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In situ Ruminal Degradation and *in vitro* Gas Production of Chemically Treated Sesame Stover

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Abstract: This study was conducted to determine the effect of chemical treatment on fermentative activity and nutritive value of Sesame stover by *in situ* and *in vitro* gas production techniques. Treatments were untreated Sesame Stover (SS), SS+Sulphuric Acid (SSA), SS+Urea (SSU), SS+NaOH (SSN) and SS+Urea+NaOH (SSUN). Nylon bags (9×17 cm) each containing 5 g sample (2 mm screen) were incubated in the rumen of fistulated sheep for 0.0, 4, 8, 16, 24, 36, 48, 72 and 96 h to determine the ruminal disappearance. Cumulatve gas production was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h of incubation and the equation of $P = A (1-e^{-ct})$ was used to describe the kinetics of gas production. Results showed that NDF concentration of the samples was significantly ($p<0.05$) decreased by sulphuric acid treatment also the urea caused a significant ($p<0.05$) increase in CP content of sesame stover. Dry matter ruminal disappearance was significantly ($p<0.05$) increased by NaOH treatment. Moreover, the *in situ* quickly degradable fraction of CP was increased (0.625±024) when urea applied. The b fraction and rate of gas production were significantly ($p<0.05$) increased (72.32±2.88) and decreased (0.029±0.002), respectively by adding the NaOH to SS.

Key words: Sesame stover, chemical treatment, *in vitro* gas production, *in situ*, seed oil, Iran

INTRODUCTION

Sesame (*Sesame indicun* L.) is an annual plant, broadleaf that cultivated after wheat in arid and semi-arid regions of Iran for its seed oil and extensively used for medicinal and food purposes. Sesame stover is the most abundance residual of Sesame cultivation in Iran and traditionally used as a basal feed in ruminants (Mesgaran *et al*., 2009). A number of studies (Weixian *et al*., 1995) have also proven that crop residues are low in available nutrients taking longer lag time and slow in rate of microbial fermentation. These characteristics of straw limit its intake and digestibility; thereby hamper the productivity of farm animals.

Treatment of straw with chemicals like ammonia and ammonia precursors as urea to increase the digestibility is used in many parts of the world. Such treatments generally increase both the rate and extent of fiber digestion in the rumen which leads to higher energy value of the treated material as well as to a higher intake (Celik *et al*., 2003). There are a number of chemical reactions taking placed during alkali treatment. Saponification of ester linkages between acetic acid and phenolic acids, polysaccharides and/or lignin as well as such linkages between uronic acid residues of xylan in hemicelluloses and lignin would be expected (Ribeiro, 1991; Chaudhry, 1998) reported significant increase of *in vitro* dry matter digestibility of wheat straw when it was treated with sodium hydroxide. The processing of fibrous feed with urea leads to the production of ammonia which caused an increase in the rumen microbiota accessing to the cell wall polysaccharides as well as favoring the degradative action of the bacterial and fungal enzymes in the rumen (Horn *et al*., 1989). In addition, urea increases the nutritional value of fibrous materials by making more digestible cellulose and hemicellulose available (Silva and Qrskov, 1988). This creates favorable condition in the rumen for the development of the cellulytic bacteria which degrade the plant cell wall (Silva and Qrskov, 1988). The aims of the present study were to evaluate the effect of chemical treatment of sesame stover with NaOH and urea or sulphuric acid on chemical composition, *in vitro* gas production and *in situ* ruminal degradation parameters of Dry Mater (DM) and Crude Protein (CP).

MATERIALS AND METHODS

Experimental samples and chemical analysis: Sesame Stover (SS) was obtained from the Iranian plant varieties adapted to grow in semi-arid condition. The samples were

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manually chopped (5 cm length) and used as untreated or treated with sulphuric acid (SSA, 2 mL/100 g DM), urea (SSU, 3 g/100 g DM), NaOH (SSN, 4 g/100 g DM) or both NaOH and urea [SSUN, NaOH as 4 g/100 g DM was sprayed on the stover and kept for 48 h then urea (3 g/100 g of initial DM) was added]. Treated SS were ensiled under un-aerobic condition for 4 weeks. Feed samples were dried at 60°C in oven dryer for 48 h and then ground to pass through a 2 mm screen. Samples were analyzed for Crude Protein (CP) (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden), Organic Matter (OM) and ash. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined using the method of Van Soest *et al*. (1991).

