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Effect of Sulfate-Containing Compounds on Methane Production by Using an *In vitro* Gas Production Technique

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Abstract: The present study demonstrated the effect of levels of sulfate-containing compounds on methane production using *in vitro* gas production technique. Treatments were TMR without supplement (control) and TMR with 0.2 or 0.4% of ammonium sulfate, copper sulfate and sodium lauryl sulfate in a 3×2 Factorial in CRD with one control. Rumen fluid was collected from two rumen-fistulated beef cattle fed on a based diet. During the incubation, the gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72 and 96 h. The results revealed that increased the levels of sulfate-containing compounds in the TMR diet decreased the gas accumulation and CH_4 accumulation all the time. Although, the sum of the MRP did not differ among treatments, the MRP at 24 h after incubation was greatly appreciated by 0.2% of ammonium sulfate (14.53%). In addition, ammonium sulfate led to the highest IVOMD followed by the copper sulfate. Therefore, this study suggested that ammonium sulfate and copper sulfate and sodium lauryl sulfate at 0.2 and 0.4% in the TMR diet have the potential to reduce methane emission.

Key words: *In vitro* gas production, methane production, sulfate-containing compound

INTRODUCTION

Methane (CH_4) is a greenhouse gas that has been implicated in contributing to global warming 14.3% of total anthropogenic CH_4 in 2005 (IPCC, 2007). Globally, ruminant livestock produces approximately 80 million tones of CH_4 annually, accounting for approximately 28% of anthropogenic emissions of CH_4 (Beauchemin *et al*., 2008). In addition, Takahashi (2006) reported CH_4 emission from rumen fermentation of ruminant farm animals in ASEAN more than 25 Tg per year. Ruminant nutritionists have been looking for several alternative methods to reduce CH_4 production in the rumen such as decreased numbers of ruminal protozoa and the methanogenesis by using several bioactive agents, such as organic acids, plant oils and other extracts (Beauchemin *et al*., 2008; Beauchemin *et al*., 2009; McAllister and Newbold, 2008; Shibata and Terada, 2010; Goel and Makkar, 2012).

Methanogenesis can be reversed by using sulfate as the terminal electron acceptor. The coupled sulfate-CH_4 reaction is proposed to proceed according to the following equation assuming a one to one stoichiometry $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$ (Caldwell *et al*., 2008) by sulfate-reducing bacteria. Moreover, sulfate-reducing bacteria compete with methanogenic archaea for H_2 when sulfate is presented. According to the report by Raskin *et al*. (1996) who found that CH_4 production decreased immediately following the addition of sulfate. However, the most important thing to be aware of regarding sulfate-containing compound is optimum levels in the diet. Due to low ruminal sulfur concentration can also depress microbial growth (Bal and Ozturk, 2006). Despite, there has been a lot of investigations various sulfate-containing compound for improving performance and health of ruminants but lack of available data indicating sulfate-containing compound sources and levels depress methanogenesis in the rumen. Therefore, the objective of this research was investigated the sources and levels of sulfate-containing compounds in the ruminant diets on CH_4 production by using *in vitro* gas production technique.

MATERIALS AND METHODS

Treatment preparation and chemical analysis: The experiment was conducted using an *in vitro* rumen fermentation technique at various incubation time intervals with three replications per treatment. Feed ingredients were dried in a hot-air oven at 60°C for 72 h and ground to pass a 1 mm screen before mixing to make a Total Mixed Ration (TMR) with 50:50 concentrate

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Table 1: The chemical composition of TMR

Item	T1	T2	T3	T4	T5	T6	T7
Feed ingredients (%)							
Rice straw	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Soybean meal	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Cassava chip	25.0	24.8	24.6	24.8	24.6	24.8	24.6
Ammonium sulfate	-	0.2	0.4	-	-	-	-
Copper sulfate	-	-	-	0.2	0.4	-	-
Sodium lauryl sulfate	-	-	-	-	-	0.2	0.4
Chemical composition (%) of DM							
OM	89.3	90.0	89.8	90.3	89.2	89.3	90.3
CP	13.8	13.6	13.1	12.8	12.3	13.9	12.8
EE	1.0	0.7	1.0	0.8	1.0	0.7	1.0
NDF	52.8	48.6	55.9	56.7	55.6	55.6	49.3
ADF	32.8	33.8	34.8	33.0	35.0	34.7	29.8

