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Study on the effect of ensiling with or without urea on physical quality, chemical composition and *in-vitro* digestibility of maize stover

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Abstract: The study was undertaken with objectives to investigate the physical quality, chemical composition and *in vitro* digestibility of maize stover ensiled for 45, 90 and 135 days with different levels of urea and with or without water. For this purpose, six different treatment groups are arranged, where the chopped maize stovers were preserved in plastic containers under normal condition and treated as control ($T_{0)}$ (sample only), T_1 (sample ensiled without water), T_2 (sample with 20% water), T_3 (sample with 20% water plus 3% urea) and T_4 (sample with 20% water plus 5% urea), T_5 (sample with 20% water plus 7% urea) and all the treatments were replicated thrice. The results indicated that physical quality (color, smell, softness and fungal growth) of maize stover was improved with urea treatments (T_3, T_4 and T_5) compared to other treatments (T_0, T_1 and T_2). The physical quality of the maize stover was improved with the increased of ensiling period from 45 to 135 days. The crude protein (CP) content was increased (P<0.05) and dry matter (DM), organic matter (OM) and crude fibre (CF) contents were decreased (P<0.05) in all the treatments (T_1, T_2, $T_{3,}$ T_4 and T_5) compared to control (T_0). The highest DM, OM and CF content was found in T_0 (72.33%, 87.70% and 37.08%) and highest CP was found in T_4 (16.88%) but the lowest DM, OM, CF and CP was observed in T_5 (57.86% 83.89%) in T_2, 20.55% in T_4 and 6.28% in T_0. The DM content was decreased (P<0.05), the OM and CF contents were similar (P>0.05) and CP content was increased (P>0.05) with the increased of ensiling period from 45 to 135 days. The IVGP (*in vitro* gas production), OMD (organic matter digestibility) and ME (metabolizable energy) contents were increased in all the treatments (T_1, T_2, T_3 , T_4 and T_5) compared to control (T_0). The highest IVGP, OMD and ME values was observed in T_4 which were 36.30 ml, 54.75%, 8.10 MJ/kg DM, respectively, and lowest values were observed in control (T_0) which were 13.35 ml, 29.58% and 4.38 MJ/kg DM, respectively. Addition of urea improved the physical quality, nutritive value and preservation capacity of maize stover. Among the treatments T_4 was found best as there was no fungal growth and showed best color, softness, nutritional quality and longer preservation capacity compared with all other treatments. Therefore, it can be concluded that, treatment with urea improved the physical quality, nutritive value and preservation capacity of maize stover.
Key words: Maize stover, treatment, ensiling, physical and chemical quality, *in vitro* digestibility

Introduction

Livestock rearing is an integral part of the farming system in Bangladesh. It plays a vital role in agro-based economy of Bangladesh. Contribution of livestock on GDP is 2.90% and to agriculture sector is 17.70% and about 25% of the total population is fully and 50% is partially depends on livestock (Bangladesh Economic Survey, 2007). Maize stover is one of the crop residues, has a higher nutrient content and is more digestible compare to most other low quality straws and stovers. It has crude protein content of about 6% and metabolizable energy (ME) value of about 9 MJ/kg DM (McDonald *et al.*, 1995). In some developed and also developing countries maize stover are being used as ruminant feed. Maize stover also frequently used as major part of the diet for dry, pregnant beef cows in North America. The stover may be chopped, ensiled and fed in a similar way to maize silage (McDonald *et al.*, 1995). But maize stover is characterized by a low protein, high fibre content and structurally too hard compared to other high quality roughages. It is difficult to fed the maize stover directly to the animals. Different physico-chemical and biological processing of maize stover may make it palatable, increase its nutritional and preservative quality. Then its may be utilized as feed for ruminants. Saadullah *et al*. (1981a) stated that treatment with urea has an advantage in the way of increasing nitrogen content of fibrous roughages. Ensiling of crop residue after urea treatment reduced hardness (Jayasuria and Perera, 1981) and increases nutritive value by making more digestible cellulose and hemi-cellulose (Silva and Ørskov, 1988). So, to meet the demands of animal feeds, urea may be used for improving the nutritive quality and preservation capacity of maize stover. Therefore, the present experiment was under taken to investigate the effect of ensiling with or without urea on physical quality, nutritive quality and in vitro digestibility of maize stover, to identify the appropriate method of ensiling of maize stover to use as ruminant feed.

