Urea Treated Corncobs Ensiled with or without Additives for Buffaloes: Ruminal Characteristics, Digestibility and Nitrogen Metabolism

M. A. Khan*, Z. Iqbal[1], M. Sarwar[2], M. Nisa[2], M. S. Khan[3], W. S. Lee, H. J. Lee and H. S. Kim
Dairy Science Division, National Livestock Research Institute, Korea

ABSTRACT : Influences of urea treated corncobs (UTC) ensiled with or without different additives on ruminal characteristics, *in situ* digestion kinetics, nutrient digestibility and nitrogen metabolism were examined in a 5×5 Latin square design using five ruminally cannulated buffalo bulls. Five iso-caloric and iso-nitrogenous diets were formulated to contain 30% dry matter (DM) from concentrate and 70% DM from 5% UTC ensiled without any additive (U) or with 5% enzose (EN), 5% acidified molasses (AM), 5% non-acidified molasses (NM) and 5% acidified water (AW), respectively. These diets were fed to buffalo bulls at 1.5% of their body weight daily. Ruminal NH_3-N concentration at 3 hours (h) post feeding was significantly higher in bulls fed U, NM and AW diets, however, at 6, 9 and 12 h post feeding it was significantly higher in bulls fed EN and AM diets. Ruminal total volatile fatty acids (VFA) and acetate concentrations were significantly higher with EM and AM diets compared with other diets at 3, 6, 9 and 12 h post feeding. Ruminal pH at 6 and 9 h post feeding was higher with EN and AM diets; however; it was notably lower with these diets at 3 h post feeding. Total ruminal bacterial and cellulolytic bacterial counts were higher in bulls fed EN and AM diets than in those fed the other diets. *In situ* ruminal DM and NDF degradabilities and total tract digestibilities were significantly higher with UTC ensiled with enzose and acidified molasses than those ensiled without any additive or other additives. Nitrogen balance was significantly higher in bulls fed EN and AM diets than those fed U, AW and NM diets. The UTC ensiled with enzose or acidified molasses resulted in better digestibility and N utilization than those ensiled without any additive, with non-acidified molasses and acidified water in buffaloes. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 705-712)***

Key Words : Corncobs, Fermentable Carbohydrates, Digestibility, Nitrogen Utilization

INTRODUCTION

Crop residues are cheaper, abundantly available and are extensively used as ruminant feed in the regions where grain feeding is not viable. However, they have poor feeding value for ruminants because they limit ruminal functions, intake and, thus impede ruminant productivity (Sarwar et al., 2003).

Out of various methods employed to nutritionally upgrade crop residues, urea treatment received popularity because it is economical and safer to use (Ali et al., 1994). Church (1991) reported urea treatment as one of the cost-effective means of increasing N contents of crop residues. However, escape of NH_3-N from urea treated crop residues renders whole process uneconomical and caused environmental pollution (Khan et al., 2004). Furthermore, most of the NH_3-N in urea treated crop residues was held as water-soluble that released rapidly in rumen and resulted in nutrient loss (Sarwar et al., 1994). Use of different additives like acids (Borhami et al., 1982; Dass et al., 2001) and sources of fermentable carbohydrates (Khan et al., 2004; Nisa et al., 2004a) have been reported to retain this escaped N in urea treated wheat straw. It was reported that ensiling urea treated wheat straw with corn steep liquor (CSL) resulted in better N fixation (Nisa et al., 2004a).

Enzose is a light amber color liquid derived from the enzymatic conversion of corn starch to dextrose. Enzose has enhanced the N capture in urea treated corncobs (UTC) because of its lactic acid contents (18%), low pH (4.4) and availability of reducing sugars (80% corn dextrose) for lactic acid production. Molasses, an important by-product of sugar cane industry, and a good source of fermentable carbohydrates (sucrose) can be used to capture NH_3-N in urea-treated material. Acidification of molasses may further help enhance the N capture in UTC their feeding value for sheep (Sarwar et al., 2005). In our previous study, various levels of organic acids and fermentable carbohydrates were compared for their effect on nitrogen capture in ammoniated corncobs. It was observed that UTC ensiled with 5% enzose, 5% molasses, 5% acidified molasses and 5% acidified water better retained NH_3-N and showed increased neutral detergent insoluble nitrogen (NDIN) contents than those ensiled without or with other levels of these additives (Sarwar et al., 2005). However, the information on the dietary effects of UTC ensiled with these additives is limited in ruminants.

