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Research Article

EFFECT OF DIFFERENT DIETARY LEVELS OF SALT ON RUMEN MICROBIAL POPULATION AND *IN- VITRO* FERMENTATION IN BUFFALO CALVES

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ABSTRACT

The response to dietary inclusion of sodium chloride at three different levels of intake was studied in relation to rumen microbial population and *in-vitro* fermentation in four healthy rumen fistulated male murrah buffalo calves. Animals were fed a diet comprising of wheat straw and concentrate mixture in the ratio of 70:30. The control group did not receive any dietary sodium chloride supplement. Groups T_1, T_2 and T_3 received sodium chloride supplements to provide a final dietary concentration of 1.0, 2.0 and 3.0% of total dry matter intake, respectively. The total ciliate population as well as *Entodenia* and *Holotrichs* were significantly lower in group T_3 as compared to other groups. A non-significant increase in total bacterial count was observed in group T_1, while a significant drop in bacterial number was recorded in group T_3 as compared to T_2. *In-vitro* fermentation parameters including fermentation rate, maximum fermentation rate before and after incubation. Net growth of rumen microbes increased on feeding 2% sodium chloride in the diet.

Key words: *In-vitro* fermentation, microbial population, buffalo calves

The importance of ruminal digestion and metabolic studies to improve the feed utilization efficiency and exploitation of optimum productive potential of animal has been increasingly realized in recent years. It may be possible to enhance the performance of animal fed on low quality roughage by increasing the sodium chloride content of protein or energy supplements. High levels of sodium chloride in the feed have been successfully used to enhance the flow of undegraded dietary nutrients from the rumen. High levels of sodium chloride in the diet stimulate efficiency of organic matter utilization (Croom *et al.*, 1982), gain in body weight and improved milk yield in lactating dairy cow (Philips *et al.*, 2000). Most of the research regarding manipulation of digestive functions has been confined to cattle and sheep. The information on the physiology of digestion in the rumen of buffalo is very limited particularly with respect to dietary salt supplementation. The present study therefore, was planned to investigate the possible beneficial effect of high levels of sodium chloride in the diet of buffalo calves with the objective to identify the optimal level of salt that can be safely included in the diet of a buffalo calf and its effect on *in-vitro* fermentation and rumen microbial population.

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MATERIALS AND METHODS

Four healthy, rumen-fistulated male Murrah buffalo calves, aged 1 to 1.5 years having 120 to 150 kg body weight were used. These were fed a ration comprising of roughage and concentrate mixture in the proportion of 70:30. Chopped wheat straw was provided as roughage and the concentrate mixture consisted of maize (25%), barley (40%) and mustard cake (35%) with 12.67% digestible crude protein (DCP) and 71% total digestible nutrients (TDN). The quantity of the ration for each animal was computed according to the National Research Council recommendations (1978). A single reversal feeding design (Eickelberger *et al.*, 1985) was used. The control group (C) was fed wheat straw and concentrate mixture in 70:30 ratio. The first treatment group (T_1) was kept on the basal diet supplemented with 1% sodium chloride, the second treatment group (T_2) on the basal diet supplemented with 2% sodium chloride and third

treatment group (T_3) on the basal diet supplemented with 3% sodium chloride (on dry matter basis). Feeding was done once daily in the morning with free access to clean drinking water. Three weeks adaptation period was allowed for each ration and rumen liquor samples were collected through fistula from various positions and depths for two consecutive days. Sampling was done before feeding (0 h) and at 2, 4, and 6 h post-feeding. The samples were analysed for protozoa count by the method of Boyne *et al.* (1957). The counting of total number of ruminal bacteria was made by the Nigrosine slide technique according to the method of Gall *et al.* (1949) modified by Nangia (1967). Nigrosine stained the background leaving the bacteria unstained. Fermentation rate, maximum fermentation rates (MFR) before and after incubation, and net growth of rumen microorganism (*in-vitro*) were estimated by the procedure of EL-Shazly and Hungate (1965). The values of fermentation rate and maximum fermentation rate before and after incubation were recorded in terms of rate of gas production (µl/g of rumen content / min). The analysis of variance was done by split plot design technique (Gill and Hafs, 1971).The critical difference among various means was worked out by least square difference technique.

