



## Replacing Concentrate with Wheat Straw Treated with Urea Molasses and Ensiled with Manure: Effects on Ruminal Characteristics, *In situ* Digestion Kinetics and Nitrogen Metabolism of *Nili-Ravi* Buffalo Bulls

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**ABSTRACT :** To evaluate the effects of replacing concentrate with urea molasses treated fermented wheat straw (FWS) ensiled with cattle manure (CM) on ruminal characteristics, *in situ* digestion kinetics and nitrogen (N) metabolism was studied in *Nili Ravi* cannulated buffalo bulls in a 4×4 Latin Square Design. Wheat straw treated with urea (4%) and molasses (6%) was ensiled with cattle manure (CM) (70:30) and fermented for 40 days. Four iso-nitrogenous and iso-caloric diets were formulated. In the FWS0, FWS10, FWS20 and FWS30 diets 0, 10, 20 and 30% of the concentrate was replaced with FWS, respectively. Daily intake by bulls was restricted to 1.5% dry matter (DM) of body weight. Ruminal ammonia nitrogen concentration was greater ( $p<0.05$ ) in bulls fed FWS diet than for those fed FWS0 diet at 3, 6, 9 and 12 h post-parandial. Bulls fed FWS 20 and FWS 30 diets had higher ruminal pH at 3 and 6 h post-parandial than bulls fed FWS10 and FWS0. Ruminal total volatile fatty acid (VFA) concentrations 3 h post-parandial were greater ( $p<0.05$ ) in bulls fed FWS0 than those fed FWS diets. However ruminal VFA tended to increase at 6, 9 and 12 h post-parandial as the level of FWS increased. *In situ* ruminal DM and neutral detergent fiber (NDF) degradation, rates of disappearance and extent of digestion were higher ( $p<0.05$ ) for bulls fed FWS30 diet than those fed FWS0. Ruminal DM and NDF lag time tended to decrease ( $p<0.05$ ) as FWS concentration in the diet increased. Feed intake, nitrogen intake, N-balance and blood urea-N did not differ ( $p>0.05$ ) in buffalo bulls fed different diets. Wheat straw treated with urea and molasses and ensiled with CM enhanced the nutritive value of wheat straw and improved nutrient utilization in buffalo bulls when up to 30% of the concentrate was replaced with FWS; no adverse effects on ruminal characteristics and nutrients digestibilities were detected. (Key Words : Wheat Straw, Cattle Manure, Ruminal Characteristics, Nitrogen Balance, Buffalo Bulls)

### INTRODUCTION

The increasing demand for cereal grains and increasing grain prices coupled with the reduction in land for fodder cultivation are severely reducing the nutrient supply for ruminants in developing countries (Shahzad et al., 2010), yet, supply of dry roughages often is abundant for livestock feeding throughout the year (Shahzad et al., 2009a). Because roughages remain in rumen longer due to high concentrations of indigestible fiber and lignin, intake is reduced and this impedes ruminant productivity (Sarwar et al., 2002). Ruminant productivity can be improved by

enhancing the nutritive value of low quality crop residues (Shahzad et al., 2009b). Various substances have been tested for treatment of dry roughages. These include urea/ammonia ( $\text{NH}_3$ ; Sarwar et al., 1994), alkali (Sarwar et al., 1985; Sarwar et al., 1992; Ali et al., 1993), urea plus corn steep liquor (CSL; Nisa et al., 2004) and organic acids (Sarwar et al., 2004).

Cattle manure (CM) generally is considered useless feed resource despite its high crude protein (CP; 8-18%) content (Jakhmola et al., 1988). However, recycling of animal waste in animal feed can not only conserve nutrients but also postpone the environmental pollution caused by these wastes (Mason et al., 1988). Numerous processing methods can increase nutritional value of poor quality feedstuffs. These include ensiling, dehydration, single cell protein production, pelleting, deep stacking, chemical preservation

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and chemical enhancement of digestibility, ensiling is considered the most economical process (Smith et al., 1979).

Anaerobic fermentation of poor quality crop residues with CM can improve the nutritional value of crop residues (Khan et al., 1992; Martínez-Avalos et al., 1998). Potential pathogens in animal waste are destroyed during fermentation (Caswell et al., 1975) and ensiling can enhance nutrient availability of low quality crop residues (Cornman et al., 1981; Khan et al., 1992) and improves its feeding value (Reddy and Reddy, 1989; Khan et al., 1992). Including low quality roughages fermented with CM in ruminant diet has had no adverse effects on nutrient intake (Smith and Lindahl, 1978) or digestibility (Martínez-Avalos et al., 1998).

