Effect of Feeding Chemically Treated Mustard (*Brassica campestris*) Straw on Rumen Fibre Degrading Enzymes in Sheep

S. Vaithiyanathan*, S. K. S. Raghuvanshi, A. S. Mishra, M. K. Tripathi, A. K. Misra
R. Prasad and R. C. Jakhmola
Central Sheep and Wool Research Institute, Avikanagar-304 501, India

ABSTRACT : The aim of the experiment was to study the changes in the activities of various rumen fibre degrading enzymes due to the feeding of chemically treated mustard (*Brassica campestris*) straw in sheep. Mustard straw (MS) (<5 cm particle size) was treated either with urea (4% (w/w), or with 2% sodium hydroxide (NaOH), or with alkaline hydrogen peroxide (2% NaOH and 1.5% hydrogen peroxide (H_2O_2)) and/or supplemented with 2% (w/w) urea. Seven maintenance type rations were prepared using MS (70 parts) with molasses (5 parts) and concentrate (25 parts). They were untreated MS (CMS), urea treated MS (UMS), urea supplemented MS (MS-US), alkali treated MS (AMS), alkali treated and urea supplemented MS (AMS-US), alkali H_2O_2 treated MS (AHMS) and alkali H_2O_2 treated and urea supplemented MS (AHMS-US). They were then compressed into a complete feed block with the help of block making machine. Forty two male hoggets of Malpura breed sheep were equally distributed into each treatment group and (were) offered feed and water *ad libitum*. At the end of 21 days of feeding trial, rumen liquor was collected through stomach tube from three animals in each group at 0 h, 4 h, 8 h, 12 h of post feeding. Results showed that the level of enzyme varied from 8.52 to 11.12, 40.85 to 50.37, 3.22 to 3.78, 2.09 to 2.77 and 31.44 to 44.24 units/100 ml SRL respectively for carboxymethyl cellulase (CMCase), α-amylase, microcrystalline cellulase (MCCase), filter paper (FP) degrading enzyme and α-glucosidase. Processing of MS affected the enzyme activities, in a way, that NaOH and AHP treatment significantly reduced CMCase and FP degrading enzyme. The effect of urea treatment showed an increase in the activity of MCCase and α-glucosidase. But the supplementation of urea increased the activity of CMCase, FP degrading enzyme and α-glucosidase. The CMCase, α-amylase, α-glucosidase activities were highest at 4hr whereas MCCase and FP degrading enzyme had maximum activities at 12 h post feeding Results suggested that MS might need longer time in the rumen for its effective degradation. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1610-1613)***

Key Words : Chemical Treatments, Mustard Straw, Rumen Fibre Degrading Enzyme, Sheep

INTRODUCTION

In arid and semi-arid condition, non-availability of sufficient quantity of feed and fodder necessitates the identification of newer sources of feeds. Mustard (*Brassica campestris*) straw, an agricultural waste is burnt in the field itself and not utilized in the animal feeding system due to its very poor quality. The straw contains very low amount of crude protein and large proportionof cell wall constituents (Mishra et al., 1996). The cellulose content is about 45%. But this vast amount of energy source is locked in the lignocellulose complex and remains unutilized due to lignin (Chaudhry, 1998). To improve the access of the rumen microbial hydrolytic enzyme to cellulose present inside the cell wall constituents, attempts are being made to disrupt the lignocellulose complex through physical/chemical/ biological means (Chaudhry, 1998; Misra et al., 1999; Mishra et al., 2000; Chaturvedi et al., 2002). The understanding of the status of ruminal microbial fibre degrading enzyme activity would reflect quantitatively the presence of fibre degrading microbes in the rumen (Silva et al., 1987) and the fibre degradation inside the rumen.

* Corresponding Author: S. Vaithiyanathan. Tel: +91-01437-220143, Fax: +91-01437-220163, E-mail: svaith@cswri.raj.nic.in
Received May 2, 2003; Accepted August 18, 2003

In this experiment, we intended to study the changes in the activities of various fibre degrading enzymes due to various chemical treatment of mustard straw (MS) in sheep.

