



Influence of Corn Steep Liquor on Feeding Value of Urea Treated Wheat Straw in Buffaloes Fed at Restricted Diets

Mahr-un-Nisa¹, M. Ajmal Khan^{1,2}, M. Sarwar^{1*}, W. S. Lee², H. J. Lee², K. S. Ki², B. S. Ahn² and H. S. Kim²

¹Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT : Influence of different levels of corn steep liquor (CSL) on chemical composition of urea treated wheat straw (UTWS), ruminal characteristics, digestion kinetics, nitrogen (N) utilization, and nutrient digestibility by ruminally-cannulated buffalo bulls was studied in a 4×4 Latin Square Design. The CSL was used to ensile 5% UTWS at the rate of 0, 3, 6, and 9% on a dry matter (DM) basis. Total N and neutral detergent fibre (NDF) were increased with increasing level of CSL. Increased NDF content was attributable to increased neutral detergent insoluble nitrogen. Four diets were formulated to contain 20% concentrate and 80% UTWS ensiled with 0, (control), 3 (CSL3), 6 (CSL6), and 9% CSL (CSL9). All diets were mixed daily and fed at 1.5% of body weight twice daily. Ruminal NH₃ concentration decreased with level of CSL used to ensile UTWS at 3 and 6 h *post prandial*, however, at 9 h *post prandial* it was similar across all diets and at 12 h *post prandial* was higher with diets containing UTWS ensiled with CSL. Concentrations of total ruminal volatile fatty acid and acetate were increased with the CSL level used to ensile UTWS. Increased rate of disappearance and reduction in lag time of DM and NDF was recorded with diets containing UTWS ensiled with CSL. Dry matter and NDF digestibilities were higher with CSL diets than on the control diet. Buffalo bulls retained more N with diets containing UTWS ensiled with CSL. The present results indicated that UTWS could be ensiled with CSL to improve its nutritive value and N utilization by ruminants. (**Key Words :** Urea, Corn Steep Liquor, Wheat Straw, Digestibility, Digestion Kinetics, Nitrogen Balance, Buffalo)

INTRODUCTION

Ruminants in developing countries are largely being fed on fibrous crop residues. Protein deficiency and low digestibility of crop residues often restrict animal productivity (Khan et al., 2006a). Physical, chemical, and biological treatments have been developed to weaken and break lingo-cellulosic bonds in crop residues, thereby increasing their nutritive value (Khan et al., 2006b; Nisa et al., 2006). Alkali, ammonia, and urea treatments have received much attention; however urea, which has high nitrogen (N) contents, was more cost effective (Sarwar et al., 2005a).

Usage of urea as a source of NH₃ is not perfect method because only 30 to 35% of NH₃ released from urea is retained in the straw (Sarwar et al., 2003; 2004a; Khan et al., 2006c). Out of the retained N much is held as water-soluble

and thus rapidly librated in the rumen and cause nutrient loss at ruminal level (Sarwar et al., 2005b). To overcome these problems, some researchers (Dass et al., 2000; Sarwar et al., 2003) have tried to fix NH₃ in straw by spraying organic acids (formic acids and acetic acid) or inorganic acids (H₂SO₄ and HCl) with different degree of NH₃ fixation. However, fixing NH₃ with acid is costly and hazardous and thus, its use by farmers is impracticable.

Corn steep liquor (CSL) may offer a solution to the problem of escaping NH₃ and poor fermentation of urea treated wheat straw (UTWS). Because, CSL not only contains easily soluble carbohydrates, which can improve fermentation, but it's acidic, pH (3.7) can also help to fix the NH₃ (Sarwar et al., 2004b). Thus, the use of this ingredient can enhance both the fermentation process and NH₃ fixation in the ensiled UTWS. However, scientific evidences regarding CSL effect on the NH₃ fixation in UTWS and its nutritional value in buffalo are limited. Therefore, objectives of this study were to establish the amount of CSL for N fixation in UTWS, and to determine its dietary effects on ruminal characteristics, digestion kinetics, N utilization and digestibility in ruminally

* Corresponding Author: M. Sarwar. Tel: +92-41-9201088, E-mail: ajmals1@hotmail.com

²Dairy Science Division, National Livestock Research Institute, Korea.

