*Research article*

# The Effect of Roughage and Urea Solution Infusion Levels on Ruminal $NH_3$-N Concentration and Nutrient Digestibility in Beef Cattle and Swamp Buffaloes

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**Abstract**

Four, fistulated, male crossbred cattle and swamp buffaloes were randomly assigned in a 2 x 2 factorial arrangement in a 4 x 4 Latin square design. There were four combination dietary treatments; T1) UTS as a roughage and urea infusion at 50 g/h/d (50U), T2) UTS as a roughage and urea infusion at 100 g/h/d (100U), T3) CH as a roughage and urea infusion at 50 g/h/d (50U) and T4) CH as a roughage and urea infusion at 100 g/h/d (100U). Rumen fluid and blood were collected at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h-post feeding and then analyzed for total bacteria and protozoal, $NH_3$-N, VFA and BUN, respectively. It was found that TDMI in buffoloes fed with UTS as a roughage source with both urea solution infusion were highest ($P<0.05$). While, digestibility of OM was higher in cattle fed with UTS as a roughage and urea solution infusion in both levels, CP, NDF and ADF digestibility were affected by type of roughage ($P<0.05$). With regards to digestible nutrient intake, the values of OM, CP, NDF and ADF (kg/d) were significantly different among treatments ($P<0.05$). Higher levels of ruminal pH were found in buffaloes fed with CH as a roughage and urea infusion in both levels ($P<0.05$). $NH_3$-N concentrations were higher ($P<0.05$) in buffaloes fed with CH as a roughage and urea in fusion at 100 g/h/d (34.3 mg%) and lower in cattle fed with both roughage sources and urea infusion at 50 g/h/d (20.0 and 23.5 mg%, respectively). TVFA was affected by type of roughage ($P<0.05$) and urea infusion at 100 g/h/d. Proportion of $C_2$, $C_3$ and $C_4$ were significantly different ($P<0.05$) among treatments.

**Key Words**: Urea infusion; $NH_3$-N, Rumen Microbes; Beef Cattle; Swamp Buffaloes; Digestibility

## Introduction

Ruminal ammonia nitrogen ($NH_3$-N) has been reported to be an important compound in supporting efficient rumen fermentation and it is the major nitrogen source for microbial protein synthesis and growth (Wanapat and Pimpa, 1999). Erdman et al. (1986) found that a higher level of $NH_3$-N can increase the rate of fermentation *in vivo*, depending on the potential fermentation of feed. Perdok and Leng (1990) found that a higher level of ruminal $NH_3$-N (15-20 mg%) increased dry matter intake and digestibility in cattle fed with low quality roughage. Urea is used extensively as a substitute for protein in ruminant diets, because bacteria in the rumen can synthesize protein from ammonia produced through hydrolysis of urea. The extent to which urea can substitute for dietary protein depends on how much the bacteria can utilize ammonia for protein synthesis (Satter and Slyter, 1974).

The objective of this experiment was to study the effect of roughage and the level of urea solution infusion on rumen ecology, feed intake and nutrient digestibility in beef cattle and swamp buffaloes.

**Materials and Methods**

Four, fistulated male crossbred cattle and swamp buffaloes were randomly assigned in a 2 x 2 factorial arrangement in a 4 x 4 Latin square design. There were four combination dietary treatments; T1) Urea-treated rice straw (UTS) as a roughage and urea (U) infusion at 50 g/h/d (50U), T2) UTS as a roughage and urea infusion at 100 g/h/d (100U), T3) Cassava hay (CH) as a roughage and urea infusion at 50 g/h/d (50U) and T4) CH as a roughage and urea infusion at 100 g/h/d (100U). During the first 14 days, all animals were fed on respective diets on *ad libitum* basis and for the last 7 days animals were intraruminally infused by Gilson Autoanalyzer proportioning pump with urea solution at 50 and 100 g/h/d, each in 5 litre of distilled water/day continuously. All feeds and fecal were sampled for the analysis of chemical composition in terms of dry matter (DM), ash, crude protein (CP) (AOAC, 1985), neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) (Goering and Van Soest, 1970). Rumen fluid and blood were collected at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h-post feeding and then analyzed for $NH_3$-N concentration (Bromner and Keeney, 1965) and blood-urea nitrogen (BUN) (Crocker, 1967), respectively. Analysis of total bacteria, protozoal count (Galyean, 1989), and volatile fatty acids (VFA) concentration were analysed at 0 and 4 h-post feeding, respectively, by using HPLC technique. All data were subjected to analysis of variance using Proc. GLM (SAS, 1999) and compared between animal species according to procedure of Kennedy et al. (1992). Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

