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Effects of organic acid salts on ruminal biogas production and fermentation kinetics of total mixed rations with different maize silage to concentrate ratios

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ABSTRACT

Ruminants are one of the major generators of methane, a greenhouse gas (GHG) with a global warming potential, 25-fold that of carbon dioxide. Methane production by ruminants also reduces the gross feed energy intake utilization by about 2–12%. The present study aimed to test the effects of different levels of a ruminal fermentation modulator (RFM) on *in vitro* ruminal fermentation and GHG production of five total mixed rations (TMR) with different silage (S) to-concentrate (C) ratios (0S:100C, 25S:75C, 50S:50C, 75S:25C, and 100S:0C). The RFM contained mainly calcium propionate and malate, and monopropylene glycol. The rumen inoculum was collected from a Brown Swiss cow fed a TMR of concentrate and alfalfa hay (1:1 dry matter (DM)) *ad libitum*. Gas production (GP) measurements were recorded up to 72 h of incubation. There were interactions ($P < 0.05$) between ration type and RFM dose for GP until 18 h and for partitioning factor and gas yield at 24 h of incubation. The 100S:0C TMR had the highest asymptotic GP (linear and quadratic effects; $P < 0.05$) compared with other TMR. The 0S:100C TMR had the lowest GP rate (linear effect; $P = 0.003$). Ration type and RFM inclusion had no effect ($P > 0.05$) on methane production. The DM digestibility increased (linear effect; $P = 0.003$) as silage level increased. Overall, increasing silage in the TMR lowered the asymptotic GP and DM digestibility. The asymptotic GP was higher with the addition of the RFM without any effect on fermentation kinetics. These results suggest that the RFM can be used as an environmental cleaner product in animal farming due to its ability to improve ruminal fermentation of feedstuffs and to reduce methane emissions.

1. Introduction

Methane is a major greenhouse gas (GHG) produced during the normal digestive process in ruminant animals (Blaxter and Clapperton, 1965) with a global warming potential, 25-fold that of carbon dioxide (IPCC, 2007). In addition to environmental implications, ruminant methanogenesis represents a loss of 2–12% of the gross energy intake (Johnson and Johnson, 1995; Soltanali et al., 2015) with a greater environmental impact from the confinement system compared with pasture-based system (O'Brien et al., 2012). A cow can produce 250 to 500 L of methane (CH_4) per day depending on the quantity and quality of the feed which affects rate of digestion and rate of passage in the fermentation process (Johnson and Johnson, 1995). Furthermore, cattle consuming high fibre diets typically lose about 6% of gross dietary energy as methane (CH_4), whereas those on high concentrate rations generally lose about 3% of dietary gross energy as CH_4 (Mc Geough et al., 2012). According to the Food and Agriculture Organization of the United Nations, the livestock sector is responsible for about 18% of total global anthropogenic GHG emissions (Gerber et al., 2013).

Abbreviations: b, the asymptotic gas, carbon dioxide or methane production; *c*, the fractional rate of fermentation; CH_4, methane; CO_2, carbon dioxide; DM, dry matter; DMD, DM degradability; GHG, greenhouse gas; GP, gas production; *Lag*, the initial delay before gas, CO_2 or CH_4 formation; RFM, ruminal fermentation modulator; TMR, total mixed rations.

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After the Kyoto Protocol and because of increased public and political outcry, there is increased concern and effort to reduce CH_4 production from the livestock industry.

One strategy to reduce CH_4 production from livestock is through dietary manipulation. Digestion in ruminants is optimized by providing an assortment of essential nutrients for microbes to support optimum efficiency of utilization of the basal feed resource. Additionally, balancing the roughage to concentrate ratio is a critical factor for efficient feed utilization (Elghandour et al., 2015a; Kholif et al., 2017b). In-feed and/or natural feed additives have been shown to be a safe feeding strategies for improving the efficiency of feed utilization in ruminants (Rojo et al., 2015), carcasses quality (Velázquez-Garduño et al., 2015), while reducing the production of GHG (Kholif et al., 2017a). Exogenous enzymes (Elghandour et al., 2015b; Togtokhbayar et al., 2015), yeast cultures (Rodriguez et al., 2015), phytogenic extracts (Elghandour et al., 2015a), essential oils (Hernandez et al., 2017), and organic acid salts (Elghandour et al., 2016a,b) are some common feed additives used in ruminant nutrition. Improved feed intake and nutrient digestibility (Salem et al., 2015), increased daily gain (Cedillo et al., 2014), increased milk production (Morsy et al., 2016) and improved health status (Cedillo et al., 2015) are some benefits of supplementing ruminant diets with these feed additives.

Most of the research on the supplementation of organic acid salts to ruminant diets has been carried out using concentrate feeds or high-concentrate diets as feed or substrates for *in vitro* and *in vivo* experiments, respectively. Therefore, the current study aimed to investigate the effect of organic acid salts on gas (GP), CH_4 and carbon dioxide (CO_2) production and ruminal fermentation kinetics of rations with different maize silage to concentrate ratios. The results are expected to enhance feed use efficiency concomitant with decreased CH_4 and CO_2 production for a cleaner environmental animal agriculture.

