Effect of Niacin Supplementation on Rumen Metabolites in Murrah Buffaloes (*Bubalus bubalis*)

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ABSTRACT : An experiment was conducted on 3 male rumen fistulated adult buffaloes fed on wheaten straw and concentrate mixture in a Latin square design to study the impact of niacin supplementation on rumen metabolites. Three animals were fed wheaten straw+concentrate mixture (group I, control), wheaten straw+concentrate mixture+100 ppm niacin (group II), and wheaten straw +concentrate mixture+200 ppm niacin (group III). After 21 days feeding, rumen liquor was drawn for 3 consecutive days at different time intervals (0, 2, 4, 6 and 8 h) to study the various rumen metabolites i.e., rumen pH, ammonia-N, total-N, trichloroacetic acid precipitable-N, non-protein nitrogen, total volatile fatty acids, their fractions and number of protozoa. Mean pH values in strained rumen liquor (SRL) of animals in 3 groups were 6.64, 6.71 and 6.67, indicating no statistically significant difference. Results revealed a significant ($p<0.01$) increase in TVFA concentration among the supplemented groups (group II and III) in comparison to control group. Mean TVFA concentration (meq/dl) was 9.75, 10.97 and 11.44 in 3 groups respectively. The highest concentration of TVFA was observed at 4 h and minimum at 0 h in all the 3 groups. The percentage of acetic, propionic, butyric and isobutyric acid was statistically similar among the three groups. The mean ammonia-N concentration (mg/dl SRL) was significantly ($p<0.01$) lower in group II (16.38) and group III (15.42) than group I (18.14). Ammonia-N concentration was higher ($p<0.01$) at 4 h as compared to all the time intervals. The mean total-N concentration (mg/dl SRL) was higher ($p<0.01$) in group II (74.16) and group III (75.47) as compared to group I (62.04). Total-N concentration was higher ($p<0.01$) at 4 h as compared to other time intervals and lowest value was recorded at 0 h.Concentration of TCA-ppt-N (mg/dl SRL) was significantly ($p<0.01$) lower in control group as compared to niacin supplemented groups. Mean value of NPN (mg/dl SRL) was significantly ($p<0.01$) lower in group III (23.21) as compared to group I (25.71), whereas groups I and II, and groups II and III were similar to each other. Total protozoa number ($\times 10^4$/ml SRL) ranged from 18.06 to 27.41 in group I, 20.89 to 38.44 in group II and 27.61 to 39.45 in group III. The mean protozoa number was significantly ($p<0.01$) higher in SRL of group II (27.60) and III (30.59) as compared to group I (22.48). It can be concluded from the study that supplementation of niacin in the diet of buffaloes had improved the rumen fermentation by decreasing the concentration of ammonia-N and increasing protein synthesis. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 1 : 38-41)***

Key Words : Niacin, Buffalo, Rumen Metabolites, Rumen Protozoa

INTRODUCTION

In ruminants, niacin is synthesized by rumen microorganisms and its synthesis has been considered to be adequate for their optimum performance (Hungate, 1966). But, recent research findings suggests that microbial production of niacin in the rumen does not meet requirements of growing calves (Girard, 1998) and oral administration of niacin has resulted in an increased microbial protein synthesis (Riddell et al., 1980,1981; Shields et al., 1983; Flachowsky, 1993) and volatile fatty acids (Doreau and Ottou,1996; Ottou and Doreau, 1996). The increase in synthesis of microbial protein resulted chiefly from protozoa (Dennis et al., 1982), as addition of niacin increased protozoa number (Doreau and Ottou, 1996) and which has been associated with simultaneous increase in ruminal ammonia and propionic acid (Abou Akkada and el-Shazly, 1964; Christiansen et al., 1965). *In vitro* studies also showed beneficial effect of niacin supplementation on microbial protein synthesis and total volatile fatty acid (TVFA) concentration. Information is lacking on the effect of niacin supplementation on rumen fermentation patterns in buffaloes. The present study was conducted to see the effect of different levels of niacin supplementation on rumen fermentation in adult Murrah buffaloes (*Bubalus bubalis*).