**Results**

Concentrate, UTS and CH contained 12.9, 7.3 and 24.7% CP, respectively (Table 1). The effect of roughage sources and urea solution infusion on ruminal pH, temperature, ammonia-nitrogen ($NH_3$-N) and blood-urea nitrogen (BUN) were shown in means from 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h-post feeding, while total volatile fatty acids (TVFA), acetic acid ($C_2$), propionic acid ($C_3$), butyric acid ($C_4$) concentration, total bacteria and protozoal population were shown in means from 0 and 4 h-post feeding (Table 2). Total DM intake and roughage intake were presented in Table 3, while digestibility coefficient and digestible nutrient intake of beef cattle and swamp buffaloes were shown in Table 4.

Higher levels of ruminal pH were found in buffaloes fed with CH as a roughage and urea infusion in both levels (50 & 100 g/h/d) ($P<0.05$) when compared with other treatments. These results were affected by species ($P<0.01$) and type of roughage ($P<0.05$), while the level of urea had no effect ($P>0.05$). Ruminal $NH_3$-N concentrations were higher ($P<0.05$) in buffaloes fed with CH as a roughage and urea infusion 100 g/h/d (34.3 mg%) and lower levels in cattle fed with both roughage sources and urea

**Table 1** Chemical composition of concentrate, urea-treated (5%) rice straw (UTS), cassava hay (CH).

| Feedstuffs | Concentrate[1] | UTS | CH |
|---|---|---|---|
| DM, % | 92.1 | 55.0 | 87.0 |
| | -----------------------% of DM------------------------------------------------------ | | |
| OM | 93.2 | 87.4 | 92.4 |
| Ash | 6.8 | 12.6 | 7.6 |
| **CP** | **12.9** | **7.3** | **24.7** |
| NDF | 33.4 | 64.4 | 44.7 |
| ADF | 7.7 | 44.4 | 32.2 |

UTS = urea-treated rice straw, CH = cassava hay, FCF = fresh cassava foliage, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral-detergent fiber, ADF = acid detergent fiber.
[1]concentrate : cassava chip 60%, rice bran 15%, palm meal 20%, molasses 2%, salt 1%, mineral mix 1%, and sulphur 1% (DM basis).

infusion at 50 g/h/d (20.0 and 23.5 mg%, respectively). Therefore, high urea solution infusion (100g/h/d) gave rise to the high $NH_3$-N concentration in both species (P<0.05). The pattern of BUN concentration was similar to the pattern of ruminal $NH_3$-N concentration, while BUN concentration was increased as urea solution infusion increased (P<0.05). The concentration of TVFA was affected by the type of roughage (P<0.05) and urea infusion at 100 g/h/d. Proportion of $C_2$, $C_3$ and $C_4$ were significantly different (P<0.05) among treatments. $C_2$ concentration was affected by the interaction between type of roughage and urea levels infusion (P<0.05), while animal species were affected by the $C_3$ concentration. Butyric acid concentrations were affected by animal species. The results showed that swamp buffaloes fed with UTS as a roughage source increased butyric acid concentration in the rumen fluid.

Bacterial population in swamp buffaloes, fed with CH as a roughage source and urea infusion at 100 g/h/d (P<0.05) was higher than those in cattle fed with both roughage sources and urea infusion levels. However, roughage sources and urea solution infusion had no effect (P>0.05) on this values. In addition, protozoan population had not been affected (P>0.05) by animal species, type of roughage and urea solution infusion levels.

It was also found that roughage intakes (kg/d, %BW and g/kg$W^{0.75}$) were affected by animal species (P<0.05). Swamp buffaloes fed with UTS, as roughage source and urea solution infusion in both levels, had higher response to feed intakes. Total dry matter intakes in terms of %BW and g/kg$W^{0.75}$ in swamp buffaloes were increased at both urea solution infusions (P<0.05). Digestibility of organic matter (OM) was higher in cattle fed with UTS as a roughage and urea solution infusion (in both levels). The values of CP, NDF and ADF digestibility were affected by type of roughage (P<0.05). The values of OM, CP NDF and ADF (kg/d) were significantly different among treatments (P<0.05) and were affected by animal species and the type of roughage sources (P<0.05).