Materials and Methods

The experiment was conducted in the Laboratory of the Department of Animal Science, Bangladesh Agricultural University, Mymensingh, Bangladesh. In this experiment, maize stover was processed and ensiled with water and urea at different levels, and then it was stored in airtight plastic container for 45, 90 and 135 days under normal condition and ensiling method was replicated thrice. After end of each stipulated period, the preserved materials were observed for physical characteristics and sub-samples were made for chemical and *in vitro* digestibility.

The experimental treatments are T_0 (Maize stover); T_1 (Maize stover + 0% water); T_2 (Maize stover + 20% water); T_3 (Maize stover + 20% water + 3% urea); T_4 (Maize stover + 20% water + 5% urea); and T_5 (Maize stover + 20% water + 7% urea).

Collection of experimental materials: The maize stover samples were collected from the field of Sherpur upazila of Bogra district just after harvesting of cobs. The samples were collected on 5th May, 2006. Commercial fertilizer grade granulated urea (NH_2-CO-H_2N; 46% N) was purchased from the local market. The composition of urea was-DM-98.0%, CP-266.0%. The plastic containers used for ensiling were washed, cleaned and dried in the sun properly.

Processing and preservation of materials: After collection, the maize stover samples were chopped into 3-4 cm. About 3 kg of chopped maize stovers was placed in a plastic bowl, mixed well and then treated with 20%

water and with or without 3, 5 and 7% urea. The properly mixed samples were placed in separate plastic container and was pressed and squeezed sufficiently to make airtight by hand pressure and finally tightly closed with cover. Each container was labeled according to treatment number. The ensiled materials were preserved at room temperature in the laboratory for 45, 90 and 135 days (4.5 months) from June to September.

Observation and collection of samples: The preserved materials of the plastic container were opened at three definite intervals of 45, 90 and 135 days. The physical changes of all preserved samples were examined and recorded. During the examination, 100g of samples were taken out from each treatment for chemical analysis. Maize stover sample was also taken for chemical analysis before ensiling. All the samples were air dried and ground with the help of grinding machine of about 1mm in sieve for chemical analysis and *in vitro* digestibility technique.

Chemical analysis: Chemical composition of the air dried samples of untreated and treated maize stover were analyzed for DM, Ash, OM, CP and CF according to the methods of AOAC (1984).

Measurement of *in vitro* gas production (IVGP), organic matter digestibility (OMD) and metabolizable energy (ME): To determine the *in vitro* gas production (IVGP), organic matter digestibility (OMD) and metabolizable energy (ME) contents of maize stover, hay was used as standard and blank was used for correction of gas measurement. The method *in vitro* gas production technique described by Menke *et al.* (1979), Menke and Steingass (1988) and Hohenheim gas test (Menke's method) was used developed by Menke et al. (1979).

Calculation of IVGP, OMD and ME: The mean gas production of blank tests (Gp_0) was determined by using the formula Gp_{9s}-Gp_0/3. The net gas production was corrected for differences in sample weight (mg DM), if different from 200 mg DM. As the piston of the syringes was moved back to 40 ml at 9 h of incubation (V_9), the following formula was used. Gp (ml/200 mg DM) = {(Gp_9-Gp_0) + (Gp_{24}-40) – Mean blank gas production × 200}/weight of samples (mg DM). The organic matter digestibility (OMD) (%) and metabolizable energy (ME MJ/kg DM) were calculated from the gas volume (Gv) and crude protein value (% CP) using the following equations (Menke and Steingass, 1988).

