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Effect of Chemical Treatment of Sesame Stover with NaOH and Urea on Chemical Composition and *In vivo* Rumen Digestion in Sheep

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Abstract

This study tested the effect of urea, urea sodium hydroxide (NaOH) and NaOH + urea on chemical composition and total tract nutrition in sheep. Treated stovers were prepared by mixing stovers with aqueous solution of urea (SU, 3g in 100 ml water /100 g DM), NaOH (SN, 4g in 100 ml water /100g DM) and urea + NaOH (SUN). Five male Balouchi sheep (30 ±1.4 kg) were used in a change-over design. *In vivo* experimental diets were as follows: alfalfa hay (AH), alfalfa hay + S (AHS), alfalfa hay + SU (AH SU), alfalfa hay + SN (AHSN) and alfalfa hay + SUN (AHSUN). The NDF content significantly ($P < 0.05$) decreased when sesame stover was treated by urea and NaOH. However, the change in ADF and OM was not significant ($P > 0.05$) in urea and NaOH. The urea caused a significant ($P < 0.05$) increase in CP content of sesame stover. The digestibility coefficients of DM, OM, NDF and ADF increased with chemically treatment of sesame stover. Furthermore, rumen pH was not affected by the chemical treatment, while rumen NH_3- N ($P < 0.05$) concentration was enhanced by urea + NaOH treatment. These results suggest that digestibility of DM, OM, CP, NDF and ADF could be improved by chemical treatment with urea and NaOH.

Keywords: *in vivo* digestibility, NaOH, sesame stover, urea

Introduction

Sesame (*Sesamum indicum* L.) is an erect annual plant of numerous types and varieties belonging to the *Pedaliacea* family. It is cultivated since antiquity for its seed, used as food and flavoring and from which prized oil is extracted. Depending on conditions, varieties grow from about 0.5 to 2. 5 m tall; some have branches, others do not. One to three flowers appear on the leaf axils. The seed capsules opened when dry, allowing the seed to scatter (Rahnama and Bakhshandeh, 2006). Sesame stover is the most abundance residual of sesame cultivation in semi- arid countries and traditionally used as a basal feed in ruminants (Danesh Mesgaran *et al.,* 2010). The quantity and quality of available feedstuffs are major factors influencing productivity of ruminants in many parts of the word, especially regions with high animal numbers (Prasad *et al.,* 1993).

Ruminants in such areas depend largely on crop residues at least during the long dry period of year for maintenance as well as production of meat, milk and skin. However, animal performance with such feedstuffs can be poor due to low voluntary intake and digestibility, which result from low protein concentration and high level of indigestible or slowly degradable fiber (Prasad *et al.,* 1993). Various physical, chemical and biological treatments have been used to improve utilization of low quality forage such as crop residues (Abebe *et al.,* 2004). The most popular alkali for treatment has been sodium hydroxide, but its use is related with health hazards. In parts of the world where small farms predominate, treating with urea solution followed by a period of storage under air- tight condition may be more practical (Abebe *et al.,* 2004). 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 accessible (Silva and Qrskov, 1988). Although several studies reported improving in nutritive value of stover due to alkali and urea treatment, most studies have concentrated on determining intake and digestibility (Sahoo *et al.,* 2002; Silva and Qrskov, 1988). There is, however, little work done on changes in the ruminal environment. Therefore, the aim of the present study was to evaluate the effect of chemically treatment of sesame stover with NaOH and urea on chemical composition and in vivo total tract nutrient digestion in sheep.

Materials and methods

Sesame plant was harvested by hand cutting from Jovein farms (Khorasan Razavi Province, Iran) which is located at 56°30' North latitude and 36°15' west longitude. The climate of the area is described as semi-arid with average annual rainfall of 218 mm and mean temperature of 16.7°C. Sesame stover was collected after sun-drying and separate seeds of sesame plant, and then chopped (5cm lengths). The seasame stover was treated by aqueous solution of urea (SU, 3g in 100 ml water /100 g DM), NaOH (SN, 4g in 100 ml water /100g DM) and urea + NaOH [SUN, NaOH as 4g in 100 ml water/ 100g DM was sprayed on the samples and kept for 48h, then urea (3g in 100 ml water/100g of initial DM) was added]. The chemically treated stovers were filled into plastic bags with 0.5 mm thickness, then tied up and kept at room temperature on cemented floor.