Received March 3, 2005; Accepted May 24, 2006

Table 1. Chemical composition of corn steep liquor (DM basis)

Items	Concentrations
Dry matter (%)	50±2.31
Crude protein (%)	40±2.10
Ash (%)	10±0.43
Nitrogen free extract (%)	16±1.10
pH	3.7±0.11
Lactic acid (%)	21±1.22
Specific gravity	1.25±0.01

cannulated buffalo bulls.

MATERIALS AND METHODS

Corn steep liquor

The CSL for this study was procured from Rafan Maize Products Ltd. Faisalabad, Pakistan. CSL derived from the enzymatic conversion of cornstarch. The steeping of corn is a necessary prerequisite to the fractionation of corn components in the wet-milling process. It involves the countercurrent flow of water, initially containing some SO₂, and dried corn in a number, around 10, of steeping tanks at 50-55°C over a period of approximately 30 h. It follows that the fresh, dried corn enters the tank that contains the steep water from exposure to the partially steeped corn in the previous tanks. The volume of steep water produced in the corn wet-milling industry is large. It is primarily handled by evaporation to concentrated thick liquor that is a complex mixture of carbohydrates, amino acids, peptides, organic compounds, inorganic ions, and *myo*-inositol phosphates (Table 1).

Treatment of wheat straw

Wheat straw was treated with 5 kg urea (50% moisture level) and was ensiled with 0, 3, 6, and 9% CSL on DM basis in four cemented pits (each pit was 3×10×2 m) at 30-35°C for fifteen days. Each pit was covered with a 10-cm-thick layer of rice straw, followed by a plastic film covering, which was plastered with a blend of wheat straw and mud to avoid any cracking on drying. When the feed was used, the plastic film was removed and the feed was withdrawn starting with the upper layer and working downwards to the lower layers. An amount of the fermented straw was taken out just sufficient for one day's feeding and the plastic film was put back to keep the pit sealed. The samples of this fermented wheat straw were analyzed for dry matter (DM), organic matter (OM), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), N and ash by the methods of AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by methods described by Van Soest et al. (1991).

Table 2. Ingredients and chemical composition of the diets (DM basis)

Ingredients	Diets			
	Control	CSL3	CSL6	CSL9
Wheat straw	80	80	80	80
Molasses	8	8.5	9	9
Wheat screenings	8	8.5	8.5	9
Mineral mixture ¹	2	2	2	2
Urea	2	1	0.5	0
Chemical composition ²				
Crude protein (%)	12.16	12.16	12.15	12.16
Neutral detergent fibre (%)	69.52	69.61	69.61	69.71
Acid detergent fibre (%)	45.0	45.2	45.5	45.8
Ash (%)	13.37	13.51	14.63	14.53
NE _L (MJ/kg)	4.59	4.61	4.62	4.64

Control, CSL3, CSL6 and CSL9 diets contain 5% urea treated wheat straw ensiled with 0, 3, 6, and 9% corn steep liquor, respectively.

¹ Mineral mixture contained per gram 226 mg Ca, 100 mg P, 91 mg Mg, 45 mg Zn, 14 mg Cu, 0.6 mg Co, and 1 mg I, 0.4 mg Se.

² Chemical composition is calculated.

Animals and diets

Four buffalo bulls of average weight 350 kg fitted with ruminal cannulae were used in a 4×4 Latin Square Design. Four diets were formulated to contain 20% concentrate and 80% UTWS ensiled with 0 (control), 3 (CSL3), 6 (CSL6), and 9% CSL (CSL9). Urea was included to make all the diets iso-nitrogenous (Table 2). All diets were mixed daily and fed twice daily at 1.5% of body weight per day in two frequencies. During the experimental period, the animals were housed on a concrete floor in separate pens.

