Effect of sulfur-containing compounds on methane production by *in vitro* gas production

ผลของสารประกอบกำมะถันต่อการผลิตแก๊สเมทเธนโดยวิธี in vitro gas production

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บทคัดย่อ: การศึกษาผลของสารประกอบกำมะถันต่อการผลิตแก๊สเมทเธนในสัตว์เคี้ยวเอื้องโดยใช้วิธีการ in vitro gas production ปัจจัยในการทดลองประกอบด้วย ฟางข้าวที่ไม่ได้รับสารเสริม (ควบคุม) ฟางข้าวเสริมกำมะถัน แอมโมเนียมซัลเฟต แคลเซียมซัลเฟต คอปเปอร์ซัลเฟต และโซเดียมลอริลซัลเฟต ที่ระดับ 0.2 เปอร์เซ็นต์ ในแผนการทดลองแบบสุ่มสมบูรณ์ ใช้โคเนื้อเจาะกระเพาะรูเมนที่ได้รับฟางข้าวเป็นอาหารหยาบ จำนวน 2 ตัว เป็นแหล่งของของเหลวจากกระเพาะรูเมน จากการศึกษาพบว่าทุกปัจจัยการทดลองไม่ส่งผลกระทบต่อ in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD) และแอมโมเนียไนโตรเจน ณ ชั่วโมงที่ 24 แต่พบว่า IVDMD และ IVOMD ณ ชั่วโมงที่ 48 ในปัจจัยการทดลองที่ได้รับคอปเปอร์ซัลเฟตเพิ่มสูงขึ้น นอกจากนี้ ณ ชั่วโมงที่ 24 พบว่า ปัจจัยการทดลองที่ได้รับโซเดียมลอริลซัลเฟตมีประชากรโปรโตซัวต่ำสุด (0.85×10^4 cells/ml) แต่ทุกปัจจัยทดลองไม่ส่งผลกระทบต่อประชากรของโปรโตซัว ณ ชั่วโมงที่ 48 จากผลการคำนวณศักยภาพในการลดการผลิตแก๊สเมทเธน (methane production reduction potential, MRP) พบว่า แอมโมเนียมซัลเฟตมีศักยภาพในการลดการผลิตแก๊สเมทเธนมากกว่า 50 เปอร์เซ็นต์ จากการศึกษาครั้งนี้สามารถแนะนำได้ว่า แอมโมเนียมซัลเฟต คอปเปอร์ซัลเฟต และโซเดียมลอริลซัลเฟต มีศักยภาพในการลดการปลดปล่อยแก๊สเมทเธนในสัตว์เคี้ยวเอื้อง

คำสำคัญ: เมทเธน สัตว์เคี้ยวเอื้อง ซัลเฟต ซัลเฟอร์

Abstract: The effect of inclusion of sulfur-containing compounds on gas and methane production was studied using in vitro gas production technique. Treatments were rice straw without supplement (control) and rice straw with 0.2 % of sulfur, ammonium sulfate, calcium sulfate, copper sulfate and sodium lauryl sulfate in a completely randomized design. Rumen fluid was collected from two rumen-fistulated beef cattle fed on a straw-based diet. Treatments had no significant effect on in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD) and NH_3-N at 24 h. However, IVDMD and IVOMD at 48 h. were significantly increased ($p<0.05$) by the addition of copper sulfate. Inclusion of sodium lauryl sulfate in

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the diet resulted in a significant reduction in protozoa count at 24 h (0.85 x 10^4 cells/ml), however, there was no significant difference observed among treatments in protozoa count at 48 h. The methane production reduction potential (MRP) of ammonium sulfate was more than 50 percent. Based on the results in this study suggested that ammonium sulfate, copper sulfate and sodium lauryl sulfate have a potential to mitigate CH_4 emission in ruminants.

Key words: methane, ruminant, sulfate, sulfur

Introduction

Methane (CH_4) is a greenhouse gas that has been implicated in contributing to global warming 14.3 percent of total anthropogenic methane in 2005 (IPCC, 2007). Machmuller and Clark (2006) reported the average daily CH_4 emission for cattle and sheep in New Zealand, was 144-308 and 24-36 g, respectively. Moss et al. (2000) reported CH_4 from enteric fermentation in animal contributes approximately 80 million tones per year and represents a partial loss of feed energy varying from 2 percent by animals feeding on high grain diets and to 12 percent when low quality forage is fed (Johnson et al., 2007). Scientists have been looking for ways to reduce CH_4 production in the rumen such as decreased numbers of ruminal protozoa and methanogensis by using several bioactive agents, such as organic acids, plant oils and other extracts. 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. Therefore, the objective of this experiment was to study effect of sulfur-containing compounds on CH_4 production by in vitro technique.

Materials and Methods

Treatments

Treatments were rice straw without supplement (control) and rice straw with 0.2 % of sulfur, ammonium sulfate, calcium sulfate, copper sulfate and sodium lauryl sulfate. After 24 and 48 h, the incubation was stopped and the inoculants were determined for dry matter digestibility (DMD), organic matter digestibility (OMD) and ammonia-nitrogen (NH_3-N) and protozoal counts.

In vitro gas production and methane measurement

Rumen fluid was collected from two rumen-fistulated beef cattle fed on a straw-based diet**.** Gas production was determined according to the procedure described by Makkar et al. (1995). The biogas was measured by water displacement with a saturated NaCl solution (Take et al., 2006) until 96 h after incubation. Gas sample was collected at 24 h and 48 h after incubation for methane analysis. Kinetics of gas production was determined according to Orskov and McDonald (1979). Methane concentrations were analyzed using a gas chromatography equipped with a flame ionization detector (Shimadzu gas chromatograph GC-14B).