After 30 days storage, silages were opened. The DM of silages was determined by drying the samples at 60°C in air forced- dry oven (Memmert 854) for 48 h. After drying, the samples were ground through a 1- mm screen (Cyclotec 1883; Sample Mill) for chemical analyses. Five male Balouchi sheep (30±1.4 kg) were used in a Change-over design. Sheep were fitted with ruminal fistula and confined individually in metabolic cages (1.5 m^2), with fresh water and feed available at all time. Before the beginning of the experiment, animals were drenched with albendazole to remove endoparasites. Total daily feed was offered in two equal portions during the day (07.00 and 17.00). Feed offered was based on the intake of previous day plus a 10% additional, in order to reduce the selection of feed components by animals. Experimental diets were: alfalfa hay (AH), alfalfa hay + S (AHS), alfalfa hay + SU (AHSU), alfalfa hay + SN (AHSN) and alfalfa hay + SUN (AHSUN); also the ratio alfalfa hay to silage was kept at 1:1 based on the dry matter.

There were 5 experimental periods (21-d), including a 14-d adaption and a 7-d collection period. Representative 100 g samples of each ingredients of the experimental diets were collected at the beginning of each period and stored (-25°C). Total faeces output of each sheep was collected from d 15 to d 21. After each individual collection, faces were weighed, homogenized, and only 100 g/kg of each treatment was kept frozen for apparent digestibility of Dry matter, Organic matter, Crude protein, Neutral detergent fiber and Acid detergent fiber. The apparent digestibility (AD) of dry matter and nutrients were calculated as follows: AD = [(ingested nutrient – nutrient found in faeces)/ingested nutrient].

Ruminal fluid was collected on the last day of each experimental period to determined ruminal pH and ammonia-N concentrations. Times of pH evaluation were 00:00 (before the morning feeding) and 00:15, 00:30, 00:45, 01:00, 01:15, 01:30, 02:00, 02:30, 03:00, 04:00, 05:00, 06:00, 07:00 and 08:00 after the morning feeding. Fluid content was obtained via the rumen fistula, and filtered through four layers of cheese cloth. Digesta pH was measured with a pH meter (Metrohm 691) immediately after obtaining the rumen content samples. In addition, 5 ml of rumen fluid obtained at times 0.0, 02:00, 04:00 and 06:00 after the morning feeding was transmitted into tubes containing 1 ml of 1 *N* HCl for determining ammonia-N (Weatherburn, 1967).

Feed (*alfalfa* hay, untreated and treated sesame stover) and faecal samples were dried for 48h at 60°C in air forced-dry oven (Memmert 854), milled through a 1.0 mm screen (Cyclotec 1883; Sample Mill). Samples were analysed for Kjeldahl N (AOAC, 1990) and crude protein was calculated as Kjeldahl N×6.25; DM, OM and fibrous component contents were determined according to methods described by Van Soest *et al.* (1991). No sulfite and heat stable amylase were included in procedure for neutral detergent fiber. Both neutral and acid detergent fibers were excluded of residual ash and expressed as NDF and ADF, respectively.

Data on chemical composition was subjected to a statistical analysis using the GLM procedure of SAS (9.1), based on the statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Yij is the general observation on chemical composition, μ is the general mean, Ti is the effect of treatments and eij is the standard error term.

Apparent digestibility data were analysed using SAS system by means of change over design by following statistical model:

$$Y_{ijk} = \mu + T_i + A_j (P_k) + e_{ijk}$$

where Yijk = observation of the effect of treatment i in period k, the animal j, μ= overall mean, Ti = effect of treatment i; i = 1 (AH), 2 = (AHS), 3 (AHSU), 4 (AHSN) and 5 (AHSUN); Aj = random effect of animal j (j = 5 animals); Pk = effect of period k (k = 5 periods); and eijk = random error associated with each observation. Data of ruminal pH and NH3- N concentration were analysed using the MIXED procedure of SAS with a repeated measures statement. The model included treatment, time and the treatment × time intraction. The Least squares means procedure (LSMEANS) was used to differentiate between treatment means at each time of rumen at a significance level of 5%.

Results and discussions

The chemical composition of the silages as untreated or chemically treated is presented in Tab. 1. The chemical composition (% DM) of alfalfa hay was including: DM, 89.6; OM, 96.7; CP, 17.0.4; NDF, 54.0; ADF, 38.0. Results of this study demonstrated that the concentration of CP and NDF was statistically affected by the ensiling procedure. By using urea, the content of CP was considerably higher ($p<0.05$) in ensiled sesame stover compared with those of the untreated or NaOH treated samples. Data also indicated that the concentration of NDF was significantly decreased when sesame stover was chemically treated by urea or NaOH ($P<0.05$). However, no significant ($p>0.05$) effect was found when concentration of ADF was considered.

Mean (±SE) DM, OM, NDF, ADF and CP apparent digestibilities are presented in Tab. 2. Results of the present experiment indicated that DM digestibility was significantly increased (P<0.05) by chemical treatment than untreated. The digestibility coefficients of NDF and ADF were significantly (P<0.05) higher in AHSU, AHSN and AHSUN than to AHS. However, OM digestibility was not affected by chemical treatment. In addition, data were showed that CP digestibility of the silages was significantly (P<0.05) enhanced by urea + NaOH (AHSUN) compared to other treatments.