**Table 2** Effect of roughage sources and urea solution infusion on ruminal ecology in beef cattle and swamp buffaloes.

| Items | Cattle | | | | Buffaloes | | | | SEM | Interactions | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UTS | | CH | | UTS | | CH | | | S | R | U | S*R | S*U | R*U | S*R*U |
| | 50U | 100U | 50U | 100U | 50U | 100U | 50U | 100U | | | | | | | | |
| pH | 6.6[a] | 6.6[a] | 6.6[a] | 6.6[a] | 6.7[ab] | 6.7[ab] | 6.9[b] | 6.9[b] | 0.06 | ** | * | ns | ns | ns | ns | ns |
| Temp, °C | 40.1 | 39.9 | 40.1 | 40.0 | 40.1 | 40.0 | 40.1 | 39.9 | 0.16 | ns | ns | ns | ns | ns | ns | ns |
| $NH_3$-N, mg% | 20.0[a] | 26.6[ab] | 23.5[a] | 27.8[ab] | 29.7[ab] | 27.7[ab] | 27.5[ab] | 34.3[b] | 4.13 | * | ns | * | ns | ns | ns | ns |
| BUN, mg% | 19.7[a] | 24.8[ab] | 22.6[ab] | 26.3[ab] | 27.5[ab] | 25.9[ab] | 25.8[ab] | 31.6[b] | 3.67 | ns | ns | * | ns | ns | ns | ns |
| TVFA, mM | 120.2[a] | 111.4[ab] | 81.2[b] | 94.4[b] | 96.7[b] | 122.0[a] | 103.1[ab] | 102.2[ab] | 9.06 | ns | * | ns | ns | ns | ns | ns |
| C2, mol % | 69.9[a] | 66.7[a] | 65.3[a] | 65.5[a] | 65.8[a] | 61.9[b] | 58.5[b] | 62.0[b] | 1.43 | * | * | ns | ns | ns | * | ns |
| C3, mol % | 17.1[a] | 20.6[ab] | 18.7[a] | 21.2[ab] | 21.8[ab] | 21.8[ab] | 24.8[b] | 20.6[ab] | 1.20 | * | ns | ns | ns | * | ns | ns |
| C4, mol% | 12.6[a] | 13.1[a] | 15.2[ab] | 13.2[a] | 12.4[a] | 16.2[ab] | 16.7[ab] | 17.3[b] | 1.11 | * | * | ns | ns | ns | ns | ns |
| Bacteria, x $10^{12}$cells/ml | 3.8[ab] | 3.7[ab] | 3.5[a] | 3.4[a] | 4.2[ab] | 4.4[ab] | 3.6[a] | 5.2[b] | 0.54 | * | ns | ns | ns | ns | ns | ns |
| Protozoa, x $10^{5}$cells/ml | 1.7 | 1.9 | 1.3 | 1.5 | 2.6 | 1.3 | 2.4 | 2.9 | 5.52 | ns | ns | ns | ns | ns | ns | ns |

[a,b,c] values on the same row with different superscripts differ (P<0.05),
UTS = urea-treated rice straw, CH = cassava hay, S = animal species (cattle and swamp buffaloes), R = roughage sources (UTS and CH),
U = urea levels infusion (50 and 100 g/d), Temp = temperature, TVFA = total volatile fatty acid, C2 = acetic acid, C3 = propionic acid,
C4 = butyric acid, BUN = blood-urea nitrogen, ns = not significant; * = p<0.05; ** = p<0.01, SEM =standard error of the mean

**Table 3** Effect of roughage sources and urea solution infusion on voluntary feed intake in beef cattle and swamp buffaloes.

