Nitrogen Retention and Chemical Composition of Urea Treated Wheat Straw Ensiled with Organic Acids or Fermentable Carbohydrates

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ABSTRACT : The influence of varying levels of urea and additives on nitrogen (N) retention and chemical composition of wheat straw was studied. The wheat straw was treated with 4, 6 and 8% urea and ensiled with 1.5, 2 and 2.5% of acetic or formic acid and 2, 4 and 6% of corn steep liquor (CSL) or acidified molasses for 15 days. The N content of wheat straw was significantly different across all treatments. The N content of urea treated wheat straw was increased with the increasing level of urea. The N content was higher in urea treated wheat straw ensiled with acetic or formic acid as compared to urea treated wheat straw ensiled without these organic acids. The N content of urea treated wheat straw was further enhanced when it was ensiled with CSL or acidified molasses. This effect was significant across all levels of urea used to treat the wheat straw. Nitrogen retention in urea treated wheat straw was decreased linearly as the urea level was increased to treat the wheat straw. The N content was increased linearly when higher levels of CSL or acidified molasses were used to ensile the urea treated wheat straw. Most of the N in urea treated wheat straw was held as neutral detergent insoluble N (NDIN). The NDIN content was increased linearly with the increasing levels of urea and additives. The neutral detergent fiber (NDF) contents were higher in urea treated wheat straw ensiled with acetic or formic acid as compared to urea treated wheat straw ensiled without additive. The NDF content further increased in urea treated wheat straw ensiled with CSL and acidified molasses. The entire increase in NDF content was because of fiber bound N. The hemicellulose content of urea treated wheat straw ensiled with CSL or acidified molasses was higher as compared to urea treated wheat straw ensiled with acetic or formic acid. The acid detergent fiber content of urea treated wheat straw ensiled with or without additives remained statistically non-significant. The cellulose contents of wheat straw was linearly reduced when urea level was increased from 4 to 6 and 8% to treat the wheat straw. This effect was further enhanced when urea treated wheat straw was ensiled with different additives. The results of the present study indicated that fermentable carbohydrates might improve the Nitrogen retention and bring the favorable changes in physiochemical nature of wheat straw. However, biological evaluation of urea treated wheat straw ensiled with fermentable carbohydrates is required. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1583-1592)***

Key Words : Wheat Straw, Urea, Organic Acids, Fermentable Carbohydrates, Nitrogen Retention, Chemical Composition

INTRODUCTION

Ruminants in developing regions of the globe are heavily relying upon fibrous crop residues (Sarwar and Nisa, 1999; Mehra et al., 2001; Sarwar et al., 2002). It is well documented that protein and energy deficiency in combination with low digestibility of crop residues often restrict ruminant productivity (Sarwar et al., 1996; Sarwar and Nisa, 1998; Man and Wiktorsson, 2001; Khan et al., 2002). A number of experiments (Borhami et al., 1982; Hartley et al., 1985; Dias-Da-Silva et al., 1988; Mason et al., 1988; Cann et al., 1991; Zhen et al., 1995) have been undertaken to investigate the effect of ammoniation through urea or NH_3 treatment on the chemical composition of fibrous crop residues. Many workers adopted various procedures, moisture levels, temperature ranges, urea levels and additives to optimize the ammoniation process for maximum urea-nitrogen retention and other favorable nutritive changes in the treated fibrous material that best suit to improve ruminal functions. Liu et al. (1991, 1995) and Xu et al. (1994) reported that rice straw treated with NH_3 almost doubled its nitrogen (N) contents.

Cann et al. (1991) reported that ammoniation had increased the fiber-bound N. However, the largest N portion of the treated straw was free NH_3-N, which accounted for about 72% of the retained N. The increase in fiber-bound N and total N were consistent as reported by Nelson et al. (1984), Cann et al. (1991) and Brown et al. (1987). However, it was well documented that the urea treated wheat straw only retained about 30-35% of NH_3 added to the straw during treatment and the remaining 65-70% was lost to the atmosphere (Saadullah et al., 1981; Ali et al., 1997). To overcome this problem, some researchers have tried to fix the excess NH_3 in the straw by spraying some organic acids (Borhami et al., 1982) or inorganic acids (Dass et al., 2001) and more recently with fermentable sugar sources with different degree of NH_3 fixation (Nisa et al., 2002).

However, the scientific evidence regarding the effect of organic acids and fermentable sugar resources on N retention and chemical composition of urea treated wheat straw was limited. This study was planned to see the effect of varying levels of urea application to wheat straw and

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Table 1. Total nitrogen content* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	1.11±0.00[V]	1.38±0.01[U]	1.49±0.01[S]
Acetic acid	1.5	1.64±0.04[QR]	1.71±0.01[OPQ]	1.70±0.01[OPQ]
	2	1.74±0.04[OP]	2.24±0.01[FG]	2.21±0.02[HI]
	2.5	1.81±0.00[MN]	2.42±0.01[FG]	2.70±0.01[C]
Formic acid	1.5	1.63±0.02[R]	1.76±0.01[NO]	1.67±0.01[PQR]
	2	1.64±0.04[QR]	2.19±0.01[HIJ]	2.24±0.01[H]
	2.5	1.76±0.01[NO]	2.47±0.01[F]	2.63±0.02[D]
Molasses	2	1.82±0.02[MN]	1.83±0.01[M]	1.97±0.01[L]
	4	1.97±0.01[L]	2.40±0.01[G]	2.60±0.02[D]
	6	2.13±0.01[J]	2.80±0.01[B]	2.91±0.01[A]
Corn steep liquor	2	1.85±0.03[M]	1.97±0.01[L]	2.06±0.01[K]
	4	2.13±0.02[IJ]	2.53±0.00[E]	2.66±0.01[CD]
	6	2.16±0.01[G]	2.90±0.01[A]	2.97±0.02[A]

* Values are expressed as mean± SD.
Means within the same row and column bearing at least one common superscript do not differ significantly (p<0.05).

ensiling the urea treated material with different levels of organic acids and fermentable sugar sources on chemical composition of wheat straw.