Ruminal degradability: *In situ* degradation of DM and CP was studied following the nylon bag technique described by Mehrez and Orskov (1977). About 4 sheep (49.6±2 kg) fitted with ruminal fistulae were used. The animals were fed with 1.5 kg DM alfalfa hay and 0.4 kg DM concentrates (165 g CP kg^{-1} DM) per head per day at 8.00 and 17:00 h. Approximately, 5 g DM of each sample (10 bags per each feed) was placed in a polyester bag (9×17 cm; pore size of 52 μm) and incubated in the rumen for 0.0 (bags were washed with cold tap water) 2, 4, 8, 16, 24, 36, 48, 72 and 96 h. After removal from the rumen, bags were washed with tap water and subsequently dried using oven dryer (60°C, 48 h) then weighed to determine DM disappearance. Crude protein concentration of un-incubated and rumen incubated samples were then determined.

***In vitro* gas production:** *In vitro* gas production was determined according to Menke and Steingass (1988) procedures. Rumen content was collected from 4 sheep (49.6±2 kg) fitted with ruminal fistulae. The animals were fed with 1.5 kg DM alfalfa hay and 0.4 kg DM concentrate (165 g CP kg^{-1} DM) per head per day at 8.00 and 17.00 h. Rumen content was strained through 4 layers of cheesecloth. The laboratory handling of rumen liquor was carried out under a continuous flow of CO_2. About 200 mg of each sample was measured into each syringe (4 replicates per each sample). Then, it was filled with 30 mL of medium consisting of 10 mL of rumen fluid and 20 mL of buffer solution as described by Menke and Steingass (1988). Four blank syringes were also provided. The syringes were placed in an incubator (38.6°C). Gas production was recorded after 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96 h. of incubation. Total gas values of the incubated samples were corrected for blank incubation.

Calculations and statistical analysis: The data from *in situ* studies were fitted into an exponential model $\{p = a+b(1 - e^{-ct})\}$ of Orskov *et al*. (1980) by using the Maximum Likelihood Programme to obtain estimates of a, b and c for each sample in each sheep. Here, a is quickly degradable fraction, b is slowly degradable fraction, c is constant fractional degradation rate and t is the hours of incubation.

Gas production in each syringe was measured directly at 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation:

$$Y = a + b\,(1-e^{-ct})$$

Where:

- b = The gas production from the fermentable fraction (mL)
- c = The gas production rate constant (mL h^{-1})
- t = The incubation time (h)
- y = The gas production at time t

The values of Organic Matter Digestibility (OMD) and Metabolisable Energy (ME) of the samples were calculated by the equation of Menke and Steingass (1988):

$$\text{OMD (g/100 g DM)} = 14.88+0.889\times GP+0.45\times CP+0.0651\times XA$$

$$\text{ME (MJ kg}^{-1}) = 2.20+0.136\ GP+0.057\ CP+0.0029\ CP^2$$

Where:

- GP = Total 24 h net gas production (mL/ 200 mg)
- CP = Crude Protein (%)
- X = Ash content (%)

Data were statistically analyzed using GLM of SAS.

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of the untreated and the chemically treated of sesame stover are shown in Table 1. Chemical composition of the samples was effected by the chemical treatment. Neutral Detergent Fiber (NDF) was significantly ($p<0.05$) decreased by sulphuric acid treatment. The main difference between untreated and treated sesame stover was observed in crude protein content as a result of the addition of urea. Urea caused a significant ($p<0.001$) increase in CP content of SS than the other treatments. Vadiveloo (2003) showed that the treatment Malaysian rice straw with 4% urea increased CP content from (6.0%) for the untreated to (9.3%) for treated rice straw. This was agreed with the finding of Al-Shami and Al-Sultan (2006) who reported crude protein content was increased to 8% for urea treated straw.

