Effect of Feeding Urea and Acetic Acid Treated Wheat Straw on the Digestibility of Nutrients in Adult Male Murrah Buffaloes (*Bubalus bubalis*)

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ABSTRACT : Wheat straw was treated on laboratory scale with 4% urea at a moisture level of 50% along with different amounts of acetic acid (AA) to fix various levels of ammonia nitrogen (15, 30, 45 and 60%) evolved from urea and stored for 4 weeks. Chemical composition of the treated samples revealed a significant ($p<0.01$) increase in N content of the samples where AA was added. The N content of the ammoniated straw was only 1.21% which increased to 2.58 with the addition of AA to trap 30% NH_3-N. The concentration of free NH_3-N in the straw was significantly ($p<0.01$) less when more than 15% NH_3-N was trapped with AA. There was significant increase ($p<0.01$) in N disappearance and depression in NDF and hemicellulose disappearance, when AA was used to trap 30% NH_3-N. Large scale treatment of wheat straw with 4% urea at a moisture level of 50% along with AA (to trap 30% NH_3-N) increased the N content, but not as much as in laboratory scale treatment. *In vivo* experiment conducted on nine adult male buffaloes divided into three groups revealed no difference in the intake of DM, OM, NDF, ADF, cellulose and hemicellulose among group I (ammoniated straw), group II (AA treated ammoniated straw) and group III (AA treated ammoniated straw +1 kg barley grain), but the intake was significantly ($p<0.05$) more in groups where AA treated straw was fed as compared to only ammoniated straw fed group. However EE digestibility was depressed in group II. The digestibility of cellulose and hemicellulose both depressed significantly ($p<0.05$) in group II and III as compared to group I. Animals in all the 3 groups showed positive nitrogen balance and it was significantly more in group II and III as compared to group I. DCP intake was significantly ($p<0.05$) more in group II and III as compared to group I, but there was no significant difference among the three groups in TDN intake. It can be concluded that AA (to trap 30% NH_3-N) is effective in capturing the excess ammonia released during urea ammoniation of straw and improving its nutritive value, as well as animal performance. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1690-1695)***

Key Words : Wheat straw, Urea, Acetic Acid, Nitrogen, Murrah Buffaloes

INTRODUCTION

Poor quality roughages such as pasture hays and crop residues form a major part of feed resources for ruminants. Protein deficiency and low digestibility often restrict animal performance (Klopfenstin et al., 1991; McAllan, 1991). Methods to improve intake and utilization of poor quality roughages included physical processing, chemical treatment or a combination of both (Jackson, 1977; Sundstol et al., 1978; Sharma et al., 1993).

Ammonia treatment of low quality roughage is simple and inexpensive, improves the nutritive value and acts as a preservative of high moisture materials. However, gaseous ammonia is not only expensive but also corrosive in nature (Sundstol et al., 1978; Solaiman et al., 1979).

Extensive information exists on the treatment of straws using ammonia released by the hydrolysis of urea (Jayasuriya and Pearce, 1983; Dass et al., 1984; Makkar and Singh, 1987; Hart and Wanapat, 1992). Other beneficial methods of ammoniation of straw have been discussed (Davies, 1983; Khan et al., 1999). However, a major drawback of this process is the loss of nitrogen (N) in the form of ammonia after treatment is completed. Attention was given to this problem by Borhami et al. (1982) who sprayed ammoniated straw with organic acids to fix the excess free ammonia. Subsequently inorganic acids like hydrochloric, sulphuric and boric acid were used with various degrees of success (Cloette and Kritzinger, 1984; Yadav and Virk, 1994a,b; Dass et al., 2000). Earlier workers (Borhami et al., 1984; Cloette and Kritzinger, 1984; Yadav and Virk, 1994a,b) sprayed organic/inorganic acids after urea ammoniation of straw samples which were stored for either 8 weeks (Borhami et al., 1982) or 2 weeks (Yadav and Virk, 1994a,b) Thus spraying of acids enhances the treatment period of the straw but does involve extra labour. With reference to the drawbacks of earlier methods an experiment was conducted to examine the effect of simultaneous treatment of wheat straw with urea and acetic acid on nitrogen fixation and chemical composition of wheat straw. A comparative study was also made with nine adult male buffaloes to study the effect of feeding ammoniated straw and acetic acid treated ammoniated straw on nutrient intake and digestibility.