Therefore, the present study was conducted to evaluate

* Corresponding Author: M. A. Khan. E-mail: ajmals1@yahoo.com
[1] Livestock and Dairy Development Department, Punjab, University of Agriculture, Faisalabad 38040, Pakistan.
[2] Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad 38040, Pakistan.
[3] Department of Animal Breeding and Genetics, University of Agriculture, Faisalabad 38040, Pakistan.
Received September 29, 2005; Accepted December 1, 2005

Table 1. Chemical composition of corncobs and additives on dry matter basis

Items (%)	Corncobs	Cane molasses	Acidified cane molasses[1]	Enzose[2]
Dry matter	91.3	68.0	68.0	69.1
Crude protein	1.72	4.70	4.70	4.00
Ash	2.03	11.1	11.1	10.0
pH	-	6.00	4.40	4.40
Lactic acid (g/L)	-	-	-	180
Dextrose	-	11.2	11.0	78.1
Sucrose	-	35.6	35.3	-
Fructose	-	5.4	5.3	1.01
Neutral detergent fiber	78.4	-	-	-
Acid detergent fiber	46.1	-	-	-
Hemicellulose	32.3	-	-	-
Cellulose	34.6	-	-	-
Lignin	11.0	-	-	-

[1] Molasses were acidified to attain pH 4.4 similar to that of enzose using HCl.
[2] Enzose is a light amber color liquid derived from the enzymatic conversion of corn starch to dextrose.

Table 2. Chemical composition of urea treated corncobs[1] on dry matter basis ensiled without and with different additives in trench silos for feeding trial

Parameters (%)	None	Enzose	Acidified molasses	Acidified water	Non-acidified molasses
Crude protein	7.30	15.0	14.0	10.0	12.0
Neutral detergent insoluble N	0.30	0.90	0.76	0.62	0.68
Acid detergent insoluble N	0.36	0.38	0.39	0.35	0.37
Ammonia-N	0.62	0.80	0.68	0.70	0.73
Neutral detergent fiber	80.9	84.4	84.3	81.2	82.0
N-free neutral detergent fiber[2]	80.6	79.5	79.2	80.2	80.4
Acid detergent fiber	53.0	53.9	53.9	53.9	53.9
Hemicellulose	27.2	30.8	30.4	28.2	28.6
N-free hemicellulose[3]	27.0	26.9	26.5	25.9	26.0
Cellulose	47.4	48.8	49.0	47.9	48.2
Acid detergent lignin	6.12	4.17	4.25	4.36	4.10

[1] 5% urea treated corncobs ensiled without any additive and with 5% enzose, 5% acidified molasses, 5% acidified water and 5% non-acidified molasses.
[2] N-free neutral detergent fiber was calculated as (NDF-NDIN×6.25). [3]N-free Hemicellulose was calculated (NDF-NDIN×6.25)-ADF-(ADIN×6.25).

the influences of UTC ensiled without and with different additives on ruminal fermentation characteristics, *in situ* digestion kinetics, nitrogen metabolism and nutrient digestibility in ruminally cannulated buffalo bulls fed restricted diets.

MATERIALS AND METHODS

Treatment of corncobs

Whole corncobs were procured from Rafhan Maize Products Co. Ltd. Faisalabad, Pakistan and were crushed to 2 cm particle size by mechanical crusher. These crushed corncobs were treated with 5% fertilizer grade urea containing 46% N (5 kg urea per 100 kg of corncobs DM) in bulk. The urea was dissolved in water (50 liter per 100 kg of corncobs DM) to achieve 50% moisture in corncobs at the time of ensiling. Four additives, enzose, cane molasses, acidified cane molasses and acidified water (having 4.4 pH similar to enzose that was achieved by HCl addition) each at the rate of 5% of corncobs DM were separately dissolved and sprayed with 5% urea solution on the crushed corncobs. The chemical composition of corncobs and additives is given in Table 1. For control treatment only 5% urea was sprayed on the crushed corncobs. These corncobs were ensiled for feeding trial in five different cemented trench (15×4×6 m) silos for 15 days.

The DM of fermented corncobs were determined by drying at 105°C for 6 h (AOAC, 1999; ID 930.5), and organic matter (OM) was calculated as weight loss upon ignition at 600°C. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined with the ANKOM fiber analyzer using reagents described by Van Soest et al. (1991). The N, NDIN, and acid detergent insoluble N (ADIN) were determined by a Kjeldahl method (AOAC, 1999; ID 984.13). The NDF was assayed with sodium sulfite and without alpha amylase. The NDF and ADF contents were expressed without residual ash. The NH_3-N in corncobs was determined by the modified micro diffusion method developed by Conway and O'Malley (1942). The pH of additives was recorded using a pH-mV meter (HM-21P, TOA Corporation, Tokyo, Japan). Chemical composition of UTC ensiled with additives is given in Table 2.