Statistical analysis

Data were analyzed by using the GLM procedures (SAS, 1985) as a completely randomized design. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

The results of gas production as affected by different sulfur-containing compound are presented in Table 1. No significant effect on in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD) and NH_3-N at 24 h of incubation was observed among treatments. However, IVDMD and IVOMD at 48 h of incubation were significantly increased (P<0.05) by the addition of copper sulfate. Furthermore, the NH_3-N concentration at 48 h of incubation were higher in the control and sodium lauryl sulfate supplement (9.32 mg%). There was no significant difference observed among treatments in protozoal count at 48 h. Inclusion of sodium lauryl sulfate in the diet resulted in a significant reduction in protozoal count at 24 h according to Santra and Karim (2000) who found that lambs administrate of 8 g sodium lauryl sulfate/100 kg body weight for 2 consecutive days was maintained free of rumen protozoa for 47 days.

The methane production reduction potential (MRP) by inclusion of ammonium sulfate was more than 50 percent within 6 h of incubation (Table 2).

Conclusion

Based on this study, it could be suggested that ammonium sulfate, copper sulfate and sodium lauryl sulfate could modify the 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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Table 1 Effect of sulfur-containing compounds on IVDMD, IVOMD, gas production characteristic, gas volume and methane production

Item	T1	T2	T3	T4	T5	T6	SEM
In vitro digestibility , %							
IVDMD at 24 h.	32.32	37.52	37.53	29.71	34.92	29.71	1.26
IVOMD at 24 h.	36.63	38.01	39.39	36.63	39.39	29.75	1.39
IVDMD at 48 h.	42.73 [b]	42.73 [b]	42.73 [b]	42.73 [b]	50.54 [a]	34.92 [c]	1.43
IVOMD at 48 h.	47.65 [ab]	47.65 [ab]	50.41 [ab]	53.16 [a]	53.16 [a]	42.14 [b]	1.37
Gas production (GP) characteristic							
Lag time, h. [2]	0.47 [ab]	0.99 [a]	0.87 [ab]	0.56 [ab]	0.85 [ab]	0.32 [b]	0.19
b [3]	46.80 [ab]	46.73 [ab]	46.05 [ab]	43.60 [b]	47.75 [ab]	50.87 [a]	1.66
c	0.033 [a]	0.031 [a]	0.028 [ab]	0.030 [a]	0.023 [bc]	0.017 [c]	0.002
potential GP, ml/g [2]	45.14 [ab]	43.45 [b]	43.51 [b]	41.95 [b]	45.62 [ab]	50.04 [a]	1.90
Methane production, ml/ g DM [3]							
6 h. after incubation	0.50 [a]	0.26 [de]	0.23 [e]	0.35 [c]	0.44 [b]	0.31 [cd]	0.02
24 h. after incubation	8.06 [a]	7.59 [ab]	6.46 [b]	7.82 [ab]	6.63 [b]	6.56 [b]	0.21
48 h. after incubation	10.29 [ab]	10.76 [a]	9.65 [ab]	9.64 [ab]	9.41 [ab]	9.08 [b]	0.20
72 h. after incubation	12.16	12.23	11.37	11.19	10.86	11.29	0.29
Ammonia nitrogen (NH_3-N), mg%							
24 h. after incubation	7.32	7.32	8.32	7.65	7.32	9.65	0.32
48 h. after incubation	9.32 [a]	7.32 [b]	7.32 [b]	7.99 [ab]	8.98 [a]	9.32 [a]	0.28
Protozoa population, x10^4 cells/ml							
24 h. after incubation	1.06 [bc]	1.06 [bc]	1.06 [bc]	2.43 [a]	1.62 [b]	0.85 [c]	0.11
48 h. after incubation	0.93	1.06	1.06	0.93	0.68	0.68	0.05

[1] T1 = rice straw without supplement, T2 = rice straw with 0.2 % sulfur, T3 = rice straw with 0.2 % ammonium sulfate, T4 = rice straw with 0.2 % calcium sulfate, T5 = rice straw with 0.2 % copper sulfate, T6 = rice straw with 0.2 % sodium lauryl sulfate

[2] lag time (h) = (1/c)ln[b/(a + b)] (Chen et al., 2008)

[3] Methane (CH_4, ml/ g DM) = (HS + GP)CH4 %, HS = head-space, GP = gas production (Longo et al., 2006)

Table 2 Methane production reduction potential (%) with sulfur-containing compounds

Item	Time after incubation, h			
	6	24	48	72
Methane production reduction potential, % [1]				
sulfur	46.91 [b]	14.32 [a]	1.90 [b]	5.16
ammonium sulfate	52.81 [a]	12.33 [ab]	3.56 [b]	9.86
calcium sulfate	30.10 [d]	7.11 [b]	10.50 [a]	4.20
copper sulfate	11.74 [e]	12.05 [ab]	3.21 [b]	6.97
sodium lauryl sulfate	38.10 [c]	16.20 [a]	13.17 [a]	14.42
SEM	3.89	1.20	1.38	1.60

[1] Methane production reduction potential (MRP) was calculated by taking net methane values for the control (without supplement) as 100% (Javanegara et al., 2009)