

## Effect of Ruminant $\text{NH}_3\text{-N}$ Levels on Ruminant Fermentation, Purine Derivatives, Digestibility and Rice Straw Intake in Swamp Buffaloes

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**ABSTRACT** : The experiment was aimed at studying the effect of ruminal  $\text{NH}_3\text{-N}$  levels on ruminal fermentation, microbial population, urinary purine derivative excretion, digestibility and rice straw intake in swamp buffaloes. Five, 3 to 4 years old, rumen fistulated swamp buffaloes were randomly assigned according to a  $5 \times 5$  Latin square design to receive five different intraruminal infusions of  $\text{NH}_4\text{HCO}_3$  (0, 150, 300, 450 and 600 g/d) on a continuous daily basis. Rice straw as a roughage was offered *ad libitum* while concentrate was given at 0.8% BW daily. The results were that as levels of  $\text{NH}_4\text{HCO}_3$  increased, ruminal  $\text{NH}_3\text{-N}$  concentrations increased from 7.1 to 34.4 mg%. The highest digestibility and voluntary straw intakes were found at 13.6 to 17.6 mg% ruminal  $\text{NH}_3\text{-N}$  levels; straw intake was highest at 13.6 mg%. Total bacterial and protozoal counts linearly increased as the ruminal  $\text{NH}_3\text{-N}$  increased and were highest at 17.6 mg%. Total urinary purine derivatives and allantoin excretion were highest (44.0, 37.4 mM/d) at 17.6 mg% ruminal  $\text{NH}_3\text{-N}$ . Highest total VFAs (115 mM) were obtained at 13.6 mg% ruminal  $\text{NH}_3\text{-N}$  while blood urea nitrogen significantly increased as ruminal  $\text{NH}_3\text{-N}$  increased. The results from this experiment suggest that optimum ruminal  $\text{NH}_3\text{-N}$  in swamp buffaloes is higher than 13.6 mg%, for improving rumen ecology, microbial protein synthesis, digestibility and straw intake. (*Asian-Aus. J. Anim. Sci.* 1999. Vol. 12, No. 6 : 904-907)

**Key Words** : Swamp Buffalo, Ruminal  $\text{NH}_3\text{-N}$ , VFA, Purine Derivatives, Microbial Population, Rice Straw

### INTRODUCTION

Ruminal ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) has been reported to be an important nutrient in supporting efficient rumen fermentation. Satter and Slyter (1974) earlier reported that 5 mg% ruminal  $\text{NH}_3\text{-N}$  was optimum for microbial fermentation in a mixed culture in a closed system while Erdman et al. (1986) found that a higher level would be required to achieve a maximum rate of fermentation *in vivo*, depending on the potential fermentability of feeds. In cattle fed low quality roughage, Boniface et al. (1986) and Perdok and Leng (1990) found a higher level of ruminal  $\text{NH}_3\text{-N}$  (15 to 20 mg%) increased digestibility and intake. Although a number of researchers including Devendra (1985) and Wanapat et al. (1994) showed that swamp buffaloes were more efficient than cattle in many aspects, namely N-recycling and fiber digestion, ruminal  $\text{NH}_3\text{-N}$  level in relation to efficient fermentation and intake has not been defined. Suwanlee and Wanapat (1994) reported that when ruminal  $\text{NH}_3\text{-N}$  increased, from 1.7 to 5.6 mg%, total bacterial count, digestibilities of DM, NDF, ADF, increased, however optimal ruminal  $\text{NH}_3\text{-N}$  levels in swamp buffaloes have not yet been substantiated. It was therefore the objective of this experiment to study effects of various levels of ruminal  $\text{NH}_3\text{-N}$  on rumen fermentation, microbial population, urinary purine derivative excretion, digestibility and rice straw intake

in swamp buffaloes.

### MATERIALS AND METHODS

#### Animals and treatments

Five, 3-4 years old male swamp buffaloes, averaging  $325 \pm 24.9$  kg live weight, fitted with rumen fistulae, were used in the experiment according to a  $5 \times 5$  Latin square design. Animals were individually maintained in metabolism crates during the experimental periods.