MATERIALS AND METHODS

Wheat straw was ground through a Wiley mill (2-mm screen) and was treated with 4, 6 and 8% urea on dry matter basis. The corn steep liquor (CSL) and acidified molasses (having equal pH to that of CSL) was added to the urea treated wheat straw at 0, 2, 4 and 6% on DM basis. The acetic and formic acids were applied at the rate of 1.5, 2 and 2.5% to urea treated wheat straw to achieve the similar pH as that of CSL. A ratio of wheat straw DM: water (100:50) was maintained across all treatments. This treated wheat straw was ensiled in laboratory silos for 15 days. Three laboratory silos were prepared for each treatment, sealed and stored in the incubator at 35°C. This temperature was chosen as corresponding approximately to the outside summer temperature in Pakistan. The samples of this fermented wheat straw were analyzed for dry matter (DM), organic matter (OM), neutral detergent insoluble N (NDIN), acid detergent insoluble N (ADIN), N and ash by the methods of AOAC (1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) by methods described by Van Soest et al. (1991).

Statistical Design

Data were analyzed as a completely randomized design with factorial arrangement for levels of urea and additives using the GLM procedure of SAS (1988). In case of an interaction, means were separated by Duncan's multiple range test (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Nitrogen fractions

The total N content of wheat straw treated with varying levels of urea and ensiled with or without additives was significantly different across all treatments (Table 1). The total N content of the urea treated wheat straw ensiled without additive was increased linearly with the level of urea applied to treat the wheat straw (Table 1). Present findings were in consistent with those of Hartley et al. (1985), Mason et al. (1988) and Cann et al. (1991). Weiss et al. (1982) treated rice straw with 5% aqueous NH_3 and ensiled for 30 d and they reported that N content were increased by 133% as compared to untreated wheat straw. Xu et al. (1994) and Zhen et al. (1995) reported that rice straw treated with NH_3 almost doubled its N contents. In the present study the percent N retained of the added urea-N was different in urea treated wheat straw ensiled with or without additives. The minimum N retention was observed in urea treated wheat straw ensiled without any additive. Percent urea-N retention in urea treated wheat straw was linearly reduced when urea level was increased from 4 to 6 and then 8% to treat the wheat straw. This effect was also seen across all levels of additives used in the present study.

A further increase in total N content was observed when urea treated wheat straw was ensiled with different additives as compared to urea treated wheat straw ensiled without any additive; this pattern was observed across all levels (4, 6 and 8%) of urea used to treat the wheat straw. The urea treatment of straw is dependent on the urease activity in plants that release ammonia from urea (Williams et al., 1984a). The ureolytic bacteria that settle on fibrous crop residues can also cause the reaction (Khan et al., 2002). The intensity of ureolysis is closely associated with the moisture content of the residue and the urea level (Cloete and Kritzinger, 1984, 1985; Williams et al., 1984a). Hassoun et al. (1990) reported non-significant effect of treatment period on urea hydrolysis however; the moisture level had a positive effect on urea hydrolysis. In the present study 50% moisture level was used across all treatments that was reported to be optimum for maximum urea hydrolysis with in one week when urea treated wheat straw was ensiled for different periods (Nisa et al., 2002). The urea hydrolysis was very marked for 40 and 60% moisture level (Hassoun et al., 1990) and moisture level higher than 60% was reported to have no effect on urea hydrolysis (Cloete and Kritzinger, 1984). In the present study, urea treated wheat straw was ensiled with or without additives for 15 days that was previously observed optimum time for urea hydrolysis (Khan et al., 2002). However, it was reported that the ureolysis decreased with increasing urea level (Williams et al., 1984a). They also observed less urea hydrolysis when the temperature increased from 24 to 35°C.

However, in the present study a constant temperature (35°C) was maintained across all laboratory silos containing treated material that was not supposed to cause any major difference in total N content of the urea treated straw ensiled with or without additives as previously reported by Nisa et al. (2002). Silva-Da-Silva et al. (1988) reported that straw samples treated with urea were clearly alkaline as would be expected from the extensive urea hydrolysis that occurred. This increased pH leads to higher ammonia escape to the environment (Khan et al., 2002). This was consistent with earlier workers (Ashbell and Donahaye, 1986; Ashbell et al., 1987). In agreement with present findings higher N values in ammoniated straw were reported by different workers who trapped the excess free NH_3 by spraying organic acids (Borhami et al., 1982), inorganic acids (Claete and Kritzinger, 1984b, c; Yadav and Virk, 1994; Taiwo et al., 1995) or using non-structural carbohydrates (Saadullah et al., 1981; Ali et al., 1993; Sarwar et al., 1994; Nisa et al., 2002; Khan et al., 2002).