RESULTS AND DISCUSSION

Microbial population: Total protozoal number as shown in Table 1 was highest at pre-feeding (0 h) in all the groups. Thereafter, the total protozoal count decreased at subsequent intervals and attained the lowest value at 6 h post-feeding. The results also revealed that animals in group T_3 showed significantly ($P<0.01$) decreased numbers of protozoa in the rumen liquor as compared to all other groups. The value in group T_2 was lower than the total protozoal count in control and T_1 groups, although the variation was statistically non-significant. This may be due to combination of various factors. The increase in the rumen osmolality due to salt supplementation is considered to be unfavorable to many rumen protozoa. The drop in protozoal numbers after feeding may be ascribed to the dilution of rumen contents with water coming from saliva, increased water intake and from blood across the rumen wall in response to increased osmotic pressure in the rumen (Warner and Stacy, 1965). Singh and Leng (1987) also reported that increased flow of liquid ingesta decreased the protozoal number as well as production rate in the rumen of sheep by feeding common salt. Increased dilution rates associated with decrease in protozoal number had been reported in an *in-vitro* study in buffaloes by Garg and Nangia (1990). Harrison *et al.* (1975) reported that mean retention time for protozoa in the rumen is inversely proportional to the dilution rates in sheep. In the present study, the higher dilution rates and higher total rumen fluid outflow rates observed in group T_2 might have resulted in the reduction of total numbers of protozoa in the rumen. However, the drastic drop in protozoal numbers in group T_3 may be attributed to the higher osmotic pressure of rumen contents. Higher osmotic pressure of the ruminal contents has been reported as detrimental to protozoal growth in sheep (Hemsley *et al.*, 1975), which could be inhibiting the protozoal growth in the present study. The post prandially decreasing trend observed in *Entodenia* count in all the groups was similar to the one exhibited by total protozoal count. However, on percentage basis, the population of *Entodenia* did not differ significantly between the different groups as well as at various post-feeding intervals. The large population of *Entodenia* as compared to *Holotrich* may be due to the fact that they reproduce more rapidly (8-10 times) than that of large *Holotrichs* (Leng, 1982). The salt treatment decreased concentration of both *Entodenia* and *Holotrichs*. However, on percentage basis the two genera did not vary between the control and treatment groups (Table 1).

The total bacterial counts at different post feeding intervals in control and treatments groups like T_1, T_2, and T_3 were 7.41, 7.93, 8.21 and 6.65×10^{10}/ml of strained rumen liquor (SRL), respectively. A non-significant increase was observed with increments of sodium chloride in the diet from control, T_1 and T_2 and in group T_3, a significant ($P=0.05$) drop in bacterial count was observed as compared to T_2. The slight increase in bacterial numbers in groups T_1 and T_2 could