Rice straw was given on *ad libitum* basis as a major roughage. The chemical composition of rice straw was 94.3% DM, 13% ash, 2.2% CP, 70.8% NDF, 45.3% ADF. Concentrate was fed at 0.8% DM of its body weight daily and chemical composition of the concentrate was 89.2% DM, 8% ash 17.0% CP, 11.3% NDF and 7.2% ADF.

All buffaloes were intraruminally infused (by Gilson Autoanalyzer proportioning pump) with ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) at 0, 150, 300, 450 or 600 g/d, each in 2 litre of distilled water. Each experimental treatment was maintained for thirteen consecutive days and during the last five days of each experimental period, collection of rumen fluid, blood, urine and faeces were made. Feed intake was recorded daily and clean water was available throughout the experimental periods.

#### Sample preparation and chemical analysis

Feces were daily weighed, thoroughly mixed and daily sampled (5% of total) from each buffalo during

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Received November 7, 1998; Accepted December 15, 1998

the last five days of each period, dried (60°C, 48 h) and composited. Rice straw and concentrate were ground through a 1 mm screen and analyzed for dry matter (DM), crude protein (CP) according to AOAC (1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to the methods of Georing and Van Soest (1970). Digestibilities were calculated according to the method of Schneider and Flatt (1975).

Urine was weighed, collected and sampled daily (10% of excretion) during the last five days of each period (acidified with 10% H<sub>2</sub>SO<sub>4</sub> to pH<3), and three subsamples were collected and stored immediately at -20°C for later purine derivative analysis by HPLC (Balcells et al., 1992) and calculation of purine derivatives according to the method of Chen and Gomest (1992).

Ruminal samples were collected through ruminal fistulae at 0, 1, 1.5, 2, 3, 4 and 5 h post-feeding for measurement of pH, NH<sub>3</sub>-N, bacterial population (Galyean, 1989) and protozoal population (Dehority, 1984). Samples of rumen fluid during each time of each period (about 100 ml) were collected. The pH of each sample was measured immediately and the sample was strained through two layers of cheese cloth. Fifty ml of samples were acidified with 5 ml of HCl solution (6 N) and centrifuged at 2000 g for 20 minute and the supernatant stored at 20°C for NH<sub>3</sub>-N and VFA analyses according to Zinn and Owens (1986). Blood was collected from the jugular vein at the same time of rumen fluid sampling and was separated by centrifugation at 500 rpm for 10 minute, and stored at 20°C until analysis of blood urea

nitrogen (BUN) according to the method of Crocker (1967).

#### Statistical analysis

All data were subjected to ANOVA and linear (L), quadratic (Q), and cubic (C) contrasts in response to levels of NH<sub>3</sub>-N were tested using SAS (1985). The mean value of each parameter within sampling period was statistically compared across treatments.

## RESULTS AND DISCUSSION

As level of NH<sub>4</sub>HCO<sub>3</sub> increased, level of ruminal NH<sub>3</sub>-N increased from 7.1 to 8.8, 13.6, 17.6 and 34.4 mg% for lowest to highest infusion levels, respectively. Average rice straw and total intakes, and apparent digestibility percentages of DM and ADF are given in table 1. Voluntary intake increased significantly in response of ruminal NH<sub>3</sub>-N (Q) and was highest at 8.8-13.6 and lowest at 34.4 mg%. Although NH<sub>3</sub>-N was at 34.4 mg%, buffaloes did not show any toxicity. Digestibilities of DM and ADF were highest at 13.6 mg% NH<sub>3</sub>-N, however bacterial and protozoal population were found highest at 17.6 mg% NH<sub>3</sub>-N. Higher ruminal NH<sub>3</sub>-N level may serve as N source to improve rumen ecology since ruminal NH<sub>3</sub>-N was lowest (1 mg%) when cattle and swamp buffaloes were fed on untreated rice straw, and improved to 10 mg% when fed on urea-treated (5%) rice straw (Chanthai et al, 1989). Excretion of total purine derivatives was not significantly different between treatments (p>0.1), but the response was highest at 17.6 mg% NH<sub>3</sub>-N (574.8 mol/BW<sup>0.75</sup>/d). Uric acid