The animals were given 20 days for adaptation to diets at the start of each experimental period, followed by a 10-day collection period. Feed offered andorts were weighed and recorded twice daily. For first 3 days, of each collection period, the ruminal contents (liquid plus digesta) were sampled (500 ml) at 3, 6, 9, and 12 h after morning feed. Ruminal pH was measured immediately and samples were squeezed through four layers of cheesecloth. Three ml of 6 N HCl was added to terminate fermentation and samples were frozen. After thawing, these samples were used to determine ruminal NH₃ (Chaney and Marbach, 1962) and volatile fatty acids (VFA). 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 total viable and 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 faeces were made according to the procedures described by Williams et al. (1984). Faeces were collected daily, dried at 55°C, bulked and mixed at the end of each collection period and sampled for analysis. Daily collections of urine were acidified with

Table 3. Influence of varying levels of corn steep liquor (CSL) on chemical composition of urea treated wheat straw

Items	Treatments			
	Control	CSL 3	CSL 6	CSL 9
Total N	1.00	1.50	1.78	2.19
NH ₃ -N	0.68	0.80	0.87	0.88
NDIN ¹	0.42	0.68	0.77	0.94
ADIN ²	0.30	0.31	0.33	0.33
Remainder-N	0.26	0.43	0.45	0.50
NDF ³	74.0	76.6	77.1	78.8
CP-free NDF ⁴	71.7	72.5	73.2	73.1
ADF	47.9	47.7	46.7	45.8
Cellulose	36.4	36.0	35.5	35.0

Control, CSL 3, CSL 6, CSL 9 treatments represents 5% urea treated wheat straw ensiled with 0, 3, 6 and 9% CSL, respectively.

¹ NDIN neutral detergent insoluble nitrogen.

² ADIN: Acid detergent insoluble nitrogen.

³ Neutral detergent fiber.

⁴ Crude protein free NDF was calculated as (NDF-NDIN×6.25).

50% H₂SO₄, stored and then mixed and sampled at the end of each period. On day 5 of each collection period, blood

samples were taken at 3, 6, 9, and 12 h after feeding. Blood samples were analyzed for urea-N (Coulomobe and Favreau, 1963). Feed, orts and faecal samples were dried at 55°C and ground through a Wiley mill (2-mm screen). These samples were analyzed for DM, N, and OM using method described by AOAC (1990), and NDF, ADF, and ADL by methods of Van Soest et al. (1991).

Nylon bag study

The samples being incubated in the rumen were of the same treated wheat straw that was fed to bulls in experimental diets. This was done to avoid the effects of diet on the ruminal fermentation of the feedstuffs (Clark and David, 1990). Nylon bags measuring 13 by 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. Two bags were used to determine DM and NDF disappearance and the third bag served as a blank (having no sample). The bags were closed and tied with braided

Table 4. Ruminal characteristics in buffalo bulls fed diets containing wheat straw with or without corn steep liquor (CSL)

Items	Treatments				SE	Probabilities		
	Control	CSL 3	CSL 6	CSL 9		Linear	Quadratic	Cubic
----- 3 h -----								
NH ₃ -N (mg/dL)	28.8	27.4	25.1	20.3	2.8	0.00	0.04	NS
pH	7.1	6.7	6.5	6.0	0.7	0.00	NS	NS
Total VFA (mM VFA (mol/100 mol))	122.6	129.3	135.3	149.1	12.8	0.00	0.00	NS
Acetate	61.9	61.5	62.7	68.2	6.5	0.00	0.00	NS
Propionate	19.8	19.6	19.9	19.7	2.0	0.00	0.00	NS
Butyrate	7.69	8.0	7.8	8.1	0.8	NS	NS	NS
----- 6 h -----								
NH ₃ -N (mg/dL)	22.8	23.4	20.1	17.3	3.0	0.00	NS	NS
pH	6.7	6.4	5.9	5.8	0.8	0.01	NS	NS
Total VFA (mM VFA (mol/100 mol))	125.6	131.3	136.3	148.4	13.2	0.00	0.03	NS
Acetate	60.1	61.9	63.5	67.9	12.8	0.00	NS	NS
Propionate	18.1	19.2	19.7	19.2	6.5	0.00	0.00	NS
Butyrate	7.89	7.91	8.1	8.4	0.8	NS	NS	NS
----- 9 h -----								
NH ₃ -N (mg/dL)	18.9	18.1	19.7	18.3	1.8	NS	NS	0.00
pH	6.3	6.2	6.4	6.1	0.15	NS	NS	NS
Total VFA (mM VFA (mol/100 mol))	120.4	128.5	130.2	142.3	12.0	0.00	NS	0.02
Acetate	58.9	60.5	60.7	68.2	6.0	0.00	0.02	NS
Propionate	18.8	19.6	19.9	19.7	2.0	0.03	NS	NS
Butyrate	7.68	7.92	7.98	8.3	0.8	NS	NS	NS
----- 12 h -----								
NH ₃ -N (mg/dL)	13.8	15.4	16.8	18.3	1.5	0.00	NS	NS
pH	7.2	7.1	7.1	7.1	0.15	NS	NS	NS
Total VFA (mM VFA (mol/100 mol))	118.5	123.2	126.3	137.9	13.0	0.00	0.03	NS
Acetate	57.9	59.7	59.0	67.5	5.0	0.00	0.01	0.03
Propionate	16.8	17.6	18.1	19.2	2.0	0.00	NS	NS
Butyrate	7.93	7.8	7.9	8.2	0.8	NS	NS	NS