2. Materials and methods

2.1. Study location

The experiment was performed at the animal nutrition laboratory, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México (Mexico). Rumen liquor donors were cared and handled according to the official Mexican standards of animals care (NORMA Oficial Mexicana, 1995).

2.2. Substrates and treatments

Five total mixed rations (TMR) with different concentrate (C) to maize silage (S) ratios (i.e., 0S:100C, 25S:75C, 50S:50C, 75S:25C, 100S:0C) were prepared as substrates for the *in vitro* incubation (Table 1). The concentrate feed was a commercial product (Purina®, Cuautitlan, Mexico). The ruminal fermentation modulator (RFM) was used at three levels: 0, 5 and 10 mg/g dry matter (DM) of substrate TMR. The RFM preparation used contained salts of organic acids including monopropylene glycol, calcium propionate, malate, and other active compounds (Table 2).

Table 2
Composition of the ruminal fermentation modulator containing organic acid salts.

	Concentration (per kg)
Mono propylene glycol powder	118 g
Calcium propionate	372 g
Calcium malate	223 g
Silicon dioxide	43 g
Amino acid-chelate Zn	2080 mg
Zinc-L-selenomethionene Se	12 ppm
E vitamin IU/kg	500 IU

2.3. In vitro incubation

The *in vitro* incubation procedures were as previously described by Rodriguez et al. (2015) and Vallejo et al. (2016). Briefly, rumen inoculum was collected from a Brown Swiss cow, fitted with a permanent rumen cannula and fed a TMR diet containing a commercial concentrate and alfalfa hay (1:1 DM basis) *ad libitum* and fresh water *ad libitum*. The rumen liquor was flushed with CO_2, mixed and strained through four layers of cheesecloth into a flask with oxygen (O_2)-free headspace. Filtered rumen fluid was immediately transported to the laboratory where it was mixed with a buffer solution at a ratio 1:4 (v/v). The buffer solution used was that described by Goering and Van Soest (1970) containing macro- and micro-minerals, with no trypticase added. Diluted rumen fluid (50 mL containing 10 mL of rumen liquor) was dispensed to each incubation bottle, where substrates (0.5 g DM of each TMR) had been previously weighed in and RFM added.

Three incubation runs were performed in three different weeks. One hundred and thirty-five bottles (three bottles for each ration × three levels of RFM × three different runs) plus three bottles as blanks (rumen fluid only) were incubated for 72 h at 39 °C. The volume of GP was recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 36, 48 and 72 h using the Pressure Transducer Technique (Extech instruments, Waltham, USA) of Theodorou et al. (1994). Both CH_4 and CO_2 production were recorded at 2, 6, 12, 18, 24, 36, 48 and 72 h of incubation using Gas-Pro detector (Gas Analyser CROWCON Model Tetra3, Abingdon, UK).

At the end of incubation at 72 h, the fermentation process was stopped by swirling the bottles in ice. The bottles were uncapped and the pH was measured immediately using a pH meter (Conductronic pH15, Puebla, Mexico). The contents of each bottle were

Table 1
Chemical composition of five total mixed rations with different maize silage (S) to concentrate (C) ratios (Adapted from Elghandour et al., 2015a,b).

Ration[a]	g/kg DM				
	Organic matter	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin
0S:100C	927[b]	172[a]	145[e]	70[e]	8[e]
25S:75C	933[ab]	133[b]	218[d]	88[d]	10[d]
50S:50C	940[a]	139[b]	302[c]	127[c]	13[c]
75S:25C	944[a]	92[c]	372[b]	149[b]	15[b]
100S:0C	944[a]	85[c]	499[a]	229[a]	20[a]
Standard error of the mean	14.8	9.3	12.6	11.0	1.4

[abc] Means in the same column with different superscripts differ ($P < 0.05$).
[a] The concentrate feed mixture contained (per kg DM): 200 g flacked maize, 260 g cracked maize grain, 154 g sorghum grain, 100 g molasses sugarcane, 100 g distilled dry grain, 96 g soybean meal, 70 g wheat bran, 10 g $NaCOOH_3$, 10 g mineral premix (vitamin A [12 000 000 IU], vitamin D_3 [2 500 000 IU], vitamin E [15 000 IU], vitamin K [2.0 g], vitamin B_1 [2.25 g], vitamin B_2 [7.5 g], vitamin B_6 [3.5 g], vitamin B_{12} [20 mg], Pantotenic acid [12.5 g], Folic acid [1.5 g], Biotin [125 mg], Niacin [45 g], Fe [50 g], Zn [50 g], Mn [110 g], Cu [12 g], I [0.30 g], Se [200 mg], Co [0.20 g] per kg).