T1: TMR without supplementation (control)
T2: TMR with 0.2% of ammonium sulfate
T3: TMR with 0.4% of ammonium sulfate
T4: TMR with 0.2% copper sulfate
T5: TMR with 0.4% copper sulfate
T6: TMR with 0.2% sodium lauryl sulfate
T7: TMR with 0.4% sodium lauryl sulfate

to roughage ratio without supplementation (control, T1) and TMR with 0.2% of ammonium sulfate (T2), 0.4% of ammonium sulfate (T3), 0.2% copper sulfate (T4), 0.4% copper sulfate (T5), 0.2% sodium lauryl sulfate (T6) and 0.4% sodium lauryl sulfate (T7) (Table 1). After 24 and 48 h, the incubation was stopped. The rumen fluid samples were collected and divided into 2 portions; the first portion of rumen fluid samples was filtered through four layers of cheesecloth and centrifuged at 15,000×g for 15 min at 4°C, finally the supernatant was stored at -20°C before NH_3-N analysis using the micro-Kjeldahl methods (AOAC, 1990). The second portion of rumen fluid samples was fixed with absolute alcohol for protozoa count. The total direct count of protozoa according to the methods of Galyen (1989) based on the use of a hemocytometer (Boeco, Hamburg, Germany).

Total mixed ration samples were dried in a hot-air oven at 60°C for 48 h and ground to pass a 1 mm screen. Dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were analyzed according to AOAC (1990). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed by the methods of Goering and Van Soest (1970).

***In vitro* gas production technique and methane measurement:** The experimental design was 3×2 Factorial in CRD plus one control (without supplementation). The main effect A was sulfate-containing compounds sources (ammonium sulfate, copper sulfate and sodium lauryl sulfate). The main effect B was 2 levels of sulfate-containing compounds (0.2 and 0.4%). Rumen fluid was collected from two rumen-fistulated beef cattle fed on a basal diet. Gas production was determined according to the procedure modified described by Makkar *et al.* (1995). During the incubation, the gas production was recorded at 0, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72 and 96 h. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows:

y = a+b [1-Exp (-ct)] and Estimation of lag time as proposed by Chen *et al.* (2008) as follows:

$$\text{lag time (h)} = (1/c)\ \ln[b/(a+b)]$$

where: a = the intercept and ideally reflects the fermentation of the soluble fraction (mL), b = the fermentation of the insoluble (but with time fermentation, mL), c = rate of gas production (mL/h.), d = |a| + b, t = incubation time and y = gas produced at time "t".

The biogas was collected by water displacement with a saturated NaCl solution modified from Take *et al.* (2006). Methane concentrations were analyzed using a gas chromatography equipped with a flame ionization detector (Shimadzu gas chromatograph GC-14B). Methane production reduction potential (MRP) was calculated by taking net CH_4 values for the control (without supplementation) as 100%, described by Jayanegara *et al.* (2009):

$$MRP = \frac{(\%NetCH_4 \text{ in control} - \%\ Net\ CH_4 \text{ in the test}) \times 100}{\%\ Net\ CH_4 \text{ in control}}$$

Statistical analysis: Data were analyzed by using the General Linear Models (GLM) procedures (SAS Inst. Inc., Cary, NC) with two factors with interaction: sulfate source and sulfate levels as a (3×2) Factorial in CRD with one control. Statistical significance for each term was assessed using an orthogonal contrast.

RESULTS AND DISCUSSION

Gas production and gas characteristics: The gas production curves of all treatments are given in Fig. 1.

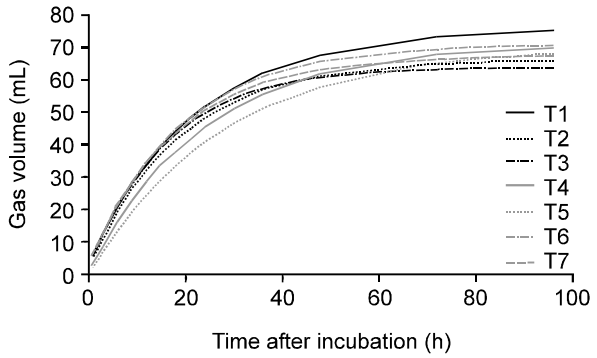

Fig. 1: Effect of levels of sulfate-containing compounds on gas accumulation. Gas accumulation (0.2 g substrate)