Table 3. Ingredients and chemical composition of experimental rations on dry matter basis

Ingredients	Diets[1]				
	U	EN	AM	NM	AW
Corncobs	70.0	70.0	70.0	70.0	70.0
Wheat screenings	12.1	13.0	13.0	13.0	13.0
Molasses	15.0	15.0	14.9	14.8	14.6
Mineral mixture	2.00	2.00	2.00	2.00	2.00
Urea	0.9	0.00	0.10	0.20	0.40
Chemical analysis					
Dry matter	76.8	77.1	77.2	77.0	77.1
Crude protein	12.2	12.4	12.4	12.3	12.3
Neutral detergent fiber	68.9	63.2	63.2	63.2	63.2
Acid detergent fiber	43.1	39.8	40.2	40.2	40.2
Acid detergent lignin	4.78	3.84	3.84	3.84	3.84
NE_L (Mcal kg^{-1})	1.24	1.26	1.26	1.25	1.24

[1] U, EN, AM, NM and AW diets were contained 70% DM from 5% urea treated corncobs ensiled without any additive and with 5% enzose, 5% acidified molasses, 5% non-acidified molasses and 5% acidified water, respectively.
NE_L was calculated according to Conard et al. (1984).

Metabolic study

Five *Nili-Ravi* buffalo bulls of average weight 350±10 kg fitted with ruminal cannulae were fed at restricted intake in a 5×5 Latin Square Design. Five iso-caloric and iso-nitrogenous diets were formulated to contain 30% DM from concentrate and 70% DM from UTC ensiled without any additive (U) and with 5% enzose (EN), 5% acidified molasses (AM), 5% non-acidified molasses (NM) and 5% acidified water (AW), respectively. Chemical composition of experimental diets is presented in Table 3. All diets were mixed daily and fed at 1.5% of body weight daily in two frequencies. During the experimental period, the bulls were housed on a concrete floor in separate pens.

In metabolic study the bulls were fed for 150 days. Each period lasted for 30 days, the first 20 days of which were adaptation and the later 10 days for determinations. Feed offered and orts were weighed and recorded twice daily. For first 3 days of each collection period, the ruminal contents (liquid plus digesta) were sampled (500 ml) manually at 3, 6, 9 and 12 h after morning feed. After strained through six layers of cheesecloth, the rumen samples were immediately measured for pH (Cole Parmer portable pH meter). Rumen sample (100 ml) was acidified with 0.5 ml of 1 N H_2SO_4 and frozen (-24°C). Samples were later thawed and centrifuged at 15,000×g for 10 min (4°C), and the supernatants were analyzed for concentrations of NH_3-N (Autoanalyser Technicom, Model 2, 1970) and volatile fatty acids (VFA). The VFAs were determined in a gas chromatograph (Shimadzu GC-14) using N_2 as a carrier gas at a flow rate of 50 ml min^{-1} and a glass column (2 m length×2 mm diameter) packed with Chromosorb AW, 100 g kg^{-1} polyethylene-glycol and 30 g kg^{-1} H_3PO_4 (Supelco Inc., Bellefonte, PA, USA). The oven, injector port and detector port temperatures were 155, 185 and 190°C, respectively. On day 4, the ruminal content were sampled just before the morning feed and strained. One portion of this strained rumen fluid was used to enumerate cellulolytic bacterial counts (Olumeyan et al., 1986). Another portion was blended with a volume of saline solution containing 20% formaline for total bacterial count by microscopic examination (Suto, 1973).

Complete collections of urine and feces were made for 7 days according to the procedures described by Williams et al. (1984). Feces were collected daily, weighed and 20% aliquot fecal samples were dried at 55°C, bulked and mixed at the end of each collection period and sampled for analysis. Daily collections of urine were measure and 20% aliquot samples of urine were acidified with 50% H_2SO_4, stored and then mixed and sampled at the end of each period. The urine samples were analyzed for N as described above. On day 3 of each collection period, blood samples were taken at 3, 6, 9 and 12 h after feeding. Plasma urea N was measured by a Urease-Indophenol Method using the Wako kit (Urea N B, code No. 279-36201, Wako Pure Chemical Industries, Ltd. Osaka, Japan). Feed, orts and fecal samples were dried at 55°C and ground through a Wiley mill (2-mm screen). These samples were analyzed for DM, N, and OM and NDF, ADF, and ADL by methods described above. Apparent total tract nutrient digestibilities and N balance were calculated as described by Sarwar et al. (2004).

Nylon bag study

In situ digestion kinetics study was conducted with metabolic trial using five *Nili-Ravi* buffalo bulls fitted with ruminal cannulae in a 5×5 Latin Square Design. The samples being incubated in the rumen were of the same treated corncobs (5% UTC ensiled without any additive or with 5% enzose, 5% molasses, 5% acidified molasses and 5% acidified water) that were fed to bulls in experimental diets for metabolic trial. This was done to avoid the effects of diet on the ruminal fermentation of the feedstuffs (Clark

Table 4. Ruminal fermentation characteristics of buffalo bulls fed restricted diets[1] containing urea treated corncobs ensiled with and without different additives

