level of NaOH (2%) in AHP treatment was of lesser magnitude and that too during initial 24 h of incubation.

Nutrient intakes and their utilization

Maximum intakes of straw were attained in 21 days and remained fairly constant thereafter (Figure 4). There was no any apparent ill effect of AHP treated straw feeding on sheep. However, the consistency of faeces was loose in these two groups due to higher sodium intake, which in turn increased the water consumption and thus resulting higher faecal moisture content. Similar observations were seen by Arnason (1980) in young bulls fed chopped NaOH treated straw (2.7 g Na kg^{-1} $W^{0.75}$) and Jaysuriya and Owen (1975) in sheep (0.84 to 1.57 g Na kg^{-1} $W^{0.75}$) fed barley straw treated with varying levels of NaOH (4.5 to 9.0 g Na kg^{-1} straw). The Na intakes of 0.44 and 0.84 g Na kg^{-1} $W^{0.75}$ in present study were comparatively lower than those of 2.2 to 2.4 g Na kg^{-1} $W^{0.75}$ being suggested as the upper limits for tolerance of Na intake in sheep fed alkali treated straw (Ali et al., 1977). The diuresis pattern in these animals remains

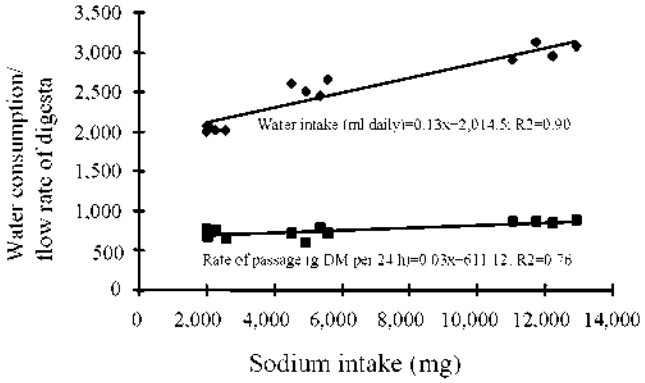

Figure 5. Effect of sodium intake on daily water consumption and rate of passage of digesta in sheep fed mustard straw based diets with concentrate supplement (200 g per sheep daily).

unchanged and excess quantity of Na was excreted through faeces only. However, it is contrary to the reports where extra Na ingested is entirely excreted in the urine; excretion through faeces is not increased; nor is the amount secreted in milk (Maeng et al., 1971; Pitchaiah, 1971; Voigt and Piatkowski, 1974; Choung and Mc Marnus, 1976). The alteration in Na excretion pattern may largely be due to a comparatively lesser intake of Na on AHP treated MS diets than those could strive the animals to change the diuresis. But this may also be attributed to some extent to a faster passage rate of digesta often associated with alkali treated straw diets (Bolduan et al., 1974; Berger et al., 1979; Klopfenstein et al., 1979) that was also observed in AHP treated MS fed sheep. This would have prevented the effective absorption of Na and thus concentrating more sodium in faeces.

Despite the larger in sacco nutrient disappearance from AHP treated straw, the *in vivo* digestibility of nutrients was not improved when fed to sheep. The possibility is that since water intake increased with alkali treated, straw diets rate of passage through rumen also increases (Figure 5), thus limiting digestibility to lower than what would be possible with slower passage (Maeng et al., 1971). Further the variability in digestibility responses to chemical processing of straw and other materials other than straws have been referred by Jackson (1977). For example, forage sorghum hay responded much less to treatment (spray 5% NaOH) than did sorghum stover, which had a lower initial digestibility. It would seem that chemical treatments result in greater responses when applied to straws of lower digestibility. However, the potential digestibility of nutrients observed for treated straw is reasonably close to that observed for MS treated with alkali alone (20 g NaOH kg^{-1} DM; Mishra et al., 2001) or pre treatment with urea (Misra et al., 2001). The AHP treatment of a more digestible straw would probably have masked the effect of chemical processing in improving the digestibility. The greater intake of digestible nutrients on 2 AHPMS diet was a consequence of higher DMI i.e. 38% higher than UMS with similar digestibility.

Treatment of MS also improved the N economy, as the N retention was higher ($p<0.05$) in sheep fed 1 AHPMS and 2 AHPMS diets due to lower (32.1 and 44.6%) urinary N losses. Comparatively greater availability of digestible energy for each g of digested protein (kcal DE g^{-1} DCP) on treated straw diets (102.2 and 134.4) than untreated straw (83.8) was the major reason for decreased urinary N excretion, because there is a requirement of energy for both protein synthesis and degradation (Kelly et al., 1993). The intakes of digestible CP were 2.26, 2.63 and 2.44 g and of DE were 221.1, 251.68 and 317.67 kcal kg^{-1} $W^{0.75}$ in UMS, 1 AHPMS and 2 AHPMS based diets. This could be considered adequate for maintenance and slow rate of growth (Ranjhan, 1998).

Ruminal fermentation

Although the pH declined with concomitant increases in total VFA concentration, as the fermentation progressed but remained greater than those of 6.2-6.5, being suggested as minimum for effective fibre digestion (Mertens, 1979). An increase in total VFA of sheep fed untreated straw diet was probably due to an increased fermentation rate in the rumen (Andries et al., 1987) and occurred primarily due to the faster rate of degradation (c) observed for the same material (Table 2). The rumen ammonia levels in sheep fed these diets were >20 mg dl^{-1}, the level suggested by Hogan (1982) as sufficient to maintain normal rumen microbial population. These were also >5.0 mg dl^{-1} suggested as necessary for maximum microbial growth (Satter and Slyter, 1974). At no time did the ammonia concentration declined below this range indicating that digestion rates in any case would have not limited by nitrogen. In this study, feeding of AHP treated straw based diets favoured the proliferation of ciliate protozoal population, specially the large holotrichs, due to slightly alkaline rumen environment (Hungate, 1966). Feed offer was associated with an abrupt increase in the total as well as differential protozoal population in present study within 3 h post feeding which could be due to migration of ciliate protozoa from reticulo rumen wall from where they migrate back to the rumen medium in response to chemical stimuli originating from the diet (Kamra et al., 1991). After the feed was utilized the protozoa, gradually migrated back to the reticulo-ruminal wall resulting in observed drop in their numbers in the rumen liquor at 6 h post feeding.

CONCLUSIONS

Alkaline hydrogen peroxide treatment with 1 and 2 per cent NaOH resulted in a decrease ($p<0.01$) in NDF, hemicellulose and phenolic acids ($p<0.001$) and increase in

cellulose and lignin content ($p<0.01$). Both treatments improved in sacco degradation of dry matter, organic matter and neutral detergent fibre of mustard straw. However, upon feeding, the effects were not apparent. This was because of 1) higher sodium intake on AHP treated diets which resulted in greater water consumption, thus increasing rate of passage of solid digesta and 2) wider straw to concentrate ratio in 1 AHPMS and 2 HPMS than that of UMS diet.

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