Table 3
In vitro rumen gas, methane and carbon dioxide kinetics, and dry matter degradability (DMD) of five total mixed rations with different maize silage (S) and concentrate (C) ratios in the presence of different levels of a ruminal fermentation modulator.

Ration	Dose (mg/g DM)	Fermentation kinetics		Gas production (mL/g DM)[a]			CH_4 production (mL/g DM)[b]			CO_2 production (mL/g DM)[c]		
		pH	DMD (mg/kg DM)	*b*	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
0S:100C	0	5.71	787	226	0.042	1.32	73.6	0.032	14.1	6.08	0.080	15.7
	5	6.55	809	207	0.032	1.17	67.0	0.037	18.3	4.81	0.058	18.1
	10	6.51	811	207	0.044	1.55	51.0	0.037	14.6	6.13	0.075	18.0
25S:75C	0	5.67	738	169	0.056	1.69	53.0	0.066	12.7	4.64	0.064	14.5
	5	5.8	717	141	0.056	1.52	43.7	0.050	13.9	4.71	0.053	14.7
	10	5.9	729	144	0.045	1.2	52.1	0.020	16.4	4.83	0.056	13.9
50S:50C	0	6.46	649	135	0.051	1.99	40.9	0.053	14.4	5.36	0.068	15.4
	5	6.77	643	141	0.061	1.92	65.1	0.045	16.6	3.98	0.078	13.4
	10	6.8	683	166	0.068	2.23	64.0	0.042	12.3	3.91	0.091	12.3
75S:25C	0	6.03	508	188	0.074	2.31	69.7	0.023	13.4	7.22	0.094	12.6
	5	6.42	555	174	0.069	2.33	67.6	0.016	13.8	3.76	0.101	13.0
	10	6.61	514	179	0.075	2.36	79.6	0.036	9.5	4.87	0.167	12.1
100S:0C	0	6.87	429	115	0.091	2.53	69.7	0.047	9.0	2.51	0.091	10.7
	5	6.97	449	146	0.075	1.78	91.6	0.058	11.3	2.43	0.128	14.5
	10	6.93	476	95	0.091	2.15	81.6	0.017	11.8	5.61	0.085	11.0
Standard error of the mean		0.265	31.0	10.7	0.0062	0.24	7.92	0.0162	2.47	0.937	0.0161	1.73
Ration effect												
Linear		0.043	<0.001	<0.001	0.003	0.001	0.276	0.385	0.544	0.111	0.549	0.016
Quadratic		0.001	0.913	0.015	0.525	0.186	0.067	0.719	0.685	0.634	0.152	0.363
Dose effect												
Linear		0.043	0.533	0.033	0.288	0.015	0.273	0.782	0.194	0.048	0.689	0.395
Quadratic		0.759	0.400	0.473	0.275	0.753	0.741	0.170	0.533	0.320	0.146	0.422
Ration × Dose		0.149	0.928	0.302	0.275	0.552	0.105	0.573	0.756	0.194	0.071	0.796

[a] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (1/h); *Lag* is the initial delay before gas formation (h).
[b] *b* is the asymptotic methane production (mL/g DM); *c* is the rate of methane production (1/h); *Lag* is the initial delay before methane formation (h).
[c] *b* is the asymptotic carbon dioxide production (mL/g DM); *c* is the rate of carbon dioxide production (1/h); *Lag* is the initial delay before carbon dioxide formation (h).

then filtered under vacuum through glass with a sintered filter to obtain the non-fermented residue for determination of DM degradability (DMD) after drying at 65 °C overnight.

2.4. Chemical analyses and calculations

Samples of the rations were analysed for DM (#934.01), ash (#942.05), nitrogen (N; #954.01) and ether extract (#920.39) according to AOAC (1997). Ration neutral detergent fibre content (Van Soest et al., 1991), acid detergent fibre and lignin content (AOAC, 1997; #973.18) analyses were carried out using an ANKOM^{200} Fibre Analyser Unit (ANKOM Technology Corp., Macedon, NY, USA) with the use of an alpha-amylase and sodium sulphite.

For estimation of GP kinetic, recorded gas volumes (mL/g DM) were fitted using the NLIN procedure of SAS (2002) according to France et al. (2000).

2.5. Statistical analyses

Data from each of the three runs within the same sample of each of the three individual samples of TMR were averaged before statistical analysis and mean values of each individual sample were used as the experimental unit. Results of *in vitro* GP and rumen fermentation parameters were analysed as a factorial experiment using the PROC GLM option of SAS (2002) as:

$$Y_{ijk} = \mu + R_i + D_j + (R \times D)_{ij} + E_{ijk}$$

where: Y_{ijk} = is every observation of the *i*th TMR type (R_i) with *j*th RFM dose (D_j); μ is the general mean; $(R \times D)_{ij}$ is the interaction between TMR type and RFM dose; E_{ijk} represents the experimental error, normally distributed with the average 0 and constant variance. Linear and quadratic polynomial contrasts were used to examine responses of different silage-to-concentrate ratios for increasing levels of the RFM. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

