Effect of Ruminal NH_3-N Levels on Ruminal 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 NH_3-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 5×5 Latin square design to receive five different intraruminal infusions of NH_4HCO_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 NH_4HCO_3 increased, ruminal NH_3-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 NH_3-N levels; straw intake was highest at 13.6 mg%. Total bacterial and protozoal counts linearly increased as the ruminal NH_3-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 NH_3-N. Highest total VFAs (115 mM) were obtained at 13.6 mg% ruminal NH_3-N while blood urea nitrogen significantly increased as ruminal NH_3-N increased. The results from this experiment suggest that optimum ruminal NH_3-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 NH_3-N, VFA, Purine Derivatives, Microbial Population, Rice Straw

INTRODUCTION

Ruminal ammonia nitrogen (NH_3-N) has been reported to be an important nutrient in supporting efficient rumen fermentation. Satter and Slyter (1974) earlier reported that 5 mg% ruminal NH_3-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 NH_3-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 NH_3-N level in relation to efficient fermentation and intake has not been defined. Suwanlee and Wanapat (1994) reported that when ruminal NH_3-N increased, from 1.7 to 5.6 mg%, total bacterial count, digestibilities of DM, NDF, ADF, increased, however optimal ruminal NH_3-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 NH_3-N on rumen fermentation, microbial population, urinary purine derivative excretion, digestibility and rice straw intake in swamp buffaloes.

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MATERIALS AND METHODS

Animals and treatments

Five, 3-4 years old male swamp buffaloes, averaging 325±24.9 kg live weight, fitted with rumen fistulae, were used in the experiment according to a 5×5 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 (NH_4HCO_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

the last five days of each period, dried (60℃, 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_2SO_4 to pH<3), and three subsamples were collected and stored immediately at -20℃ 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_3-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℃ for NH_3-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 centifugation at 500 rpm for 10 minute, and stored at 20℃ 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_3-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_4HCO_3 increased, level of ruminal NH_3-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_3-N (Q) and was highest at 8.8-13.6 and lowest at 34.4 mg%. Although NH_3-N was at 34.4 mg%, buffaloes did not show any toxicity. Digestibilities of DM and ADF were highest at 13.6 mg% NH_3-N, however bacterial and protozoal population were found highest at 17.6 mg% NH_3-N. Higher ruminal NH_3-N level may serve as N source to improve rumen ecology since ruminal NH_3-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_3-N (574.8 mol/$BW^{0.75}$/d). Uric acid

Table 1. Effects of ruminal NH_3-N on rice straw intake, digestibility, total ruminal bacterial, protozoal population and urinary excretion of purine derivatives

Item	Average ruminal NH_3-N concentration mg%[a]					SEM	Contrast[b]
	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, $\times 10^8$ cell/ml	1.4	1.7	2.6	3.7	1.5	0.24	L/Q
Total protozoa, $\times 10^5$ 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

[a] Solutions of NH_4HCO_3 were infused intraruminally into the rumen throughout 24 h.
[b] Orthogonal contrasts where L=linear, Q=quadratic, NS=not significant ($p>0.1$).

increased as level of ruminal NH_3-N increased ($p<0.09$), while allantoin concentrations were not different ($p>0.1$), averaging 292.6 to 488.6 mol/$BW^{0.75}$/d; the allantoin excretion was found highest ($p>0.1$) at 17.6 mg% NH_3-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 NH_3-N at 17.6 mg% resulted in the highest total purine derivatives, indicating highest microbial protein synthesis. It is postulated that at higher ruminal NH_3-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 NH_3-N concentration and urinary allantoin excretion at the same NH_3-N concentration was lower than 14 mg% and were in the same range with that reported by Balcells et al. (1993).

Ruminal NH_3-N as measured from 0 to 5 h post-feeding exhibited similar patterns among treatments. As clearly shown, high levels of NH_4HCO_3 infusion increased ruminal NH_3-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 NH_4HCO_3 infusion. Rumen pH was very stable throughout the sampling periods (6.2 to 6.9) but significantly increased with increasing levels of NH_3-N concentration (Q). In this experiment the highest pH was 6.7 at a ruminal NH_3-N concentration of 34.4 mg%. Infusion of NH_4HCO_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 NH_3 concentration, Butyric and isobutyric acids (molar %) also increased by the levels of NH_4HCO_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 NH_4HCO_3 when ruminal NH_3-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 NH_3-N level found in swamp buffaloes may imply that non-protein nitrogen (NPN) like urea could be efficiently used at higher concentrations.

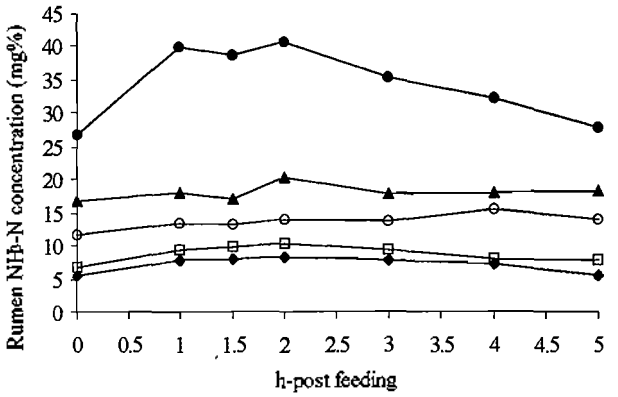

Figure 1. Rumen NH_3 concentration(mg%) in swamp buffalo at 0, 1, 1.5, 2, 3, 4 and 5 h post feeding as a result of intraruminally infusion of 0 (◆), 150 (□), 300 (○), 450 (▲) and 600 (●)g NH_4-HCO_3 per day

In conclusion, the results demonstrated that ruminal NH_3-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 NH_3-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 NH_4-HCO_3 on ammonia concentration, pH, volatile fatty acids and blood urea nitrogen (BUN) in swamp buffaloes

Item	Levels of NH_4-HCO_3 g/d[a]					SEM	Contrast[b]
	0	150	300	450	600		
Ruminal NH_3-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

[a] Solutions of NH_4-HCO_3 were infused intraruminally into the rumen throughout 24 h.
[b] Orthogonal contrasts where L=linear, Q=quadratic, NS=non significant ($p>0.01$).

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