Parameters	Diets[1]					
	U	EN	AM	NM	AW	SE
3 h						
Ammonia nitrogen (mg dl^{-1})	29.4^{a}	24.2^{c}	24.1^{c}	26.5^{b}	26.8^{b}	0.61
pH	7.2^{a}	6.40^{c}	6.20^{c}	7.10^{b}	7.20^{a}	0.11
Total VFA (mM L^{-1})	122.1^{c}	166.4^{a}	158.3^{a}	139.9^{b}	130.3^{b}	1.54
Acetate (mol 100 mol^{-1})	61.4^{b}	73.4^{a}	71.2^{a}	66.4^{b}	62.7^{b}	0.96
Propionate (mol 100 mol^{-1})	21.8	22.1	22.5	22.5	21.8	0.70
Butyrate (mol 100mol^{-1})	8.69	10.9	11.0	9.90	10.1	0.98
6 h						
Ammonia nitrogen (mg dl^{-1})	20.6^{c}	25.3^{a}	25.1^{a}	22.2^{b}	22.5^{b}	0.64
pH	6.40^{b}	6.80^{a}	6.90^{a}	6.40^{b}	6.30^{b}	0.15
Total VFA (mM L^{-1})	125^{b}	159^{a}	155^{a}	138^{b}	129^{b}	1.32
Acetate (mol 100 mol^{-1})	53.1^{b}	65.3^{a}	63.9^{a}	55.7^{b}	52.9^{b}	0.73
Propionate (mol 100 mol^{-1})	17.9^{b}	21.9^{a}	21.7^{a}	18.1^{b}	17.0^{b}	0.90
Butyrate (mol 100 mol^{-1})	7.89	9.50	9.40	9.00	9.40	0.70
9 h						
Ammonia nitrogen (mg dl^{-1})	14.9^{b}	19.1^{a}	19.4^{a}	16.9^{b}	16.2^{b}	0.78
pH	6.5^{b}	6.90^{a}	6.91^{a}	6.50^{b}	6.50^{b}	0.11
Total VFA (mM L^{-1})	120^{b}	141^{a}	139^{a}	121^{b}	121^{b}	1.00
Acetate (mol 100 mol^{-1})	53.9^{b}	62.7^{a}	61.9^{a}	50.9^{b}	49.1^{b}	0.91
Propionate (mol 100 mol^{-1})	17.8	19.3	19.8	20.1	20.1	0.86
Butyrate (mol 100 mol^{-1})	7.68	9.10	8.97	7.80	7.80	0.65
12 h						
Ammonia nitrogen (mg dl^{-1})	12.8^{c}	16.0^{a}	16.4^{a}	11.2^{b}	14.2^{b}	0.67
pH	7.2	6.92	6.98	7.20	7.10	0.19
Total VFA (mM L^{-1})	115^{b}	136^{a}	137^{a}	119^{b}	122^{b}	1.32
Acetate (mol 100 mol^{-1})	50.9^{b}	58.2^{a}	59.4^{a}	50.8^{b}	50.5^{b}	0.69
Propionate (mol 100 mol^{-1})	16.8	19.0	19.1	16.5	17.8	0.97
Butyrate (mol 100 mol^{-1})	7.93	7.90	7.80	7.90	7.90	1.00

[a, b, c] Means within row bearing different superscripts differ significantly ($p<0.05$).

[1] U, EN, AM, NM and AW diets were contained 70% DM from 5% urea treated corncobs ensiled without any additive and with 5% enzose, 5% acidified molasses, 5% non-acidified molasses and 5% acidified water, respectively.

and David, 1990). Corncobs samples were ground in a meat mincer machine with 1 cm diameter holes. Nylon bags measuring 13×21 cm, with an average pore size of 50 μm, were used to determine the rate and extent of DM disappearance. For each time point, 5 grams DM of each sample was weighed into bags, in triplicate. The bags were closed and tied with braided nylon fishing line. On day 6 of each collection period at 08.00 h 3 bags for each fermentation time were incubated in the rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 h, in reverse order and were removed all at the same time. After removal from the rumen, bags were washed in running tap water until the rinse was clear. Washing time averaged 2.5 min per bag. Bags were then dried in oven at 55°C for 48 h.

After equilibration with air for 8 h, the bags were weighed and the residues were transferred to 100 ml cups and stored for later analyses. The extent of digestion, rate of digestion and lag time, were determined for each incubation period individually. Degradation rates were determined by subtracting the indigestible residue, i.e. the 96 h of ruminal incubation, from the amount in the bag at each time point and then regressing the natural logarithm of that value against time (Sarwar et al., 1991) after correcting for lag time (Mertens, 1977). The lag time was calculated according to Mertens and Loften (1980) as described below.

$$\text{Lag time} = \frac{\ln 100\text{-intercept}}{\text{Rate of digestion}}$$

Where, rate of digestion is equal to the regression slope of ruminal digestibility values against time.

Statistical analysis

Data on various parameters from metabolic and *in situ* studies were analyzed using 5×5 Latin square design with GLM procedure of SAS (1988). The sums of squares were partitioned into animal, treatment and period. Means were tested by Duncan's multiple range test (Steel and Torrie, 1984).