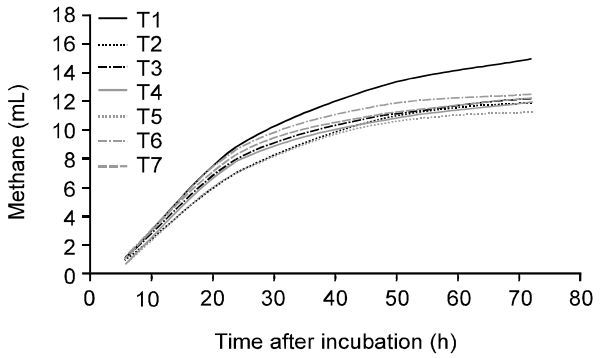

Fig. 2: Effect of levels of sulfate-containing compounds on CH_4 accumulation. Methane cumulation (mL/0.25 g DM)

The parameters of the gas production characteristics are presented in Table 2. The fermentation of the insoluble fraction (b) was significantly higher in TMR containing sulfate at 0.2% DM. The rates of gas production (c) ranged from 0.04 to 0.06 mL/h. in accordance with Khejornsart and Wanapat (2010) who reported that the rate of gas production of rice straw based diet was 0.04 mL/h. The gas produced after 96 h incubation of TMR without supplementation, TMR with 0.2% ammonium sulfate, TMR with 0.4% ammonium sulfate, TMR with 0.2% copper sulfate, TMR with 0.4% copper sulfate, TMR with 0.2% sodium lauryl sulfate and TMR with 0.2% sodium lauryl sulfate was 74.7, 65.6, 63.6, 69.9, 67.8, 70.1 and 67.0 ml/g DM, respectively (Table 2).

Methane accumulation and methane emission: The CH_4 accumulation and CH_4 emission at 24 h after incubation did not differ among treatments except those of the control which were significantly higher ($p<0.01$) than those of sulfate-containing compounds (Fig. 2). Moreover, increased the proportion of sulfate-containing compounds in the diet resulted in decrease ($p<0.05$) the CH_4 accumulation at all the times (Table 3). There is evidence that using sulfate-containing compounds at 0.2 and 0.4% in the diet can directly reduce CH_4 accumulation and CH_4 emissions. According to Minamikawa *et al.* (2005) who found that the CH_4 emission decreased with increases in ammonium sulfate application rate, caused by competition for substrates between sulfate-reducing bacteria and methanogens. Interestingly, a study in the Philippines found that the use of ammonium sulfate reduced CH_4 emissions by 25 to 36%. In addition, applying phosphogypsum (calcium sulfate dihydride) in combination with urea has been determined to reduce CH_4 emissions by more than 70% (Corton *et al.*, 2000).

Methane production reduction potential (MRP): The sum of the CH_4 production reduction potential (MRP) did not differ among treatments except the MRP at 24 h after incubation was greatly appreciated by 0.2% of ammonium sulfate ($p<0.05$). The present study found that the MRP of sodium lauryl sulfate at 0.2 and 0.4% was more than 30% (Table 4). The result was lower than the previous reported by Epule *et al.* (2011) who found that the maximum reduction of CH_4 emissions by using a sulfate fertilizer in rice fields is in the range 60-70%. In addition, reductions in CH_4 at 100% could be further difficult because CH_4 emissions are not controlled only by CH_4 production but also by CH_4 oxidation and by the gas transport pathways to the atmosphere such as diffusion, ebullition and also related transport (Denier van der Gon and Neue, 1994).

***In vitro* degradability:** *In vitro* dry matter degradability of the control was similar among dietary treatments. Compared to the control, inclusion of levels of sulfate-containing compounds showed no effect on the degradability of dry matter. This result was in accordance with that reported by Felix *et al.* (2012) who found that there was no effect of copper supplementation on dry matter degradability. The present study found that levels of sulfate-containing compounds were significantly ($p<0.05$) higher in *in vitro* organic matter degradability at 24 h after incubation. Addition of ammonium sulfate led to the highest *in vitro* organic matter degradability at 24 h after incubation than copper sulfate ($p = 0.05$) Table 5.