Control, CSL 3, CSL 6, and CSL 9 diets contain 5% urea treated wheat straw ensiled with 0, 3, 6, and 9% CSL, respectively.

Non significant at p = 0.05.

Table 5. Influence of varying levels of corn steep liquor (CSL) on rumen microbial counts in buffalo bulls fed diets containing urea treated wheat straw with or without corn steep liquor

Items	Treatments				Probabilities		
	Control	CSL 3	CSL 6	CSL 9	Linear	Quadratic	Cubic
Total bacterial count per ml	2.6×10^{10}	2.3×10^{10}	2.4×10^{10}	2.1×10^8	0.00	0.00	0.00
Viable bacterial count per ml	2.5×10^8	2.6×10^8	2.5×10^8	2.7×10^7	0.00	0.00	0.00
Cellulolytic count per ml	2.6×10^6	2.5×10^7	2.3×10^7	2.8×10^5	0.00	0.00	0.00

Control, CSL 3, CSL 6, and CSL 9 diets contained 5% urea treated wheat straw ensiled with 0, 3, 6, and 9% CSL, respectively.

Non significant at $p = 0.05$.

Table 6. Influence of varying levels of corn steep liquor on dry matter (DM) and neutral detergent fiber (NDF) digestion kinetics in buffalo bulls fed diets containing urea treated wheat straw with or without corn steep liquor

Items	Treatments				SE	Probabilities		
	Control	CSL 3	CSL 6	CSL 9		Linear	Quadratic	Cubic
DM degradability (%)	49.92	59.83	60.27	64.0	6.0	0.00	0.00	0.01
Rate of disappearance (%/h)	3.98	5.92	6.18	6.51	0.3	0.00	0.01	NS
Lag time (h)	3.19	2.22	1.85	1.13	0.02	0.00	NS	NS
Extent of digestion	64.42	66.64	67.0	67.91	9.0	0.01	NS	NS
NDF degradability (%)	42.12	52.96	55.32	59.30	5.0	0.00	0.04	NS
Rate of disappearance (%/h)	3.12	5.03	5.60	5.98	0.9	0.00	0.00	NS
Lag time (h)	3.62	2.51	2.28	2.16	0.02	0.00	0.01	NS
Extent of digestion	55.8	61.0	62.0	63.5	9.0	0.00	NS	NS

Control, CSL 3, CSL 6, and CSL 9 diets contained 5% urea treated wheat straw ensiled with 0, 3, 6, and 9% CSL, respectively.

Non-significant at $p = 0.05$.

nylon fishing line. To remove soluble and or 50- μ m filterable material, the bags were soaked in a specific amount of tap water for 15 min, just before being incubated. Weight loss due to soaking was expressed as pre-ruminal DM disappearance. On day 6 of each collection period at 08.00 hours 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. 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 Lofton (1980).

Statistical analysis

Data were analyzed as a 4 \times 4 Latin Square Design using the GLM procedure of SAS (1988). The ANOVA and trend comparisons were made to see the linear quadratic and cubic responses. Significance at $p = 0.05$ were used throughout unless otherwise noted.

RESULTS

Chemical composition of UTWS ensiled with or without CSL is given in Table 3. Nitrogen, NDIN and ADIN contents of UTWS were increased linearly ($p < 0.01$) with increasing level of CSL used to ensile UTWS. The percent retained of added N as NDIN was 48, 44, and 48% when UTWS was ensiled with 3, 6, and 9% CSL. The NDF and ADF contents were linearly increased with increasing CSL level. Nitrogen free NDF (NDF-NDIN \times 6.25) and cellulose contents were similar across treatments.

