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Research Article

EFFECT OF DIETARY SUPPLEMENTATION OF SODIUM FUMARATE ON RUMINAL FERMENTATION IN BUFFALO CALVES

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ABSTRACT

The ability of sodium fumarate to alter rumen fermentation was investigated in four healthy male Murrah buffalo calves fitted with rumen fistula, fed high roughage low protein diet (70:30) in single reversal trial. The basal diet was supplemented with sodium fumarate at 1% (T-1) and 2% (T-2) levels on dry matter basis. The studies revealed that rumen pH and total volatile fatty acids concentration did not vary much between the control and treatment groups. However, propionate concentration was significantly higher (P = 0.01) and butyrate concentration lower (P = 0.05) in T-2 as compared to the control group. Rumen total nitrogen concentration remained unaffected in treatment groups but lower rumen ammonia (P = 0.05) and higher microbial protein nitrogen (P = 0.05) were recorded in T-2. The total protozoa, *Entodinia* and *Holotrichs* counts were lower in T-2 group but total bacterial count was significantly higher (P = 0.01) in T-1 and T-2 groups. Increase in DM disappearance was observed in T-1 and T-2 groups (P = 0.01) as compared to the control.

Keywords: Sodium fumarate, rumen fermentation, buffalo

In the recent past most of the research has been focused on the manipulation of ruminal microbial ecosystem with antimicrobial compounds, methane inhibitors and microbial growth factors. However, because of the consumer concern about the safety of the use of antibiotics in animal feeds, the attention is now being shifted to other alternatives for rumen manipulation. Organic acids like aspartate, fumarate and malate have shown potential as alternative compounds (Lopez *et. al.*, 1999). These dicarboxylic acids can stimulate the growth of prominent lactic acid utilizing bacteria rather than inhibiting specific ruminal microbial population and can favourably alter the ruminal fermentation, remove lactate and indirectly stimulate fiber digestion. Extensive work with regard to feeding of organic acids as feed additives is mainly confined to pigs. In ruminants, their influence on rumen fermentation has usually been examined through *in-vitro* studies. Present study describes the effect of sodium fumarate on ruminal fermentation *in-vivo* in buffalo calves.

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MATERIALS AND METHODS

Experimental animals: Four healthy, rumen-fistulated male, Murrah buffalo calves, aged 1 to 1.5 years were maintained by feeding a high roughage low concentrate ration in the ratio of 70:30. 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). Chopped wheat straw was provided as roughage. The quantity of the ration for each animal was computed according to the National Research Council recommendations (1978).

Experimental design: A single reversal feeding design (Eickelberger *et al.*,1985) was used. The control group (C) was given basal diet consisting of 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 fumarate and the second treatment group (T-2) on the basal diet supplemented with 2% sodium fumarate (based on total dry matter intake). 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 thereafter for two consecutive days. Sampling was done before feeding (0 h) and at 2, 4, and 6 h post-feeding.

Methods of analysis : The samples were analysed for pH by digital pH meter, total volatile fatty acids by the method of Barnett and Reid (1957), individual VFAs by the method of Bernard and Boucque (1968) with using gas liquid chromatograph (Nucon 5700 series), total nitrogen by Mckenzie and Wallace (1954), ammonia nitrogen by Conway (1957), microbial protein by Cline *et al.* (1958), protozoa count by Boyne *et al.* (1957) and bacterial count by Gall *et al.* (1949) modified by Nangia (1967). Dry matter disappearance was also examined according to Mehrez and Orskov (1977) and the statistical analysis of the data was done by the method of Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

As presented in Table 1, the pH of rumen liquor in three groups varied between 6.85 and 7.31. There was no significant change in the pH in both the treatment groups as compared to the control group. Similar observations have been reported by Lopez *et al.*, 1999 and L'Estrange (1977) in sheep on feeding formic, acetic or propionic acid treated grass palates. This probably might be due to the ability of dicarboxylic acids to stimulate growth and activity of lactic acid utilizing bacteria *Selenomonas ruminantium* (Martin and Parker, 1996) and due to insignificant change in total volatile fatty acids(TVFA) concentration in the treatment groups and increased salivary flow to the rumen which would help in buffering the rumen and alleviate fall in the pH (Anneroth *et al.*, 1980). The peak concentration of TVFA was recorded at 4 h post-feeding and thereafter declined at subsequent

Table 1
Effect of sodium fumarate supplementation on rumen pH, volatile fatty acids and nitrogen fractions in the rumen fluid