Ammonia concentration and protozoa population: Ammonia nitrogen concentration at 24 h after incubation was significantly reduced by sulfate-containing compounds addition in both levels. However, the NH_3-N concentration at 48 h after incubation did not differ among treatments ranging from 11.3-13.3 mg% (Table 6). This result was in accordance with Hegarty *et al.* (1991) who found that sulfate-containing

Table 2: Effect of levels of sulfate-containing compounds on gas production characteristic

	Gas production characteristics (0.2 g DM)				
Item	a, ML	b, mL	c, mL/h	d, mL	Gas at 96 h
Non supplement (control)	2.8^{a}	72.8^{a}	0.05^{b}	75.6^{a}	74.7^{a}
Ammonium sulfate (0.2%)	1.8^{a}	64.2^{bc}	0.05^{b}	66.0^{b}	65.6^{b}
Ammonium sulfate (0.4%)	1.9^{a}	61.8^{c}	0.06^{a}	63.7^{b}	63.6^{b}
Copper sulfate (0.2%)	0.6^{b}	70.6^{ab}	0.04^{c}	71.2^{ab}	69.9^{ab}
Copper sulfate (0.4%)	-0.2^{b}	70.2^{ab}	0.04^{c}	70.5^{ab}	67.8^{ab}
Sodium lauryl sulfate (0.2%)	1.9^{a}	68.6^{abc}	0.05^{b}	70.5^{ab}	70.1^{ab}
Sodium lauryl sulfate (0.4%)	2.6^{a}	64.7^{bc}	0.06^{a}	67.3^{b}	67.0^{b}
SEM	0.32	2.07	<0.01	2.10	2.05
Factor A: Sulfate sources	0.02	0.17	0.63	0.09	0.09
Factor B: Levels	0.24	0.02	0.01	0.02	0.01
Interaction A×B	0.60	0.21	<0.01	0.15	0.22
Orthogonal contrast					
Control vs., others	0.52	0.12	0.69	0.14	0.19
Amm vs., Cop	0.24	0.17	<0.01	0.20	0.32
Amm vs., SLS	<0.01	0.26	0.03	0.10	0.08
Cop vs., SLS	<0.01	0.76	<0.01	0.65	0.38
Amm + Cop vs SLS	<0.01	0.62	<0.01	0.21	0.12

^{abc}Means in the same row with different in superscript differ significantly ($p<0.05$)
[1]Amm: Ammonium sulfate,
Cop: Copper sulfate,
SLS: Sodium lauryl sulfate

Table 3: Effect of levels of sulfate-containing compounds on gas and CH_4 accumulation at 24, 48 and 72 h after incubation

	Gas accumulation (mL/0.2 g DM)			CH_4 accumulation (mL/0.2 g DM)			Percentage of CH_4 (%)		
Item	24	48	72	24	48	72	24	48	72
Non supplement (control)	50.8^{a}	69.7^{a}	73.5^{a}	9.3^{a}	13.9^{a}	15.9^{a}	18.4^{a}	20.0	21.6^{a}
Ammonium sulfate (0.2%)	47.4^{bc}	61.2^{cd}	64.8^{c}	7.4^{b}	11.5^{b}	12.7^{b}	15.7^{b}	18.7	19.6^{ab}
Ammonium sulfate (0.4%)	48.9^{ab}	60.8^{cd}	63.8^{c}	8.4^{ab}	11.7^{b}	13.0^{b}	17.3^{ab}	19.2	20.4^{ab}
Copper sulfate (0.2%)	45.9^{c}	63.8^{bc}	67.4^{bc}	8.2^{ab}	11.3^{b}	12.8^{b}	18.0^{ab}	17.8	19.0^{b}
Copper sulfate (0.4%)	41.6^{d}	58.1^{d}	63.5^{c}	7.5^{b}	11.1^{b}	12.0^{b}	18.1^{ab}	19.1	18.8^{b}
Sodium lauryl sulfate (0.2%)	51.0^{a}	67.0^{ab}	69.6^{b}	9.1^{a}	12.4^{b}	13.3^{b}	18.0^{ab}	18.6	19.1^{b}
Sodium lauryl sulfate (0.4%)	49.4^{ab}	63.7^{bc}	66.6^{bc}	8.8^{a}	11.8^{b}	12.6^{b}	17.8^{ab}	18.6	19.0^{b}
SEM	0.88	1.17	1.17	0.33	0.46	0.60	0.74	0.65	0.77
Factor A: Sulfate sources	0.24	0.15	0.11	0.12	0.03	0.15	0.50	0.29	0.82
Factor B: Levels	0.07	0.02	0.01	0.03	0.02	0.01	0.53	0.73	0.44
Interaction A×B	0.82	0.36	0.25	0.38	0.75	0.73	0.28	0.44	0.63
Orthogonal contrast[1]									
Control vs., others	0.89	0.45	0.26	<0.01	0.25	0.40	0.02	0.86	0.96
Amm vs., Cop	0.35	0.88	0.47	0.94	0.58	0.63	0.62	0.60	0.35
Amm vs., SLS	0.07	0.07	0.08	0.04	0.01	0.04	0.52	0.26	0.44
Cop vs., SLS	0.02	0.09	0.24	0.04	0.03	0.02	0.89	0.53	0.10
(Amm+Cop) vs., SLS	0.02	0.05	0.09	0.02	<0.01	0.01	0.65	0.31	0.16