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MATERIALS AND METHODS

Three adult male buffaloes fitted with permanent fistula in the rumen were assigned three different diets in a Latin square design (3×3) with one animal per diet. Animals in 3 groups were fed wheaten straw+concentrate mixture (group I), wheaten straw+concentrate mixture+100 ppm niacin (group II), and wheaten straw+concentrate mixture+200 ppm niacin (group III), respectively. Niacin was mixed in the concentrate mixture offered to animals of experimental groups II and III. Concentrate mixture comprised soybean cake (25%), wheat bran (32%), maize crushed (40%), mineral mixture (2%) and common salt (1%). Niacin (nicotinic acid) was purchased from Central Drug House Pvt. Ltd., New Delhi, India. The diet supplied the crude protein (CP) and total digestible nutrients (TDN)

Table 1. Chemical composition of feeds fed to buffaloes (% on dry matter basis)

Items	Wheaten straw	Concentrate mixture
Organic matter	90.5	91.2
Crude protein	2.9	20.8
Ether extract	1.0	2.3
Total carbohydrate	86.6	68.2
Neutral detergent fibre	81.6	53.1
Acid detergent fibre	44.1	11.8
Cellulose	37.5	9.0
Hemi-cellulose	37.5	41.4

requirements as per Pathak and Verma (1993). Feed was offered once daily at 9.00 am. Wheaten straw was offered to all the animals after the complete consumption of concentrate mixture. Water was provided twice a day. After 21 days of feeding, strained rumen liquor (SRL) was drawn from these animals for 3 consecutive days, before feeding (0 h) and at different intervals of time post- prandially (2, 4, 6 and 8 h), with the help of metallic probes, whose multiple holes were wrapped with muslin cloth and located at four different sites in the rumen. On the day of rumen liquor collection, water was provided before 0h sampling and after the final collection of rumen liquor sample. About 100 ml of SRL was collected in a conical flask, which was capped with a cork and immediately brought to the laboratory for pH determination with a digital pH meter (Century CP-9, India). Then about 30 ml of SRL was preserved with 2 drops of 0.01 N H_2SO_4 in a plastic vial for the estimation of nitrogen. Another 30 ml of SRL was preserved with saturated mercuric chloride solution for the estimation of TVFA and their fractions. For fractionation of VFA, 5ml of SRL was mixed with 1 ml of 25% metaphosphoric acid to precipitate the proteins, allowed to stand for 30 minutes before it was centrifuged at 4,000 rpm for 20 min. Clear supernatant was taken for VFA fractionation. All the samples were stored frozen (-6°C) until analyzed. One ml of SRL was preserved with 1 ml formal saline with brilliant green dye in capped vials for the total protozoa counts.

Analysis of feed and rumen liquor sample

Wheaten straw and concentrate mixture were analyzed for organic matter (OM), crude protein (CP) and ether extract (EE) as per AOAC (1995) and fibre fractions were analysed by the method suggested by Van Soest et al. (1991) (Table 1). Total nitrogen in SRL was analysed by micro-Kjeldahl technique (AOAC, 1995) and total volatile fatty acid as per the method of Barnett and Reid (1957). Ammonia-N and non-protein nitrogen (NPN) in rumen liquor were estimated as per the method of Conway (1957) and Pearson and Smith (1943), respectively. Trichloro acetic acid-precipitable-nitrogen (TCA-ppt-N) was calculated by subtracting the NPN from the total-N concentration. Fractionation of volatile fatty acids (VFA) was done by gas liquid chromatography (Newcon, India) as per method of Erwin et al. (1961). Total number of protozoa in the rumen liquor were counted by using a haemocytometer as described (Kamra et al., 1991).