MATERIALS AND METHODS

Animals, feeds and experimental procedure

Forty two male hoggets of Malpura breed with uniform bodyweight (12-18 months of age) were equally distributed in seven groups of six animals each, and (were) offered feed and water *ad libitum*. Chaffed MS (<5 cm particle size) was obtained from the farmer's field. It was treated either with urea (4% (w/w), or with 2% sodium hydroxide (NaOH), or with alkaline hydrogen peroxide (AHP) (2% NaOH and 1.5% hydrogen peroxide) and/or supplemented with urea (2% w/w). Seven maintenance type rations were prepared using MS (70 parts), molasses (5 parts) and concentrate (25 parts). They were untreated MS (CMS), urea treated MS (UMS), urea supplemented MS (MS-US), alkali treated MS (AMS), alkali treated and urea supplemented MS (AMS-US), alkali H_2O_2 treated MS (AHMS) and alkali H_2O_2 treated and urea supplemented MS (AHMS-US). These were then compressed into a complete feed block with the help of block making machine. Chemical constituents of diets were analyzed for dry matter and crude protein

Table 1. Chemical composition[1] (% DM) of complete feed blocks fed to sheep

Attribute[1]	Treatments							SEM
	CMS	UMS	MS-US	AMS	AMS-US	AHMS	AHMS-US	
Dry matter	94.8	95.3	95.8	94.6	94.9	95.3	95.1	0.09
Crude protein	11.9	16.8	15.6	12.0	14.7	12.6	14.5	0.44
NDF	49.6	51.1	51.8	54.1	49.4	53.8	52.6	0.44
ADF	34.1	37.0	33.8	38.0	38.8	39.7	39.6	0.53
Hemicellulose	15.5	14.2	18.0	16.1	10.5	14.4	13.0	0.52
Cellulose	23.2	26.3	24.5	27.7	24.5	30.4	29.5	0.57
Lignin	6.5	7.7	7.1	8.4	7.6	8.1	8.0	0.15
GE (Kcal/g)	4.51	4.26	4.19	4.55	4.20	4.62	4.38	0.051

[1] Means of triplicate analysis. MS=Mustard Straw, CMS=untreated MS, UMS=urea treated MS, MS-US=urea supplemented MS, AMS=alkali treated MS, AMS-US=alkali treated and urea supplemented MS, AHMS=alkali H_2O_2 treated MS, AHMS-US=alkali H_2O_2 treated and urea supplemented MS.

Table 2. Dry matter intake and digestibility in sheep fed complete feed block diets

Attribute	Treatments							SEM	P
	CMS	UMS	MS-US	AMS	AMS-US	AHMS	AHMS-US		
Dry matter intake									
g/day	883	885	827	943	797	876	950	25.7	NS
g/100kgBW	3.55	3.57	3.40	3.61	3.23	3.34	3.75	0.086	NS
g/kg$W^{0.75}$	79.3	79.6	75.5	81.6	70.7	75.6	84.2	2.06	NS
Dry matter digestibility coefficient	0.52	0.56	0.49	0.53	0.50	0.52	0.55	0.002	NS

MS=Mustard Straw, CMS=untreated MS , UMS=urea treated MS , MS-US=urea supplemented MS, AMS=alkali treated MS, AMS-US=alkali treated and urea supplemented MS, AHMS=alkali H_2O_2 treated MS, AHMS-US=alkali H_2O_2 treated and urea supplemented MS.

following AOAC (1995) procedure, neutral detergent fiber (NDF) was determined following the procedure of Van Soest et al. (1991), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the method described by Robertson and Van Soest (1981). Gross energy was estimated according to the method described in the manual of Gallenkamp bomb calorimeter (Gallenkamp, UK.). Chemical composition of diets is given in Table 1. The feeding trial was conducted for two month followed with a digestibility trial of 7 day duration. The rumen liquor was collected at 0 h, 4 h, 8 h and 12 h post-feeding at the end of 21 days of feeding trial through stomach tube and analyzed immediately for microbial fibre degrading enzyme activities.

Determination of ruminal fibre degrading enzyme activities

The extraction of enzyme from microbial biomass and the estimation of enzyme were followed the method as described by Agarwal et al. (2000a,b) with slight modification. Ten milliliter of strained rumen liquor (SRL) was centrifuged at 14,000 rpm for 20 min and the supernatant and the pellet were collected separately. The supernatant was used as enzyme source for extra cellular portion. The pellet containing microbial biomass (bacteria, fungi and protozoa) was suspended in a solution containing 5 ml 0.1 M phosphate buffer (pH 6.8), 2 ml of carbon tetra chloride and 2 ml of 0.4% lysozyme. The suspension was incubated for 3 h at 39°C and then centrifuged at 14,000 rpm for 20 min. The supernatant was collected and used as enzyme source for cellular portion. The activities of the enzyme viz. carboxy methyl cellulase (CMCase), α-amylase, microcrystalline cellulase (MCCase), filter paper (FP) degrading enzyme, α-glucosidase were determined separately for individual animal.