Table 1
Effect of sodium chloride supplementation on rumen microbial population

Parameters	Group	Pre-feeding h (mean ± S.E.)	Post-feeding h (mean ± S.E.)			Overall (mean ± S.E.)
		0	2	4	6	
Total protozoal count** (x 10^4/ml)	C	31.46 ±1.46[a]	26.75 ±1.84[a]	23.43 ±1.64[a]	22.68 ±1.52[a]	26.12 ±2.03[a]
	T_1	32.20 ±2.04[a]	27.28 ±1.87[a]	25.13 ±1.8[a]	20.13 ±1.13[a]	26.19 ±2.50[a]
	T_2	27.25 ±2.64[b]	23.53 ±1.46[a]	20.18±1.83[a]	19.63 ±2.19[a]	22.85 ±1.68[a]
	T_3	21.48 ±1.40[c]	18.43± 1.77[b]	15.73 ±1.97[b]	13.45 ±2.68[b]	17.27 ±1.73[b]
*Entodenia*** (x 10^4/ml)	C	27.25 ±1.42[a]	23.63±1.58[a]	21.47 ±1.77[a]	20.08 ±1.58[a]	23.11 ±1.56[a]
	T_1	27.95 ±2.18[a]	23.35 ±1.46[a]	22.48 ±1.80[a]	18.13 ±1.06[a]	22.98 ±2.01[a]
	T_2	24.05 ±1.90[a]	20.35 ±1.24[a]	18.33 ±1.51[a]	17.30 ±1.85[a]	20.01 ±1.49[a]
	T_3	19.23 ±1.14[b]	16.38 ±1.44[b]	13.85 ±1.66[b]	11.95 ±2.34[b]	15.35 ±1.58[b]
*Holotrich*** (x10^4/ml)	C	4.35 ±0.61[a]	3.13 ±0.33[a]	2.83 ±0.11[a]	2.60 ±0.14[a]	3.23 ±0.39[a]
	T_1	4.25 ±0.33[a]	3.93 ±0.47[a]	2.65 ±0.32[a]	2.00 ±0.21[a]	3.21 ±0.53[a]
	T_2	3.20 ±0.76[ab]	3.18 ±0.43[a]	2.65 ±0.36[a]	2.33 ±0.39[a]	2.84 ±0.12[ab]
	T_3	2.25 ±0.36[b]	2.05 ± 0.33[b]	1.88 ±0.32[a]	1.50 ±0.37[a]	1.92 ±0.16[b]
Entodenia (%)	C	86.18 ±1.90	88.30 ±0.72	87.60 ±1.12	88.30 ±1.20	87. 60 ±0.50
	T_1	86.55 ±8.56	85.70 ±0.89	89.33 ±1.62	90.10 ±0.90	87.92 ±1.06
	T_2	88.80±1.98	86.50 ±1.38	87.50 ±0.92	88.33 ±1.08	87.78 ±0.50
	T_3	89.48 ±1.27	89.08 ±0.69	88.40 ±1.35	88.88 ±1.34	88.96 ±0.22
Holotrich (%)	C	13.83 ±1.90	11.70 ±0.72	12.40 ±1.12	11.70 ±1.20	12.40 ±0.50
	T_1	13.45 ±1.56	14.30 ± 0.89	10.68± 1.52	9.90 ± 0.90	12.08 ±1.06
	T_2	11.20 ±1.98	13.50 ±1.38	12.50 ±0.92	11.67 ±1.08	12.22 ±0.50
	T_3	10.52 ±1.27	10.92 ±0.69	11.60 ±1.35	11.12 ±1.34	11.04 ±0.22
Bacteria* count (x 10^{10}/ml)	C	7.75 ±0.34[a]	7.58 ±0.23[a]	7.35 ±0.36[a]	6.95 ±0.30[a]	7.41 ±0.17[ab]
	T_1	8.90 ±0.31[b]	7.98 ±0.50[a]	7.63 ±0.14[a]	7.20 ±0.39[a]	7.93 ±0.36[ab]
	T_2	8.80 ±0.51[b]	8.30 ± 0.53[b]	7.85 ±0.61[a]	7.88 ±0.46[b]	8.21 ±0.22[b]
	T_3	7.25 ±0.51[a]	6.90 ±0.58[c]	6.40 ±0.50[b]	6.05 ±0.40[c]	6.65 ±0.27[c]

The values bearing different superscripts in column differ significantly. * P≤0.05, **P≤0.01

be explained on the basis of the proportionate reduction in protozoal numbers observed in these groups. As protozoa are responsible for the engulfment of bacteria in the rumen, a decrease in the protozoal population is accompanied by a proportionate increase in bacterial count. However, the drop in bacterial count in group T_3 may be due to the inhibitory effect of high level of sodium chloride in the diet of animals in this group.

***In-vitro* fermentation:** The data obtained on in vitro fermentation rates including fermentation rate, maximum fermentation rate (before and after incubation) and net growth of rumen microbes has been presented in Table 2. The data on fermentation rate revealed that the values showed a decline at 2 h post-feeding and an increasing trend thereafter at subsequent intervals attaining peak value at 6 h post-feeding in all of the four groups. The animals in T_1 and T_2 groups tended to have slightly higher fermentation rates, and in group T_3, it dropped to a level even below the control group, although none of these were statistically significant. The maximum fermentation rates showed increasing trend at various post-feeding intervals in all the groups with the lowest value observed at pre-feeding (0 h) and peak value at 6 h post-feeding. The overall average on maximum fermentation rates followed almost the same trend as observed in case of fermentation rate with higher values in groups T_1 and T_2 and a slightly lower value in group T_3 but still higher when compared to control and T_1 groups.