Mean (± SE) of pH and ammonia-N concentration are given in Tab. 3. Ruminal fluid pH was similar among treated stover and was not affected by chemical treatment (P>0.05). Ruminal fluid pH decreased within 2 h of the morning feeding and then gradually increased, but significant effect of treatment × time was not observed (Fig. 1). Rumen ammonia-N concentration was significantly increased when sheep was fed AHSUN (P<0.05) compared with those of the other groups. In addition, rumen ammonia-N concentration indicated significant effect of treatment × time (Fig. 2).

Present results indicate that the content of NDF decrease with applying NaOH to make sesame stover silage (Tab. 1). The chemical compounds used might remove some linkages within hemicelluloses and thus enhanced their solubility in detergent solutions (Chaudhry, 1998). Similar losses due to NaOH (SN) have already been demonstrated by Mishra *et al.* (2000). Their investigation showed that NDF content decreased significantly in treatments where Mustard straw was soaked in solution containing either 2% NaOH or 2% NaOH + 1.5% H_2O_2. Urea treatment of seasame stover resulted in increase in crude protein (CP) about 2 times compared with the untreated stover. Similar results were found by Oji *et al.* (2007).

They determined that aqueous NH3 and urea were more effective in increasing protein content in maize stalks and husks than cobs.

The increases in digestibilities of the nutrients achieved by chemically treatments of sesame stover in present study (Tab. 2) were presumably due to the increased susceptibility of structural carbohydrates to ruminal fermentation (Chaudhry, 1998). Dry matter, NDF and ADF digestibilities in the current research were increased by both applying urea and NaOH treatments. These findings are consistent with Chaudhry (1998) who observed DM digestibility of wheat straw was enhanced by alkali treatment. Sahoo *et al.* (2002) showed that digestibility of NDF and ADF wheat straw increased when straw was treated by 3% urea + 3% calcium. They also described that it might be due to cleavage of linkages between lignin and hemicellulose

Alkali treatment causes swelling and changes in the crystalline structure of cellulose (Guggolz *et al.*, 1971; Klopfenstein, 1978) and makes it susceptible to ruminal microbial degradation. Removal or conversion of phenolic acids was another possibility which increased digestibility (Akin *et al.*, 1983).

The increase in CP digestibility with urea + NaOH treatment in the present study is consistent with Prasd *et*

Tab. 1. Dry matter and chemical composition (% DM) of sesame stover as untreated or chemically treated with NaOH and/or urea

Compound	Treatments[1]				SE	Probability
	S	SU	SN	SUN		
Dry matter	88.25	40.35	40.32	40.73	ND	-
Organic matter	96.40	96.21	96.31	96.33	0.248	0.142
Crude protein	6.70^c	12.61^a	6.64^c	11.27^b	0.206	***
NDF	75.75^a	62.35^b	54.35^c	59.80^b	0.87	**
ADF	46.20	43.65	44.75	45.35	0.45	0.152

Means in the same row with different letters (a, b, c) differ (P<0.05).
SE: standard error of the mean.
ND: not determined.
NDF; Neutral detergent fiber, ADF; Acid detergent fiber.
[1]S: sesame stover. SU: sesame stover + urea. SN: sesame stover + NaOH and SUN: sesame stover + NaOH + urea

Tab. 2. In vivo nutrient digestibilities (% DM) of experimental diets consisted of alfalfa hay plus sesame stover as untreated or treated with NaOH and/or urea in sheep

Compound	Treatments[1]					SE	Probability
	AH	AHS	AHSU	AHSN	AHSUN		
Dry matter	57.58^{bc}	53.33^c	64.28^{ab}	68.84^a	62.72^{ab}	2.70	**
Organic matter	64.13	64.32	64.65	66.43	63.43	1.15	***
Crude protein	72.01^b	70.01^b	73.39^b	71.97^b	77.76^a	1.38	***
NDF	59.90^{ab}	55.99^b	61.08^a	63.74^a	61.74^a	1.56	*
ADF	59.10^{ab}	57.0^b	59.11^{ab}	61.04^a	60.58^a	0.68	**

Means in the same row with different letters (a, b, c) differ (P<0.05)
SE: standard error of the mean.
NDF; neutral detergent fiber, ADF; acid detergent fiber.
[1]Treatments are: AH; alfalfa hay, AHS; sesame stover + alfafa, AHSU; sesame stover treated with urea 3% + alfalfa, AHSN; sesame stover treated with NaOH 4% + alfalfa and AHSUN; sesame stover treated with NaOH 4% and urea 3% + alfalfa.

al. (1998), who observed the digestibility coefficient crude protein was higher for the urea-treated rice straw than for the untreated straw. It might be because of conversion urea to NH_3 in during ensiling (Ramalho, 1991).