**Table 1.** Effects of ruminal NH<sub>3</sub>-N on rice straw intake, digestibility, total ruminal bacterial, protozoal population and urinary excretion of purine derivatives

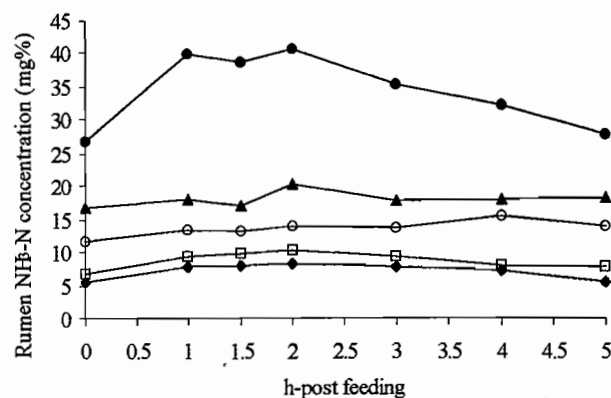
Item	Average ruminal NH <sub>3</sub> -N concentration mg% <sup>a</sup>					SEM	Contrast <sup>b</sup>
	7.1	8.8	13.6	17.6	34.4		
Rice straw, kg/d	5.1	5.6	5.6	5.3	4.7	0.42	L/Q
% BW/d	1.4	1.5	1.6	1.5	1.4	0.21	NS
Total intake, kg/d	8.0	8.5	8.5	8.1	7.4	0.41	L/Q
Digestibility, %							
DM	62.9	67.5	72.5	67.4	58.3	7.04	Q
NDF	69.6	71.3	75.8	72.0	69.3	6.27	NS
ADF	65.8	71.7	75.3	68.4	64.1	6.51	Q
CP	72.7	70.2	74.8	75.0	68.5	6.48	NS
Total bacteria, ×10 <sup>8</sup> cell/ml	1.4	1.7	2.6	3.7	1.5	0.24	L/Q
Total protozoa, ×10 <sup>5</sup> cell/ml	8.2	8.4	8.9	10.7	6.4	1.66	Q
Urinary excretion, mM/d							
Allantoin	28.3	36.1	27.6	37.4	22.4	13.20	NS
Uric acid	4.9	5.0	6.2	6.6	9.1	3.71	L
Total	33.2	41.0	33.6	44.0	31.5	15.21	NS

<sup>a</sup> Solutions of NH<sub>4</sub>-HCO<sub>3</sub> were infused intraruminally into the rumen throughout 24 h.

<sup>b</sup> Orthogonal contrasts where L=linear, Q=quadratic, NS=not significant (p>0.1).

increased as level of ruminal  $\text{NH}_3\text{-N}$  increased ( $p < 0.09$ ), while allantoin concentrations were not different ( $p > 0.1$ ), averaging 292.6 to 488.6 mol/BW<sup>0.75</sup>/d; the allantoin excretion was found highest ( $p > 0.1$ ) at 17.6 mg%  $\text{NH}_3\text{-N}$  (table 1). The increase in total purine derivative excretion was not linearly related to digestible OM intake, but the relationship was quadratic. Higher levels of ruminal  $\text{NH}_3\text{-N}$  at 17.6 mg% resulted in the highest total purine derivatives, indicating highest microbial protein synthesis. It is postulated that at higher ruminal  $\text{NH}_3\text{-N}$  level, different types of bacterial, protozoal and fungal populations may occur. However, in the present experiment the values of purine excretion (allantoin and uric acid) were different from those values reported by Vercoe (1976) but higher than those estimated from the equations developed by Liang et al. (1993) and Chen et al. (1996). The relationship between rumen  $\text{NH}_3\text{-N}$  concentration and urinary allantoin excretion at the same  $\text{NH}_3\text{-N}$  concentration was lower than 14 mg% and were in the same range with that reported by Balcells et al. (1993).