The rate of gas production is an indirect index of fermentation rate and the change in gas production reflects microbial activity since under normal favourable conditions maximum fermentation rates are a linear function of rumen microbial population (El–Shazly and Hungate, 1965). Thus, the higher fermentation rate and also maximum fermentation rate observed in group T_2 may be attributed to higher microbial

Table 2
Effect of sodium chloride supplementation on in-vitro fermentation rates and net growth of rumen microbes

Parameters	Group	Pre-feeding h (mean ± S.E.)	Post-feeding h (mean ± S.E.)			Overall (mean ± S.E.)
		0	2	4	6	
Fermentation rate (μl/g/min)	C	4.63 ± 0.20	3.96 ± 0.12	5.38 ± 0.25	6.06 ± 0.06	5.00 ± 0.46
	T_1	4.72 ± 0.18	4.34 ± 0.17	5.69 ± 0.30	6.,24 ± 0.20	5.25 ± 0.44
	T_2	4.67 ± 0.20	4.13 ± 0.18	6.16 ± 0.31	6.71 ± 0.23	5.42 ± 0.61
	T_3	4.32 ± 0.17	3.67 ± 0.26	5.47 ± 0.21	6.07 ± 0.32	4.88 ± 0.54
MFR before incubation (μl/g/min)	C	30.84 ± 1.36	33.52 ± 1.10	44.32 ± 1.31	53.84 ± 1.39	40.63 ± 5.27
	T_1	31.88 ± 2.26	34.88 ± 1.23	45.69 ± 1.85	54.76 ± 0.34	41.80 ± 5.25
	T_2	33.37 ± 1.64	37.96 ± 1.07	48.04 ± 1.89	58.62 ± 1.76	44.49 ± 5.62
	T_3	32.56 ± 0.92	37.66 ± 0.74	47.86 ± 1.17	57.29 ± 1.02	53.84 ± 5.50
MFR after incubation (μl/g/min)	C	31.72 ± 1.31	35.20 ± 1.29	46.32 ± 2.02	58.08 ± 0.97	42.83 ± 5.96
	T_1	33.39 ± 2.11	36.77 ± 1.54	48.94 ± 0.68	59.70 ± 2.63	47.70 ± 6.01
	T_2	35.22 ± 2.26	40.91 ± 1.13	53.61 ± 1.94	63.54 ± 3.47	48.32 ± 6.30
	T_3	34.08 ± 1.15	39.96 ± 0.55	49.96 ± 1.32	62.30 ± 1.53	46.58 ± 6.18
Net growth of rumen microbes (% h)	C	2.85 ± 1.26	5.01 ± 0.67	4.51 ± 1.92	7.88 ± 1.82	5.06 ± 1.05
	T_1	4.73 ± 1.21	5.42 ± 2.72	7.11 ± 1.25	9.02 ± 0.96	6.57 ± 0.96
	T_2	5.54 ± 0.31	7.77 ± 1.64	11.59 ± 1.07	8.39 ± 0.83	8.32 ± 1.25
	T_3	4.67 ± 0.43	6.11 ± 0.62	4.38 ± 0.81	8.74 ± 1.13	5.98 ± 1.00

The values bearing different superscripts in column differ significantly. * P≤0.05, **P≤0.01, MFR - Maximumm fermentation rate.

activity. The rate of net growth of rumen microbes increased post prandially in all the groups. However, maximum growth was obtained at 6 h post prandially in all the groups with the exception of T_2 where the peak value was noticed at 4 h post prandially. In control and group T_3, there was a drop in net growth of rumen microbes after 2 h before reaching their peak values at 6 h post prandially.