Ruminal NH₃, total volatile fatty acid concentrations and pH were shown in Table 4. Ruminal NH₃ concentration in bulls at 3 and 6 h post parandial was linearly decreased ($p > 0.01$) with increasing level of CSL used to ensile UTWS; at 9 h post parandial it was similar, however, at 12 h post parandial ruminal NH₃ was linearly ($p < 0.01$) increased with increasing CSL level. Ruminal pH values were linearly reduced ($p < 0.01$) at 3 and 6 h post parandial with higher CSL level there after ruminal pH was noted similar across bulls. Concentrations of total ruminal VFA, acetate and propionate were linearly increased ($p < 0.01$) at all sampling times in bulls fed diets containing UTWS ensiled with higher CSL levels. Concentrations of total ruminal, viable and cellulolytic bacteria were linearly increased ($p < 0.01$) with diets containing UTWS ensiled with CSL (Table 5).

Ruminal DM and NDF degradability and rate of disappearance were linearly increased ($p < 0.01$) with increasing CSL level (Table 6). The extent of DM and NDF

Table 7. Nutrient intake and digestion by buffalo bulls fed diets containing urea treated wheat straw with or without corn steep liquor (CSL)

Items	Treatments				SE	Probabilities		
	Control	CSL 3	CSL 6	CSL 9		Linear	Quadratic	Cubic
DM ¹ intake (g/day)	5,110	5,120	5,111	5,112	42.5	NS	0.01	0.00
Apparent DM digestibility	55.12	59.21	62.45	68.12	5.4	0.00	0.00	0.00
OM ² Intake (g/day)	629	623	620	644	16.9	NS	NS	NS
Apparent OM digestibility	64.12	65.45	65.89	68.12	5.6	NS	NS	NS
CP ³ Intake (g/day)	3,552	3,564	3,558	3,564	32.3	NS	NS	NS
Apparent CP digestibility	50.05	55.0	58.12	65.09	5.0	0.00	NS	NS
NDF ⁴ Intake (g/day)	2,299	2,314	2,326	2,341	21.8	NS	0.00	NS
NDF digestibility (%)	45.8	51.7	54.8	61.9	5.8	0.00	NS	NS

Control, CSL 3, CSL 6, and CSL 9 diets contain 5% urea treated wheat straw ensiled with 0, 3, 6, and 9% CSL, respectively.

Non-significant at $p = 0.05$.

¹ Dry matter. ² Organic matter. ³ Crude protein. ⁴ Neutral detergent fibre.

Table 8. Utilization of nitrogen (N) and plasma urea N by buffalo bulls fed diets containing urea treated wheat straw with or without corn steep liquor (CSL)

Items	Treatments				SE	Probabilities		
	Control	CSL 3	CSL 6	CSL 9		Linear	Quadratic	Cubic
Plasma urea-N (mg/dL)	17.2	16.1	15.2	12.2	1.8	0.03	NS	NS
N Intake (g/day)	100.64	99.68	99.21	103.0	0.8	NS	0.01	NS
Fecal N (g/day)	36.16	34.72	33.92	32.8	3.4	NS	NS	NS
Urinary N (g/day)	46.24	43.30	41.80	37.2	3.3	0.00	NS	0.05
N Balance (g/day)	18.24	21.66	23.49	33.0	3.7	0.00	NS	NS

Control, CSL 3, CSL 6, and CSL 9 diets contained 5% urea treated wheat straw ensiled with 0, 3, 6, and 9% CSL, respectively.

Non-significant at $p = 0.05$.

disappearance at 96 h of ruminal incubation (an estimate of extent of digestion) was higher ($p < 0.01$) with higher levels of CSL used to ensile CSL.

Apparent DM digestibility was linearly increased ($p < 0.01$) with diets containing UTWS ensiled with higher CSL level (Table 7). Crude protein digestibility was noted similar across all experimental diets. Linear increase ($p < 0.01$) in NDF and ADF digestibilities were noted with diets containing UTWS ensiled with higher CSL levels.

