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Effects of salinomycin and sodium fumarate on rumen fermentation using rumen simulation technique

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ABSTRACT

The objective of this study was to determine the effect of salinomycin and sodium fumarate on rumen fermentation. The apparent digestibility of basal ration was reduced by salinomycin appreciably at low doses, whereas, sodium fumarate tended to increase it without any change in pH, bacterial count and ammonia nitrogen level. Salinomycin produced a dose-dependent decrease in protozoal count whereas sodium fumarate did not produce any significant change. Microbial protein increased with the addition of salinomycin, whereas sodium fumarate did not produce any significant change in microbial protein synthesis. Salinomycin increased the molar proportion of propionate and reduced the acetate, butyrate and acetate: propionate ratio. Sodium fumarate also reduced acetate: propionate ratio, but increased both acetate and propionate concentrations. Salinomycin and sodium fumarate reduced methane production, however the reduction brought about by sodium fumarate was not comparable with ionophore. Thus, it was concluded that salinomycin and sodium fumarate may be a useful dietary additive for ruminants.

Key words: Rumen fermentation, Salinomycin, Sodium fumarate

The rumen is inhabited by a diverse and interdependent population of bacteria, protozoa and fungi. Much interest has been generated over the past few years aimed at evaluating alternative means to manipulate the gastrointestinal microflora in livestock to improve the productivity and to compensate for the continuously declining fodder resources (Russell and Rychlick 2001). Bharat Kumar *et al.* (2008) reported that organic acids stimulate growth of the prominent ruminal bacterium, favorably alter the mixed ruminal microorganism fermentation and improve the performance. The reduction in methane production is of emerging importance due to the role of methane in global warming phenomenon and in the destruction of ozone layer. The present study was, therefore performed using rumen simulation technique (Rusitec) in which the fermentation of mixed ration of hay and rice bran by rumen microorganisms was compared with the fermentation of the same diet with different doses of salinomycin and sodium fumarate.

MATERIALS AND METHODS

The *in vitro* rumen fermentation study using salinomycin as 12% premix as was used for the study was carried out using Rusitec apparatus (Czerkawski and Breckenridge 1977).

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Experimental trials

The trial consisted of a 6-day adaptation period followed by a 6-day collection period, in which the sample was collected to determine the rumen fermentation parameters.

Incubation procedure: The rumen liquor for the fermentation vessels were obtained from a pooled sample of ruminal contents of 6 cattle for the first 3 trials and from 15 sheep for the fourth trial, from the slaughterhouse. On the first day of the experiment, the reaction vessel was loaded with 500 ml of SRL and 200 ml of artificial saliva buffer. 80 g of solid rumen contents were weighed and put into a nylon bag (203 mm × 102 mm × 40 mm) and placed inside the food container in each vessel together with a bag of basal feed on the first day of the experiment. The basal ration was prepared with 12 g of hay and 3 g of rice bran, on dry matter basis. The perforated cage with solid digesta was placed in the vessel and the remaining space was filled with distilled water and closed. After filling the inoculum, carbon dioxide was passed through the gas valve for 5. Artificial saliva with pH adjusted to 7.0 by infusing carbon dioxide was infused @ 600 ml for 24 h.

Maintenance of rumen culture in vitro: After 24 h of

incubation, the bags were removed and washed with 40 ml of artificial saliva. They were flushed with carbon dioxide and the procedure was repeated every 24 h. The bag which had been in the vessel for 2 days was removed and replaced with a bag of feed.

Collection of samples: The effluents of the Rusitec were collected in the effluent flask containing few drops of saturated mercuric chloride. The effluent samples were collected every day before placing the bags, and the total volume of effluent was measured. For the fourth trial, samples were taken for estimation of VFA and lactic acid every 4 h, for 48 h, from the time of feeding sago on the seventh day.

Estimation of rumen fermentation parameters: The nylon bags removed from the reaction vessel after 48 h were washed under running water and dried at 70°C and weighed. *In vitro* digestibility of samples was expressed as percentage and calculated by difference in weight of bag with sample between before and after inoculation.