% OMD = 14.88 + 0.889 × Gv + 0.45 × % CP and

ME = 2.20 + 0.136 × Gv + 0.057 × % CP.

Statistical analysis: The data of proximate analysis and *in vitro* digestibility were analyzed using MSTAT statistical programme, and analysis of variance was done in completely randomized design (CRD) with three replications. The Duncan's Multiple Test was used to determine significance among the treatment means.

Results and Discussion

Physical quality of maize stover: The physical characteristics of ensiled materials are presented in Table 1. It revealed that ensiled maize stover (T_1 and T_2) without urea had unacceptable colour, smell, softness and there was much fungal growth. Urea treatment (T_3 T_4 and T_5) had acceptable colour, softness and ammonia smell but there was some fungal growth in T_3 and T_5. There was a increasing trend of fungal growth with the increasing of ensiling period from 45 to 135 days, but the colour, smell and softness were improved with increasing the ensiling period especially in T_3, T_4 and T_5 treatment groups. Similar result have been reported on poultry litter (nitrogen source) ensiled with barley straw (Hadjipanayotou, 1982). Silage with urea has good aerobic stability (Meeske *et al.,* 2002).The ammoniation process developed alkaline condition quickly inhibited the microbial fermentation and maintained the treated maize stover in a good condition (Munthali *et al.,* 1990). Therefore, the reason of increase of fungal growth with the increasing of ensiling period in the present findings may be due the same cause. In the present experiment urea and water were used, so moisture may be increased. Due to this, 7% urea ensiled maize stover was favoured higher fungal growth. Higher moisture contents may one of the cause but fungal growth in silage may be due to some other factors, such as poor management during ensiling, resulted in oxygen getting into the stored feed. Well managed silos covered properly had no significantly greater levels of fungal growth. When silage is stored for longer period could create an environment for fungal growth and toxin produced (Gotlieb, 2000).

Table 1. Effect of different ensiling methods on the physical quality of maize stover

Parameters	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
Colour	45	Blackish	Blackish	Brownish	Brownish	Brownish
	90	Black	Blackish	Brownish	Brownish	Blackish
	135	Black	Blackish	Brown	Brownish yellow	Black
Smell	45	Bad	Bad	Pungent smell of NH3	Pungent smell of NH3	Smell of NH3
	90	Bad	Bad	Smell of NH3	Smell of NH3	Smell of NH3
	135	Bad	Bad	Smell of NH3	Smell of NH3	Smell of NH3
Softness	45	Hard	Hard	Moderately soft	Moderately soft	Moderately soft
	90	Hard	Hard	Moderately soft	Soft	Soft
	135	Hard	Hard	Soft	Soft	Soft
Fungus	45	Present	Present	Absent	Absent	Absent
	90	Present	Present	Present	Absent	Present
	135	Present	Present	Present	Absent	Present

T_0 = Untreated and unensiled, T_1 = Untreated and ensiled, T_2 = Ensiled with 20% water, T_3 = T_2+ 3% urea, T_3 = T_2+ 5% urea T_3 = T_2+ 7% urea. Hard indicating not acceptable by ruminants by (cattle, sheep, goat). Soft indicating accepted by ruminants (cattle, sheep, goat).

Chemical composition: Effect of different levels of urea and ensiling time on the composition of maize stover are shown in Table 2. The highest DM content was (72.33%) found in untreated (T_0) and lowest (57.86%) in urea treated (T_5) maize stover ($P<0.05$). The DM content was decreased significantly ($P<0.05$) with the urea treated (T_3, T_4 and T_5) maize stover. The OM content was highest (87.70%) in the untreated (T_0) and lowest (83.89%) in the 20% water treated (T_2) maize stover ($P<0.05$). The CP content was highest (16.88 %) in 5% urea treated (T_4) maize stover ($P<0.05$) followed by 6.28, 6.66 and 7.65, 13.98 and 13.48% in the other treatments (T_0, T_1 , T_2, T_3 and T_5) of maize stover. The CF content was highest (37.08%) in untreated (T_0) and lowest (20.55 %) in urea treated (T_4) maize stover ($P<0.05$). It was revealed that DM, OM and CF contents were decreased by 20.10, 4.34 and 44.58%, respectively, where CP content was increased by 158.12% or 2.58 times (i.e. 2.6 fold is increased). It was observed that the DM content was decreased and CP content was increased significantly ($P<0.05$) with increasing the ensiling period from 45 to 135 days while the OM and CF content was not significantly ($p>0.05$) changed with increasing the ensiling period from 45 to 135 days).