In laboratory silos, anaerobic fermentation of wheat straw (WS) treated with urea and molasses and ensiled with CM for 40 days increased protein and decreased fiber contents of WS (unpublished data). However, scientific information regarding the feeding value of this product for buffalo is lacking. The present study was designed to evaluate the effect of replacing concentrates with different levels of this fermented (FWS) product on intake and digestibility, ruminal characteristics, *in situ* digestion kinetics and nitrogen (N) metabolism in *Nili-Ravi* buffalo bulls.

## MATERIAL AND METHODS

### Preparation of fermented wheat straw

Urea (4% as fed basis) and molasses (4% as fed basis) solutions were uniformly sprinkled and thoroughly mixed with WS. Once the wheat straw was treated with urea and molasses it was thoroughly mixed with CM in the ratio of 70:30. This mixture was packed in cement pits ( $5 \times 2 \times 3$  m) and allowed to ferment for minimum of 40 days prior to feeding. Pits were covered with a layer of rice straw 10 cm thick and a plastic film that was plastered with a blend of WS and mud to avoid cracking during drying. The plastic film and mud plastering ensured anaerobic fermentation conditions. After 40 days, the plastic film was removed and a sample of FWS was withdrawn. Following sampling, the

plastic film was replaced to keep the pit sealed. The sample of FWS was analyzed for dry matter (DM), crude protein (CP), true protein (TP), NPN, ash (AOAC, 1990), acid detergent fiber (ADF) (Goering and Van Soest, 1970) and neutral detergent fiber (NDF; Van Soest et al., 1991). The net energy for lactation (NE<sub>L</sub>) was calculated by the equations developed by Conrad et al. (1984). Chemical compositions of WS, CM, molasses and FWS are given in Table 1.

### Animals, diets and data collection

Four *Nili-Ravi* buffalo bulls weighing  $350 \pm 30$  kg, fitted with ruminal cannulae were used to evaluate the effects of FWS in a  $4 \times 4$  Latin Square Design (LSD). Four iso-nitrogenous and iso-caloric diets were formulated. To form FWS 0, FWS 10, FWS 20 and FWS 30 diets, concentrate was replaced with FWS at the rate of 0, 10, 20 and 30%, respectively (Table 2). Intake by animals was restricted to 1.5% of body weight with feed offered twice each day. During the experiment, bulls were housed on a concrete floor in separate pens.

Animals were given 10 days for adaptation at the start of each period, followed by a 7-day fecal collection period. Feed offered andorts were weighed and recorded daily. Feces, collected daily, were combined and mixed at the end of each collection period and sampled (10% aliquot) for analysis. Urine acidified with 50% H<sub>2</sub>SO<sub>4</sub> was collected daily, mixed and sampled at the end of each period (Sarwar et al., 2004). Feed,orts and fecal samples were dried at 55°C and ground through a Wiley mill (Arthur H. Thomas, Philadelphia) with a 2 mm screen.

On days 11 and 12 of each collection period, ruminal contents were sampled (500 ml) at 3, 6, 9, and 12 h after the morning feeding, samples were strained through four layers of cheesecloth. After mixing, 50 ml was retained and the remaining 450 ml was returned to the rumen. The ruminal liquor sample was chilled to 5°C and transported to the laboratory for ruminal pH (Ruiz et al., 2001), NH<sub>3</sub> (Broderick and Kang, 1980) and volatile fatty acids (VFA) analyses. On day 11 of each sampling period, blood samples

**Table 1.** Chemical composition of wheat straw, molasses, cattle manure and fermented wheat straw

Nutrients (%)	Wheat straw	Molasses	Cattle manure	Fermented wheat straw
Dry matter	90.70	70.24	20.10	56.96
Crude protein	2.90	3.00	8.21	15.18
True protein	0.28	-	2.97	10.11
Non-protein-N	0.42	-	0.84	0.81
Neutral detergent fiber	85.10	-	47.21	38.22
Acid detergent fiber	51.21	-	39.54	26.45
Ash	5.15	8.11	11.16	16.53
NE <sub>L</sub> (Mcal/kg) <sup>1</sup>	1.09	1.11	1.30	1.52

<sup>1</sup> Net energy for lactation.

