In vitro evaluation of Salinomycin addition in wheat straw based total mixed diets on rumen fermentation, methanogenesis and dry matter degradability in buffalo

Sunil K. Sirohi, Navneet Goel

Nutrition Biotechnology Lab,
Animal Biotechnology Centre, Dairy Cattle Nutrition Division
National Dairy Research Institute, Karnal-132001, India
Corresponding author: S K Sirohi, email: sirohisk@gmail.com
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Abstract

Aim: The aim of the current study was to evaluate the effect of salinomycin *in vitro on* methanogenesis and rumen fermentation.

Materials and Methods: Different levels of (0,10, 15 and 20 ppm) salinomycin were checked for their effect on *in vitro* methanogenesis and rumen fermentation on three wheat straw based diets i.e. low fiber diet (LFD, 40R:60C), medium fiber diet (MFD, 50R:50C) and high fiber diet (HFD, 60R:40C). Evaluation of salinomycin was carried out using *in vitro* gas production technique. Methane production and individual fatty acids were estimated by Gas Chromatography.

Results: Results of different levels of salinomycin on *in vitro* methanogenesis indicated that the maximum methane reduction (38.14% in term of mM/gDM) was noticed in HFD at 20 ppm level. IVDMD showing increasing trend with an increasing concentration of salinomycin with HFD and LFD, while shown decreasing trend with MFD respectively. Protozoal population significantly decreased by addition of salinomycin in all diets.

Conclusion: The results of salinomycin evaluation in the current study can be implicated to mitigate the methane production, thus saving the feed energy loss and the accumulation of green house gases in environment.

Key Words: In vitro gas production technique, IVDMD, Methanogenesis, Salinomycin

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Introduction

Ionophores are antimicrobial compounds that are commonly used in the diets of monogastric and poultry for increasing the feed conversion efficiency and prevention of some diseases. Commonly used ionophores are monensin, lasalocid, laidlomycin, salinomycin and narasin. Salinomycin is a polyether antibiotic and produced by a strain of streptomyces albus (ATCC 21838). The main physiochemical characteristics of salinomycin are, molecular weight, 750; molecular formula, $C_{42}H_{70}O_{11}$; melting poin, 112.5 to 113.5^{0}C; pKa, 6.40; cation affinity, K>Na>Ca,Mg; cation transport activity, Na>K> Ca,Mg [1, 2]. Salinomycin has been shown the activity against gram-positive bacteria, including myco-bacteria and some filamentous fungi. However, no activity has been found against gram-negative bacteria or yeasts [1].

A number of study have found that salinomycin to be effective in enhancing both rate of gain and feed efficiency in feedlot cattle [3,4,5]. It is also effective in the treatment of coccidial infections of poultry and cattle [6].

Ionophores inhibit gram positive bacteria, which subsequently alters ruminal fermentation. When the gram negative bacteria predominate in the rumen, less methane is produced and more propionate is produced as reported by several workers [7, 8]. Methane is one of the most potent GHG which is produced from enteric fermentation in ruminants. Therefore, present study was planned to investigate the effect of salinomycin supplementation in different diets on rumen fermentation and methanogenesis *in vitro*.

Materials and methods

Preparation of Diets: To study the effect of salinomycin on rumen fermentation and methane production three diets were prepared with different roughage concentrate ratio i.e. high fiber diet (HFD,

60R:40C), medium fiber diet (MFD, 50R:50C) and low fiber diet (LFD, 40R:60C) and milled to pass through 1 mm sieve and used as substrate. Source of roughage and concentrate were wheat straw and normal farm concentrate having about 20 % crude protein and 70% total digestible nutrients (TDN). The concentrate part comprised of maize (33%), GNC (21%), mustard cake (12%), wheat bran (20%), deoiled rice bran (11%), mineral mixture (2%) and salt (1%).

Treatments and experimental design: Four levels i.e. 0ppm, 10ppm, 15ppm and 20ppm of salinomycin were added to the different wheat straw based HFD, MFD and LFD diets. All the treatment combinations were arranged in 4 x 3 factorial arrangements in randomized block designs with three replicates. Set was also incubated devoid of substrate with and without additive, which served as blanks for particular treatment and values were corrected for different parameters with these blanks.

Preparation of Inoculums and *In Vitro* Gas Production: Rumen liquor (RL) was obtained from fistulated adult male buffaloe (*Bubalus bubalis*) kept on standard diet (60 parts roughage: 40 parts concentrate) before one hour of morning feeding into a pre-warmed insulated flask and brought into the laboratory. Permission for taking rumen liquor from male fistulated buffaloes already taken from Animal ethics committee of institute. Then rumen liquor filtered through four layers of muslin cloth and the required amount of strained rumen liquor used as a source of inoculum.

The incubation medium was prepared as per previously described method [9]. *In vitro* studies were carried out in 100ml calibrated glass syringes containing 200±10 mg of milled (1mm) three type wheat straw based diet [9].