Table 2. Effect of different treatments and ensiling on the composition of maize stover (% on DM basis)

Parameters	Days	Treatments						
		T_0	T_1	T_2	T_3	T_4	T_5	Overall
DM	45	72.40	71.11	64.69	58.30	59.63	58.53	64.11a
	90	72.94	69.77	62.44	59.42	60.14	59.02	63.96a
	135	71.66	67.84	62.38	56.11	57.34	56.03	61.89b
	Mean	72.33a	69.57b	63.17c	57.94d	59.03e	57.86d	
	Std.	0.64	1.64	1.32	1.68	1.49	1.60	
OM	45	87.31	86.52	84.28	85.27	86.07	85.50	85.83
	90	87.07	84.37	83.39	84.68	86.46	84.05	85.00
	135	88.74	85.36	83.99	83.26	86.50	85.02	85.48
	Mean	87.70a	85.42b	83.89c	84.41d	86.34e	84.86d	
	Std.	0.90	1.08	0.45	1.03	0.24	0.74	
CP	45	6.34	6.31	7.15	15.40	15.49	12.04	10.46a
	90	6.24	6.55	8.34	14.31	16.57	13.88	10.98a
	135	6.25	7.12	7.47	12.23	18.57	14.51	11.03b
	Mean	6.28a	6.66a	7.65b	13.98c	16.88d	13.48c	
	Std.	0.06	0.42	0.62	1.61	1.56	1.28	
CF	45	36.73	35.06	33.85	22.52	20.40	21.97	28.42a
	90	37.64	34.41	33.36	21.38	21.47	21.08	28.22a
	135	36.88	36.15	32.44	19.82	19.78	19.98	27.51b
	Mean	37.08a	35.21b	33.22c	21.24d	20.55d	21.01e	
	Std.	0.49	0.88	0.72	1.36	0.85	1.00	

Values in the different supercripts within same row and column differ significantly ($p<0.05$)

Effect of ensiling on DM: In the present study, DM content was decreased from 72.33 to 57.86% which is supported by previous reports that DM was decreased from 92.6 to 90.5% in maize stover with 5% urea treatment (Smith *et al.,* 1989), from 91.7 to 35.9% in maize stover with 5% urea treatment (Munthali, *et al*., 1991) and from 89.0 to 50.6% in urea treated wheat straw (Wexian *et al.*, 1995). The DM at 45, 90 and 135 days of ensiling period were found 64.11, 63.96 and 61.89%, respectively. It was observed that DM content was decreased with ensiling time from 64.11 to 61.89% with the increase of duration from 45 to 135 days ($P<0.05$). The previous results support the present findings. Further, Otieno *et al.*, (1986) also observed that DM decreased from 22.58 to 20.83% in ensiled maize stover.

