DIETARY SUPPLEMENTATION OF OIL AND NON-PROTEIN NITROGEN TO MITIGATE METHANE EMISSIONS FROM GROWING CATTLE

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ABSTRACT

A two factorial experiment was carried out in three months (June to August, 2012) at the experimental station of Viet Nam National University of Agriculture to determine the effect of dietary supplementation with four different levels of sunflower oil (SFO) and two different kinds of non-protein nitrogen (NPN) on enteric methane emissions and performance of growing cattle. Twenty-four growing Lai Sind cattle (170 kg on average) were randomly divided into 8 blocks corresponding to 8 diets. Each diet includes 2% NaOH-treated rice straws and cassava leaf meal (1% BW - body weight, dry matter basis) as a basal diet supplemented with one of four SFO levels (1.5%, 3.0%, 4.5%, 6.0%) in combination with 4% calcium nitrate or 1.5% urea as NPN source supplement. Methane emissions was determined by using CH_4 to CO_2 ratio method. Results showed that methane emissions intensity (l/kg DMI - dry matter intake) was reduced by 26% when using nitrate supplement instead of urea supplement. The increase in oil level in the diet nonlinearly reduced methane emissions. The best level of SFO supplement was 3.0%. However, the best dietary treatment was supplementation with 4% calcium nitrate and 1.5% SF oil. It was also shown that the estimated energy losses as CH_4 emissions from the experiment diet ranged from 5-8% gross energy intake, compared with around 12% potential energy loss from diet without supplement. In conclusion, it is suggested that the diets of growing cattle should be supplemented with 4% calcium nitrate and 1.5% oil to mitigate methane emissions.

Keywords: Calcium nitrate, growing cattle, methane emission, sunflower oil.

Bổ sung dầu và nitơ phi protein vào khẩu phần để giảm phát thải khí mêtan của bò sinh trưởng

TÓM TẮT

Thí nghiệm hai nhân tố được tiến hành trong ba tháng (tháng Sáu đến tháng Tám năm 2012) tại trại thí nghiệm - Học viện Nông nghiệp Việt Nam để xác định ảnh hưởng của việc bổ sung vào khẩu phần 4 mức dầu hướng dương (SFO) và một trong hai loại nitơ phi protein (NPN) đến sự phát thải khí mêtan do lên men ở dạ cỏ và năng suất của bò sinh trưởng. 22 bò Lai Sind (khối lượng trung bình 170kg) được chia ngẫu nhiên vào 8 ô thí nghiệm tương ứng với 8 khẩu phần ăn. Mỗi khẩu phần ăn gồm khẩu phần cơ sở là rơm đã xử lý với 2% NaOH và bột lá sắn (1% BW - khối lượng cơ thể, tính theo vật chất khô). Khẩu phần cơ sở được bổ sung với một trong 4 mức SFO (1,5%, 3,0%, 4,5%, 6,0 %, tính theo vật chất khô) kết hợp với một trong hai loại NPN (hoặc 4% canxi nitrat hoặc 1,5%). Lượng phát thải khí mêtan được xác định bằng phương pháp sử dụng tỷ lệ CH_4/CO_2. Kết quả cho thấy cường độ phát thải khí metan (l/kg DMI - chất khô thu nhận) giảm 26% ở khẩu phần bổ sung canxi nitrat so với khẩu phần bổ sung urê. Tăng mức SFO trong khẩu phần làm giảm lượng phát thải mêtan một cách không tuyến tính. Mức bổ sung SFO tốt nhất là 3,0%. Tuy nhiên, tỷ lệ bổ sung kết hợp vào khẩu phần tốt nhất là 4% canxi nitrat và 1,5% SFO. Kết quả cũng chỉ ra rằng sự mất năng lượng dưới dạng CH_4 ở các khẩu phần thí nghiệm ước tính chỉ chiếm khoảng 5-8% năng lượng thô ăn vào, so với mức độ thất thoát khoảng 12% ở khẩu phần không bổ sung. Kết luận, khẩu phần của bò sinh trưởng nên được bổ sung với 4% canxi nitrat và 1,5% dầu hướng dương để giảm lượng phát thải khí mêtan.

Từ khóa: Canxi nitrat, dầu hướng dương, gia súc sinh trưởng, phát thải khí mêtan.

1. INTRODUCTION

Ruminants are one of the main sources of methane emissions to the atmosphere, contributing to greenhouse effect. Ruminants contribute about 22% of the total anthropic sources of methane in the world, or 80 Tg/year (USEPA, 2000). Methane production results from the digestive process of herbivore ruminants in the rumen, during anaerobic fermentation of soluble and structural carbohydrates, mainly in grass forage, and corresponds to an energy loss of around 6% (in temperate climate) or 10% (in tropical climate) of gross energy intake (USEPA, 2000).