Plungers of syringes applied with petroleum jelly for smooth movement and stop any leakage. The 30 ml incubation medium was dispensed anaerobically in each syringe and before incubation, different levels of salinomycin was added by small syringe into 100ml syringes individually and mixed uniformly. Syringes were closed using clamps and were incubated at 39±0.5^{0}C for 24h in temperature controlled incubator cum shaker.

Total gas production and methane estimation: After 24 h incubation, gas production was recorded with the displacement of piston during incubation. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe (containing no substrate, but only the inoculum and buffer) from total gas produced in the syringe containing substrate and inoculum.

For methane estimation, representative of gas was sampled from the headspace of syringe in an airtight syringe and injected into Nucon-5765 gas chromatograph equipped with flame ionization detector (FID) and stainless steel column packed with Porapak-Q (length 6';o.d.1/8" i.d. 2 mm; mesh range 80-100). The gas flow rates for carrier gas (nitrogen), hydrogen and air were 30, 30 and 300 ml/min, respectively. Temperature of injector oven, column oven and detector were 40, 50 and 50^{0}C, respectively. For methane estimation, each gas sample (250µl) was manually injected using Hamilton airtight syringe.

Methane content in sample was calculated by external calibration, using a certified gas standard mixture of 50% CH_4 and 50% CO_2 (Spantech calibration gas, Surrey, England).

Methane production (ml) = Total gas produced (ml) x % methane in the sample

In vitro true Dry matter degradability: To estimate true DM degradability of feed sample of each syringe containing residues after incubation was estimated as per method [10].

Proximate analyses and cell wall constituents: The proximate analysis of substrate was carried out as per the methods of [11]. The cell wall constituents of substrates were determined according to suggested method [10].

Estimation of ammonia nitrogen and individual volatile fatty acid (IVFA): After the end of incubation, fermented material was transferred into centrifuge tubes and centrifuge at 3000 rpm for ten minutes, than supernatant of each syringe including that of blank was collected in vials used for ammonia nitrogen (NH_3-N) and individual volatile fatty acid (IVFA).

For NH_3-N estimation, 5 ml of supernatant was taken in tube mixed with 12 ml 1 N NaOH and steam passed using KEL PLUS-N analyzer (Pelican, India) and the NH_3 evolved was collected in conical flask containing boric acid solution having mixed indicator and titrated against N/100 H_2SO_4.

Individual volatile fatty acids (IVFAs) were estimated by taking 1 ml of the supernatant treated with 25% meta-phosphoric (4 ml) prepared in 1N sulphuric acid and kept for 3-4 h at ambient temperature [12]. Thereafter, it was centrifuged (3,000 rpm for 10 min) and clear supernatant was collected and stored at -20^{0}C until analyzed. IVFAs were

Table-1. Physical and chemical composition of wheat straw based diets used as substrate in *in vitro* incubation

Diets	Ingredient of diets g/kg on DM basis		Ingredient of concentrate	
	Wheat straw	Concentrate	Particulars	g/kg on DM basis
HFD	600	400	Maize	330
MFD	500	500	Ground nut cake	210
LFD	400	600	Mustard cake	120
			Wheat bran	200
			Deoiled rice bran	110
			Mineral mixture	20
			Salt	10

Diets	Chemical constituents of diets (g/kg on DM basis)						
	OM	CP	EE	NDF	ADF	HC	TA
HFD (60R:40C)	867.6	108.6	23.4	623.1	372.0	251.1	132.4
MFD (50R:50C)	878.4	125.3	30.4	604.5	329.5	275.0	121.6
LFD (40R:60C)	875.6	142.7	34.8	538.7	298.7	240.0	124.4

HFD, high fiber diet; MFD, medium fiber diet; LFD, low fiber diet; R, roughage; C, concentrate; OM, organic matter; CP, crude protein; EE, ether extract; NDF, natural detergent fiber; ADF, acid detergent fiber; HC, hemicelluloses; TA, total ash

estimated using gas chromatograph (Nucon 5700, India) equipped with flame ionization detector (FID) and stainless steel column (length 4'; o.d 1/8"; i.d 2mm) packed with Chromosorb–101.

Temperature of injection port, column and detector was set at 200, 180 and 210^{0}C, respectively. The flow rate of carrier gas (nitrogen) through the column was 40 ml/ min and the flow rates of hydrogen gas and air through FID were 30 and 300 ml/ min. respectively.

Sample (2µ) was injected through the injection port using Hamilton syringe (10µ). Individual VFA's of the samples were identified on the basis of their retention time and their concentration (mM) calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values.

Protozoa counting: The stained protozoa was diluted (if needed) and counted by Haemocytometer as per prescribed method [13].

Statistical analysis: Experimental data of different parameters were analyzed in complete randomized block design for analysis of variance and standard error of means [14].

Results and Discussion

The physical and chemical composition of all the three diets was given in table 1. Results of different levels of salinomycin on *in vitro* rumen fermentation and methanogenesis were shown in Table 2.