Table 5. Bacterial count of rumen liquor in buffalo bulls fed restricted diets[1] containing urea treated corncobs ensiled with different additives

Parameters	U	EN	AM	NM	AW	SE
Total bacterial count	1.5×10^{9c}	$1.9\times10^{10\ a}$	$1.8\times10^{10\ a}$	$1.5\times10^{9\ b}$	$1.6\times10^{9\ b}$	0.71×10^{8}
Cellulolytic bacterial count	2.6×10^{6c}	$2.7\times10^{7\ a}$	$2.6\times10^{7\ a}$	$2.4\times10^{7\ b}$	$2.5\times10^{7\ b}$	$0.12\times`10^{7}$

[a, b, c] Means within row bearing different superscripts differ significantly ($p<0.05$).

[1] U, EN, AM, NM and AW diets were contained 70% DM from 5% urea treated corncobs ensiled without any additive and with 5% enzose, 5% acidified molasses, 5% non-acidified molasses and 5% acidified water, respectively.

Table 6. *In situ* digestion kinetics of urea treated corncobs ensiled with different additives in buffalo bulls fed restricted diets[1]

Parameters	U	EN	AM	NM	AW	SE
Dry matter						
Degradability (%)	49.9[c]	65.4[a]	63.6[a]	55.8[b]	54.3[b]	1.80
Rate, (% h^{-1})	3.98 [c]	6.50 [a]	6.30 [a]	5.50 [b]	5.40 [b]	0.35
Lag (h)	1.79	1.40	1.60	1.71	1.65	0.39
Extent of digestion (%)[2]	64.42 [b]	78.9 [a]	77.9 [a]	77.3 [a]	77.8 [a]	0.88
Neutral detergent fiber						
Degradability (%)	42.12 [c]	57.1[a]	57.9[a]	52.5[b]	52.6[b]	1.31
Rate (% h^{-1})	3.12 [c]	5.50	5.60	4.50	4.00	0.48
Lag (h)	3.62	3.00	3.05	3.65	3.70	0.21
Extent of digestion (%)[2]	55.8 [b]	66.8 [a]	66.0 [a]	65.1 [a]	64.7 [a]	1.23

[a, b, c] Means within row bearing different superscripts differ significantly ($p<0.05$).

[1] U, EN, AM, NM and AW diets were contained 70% DM from 5% urea treated corncobs ensiled without any additive and with 5% enzose, 5% acidified molasses, 5% non-acidified molasses and 5% acidified water, respectively.

[2] Extent of digestion was calculated at 96 h ruminal incubation as an indicator of maximum ruminal degradation.

RESULTS

Ruminal fermentation characteristics

Ruminal NH_3-N concentration at 3 h post prandial was higher ($p<0.05$) in bulls fed UTC ensiled without any additive, with acidified water and non-acidified molasses compared to those fed UTC ensiled with other additives (Table 4). However, ruminal NH_3-N at 6, 9 and 12 h post prandial was significantly ($p<0.05$) higher in bulls fed UTC ensiled with enzose and acidified molasses than those fed UTC ensiled without any additive or with non-acidified molasses and acidified water.

Ruminal total VFA and acetate concentrations were significantly higher in bulls fed UTC ensiled with enzose or acidified molasses than those fed UTC ensiled without any additive, with non-acidified molasses and acidified water at 3, 6, 9 and 12 h post prandial (Table 4). Ruminal propionate and butyrate concentrations were similar in bulls fed UTC ensiled without any additive and with different additives.

Ruminal pH at 3 h post prandial was higher in bulls fed UTC ensiled without any additive, with non-acidified molasses and acidified water than those fed UTC ensiled with other additives (Table 4). However, it was significantly higher in bulls fed UTC ensiled with either enzose or acidified molasses at 6 and 9 h post prandial compared with those fed UTC ensiled without any additive, with non-acidified molasses and acidified water. At 12 h post prandial ruminal pH was not significantly different in bulls fed UTC ensiled without or with additives.

Total ruminal bacterial and cellulolytic bacterial counts were higher in bulls fed UTC ensiled with enzose and acidified molasses than those fed UTC ensiled without any additive, with acidified water or non-acidified molasses (Table 5).

In situ ruminal digestion kinetics

Urea treated corncobs ensiled with enzose and acidified molasses had significantly ($p<0.05$) higher ruminal DM and NDF degradabilities and rate of digestion than those ensiled without any additive, with acidified water and non-acidified molasses (Table 6). Urea treated corncobs ensiled with additives had higher ruminal extent of digestion of DM and NDF compared to those ensiled without any additives.

Digestibility

Dry matter, NDF and CP intakes by bulls fed UTC ensiled with additives were not significantly different because of restricted feeding in this study (Table 7).