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MATERIALS AND METHODS

Experiment 1

In sacco dry matter disappearance of wheat straw treated with urea and acetic acid : Wheat straw (200 g) was weighed and treated with fertilizer grade urea (4 percent) at a moisture level of 50 percent. Acetic acid (0, 1.35, 2.7, 4.05 and 5.4 ml) was added to trap 0, 15, 30, 45 and 60% of ammonia-N. These samples of wheat straw were placed in polythene bags, which were sealed to maintain the anaerobic conditions. The bags were opened after 4 weeks and the treated samples were air dried and milled to pass through a 1 mm sieve before determining the chemical composition using methods recommended by AOAC (1985) and cell wall constituents (Van Soest et al., 1991). Hemicellulose and cellulose were calculated as NDF (Neutral detergent fibre)-ADF (Acid detergent fibre) and ADF-ADL (Acid detergent lignin), respectively. Samples were also analysed for NH_3-N (Ammonia-Nitrogen) according to Markham (1942). The *in sacco* degradability of dry matter (DM), nitrogen (N) and cell wall constituents (NDF and ADF) was determined by the polyester bag (Size of bag 9 cm × 13 cm, pore size 44 µm) technique with 5 g of dry sample (Mehrez and Orskov, 1977) in 3 rumen fistulated buffalo bulls (3.5 years of age, 400 kg body weight).

Animals and their diets

Three rumen fistulated buffalo bulls were fed *ad libitum* wheat straw and a concentrate mixture containing equal parts of ground nut cake, maize and wheat bran alongwith salt, mineral mixture and vitablend (a source of vitamin A and D_3) as specified by Kearl (1982). Three bags for each animal/treatment were suspended for 48 h in the rumen of each animal (i.e. 9 bags for each sample).

Statistical analysis

Analysis of variance was performed as per Snedecor and Cochran (1967), and significant F values were determined. Means were separated using Duncan's multiple range test.

Experiment 2

Treatment of wheat straw on large scale : On the basis of the results of experiment 1, wheat straw was treated on a large scale with 4 percent urea at a 50 percent moisture level. Acetic acid (commercial grade 2.7 liter per quintal) was added simultaneously to trap approximately 30 percent of the ammonia released from 4% urea during urea ammoniation of wheat straw. The straw was covered with a polythene sheet as described by Dass et al. (1984). After 4 weeks the polythene sheet was removed and straw was aerated for 4-5 days before feeding to the animals.

An experiment was conducted with 9 adult male Murrah buffaloes divided into 3 groups of 3 animals in each group. Animals were fed *ad libitum* ammoniated wheat straw (group I), acetic acid treated ammoniated straw (group II) and acetic acid treated ammoniated straw +1 kg barley grain (group III). After adaptation on the above diet for a period of 21 days, a metabolic trial was conducted to determine the digestibility of nutrients and their retention. Feed and faeces were analysed for proximate principles and fibre constituents by the method as described in experiment 1. The data were analysed statistically also as described in experiment 2.