The total N content was higher in urea treated wheat straw ensiled with acetic or formic acid as compared to urea treated wheat straw ensiled without acetic and formic acid. Similarly increased N values in ammoniated straw were reported by different workers who trapped the excess free NH_3 by spraying organic acids (Borhami et al., 1982; Mehra et al., 2001). Mehra et al. (2001) used acetic acid to increase the N retention in urea treated wheat straw. They reported that about 50-60% of the added N was retained when 2.7% acetic acid was added to 4% urea treated wheat straw (DM basis). They explained that it might be due to the formation of ammonium acetate. Borhami et al. (1982) reported that application of formic acid to urea treated material reduced the pH and this low pH inhibited the urease activity in the treated material. The present results were in agreement with Stephanie and Simon (1992) and Philip et al. (1990) who proposed that formic acid reduced the ammonia concentration in urea treated material and this naturally leads to the combination of urea with formic acid. The wheat straw treated with 4, 6 and 8% urea and ensiled with 1.5, 2 and 2.5% acetic or formic acid have had significantly different total N contents. The higher total N contents were observed in 8% urea treated wheat straw ensiled with 6% level of acetic or formic acid. However, the urea treated wheat straw ensiled with acetic acid has shown better total N contents than formic acid across all levels of urea treatments. Borhami et al. (1982) reported that N content was markedly increased with NH_3 treatment. In addition, spraying with formic or acetic acid showed an increase in the N content of the straw, whereas phosphoric acid seemed to have no effect. The NH_3-N as a percent of total N was increased by NH_3 treatment, and it further increased by acids. They explained that organic acids, formic and acetic, had a greater capability than phosphoric acid to fix NH_3 in the straw.

The percent N retained of added urea-N in urea treated wheat straw ensiled with acetic or formic acid increased linearly as the level of acetic or formic acid was increased from 1.5 to 2% to ensile urea treated wheat straw. This effect was further enhanced when urea treated wheat straw was ensiled with 2.5% acetic and formic acids. However, Dass et al. (2001) explained that a continuous decrease was noted in added N retention with the increase in the level of HCl. They suggested that, as there was no significant difference in N content of straw added with different levels of HCl, so HCl to trap 30% of added N was optimum. However, in the present study the percent retention of urea-N was reduced when wheat straw was treated with higher levels (6 and 8%) of urea and ensiled with 2, 4 and 6% acetic or formic acid as compared to wheat straw treated with 4% urea and ensiled with 2, 4 and 6% acetic or formic acid. The present results indicated that 4% urea treated wheat straw ensiled with acetic acid or formic acid was a feasible method of straw treatment.

Further, increase in total N content of wheat straw was observed when urea treated wheat straw was ensiled with CSL or acidified molasses as compared to urea treated wheat straw ensiled with acetic or formic acid. This effect was observed for all levels of urea applied to treat the wheat straw. These findings were consistent with previous workers who have tried to fix the urea-N in wheat straw (Nisa et al., 2002) and corncobs (Khan et al., 2002) by ensiling urea treated wheat straw and corncobs with fermentable carbohydrate sources. Williams et al. (1984a) and Shah et al. (1981) demonstrated that hydrolysis of urea occurred at least partly through bacterial activity and it was thought that addition of a soluble source of readily available carbohydrate to the straw might had enhanced the rate and degree of urea hydrolysis. Further, ensiling urea treated wheat straw with CSL (a rapidly fermentable carbohydrate source) can stimulate the growth of lactic acid producing bacteria in urea treated wheat straw and thus the higher lactic acid concentration can reduce the N escape from urea treated wheat straw by lowering its pH (Nisa et al., 2002).

In the present study, highest total N content of wheat straw was noted in wheat straw treated with 8% urea and ensiled with either 6% CSL or acidified molasses. However, the total N content was noted higher in urea treated wheat straw ensiled with CSL as compared to urea treated wheat straw ensiled with acidified molasses. This effect was observed across all levels of urea used to treat the wheat straw and ensiled with 2, 4 and 6% either CSL or formic acid. Nisa et al. (2002) reported that urea treated wheat straw ensiled with 3, 6 and 9% CSL increased 50, 78 and 111% wheat straw N when compared to urea treated wheat straw without CSL. Other workers (Dryden and Leng, 1988; Reddy et al., 1991) had also reported increased N

Table 2. Neutral detergent insoluble nitrogen contents* of wheat straw treated with different levels of urea and additives

Items		Urea levels		
Additives (%)		4%	6%	8%
Control	0	0.33±0.01^{U}	0.47±0.05^{T}	0.61±0.01^{QR}
Acetic acid	1.5	0.51±0.01ST	0.70±0.01^{N}	0.80±0.01^{IJK}
	2	0.64±0.01^{OPQ}	0.75±0.00^{LM}	0.92±0.01^{F}
	2.5	0.65±0.01^{PQ}	0.87±0.02^{GH}	1.00±0.01^{CDE}
Formic acid	1.5	0.53±0.01^{S}	0.67±0.01^{NOP}	0.74±0.01^{M}
	2	0.62±0.01^{QR}	0.80±0.01^{JK}	0.96±0.02^{E}
	2.5	0.77±0.01^{KLM}	0.90±0.02^{FG}	1.02±0.01^{CD}
Molasses	2	0.58±0.01^{R}	0.78±0.00^{KLM}	0.87±0.02^{GH}
	4	0.69±0.01^{NO}	0.98±0.00^{DE}	1.03±0.01^{C}
	6	0.84±0.01^{HIJ}	1.08±0.01^{B}	1.14±0.02^{A}
Corn steep liquor	2	0.61±0.01^{QR}	0.84±0.01^{HI}	0.88±0.01^{GH}
	4	0.70±0.01^{N}	0.98±0.00^{DE}	1.08±0.01^{B}
	6	0.79±0.01^{KL}	1.14±0.01^{A}	1.16±0.01^{A}

* Values are expressed as mean± SD.
Means within the same row and column bearing at least one common superscript do not differ significantly ($p<0.05$).

content of ensiled urea treated straws. This increase in N content of urea treated wheat straw ensiled with CSL may be that it provided readily available nutrients (carbohydrates, minerals and proteins) for the proper fermentation milieu, which might have caused a rapid drop in pH. This reduced pH probably has converted free ammonia (NH_3) into an ionic form of ammonia (NH^{+4}) that is very reactive and has the greater tendency to make bonds with fibrous materials.