Total tract DM and NDF digestibilities were significantly ($p<0.05$) higher in bulls fed UTC ensiled with either enzose or acidified molasses than those fed UTC ensiled without any additive, with acidified water and non-acidified molasses (Table 7). However, the CP digestibility was non-significant in bulls fed UTC ensiled without or with different additives.

Nitrogen metabolism

Plasma urea nitrogen was significantly ($p<0.05$) higher in bulls fed UTC ensiled without additive, with acidified water and non-acidified molasses than those fed UTC

Table 7. Nutrients intake and digestibility by buffalo bulls fed restricted diets[1] containing urea treated corncobs ensiled with different additives

Parameters	U	EN	AM	NM	AW	SE
Nutrient intake (kg/d)						
Dry matter	5.21	5.25	5.27	5.24	5.24	0.46
Neutral detergent fiber	3.41	3.30	3.30	3.30	3.30	0.29
Crude protein	0.642	0.651	0.653	0.644	0.647	0.02
Nutrient digestibility (%)						
Dry matter	52.2[c]	66.5[a]	64.9[a]	58.8[b]	57.0[b]	1.81
Neutral detergent fiber	46.7[c]	58.2[a]	58.1[a]	53.8[b]	54.0[b]	0.39
Crude protein	69.8	71.8	72.2	70.8	70.7	1.32

[a, b, c] Means within row bearing different superscripts differ significantly ($p<0.05$).

[1] U, EN, AM, NM and AW diets were contained 70% DM from 5% urea treated corncobs ensiled without any additive and with 5% enzose, 5% acidified molasses, 5% non-acidified molasses and 5% acidified water, respectively.

Table 8. Nitrogen utilization in buffalo bulls fed restricted diets[1] containing urea treated corncobs ensiled without and with different additives

Parameters	U	EN	AM	NM	AW	SE
Plasma urea nitrogen (mg/dl)	17.6[a]	11.5[b]	12.1[b]	15.2[a]	17.7[a]	0.51
Nitrogen intake (g/day)	103.7	104.2	104.5	103.0	103.5	1.30
Fecal nitrogen (g/day)	29.0	26.0	26.5	25.9	26.2	0.70
Urinary nitrogen (g/day)	57.24	40.0[c]	40.4[c]	50.8[b]	60.1[a]	1.31
Nitrogen balance (g/day)	17.46	38.2[a]	37.6[a]	26.3[b]	17.2[b]	2.79
Nitrogen balance (% DNI[2]/day)	23.4[c]	48.8[a]	48.2[a]	34.1[b]	22.3[c]	0.80

[a, b, c] Means within row bearing different superscripts differ significantly ($p<0.05$).

[1] EN, AM, NM and AW diets were contained 70% DM from 5% urea treated corncobs ensiled with 5% enzose, 5% acidified molasses, 5% non-acidified molasses and 5% acidified water, respectively.

[2] DNI refers to digestible nitrogen intake.

ensiled with enzose or acidified molasses (Table 8). Nitrogen intake and fecal N were not significantly different across all diets. However, urinary N was significantly higher in bulls fed UTC ensiled without any additive, with acidified water and non-acidified molasses, with no significant difference between EN and AM diets. Nitrogen balance either expressed as g/day or percent of digestible N intake, was significantly higher in bulls fed UTC ensiled with either enzose or acidified molasses than those fed UTC ensiled without any additive, with acidified water or non-acidified molasses (Table 8).

DISCUSSION

Ruminal fermentation characteristics

Higher concentrations of ruminal NH_3 at later sampling hours in bulls fed UTC ensiled either with enzose or acidified molasses were probably because of their higher NDIN contents (Table 2). The NH_3-N was fixed in the fiber matrix of UTC ensiled with enzose or acidified molasses better than those ensiled without any additive and with other additives (Table 2; Sarwar et al., 2005). Higher amount of soluble N in UTC ensiled without any additive, with acidified molasses and non-acidified water probably resulted in rapid release of NH_3-N in the rumen during early hours of post feeding. Present results indicated that urea N was better fixed in the matrices of cell wall of UTC ensiled with enzose or acidified molasses and released slowly with fiber fermentation in the rumen. Similar results were previously documented by Nisa et al. (2004a) who ensiled urea treated wheat straw with corn steep liquor (CSL), a fermentable sugar source having 4.4 pH.

Higher ruminal VFA concentrations in bulls fed diets containing UTC ensiled with enzose or acidified molasses than those ensiled without and with other additive were probably because of their higher ruminal DM and NDF degradabilities (Table 6). Ensiling urea treated wheat straw with fermentable carbohydrates brought physiochemical changes in straw and thus alleviated those factors that hindered fiber fermentation (Nisa et al., 2004b).