| Items | Cattle | | | | Buffaloes | | | | SEM | Interactions | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UTS | | CH | | UTS | | CH | | | S | R | U | S*R | S*U | R*U | S*R*U |
| | 50U | 100U | 50U | 100U | 50U | 100U | 50U | 100U | | | | | | | | |
| Roughage dry matter intake/day | | | | | | | | | | | | | | | | |
| kg | $9.6^{a}$ | $7.4^{b}$ | $6.7^{b}$ | $7.1^{b}$ | $7.5^{b}$ | $7.6^{b}$ | $6.5^{bc}$ | $6.2^{c}$ | 0.20 | * | * | ns | ns | ns | ns | * |
| %BW | $1.6^{a}$ | $1.5^{a}$ | $1.5^{a}$ | $2.1^{b}$ | $2.3^{c}$ | $2.3^{c}$ | $2.0^{b}$ | $1.9^{b}$ | 0.08 | * | ns | ns | * | * | * | * |
| $g/kgW^{0.75}$ | $80.4^{a}$ | $70.7^{b}$ | $70.0^{b}$ | $90.3^{ac}$ | $98.0^{c}$ | $97.2^{c}$ | $85.1^{a}$ | $80.3^{a}$ | 3.89 | * | ns | ns | * | * | * | * |
| Total dry matter intake/day | | | | | | | | | | | | | | | | |
| kg | $11.4^{a}$ | $8.9^{b}$ | $8.0^{bc}$ | $8.1^{bc}$ | $8.3^{b}$ | $8.6^{b}$ | $7.5^{bc}$ | $7.2^{c}$ | 0.41 | * | * | * | ns | * | ns | * |
| %BW | $1.9^{a}$ | $1.8^{a}$ | $1.8^{a}$ | $2.4^{bc}$ | $2.6^{a}$ | $2.6^{b}$ | $2.3^{c}$ | $2.2^{c}$ | 0.08 | * | ns | ns | * | * | * | * |
| $g/kgW^{0.75}$ | $95.1^{a}$ | $84.8^{b}$ | $83.5^{b}$ | $103.1^{ac}$ | $110.7^{c}$ | $110.0^{c}$ | $97.8^{a}$ | $91.3^{a}$ | 3.90 | * | * | ns | * | ns | * | * |

$^{a,b}$ values on the same row with different superscripts differ (P<0.05),
UTS = urea-treated rice straw, CH = cassava hay, S = animal species (cattle and swamp buffaloes), R = roughage sources (UTS and CH),
U = urea levels infusion (50 and 100 g/d), ns = not significant, * = p<0.05, SEM = standard error of the mean

**Table 4** Effect of roughage sources and urea solution infusion on digestibility coefficient and digestible nutrient intake in beef cattle and swamp buffaloes.

| Items | Cattle | | | | Buffaloes | | | | SEM | Interactions | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UTS | | CH | | UTS | | CH | | | S | R | U | S*R | S*U | R*U | S*R*U |
| | 50U | 100U | 50U | 100U | 50U | 100U | 50U | 100U | | | | | | | | |
| Digestion coefficient, % | | | | | | | | | | | | | | | | |
| DM | 71.8 | 70.6 | 64.8 | 67.4 | 67.6 | 69.0 | 70.8 | 69.5 | 2.52 | ns | ns | ns | ns | ns | ns | ns |
| OM | 76.3[a] | 75.4[a] | 67.1[b] | 70.4[ab] | 72.2[ab] | 73.7[ab] | 73.3[ab] | 72.7[ab] | 2.59 | ns | * | ns | * | ns | ns | ns |
| CP | 62.5[a] | 62.8[a] | 67.3[b] | 69.8[b] | 63.1[a] | 64.9[a] | 69.2[b] | 67.2[b] | 3.32 | ns | ** | ns | ns | ns | ns | ns |
| NDF | 68.1[a] | 66.0[ab] | 66.3[ab] | 69.3[a] | 63.5[b] | 66.7[ab] | 66.9[ab] | 65.2[b] | 3.28 | ** | * | ns | * | ns | ns | ns |
| ADF | 56.0[ab] | 54.9[a] | 58.2[ab] | 63.4[b] | 56.7[a] | 52.3[a] | 67.1[b] | 64.3[b] | 3.33 | ns | ** | ns | ns | ns | ns | ns |
| Digestible nutrient intake, kg/d | | | | | | | | | | | | | | | | |
| DM | 5.6[a] | 5.9[ab] | 5.2[a] | 5.0[a] | 6.1[b] | 6.2[b] | 5.2[a] | 5.4[a] | 0.32 | ** | ** | ns | * | ns | ns | ** |
| OM | 5.4[ab] | 5.6[ab] | 5.0[a] | 4.8[a] | 5.6[ab] | 5.9[b] | 5.0[a] | 5.3[ab] | 0.29 | ** | ** | ns | * | ns | ns | ** |
| CP | 0.4[a] | 0.4[a] | 0.8[ab] | 0.8[ab] | 0.6[ab] | 0.5[ab] | 0.9[b] | 0.9[b] | 0.03 | ** | ** | ns | * | ns | ns | * |
| NDF | 2.8[ab] | 3.0[ab] | 2.2[a] | 2.0[a] | 3.6[b] | 3.5[b] | 2.3[a] | 2.4[a] | 0.21 | ** | ** | ns | ** | ns | ns | * |
| ADF | 1.9[a] | 1.8[a] | 1.4[b] | 1.3[b] | 1.5[b] | 1.9[a] | 1.3[b] | 1.5[b] | 0.13 | ns | ** | ns | ns | ns | * | ns |