Parameter	Group	Pre-feeding hour (mean ± S.E.)	Post-feeding hour (mean ± S.E.)			Overall mean ± S.E.
		0	2	4	6	
pH	C	7.29±0.01	7.25±0.08	7.13±0.07	7.07±0.10	7.18±0.05
	T-1	7.30±0.08	7.19±0.04	6.99±0.02	6.85±0.01	7.08±0.10
	T-2	7.31±0.06	7.23±0.05	7.06±0.08	6.90±0.12	7.13±0.09
TVFA (mEq/L)	C	83.75±1.49	103.25±2.02	123.75±4.09	120.25±3.22	107.75±9.17
	T-1	89.75±4.93	110.50±1.70	134.25±1.31	120.25±1.65	113.69±9.34
	T-2	87.75±5.49	108.25±1.75	131.50±0.95	117.50±0.95	111.25±9.18
Acetate (%)	C	79.29±0.36	76.89±0.71	76.47±0.98	77.44±0.43	77.53±0.62
	T-1	79.85±0.43	77.65±0.58	77.08±1.10	78.27±0.37	78.22±0.59
	T-2	80.18±0.43	77.90±0.41	76.52±0.51	77.31±0.41	77.98±0.92
Propionate** (%)	C	11.16±0.11	14.90±0.26	16.81±0.65	15.58±0.26	14.61±1.21[a]
	T-1	11.94±0.14	15.48±0.24	17.45±0.41	15.50±0.24	15.09±1.14[a]
	T-2	12.66±0.24	17.64±0.43	18.39±0.24	17.71±0.25	16.60±1.32
[b]Butyrate*(%)	C	7.60±0.71	5.69±0.56	4.92±0.71	5.04±0.41	5.81±0.61[a]
	T-1	6.85±0.69	5.36±0.47	4.47±0.42	4.89±0.40	5.39±0.51[a]
	T-2	6.27±0.40	4.16±0.81	4.50±0.58	4.03±0.50	4.74±0.52[b]
Total Nitrogen (mg/dl)	C	110.13±4.29	150.10± 4.63	136.25±4.23	132.30±3.53	132.19±8.28
	T-1	110.98±3.38	146.33± 3.89	139.20±6.27	133.40±3.86	132.48±7.63
	T-2	112.75±3.55	150.82± 3.69	141.50±6.49	134.40±2.90	134.87±8.10
Ammonia N (mg/dl) *	C	11.40±0.57	15.80±0.86	14.93±0.81	13.23±0.46	13.84±0.97[a]
	T-1	11.50±0.99	14.68±1.64	14.00±0.39	12.83±0.37	13.16±0.77[a]
	T-2	9.10±0.23	13.52±0.78	13.35±0.55	12.12±0.13	12.02±1.02[b]
Microbial protein (TCA-N) (mg/dl) *	C	21.33±0.53	24.05±0.46	27.43±0.58	33.18±0.88	26.49±2.55[a]
	T-1	21.50±0.47	25.50±0.50	28.45±0.47	34.50±0.74	27.49±2.73[a]
	T-2	23.45±0.46	27.30±0.78	30.00±0.90	36.82±0.58	29.39±2.81[b]

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

intervals but the value still remains higher than that of prefeeding levels in control and treatments groups. Slightly higher overall TVFA concentration was recorded in T-1 (113.69 mEq/L) and T-2 (111.25 mEq/L) but the change was statistically non significant in comparison to C (107.75 mEq/L). Individual VFAs gave different results. The molar percentage of acetate did not differ among C (77.53%), T-1 (78.22%) and T-2 (77.98%) groups while the concentration of propionate was significantly higher (P = 0.01) in T-2 (16.6%) than in C (14.61%). Butyrate concentration on the other hand was significantly lower (P = 0.05) in T-2 (4.74%) than in C (5.81%). The increase in propionate production might be due to fumarate supplementation in the diet as fumarate is a metabolic precursor of propionate and can be converted into propionate and acetate following different pathway (Demeyer and Hendericks, 1967). Increase in propionate production with stoichiometric decrease in methane production has been reported by Lopez *et al.* (1999) on addition of fumarate to rumen simulating fermenter.

Rumen total nitrogen concentration, as shown in Table 1 did not differ significantly between C (132.19 mg/dl), T-1 (132.48 mg/dl) and T-2 (134.87 mg/dl). Rumen ammonia evidenced a declining trend with increasing level of sodium fumarate in diet where the concentration in T-2 (12.02 mg/dl) in comparison to C (13.84 mg/dl) was significantly lower (P = 0.05). This decline was probably due to efficient incorporation of ammonia nitrogen into microbial cell for microbial protein synthesis as evidenced by higher microbial protein nitrogen in T-2 (29.39 mg/dl) than in C (26.49 mg/dl). Lower microbial protein nitrogen in C and T-1 find support from comparatively larger population of protozoa recorded in C (26.12 x 10^4/ml) and T-1 (27.52 x 10^4/ml) than in T-2 (23.31 x 10^4/ml) Table 2, which might contribute partly towards lower efficiency of microbial protein synthesis in C and T-1 groups. It might also be due to increased fractional outflow rate of rumen content due to higher osmotic pressure in the rumen in response to dietary inclusion of 2% sodium fumarate.