Higher ruminal pH at early sampling hours in bulls fed diets containing UTC ensiled without any additive, with non-acidified molasses and acidified water may be because of their higher water-soluble N contents (Table 2) and lower concentration of total ruminal VFA (Table 4). Further, U, NM and AW diets also contained the urea that was added to make them iso-nitrogenous (Table 3). Lines and Weiss (1996) described that soluble N increased with increasing level of urea in the diet that led to rapid release of NH_3-N in the rumen at early post prandial hours.

Higher ruminal bacteria in bulls fed diets containing UTC ensiled either with enzose or acidified molasses was probably the result of more constant supply of NH_3 for microbial growth. Ensilation of UTC with enzose or

acidified molasses increased the NDIN (Table 2) that was released slowly in the rumen (Table 4) and thus might have synchronized with fiber fermentation to maximize the ruminal bacterial growth in bulls. However, availability of carbon skeleton or energy (VFA) for microbial growth and multiplication might have differed much in bulls fed UTC ensiled with different additives because of the difference in their ruminal degradability (Table 6). It may be suggested that changes occurred in the cell wall structure of corncobs like de-lignification and solubilisation of hemicellulose (Sarwar et al., 2004) after ensilation of UTC with enzose or acidified molasses because of higher N retention might have enhanced the availability of fermentable carbohydrates for microbial fermentation and thus their counts in the rumen.

***In situ* digestion kinetics**

Higher DM and NDF ruminal degradabilities and rate of digestion of UTC ensiled with enzose or acidified molasses in bulls were probably because of higher particle disintegration due to increased N retention (Sarwar et al., 2005), which might have provided better adhesion sites for microbial attachment and activity (Grenet and Barry, 1990). Increased N retention in UTC ensiled with enzose and acidified molasses might have increased their fragility and lowered the number of residual ester linkages (Dias-Da-Silva et al., 1988), and thus increased the fiber degradability and rate of disappearance (Khan et al., 2004).

Digestibility

Higher DM and NDF digestibilities in bulls fed diets containing UTC ensiled with enzose and acidified molasses may be because of their higher ruminal rate of disappearance (Table 6), slow release of NH_3 (and sufficient production of VFA (Table 4) leading to better utilization of the nutrients for rumen microbial growth (Table 5) that resulted in improved digestibility. Increased surface area of lingo-cellulose due to higher N retention (Khan et al., 2004) might have resulted in increased accessibility to microbial attack in corncobs ensiled with enzose and acidified molasses. Similar results were reported by Nisa et al. (2004a, b) who fed urea treated wheat straw ensiled with CSL to buffalos. They explained that increased rate of ruminal degradation, shorter ruminal lag time, increased surface area fermentability index and fragility of straw resulted in higher digestibility of urea treated wheat straw ensiled with fermentable sugars.

Nitrogen utilization

In present study, enzose or acidified molasses have helped in binding of NH_3-N to the fibrous portion of ensiled (Sarwar et al., 2005), causing a slow release of NH_3 at ruminal level (Table 4), which was probably utilized more efficiently by the ruminal microbes. Thus, less NH_3 was absorbed through the ruminal walls that consequently led to lower plasma urea N, reduced the urinary N loss and improved the N balance in bulls fed diets containing UTC ensiled with either enzose or acidified molasses. Ensilation of urea treated wheat straw with CSL increased N fixation in the matrix of cell wall fiber, thus slow down its release in the rumen, which maximized N synchronization with carbon skeleton and this consequently minimized N loss (Nisa et al., 2004b; Sarwar et al., 2004).

CONCLUSIONS

Ensiling urea treated corncobs with enzose or acidified molasses compared with non-acidified molasses or acidified water better captured the urea nitrogen and increase the fiber bound nitrogen, their ruminal degradability, and total tract nutrients digestibility in buffaloes. Increased fiber bound nitrogen in urea treated corncobs ensiled with fermentable carbohydrates (having acidic pH) released slowly in the rumen and, thus minimize the nitrogen loss by enhancing the nitrogen utilization by ruminal microbes and animal body. Further studies are warranted to examine the influence of urea treated corncobs ensiled with enzose or acidified molasses on intake, digestibility, milk yield and its composition in lactating buffaloes.

REFERENCES

Ali, A., A. H. Gilani and A. S. Hashmi. 1994. Nutritional value of rice straw as influence by different methods of urea and alkali treatment and storage periods. J. Scientific Res. 46:35-42.

AOAC. 1999. Official Methods of Analysis, 16th ed. Official Methods of Analysis of AOAC International, Gaithersburg, MD, USA.

Borhami, B. E. A., F. Sundstol and T. H. Garmo. 1982. Studies on ammonia treated straw. II fixation of ammonia in treated straw by spraying with acids. Anim. Feed Sci. Technol. 7:53-59.

Church, D. C. 1991. Livestock Feeds and Feeding. (3rd.Ed), Prentice-Hall International Edition, USA.

Clark, J. H. and C. L. David. 1990. Some aspects of feeding high producing dairy cows. J. Dairy Sci. 68:873-880.