Nitrogen balance was positive in all bulls; however, the bulls fed diets containing UTWS ensiled with CSL retained higher amount of N (Table 8). Urinary N excretion was reduced with higher CSL level than those; however, fecal N excretion was similar across treatments. Nitrogen balances whether expressed as grams per day, as a percentage of N intakes or, as a percentage of digestible N intakes were linearly ($p < 0.01$) improved with CSL level used to ensile UTWS. When expressed as a percentage of N intakes, plasma urea-N was also affected by treatment.

DISCUSSION

Chemical composition of wheat straw

Higher N content of UTWS ensiled with CSL was because of high lactic acid content of CSL (Table 1). However, the provision of readily available nutrients (carbohydrates, minerals and proteins) for proper

fermentation milieu by CSL might have caused a further drop in pH of UTWS. This reduced pH probably has changed free ammonia (NH_3) released from urea into an ionic form of ammonia (NH_4^+) that is very reactive and has the greater tendency to make bonds with fibrous materials. Consistent with the present findings higher N values in ammoniated straw were reported by different workers who trapped the excess free NH_3 by spraying organic acids (Sarwar et al., 2003), inorganic acids (Taiwo et al., 1995) or using non-structural carbohydrates (Khan et al., 2004; Sarwar et al., 2004a). Increase in NDF content of UTWS was due to increased NDIN because when NDF was calculated on a crude protein (CP) free basis ($\text{NDF} - \text{NDIN} \times 6.25$), its concentration was similar between UTWS ensiled with different levels of CSL. Similar results regarding hemicellulose were obtained when it was calculated on CP-free basis ($\text{NDF} - \text{NDIN} \times 6.25$)-ADF- ($\text{ADIN} \times 6.25$). In this study, approximately one half of the added N was bound in some form. Consistent with present findings, Lines and Weiss (1996) have reported that ammoniation increased the concentration of NDF and its entire increase was in the hemicellulose fraction and was because of increased NDIN.

Ruminal characteristics

Higher ruminal NH_3 values at 3 and 6 h post prandial with control diet indicated rapid release of N from the

UTWS ensiled without CSL. Increased utilization of urea N by rumen micro-flora and ruminal fermentation explained the decrease in ruminal pH at 9 and 12 h post-pancreal (Nisa et al., 2004). Present results indicated that urea N was fixed in the matrices of cell wall of UTWS ensiled with CSL and released slowly in the rumen. The slow release of ammonia N from UTWS ensiled with CSL was beneficial in keeping the ruminal NH_3 concentrations below wasteful levels (Nisa et al., 2004; Sarwar et al., 2004b). Increased ruminal VFA concentration with diets containing UTWS ensiled with CSL was because of increased degradability of DM and NDF in the rumen (Table 6). However, it may be suggested that changes occurred in the cell wall structure after ensiling UTWS with CSL, making more structural carbohydrates available for microbial fermentation in the rumen (Sarwar et al., 2006). Ensiling UTWS with fermentable carbohydrates brought physiochemical changes in straw and thus alleviated those factors that hindered fibre fermentation (Khan et al., 2006b).

Microbial count

Ruminal microbial response indicated more constant supply of NH_3 for ruminal microbial growth with diets containing UTWS ensiled with CSL. However, availability of carbon skeleton or energy for microbial growth might have differed in bulls fed different experimental diets. It has been proposed that the energy usually was a limiting nutrient for growth of ruminal microbes (Sarwar et al., 1991). It may be suggested that changes occurred in the cell wall structure after ensilation of UTWS with CSL, making more structural carbohydrates available for microbial fermentation in the rumen. Moreover, ensilation of UTWS with CSL retained most of the urea N as NDIN that was released slowly (Table 3) and thus synchronized with fibre fermentation to maximize the bacterial growth in bulls fed UTWS ensiled with CSL.

Digestion kinetics

Higher ruminal DM and NDF degradabilities and rate of disappearance of UTWS ensiled with CSL was due to increased surface area fermentability index (SAF_i) when calculated according to Fisher et al. (1989). Bhat and Bansil (1999) reported one of the constraints to NDF degradation either by enzymatic or chemical systems was the extensive hydrogen bonding that occurred in micro fibrils to give cellulose a crystalline nature. Increased NH_3 -N retention in UTWS ensiled with CSL might have sponify esters bonds between lignin and hemicellulose and saturates H-bonds linking the matrix polysaccharides. The NH_3 may affect the bonds linking the macromolecules, thus altering the physical rather than the chemical structure of cell wall. Similar results were reported by Sarwar et al. (2003) when they fed UTWS ensiled with CSL or acidified molasses to buffalo bulls.