The total and individual VFA concentrations were estimated by gas chromatography as per Chase (1990). Netel gas chromatograph, was used for this purpose. The microbial protein was estimated by using a protein estimation kit and ammonia nitrogen content was measured as per Weatherburn (1967). Lactic acid was estimated as described by Barker and Summerson (1941). Total bacterial and protozoa count were quantified according to Gall *et al*. (1949). The stoichiometry of the rumen fermentation as acetate: propionate ratio, non glycogenic ratio and energy efficiency of VFA were calculated according to Orskov *et al.* (1968).

The gas produced during fermentation was collected by attaching a gas collection bag to the effluent vessel. The amount of gas produced was measured by water displacement method. The gases were fractionated to methane and carbon dioxide by passing 4 ml of the fermentation gas into saturated potassium hydroxide. Carbon dioxide was absorbed by potassium hydroxide and the remaining was methane gas.

The data obtained were analysed by completely randomized design (Snedecor and Cochran 1994) and results are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Rumen is often considered as a 'black box', because of the complex and diverse ecosystem. A reduction in acetate: propionate ratio, prevention of wasteful degradation of dietary protein, maximisation of microbial protein and reduction of wasteful methane production helps in the maximisation of productivity.

The significant ($P<0.05$) reduction in the digestibility per cent observed in the present study with the addition of ionophore is in accordance with our previous finding (Bharat Kumar *et al.* 2008). However, the present result is contrary to the findings of Maas *et al*. (2001), who observed no effect on apparent digestibility of dry matter, which might be due to high fibre content in the diet. The digestibility per cent in this study was low at lower dose of salinomycin (0.5 mg), as compared to the digestibility at higher doses (1.5 mg and 3 mg), which might be due to higher doses of ionophore replacing or reducing the number of sensitive bacteria and thereby probably establishing a new ecosystem. The increase in digestibility per cent observed with addition of organic acid sodium fumarate was in assessment with the findings of Lopez *et al.* (1999).

Salinomycin did not significantly alter the rumen pH similar to the observations of Ruiz *et al.* (2001) whereas sodium fumarate caused a slight nonsignificant increase in pH, which concurred with the observation of Lopez *et al.* (1999). Salinomycin and sodium fumarate does not produce any consistent effect on ruminal ammonia production or microbial protein synthesis, similar to the results of Lopez *et al.* (1999). Salinomycin did not significantly alter the total bacterial count, which is in agreement with the observations of Olumeyan *et al.* (1986) whereas a dose dependent reduction in the number of protozoa was observed. Sodium fumarate did not affect the total protozoal count. This resembles with the *in vitro* finding of Bogaert *et al.* (1990).

Salinomycin and sodium fumarate decreased the total gas and methane production, as also reported by Jalc and Laukova (2002). The decrease in methane production might not have

Table 1. Effect of salinomycin and sodium fumarate on rumen fermentation in Rusitec

Parameters	Salinomycin (mg/day)				Sodium fumarate (1 g/day)
	0	0.5	1.5	3.0	
Apparent digestibility of feed (%)	65.69^{b}±3.56	53.10^{a}±2.81	63.72^{b}±2.65	65.33b±2.21	70.07±2.78
pH	6.74±0.03	6.78±0.03	6.69±0.04	6.73±0.03	6.81±0.03
Ammonia nitrogen (ml/day)	66.18±11.18	56.64±7.68	48.09±8.94	60.09±6.96	67.32^{b}±7.74
Microbial protein (mg/day)	134.17^{a}±10.44	130.88^{a}±4.37	148.33^{a}±8.43	168.17^{b}±8.31	160±11.12
Total bacterial count ($\times10^{9}$/ml)	4.65±0.08	4.42±0.06	4.72±0.14	4.66±0.08	5.01±0.11
Total protozoal count ($\times10^{3}$/ml)	2.52^{c}±0.19	1.87^{bc}±0.20	1.52^{ab}±0.24	1.02^{a}±0.11	2.48^{b}±0.21
Total gas produced (ml/day)	2565^{b}±91	2047^{a}±71	1788^{a}±95	2480^{b}±74	2572^{b}±54
Methane (ml/day)	1154^{c}±40	716^{a}± 25	626^{a}± 33	868^{b}±26	900^{b}±19
Carbon dioxide (ml/day)	1410^{b}±50	1330^{ab}±46	1162^{a}±62	1612^{c}±47	1671^{c}±35

Means bearing different superscripts between columns within a row differ significantly ($P<0.05$).