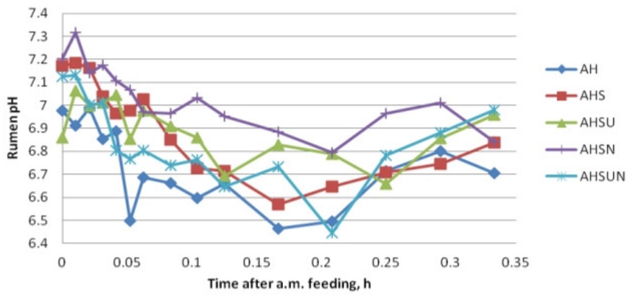

Fig. 1. Effects sesame stover treated with NaOH or urea pluss alfalfa hay on ruminal pH in sheep. Effect of Treatment x Time, p >0.668

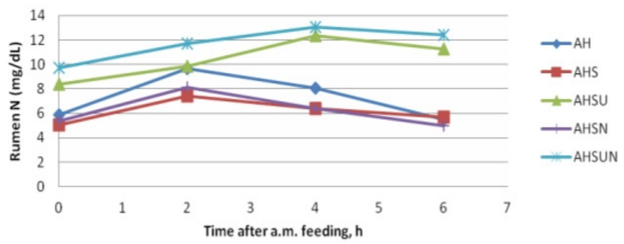

Fig 2. Effects sesame stover treated with NaOH or urea pluss alfalfa hay on ruminal ammonia concentration in sheep. Effect of Treatment x Time, p<0.0001

Goto *et al.* (1993) reported that ammonia treatment cause an increase in the ability of NH_3 to form an ammonia-N cellulose complex and to decline cellulose crystallinity (Isogai and Usauda, 1992). Goto and Yokoe (1996) argued that a more rapid fragmentation of ingested treated material would increased the surface area available for microbial attack and the rate of breakdown would increase the rate of passage of treated straw through the digestive tract.

In this experiment, Ruminal fluid pH was not affected by chemical treatment (Tab. 3). However, it was relatively high, which probably was related to adequate fiber content of all diets. Ruminal NH3 - N concentration is an important N source for microbial protein synthesis (Chamberlain and Thomas, 1979). Concentration of ammonia-N in ruminal fluid is a function of ammonia production (deamination of amino acids, intensified when energy is limited; Russell *et al.,* 1983), ammonia uptake by the ruminal microorganisms and diffusion through the rumen wall.

In the present study the NH3-N concentration ranged from 6.12 to 12.85 mg/ dl and was higher than that of 5 mg/ dl required for optimal microbial protein synthesis (Satter and Slyter, 1974). Also, the increase in the rumen ammonia-N concentration observed in sheep fed urea and urea + NaOH treated stover, was presumably a consequence of decrease in available energy and also most likely enhance protein digestibility. These findings confirmed the results of Abeb *et al.* (2004) who indicated ruminal ammonia-N concentration remarkably improved by urea-treated wheat straw. Boucher *et al.* (2007) showed that ruminal ammonia-N concentrations increased quadratically with increasing urea supplementation in a corn silage-based diet: 9.0, 11.9, 12.8, and 17.4 mg/dl for the 0, 0.3, 0.6, and 0.9% urea diets, respectively.

Conclusions

It was concluded that the chemically treatments of sesame stover improved the digestion of untreat in sheep through modification of cell wall. In addition, sodium hydroxide cause to enhance the fermentability of the fibrous feed evaluated. On the other hand, urea applied at the present study provides condition to improve the ruminal degradation of CP. The urea treatment improved ruminal of ammonia-N.

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Tab. 3. Mean pH ruminal and ammonia-N concentration of experimental diets consisted of alfalfa hay plus sesame stover as untreated or treated with NaOH and/or urea in sheep

Trait	Treatments[1]					SE	Probability
	AH	AHS	AHSU	AHSN	AHSUN		
Ruminal pH	6.72	6.88	6.89	7.02	6.84	0.064	0.110
NH_3-N, mg/dl	7.27[c]	6.12[c]	10.4[b]	6.21[c]	12.85[a]	0.55	***

Means in the same row with different letters (a, b, c) differ (P<0.05)
SE: standard error of the mean.
[1]Treatments are: AH; alfalfa hay, AHS; sesame stover + alfafa, AHSU; sesame stover treated with urea 3% + alfalfa, AHSN; sesame stover treated with NaOH 4% + alfalfa and AHSUN; sesame stover treated with NaOH 4% and urea 3% + alfalfa.

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