No interaction ($P > 0.05$) between ration type and the RFM dose was observed for all measured parameters (Table 3). Higher fermentation pH ($P = 0.04$) was observed with the 5 mg/g DM of TMR dose. Fermentation parameters of pH and DMD differed ($P < 0.05$) between rations. Higher fermentation pH ($P < 0.05$) was observed with the 100S:0C TMR compared to the other TMR; however, a lower pH was observed with the 25S:75C TMR. Higher DMD was observed with the 0S:100C TMR (linear effect, $P \leq 0.009$). The lowest DMD was observed (linear effect, $P \leq 0.009$) with the 100S:0C TMR. Rations with high concentrate levels had higher DMD. This may be related to increased ruminal activity due to more nutrients being available with these rations. Higher fermentation pH was observed with RFM at 5 and 10 mg/g DM. Higher ruminal pH has been reported in many experiments with malate supplementation both *in vitro* and *in vivo* (Montaño et al., 1999). A linear increase in rumen pH was reported by Martin et al. (1999) with increasing malate supplementation in diets for steers. Higher fermentation pH is favourable for higher fibre digestibility; however, this effect was not observed in the current trial as DMD was not affected by the RFM addition. Similar results were reported by Elghandour et al. (2016b) with the addition of RFM to rations containing soybean hulls.

Although ruminal DMD was not affected by RFM addition, several reports have shown increased DMD and fibre digestibility resulting in increased nutrient availability and increased microbial N production and efficiency with malate inclusion in the diet. In agreement with the current study, Montaño et al. (1999) observed no effect on *in vivo* ruminal digestion with malic acid supplementation. The inconsistency of results between *in vivo* and *in vitro* experiments may be due to the diluted microbial concentrations in the rumen compared with the *in vitro* systems and different concentrations of DM content in both *in vitro* and *in vivo* systems.

Fig. 1 shows the *in vitro* GP of different TMR as affected by different levels of RFM at different incubation hours. Comparing