The protozoal number was highest before feeding in all the groups and thereafter the count decreased at subsequent intervals and attains the

Table 2
Effect of sodium fumarate supplementation on rumen microbial population.

Parameter	Group	Pre-feeding hour (mean ± S.E.)	Post-feeding hour (mean ± S.E.)			Overall mean ± S.E.
		0	2	4	6	
Protozoa count ($X10^4$/ml)	C	31.60±1.46	26.75±1.84	23.43±1.64	22.68±0.52	26.12±2.03
	T-1	33.83±0.92	27.35±1.65	26.72±1.58	22.17±1.30	27.52±2.39
	T-2	29.48±0.93	23.30±0.29	20.33±0.29	20.15±0.95	23.31±2.17
Entodenia ($X10^4$/ml)	C	28.90±0.02	24.20±1.92	21.60±2.08	21.07±1.22	23.94±1.79
	T-1	30.37±0.05	24.70±1.80	24.57±1.61	21.00±1.35	25.16±1.93
	T-2	27.07±0.06	21.70±0.15	18.95±0.98	18.97±0.91	21.67±1.91
*Holotrichs** ($X10^4$/ml)	C	2.70±0.42	2.60±0.34	1.82±0.46	1.60±0.30	2.17±.0.27[a]
	T-1	3.45±0.13	2.65±0.42	2.15±0.29	1.15±0.09	2.35±.0.46[a]
	T-2	2.40±1.52	1.60±0.15	1.48±0.20	1.18±0.07	1.66±.1.26[b]
Entodenia (%)	C	91.43±0.38	90.22±1.47	91.68±2.54	93.09±.0.83	91.60±.0.58
	T-1	89.79±0.48	90.31±1.75	91.89±1.19	94.73±.0.55	91.68±.1.11
	T-2	91.84±0.20	93.78±1.09	93.23±0.96	94.16±.0.25	93.25±.0.50
Holotrichs (%)	C	09.53±0.38	9.80±1.46	8.30±2.54	6.90±.0.83	8.38±.0.59
	T-1	10.17±0.48	9.80±1.85	8.00±1.20	5.20±.0.55	8.29±.1.13
	T-2	8.10±0.20	6.83±0.54	7.24±0.94	5.83±.0.26	7.00±.0.47
Bacterial count** ($X10^{10}$/ml)	C	7.75±0.34	7.58±0.23	7.35±0.38	6.95±.0.30	7.04±.0.17[a]
	T-1	10.17±0.48	8.68±0.38	8.48±0.38	8.08±.0.27	8.58±.0.20[ab]
	T-2	8.10±0.20	10.05±0.28	9.90±0.17	9.55±.0.16	9.96±.0.17[b]

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

lowest value at 6 h post-feeding. The drop in protozoal number after feeding may be due to the dilution of rumen contents with water coming from saliva and from blood across the rumen wall in response to increased osmotic pressure in the rumen (Warner and Stacy, 1965). The drop in protozoal counts in group T-2 may be attributed to higher osmotic pressure in rumen content due to feeding of sodium fumarate, which is detrimental to protozoa (Hemseley *et. al.*, 1975). As shown in Table 2, sodium fumarate treatment resulted in decline in the population of both *Entodinia* and *Holotrichs*, though the proportion of the two did not differ in C, T-1 and T-2 groups. Significantly higher (P = 0.01) bacterial count was recorded in T-2 (9.96 x 10^{10}/ml) as compared to C (7.04 x 10^{10}/ml). This may be attributed to the reduction in protozoal numbers in T-2 group and also to the organic acid-effected growth stimulation of prominent ruminal bacteria like *Selenomonas ruminantium* (Callaway and Martin, 1996, Martin *et al.*, 1999) which accounts for 51% of total viable bacteria in the rumen (Caldwell and Bryant, 1966).

The increasing level of sodium fumarate supplementation resulted in significantly higher (P = 0.01) values of dry matter disappearance in T-1 (31.39%) and T-2 (36.38%) than in C (26.43%). These observations are in agreement with earlier reports of Lopez *et al.* (1999) and Carro *et al.* (1999). The higher DM disappearance in the treatment group might be because of the ability of fumarate to promote lactate utilization and stimulate growth of *Selenomonas ruminantium* that removes lactate and prevents acidosis and indirectly stimulates fiber digestion (Nisbet and Martin, 1990, 1993). Another reason may be the decline in protozoa population associated with rise in bacterial counts on sodium fumarate supplementation, as bacteria and fungi are responsible for 80% of total fiber digestion while protozoal contribution is only 17% to 20% (Dijkstra and Tamminga, 1995). In the present study, the decrease in total protozoal population (group T-2) associated with an increase in direct bacterial count (T-1 and T-2) might be the possible cause of higher dry matter disappearance in groups T-2 and T-1. The studies thus demonstrated that dietary supplementation of sodium fumarate in high roughage and low concentrate diet at 2% level of total DM intake was able to alter microbial population, improve microbial protein synthesis and increase DM degradation in buffalo calves.