RESULTS

Experiment 1

Impact of adding AA along with urea-ammoniation of wheat straw on N fibre constituents and the concentration of free ammonia-N (mg/100 g feed) at the time of opening of bags is presented in table1. Results revealed a significant ($p<0.01$) increase in N content of wheat straw as a result of AA addition during urea-ammoniation of wheat straw. The N content of wheat straw after urea ammoniation was

Table 1. Chemical composition of wheat straw treated with urea and acetic acid

Treatment	N	NDF	ADF	Cellulose	Hemi-cellulose	Lignin	NH_3-N (mg/100g)	Calculated Trapped NH_3-N (%)
WS	0.59**	83.4*^{b}	54.4**	44.20**^{b}	29.0**^{b}	10.4^{b}	-	-
	±0.60	±0.72	±0.31	±0.41	±0.3	±0.7		
AWS	1.21^{a}	79.93^{a}	56.56^{b}	45.38^{b}	23.47^{a}	5.88^{a}	295**^{c}	-
	±0.20	±0.56	±0.51	±0.62	±0.56	±0.81	±8.72	
AWS+AA (1.35 ml)	1.24^{a}	78.90^{a}	56.72^{b}	45.28^{b}	22.18^{a}	5.79^{a}	202.66^{b}	31.3
	±0.07	±0.13	±0.23	±0.14	±0.36	±0.04	±8.35	
AWS+AA (2.70ml)	2.58^{b}	79.83^{a}	52.34^{a}	41.18^{a}	27.49^{b}	5.56^{a}	43.00^{a}	85.4
	±0.05	±0.35	±0.16	±0.28	±0.31	±0.03	±1.53	
AWS+AA (4.05 ml)	2.50^{b}	79.13^{a}	50.74^{a}	40.70^{a}	28.39^{b}	5.65^{a}	35.67^{a}	87.9
	±0.06	±0.08	±0.04	±0.08	±0.04	±0.07	±0.67	
ASW+AA (5.40 ml)	1.57^{a}	79.04^{a}	51.65^{a}	41.95^{a}	27.39^{b}	5.69^{a}	36.60^{a}	87.6
	±0.16	±0.20	±0.10	±0.23	±0.25	±0.06	±2.67	

WS - Wheat straw, AWS- Ammoniated wheat straw, AA- Acetic acid * $p<0.05$ ** $p<0.01$.

[a,b,c] Different superscripts in the same column indicate significance.

1.21%, which increased to1.24, 2.58, 2.50 and 1.57% with the addition of AA to trap 15, 30, 45 and 60% respectively of the ammonia evolved from urea. Statistically the N content was significantly ($p<0.01$) different in various treatment and there was maximum increase at 30% level, afterwards it remained constant. There was significant ($p<0.01$) depression in various fractions of cell wall constituents. The concentration of ammonia-N (mg/100 g feed sample) was significantly ($p<0.01$) more in wheat straw sample (T_1) where no AA was added. There was continuous decrease in the ammonia-N concentration in wheat straw samples with the increase in the level of AA.

In sacco disappearance of dry matter of wheat straw after incubation for 48 h increased significantly ($p<0.01$) due to ammoniation and AA treatment (table 2) which has also been documented earlier (Horton, 1978; Sehneider and Flachowsky, 1990; Coneque et al., 1998). Similarly there was a significant ($p<0.01$) increase in N, NDF, ADF and hemicellulose disappearance due to ammoniation of straw, although no effect was seen due to AA treatment. These results are in line with earlier observations (Dass et al., 1993 a,b).