Effect of ensiling on OM: The OM content of the treatments (T_0, T_1, T_2, T_3 , T_4 and T_5) were 87.70, 85.42, 83.89, 84.41, 86.34 and 84.86% respectively. In the present experiment the OM content was decreased ($P<0.05$) from 87.70% (T_0) to 83.89% (T_2) among the treatments. The OM content was decreased from 87.70 (T_0) to 84.41% (T_3) in urea treated group. Some findings also revealed that, OM is decreased with the urea treatment of maize stover (Smith *et al.*, 1989), reduced due to treatment of wheat straw with urea (Wexian *et al.*, 1995), with caged layer waste (nitrogen source). Ensiling with caged layer waste (nitrogen source) of maize stover, wheat straw and maize cobs reduced the OM content was observed by Kayongo *et al.* (1986). The OM content in different ensiling time (45, 90 and 135 days) were 85.83, 85.00 and 85.48 % respectively. The present study indicates that there were no effect of ensiling time on the OM content ($P>0.05$), as the OM content were similar in 45, 90 and 135 days of ensiling.

Effect of ensiling on CP: The CP content of the treatments (T_0, T_1, T_2, T_3, T_4 and T_5) were 6.28, 6.66, 7.65, 13.98, 16.88 and 13.48 % respectively. It was observed that the CP content was highest in the 5% urea treated (T_4) maize stover (16.88 %) followed by other treatments (T_0, T_1, T_2 , T_3 and T_5) of maize stover (6.28, 6.66 and 7.65 13.98 and 13.48%) significantly ($P<0.05$). So, the CP content increased from 6.28 to 16.88 % in urea treatment (T_4). The similar type of findings were also observed by the other experimenter, such as treatment with urea (5% and 7%) increase the CP content of maize stover from 3.63% to 11.25 and 13.63% respectively (Smith *et al.*, 1989). Ensiling with urea increase the CP content of maize stover (Munthali *et al.*, 1990; Altaf-ur-Rahman *et al.* 2001). A two-fold increase in CP content was reported by Man and Wiktorsson (2003) in urea-treated sample. Where in the present experiment 2.6 fold was increased. Urea increases the nitrogen content as well as CP content and exerts no substantial influence on fermentation. The increase of CP content due to urea treatment is supported by Saadullah *et al.* (1981a); Haque and Akbar (1986), they stated that the urea treatment of straw has an advantage that, it increases the nitrogen content of roughages. In the present experiment the CP content in different ensiling time (45, 90 and 135 days) were 10.46, 10.37 and 11.15% respectively where the CP content increased with increasing the ensiling time (45 to 135 days)(P>0.05). Chauhan (1985) supported that there is an increase in CP content with 0.5 or 1% urea (ensiling for 3 months) from 4.6 to 10.0 and 12.7%. Mohanta (2005) stated that, in different days (7, 15 and 21 days) of ensiling CP content were different and was increased with the increased of ensiling time.

Effect of ensiling on CF: The CF content of the treatments (T_0, T_1, T_2, T_3 ,T_4 and T_5) were 37.08, 35.21, 33.22, 21.24, 20.55 and 21.01% respectively. In the present experiment the value of CF was significantly (P<0.05) higher in untreated (T_0) maize stover than the treated (T_1 to T_5) maize stover. In the present experiment, in urea treated (T_4) maize stover the CF content was 20.55% which was significantly lowest (P<0.05) among the treatments. Similar reduction was reported by Sudesh-Rodortra (2004) who observed that a decrease of CF content of wheat straw from 42.74% to 35.66% when wheat straw was treated with 4% urea.

***In vitro* gas production (IVGP), organic matter digestibility (OMD) and metabolizable energy (ME) content of maize stover:** The *in vitro* gas production (IVGP), predicted organic matter digestibility (OMD) and predicted metabolizable energy (ME) contents of ensiled maize stover are presented in Table 3. It was evident that IVGP was the highest (34.97 ml) in 5% urea treated (T_4) maize stover (P<0.05), where as, other treatments (T_5, T_3, T_2, T_1 and T_0) showed the values of 30.86, 29.83, 17.82, 15.44 and 13.35 ml, respectively. The predicted OMD was the highest (P<0.05) in 5% urea treatment (T_4), where the value was 54.75 %. The predicted OMD of the other treatments (T_5, T_3 ,T_2, T_1 and T_0) were 48.38, 47.71, 34.16, 31.60 and 29.58 %, respectively. In the present study, the predicted ME value was increased significantly (P<0.05) with treatment and ensiling. The highest predicted ME content (8.10 MJ/kg DM) was observed in 5% urea treated (T_4) maize stover , followed by 7.17, 7.06, 5.06, 4.68 and 4.38 MJ/kg DM for T_5, T_3 T_2, T_1 and T_0, respectively. It was also revealed that the IVGP, OMD and ME content was increased by 161.95, 76.60 and 91.40 %, respectively.