Nevertheless, understanding the relationship between diets and enteric methane production is essential to reduce uncertainty in greenhouse gas emission inventories and to identify viable greenhouse gas reduction strategies. For cattle, reducing methane means an improvement in feed quality. Dietary changes can impact methane emissions by decreasing the fermentation of organic matter in the rumen, shifting the site of digestion from the rumen to the intestines, diverting H away from methane production during fermentation, or by inhibiting methanogenesis by rumen bacteria (Johnson and Johnson 1995; Benchaar et al 2001). Diets that restrict the hydrogen available in the rumen can make methane hygienic bacteria generating less enteric CH_4.

When rumen microorganisms ferment feed organic matter, they generate the reduced cofactor NADH which is in equilibrium with rumen H_2. In rumen, the H_2 generated during fermentation is normally removed by the reduction of CO_2 to form methane. Therefore, in order to reduce methane emission from rumen, one of the solution is that H_2 generated in the rumen need to be used in other pathways. Dietary supplementation of nitrate (NO_3^-) can be such that solution because it can act as an alternative hydrogen sink in the rumen. NO_3^- has a higher affinity for H_2 than CO_2. So when it is present, H_2 is first used in the reduction of NO_3^- to NO_2^- and then NO_2^- to NH_3 thereby reducing the production of methane from CO_2 (Ungerfeld and Kohn 2006). Zhou et al. (2011) reported that when rumen fluid of a Jersey cattle was incubated with sodium nitrate (12 mM) in vitro, methane production was reduced up to about 70% compared with the control.

Similar to nitrate, dietary addition of some plant oils rich in unsaturated fatty acids such as canola oil, coconut oil, linseed oil or sunflower oil can also reduce methane emissions from the ruminant because some microorganisms in the rumen can use H_2 to hydrogenate the double bonds of unsaturated fatty acids in this oils and therefore reduce the formation of methane in the rumen (Beauchemin et al., 2008). According to McGinn et al. (2004), the inclusion of sunflower oil to the diet of cattle resulted in 22% decrease of methane emissions.

So, providing nitrate and oil sources is expected to reduce methane production and emissions from ruminants. However, interaction effect of both nitrate and oil on the methane emissions of growing cattle is not well-documented, especially with typical cattle diets in Viet Nam.

2. MATERIALS AND METHODS

2.1. Location

The *invivo* experiment was done at the experimental station of Faculty of Animal Sciences, Viet Nam National University of Agriculture (FAS-VNUA).

2.2. Animals

Experiment involved 24 growing male cattle which have the weight of around 170 kg and age of around 12-15 months. Each young bull cattle was housed in a tie-stall to allow individual intake measurement and methane collection (Photo 1).

2.3. Experimental design

With regard to the objective of evaluating effect of oil and non-protein nitrogen (NPN) on methane emissions of growing cattle, the

Photo 1. Growing cattle involved in the experiment

Table 1. Levels of sunflower oil (SFO) and NPN supplement in the basal diet

Factor 1: SFO supplementation	Factor 2: non-protein nitrogen supplementation	
	1.5% Urea	4% Calcium nitrate
1.5%	D1	D3
3.0%	D2	D4
4.5%	D5	D7
6.0%	D6	D8

Note: D1÷ D8 are experimental diets supplemented with different levels of SFO and NPN source Diets

experiment followed a 2*4 factorial design (table 1) with calcium nitrate (4%DM) or urea (1.5% DM) as sources of NPN and 4 levels of sunflower oil (SFO) (1.5%, 3.0%, 4.5% and 6.0% DM). 24 growing cattle were blocked into 3 blocks with 8 cattle/block based on their body weight, age and sex. Then, the cattle in each block were randomly allocated to 8 treatments (8 diets). The experiment lasted for 4 weeks (one week for adaptation and 3 weeks for data collection).

Experimental diets were a representative for almost dairy systems, diets were thus formulated using main forages and by-products in northern Viet Nam. The basal diet included: 2% NaOH-treated rice straws *ad libitum* + cassava leaves at 1% body weight (BW) on dry matter (DM) basis. This basal diet was supplemented with different levels of SFO in combination with urea or calcium nitrate (table 1). The chemical compositions of the diets from 1 to 8 was presented in table 2.