Experiment 2

The chemical composition of various feeds offered to the experimental animals in different groups is given in table 3. Although the N content of AA treated ammoniated wheat straw was higher than the ammoniated straw but the increase was not as much as obtained in experiment 1, where AA was added in the same proportion to trap 30% of free ammonia-N. Digestibility of nutrients and their intake is presented in table 4. The palatability of AA treated straw was as that of ammoniated wheat straw, however, Borhami et al. (1982) found a depressing effect on the palatability of AA treated straw in sheep, as the level of acid used was to neutralize 100% of the ammonia-N into the straw stack, but in the present experiment the acid used was only to trap 30% of the ammonia released. Intake of dry matter (DM), organic matter (OM), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and hemicellulose were statistically alike in 3 groups. However, the N intake was significantly ($p<0.05$) more in AA treated groups, but there was no significant difference between the two groups. The digestibility coefficient of DM, OM, NDF, ADF were statistically alike in three groups, but the digestibility coefficient of N was significantly ($p<0.05$) more in group II and III, as compared to group I. The digestibility coefficient of EE was statistically alike in group I and III, but it was significantly ($p<0.05$) depressed in group II. The digestibility coefficient of cellulose and hemicellulose depressed ($p<0.05$) significantly in group II and III as compared to group I. Intake of nitrogen and its balance is presented in table 5. Intake was significantly ($p<0.05$) higher in group II and III as compared to group I. Excretion of N through faeces and urine was more in group II and III as compared to group I, although non significant. Animals in all the three groups were in +ve N balance, and it was significantly ($p<0.05$) more in group II and III as compared to group I. Improved recovery of nitrogen in the straw resulted in a significant ($p<0.05$) increase in N (DCP) intake in group II and III. The % DCP in the ration was 4.72, 8.45 and 7.79 respectively in group I, II, III and the same was significantly ($p<0.05$) different in AA treated groups i.e. in group II and III as compared to group I. Increased DOM caused a higher total digestible nutrients in group II and III as compared to group I. The TDN (%) of the ration was not different significantly ($p<0.05$) among the three groups.

Table 2. *In sacco* DM, N, NDF, ADF and hemicellulose disappearance from WS, AWS and AWS+varying quantities of AA

Treatment	DM	N	NDF	ADF	Hemicellulose
WS	42.5**^{a}±1.2	-	44.1**^{a}±2.6	44.2^a±2.4	44.0^b±2.5
AWS	56.6^b ±0.3	8.57**^{a}±3.6	56.9^b ±1.3	52.6^b±2.8	54.2^c±1.4
AWS+AA (1.35 ml)	50.6^b ±5.1	9.88^b ±2.7	44.8^a ±2.9	53.0^b±0.9	57.6^c±2.5
AWS+AA (2.70 ml)	60.0^c ±1.8	11.04^b ±2.0	42.6^a ±2.4	54.1^b±1.6	45.1^c±2.6
AWS+AA (4.05 ml)	54.2^b ±0.2	11.44^c ±1.1	44.0^a ±0.1	55.7^b±2.8	41.7^a±0.8
AWS+AA (5.40 ml)	52.1^b ±0.7	10.81^c ±0.6	43.5^a ±1.4	56.1^b±5.6	41.7^a±2.0

* $p<0.05$, ** $p<0.01$.
[a,b,c] Different superscripts in the same column indicate significance - Not detected

Table 3. Chemical composition (%) of feeds offered

Feed	DM	CP	EE	NDF	ADF	Cellulose	Hemi-cellulose
Urea treated wheat straw	89.3	9.2	1.1	83.8	57.2	44.5	26.6
Urea + AA treated wheat straw	88.4	12.5	1.1	83.7	53.6	41.0	30.1
Barley grain	94.0	10.5	2.1	45.9	14.4	11.0	31.5

Table 4. Nutrients intake (g/d) and digestibility (%) in buffalo bulls offered WS, AWS, AWS + barley

Parameters	Treatment			SEM
	I	II	III	
DM	3883.3	4612.2	4805.5	494.8
DM/kg $W^{0.75}$	55.7	63.0	62.0	4.5
Digestibility	50.8	47.4	49.1	2.5
OM	3477.5	4090.4	4327.5	428.1
Digestibility	54.4	50.8	53.4	2.4
N*	57.2[a]	93.8[b]	96.3[b]	6.70
Apparent digestibility (%)*	51.0[a]	66.4[b]	61.8[b]	2.4
EE	44.1	53.4	64.4	5.7
Digestibility*	45.7[b]	34.1[a]	43.3[b]	1.2
NDF	3266.2	3864.6	3695.3	411.6
Digestibility	60.4	54.5	52.81	2.4
ADF	2232.1	2479.3	2231.1	268.4
Digestibility	53.7	46.3	46.1	2.8
Cellulose	1736.4	1892.4	1705.2	204.6
Digestibility	71.4[b]	62.5[a]	64.3[a]	2.2
Hemicellulose	1034.1	1388.6	1464.0	144.0
Digestibility	75.0	69.0	62.8	1.98

[a,b] Different superscripts in the same row differ significantly. * p<0.05.