For the estimation of CMCase and α-amylase, the reaction mixture contained 0.1 M phosphate buffer (pH 6.8) 1 ml, carboxy methyl cellulose (1%) 0.5 ml, enzyme 0.5 ml, buffer 0.5 ml, soluble starch (1%) 0.25 ml, enzyme 0.25 ml and incubated for 60 and 30 min respectively. For the estimation of MCCase, the 1% substrate (microcrystalline cellulose) suspension in the buffer was incubated at 10°C for 48 h prior to the use in the enzyme assay. The 2 ml assay mixture containing 1 ml substrate suspension and 1 ml enzyme solution was incubated for 60 min, with intermittent shaking. For the estimation of FP degrading enzyme activities, the assay mixture containing 1 ml buffer, 50 mg whatman No.1 filter paper and 1ml enzyme was incubated of 60 min with intermittent shaking. In all above assays, the reaction was stopped by adding 3 ml of dinitrosalicylic acid solution. The reducing sugars liberated due to the enzyme activities were estimated by dinitrosalicylic acid reagent (Miller, 1959). The enzyme activities were expressed as μmole of reducing sugars produced per minute per ml under the assay conditions. In the estimation of α-glucosidase, the substrate was p-nitrophenol-β-D-glucopyranoside (PNPG) and the enzyme activity was determined by measuring the amount of p-nitrophenol released during incubation of substrate with enzyme. The assay mixture contained 0.1 ml enzyme and

0.9 ml PNPG (0.1%) solution and incubated for 10 min. The reaction was stopped by adding 1ml sodium carbonate, and the released p-nitrophenol was measured at 400 nm. In the entire enzyme assay, the incubation was done at 39°C and the enzyme solutions were kept at 4°C.

After estimating the activities of enzyme in the two portions, they were pooled for presentation and statistical analysis. Data were analyzed using SPSS Base 10.0 (SPSS software products, Marketing department, SPSS Inc. Chicago, IL, 60606-6307, USA).

RESULTS AND DISCUSSION

Dry matter intake of animals ranged from 827 g to 950 g per day amounting to 3.23 to 3.75% of body weight (Table 2) and considered optimum for adult sheep (Misra et al., 2000). Dry matter intake and its digestibility coefficients were similar among different treatments.

Overall results showed that the enzyme activities varied from 8.52 to 11.12, 40.85 to 50.37, 3.22 to 3.78, 2.09 to 2.77 and 31.44 to 44.24 units/100 ml SRL respectively for CMCase, α-amylase, MCCase, FP degrading enzyme and α-glucosidase (Table 3). The levels of enzyme activities presented in this study (represents for SRL) account for about 15% of total enzyme activities of the rumen content (Agarwal et al., 2000a). The activities of CMCase, α-amylase and FP degrading enzyme in the SRL of sheep fed with chemically treated MS containing complete feed block showed significant ($p<0.05$) variation between groups. The higher amount of α-amylase activity amongst other enzyme in this study was possibly due to the presence of easily digestible starch in the concentrate. The comparatively low level of MCCase and FP degrading enzyme activities suggest that the cellulose present in the MS seems to be in the crystalline nature and/or in the ligno-cellulose complex. Hence, the MS is not being utilized to meet the maintenance requirements of sheep (Mishra et al., 2000).

The levels of enzyme activities determined at different hours of post-feeding (Table 4) showed that there was a difference in the time required to attain the peak level. CMCase, α-amylase and α-glucosidase reached the peak level at 4 h post-feeding while the MCCase and FP degrading enzyme reached the peak level at 12 h post-feeding. These higher levels of MCCase and FP degrading enzyme activities at 12 h post feeding further suggest that a substantial amount of crystalline cellulose may be present in the straw, and they require longer time inside the rumen millieu for the rumen microbes to gain access to the crystalline cellulose vis-à-vis for its solubility.