The N retention in urea treated wheat straw ensiled with CSL or acidified molasses was observed higher when compared to urea wheat straw ensiled with acetic or formic acid. However, maximum N retention was noted in 4% urea treated wheat straw ensiled with CSL and acidified molasses as compared to all other treatments in the study. The observations in the present study indicated that by increasing urea level beyond 4% for the treatment of straw may render the ammoniation process uneconomical by increasing the rate of escaped N from treated material. Khan et al. (2002) reported that enzose was applied to the urea treated corncobs as a source of readily fermentable sugars to fix the urea-N in urea treated corncobs. The N contents increased linearly with the level of enzose, applied to the corncobs. The highest N content was observed in corncobs treated with urea and 6% enzose. It was observed that the application of 5% urea and 6% enzose (DM basis) to corncobs increased the total N content of corncobs about 5 times than untreated corncobs and almost 2 times than urea treated corncobs without enzose. They explained that sugars present in the enzose might have provided the carbon skeleton or/and energy for the microbial growth during ensilation of urea treated corncobs. The increased microbial population in the ensiled material might have increased the urease production and ultimately the degradation of urea. They suggested that enzose was expected to provide the sugars to anaerobic bacteria for production of lactate that might have resist the change in pH of the ensiled material due to extensive urea hydrolysis. Moreover, the low pH (3.3) of enzose might resist the change in pH of the treated material and thus improved the N content of urea treated corncobs ensiled with enzose.

The NDIN contents of urea treated wheat straw were different across all treatments in the study (Table 2). Neutral detergent insoluble content increased in urea treated wheat straw as the level of urea increased from 4 to 8%. The NDIN content increased further in urea treated wheat straw when urea treated wheat straw was ensiled with acetic or formic acid. The NDIN content was noted high in urea treated wheat straw ensiled with 2.5% acetic or formic acid as compared to urea treated wheat straw ensiled with 2 and 1.5% of acetic or formic acid. The urea treated wheat straw ensiled with CSL or acidified molasses have shown higher NDIN contents when compared to urea treated wheat straw ensiled with acetic or formic acid. This effect was noted across all the urea levels studied. The highest values of NDIN were noted in urea treated wheat straw ensiled with 6% CSL or acidified molasses. The present values of NDIN reflected that ensilation of urea treated wheat straw with fermentable carbohydrate source could improve the fiber bound N. Nisa et al. (2002) reported that the usage of CSL has not only increased the total N concentration of the wheat straw but it has also improved the NDIN compared to the control treatment. The percent retained of the added N as NDIN was 48, 44, 48% when urea treated wheat straw was ensiled with 3, 6 and 9% CSL. The urea treated wheat straw ensiled with 0, 3, 6 and 9% CSL had 70, 50, 46 and 39% NH_3 of total N, respectively, whereas, at 3, 6 and 9% CSL, the N recovered as NH_3 was 11.54, 16.25 and 11.40%, respectively, of the added N. In this study, approximately one half of the added N was bound in some form. The concentration of NDIN was approximately 1.6 (0.25 percentage units), 1.8 (0.35 percentage units) and 2.4 (0.55 percentage units) times higher in 3, 6 and 9% CSL compared to urea treated wheat straw without CSL. The increase in NDIN was about twice as large as the increase in bound N, which can be directly attributed to NH_3 addition. The ammoniation had increased the fiber-bound N (NDF-N and ADF-N) (Cann et al., 1991). However, the largest N portion of the treated straw was free NH_3-N, which accounted for about 72% of the retained N (Garret et al., 1979). The increase in fiber-bound N and total N was consistent with Liu et al. (1995); Zhen et al. (1995); Xu et al. (1994); Nelson et al. (1984), and Brown et al. (1987). Zhang (1995a) reported that crude protein (CP) content of wheat straw was increased from 3.4 in control to 12.9 and 9.9% in anhydrous NH_3 treated and urea treated wheat straw, respectively. Acid detergent fiber content was increased and NDF content decreased after the application of both