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

	FWS0	FWS10	FWS20	FWS30
Ingredients	----- Ingredients, % -----			
Wheat straw	30.0	30.0	30.0	30.0
Fermented wheat straw	0.0	10.0	20.0	30.0
Canola meal	20.0	17.0	12.0	8.0
Rice polish	21.0	14.0	14.0	10.0
Molasses	21.0	21.0	16.0	14.0
Wheat bran	7.0	7.0	7.0	7.0
Dicalcium phosphate	0.5	0.5	0.5	0.5
Urea	0.5	0.5	0.5	0.5
Chemical analysis (% DM)				
Dry matter	87.39	83.97	81.29	78.17
Organic matter	79.65	79.67	79.63	79.70
Crude protein	16.06	15.87	16.14	15.97
True protein	9.89	10.91	12.21	13.20
Neutral detergent fiber	41.63	41.21	42.98	42.87
Acid detergent fiber	21.13	19.99	20.82	20.97
NE <sub>L</sub> (Mcal/kg) <sup>1</sup>	1.61	1.63	1.64	1.63

FWS0, FWS10, FWS20 and FWS30 contained 0, 10, 20 and 30% DM from FWS as a replacement of concentrate; Fermented wheat straw contain 4% urea and 4% molasses treated WS fermented anaerobically with CM (70:30 DM) for 40 days interval.

<sup>1</sup> Net energy for lactation.

were taken from the jugular vein at 3, 6, 9 and 12 h after morning feeding and transferred on ice immediately to the laboratory for blood urea-N (BUN) analysis (Broderick and Kang, 1980).

#### Nylon bag experiment

Four *Nili-Ravi* ruminally cannulated buffalo bulls were used in this 4×4 LSD to evaluate *in situ* DM and NDF digestion kinetics. Various ratios of untreated WS to FWS (100:0, 50:50, 25:75 and 0:100) samples were chopped manually to a particle length of approximately 1 cm and weighed (10 g fresh weight per bag) into 13×21 cm polyester bags (50±5 µm pore size). The bags were closed and tied with braided nylon fishing line. To remove soluble and or 50 µm filterable material, bags were soaked in tap water for 15 minutes immediately before ruminal incubation. Triplicate bags were incubated in ventral rumen of each buffalo bull for specific time intervals (0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 h), in reverse order, all were removed all at the same time. Two bags were used to determine DM and NDF disappearance whereas the third bag served as blank.

After removal from the rumen, bags were washed in running tap water until the rinse water was clear. The bags were dried in a forced air oven at 55°C for 48 h. After equilibration with air for 8 h, bags were weighed and residues were transferred to 100 ml cups and stored for later analysis (Sarwar et al., 2004). *In situ* digestion kinetics parameters, i.e., extent of digestion, rate of digestion and

lag time, were determined for each period individually. Degradation rates were determined by subtracting the indigestible residue, i.e., 96 h residue, from the amount in each bag at each time point and regressing natural logarithm of that value against time (Sarwar et al., 1991) after correcting for lag (Mertens, 1977). Lag was calculated according to the method proposed by Mertens and Lofton (1980).

#### Laboratory analysis

Dry matter was determined by drying at 135°C until a constant weight was reached (method 930.15; AOAC, 1990). Protein-N of FWS was analyzed using an acidified extract (20 g of fresh sample in 200 ml of 0.01N HCl, agitated at 21°C for 22 h) that was deproteinized with trichloroacetic acid (TCA; Novozamsky et al., 1974). Nitrogen fractions (Total-N, TCA insoluble-N) followed the Kjeldahl method (955.04; AOAC, 1990). Crude protein was calculated by multiplying %-N by 6.25 (method 984.13; AOAC, 1990). Non protein-N and TP contents of other than FWS were analyzed following methods 991.21 and 991.23, respectively, of AOAC (1990).

Ash was determined as the residue after incineration at 600°C (method 942.05; AOAC, 1990). The OM was calculated as the difference between DM and ash contents. Acid detergent fiber was determined using acetyl-trimethyl ammonium bromide detergent in 0.5 M sulfuric acid (Goering and Van Soest, 1970). Neutral detergent fiber was determined using sodium sulfite and amylase (Van Soest et

al., 1991(method A for NDF)).