^{abc}Means in the same row with different in superscript differ significantly ($p<0.05$)
[1]Amm: Ammonium sulfate,
Cop: Copper sulfate,
SLS: Sodium lauryl sulfate

compound supplementation increased the H_2S concentration but tended to reduce NH_3-N concentration (7.8 vs., 8.8%) in the umen fluid of fauna free sheep. However, NH_3-N concentration at 24 h was higher in the control (10.0 mg%). Importantly, Satter and Slyter (1974) demonstrated that 5 mg% ruminal NH_3-N was optimum for microbial fermentation in mixed culture in a closed system.

Protozoa populations in TMR with 0.4% ammonium sulfate was increased ($p<0.01$) higher than in TMR with copper sulfate and sodium lauryl sulfate. Importantly, protozoa population was the lowest ($p<0.05$) in TMR with sodium lauryl sulfate (Table 6). These results were in accordance with Santra and Karim (2000, 2002) and Santra *et al.* (2007) who reported that sodium lauryl sulfate can effectively defaunate the animal and as a

Table 4: Effect of levels of sulfate-containing compounds on CH4 production reduction potential (MRP) at 6, 24, 48 and 72 h after incubation

	Methane production reduction potential (%)				
Item	6	24	48	72	Sum
Ammonium sulfate (0.2%)	6.6	14.5	6.1	9.3	36.6
Ammonium sulfate (0.4%)	6.7	6.2	3.7	5.7	22.3
Copper sulfate (0.2%)	14.2	2.3	11.0	12.2	39.7
Copper sulfate (0.4%)	4.3	1.8	4.3	13.0	23.5
Sodium lauryl sulfate (0.2%)	12.3	2.6	6.9	11.8	33.6
Sodium lauryl sulfate (0.4%)	13.8	3.3	7.0	12.2	36.3
SEM	3.90	4.33	3.49	2.93	9.90
Factor A: Sulfate sources	0.29	0.15	0.72	0.21	0.86
Factor B: Levels	0.40	0.46	0.31	0.75	0.27
Interaction A×B	0.32	0.55	0.63	0.71	0.59
Orthogonal contrast					
Amm 0.2 vs., Amm (0.4%)	0.99	0.20	0.63	0.40	0.33
Cop 0.2 vs., Cop (0.4%)	0.09	0.94	0.20	0.85	0.27
SLS 0.2 vs., SLS (0.4%)	0.76	0.92	0.98	0.92	0.85
Amm vs., Cop, SLS at (0.2%)	0.19	0.04	0.52	0.48	0.99
Amm vs., Cop, SLS at (0.4%)	0.63	0.50	0.65	0.80	0.55
Cop vs., SLS at (0.2%)	0.73	0.96	0.43	0.93	0.67
Cop vs., SLS at (0.4%)	0.11	0.82	0.60	0.85	0.37

[1]Amm: Ammonium sulfate, Cop: Copper sulfate, SLS: Sodium lauryl sulfate

Table 5: Effect of levels of sulfate-containing compounds on *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) at 24 and 48 h after incubation