Table 3. Acid detergent insoluble nitrogen content* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	0.29 ± 0.01^{K}	0.41 ± 0.01^{H}	0.48 ± 0.01^{CDEF}
Acetic acid	1.5	0.30 ± 0.01^{JK}	0.43 ± 0.01^{FGH}	0.43 ± 0.01^{FGH}
	2	0.34 ± 0.01^{IJ}	0.45 ± 0.01^{EFGH}	0.45 ± 0.01^{DEFGH}
	2.5	0.35 ± 0.01^{I}	0.48 ± 0.01^{CDEF}	0.47 ± 0.01^{CDEF}
Formic acid	1.5	0.32 ± 0.01^{IJK}	0.42 ± 0.01^{GH}	0.47 ± 0.01^{CDEF}
	2	0.32 ± 0.02^{IJK}	0.44 ± 0.01^{EFGH}	0.47 ± 0.02^{CDEF}
	2.5	0.34 ± 0.01^{IJ}	0.47 ± 0.01^{CDEF}	0.49 ± 0.02^{BCDE}
Molasses	2	0.31 ± 0.01^{IJK}	0.44 ± 0.00^{EFGH}	0.49 ± 0.02^{BCDE}
	4	0.34 ± 0.01^{IJ}	0.47 ± 0.01^{DEFG}	0.51 ± 0.02^{ABC}
	6	0.36 ± 0.01^{I}	0.48 ± 0.01^{CDEF}	0.53 ± 0.02^{AB}
Corn steep liquor	2	0.32 ± 0.01^{IJK}	0.46 ± 0.01^{DEFE}	0.50 ± 0.02^{BCD}
	4	0.33 ± 0.01^{IJK}	0.48 ± 0.01^{CDEF}	0.52 ± 0.02^{ABC}
	6	0.33 ± 0.01^{IJK}	0.47 ± 0.01^{CDEF}	0.54 ± 0.01^{A}

* Values are expressed as mean±SD.
Means within the same row and column bearing at least one common superscript do not differ significantly (p<0.05).

treatments to wheat straw.

The ADIN contents of urea treated wheat straw were significantly different among the urea levels studied (Table 3). Weixian (1995) reported the similar findings. The ADIN contents of 8% urea treated wheat straw were significantly different among additives used to ensile urea treated wheat straw. The ADIN contents were observed higher in urea treated wheat straw ensiled with CSL when compared to all other treatments. The highest ADIN contents were observed in urea treated wheat straw ensiled with 6% CSL. The increases in cell wall N reported in our study have been recorded previously (Dryden and Kempton, 1983; Van Soest et al., 1984). They suggested that an NH_3-lignin complex was formed in ammoniated straw. The reason for the increased lignin content following ammoniation in this experiment was not known, as it cannot be totally explained by the complexing of additional N with lignin. Khan et al. (2002) reported that the ADIN content increased with the increasing level of enzose in urea treated corncobs. However, this fraction of N in urea treated corncobs ensiled with or without enzose remained statistically non-significant. The present results were consistent with earlier workers (Oji and Mowat, 1979; Nisa, 2002). In contrast to our findings, Solaiman et al. (1979) and Itoh et al. (1981) suggested that most of N was held either as water soluble N (NH_3 and amide-N) or water insoluble, neutral detergent soluble N, together with smaller amounts bound to cell wall. Ammoniation resulted in an increase in total N content, more than 90% of the retained N was detected as water-soluble N, most of which was NH_3-N. N bound to ADF residue showed a negligible increase from the urea treatment. Oji and Mowat (1979); Williams et al. (1984b) reported that 43% of the N added as urea was lost by diffusion, more than 90% of the retained N was analyzed as water soluble N, a figure higher than those was reported by Dryden and Kempton (1983) with barley straw treated with anhydrous NH_3. The amount of N bound to the ADF remained essentially unchanged. From these results, it appears that, following urea treatment of maize stover, the retained N represents a source of readily available N for rumen microbes.

The ammonia N of wheat straw treated with 4, 6 and 8% urea and ensiled with or without additives was noted significantly different (Table 4). Ammonia N was increased in urea treated wheat straw ensiled with different additives as compare to urea treated wheat straw ensiled without additive. However, ammonia N was noted statistically non-significant when 4 and 6% urea treated wheat straw was ensiled with organic acids or fermentable sugar sources. The 8% urea treated wheat straw has shown the additive effect on ammonia N contents. The ammonia N content of

Table 4. NH_3-N contents* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	0.730 ± 0.015^{L}	0.880 ± 0.010^{G}	0.880 ± 0.15^{G}
Acetic acid	1.5	0.770 ± 0.010^{KL}	0.905 ± 0.015^{EFG}	0.935 ± 0.015^{BCDE}
	2	0.780 ± 0.010^{GK}	0.920 ± 0.010^{DEFG}	0.945 ± 0.015^{ABCDE}
	2.5	0.800 ± 0.010^{IGK}	0.935 ± 0.015^{BCDE}	0.955 ± 0.015^{ABCD}
Formic acid	1.5	0.790 ± 0.010^{IJK}	0.915 ± 0.015^{DEFG}	0.925 ± 0.015^{CDEF}
	2	0.820 ± 0.010^{HIJ}	0.925 ± 0.015^{CDEF}	0.935 ± 0.015^{BCDE}
	2.5	0.820 ± 0.020^{HIJ}	0.935 ± 0.015^{BCDE}	0.965 ± 0.015^{ABC}
Molasses	2	0.760 ± 0.010^{KL}	0.905 ± 0.015^{EFG}	0.935 ± 0.015^{BCDE}
	4	0.770 ± 0.020^{KL}	0.890 ± 0.010^{FG}	0.965 ± 0.015^{BCDE}
	6	0.785 ± 0.015^{IJK}	0.915 ± 0.015^{DEFG}	0.975 ± 0.005^{AB}
Corn steep liquor	2	0.800 ± 0.010^{IJK}	0.920 ± 0.010^{DEFG}	0.965 ± 0.015^{ABCE}
	4	0.840 ± 0.010^{H}	0.935 ± 0.015^{BCDE}	0.975 ± 0.015^{AB}
	6	0.825 ± 0.015^{HI}	0.950 ± 0.010^{ABCD}	0.985 ± 0.005^{A}

* Values are expressed as mean±SD.
Means within the same row and column bearing at least one common superscript do not differ significantly (p<0.05).