a,b values on the same row with different superscripts differ (P<0.05), UTS = urea-treated rice straw, CH = cassava hay, S = animal species (cattle and swamp buffaloes), R = roughage sources (UTS and CH), U = urea levels infusion (50 and 100 g/d), DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral-detergent fiber, ADF = acid detergent fiber, ns = not significant, * = p<0.05, ** = p<0.01, SEM = standard error of the me

**Discussion**

These results of ruminal pH pattern in this trial were similar to those reported by Rihani et al. (1993). Ruminal pH was maintained within the values of 6.6 to 6.9, the optimal value for microbial growth and digestion of fiber (pH 6-7) (Weimer, 1996). Mould and Orskov (1984) demonstrated that cellulose digestion was limited when ruminal pH was below 6.0. In this study, the pH was much higher than this threshold value. Moreover, the rumen pH may be affected by $NH_3$-N concentration in the rumen fluid when urea is hydrolyzed by urea utilizing bacteria into $NH_3$. Slyter et al. (1979) and Pan et al. (2003) demonstrated that increased ruminal $NH_3$-N (22.5 mg%) might increase ruminal pH, TVFA production and stimulated cellulolytic bacteria activity in the rumen. A similar trend of ruminal total bacteria and protozoan population were also reported by Song and Kennelly (1990). Wanapat and Pimpa (1999) also found that higher levels of ruminal $NH_3$-N at 17.6 mg% resulted in the highest total purine derivatives, indicating highest rumen microbial protein synthesis. Kajanapruthipong and Leng (1998) showed that protozoan, fungal and bacterial populations in the rumen were influenced by the levels of ruminal $NH_3$-N. Wanapat et al. (2000) found that the rumen samples of microbial populations, obtained from animals kept under traditional village conditions in the Northeast of Thailand, had similar pH value for both species, but had significantly difference in the numbers of microorganism. However, there was a trend for a higher population of bacteria, a lower population of protozoa and more fungal zoospores in the ruminal fluid of buffaloes as compared with those of cattle. Wattanachant et al. (1990) and Wora-anu et al. (2000) found that total bacterial count and cellulolytic bacteria in swamp buffalo were higher than those of the cattle. In the present study where animals were subjected to similar feeding, a higher bacterial and lower protozoan counts were detected.

The pattern of BUN concentration was similar to the pattern of ruminal $NH_3$-N concentration, while BUN concentration was increased as urea solution infusion increased and maximum levels shown in swamp buffaloes fed on cassava hay with urea solution infusion at 100 g/h/d.

The values of DM, CP and NDF were enhanced by urea solution infusion in both levels. These results were in agreement with the report of Winter and Pigden (1971), who found that the increase in DM and cellulose digestibility of oat straw diet responded to continuous urea infusion into the rumen. The values of OM, CP, NDF and ADF were affected by animal species and the type of roughage sources.

**Conclusion**

Based on this study, it can be concluded that the use of urea-treated rice straw as a roughage source is sufficient for ruminant production, providing effective fiber, maintaining higher pH, improving rumen $NH_3$-N, VFA and increasing bacteria population. CH should be recommended as high quality roughage and as a protein supplement during the long dry season for improving rumen ecology and protein supply for animal. Urea was the most common non-protein nitrogen (NPN) source used in ruminant feeding. Urea can be used to provide optimal rumen $NH_3$-N which would influence microorganisms, particularly total bacteria in the rumen as well as digestibility of fibrous fractions and feed intake in beef cattle and swamp buffaloes. However, further studies should be conducted using specific techniques as Real Time PCR and Denaturing Gradient Gel Electrophoresis (DGGE) with specific probes/primers to identify and quantify this bacterial population, particularly predominant ruminal cellulolytic bacteria.

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