Ruminal liquor (50 ml) was centrifuged at 500×g (5 minutes, 5°C) to remove feed particles and protozoa. The sample then was centrifuged at 10,000×g (15 minutes, 5°C) to remove bacteria. A portion of the clarified ruminal fluid (10 ml) was frozen for NH<sub>3</sub> analysis. The remaining clarified ruminal fluid was placed in a 39°C water bath and purged slowly with carbon dioxide (CO<sub>2</sub>) for 15 minutes. The pH of the clarified and CO<sub>2</sub> equilibrated ruminal fluid was determined with a combination electrode (Ruiz et al., 2001). Samples for NH<sub>3</sub> were precipitated with 65% TCA (5% final concentration) and stored on ice for 30 minutes and then centrifuged at 28,000×g (15 minutes, 4°C); the supernatant fluid was frozen until analyzed for NH<sub>3</sub> (Broderick and Kang, 1980). Ruminal VFA were quantified by the method described by Sarwar and Nisa (1999).

Plasma was collected by centrifuging heparinized blood at 1,500×g for 40 minutes and stored at -4°C until being analyzed for urea. Urea-N in the plasma was determined following conversion of urea to NH<sub>3</sub> with urease. Plasma (0.25 ml) was thawed at room temperature (20°C) and incubated for 10 minutes with 1.5 ml of a urease solution (4.03 U/ml, Sigma Chemical Co). The reaction was terminated by vortexing with 0.15 ml of 65% (wt/vol) TCA and incubation on ice for 30 minutes. After centrifugation at 21,000×g for 10 minutes, urea in the supernatant was determined (Broderick and Kang, 1980).

### Statistical analysis

Data were analysed as 4×4 LSD using the GLM procedure of SAS (1988). The sum of squares of the model was separated into animal and treatment effects. When treatment effects were detected, means were separated by Duncan's multiple range test (Steel and Torrie, 1984).

## RESULTS

### Nutrient digestibilities

Apparent digestibilities of DM, OM, NDF and ADF increased ( $p<0.05$ ) with increased FWS in the diet (Table 5). However, the apparent CP digestibility remained unaffected ( $p>0.05$ ) by level of FWS. The highest DM (64.30%), OM (67.85%), NDF (61.51%) and ADF (54.96%) digestibilities were observed for bulls fed the FWS30 diet.

### Ruminal characteristics

The ruminal NH<sub>3</sub>-N concentrations were higher ( $p<0.05$ ) for bulls fed diets containing FWS than those fed FWS0 at 3, 6, 9 and 12 h post-parandial (Table 3). Ruminal NH<sub>3</sub>-N concentration decreased as post-parandial time increased. The highest ruminal NH<sub>3</sub>-N concentrations were observed for bulls fed FWS20 and FWS30 diets at all hours post-parandially. Ruminal NH<sub>3</sub>-N in bulls fed FWS0 and

FWS10 diets at 9 h post-parandial were not significantly different ( $p>0.05$ ).

Ruminal pH was higher for bulls fed FWS20 and FWS30 diets than those fed FWS 0 and FWS 10 diets at 3 and 6 h post feeding. Ruminal pH remained unaffected for bulls fed different levels of FWS at 9 and 12 h after feeding. Ruminal pH in all bulls fed increased with increasing post parandial time.

Ruminal total VFA concentration was higher ( $p<0.05$ ) at 3 h post-parandial for bulls fed FWS0 than those fed FWS 10, FWS 20 and FWS 30 diets (Table 3). Ruminal total VFA and acetate concentration increased ( $p<0.05$ ) with increasing level of FWS at 6, 9 and 12 h post feeding. Ruminal propionate and butyrate concentrations remained unaltered by FWS level, at all sampling hours.

### In situ digestion kinetics

Ruminal DM and NDF degradabilities, rate of disappearance and extent of digestion were higher ( $p<0.05$ ) for bulls fed FWS 30 compared with those fed FWS 0, FWS 10 and FWS 20 diets (Table 4).

### Nitrogen balance

Nitrogen intake, N-output, N-balance, N-balance as a percent of digestible N-intake and BUN did not differ ( $p>0.05$ ) with FWS levels (Table 6). All bulls were in positive N-balance.

## DISCUSSION

### Nutrients intake and digestibilities

Nutrient intake by bulls fed varying level of FWS was not different because intake was restricted. High apparent DM and NDF digestibilities for bulls fed high levels of FWS reflect high ruminal degradability (Sarwar et al., 1996).