The results on the effect of processing by NaOH/AHP treatment or no treatment showed significant variation in the enzyme activities. CMCase and FP degrading enzyme activities of 10.83 and 2.65 units/100 ml SRL, respectively were found to be significantly ($p<0.05$) higher in animals fed with untreated MS as compared to treated either with NaOH or AHP. However, there was little effect on α-

Table 3. Effect of feeding of chemically treated MS on rumen microbial enzyme in sheep

Groups	Enzyme units/100 ml SRL				
	CMCase	α-Amylase	MCCase	FP degrading enzyme	α-Glucosidase
CMS	10.55 ± 0.76^{ab}	49.84 ± 2.89^{ab}	3.74 ± 0.42	2.77 ± 0.17^{a}	36.38 ± 5.66
UMS	10.39 ± 0.51^{ab}	44.60 ± 3.22^{cd}	3.78 ± 0.28	2.48 ± 0.16^{bcd}	36.51 ± 6.09
MS-US	11.12 ± 0.77^{a}	50.29 ± 3.82^{a}	3.22 ± 0.19	2.53 ± 0.15^{ab}	44.24 ± 5.84
AMS	8.52 ± 0.36^{c}	45.43 ± 2.94^{bcd}	3.43 ± 0.28	2.09 ± 0.03^{f}	31.44 ± 6.02
AMS-US	9.87 ± 0.76^{b}	40.85 ± 3.58^{d}	3.36 ± 0.21	2.27 ± 0.11^{edf}	32.72 ± 5.64
AHMS	9.62 ± 0.63^{bc}	50.37 ± 4.15^{a}	3.29 ± 0.35	2.22 ± 0.10^{ef}	34.11 ± 5.84
AHMS-US	10.20 ± 0.63^{ab}	47.71 ± 3.73^{abc}	3.67 ± 0.41	2.30 ± 0.08^{cdef}	33.91 ± 3.93
CD	1.2356	4.5840		0.2160	

Superscripts with different letters in a column differ significantly at $p<0.05$. MS=Mustard Straw, CMS=untreated MS, UMS=urea treated MS, MS-US=urea supplemented MS, AMS=alkali treated MS, AMS-US=alkali treated and urea supplemented MS, AHMS=alkali H_2O_2 treated MS, AHMS-US=alkali H_2O_2 treated and urea supplemented MS, CMCase=carboxymethyl cellulase, MCCase=microcrystalline cellulase, FP degrading enzyme=filter paper degrading enzyme, CD=critical difference, SRL=strained rumen liquor.

Table 4. Effect of hours of post- feeding of chemically treated MS on rumen microbial enzyme in sheep

Hours	Enzyme units/100 ml SRL				
	CMCase	α-Amylase	MCCase	FP degrading enzyme	α-Glucosidase
0	9.27 ± 0.47^{b}	35.99 ± 1.57^{d}	3.16 ± 0.10^{bcd}	2.11 ± 0.04^{c}	20.45 ± 4.04^{c}
4	11.47 ± 0.41^{a}	60.26 ± 1.33^{a}	2.93 ± 0.10^{d}	2.37 ± 0.07^{ab}	50.28 ± 3.26^{a}
8	11.39 ± 0.34^{a}	51.19 ± 1.91^{b}	3.06 ± 0.09^{cd}	2.22 ± 0.04^{bc}	39.28 ± 2.82^{b}
12	8.03 ± 0.12^{c}	40.60 ± 1.15^{c}	4.85 ± 0.25^{a}	2.82 ± 0.13^{a}	32.46 ± 2.88^{bc}
CD	0.93	3.47	0.41	0.16	9.12

Superscripts with different letters in a column differ significantly at $p<0.05$. CMCase=carboxymethyl cellulase, MCCase=microcrystalline cellulase, FP degrading enzyme=filter paper degrading enzyme, CD=critical difference, SRL=strained rumen liquor.

amylase, MCCase, α-glucosidase activities due to processing. It has been reported that NaOH treatment of MS reduced titrable groups and residual phenolic in the MS (Chaturvedi et al., 2002). Yet, the feeding trial with NaOH/AHP treated straw did not reveal improvement in the ruminal fibre degrading enzyme activities. It suggests that release of phenolic acids into the rumen through breaking of lignocellulosic complex by NaOH and AHP treatment (Tarkow and Feist, 1969) of straw might have caused the inhibition of microbes/enzyme by forming complexes. Further, the reported effect of NaOH on swelling of crystalline cellulose (Klopfebstein, 1978) did not help in the improvement of activities of MCCase and FP degrading enzyme.