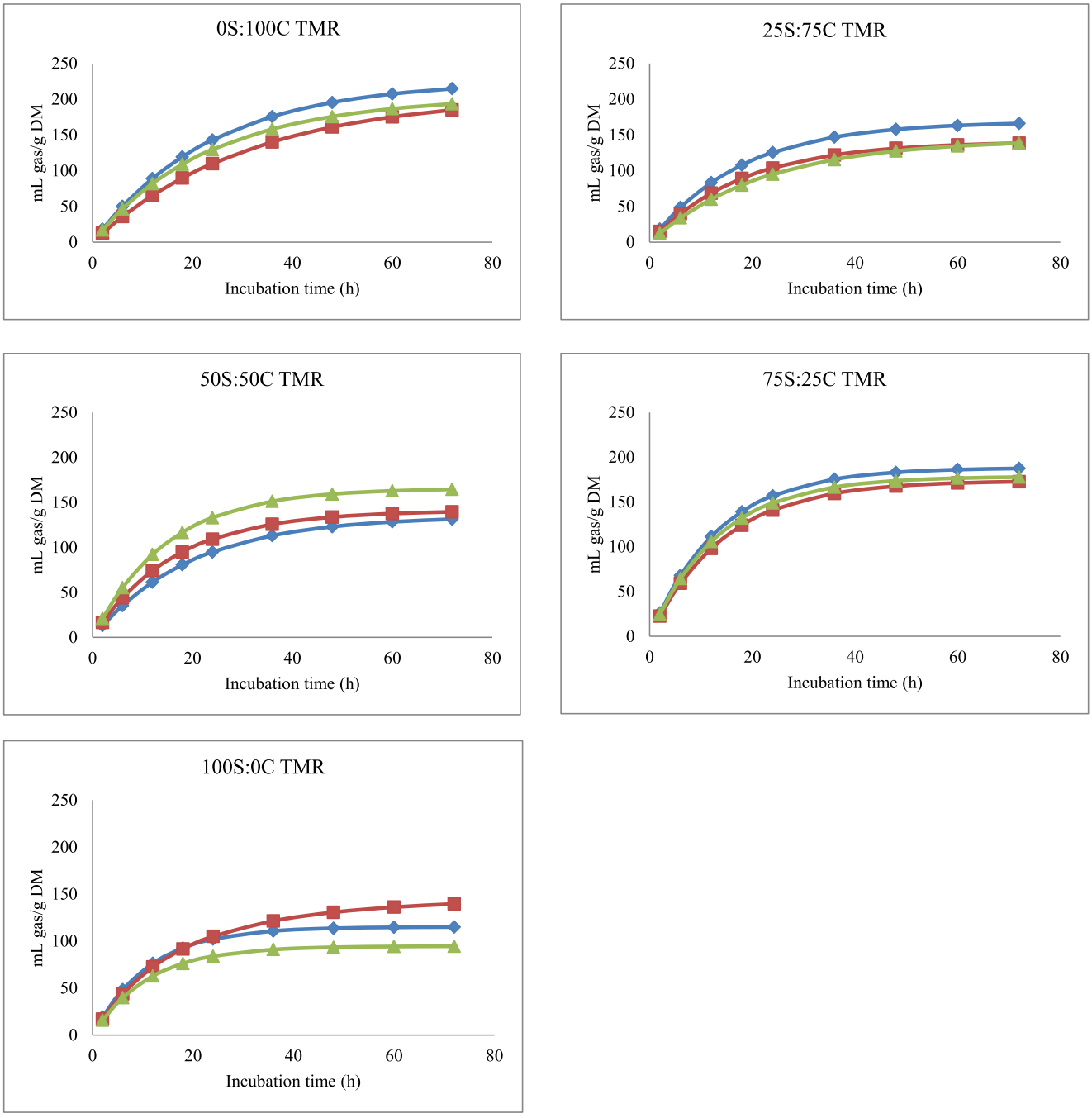

Fig. 1. *In vitro* rumen gas production (mL/g incubated DM) of five total mixed rations (TMR) with different maize silage (S) and concentrate (C) ratios in the presence of different levels of a ruminal fermentation modulator at 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the TMR.

different diets without the RFM addition (i.e. at 0 mg/g DM) showed a linear increase ($P < 0.001$) of asymptotic GP with a lower rate of GP ($P = 0.003$) with the 0S:100C TMR. Elghandour et al. (2016b) observed no effect on asymptotic GP with the addition of RFM (the same preparation used in the present experiment) at different levels to TMR containing different levels of prickly pear cactus. The difference between their results and those of the current study may be related to the use of different substrates. The lag time of GP increased linearly ($P = 0.001$) with the 75S:25C TMR. Moreover, RFM increased the asymptotic GP while the 0S:100C TMR decreased the rate of GP. The 0S:100C TMR produced more gas compared with the 100S:0C. Diets with high concentrate contents contain more available energy and protein for ruminal fermentation activity and GP (Kholif et al., 2017b). Consequently, the amount of gas produced is a good indicator of digestibility, fermentability and rumen microbial protein synthesis compared to fibrous rations which may decrease nutrient availability because of increased structural carbohydrate content which would reduce microbial fermentation (Kumari et al., 2012). The higher GP may be due to increased propionate and CO_2 production (Kumari et al., 2012).

RFM did not alter the rate of GP but affected both the asymptotic GP ($P = 0.033$) and lag time ($P = 0.015$). The RFM linearly increased