Ruminal  $\text{NH}_3\text{-N}$  as measured from 0 to 5 h post-feeding exhibited similar patterns among treatments. As clearly shown, high levels of  $\text{NH}_4\text{HCO}_3$  infusion increased ruminal  $\text{NH}_3\text{-N}$ , peaked at 2 h post-feeding, and gradually declined thereafter until 5 h post-feeding (figure 1). Mean values increased from 7.1 to 34.4 mg% in response to the increased level of  $\text{NH}_4\text{HCO}_3$  infusion. Rumen pH was very stable throughout the sampling periods (6.2 to 6.9) but significantly increased with increasing levels of  $\text{NH}_3\text{-N}$  concentration (Q). In this experiment the highest pH was 6.7 at a ruminal  $\text{NH}_3\text{-N}$  concentration of 34.4 mg%. Infusion of  $\text{NH}_4\text{HCO}_3$  solution increased BUN linearly and quadratically ( $p < 0.01$ ) from 13 to 39.3 mg%. The total VFA concentrations increased with the level of ruminal  $\text{NH}_3$  concentration, Butyric and



**Figure 1.** Rumen  $\text{NH}_3$  concentration (mg%) in swamp buffalo at 0, 1, 1.5, 2, 3, 4 and 5 h post feeding as a result of intraruminally infusion of 0 (◆), 150 (□), 300 (○), 450 (▲) and 600 (●) g  $\text{NH}_4\text{-HCO}_3$  per day

isobutyric acids (molar %) also increased by the levels of  $\text{NH}_4\text{HCO}_3$  infusion, however the proportions of propionic and acetic acids were not different among treatments ( $p > 0.05$ ). Highest total VFA were obtained at infusion of 300-450 g/d  $\text{NH}_4\text{HCO}_3$  when ruminal  $\text{NH}_3\text{-N}$  was 13.6-17.6 mg% (table 2) (figure 1). This finding was similar to that reported by Perdok and Leng (1990). Higher ruminal  $\text{NH}_3\text{-N}$  level found in swamp buffaloes may imply that non-protein nitrogen (NPN) like urea could be efficiently used at higher concentrations.

In conclusion, the results demonstrated that ruminal  $\text{NH}_3\text{-N}$  level between 13.6-17.6 mg% (15 mg%) improved rumen ecology, digestibility and intake of rice straw in swamp buffaloes. However, further studies of higher levels of ruminal  $\text{NH}_3\text{-N}$  on bacterial, protozoal and fungal population changes and their ruminal fermentation should be conducted, especially on straw based feeding.

**Table 2.** Effect of intraruminal infusion of  $\text{NH}_4\text{-HCO}_3$  on ammonia concentration, pH, volatile fatty acids and blood urea nitrogen (BUN) in swamp buffaloes

Item	Levels of $\text{NH}_4\text{-HCO}_3$ g/d <sup>a</sup>					SEM	Contrast <sup>b</sup>
	0	150	300	450	600		
Ruminal $\text{NH}_3\text{-N}$ , mg%	7.1	8.7	13.6	17.6	34.4	8.05	L/Q
pH	6.5	6.5	6.2	6.4	6.7	0.27	Q
Volatile fatty acids							
Acetic, %	74.3	74.4	73.8	73.9	74.2	1.39	NS
Propionic, %	16.4	16.2	16.7	16.4	16.4	1.14	NS
Isobutyric, %	0.9	0.9	0.8	0.8	0.8	0.12	L
Butyric, %	6.5	6.6	6.8	6.8	6.8	0.59	L
Valeric, %	0.6	0.6	0.6	0.6	0.6	0.09	NS
Total VFA, mM	96.7	98.1	115.3	113.4	110.1	15.45	L/Q
BUN, mg%	13.0	17.8	23.4	29.3	39.3	2.82	L/Q

<sup>a</sup> Solutions of  $\text{NH}_4\text{-HCO}_3$  were infused intraruminally into the rumen throughout 24 h.

<sup>b</sup> Orthogonal contrasts where L=linear, Q=quadratic, NS=non significant ( $p > 0.01$ ).

## ACKNOWLEDGMENTS

The authors were sincerely thank the staff and M.S. students in Ruminant Nutrition Group, Khon Kaen University, Thailand and to Drs. David Parker and Peter Rowlinson, University of Newcastle upon Tyne, United Kingdom for all their full practical assistance and technical support. Financial support of this project was kindly made available by the National Research Council (NRC) of Thailand and the Thailand Research Fund (TRF).

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