Table 2. Effect of niacin supplementation on rumen metabolites in buffaloes

Items	G-I	G-II	G-III	SEM
pH	6.64	6.71	6.67	0.05
NH_3-N (mg/dl SRL)**	18.1^{x}	16.4^{y}	15.4^{y}	1.29
TN (mg/dl SRL)**	62.0^{y}	74.2^{x}	75.5^{x}	3.24
TCA-ppt N (mg/dl SRL)**	36.3^{y}	49.8^{x}	52.3^{x}	3.09
NPN (mg/dl SRL)**	25.7^{x}	24.4$^{x\,y}$	23.2^{y}	0.43
Protozoa ($\times 10^4$/ml SRL)**	22.5^{y}	27.6^{x}	30.6^{x}	1.59
TVFA (meq/dl SRL) **	9.8^{y}	11.0^{x}	11. 4^{x}	0.41
Fractions of VFA (%)				
Acetic acid	72.7	72. 9	73.2	0.47
Propionic acid	17.0	17.4	17.5	0.20
Butyric acid	9.6	8.9	8.7	0.47
Isobutyric acid	0.8	0.8	0.6	0.06

$^{x, y}$ Means with different superscripts in a row differ significantly ($p<0.01$).
Treatments: G-I wheaten straw+concentrate mixture, G-II wheaten straw+concentrate mixture+100 ppm niacin and G-III Wheaten straw+concentrate mixture+200 ppm niacin.

Statistical analysis

The data were subjected to a test of significance between the diets and hours using two way analysis of variance techniques (Snedecor and Cochron, 1989) and means were compared using Duncan's multiple range test (Steel and Torrie, 1980). All this statistical analysis was done by SPSS 7.5 programme using computer.

RESULTS AND DISCUSSION

The mean concentration of rumen metabolites is presented in Table 2 and depicted in Figure 1 and 2. The mean pH value was 6.64, 6.71 and 6.67 in groups I to III, respectively with no effect of niacin supplementation, which is in concurrent with the findings of earlier workers (Shields et al., 1983; Horner et al., 1988; Samanta et al., 2000 a,b). In general, pH is inversely proportional to TVFA concentration in the rumen liquor (Phillipson, 1982) and the lowest pH value observed at 4 h in all the 3 groups coincided with the maximum concentration of TVFA in the rumen liquor. Results showed a significant ($p<0.01$) increase in TVFA concentration due to niacin supplementation with the mean TVFA values (mg/dl, SRL) of 9.75, 10.97 and 11.44 in three groups, respectively. These results are similar to the findings of Nangia et al. (2000) and Ghosh and Kewalramani (2002 a,b), who also observed an increase in TVFA concentration due to niacin supplementation in the diet of buffaloes. Increase in TVFA concentration may be due to increase in total number of protozoa as reported earlier (Doreau and Ottou, 1996) and also observed in this study. Mean percentage of acetate, propionate, butyrate and isobutyrate in rumen liquor were

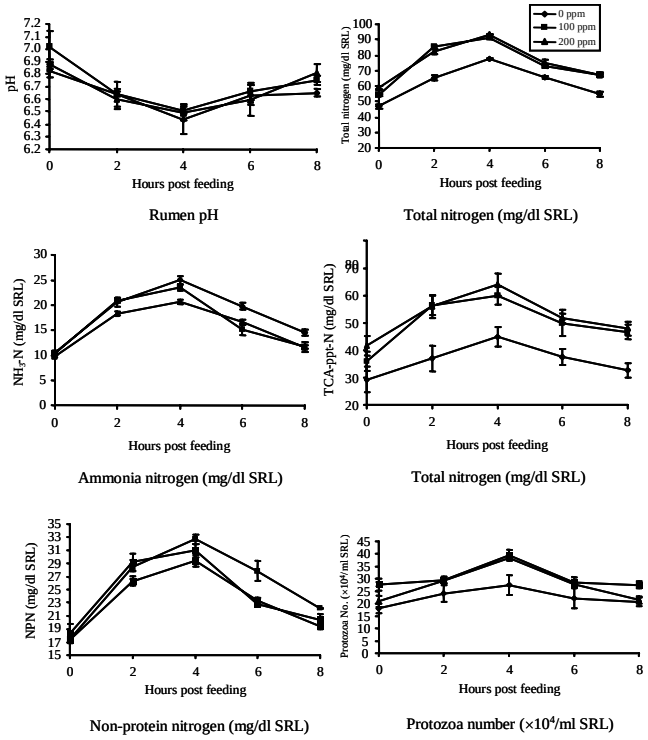

Figure 1. Rumen fermentation pattern in buffalo.