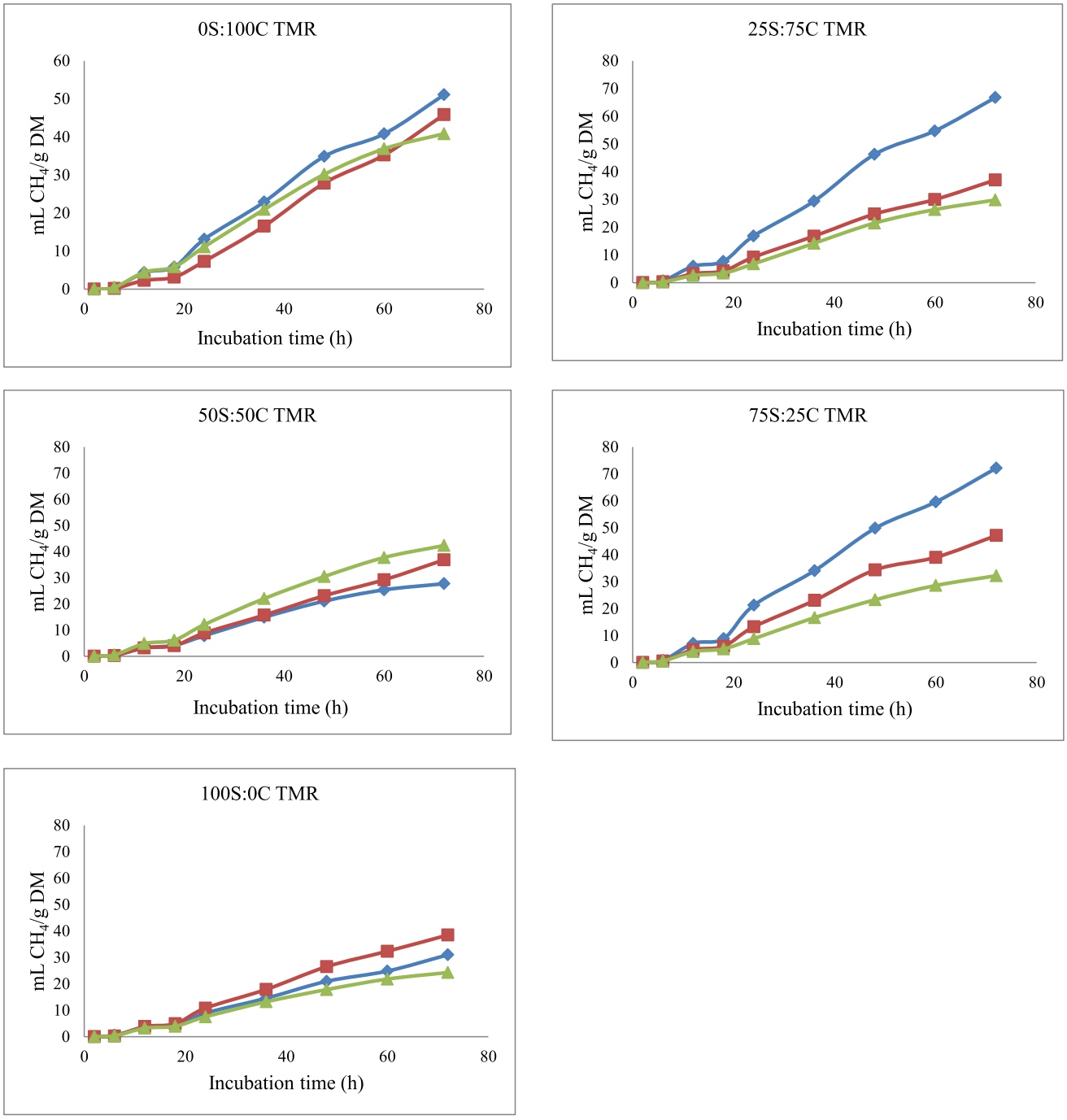

Fig. 2. *In vitro* methane (CH_4) production (mL/g incubated DM) of five total mixed rations (TMR) with different maize silage (S) and concentrate (C) ratios in the presence of different levels of a ruminal fermentation modulator 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the TMR.

($P < 0.05$) the GP from 4 to 24 h of incubation. The greatest effect was observed with the 10 mg/g DM when the RFM was added to the 50S:50C TMR. In contrast, RFM had a negative effect with the 0S:100C ration. Ration type and RFM dose did not affect ($P > 0.05$) the asymptotic CH_4 and CO_2 production, the rate of CH_4 and CO_2 production and the lag time of CH_4 and CO_2 production. Higher GP with the RFM addition may be due to its calcium malate content (about 37%) which has the ability to increase the number of *Selenomonas ruminantium* by direct oxidation of lactate (Paynter and Elsden, 1970). The addition of RFM increased GP at 10 mg/g DM of the 50S:50C TMR, with a negative effect on the 0S:100C TMR indicating that the effect is substrate-dependent. It has been suggested that malate can stimulate or inhibit ruminal microbial activity at different doses (Liu et al., 2009). Besides, the presence of calcium propionate at about 390 g/kg of the product may also be responsible for the increased GP with RFM. Propionate constitutes an important energy source for ruminants and can provide ruminal microflora with about 60% of its energy requirements (Drackley et al., 2001).