### Ruminal characteristics

High ruminal NH<sub>3</sub>-N in bulls fed FWS diets at all post-parandial sampling times indicated a continual release of NH<sub>3</sub>-N from FWS due to gradual release of fiber bound-N from urea treated straw. Similar results were observed by Sarwar et al. (2004) who stated further that urea-N was fixed in the matrices of cell wall, when urea treated WS was ensiled with corn steep liquor (CSL), N was released slowly in the rumen so that ruminal NH<sub>3</sub>-N concentration remained high even 9 and 12 h post-parandially. High ruminal NH<sub>3</sub>-N concentration at 3 h for bulls fed FWS might reflect reduced conversion of NH<sub>3</sub>-N into bacterial proteins due to rapid release of NH<sub>3</sub>-N by urea hydrolysis. Increased ruminal NH<sub>3</sub>-N concentration in ruminants fed urea treated wheat straw had been reported by several researchers (Manyuchi et al., 1992; Nisa et al., 2004; Sarwar et al., 2004).

**Table 3.** Ruminal characteristics of cannulated buffalo bulls fed diets containing different levels of fermented wheat straw at 3, 6, 9 and 12 h

	FWS0	FWS10	FWS20	FWS30	SE
3 h					
NH <sub>3</sub> -N <sup>1</sup> , mg/dl	18.29 <sup>c</sup>	21.10 <sup>b</sup>	24.35 <sup>a</sup>	26.91 <sup>a</sup>	2.40
pH	6.10 <sup>c</sup>	6.30 <sup>b</sup>	6.60 <sup>a</sup>	6.70 <sup>a</sup>	0.67
Total VFA <sup>2</sup>	149 <sup>a</sup>	139 <sup>b</sup>	135 <sup>b</sup>	132 <sup>b</sup>	12.70
Acetate	66.10 <sup>a</sup>	65.12 <sup>a</sup>	61.30 <sup>b</sup>	60.10 <sup>b</sup>	5.80
Propionate	20.92	19.32	19.01	19.30	1.80
Butyrate	7.90	7.70	7.80	7.70	0.80
6 h					
NH <sub>3</sub> -N, mg/d	17.82 <sup>c</sup>	19.14 <sup>b</sup>	22.40 <sup>a</sup>	23.10 <sup>a</sup>	2.50
pH	6.10 <sup>c</sup>	6.30 <sup>b</sup>	6.60 <sup>a</sup>	6.70 <sup>a</sup>	0.63
Total VFA, mM	145 <sup>b</sup>	140 <sup>b</sup>	143 <sup>b</sup>	147 <sup>a</sup>	11.70
Acetate, mM	61.90 <sup>b</sup>	62.30 <sup>b</sup>	65.90 <sup>a</sup>	66.80 <sup>a</sup>	5.90
Propionate, mM	21.94	18.01	19.11	20.00	2.90
Butyrate, mM	7.80	7.90	8.00	8.10	0.70
9 h					
NH <sub>3</sub> -N, mg/dl	17.32 <sup>b</sup>	19.59 <sup>b</sup>	22.94 <sup>a</sup>	23.50 <sup>a</sup>	2.70
pH	6.40	6.50	6.60	6.50	0.70
Total VFA, mM	138 <sup>c</sup>	140 <sup>b</sup>	147 <sup>b</sup>	149 <sup>a</sup>	11.40
Acetate, mM	60.39 <sup>b</sup>	63.00 <sup>a</sup>	63.91 <sup>a</sup>	64.32 <sup>a</sup>	6.10
Propionate, mM	20.11	19.42	20.11	20.91	2.20
Butyrate, mM	7.70	7.80	7.80	8.00	0.90
12 h					
NH <sub>3</sub> -N, mg/dl	15.00 <sup>c</sup>	18.29 <sup>b</sup>	20.76 <sup>a</sup>	21.00 <sup>a</sup>	1.80
pH	6.50	6.60	6.60	6.70	0.80
Total VFA, mM	125 <sup>b</sup>	138 <sup>a</sup>	141 <sup>a</sup>	144 <sup>a</sup>	12.91
Acetate, mM	59.05 <sup>b</sup>	61.90 <sup>a</sup>	61.77 <sup>a</sup>	62.30 <sup>a</sup>	5.80
Propionate, mM	18.09	18.10	18.46	18.80	2.10
Butyrate, mM	8.00	7.98	8.10	8.04	0.80

Means in the same row followed by the same letter are not significantly different at p = 0.05.

<sup>1</sup> Ammonia nitrogen. <sup>2</sup> Volatile fatty acids.