Table 5. Reaming nitrogen contents* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	0.235±0.015^{O}	0.300±0.010^{NO}	0.600±0.200^{ABCDEFG}
Acetic acid	1.5	0.365±0.015^{LMN}	0.515±0.015^{EFGHIJ}	0.535±0.015^{ABCDEF}
	2	0.400±0.010^{KLMN}	0.575±0.015^{ABCDEFG}	0.580±0.010^{KLMN}
	2.5	0.445±0.015^{IJKLM}	0.620±0.010^{ABCDE}	0.595±0.015^{BCDEFGH}
Formic acid	1.5	0.345±0.015^{MN}	0.499±0.011^{FGHIJK}	0.555±0.015^{BCDEFGH}
	2	0.425±0.015^{JKLM}	0.545±0.015^{CDEFGHI}	0.560±0.010^{ABCDE}
	2.5	0.465±0.015^{HIJKL}	0.640±0.020^{BCDEFGH}	0.600±0.010^{KLMN}
Molasses	2	0.385±0.015^{KLMN}	0.540±0.020^{AB}	0.575±0.015^{ABCDEF}
	4	0.440±0.010^{IJKLM}	0.560±0.010^{IJKLM}	0.585±0.015^{ABC}
	6	0.475±0.015^{GHIJK}	0.655±0.015^{ABCDEF}	0.645±0.015^{KLMN}
Corn steep liquor	2	0.400±0.010^{KLMN}	0.445±0.015^{ABCDE}	0.585±0.015^{ABCDEF}
	4	0.425±0.015^{JKLM}	0.599±0.011^{ABCDE}	0.610±0.020^{ABCDE}
	6	0.440±0.010^{IJKLM}	0.610±0.020^{DEFGHI}	0.670±0.020^{A}

* Values are expressed as mean±SD
Means within the same row and column bearing at least one common superscript do not differ significantly (p<0.05).

Table 6. Neutral detergent fiber content* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	78.2±0.02^{X}	76.1±0.00^{Z}	76.8±0.00^{Z}
Acetic acid	1.5	78.9±0.05^{V}	78.9±0.03^{V}	79.9±0.00^{Q}
	2	79.3±0.04ST	80.9±0.00^{N}	83.0±0.02^{G}
	2.5	80.2±0.02^{0}	82.9±0.02^{G}	83.5±0.00^{D}
Formic acid	1.5	78.7±0.04^{W}	79.6±0.03^{R}	80.1±0.03^{P}
	2	79.1±0.03^{U}	81.9±0.03^{J}	83.1±0.00^{F}
	2.5	80.2±0.02^{O}	83.9±0.03^{BC}	83.5±0.00^{D}
Molasses	2	79.3±0.00^{T}	80.1±0.03^{P}	79.4±0.00^{S}
	4	81.0±0.03^{M}	82.2±0.03^{I}	82.9±0.03^{H}
	6	83.9±0.03^{B}	84.0±0.03^{A}	83.3±0.00^{E}
Corn steep liquor	2	77.1±0.00^{Y}	79.9±0.03^{Q}	79.3±0.03^{T}
	4	78.7±0.05^{W}	81.4±0.03^{L}	81.8±0.00^{K}
	6	80.1±0.02^{P}	83.8±0.03^{C}	83.2±0.00^{E}

* Values are expressed as mean± SD.
Means within the same row and column bearing at least one common superscript do not differ significantly (p<0.05).

Table 7. Cellulose content* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	43.2±0.02^{A}	33.9±0.01^{V}	33.6±0.01^{X}
Acetic acid	1.5	40.8±0.02^{D}	31.7±0.02^{Y}	32.6±0.01^{Z}
	2	40.1±0.02^{G}	32.9±0.02^{Z}	33.1±0.01^{Y}
	2.5	39.9±0.01^{H}	34.2±0.01^{TU}	34.2±0.02^{T}
Formic acid	1.5	41.8±0.02^{B}	32.9±0.02^{Z}	33.7±0.01^{W}
	2	40.9±0.01^{C}	34.1±0.01^{U}	34.1±0.02^{TU}
	2.5	40.2±0.020^{G}	34.5±0.01^{S}	35.8±0.01^{N}
Molasses	2	39.3±0.02^{J}	34.1±0.02^{TU}	34.5±0.00^{R}
	4	40.9±0.02^{C}	35.3±0.02^{P}	35.8±0.00^{MN}
	6	40.6±0.01^{E}	36.2±0.02^{L}	36.1±0.01^{L}
Corn steep liquor	2	38.9±0.01^{K}	35.9±0.02^{M}	34.5±0.02^{S}
	4	39.7±0.13^{I}	34.2±0.01^{TU}	34.8±0.01^{Q}
	6	40.4±0.02^{F}	35.4±0.02^{O}	25.6±0.01^{Z}

* Values are expressed as mean± SD.
Means within the same row and column bearing at least one common superscript do not differ significantly (p<0.05).

8% urea treated wheat straw ensiled with CSL was higher as compared to all other treatments in the study. Dryden and Leng (1988) reported that most of the increase in N content (66%) was due to an improved retention of water-soluble N (WSN) and 55% of the additional WSN was NH_3-N. The increase in the cell wall N and the detergent soluble water insoluble N contents accounted for 12.5 and 21% of the increase in total N content following ammoniation.