REFERENCES

Anneroth, G., Nordenram,G. and Bengtsson, E, (1980). Effect of saliva stimulants (Hybrin and malic acid) on cervical root surfaces in-vitro. *Scand. J. Dent. Res.* **88**: 214-218.

Bernard, G.C. and Boucque, C.V. (1968). Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *J. Agric. Food Chem.* **16**: 192-201.

Barnett, A.J.G. and Reid, R.C. (1957). Studies on the production of volatile fatty acids from grass and rumen liquor in an artificial rumen. *J. Agric. Sci. Camb.* **48**: 315-318.

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.

Caldwell, D.R. and Bryant, M.P. (1966). Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Appl. Microbiol.* **14:** 797-801.

Callaway, T.R. and Martin, S.A. (1996). Effect of organic acid and monensin treatment on in-vitro mixed microorganism fermentation of cracked corn. *J. Anim. Sci.* **74** : 1982-1989.

Carro, M.D., Lopez, S., Valdes, C. and Ovejero, F.J. (1999). Effect of DL-malate on mixed ruminal microorganisms fermentation using the rumen simulation technique (Rusitec). *Anim. Feed Sci. Technol.* **79** : 279-288.

Cline, J.H., Hershberger, T.V. and Bentley, O.G. (1958) .Utilization and/or synthesis of valeric acid during the digestion of glucose, starch and cellulose by rumen microorganisms in-vitro. *J. Anim. Sci.* **17**: 284-292.

Conway, H.J. (1957). Microdiffusion analysis and volumetric error, (4th edn.) Crossby Lockwood, London,U.K.

Demeyer, D.I. and Henderick, H.K. (1967). Competitive inhibition of in-vitro methane production by mixed rumen bacteria, *Arch. Intnl. de Physiologic et de Biochemie.* **75**: 157-159.

Dijkstra, J. and Tamminga, S. (1995). Simulation of the effect of diet on the contribution of rumen protozoa to degradation of fiber in the rumen. *Br. J. Nutr.* **74**: 617-634.

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.

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.

Hemseley, J.A. Hogan, J.P. and Weston, R.H. (1975). Effect of high intakes of sodium chloride on the utilization of a protein concentrate by sheep. II. Digestion and

absorption of organic matter and electrolytes. *Australian J. Agric. Res.* **26**: 715-727.

L'Estrange, J.L., Mayes, R.W., Ryan, D. and Estrange, J.L.L. (1977). Effects of organic acids and ammonium salts on food intake and metabolism of sheep, Proceedings: International meeting on animal production from temperate grassland, Dublin, pp. 159-160.

Lopez, S., Valdes, C., Newbold, C.J. and Wallace, R.J. (1999). Influence of sodium fumarate addition on rumen fermentation in-vitro. *Br. J. Nutr.* **81**: 59-64.

Martin, S. A. and Parker, C.M. (1996). Effect of intracellular hydrogen on organic acid utilization by the ruminal bacterium *Selenomonas ruminantium*. *Curr. Microbiol.* **32**: 327-331.

Martin, S.A., Streeter, M.N., Nisbet, D.J., Hill, G.M. and Williams, S.E. (1999). Effects of DL-malate on ruminal metabolism and performance of cattle fed a high concentrate diet. *J. Anim. Sci.* **77**:77.

McKenzie, H.A. and Wallace, H.S. (1954). The Kjeldahl determination of nitrogen. *Australian J. Chem.* **7**:55.

Mehrez, A.Z. and Orskov, E.R. (1977). A study of the artificial bag technique for determining the digestibility of feeds in rumen. *J. Agric. Sci. Camb.* **88**: 645-650.

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

Nisbet, D.J. and Martin, S.A. (1990). Effect of dicarboxylic acids and *Aspergillus oryzae* fermentation extract on lactate uptake by the ruminal bacterium *Selenomonas ruminantium*. *Appl. Environ. Microbiol.* **56**(11): 3515-3518.

Nisbet, D.J. and Martin, S.A. (1993). Effects of fumarate, L-malate and *Aspergillus oryzae* fermentation extract on D-lactate utilization by the ruminal bacterium *Selenomonas ruminantium*.*Curr. Microbiol.***26**: 133-136.

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

Snedecor, G.W. and Cochran, W.G. (1968). Statistical methods. 6th edition Allied Pacific, Bombay, India.

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