Effect of salinomycin on IVDMD was significant for all three diets. In HFD, digestibility was maximum increased (10.18%) at 20 ppm level than 7.80% in LFD at 10ppm level, while decreased slightly with increasing concentration of salinomycin in case of MFD. A reduction in methane production (ml/gDM, mM/gDM, mM/gDDM) was seen in all the diets with addition of salinomycin. Methane production percentage decreased with increasing concentration of salinomycin in all diets. Results showed the highest methane reduction (38.14, 30.14 and 27.68%) at 20 ppm level in HFD, MFD and LFD, when expressed in mM/gDM respectively. Similar effect of salinomycin on methane production was also found by other workers [15,16].

The propionic acid concentration (mM/l) increased with increasing dosage in all the three diet. The increase in propionic acid concentration was 48.43% at 15 ppm level in MFD, 19.95% at 20 ppm level in HFD and slightly changes in LFD. A non significant ($P \leq 0.05$) change in acetate concentration was observed in all diets. Acetate concentration increased in almost all cases except HFD, although the increase was non significant. A significant effect of salinomycin on butyrate concentration was seen in present study. Slight change in A/P ratio was observed in all cases except 20 ppm level in HFD, where the maximum reduction (47.20%) was found. The similar results were observed during salinomycin supplementation were consistent with the earlier findings of numerous researchers [17-19]. A non significant ($P \leq 0.05$) reduction in NH_3-N was observed due to salinomycin supplementation. The concentration of ammonia nitrogen increased with increasing concentrations of salinomycin in HFD and LFD while, decreased in MFD. The maximum reduction was (20%) found in MFD at 15 ppm level.

In present experiment, a significant ($P \leq 0.05$)

Table-2. Rumen fermentation parameters as affected by Salinomycin supplementation in different diets

Diets	Dose (ppm)	IVDMD (%)	CH4 (ml/gDM)	CH4 (mM/gDM)	CH4 (mM/gDDM)	Acetate (mM/l)	Propionate (mM/l)	Butyrate (mM)	A:P	NH_3-N (mg/100ml)	Protozoa (x10^5cells/ml)
HFD	00	60.50	34.74	3.45	2.86	72.90	11.68	8.20	6.25	14.00	5.50
(60R:40C)	10	63.00	27.83	2.77	1.75	68.09	13.84	3.50	5.15	14.00	4.60
	15	61.50	23.79	2.37	1.70	65.15	10.87	2.10	6.30	14.00	2.50
	20	66.66	21.38	2.13	1.25	71.95	14.01	6.20	3.30	14.93	2.00
MFD	00	68.66	35.61	3.54	2.13	64.60	10.88	5.10	6.34	14.00	3.50
(50R:50C)	10	65.83	36.36	3.62	2.25	94.36	11.26	4.69	5.23	11.66	1.00
	15	64.83	33.11	3.29	2.10	83.84	16.15	7.20	4.78	11.20	1.00
	20	64.66	24.85	2.47	1.53	58.26	14.21	6.98	5.01	12.60	0.50
LFD	00	70.50	36.71	3.65	1.95	63.90	14.01	8.34	4.21	10.26	3.50
(40R:60C)	10	76.00	27.55	2.74	1.60	75.60	13.17	4.82	4.54	12.13	2.50
	15	71.00	26.69	2.65	1.61	60.70	11.62	8.21	4.73	13.53	3.00
	20	70.50	25.65	2.55	1.47	79.20	14.72	11.27	4.67	13.06	1.50
SEM	**Dose**	2.08	1.09	0.04	0.07	NS	NS	0.05	0.26	NS	0.46
	Diet	2.40	1.26	0.04	0.09	NS	NS	0.05	0.29	NS	0.52
	D*D	**	**	**	**	NS	NS	**	**	NS	**

* Significant at P $\leq$ 0.05; **Significant at P $\leq$ 0.01; SEM, Standard Error of Means; IVDMD, in vitro dry matter digestibility; CH4, methane; A:P, acetate to propionate ratio; NH3-N, ammonia nitrogen

reduction in protozoal number was observed with the increasing concentration of salinomycin. At 20 ppm level of salinomycin, maximum reduction in protozoa number was found i.e. 63.63, 85.71 and 57.15% in HFD, MFD and LFD, respectively.

Conclusions

On comparing the effects of salinomycin on three diets, it was observed that the propionate production increased with the increasing dosage of salinomycin. On the other hand the methane production was reduced significantly and highest decrease was noticed at 20ppm. This indicates a shift in ruminal fermentation pattern. The hydrogen was shifted towards propionate production. IVDMD was increased with the increasing concentration of salinomycin on high and low fibre diets but decreased slightly in medium fibre diet.

Author's contribution

N. Goel conducted experiment, prepared tables and S.K. Sirohi planned the experiment, involved in statistical analysis and drafting of the paper. Both the authors read and approved the final manuscript.

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Competing interest

Authors declares that they have no competing interest.

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