The remaining N contents of urea treated wheat straw ensiled with or without additives remained non-significant across all treatments in the present study (Table 5). However the remaining N contents was improved as the level of urea was increased to treat the wheat straw.

Fiber fractions

Neutral detergent fiber content of urea treated wheat straw ensiled with or without additives was observed significantly different across all treatments (Table 6). The NDF content of urea treated wheat straw was decreased linearly with the increasing level of urea. The cellulose contents of wheat straw was linearly reduced when urea level was increased from 4 to 6 and then 8% to treat the wheat straw (Table 7). However, the hemicellulose contents remained similar in wheat straw treated with 4, 6 and 8% urea and ensiled without additive. In agreement with present findings, Nelson et al. (1985); Brown et al. (1987); Zhen et al. (1995) reported reduced NDF concentrations in urea treated straw when compared to untreated straw. This decrease has been attributed to hemicellulose solubilization during urea treatment of straws (Khan et al., 2002; Nisa et al., 2002). The decrease in cell wall constituents in urea treated straw was also reported by many workers (Reddy

Table 8. Crude protein free neutral detergent fiber content* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	75.1±0.02^{X}	75.0±0.02^{Y}	75.8±0.01^{V}
Acetic acid	1.5	74.8±0.01^{Z}	76.8±0.01^{OP}	75.9±0.00^{U}
	2	74.3±0.00^{Z}	77.1±0.03^{JK}	77.6±0.01^{H}
	2.5	74.5±0.02^{Z}	77.5±0.03^{I}	79.1±0.01^{A}
Formic acid	1.5	76.4±0.02^{Q}	76.9±0.02^{MN}	76.9±0.00^{NO}
	2	76.8±0.02^{P}	78.2±0.03^{E}	78.9±0.00^{B}
	2.5	75.4±0.00^{W}	78.5±0.00^{F}	77.8±0.02^{G}
Molasses	2	76.8±0.00^{P}	76.9±0.01^{LM}	76.2±0.03^{S}
	4	77.0±0.04^{L}	77.1±0.03^{K}	76.0±0.00^{T}
	6	78.8±0.00^{C}	78.4±0.01^{D}	77.1±0.02^{K}
Corn steep liquor	2	74.4±0.00^{Z}	76.3±0.00^{R}	75.9±0.03^{V}
	4	73.6±0.03^{Z}	76.2±0.02^{S}	76.4±0.01^{Q}
	6	74.1±0.00^{Z}	76.8±0.01^{OP}	77.2±0.03^{J}

* Values are expressed as mean± SD.
Means within the same row and column bearing at least one common superscript do not differ significantly ($p<0.05$).

Table 9. Crude protein free hemicellulose content* of wheat straw treated with different levels of urea and additives

Items		Urea level		
Additives (%)		4%	6%	8%
Control	0	21.9±0.01^{T}	23.1±0.02^{P}	22.2±0.02^{S}
Acetic acid	1.5	22.9±0.01^{Q}	24.6±0.01^{H}	24.1±0.02^{K}
	2	23.4±0.02^{N}	24.6±0.01^{I}	25.1±0.02^{D}
	2.5	23.9±0.02^{L}	25.1±0.02^{D}	25.2±0.02^{C}
Formic acid	1.5	24.6±0.01^{HI}	24.8±0.02^{G}	23.4±0.02^{N}
	2	24.2±0.01^{J}	25.2±0.02^{C}	25.6±0.03^{B}
	2.5	23.9±0.02^{L}	25.8±0.02^{A}	25.0±0.01^{E}
Molasses	2	19.9±0.02^{X}	21.5±0.02^{V}	24.8±0.01^{FG}
	4	20.8±0.01^{W}	21.9±0.02^{U}	24.5±0.01^{I}
	6	21.5±0.02^{V}	22.1±0.02^{S}	25.1±0.01^{C}
Corn steep liquor	2	23.2±0.02^{O}	22.8±0.02^{R}	23.6±0.02^{M}
	4	24.2±0.02^{JK}	23.4±0.02^{N}	23.1±0.01^{O}
	6	23.9±0.01^{L}	23.9±0.02^{L}	24.9±0.02^{F}

* Values are expressed as mean±SD.
Means within the same row and column bearing at least one common superscript do not differ significantly ($p<0.05$).

and Reddy, 1985; Reddy et al., 1991; Weixian, 1995). This decrease in NDF might be because of both of their solubilization during ensiling (Claete and Kritzinger, 1984a; Yadav and Virk, 1994) and utilization by microbial fermentation. Similar effects have been noted previously (Ali et al., 1977) and were caused by alkali induced “peeling” reactions, in which degradation of sugar moieties occurred at the reducing end of hemicellulose chains.

The NDF content of urea treated wheat straw ensiled with acetic or formic acid was increased as compared to urea treated wheat straw ensiled without acetic or formic acid. These results were consistent with those previously reported by Cann et al. (1991); Lines and Weiss (1996); Nisa et al. (2002). The NDF content of urea treated wheat straw ensiled with acetic and formic acid was affected both by the level of urea and organic acids. Neutral detergent fiber content of wheat straw was increased when urea and organic acid levels were increased to treat the wheat straw. Higher value of NDF was observed when wheat straw was treated with 8% urea and ensiled with 2.5% of acetic or formic acid as compared to wheat straw treated with 6 and 4% urea level and ensiled with 1.5 and 2% level of acetic or formic acid.