Figs. 2 and 3 show the *in vitro* CH_4 and CO_2 production of

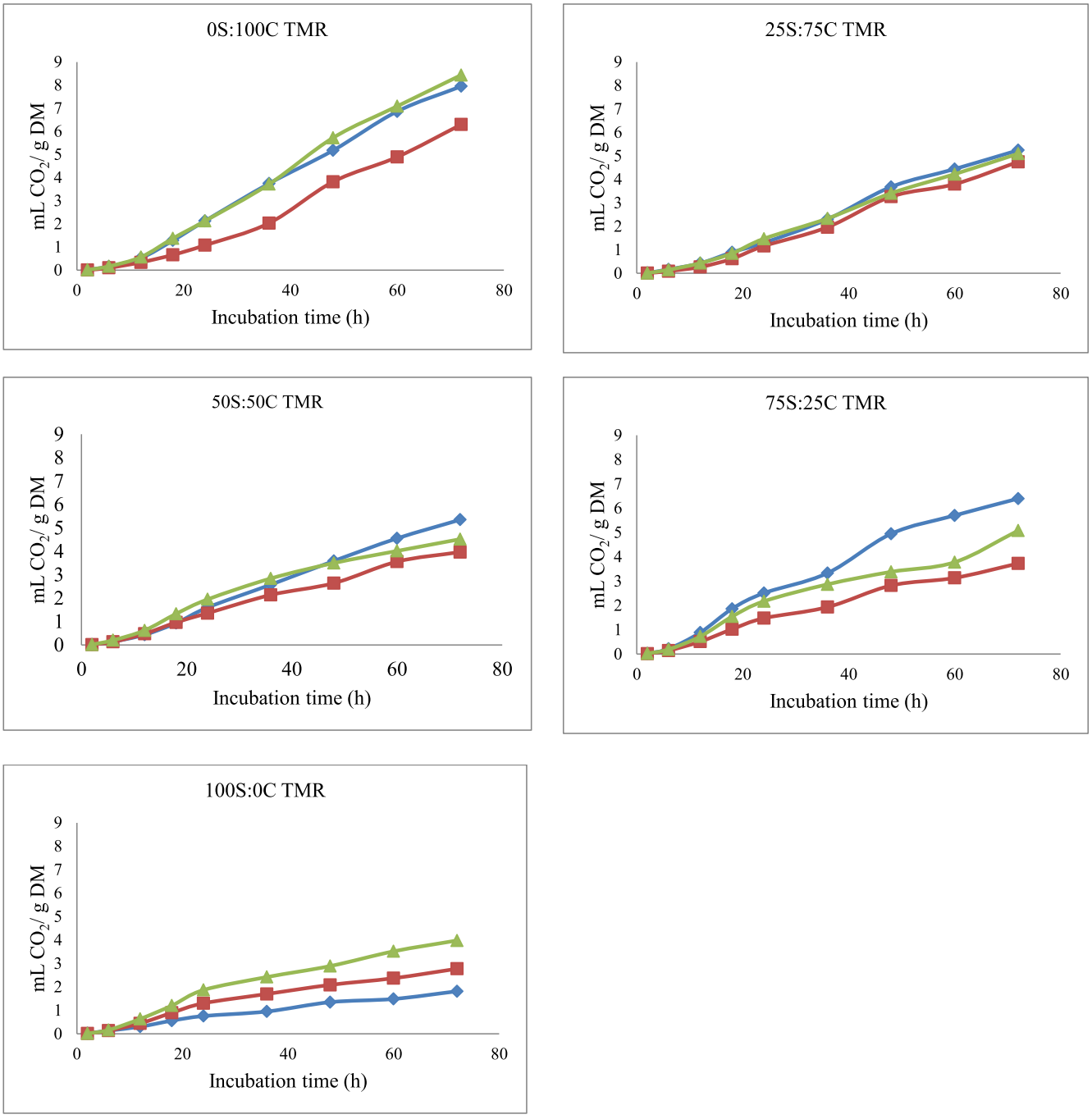

Fig. 3. *In vitro* carbon dioxide (CO_2) production (mL/g incubated DM) of five total mixed rations (TMR) with different maize silage (S) and concentrate (C) ratios in the presence of different levels of a ruminal fermentation modulator 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the TMR.

different TMR as affected by different levels of RFM at different incubation hours. Ration type did not affect ($P > 0.05$) CH_4 and CO_2 production (Tables 4 and 5). Compared with the 0S:100 C TMR, RFM lowered ($P < 0.05$) CH_4 production with the 100S:0C TMR at 5 mg/g DM of substrate. Elghandour et al. (2016b) observed an increased CH_4 production with the inclusion of the RFM at 5 and 10 mg/g DM of TMR containing prickly pear cactus. The addition of the RFM had no effect ($P > 0.05$) on CO_2 production during incubation in agreement with Elghandour et al. (2016a) who also observed no effect on CO_2 production with the inclusion of the RFM to rations containing different levels of soybean hulls.

It has been documented that fibrous feeds boost acetic acid and CH_4 production since acetic acid generation in the rumen is associated with the release of hydrogen that is used by methanogens for CH_4 production (Stewart et al., 1997). Addition of RFM at 5 mg/g DM lowered CH_4 production from most rations tested. In ruminant animals, about 2–12% of gross dietary energy is lost as CH_4 (Johnson and Johnson, 1995) causing a negative effect on animal performance and contributing to the GHG effect and global warming. Many experiments have shown that malate can reduce CH_4 production within the rumen by modulating the activity of specific rumen microbial populations and by increasing hydrogen

Table 4
In vitro methane production as a percent of total gas production of five total mixed rations with different maize silage (S) and concentrate (C) ratios in the presence of different levels of a ruminal fermentation modulator.