Digestibility

Increased DM and NDF digestibilities with CSL diets were because of increased rate of degradation and shorter lag time of these fractions (Table 7) or increased SAF_i of UTWS ensiled with CSL that had increased the fragility of UTWS. The increased surface area of lingo-cellulose resulted in increased accessibility to microbial attack (Sarwar et al., 2004) and ammonolysis of uronic ester cross-links in the cell wall. In present study, increased NH_3 retention in UTWS ensiled with CSL might have broken either linkages between lignin and cellulose or hemicellulose, and increase extent and rate of cellulose and hemicellulose digestion (Nisa et al., 2005).

Nitrogen utilization

Higher plasma urea-N concentration with control diet was because of higher concentration of soluble N in UTWS ensiled without CSL (Table 3) that released rapidly in the rumen (Table 4). The lower blood urea N in buffalo bulls fed diets containing UTWS ensiled with CSL implies slower release of NH_3 . The slower release of fibre bound N from UTWS ensiled with CSL might have synchronized with fibre fermentation and thus utilized by the rumen micro-flora. The present results indicated that UTWS ensiled with CSL was effective in enhancing utilization of N by minimizing N loss.

CONCLUSION

Ensiling UTWS with CSL increased the N fixation in the matrix of cell wall thus slowing its release at ruminal NH_3 that maximized N synchronization with carbon skeleton, which consequently minimized N loss. Ensilation of UTWS with 9% CSL seems more effective to improve nutritive value of wheat straw. However, further research is warranted to see the effect of urea plus CSL treated wheat straw in lactating animals.

ACKNOWLEDGMENT

Authors are highly thankful to Rafan Maize Products Limited, Faisalabad, Pakistan on providing CSL for this project.

REFERENCE

- AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists. Arlington, Virginia, USA.
- Bhat, P. N. and P. C. Bansil. 1999. Grains, roughage production, and thier utilization in Asian Australian region. *Asian-Aust. J. Anim. Sci.* 12:481-486.
- Chaney, A. L. and L. P. Marbach. 1962. Modified reagent for the determination of urea and ammonia. *Clin. Chem.* 8:130-137.
- Clark, J. H. and C. L. David. 1990. Some aspects of feeding high