The NDF content was further increased when urea treated wheat straw was ensiled with CSL or acidified molasses as compared to urea treated wheat straw ensiled with acetic or formic acid. Neutral detergent fiber content of urea treated wheat straw ensiled with CSL or acidified molasses was increased linearly as the level of CSL or acidified molasses was increased to ensile the wheat straw. In the present study, NDF content was increased both with the increasing levels of urea and fermentable carbohydrate sources applied to treat the wheat straw. Highest value of NDF was noted in wheat straw treated with 6% urea and ensiled with CSL as compared to all other treatments in the study. However, in the present study, CP free NDF (NDF-NDIN×6.25) contents remained unchanged across all treatments (Table 8). Lines and Weiss (1996) have reported that ammoniation increased the concentration of NDF and its entire increase was in the hemicellulose fraction. They attributed this increase in NDF to increased NDIN. In the present study, the hemicellulose content of urea treated wheat straw ensiled with or with out additives was noted significantly different. The presents results were in agreement with, Khan et al. (2002) who reported that the NDF and hemicellulose contents of urea treated corncobs ensiled with 0, 2, 4 and 6% enzose (a fermentable sugar source) showed a linear increase as the level of enzose applied increased to ensile urea treated corncobs. However the CP free NDF content remained similar across all treatments (Table 8). The increased NDF content in urea treated corncobs ensiled with enzose as compared to control was the function of increased NDIN contents when compared to urea treated corncobs ensiled without enzose. Similar results have been reported by Nisa et al. (2002). However, some workers (Buettner et al., 1982; Zorrilla-Rios et al., 1985; Zhang, 1995a) reported contrasting results.

The present results indicated that entire increase in NDF contents in urea treated wheat straw ensiled with different additives was due to binding of N with hemicellulose moieties. It may be supported by the fact that the CP free hemicellulose content of urea treated wheat straw ensiled with or without additives remained similar across all treatments in the study (Table 9). However, the hemicellulose content was increased when urea treated wheat straw was ensiled with acetic or formic acid as compared to urea treated wheat straw ensiled without any additive. In the present study, this effect was noted across all levels of urea used to treat the wheat straw. Hemicellulose contents was increased linearly when urea treated wheat straw was ensiled with 1.5, 2 and 2.5% acetic or formic acid. The hemicellulose contents of urea treated wheat straw ensiled with formic acid was observed higher than urea treated wheat straw ensiled with acetic acid. This effect was noted for all levels urea and organic acids used.

The hemicellulose content of urea treated wheat straw ensiled with CSL or acidified molasses was higher as compared to urea treated wheat straw ensiled with acetic or formic acid. Urea treated wheat straw ensiled with CSL or acidified molasses exhibited a linear increase in hemicellulose contents as the level of CSL or acidified molasses was increased to ensile urea treated wheat straw. This effect was seen in wheat straw treated with 4, 6 and 8% urea levels. Highest hemicellulose contents were observed in wheat straw treated with 8% urea and ensiled with CSL or acidified molasses as compared to all other treatments in the present study. The ADF content of urea treated wheat straw ensiled with or without additives remained statistically non-significant. These results supported by the findings of Dryden and Kempton (1983), Liu et al. (1991, 1995) and Xu et al. (1994).

The cellulose contents of urea treated wheat straw ensiled with or without additives were significantly different. The cellulose contents of wheat straw was linearly reduced when urea level was increased from 4 to 6 and 8% to treat the wheat straw. This effect was further enhanced when urea treated wheat straw was ensiled with different additives. A lower cellulose contents of wheat straw was noted when urea treated wheat straw was ensiled with acetic or formic acid as compared to urea treated wheat straw ensiled without any additive. The cellulose contents were further reduced when urea treated wheat straw was ensiled with 2.5% acetic or formic acid as compared to 2 and 1.5% acetic or formic acid.

Contrary to present findings, Dryden and Leng (1988) reported that after ammoniation the organic matter content was reduced by ammoniation from 75.4 to 66.2%, reduction in organic matter content due to NH_3 was achieved by solubilization of hemicellulose with no effect on the cellulose content. Ammoniation also influenced the recovery and composition of cell walls. About 8 to 9 % of the cell wall of untreated straws was rendered soluble in neutral detergent solution by the NH_3 treatment, producing proportionate increase in cellulose and lignin relative to hemi cellulose. However, in the present study, decrease in cellulose contents may be attributed to propionate increase in hemicellulose contents of urea treated wheat straw ensiled with different levels of organic acids or fermentable carbohydrate sources. A further reduction in cellulose contents of urea treated wheat straw was observed when urea treated wheat straw was ensiled with CSL or acidified molasses. This effect was more pronounced when higher levels of CSL or acidified molasses were used to ensile urea treated wheat straw. Maximum reduction in cellulose contents was noted in 8% urea treated wheat straw ensiled with 6% CSL or acidified molasses as compared to 4 and 6% urea treated wheat straw ensiled with 2 or 4% of CSL or acidified molasses.

CONCLUSION

The present results revealed that 4% urea treated wheat straw ensiled with CSL or acidified molasses have better fix the N in wheat straw and brought favorable physiochemical changes in wheat straw as compared to urea treated wheat straw ensiled with organic acids. However, before recommending its use further research is warranted to explore the effect of urea treated wheat straw ensiled with fermentable carbohydrate sources on ruminal digestion, N metabolism, and productivity of ruminants through biological trials.

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