Table 5. Nitrogen intake and excretion in buffaloes

Parameters	Group			SEM
	I	II	III	
N intake (g/d)*	57.2[a]	93.8[b]	96.3[b]	6.7
N excreted (g/d)				
Faeces	28.3	31.4	36.4	4.1
Urine	17.8	28.1	26.5	6.2
Apparent digestibility*	51.0[a]	66.4[b]	61.8[b]	2.4
N retention*	11.0[a]	34.2[b]	33.4[b]	2.1

[a,b] Different superscripts in the same row differ significantly.
* p<0.05.

DISCUSSION

The N content of wheat straw increased with the increase in fixation level of AA. This might be due to the formation of ammonium acetate. Borhami et al. (1982), Cloete and Kritzinger (1984) and Taiwo et al. (1995) also observed significant increase in N content of straw after the addition of acids to ammoniated straw. Cell wall fractions like NDF and hemicellulose were not declined at 30% AA level, however, there was decreasing trend in ADF and cellulose values. This decreasing trend might be due to solubilization of cell wall constituents, Yadav and Virk (1994a,b) also reported that ammoniation decreases cell wall constituents of gram straw and wheat straw respectively. Among various AA fixation levels, DM and N disappearance was significantly more at 30% level, which indicated that beyond this level there will be no beneficial use of adding AA. This may be due to better utilization of compounds formed due to AA treatment by the rumen microbes.

The results of experiment 2, where DM intake although more in group II and III was statistically alike in all the 3 groups. The results were similar with the findings of Yadav and Virk (1994a,b); and Dass et al. (2001), who observed no difference in dry matter intake in buffalo calves fed on ammoniated straw and ammoniated straw treated with hydrochloric acid. In earlier findings Borhami et al. (1982) also did not find any significant difference in dry matter intake in sheep fed on ammoniated straw or ammoniated straw sprayed with acetic or formic acid. Contrary to the above L'estrange and Murphy (1972) observed an adverse effect on voluntary food intake of the sheep by 19% and 30% due to treatment of wheat straw with HCl and H_2SO_4 respectively. The cause of this reduced intake could be related to rumen pH, rumen acid concentration, prolonged food retention and metabolic disturbance. The digestibility of OM and DM was not affected in group II and III, but the digestibility of N was significantly (p<0.05) more in group II and III, as compared to group I. Fahmy and Orskov (1984), Torotich (1992) and Yadav and Virk (1994a,b) reported significant improvement in DM digestibility due to ammonia and acid treatment. It was reported (Yadav and Virk, 1994a,b) that buffalo calves fed on ammoniated straw treated with hydrochloric acid and sulphuric acid, and sheep fed on ammoniated straw sprayed with acetic and formic acid (Borhami et al., 1982) increased (p<0.01) apparent digestibility of nitrogen. The EE digestibility was equivalent in group I and III, but it depressed in group II, due to significant loss of EE in faeces. Borhami et al.

(1982) also noticed slight depression in EE digestibility due to feeding of AA treated ammoniated straw. Although there was depression in NDF and ADF digestibility but not significant ($p<0.05$). Depression in hemicellulose and cellulose digestibility, may be due to lower pH in the rumen, which might have depressed the growth of cellulolytic bacteria. Yadav and Virk (1994a) also reported depression in cellulose digestibility in adult buffaloes due to feeding of sulphuric acid treated ammoniated straw. Improved recovery of nitrogen in acid treated groups II and III resulted in a significant ($p<0.05$) increase in digestible CP (DCP).

CONCLUSION

It can be concluded that AA (to trap 30% NH_3-N) is effective in capturing the excess ammonia released during urea ammoniation of straw and improving its nutritive value, as well as animal performance.

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