Table 1: Chemical composition of sesame stover as untreated or treated treated with NaOH, urea or sulphuric acid

	Treatments*						
Items	SS	SSA	SSU	SSN	SSUN	SEM	p-value
NDF	75.7^{a}	66.6^{d}	71.7^{b}	69.9^{c}	73.6^{ab}	0.85	<0.05
ADF	46.2	43.8	45.4	45.4	45.8	0.55	NS
CP	6.7^{c}	6.7^{c}	11.8^{a}	6.3^{c}	8.9^{b}	0.13	<0.05
OM	96.4^{ab}	96.1^{b}	94.4^{d}	96.5^{a}	95.7^{c}	0.09	<0.05

*SS: Sesame Stovet, SSA: Sesame Stover+Sulphuric Acid, SSU: Sesame Stover+Urea, SSN: Sesame Stover+NaOH, SSUN: Sesame Stover+NaOH+Urea; Means within a row with different superscripts differed at $p<0.05$

Table 2: *In situ* ruminal degradation of Dry Matter (DM) and Crude Protein (CP) of sesame stover as untreated or treated with NaOH, urea and sulphuric acid (mean±SE)

	DM			CP			
Samples*	a	b	c	a	b	c	p-value
SS	0.22±0.015^{a}	0.36±0.01^{a}	0.07±0.010^{a}	0.30±0.028^{a}	0.46±0.030^{a}	0.10±0.017^{a}	<0.05
SSA	0.28±0.01^{ab}	0.29±0.01^{b}	0.06±0.008^{b}	0.42±0.040^{b}	0.37±0.051^{b}	0.04±0.017^{bc}	<0.05
SSU	0.21±0.03^{ab}	0.35±0.03^{ac}	0.05±0.016^{bc}	0.63±0.024^{c}	0.21±0.032^{c}	0.05±0.021^{b}	<0.05
SSN	0.30±0.019^{c}	0.38±0.02^{d}	0.05±0.008^{cd}	0.44±0.032^{b}	0.35±0.052^{d}	0.038±0.014^{d}	<0.05
SSUN	0.23±0.024^{b}	0.36±0.02^{ac}	0.05±0.011^{d}	0.53±0.026^{d}	0.25±0.031^{e}	0.049±0.017^{c}	<0.05

*SS (Sesame Stovet), SSA (Sesame Stover+sulphuric acid), SSU (Sesame Stover+Urea), SSN (Sesame Stover+NaOH), SSUN (Sesame Stover+NaOH+Urea); Means within a row with different superscripts differed at $p<0.05$

Table 3: Gas production parameters of sesame stover treated with NaOH, urea or sulphuric acid (mean±SE)

	Treatment*					
Parameters	SS	SSA	SSU	SSN	SSUN	p-value
b (mL/0.2 g)	59±2.3^{a}	51±2.5^{b}	60±2.8^{ac}	72±2.9^{d}	62±2.5^{c}	<0.05
c (/h)	0.04±0.004^{a}	0.04±0.006^{a}	0.03±0.003^{b}	0.02±0.002^{c}	0.03±0.003^{b}	<0.05
ME (MJ kg^{-1})	7.8	7.23	8.02	7.98	7.78	NS
OMD (g/100 g DM)	54.48	50.6	56.78	56.02	52.58	NS

*SS: Sesame Stovet, SSA: Sesame Stover+Sulphuric Acid, SSU: Sesame Stover+Urea, SSN: Sesame Stover+NaOH, SSUN: Sesame Stover+NaOH+Urea; Means within a row with different superscripts differed at $p<0.05$

***In situ* degradation:** The values of *in situ* ruminal degradation coefficients of DM and CP of the experimental samples are shown in Table 2. Results showed that the chemical treatments applied in the present study did alter the degradation coefficients of SS. Quickly and slowly degradable fractions for DM and CP were significantly affected by the treatments ($p<0.05$). DM degradation rates (both a and b fractions) of SSUN was significantly ($p<0.05$) higher than those of the other treatments.