	IVDMD			IVOMD		
Item	24	48	Mean	24	48	Mean
Non supplement (control)	62.2	69.4	65.8	67.6	74.5	71.1
Ammonium sulfate (0.2%)	61.0	68.6	64.8	72.2	73.6	72.9
Ammonium sulfate (0.4%)	61.7	69.3	65.5	72.6	77.3	75.0
Copper sulfate (0.2%)	60.7	67.9	64.3	67.3	74.0	70.7
Copper sulfate (0.4%)	55.7	59.9	57.8	68.8	76.2	72.5
Sodium lauryl sulfate (0.2%)	60.8	67.1	64.0	68.0	75.2	71.6
Sodium lauryl sulfate (0.4%)	54.9	61.5	58.2	69.4	70.0	69.7
SEM	1.67	1.96	1.70	1.60	1.33	3.08
Factor A: Sulfate sources	0.16	0.41	0.13	0.07	0.11	0.24
Factor B: Levels	0.06	0.14	0.90	0.03	0.94	0.19
Interaction A×B	0.17	0.61	0.71	0.11	0.75	0.63
Orthogonal contrast[1]						
Control vs., others	0.40	0.12	0.38	0.18	0.66	0.08
Amm vs., Cop	0.20	0.45	0.35	0.05	0.97	0.48
Amm vs., SLS	0.20	0.47	0.12	0.14	0.04	0.22
Cop vs., SLS	0.89	0.97	0.46	0.32	0.04	0.57
(Amm+Cop) vs., SLS	0.46	0.69	0.19	0.71	0.03	0.30

[1]Amm: Ammonium sulfate, Cop: Copper sulfate, SLS: Sodium lauryl sulfate

Table 6: Effect of levels of sulfate-containing compounds on ammonia nitrogen (NH_3-N) and protozoa population at 24 and 48 h after incubation

	NH_3-N			Protozoa population, $\times 10^4$		
Item	24	48	Mean	24	48	Mean
Non supplement (control)	10.0^{a}	13.0^{a}	11.5^{a}	2.8	2.5	2.6
Ammonium sulfate (0.2%)	6.7^{b}	13.3^{a}	10.0^{b}	3.0	3.3	3.1
Ammonium sulfate (0.4%)	8.0^{ab}	13.3^{a}	10.7^{ab}	4.3	4.3	4.3
Copper sulfate (0.2%)	9.3^{a}	11.3^{bc}	10.3^{ab}	2.3	2.3	2.3
Copper sulfate (0.4%)	9.3^{a}	11.0^{c}	10.2^{ab}	2.0	2.0	2.0
Sodium lauryl sulfate (0.2%)	8.7^{a}	13.3^{a}	11.0^{ab}	1.8	1.5	1.6
Sodium lauryl sulfate (0.4%)	9.3^{a}	12.7^{ab}	11.0^{ab}	1.5	1.3	1.4
SEM	0.56	0.47	0.39	0.38	0.29	0.34
Factor A: Sulfate sources	0.16	0.53	0.17	<0.01	<0.01	<0.01
Factor B: Levels	0.37	0.58	0.33	0.29	0.09	0.05
Interaction A×B	0.11	0.53	0.40	0.15	0.15	0.02
Orthogonal contrast[1]						
Control vs., others	<0.01	0.12	0.11	0.17	<0.01	0.02
Amm vs., Cop	0.57	0.73	0.84	<0.01	<0.01	<0.01
Amm vs., SLS	0.12	0.32	0.10	<0.01	<0.01	<0.01
Cop vs., SLS	0.28	0.20	0.13	0.52	0.66	0.31
(Amm+Cop) vs., SLS	0.13	0.19	0.07	0.20	0.02	0.03

[abc]Means in the same row with different in superscript differ significantly ($p<0.05$), [1]Amm: Ammonium sulfate, Cop: Copper sulfate SLS: Sodium lauryl sulfate

defaunating agent it had no apparent adverse effect on the performance of sheep as evident from similar nutrient degradability, plane of nutrition, rumen fermentation pattern and ciliate protozoa population between refaunated and faunated sheep.

Conclusion: The present study found that added sulfate-containing compounds in the diet decreased the gas accumulation and CH_4 accumulation. The sum of the CH_4 production reduction potential did not differ among treatments but the MRP at 24 h after incubation was greatly reduced by 0.2% of ammonium sulfate. In addition, ammonium sulfate led to the highest *in vitro* organic matter degradability at 24 h. Therefore, this study suggested that ammonium sulfate and copper sulfate and sodium lauryl sulfate at 0.2 and 0.4% in the diet could modify rumen fermentation and have a potential to mitigate CH_4 emission in ruminants. Further research should be completed *in vivo* study to elucidate their effects on CH_4 emission and animal performances.

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