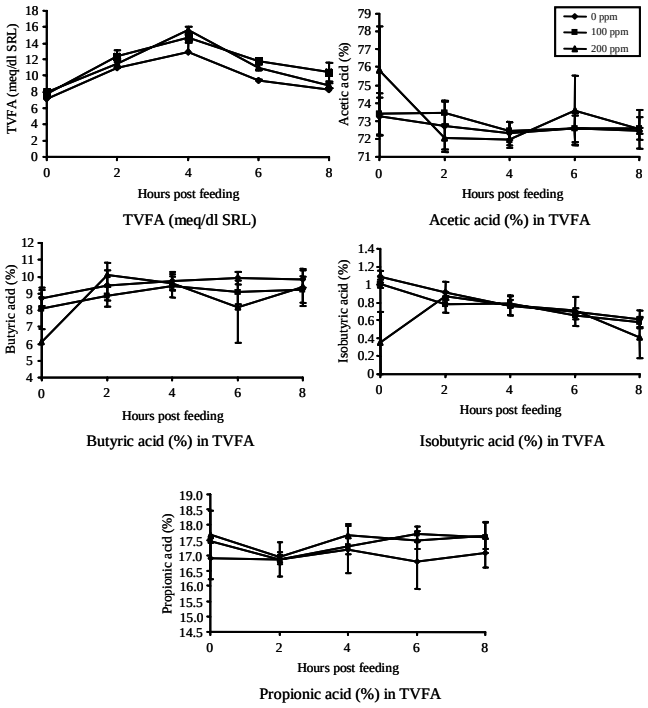

Figure 2. Total VFA and its fractionation in rumen liquor.

statistically similar in 3 groups, without showing any effect of niacin.

In all the three groups there was an increase ($p<0.01$) in the concentration of ammonia-N with time after feeding until 4 h, followed by a decline up to 8 h post feeding. Mean ammonia-N concentration (mg/dl SRL) was significantly ($p<0.01$) lower in group II (16.38) and group III (15.42) as compared to group I (18.14). Similar lower ammonia-N concentration was reported earlier in cattle (Riddell et al., 1980,1981; Horner et al., 1988) and buffaloes (Nangia et al., 2000) that received supplemental niacin in their diet. The mean ammonia-N concentration recorded in this study was higher than the minimum threshold of 5-8 mg/100 ml SRL, as proposed by Maynard et al. (1979) for optimum microbial growth in all the three groups. Total N concentration (mg/100 ml SRL) in niacin supplemented groups was significantly ($p<0.01$) higher than non-supplemented group. In all the 3 groups, the total-N concentration increased ($p<0.01$) from 0 h to 4 h post feeding followed by a decline up to 8 h post feeding. TCA precipitable-N mainly represents microbial-N and results revealed that supplementation of niacin significantly ($p<0.01$) increased the concentration of TCA-ppt-N (mg/100 ml) in group II (49.8) and group III (52.3) above the control group (36.3). Niacin serves as the precursor for the synthesis of pyridine nucleotides, which in turn increase the use of ammonia by rumen microbes for their protein synthesis (Allison et al., 1969). Increase in synthesis of microbial protein resulted chiefly from protozoa (Dennis et al., 1982). These findings are in accordance with the results of Horner et al. (1988) and Samanta et al. (2000a, b). Non-protein nitrogen concentration (mg/100ml SRL) was also significantly ($p<0.01$) lower in group III as compared to other 2 groups.

The protozoa number was significantly ($p<0.01$) greater in group II and III in comparison to group I. This might be because bacteria can synthesize niacin from tryptophan but rumen protozoa can not (Jones, 1974) and niacin present in feeds was in bound form i.e. peptide niacinogens and carbohydrate complex niacytin, which are not available to rumen protozoa (Ghosh, 1963) and thus niacin supplementation enhanced the proliferation of rumen protozoa. Similar higher protozoa numbers on niacin supplementation under *in vitro* and *in vivo* conditions were reported by Doreau and Ottou (1996) in cattle and Nangia et al. (2000) in buffaloes. Number of total protozoa observed in this study were within the normal range (10^4-10^5/ml, Hungate, 1966).

CONCLUSION

Supplementation of niacin had a stimulating effect on rumen fermentation in buffaloes with enhanced synthesis of microbial protein and TVFA. Positive effect of niacin supplementation on rumen protozoa number has also been confirmed.

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