FWS0, FWS10, FWS20 and FWS30 contained 0, 10, 20 and 30% DM from FWS as a replacement of concentrate.

**Table 4.** Associative effects of untreated wheat straw and fermented wheat straw on dry matter digestion kinetics in buffalo bulls

	WS100	FWS50	FWS75	FWS100	SE
Dry matter					
Degradability (%)	42.30 <sup>c</sup>	54.40 <sup>b</sup>	55.10 <sup>b</sup>	58.92 <sup>a</sup>	5.40
Rate of disappearance (%/h)	3.00 <sup>c</sup>	4.90 <sup>b</sup>	5.10 <sup>b</sup>	5.80 <sup>a</sup>	0.04
Lag time (h)	4.10 <sup>a</sup>	2.90 <sup>b</sup>	3.00 <sup>b</sup>	2.40 <sup>b</sup>	0.03
Extent of digestion <sup>1</sup>	49.80 <sup>b</sup>	65.29 <sup>a</sup>	66.20 <sup>a</sup>	67.98 <sup>a</sup>	6.30
Neutral detergent fiber					
Degradability (%)	41.44 <sup>c</sup>	51.65 <sup>b</sup>	53.15 <sup>b</sup>	57.60 <sup>a</sup>	2.26
Rate of disappearance (%/h)	2.45 <sup>c</sup>	3.65 <sup>b</sup>	4.05 <sup>a</sup>	3.95 <sup>a</sup>	0.25
Lag time (h)	4.50 <sup>a</sup>	3.05 <sup>b</sup>	2.97 <sup>b</sup>	2.45 <sup>c</sup>	0.29
Extent of digestion	49.05 <sup>b</sup>	62.25 <sup>a</sup>	64.15 <sup>a</sup>	66.35 <sup>a</sup>	2.57

<sup>1</sup> Extent of digestion was calculated at 96 h after rumen incubation.

Means in the same row followed by the same letter are not significantly different at p = 0.05.

FWS0, FWS10, FWS20 and FWS30 contained 0, 10, 20 and 30% DM from FWS as a replacement of concentrate.

**Table 5.** Nutrients intake and their digestibilities in buffalo bulls fed diets containing different levels of fermented wheat straw

	FWS0	FWS10	FWS20	FWS30	SE
Intake (kg/d)					
Dry matter	6.00	6.05	6.04	6.01	0.33
Organic matter	4.78	4.82	4.81	4.79	0.45
Crude protein	0.96	0.96	0.97	0.96	0.07
Neutral detergent fiber	2.40	2.42	2.42	2.40	0.13
Acid detergent fiber	1.26	1.21	1.27	1.26	0.07
Digestibility (%)					
Dry matter	59.23 <sup>b</sup>	61.50 <sup>b</sup>	64.32 <sup>a</sup>	64.30 <sup>a</sup>	2.93
Organic matter	62.09 <sup>b</sup>	62.95 <sup>b</sup>	68.00 <sup>a</sup>	67.85 <sup>a</sup>	2.01
Crude protein	66.60	67.08	67.91	68.09	3.65
Neutral detergent fiber	49.21 <sup>c</sup>	56.90 <sup>b</sup>	59.21 <sup>a</sup>	61.51 <sup>a</sup>	2.01
Acid detergent fiber	46.35 <sup>c</sup>	50.79 <sup>b</sup>	53.20 <sup>a</sup>	54.96 <sup>a</sup>	3.43

Means in the same row followed by the same letter are not significantly different at p = 0.05.

FWS0, FWS10, FWS20 and FWS30 contained 0, 10, 20 and 30% DM from FWS as a replacement of concentrate.

Ruminal pH reflects a balance between ruminal volatile fatty acids (VFAs) and NH<sub>3</sub>-N concentration (Mir et al., 1980). Higher ruminal NH<sub>3</sub>-N concentration increased ruminal pH in bulls fed diets containing more FWS. In the present study, replacement of concentrate with FWS in the diet of bulls fed FWS20 and FWS30 diet also might have increased chewing time that also could increase ruminal pH. Increased dietary effective fiber has also been reported to increase rumen pH by increasing salivation rate (Sarwar et al., 1991). Lower rumen pH values in bulls fed FWS0 diet compared to those fed FWS30 diet may be attributed to high level of concentrate (70%) and untreated WS in that diet.