These results confirmed the finding of Chaudhry (1998) who observed that the digestibility of DM of wheat straw was increased by treatment with NaOH. Chaudhry (2000) described that the treatment with NaOH+$Ca(OH)_2$ caused the greatest disappearance of DM, OM and NDF. In addition, he showed that the quickly degradable fractions of DM, OM and NDF were greatest for NaOH+$Ca(OH)_2$ treated straw compared with the untreated.

It may be due to the greater effect of NaOH on the lignin molecule to release phenolics to inhibit rumen microbes and consequently rumen degradation of straw (Marvin *et al*., 1996). Results of the present study demonstrated that the slowly degradable fraction of DM was the greatest for the sample treated by sulphuric acid compared with those of the untreated or the other samples. The degradation constant rate of the treated samples was significantly higher than that of the untreated sample ($p<0.05$). In the present study the quickly degradable fraction (a) of CP was significantly ($p<0.05$) increased when urea was applied. Urea affects the cuticle wax to enhance the digestibility of parenchyma tissue through swelling of the wall of parenchyma cells and cracking of the wall of vascular tubes. Therefore, urea causes both physical and chemical changes in fibrous feed tissues and cell walls (Shen *et al*., 1999) facilitating degradation by rumen bacteria and an increase in degradability especially of the leaf fraction (Vadiveloo, 2000). Prasad *et al*. (1998) observed that DM intake was significantly higher in urea treated rice straw than on untreated straw which might be due to improved palatability.

***In vitro* gas production:** *In vitro* gas production parameters (b and c), OMD and ME values of the experimental samples are shown in Table 3. The b fraction of gas production was significantly ($p<0.05$) higher when seasame stover was treated with NaOH than those of the others. NaOH caused a significant decrease in c fraction of SS compared with the other treatments ($p<0.05$). While there was an increase in b fraction of gas production of SS when was treated by NaOH ($p<0.05$). The present effect of

NaOH on gas production parameters of sesame stover confirmed the results of Liu *et al*. (2002) who found that NaOH improved gas production of rice straw.

Chaji *et al*. (2010) observed that processing sugarcane pith with high steam+NaOH caused an increase in gas produced parameters (b and c). They described that treating sugarcane pith with NaOH had the highest cell wall degradability. Treatment of wheat straw with NaOH may have removed some chemical linkages within hemicelluloses and thus enhanced their solubility in detergent solutions (Chaudhry, 1998). The reduction in NDF of wheat straw by NaOH treatment was mainly due to decrease hemicelluloses content (Haddad *et al*., 1995).

It was reported that an alkali solution solubilizes the inhibitory phenolic compounds and hemicelluloses (Chen *et al*., 2007). Gould (1984) proposed that alkali react with lignocelluloses to yield partially delignified products that are highly susceptible to enzymatic and microbial attach, about 20% loss in the initial DM following sodium hydroxide treatment. Kamalak *et al*. (2005) observed that gas produced at all incubation times of corn silage was significantly higher than those of wheat straw and barley straw. Therefore, the estimated parameters (c and b) of corn silage were significantly higher than the others. They also expressed that the cell wall content (NDF and ADF) had negatively correlated with gas production at all incubation times.

The negative correlation between gas production and the cell wall content might be a result of the reduction of microbial activity from increasingly adverse environmental conditions as the incubation time progresses. Under present experimental condition, both Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) was not affected by the chemical treatments applied (Table 3). These results did not confirm the finding of Chaji *et al*. (2010). They showed that high steam and NaOH caused to increase *in vitro* cell wall degradation, OMD and ME of sugarcane pith.

CONCLUSION

In conclusion, present results indicate that chemical treatment such as sodium hydroxide is highly effective chemical treatment for improving degradability of the sesame stover. In addition, sodium hydroxide cause to enhance the fermentability of the fibrous feed evaluated. On the other hand, urea applied at the present rate to treat the seame stover creat a suitable condition to improve the ruminal degradation of CP. Therefore, it has been suggested that sodium hydroxide and urea might use as appropriate agents for improving digestion and nutrition value of sesame stover.

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