Conrad, H. R., W. P. Weiss, W. O. Odwongo and W. L. Shockey. 1984. Estimating net energy lactation from components of cell solubles and cell walls. J. Dairy Sci. 67:427-433.

Conway, E. J. and O'Malley. 1942. Micro diffusion methods: ammonia and urea using buffered absorbents (revised methods of ranges greater than 10 μg N). Biochem Journal. 36:655-666.

Dass, R. S., A. K. Verma, U. R. Mehar and D. S. Shu. 2001. Nutrient utilization and rumen fermentation pattern in murrah buffaloes (*Bubalus bubalies*) fed urea and urea plus hydrochloric acids wheat straw. Asian-Aust. J. Anim. Sci. 14:1542-1548.

Dias-Da-Silva, A. A., A. Mascarenhas Ferriera and C. V. M. Cudedes. 1988. Effect of moisture level, treatment time and soybean addition on the nutritive value of urea treated maize stovers. Anim. Feed Sci. Technol. 16: 67-74.

Grenet, E. and P. Barry. 1990. Microbial degradation in the rumen of wheat straw and anhydrous ammonia treated straw observed

electron microscopy. Reprod. Nut. Devel. 30:533-541.

Khan, M. A., M. Sarwar, M. Nisa and M. S. Khan. 2004. Influence of enzose on feeding value of urea treated corncobs in lactating crossbred cows. Asian-Aust. J. Anim. Sci. 17:70-74.

Lines, L. W. and W. P. Weiss. 1996. Use of nitrogen from ammoniated alfalfa hay urea soybean meal and animal protein meal by lactating cows. J. Dairy Sci. 79:1992-1999.

Mertens, D. R. 1977. Dietary fiber components: relationship to the rate and extent of ruminal digestion. Feed. Proc. 36:187.

Mertens, D. R. and J. R. Loften. 1980. The effect of starch on forage fiber digestion kinetics *in vitro*. J. Dairy Sci. 63.1437-1444.

Nisa, M., M. Sarwar and M. A. Khan. 2004a. Influence of *ad libitum* feeding of urea treated wheat straw with or without corn steep liquor on intake, *in situ* digestion kinetics, nitrogen metabolism and nutrient digestion in ruminally cannulated buffalo bulls. Aust. J. Agric. Reas. 55:229-234.

Nisa, M., M. Sarwar and M. A. Khan, 2004b. Nutritive value of urea treated wheat straw ensiled with or without corn steep liquor for lactating *Nili-Ravi* buffaloes. Asian-Aust. J. Anim. Sci. 17:825-829.

Sarwar, M., J. L. Firkins and M. Eastridge. 1991. Effect of replacing NDF of forage with soyhulls and corn gluten feed for dairy heifers. J. Dairy Sci. 74:1006-1015.

Sarwar, M., M. A. Iqbal, C. S. Ali and T. Khaliq. 1994. Growth performance of buffalo male calves as affected by using cowpeas and soybean seeds as a source of urease during urea treated wheat straw ensiling process. Egyptian J. Anim. Prod. 2:179-185.

Sarwar, M., M. A. Khan and M. Nisa. 2003. Nitrogen retention and chemical composition of urea treated wheat straw ensiled with organic acids or fermentable carbohydrates. Asian-Aust. J. Anim. Sci. 16:1583-1589.

Sarwar, M., M. A. Khan and M. Nisa. 2004. Effect of organic acids or fermentable carbohydrates on digestibility and nitrogen utilization of urea treated wheat straw in buffalo bulls. Austr. J. Agric. Res. 55:234-240.

Sarwar, M., M. A. Khan and M. Nisa. 2005. Chemical composition and feeding value of urea-treated corncobs ensiled with additives for sheep. Austr. J. Agric. Res. 56:685-690.

SAS. 1988. Statistical Analysis System. SAS user's guide: Statistics, SAS Inst. Inc., Carry, NC.

Steel, R. G. D. and J. H. Torrie. 1984. Principles and Procedures of Statistics. A Biometrical Approach (2nd Ed). McGraw Hill Book Co. Inc. New York, USA.

Suto, T. 1973. Rumen no kensa (Examination of the rumen). In (Ed. R. Nakamura, T. Yonemura and T. Suto). Ushino Rinsho Kensaho (Methods in the Clinical Examination of the Bovine). Nosangyoson BunkaKyokai. Tokyo. p. 39.

Van Soest, P. J., H. B. Robertson and B. A. Lewis. 1991. Methods of dietary fiber, NDF and non-starch polysaccharides in relation to animal material. J. Dairy Sci. 74:3583-3595.

Williams, P. E. V., G. M. Innes and A. Brewer. 1984. Ammonia treatment of straw via the hydrolysis of urea 1. Effect of dry matter and urea concnetration on the rate of hydrolysis by urea. Anim. Feed Sci. Technol. 11:103-111.