- producing dairy cows. *J. Dairy Sci.* 68:873-879.
- Coulombe, J. J. and L. Favreau. 1963. A new semi micro method for colorimetric determination of urea. *Clin. Chem.* 9:102-108.
- Dass, R. S., U. R. Mehra and A. K. Verma. 2000. Nitrogen fixation and *in situ* dry matter and fiber constituent disappearance of wheat straw treated with urea and boric acid in murrh buffaloes. *Asian-Aust. J. Anim. Sci.* 13:1113-1118.
- Fisher, D. S., J. C. Burns and K. R. Pond. 1989. Kinetics of *in vitro* cell wall disappearance and *in vivo* digestion. *Agronomy J.* 81:25-32.
- Khan, M. A., M. Sarwar, M. Nisa and M. S. Khan. 2004. Feeding value of urea treated corncobs ensiled with or without enzyme (corn dextrose) for lactating cross cows. *Asian-Aust. J. Anim. Sci.* 17:1093-1097.
- Khan, M. A., M. Sarwar, M. Nisa, S. A. Bhatti, Z. Iqbal, W. S. Lee, H. J. Lee, H. S. Kim and K. S. Ki. 2006a. Feeding value of urea treated wheat straw ensiled with or without acidified molasses in *Nili-Ravi* buffaloes. *Asian-Aust. J. Anim. Sci.* 19:645-650.
- Khan, M. A., Z. Iqbal, M. Sarwar, M. S. Nisa, M. Khan, H. J. Lee, W. S. Lee, H. S. Kim and K. S. Ki. 2006b. Urea treated corncobs ensiled with or without additives for buffaloes: Ruminal characteristics, digestibility and nitrogen metabolism. *Asian-Aust. J. Anim. Sci.* 19:705-712.
- Khan, M. A., M. Sarwar, M. Nisa, Z. Iqbal, M. S. Khan, W. S. Lee, H. J. Lee and H. S. Kim. 2006c. Chemical composition, *in situ* digestion kinetics and feeding value of Oat grass (*Avena sativa*) ensiled with molasses for *Nili-Ravi* Buffaloes. *Asian-Aust. J. Anim. Sci.* 19:1127-1133.
- 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.* 79:1992-1998.
- Mertens, D. R. 1977. Dietary fiber components: relationship to the rate and extent of ruminal digestion. *Feed Proc.* 36:187-199.
- Mertens, D. R. and J. R. Lofton. 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. 2004. 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 *Nili-Ravi* buffalo bulls. *Austr. J. Agric. Res.* 55:229-236.
- Nisa, M., N. A. Touqir, M. Sarwar, M. A. Khan and M. Akhtar. 2005. Effect of additives and fermentation periods on chemical composition and *in situ* digestion kinetics of Mott grass (*Pennisetum purpureum*) silage. *Asian-Aust. J. Anim. Sci.* 18:812-815.
- Nisa, M., M. A. Khan, M. Sarwar and M. Mushtaque. 2006. Influence of re-growth interval on chemical composition, herbage yield, digestibility and digestion kinetics of *Setaria sphacelata* and *Cenchrus ciliaris* in Buffaloes. *Asian-Aust. J. Anim. Sci.* 19:381-385.
- Olumeyan, D. B., T. G. Nagaraja, G. N. Miller, R. A. Frey and L. E. Boyer. 1986. Ruminal changes in cattle feed diets with or without salinomycin. *Applied Environ. Micro.* 51:340.
- Sarwar, M., J. L. Firkins and M. L. Estridge. 1991. Effect of replacing neutral detergent fibre of forage with soy hulls and corn gluten feed for dairy heifers. *J. Dairy Sci.* 74:1006.
- 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.
- Sarwar, M., M. A. Khan and M. Nisa. 2004a. Effect of organic acids or fermentable carbohydrates on digestibility and nitrogen utilization of urea treated wheat straw in buffalo bulls. *Aust. J. Agric. Res.* 55:223-228.
- Sarwar, M., M. A. Khan and M. Nisa. 2004b. Influence of ruminally protected fat and urea treated corncobs on nutrient intake, digestibility, milk yield and its composition in *Nili-Ravi* buffaloes. *Asian-Aust. J. Anim. Sci.* 17:86-93.
- Sarwar, M., M. A. Khan and M. Nisa. 2005a. Chemical composition and feeding value of urea-treated corncobs ensiled with additives for sheep. *Aust. J. Agric. Res.* 56:685-690.
- Sarwar, M., M. A. Khan, M. Nisa and N. A. Touqir. 2005b. Influence of berseem and lucerne silages on feed intake, nutrient digestibility and milk yield in lactating *Nili* Buffaloes. *Asian-Aust. J. Anim. Sci.* 18:475-478.
- Sarwar, M., M. Nisa, M. A. Khan and M. Mushtaque. 2006. Chemical composition, herbage yield, and nutritive value of *Panicum antidotale* and *Pennisetum orientale* for *Nili* buffaloes at different clipping intervals. *Asian-Aust. J. Anim. Sci.* 19:176-180.
- Statistical Analysis System (SAS). 1988. 'SAS user's guide: Statistics' (SAS Inst. Inc., Cary, 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.
- Streeter, C. L. and G. W. Horn. 1980. Crop residue management in livestock production and conservation system. The use of crop residues as feedstuffs for ruminant animals. Okla. Agric. Experiment Station Research Report. pp. 24-37.
- Suto, T. 1973. Rumen no kensa (Examination of the rumen), Proceedings of the methods in the clinical examination of the bovine (Ed. R. Nakamura, T. Yonemura and T. Suto). Ushino Rinsho Kensaho pp. 39 (Nosangyoson Bunka Kyokai, Tokyo).
- 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.
- Williams, P. E. V., G. M. Innes and A. Brewer. 1984. Ammonia treatment of straw via the hydrolysis of urea. Effect of dry matter and urea concentrations on the rate of hydrolysis of urea. *Anim. Feed Sci. Tech.* 11:103.