The validity of *in-vitro* method of estimating the growth of rumen microbial population by measuring maximal fermentation rate was indicated by El–Shazly and Hungate (1965) who reported that changes in fermentation rate under favourable conditions with excess substrate reflect changes in population size and can therefore be used to measure growth of rumen microorganisms. These workers concluded that net growth is a reflection of the turn over rate of rumen contents. The turn over rates/day (net growth % x 24) calculated from the present data were 1.21, 1.58, 1.99 and 1.44 in control and treatment groups T_1, T_2 and T_3, respectively. The higher turn over observed in treatment group T_2 may be responsible for higher net growth of rumen microorganisms. This has been shown in continuous culture studies where increasing the turnover of fluid contents markedly increased microbial cell synthesis (Isaacson *et al.*, 1975). Kennedy *et al.* (1976) suggested that efficiency of microbial synthesis was positively related to dilution rate which may be because of reduced autolysis of bacteria and reduced engulfment of bacterial mass by protozoa. The drop in the net growth of rumen microbes in T_3 group may be due to the detrimental effect of high level of sodium chloride on rumen microbial population. It may be concluded from the present study that dietary supplementation of sodium chloride in high roughage diet up to 2% level (on DM basis) is most beneficial for microbial population and beyond 2% level is detrimental to ruminal microflora.

REFERENCES

Boyne, A.W., Eadi ,J.M. and Raitt, K. (1957).The development and testing of method of counting rumen ciliate protozoa. *J. Gen. Microbiol.* **17**: 414.

Croom, W.J.Jr., Harvey, R.W., Linnerud, A.C. and Froetschel, M. (1982). High levels of sodium chloride in beef cattle diets. *Can. J. Anim. Sci.* **62**: 217-227.

Eickelberger, R.C., Muller, L.D., Sweeney, T.F. and Abrams, S.M. (1985). Addition of buffers to high quality alfalfa hay-based diet for dairy cows in early lactation. *J. Dairy Sci.* **68**:1722-1731.

El-Shazly, K. and Hungate, R.E. (1965). Fermentation capacity as a measure of net growth of rumen microorganisms. *Appl. Microbiol.* **13**: 62-69.

Gall, L.S., Burrough, W., Gerlaugh, P. and Edgington, B.H. (1949). Rumen bacteria in cattle and sheep on practical farm rations. *J. Anim. Sci.* **8:** 441-449.

Garg, S.L. and Nangia, O.P. (1990). Effect of high intake of sodium chloride in the diet on dilution rate and microbial protein synthesis in the rumen of buffaloes. *Intnl. J. Anim. Sci.* **5**: 197-202.

Gill, J.L. and Hafs, H.D. (1971). Analysis of repeated measurements of animals. *J. Anim. Sci.* **33**: 331-336.

Harrison, D.G., Beever, D.E., Thomson, D.J. and Osbourn D.F. (1975). Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. *J. Agric. Sci.* **85**: 93-101.

Hemseley J.A., Hogan, J.P. and Weston, R.H. (1975). Effect of high intakes of sodium chloride on the utilization of a protein concentrates by sheep. II. Digestion and absorption of organic matter and electrolytes. *Australian J. Agric. Res.* **26**: 715-727.

Isaacson, H.R., Hindis, F.C., Bryant, M.P. and Owens, F.N. (1975). Efficiency of energy utilization by mixed rumen bacteria in continuous culture. *J. Dairy Sci.* **58**: 1645-1659.

Kennedy, P.M., Christopherson, R.J. and Milligan, L.P. (1976). The effect of cold exposure of sheep on digestion, rumen turnover and efficiency of microbial synthesis. *Br. J. Nutr.* **36** : 231-242.

Leng, R.A. (1982). Dynamics of protozoa in the rumen of sheep. *Br. J. Nutr.* **48**: 399-415.

Nangia O.P. (1967). Studies on the functional development of rumen in bovine, Ph.D. thesis, Agra University, Agra.

National Research Council, (1978).Nutrient requirement of dairy cattle, No.3, (5th edn.) National Academy of Science Washington D. C.

Philips, C.J.C., Chiy, P.C., Arney, D.R. and Kart, O. (2000). Effects of sodium fertilizers and supplements on milk production and mammary gland health. *J. Dairy Res.* **67**: 1-12.

Singh, G.P. and Leng, R.A. (1987). Effect of altering rumen fluid outflow on the protozoal population and production rate in sheep. *Indian J. Anim. Nutr.* **4**:155-160.

Warner, A.C.I. and Stacy, B.D. (1965). Solutes in the rumen of the sheep. *Quart. J. Expt. Physiol.* **50**: 169.