Table 2. Effect of salinomycin and sodium fumarate on production of volatile fatty acids in Rusitec

Parameters (mg/day)	Salinomycin (mg/day)				Sodium fumarate (1 g/day)
	0	0.5	1.5	3.0	
Acetic acid	27.76^{b}±1.25	21.81^{a}±0.65	24.06^{ab}±0.93	25.21^{ab}±0.75	30.28^{b}±0.76
Propionic acid	8.98^{a}±0.34	9.98^{a}±0.29	12.13^{b}±0.53	12.43^{b}±0.56	11.08^{a}±0.70
Butyric acid	2.92^{b}±0.06	2.03^{a}±0.05	2.16^{a}±0.08	2.23^{a}±0.03	3.19^{c}±0.08
Isobutyric acid	0.79^{b}±0.03	0.66^{a}±0.03	0.62^{a}±0.03	0.62^{a}±0.03	0.57±0.02
Valeric acid	1.86^{b}±0.06	0.91^{a}±0.03	1.08^{a}±0.05	1.08^{a}±0.05	2.01^{b}±0.08
Isovaleric acid	1.02^{b}±0.06	0.82^{a}±0.04	0.94^{ab}±0.04	0.97^{b}±0.05	1.09±0.07
Total VFA	42.54^{b}±1.50	35.55^{a}±0.87	40.37^{ab}±1.48	41.91^{b}±1.30	47.65^{b}±0.93
Acetate: propionate ratio	3.10^{b}±0.11	2.19^{a}±0.04	1.99^{a}±0.02	2.04^{a}±0.04	2.80^{b}±0.23
Non-glucogenic ratio	3.76^{b}±0.13	2.59^{a}±0.04	2.34^{a}±0.03	2.40^{a}±0.05	3.39^{b} 0.28
Energetic efficiency (%)	69.23^{a}±0.77	76.52^{b}±0.44	78.22^{b}±0.44	77.74^{b}±0.45	71.56^{a}±1.50

Means bearing different superscripts between columns within a row differ significantly (P<0.05).

occurred due to the direct inhibition of methane producing bacteria, but may be due to the inhibition of hydrogen producing bacteria (*Lachnospira* sp. and *Ruminococcus* sp.) and formic acid producing bacteria (*Eubacterium* sp. and *Ruminococcus* sp.) (Nagaraja and Taylor 1987). However, formic acid producers like *Bacteroides* sp. and hydrogen producers like *Megasphaera elsdenii* and *Selenomonas ruminantium* are resistant to ionophore, which explains the partial inhibition of methane production due to ionophore in the present study. The reduction in methane production by sodium fumarate was due to utilization of hydrogen or formate by bacteria other than methanogens (Marounek *et al.* 1993).

Salinomycin and sodium fumarate increased the molar proportion of propionic acid and reduced the molar proportion of acetic acid and butyric acid, which is in agreement with the finding of Jalc and Laukova (2002). Increase in propionate may be due to selection for succinate producers and lactate fermenters (Nagaraja and Taylor 1987). The acetate: propionate ratio reduced from 3.10 to 1.99 with salinomycin treatment, was similar to the observation of Jalc and Laukova (2002). However, they were able to achieve acetate: propionate ratio of around one, which might be attributed to the variability in diet and also to the adaptation of the rumen ecosystem to the diet in donor animals. The non-glycogenic ratio was reduced from 3.76 to 2.34 with salinomycin treatment. The total volatile fatty acid was reduced with ionophore treatment in our study, which was in accordance with the findings of Rogers *et al.* (1997). Sodium fumarate increased acetic acid and propionic acid, however, the molar proportion of acetic acid reduced and that of propionic acid increased, almost similar to the findings of Martin and Streeter (1995) who used malate in *in vitro* mixed ruminal microorganism fermentation and Lopez *et al.* (1999) who used fumarate in their experiment.

Our results suggested that salinomycin and sodium fumarate might have a beneficial effect via decreased methanogenesis, increased propionate production and increase the nitrogen retention by increased dietary and/or microbial protein utilisation. However, detailed *in vivo* trials have to be conducted to validate the findings of present study.

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