The effect of urea treatment/supplementation while preparing the complete feed block showed negligible effect on fibre degrading enzyme activities. Urea supplementation slightly improved those activities of CMCase, FP degrading enzyme and α-glucosidase activities while it decreased activities of α-amylase and MCCase. But the urea treatment decreased the activities of CMCase, α-amylase and FP degrading enzyme while it increased the activities of MCCase and α-glucosidase. However, the differences were found to be non-significant. The idea of urea treatment/ supplementation was to provide non-protein nitrogen source as well as to improve the nutrient quality of the straw to some extent. However, they did not influence much on the nutrient quality of straw as shown by the fibre degrading enzyme activities.

CONCLUSION

MS may need longer time for effective attachment of the rumen microbes, as indicated by the highest enzyme activities achieved after 4 h and 12 h of post-feeding, respectively for CMCase, α-amylase, α-glucosidase and MCCase and FP degrading enzyme. The chemical treatment of MS did not have any major effect on activities of fibre degrading enzyme.

ACKNOWLEDGEMENTS

Authors wish to thank the Director for providing the facilities to carry out this experiment. Funding provided by NATP (NATP/AED (arid)/PAL028/99) is gratefully acknowledged.

REFERENCES

Agarwal, N. 2000. Estimation of fibre degrading enzyme. In: Feed microbiology. (Ed L. C. Chaudhary, N. Agarwal, D. N. Kamra amd D. K. Agarwal). CAS Animal Nutrition, IVRI, Izatnagar, India.

Agarwal, N., I. Agarwal, D. N. Kamra and L. C. Chaudhary. 2000. Diurnal variations in the activities of hydrolytic enzyme in different fractions of rumen content of Murrah Buffalo. J. Appl. Anim. Res. 18:73-80.

AOAC. 1995. Official methods of analysis. 16th ed., Association of Official Analytical Chemists Washington, DC.

Chaturvedi, O. H., A. Santra, A. S. Mishra, R. Prasad and R. C. Jakhmola. 1999. Effect of soaking medium and hours of soaking in nutritive value of mustard (*Brassica campestris*) straw. Indian J. Anim. Sci. 69:739-741.

Chaturvedi, O. H., A. Santra, A. S. Mishra, R. Prasad, A. K. Misra, S. Vaithiyanathan and R. C. Jakhmola. 2002. Titrable groups and soluble phenolics compounds in untreated and treated mustard (*Brassica campestris*) straw and their relation with cell wall constituents and dry matter disappearance. Indian J. Anim. Sci.71:69-73.

Chaudhry, A. S. 1998. Chemical and biological procedures to upgrade cereal straw for ruminants. Nutr. Abst and Rev. (Series B). 68:319-331.

Klopfenstein, T. J. 1978. Chemical treatment of crop residues. J. Anim. Sci. 46:841-848.

Miller, G. L. 1959. Modified DNS method for reducing sugars. Anal. Chem. 31:426-428.

Mishra, A. S., O. H. Chaturvedi, A. K. Misra and S. A. Karim. 2000. Comparative utilization of urea and fungal treated MS by sheep. Indian J. Anim. Sci. 70:854-856.

Mishra, A. S., O. H. Chaturvedi, A. K. Misra and S. A. Karim. 1996. A note on chemical composition of untreated and urea treated mustard (*Brassica campestris*) straw. Indian J. Small Rumi. 2:49-51.

Misra, A. K., O. H. Chaturvedi, A. S. Mishra and S. A. Karim. 1999. Effect of urea treatment on the cell wall constituents and dry matter digestibility of mustard (*Brassica campestris*) straw. Indian J. Anim. Sci. 69:1180-1182.

Misra, A. K., S. A. Karim, D. L. Verma, A. S. Mishra and M. K. Tripathi. 2000. Nutrient intake, its utilization, rumen fermentation pattern and blood bio-chemical constituents of sheep fed urea treated mustard (*Brassica campestris*) straw. Asian-Aus. J. Anim. Sci. 13:1674-1680.

Robertson, J. B. and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Cornell University, Ithaca, New York.

Silva, A. T., R. J. Wallace and E. R. Orskov. 1987. Use of particle bound microbial activity to predict the rate and extent of fibre degradation: A review. Br. J. Nut. 57:407-415.

Tarkow, H. and W. L. Feist. 1969. A mechanism for improving the digestibility of lignocellulose materials with dilute alkali and liquid ammonia. J. Anim. Sci. 35:451-357.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. Symposium: Carbohydrate methodology, metabolism and nutritional implications in dairy cattle. J. Dairy Sci. 74:3583-3597.