Ration	Dose (mg/g DM)	mL/g incubated DM			mL/g degraded DM			% of total gas production		
		6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
0S:100C	0	0.34	13.10	34.87	0.44	16.66	44.35	0.68	9.09	17.71
	5	0.19	7.31	27.85	0.24	9.05	34.43	0.60	7.26	18.32
	10	0.44	11.10	30.16	0.55	13.85	37.28	0.94	8.28	17.11
25S:75C	0	0.65	16.87	46.26	0.91	22.98	63.45	1.37	13.36	29.36
	5	0.34	9.22	24.75	0.47	13.14	35.10	0.85	8.68	18.68
	10	0.25	6.80	21.50	0.34	9.30	29.50	0.73	7.13	16.90
50S:50C	0	0.33	7.84	21.06	0.51	12.10	32.47	0.94	8.28	17.11
	5	0.26	8.88	23.13	0.42	14.29	36.93	0.54	7.27	16.08
	10	0.51	12.10	30.46	0.75	18.14	45.71	0.95	9.00	19.10
75S:25C	0	0.80	21.30	49.83	1.59	43.21	100.41	1.18	13.52	27.35
	5	0.56	13.29	34.39	0.94	23.22	59.12	0.97	9.70	20.85
	10	0.60	8.88	23.31	1.16	17.46	44.95	0.95	6.00	13.77
100S:0C	0	0.57	8.98	20.98	1.33	20.96	49.36	1.18	8.86	18.35
	5	0.28	10.84	26.53	0.64	24.25	59.75	0.64	10.03	18.85
	10	0.37	7.57	17.86	0.78	16.06	37.55	0.95	9.00	19.10
Standard error of the mean		0.102	2.823	6.042	0.153	4.926	11.174	0.192	1.935	3.166
Ration effect										
Linear		0.629	0.700	0.228	0.237	0.683	0.972	0.673	0.986	0.914
Quadratic		0.354	0.654	0.501	0.406	0.748	0.603	0.128	0.273	0.079
Dose effect										
Linear		0.002	0.047	0.067	0.001	0.049	0.077	0.007	0.107	0.098
Quadratic		0.997	0.121	0.066	0.695	0.073	0.049	0.950	0.114	0.087
Ration × Dose		0.226	0.125	0.091	0.120	0.105	0.122	0.499	0.387	0.167

utilization by rumen microbes (Li et al., 2009). Li et al. (2009) observed a 48% lower *in vitro* CH_4 production with malate addition to a diet containing 300 g ground alfalfa hay per 1 kg DM.

4. Future outlook

Including natural dietary additives to manipulate rumen fermentation has gained the attention of researchers, as a method for improving feed utilization and animal productivity, as well as decreasing GHG emission from livestock. *In vitro* experiments can predict the expected returns of the inclusion of feed additives in diets to conduct feeding trials. Therefore, further *in vivo* research should be conducted to validate the efficacy of organic acid salt preparations as a ruminal fermentation modulator.

5. Conclusions

Increasing the roughage portion (e.g., silage) in diets lowered the asymptotic gas production and dry matter degradability. The organic acid salts used as a ruminal fermentation modulator increased the asymptotic gas production at 5 and 10 mg/g DM of rations. Although it was expected that increasing the silage level in

Table 5
In vitro carbon dioxide production as a percent of total gas production of five total mixed rations with different maize silage (S) and concentrate (C) ratios in the presence of different levels of a ruminal fermentation modulator.

Ration	Dose (mg/g DM)	mL/g incubated DM			mL/g degraded DM			% of total gas production		
		6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
0S:100C	0	0.16	2.13	5.18	0.20	2.70	6.58	0.32	1.50	2.66
	5	0.10	1.08	3.82	0.12	1.33	4.73	0.28	0.99	2.40
	10	0.17	2.13	5.72	0.20	2.64	7.09	0.34	1.64	3.27
25S:75C	0	0.17	1.30	3.68	0.23	1.77	4.95	0.34	1.03	2.32
	5	0.09	1.16	3.26	0.13	1.65	4.63	0.22	1.10	2.45
	10	0.15	1.47	3.41	0.21	2.02	4.68	0.44	1.56	2.69
50S:50C	0	0.14	1.60	3.59	0.22	2.47	5.53	0.40	1.67	2.88
	5	0.13	1.36	2.64	0.21	2.16	4.17	0.27	1.21	1.95
	10	0.20	1.95	3.50	0.31	3.02	5.40	0.36	1.42	2.15
75S:25C	0	0.21	2.51	4.94	0.40	4.78	9.56	0.32	1.57	2.67
	5	0.14	1.47	2.82	0.25	2.58	4.90	0.24	1.04	1.67
	10	0.19	2.17	3.38	0.36	4.11	6.39	0.30	1.52	2.04
100S:0C	0	0.14	0.76	1.35	0.32	1.79	3.19	0.28	0.74	1.18
	5	0.14	1.30	2.09	0.31	2.97	4.75	0.29	1.16	1.49
	10	0.16	1.87	2.89	0.34	3.93	6.10	0.41	2.22	3.08
Standard error of the mean		0.044	0.427	0.749	0.078	0.726	1.246	0.078	0.300	0.425
Ration effect										
Linear		0.634	0.690	0.011	0.286	0.591	0.289	0.619	0.815	0.206
Quadratic		0.663	0.200	0.247	0.660	0.272	0.354	0.897	0.417	0.839
Dose effect										
Linear		0.136	0.166	0.093	0.161	0.229	0.102	0.173	0.301	0.202
Quadratic		0.193	0.064	0.288	0.288	0.078	0.360	0.093	0.008	0.049
Ration × Dose		0.983	0.654	0.567	0.991	0.500	0.370	0.947	0.243	0.138

the diet would increase methane and carbon dioxide production, these gasses were not affected by the organic acid salts. The organic acid salts lowered methane production without affecting carbon dioxide production, thus, these salts could be used as an environmental cleaner product in animal farming due to their ability to improve ruminal fermentation of diets offered to cattle.

Conflict of interest

None.

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