Effect of ensiling on IVGP, OMD and ME: In the present study, the results showed that there were significant differences among the treatments (P<0.05) of *in vitro* gas production, where gas production depends on some conditions which were found in different experiments. In the present experiment, a positive relationship was observed between gas production and CP content of the treated and ensiled maize stover. Khazaal *et al.* (1993) also noted that the cumulative gas production increased with the ensiling and maximum gas production is positively related to CP contents. *In vitro* gas production is positively related to microbial protein synthesis (Hillman *et al.*, 1993). As the maize stover was treated and ensiled, due to this, there may be higher microbial protein synthesis and higher gas production observed in the present study. However, where CF content was lower, the gas production was higher *i.e* the negative relationship. Nsahlai *et al.* (1994) also reported that cumulative gas production is negatively related with the fibre content of the materials.

In the present experiment there was increase the predicted OMD with treatment and ensiling. Predicted OMD of maize stover treated with 3, 5 and 7% urea were 53.0, 52.0 and 50% (Smith *et al.*, 1989), which were close to 49.24, 54.75 and 48.38 % found in present experiment with 3, 5 and 7% urea treatment. Hiep and Man (2003) reported that, predicted OMD was shown to increase (P<0.05) due to urea treatment of maize stalks from 63.7 to 65.7%.

Table 3. ***In vitro*** **gas production (IVGP), organic matter digestibility (OMD) and Metabolizable energy (ME) contents in maize stover after different treatments and ensiling**

Parameters	Days	Treatments						
		T_0	T_1	T_2	T_3	T_4	T_5	Mean
IVGP (ml)	45	12.75	14.05	17.79	30.85	34.52	29.82	23.30a
	90	14.22	15.12	19.31	29.88	36.67	30.91	23.85a
	135	13.09	17.15	16.55	28.77	37.72	31.85	24.02b
	Mean	13.35a	15.44b	17.82c	29.83d	36.30f	30.86g	
	Std.	0.77	1.57	1.38	1.04	1.63	1.02	
OMD(%)	45	29.07	30.21	33.74	49.24	52.54	46.81	40.27
	90	30.33	31.27	35.80	47.88	54.94	48.61	40.88
	135	29.33	33.33	32.95	46.01	56.77	49.72	41.20
	Mean	29.58a	31.60b	34.16c	47.71d	54.75e	48.38d	
	Std.	0.67	1.59	1.47	1.62	2.12	1.47	
ME (Mj/kg DM)	45	4.30	4.47	5.00	7.27	7.78	6.94	5.96
	90	4.49	4.63	5.30	7.08	8.13	7.20	6.05
	135	4.34	4.94	4.88	6.82	8.39	7.36	6.10
	Mean	4.38a	4.68a	5.06a	7.06b	8.10c	7.17b	
	Std.	0.10	0.24	0.22	0.23	0.31	0.21	

Values in the different supercripts within same row and column differ significantly (p<0.05)

Results indicated that ensiling of maize stover with urea helps to improve the physical quality, nutritive value and preservation capacity. Maize stover ensiled with 5% urea (T_4) showed best physical quality (v.z. color, softness and absence of fungal growth), nutritional quality and longer preservation capacity compared to all other treatments groups. Therefore, it can concluded that farmers may use 20% (W/W) water plus 5% urea for ensiling maize stover depending on the availability, cost of preservation and animal acceptance. Further *in vivo* feeding trial is needed with urea ensiled maize stover to justify the present findings.

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