Higher ruminal total VFA in bulls fed FWS30 diet than those fed FWS0 diet reflects greater DM degradation of FWS. Urea treatment in FWS may have increased availability of structural carbohydrates for ruminal microbial fermentation by changing the cell wall structure. These findings are supported by Nisa et al. (2004) who reported a similar trend in ruminal VFA concentration in bulls fed with or without urea treated WS fermented with CSL (3 and 6%). The shorter lag time and greater extent of digestion of these feed fractions also may explain the differences in total VFAs concentration (Sarwar and Nisa,

1999).

#### In situ digestion kinetics

The increased ruminal DM and NDF disappearance and decreased lag time with an increased level of FWS may be due to fermentation changes in WS when ensiled with CM (Z.U. Hasan, unpublished data). Extensive hydrogen bonding in micro-fibrils of WS has been reported to be one of the constraints for degradation either by enzymatic or chemical systems (Sarwar et al., 2010). Disruption of hydrogen bonds ensures better degradation (Bhat and Bansil, 1999). Ammoniation also has been reported to increase the fragility of straw (Zorilla-Rios et al., 1985). The NH<sub>3</sub> may have cleaved linkages between lignin and cellulose or lignin and hemicellulose and thus increased extent and rate of NDF digestion due to cleavage of ester bonds and acetyl groups by chemical treatment (Buettner et al., 1982). Increased DM and OM digestibilities also have been reported in ruminants when fed sugarcane bagasse treated with urea and CM (Khan et al., 1992). Moreover, higher NDF digestibility by bulls fed FWS30 diet may have been due to more optimum cellulolytic microbial activity in higher ruminal pH. These findings match those reported by Cornman et al. (1981) who indicated a linear increase

**Table 6.** Nitrogen balance and blood urea nitrogen in buffalo bulls fed diets containing different levels of fermented wheat straw

Nitrogen (g/d)	FWS0	FWS10	FWS20	FWS30	SE
Intake	153.20	153.60	154.50	153.44	11.90
Fecal-outgo	50.65	50.53	49.58	49.07	5.10
Urinary-excretion	78.88	79.90	80.20	80.50	3.30
Balance	23.67	23.17	24.72	23.87	2.50
Percent of digestible N intake	23.15	22.55	23.50	23.00	1.70
Blood urea nitrogen (mg/dl)	19.30	20.00	19.50	21.01	2.20

Means in the same row followed by the same letter are not significantly different at p = 0.05.

FWS0, FWS10, FWS20 and FWS30 contained 0, 10, 20 and 30% DM from FWS as a replacement of concentrate.

( $p<0.05$ ) *in vitro* DM digestibility when rye straw-cattle waste silage was replaced with cattle waste in diets for cows.

Higher ruminal DM and NDF rate of disappearance, extent of digestion and their shorter lag time for bulls fed more FWS might be attributed to physicochemical changes in FWS during anaerobic fermentation which decreased NDF content and increased CP content. An improved ruminal DM and NDF degradation of low quality roughages following urea/ $\text{NH}_3$  treatment is well documented (Thorlacius and Robertson, 1984; Nisa, 2006).

Extent of degradation by rumen microbes varies with cell surface structure, wall thickness and lignin (Wilson and Mertens, 1995); this can explain the longer lag time for diets containing untreated WS compared to FWS. The increased N-retention in WS ensiled with CM might have increased fiber degradation by reducing the number of residual ester linkages in straws (Dias-da-Silva and Sundstol, 1986).

### Nitrogen balance

The lack of difference in N-intake and N-output (fecal and urinary-N) for bulls fed various level of FWS may reflect restricted feed intake. Similar blood urea nitrogen (BUN) concentrations across all diets indicate efficient utilization of  $\text{NH}_3\text{-N}$  by rumen microbe with restricted feed intake. Nisa et al. (2006) reported that high DM fermentation in bulls fed urea treated WS ensiled with fermentable sugars that supplied carbon skeleton and energy for microbial growth to maximize the bacterial growth when synchronized with  $\text{NH}_3\text{-N}$  released by protein or NPN hydrolysis in the rumen. In the present study, high release of  $\text{NH}_3$  and high DM degradation for bulls fed more FWS might have caused BUN differences to be non-significant.

### CONCLUSION

Fermentation of urea molasses treated WS ensiled with CM not only increased the nutritive value of WS but also improved nutrient utilization by bulls fed diets from which up to 30% the concentrate was replaced with FWS.

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