2.4. Feed intake measurement

For each cattle, the daily forage and concentrate intake were individually determined. Forage refusals were weighed in next morning. Total DMI was calculated as the difference between the total amount of feeds offered and that refused, on DM basis.

Table 2. Chemical composition of experimental diets (%DM)

Diet	Supplement	Energy (*)	CP	NDF	ADF	ADL
D1	U1.5 O1.5	1883	10.2	60.1	42.5	4.72
D2	U1.5 O3.0	1929	10.1	59.7	42.2	4.66
D3	N4.0 O1.5	1869	10.0	59.3	42.0	4.70
D4	N4.0 O3.0	1890	9.9	59.3	41.9	4.64
D5	U1.5 O4.5	1969	10.0	59.3	41.9	4.62
D6	U1.5 O6.0	2021	9.9	59.0	41.6	4.56
D7	N4.0 O4.5	1948	9.9	58.6	41.4	4.63
D8	N4.0 O6.0	1995	9.7	58.2	41.1	4.57

Note: (*) kcal ME/kg, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin

2.5. Feed sampling

Approximately 500 g on a fresh matter basis of each ingredient was collected every methane estimating day. Samples were then dried in an oven at 70°C for 48 h, grounded into a 1 mm screen CYCLOTEC and stored in closed plastic boxes at room temperature prior to chemical analyses.

2.6. Chemical analysis

Chemical composition of each feed (ash, CP, NDF, ADF, ADL, cellulose, hemicellulose, starch and sugar) was predicted according to a large NIRS database and equations for tropical and temperate forages from Gembloux (Belgium) and CIRAD (France) databases. Chemical analysis was carried out at laboratories of FAS-VNUA.

2.6. Gas measurement and methane emissions estimation

Calculation of actual methane emissions: The total methane emissions was calculated for each cow using the equation developed by Madsen et al. (2010) as follow:

CH_4 produced (l/d) = a * (b-d)/(c-e)

where:

a is CO_2 produced by the animal, l/day

b is the concentration of CH_4 in air mix, ppm

c is the concentration of CO_2 in air mix, ppm

d is the concentration of CH_4 in background air, ppm

e is the concentration of CO_2 in background air, ppm.

The CH_4 production was estimated as shown above, based on known/calculated CO_2 production by the animal(s), measured background concentration (outdoor concentration representing atmospheric air) of CH_4 and CO_2, and measured concentration of CH_4 and CO_2 in an air sample containing a mixture of air from background and gases excreted from the animal (Photo 2). The air samples were collected two days at the end of the experiment and then measured for CH_4 and CO_2 by Gas chromatography: GC17A, Detector FID.

Photo 2. Gas collection for CO_2 and CH_4 determination

Estimation of potential methane emission: The total methane production was estimated using the equation developed by Moe and Tyrell (1979): CH_4 l/day = 86.1 + 67.0*C + 43.9*H + 12.9 * S (C: Cellulose; H: Hemicellulose; S: Starch and Sugar in kg ingested/day on DM basis).

2.7. Statistical analysis

The data were analysed using the General Linear Model option in the ANOVA program of SAS system Software (version 8.0).

3. RESULTS AND DISCUSSIONS

3.1. Feed intake

The effect of NPN source and oil level on diet intakes are shown in table 3. Results showed that nitrate supplement significantly increased DM, CP, NDF and ADF intakes compared with urea supplement. In fact, the nitrate supplement increased intake by 8%, 5%, 6% and 6% for DM, CP, NDF and ADF, respectively. This could be explained by low degradation of nitrate and therefore more efficient nitrogen utilization of rumen microbes in the rumen. Faverdin (2003) and Hoover & Stokes (1991) suggested that the efficiency of protein use depended on protein sources and their degradation rates. A rapidly degradable protein could be underutilized because the rumen microbes could not, at the same time, depose enough energy issued from the carbohydrate fermentation process. Hence, the exceeded nitrogen could provoke digestive disorder or metabolisable troubles (uraemia) and/or reduce microbial activities considerably. The nitrogen lowly reduced from nitrate is thus more important than from urea because nitrate provides the nitrogen source to microbes at the same time as the carbohydrates are fermented.

Results showed, on the other hand, that no effect of oil supplement on intake was found for all variables. Beauchemin et al. (2008) assumed that most forages have some fat content and that DMI may be suppressed at fat intakes of above 6 to 7%, and CH_4 mitigation of 10-25% was possible from an addition of dietary oils to diets of ruminants. Machmuller et al. (2000) reported that oils offer a practical approach to reducing methane in situations where animals can be given daily feed supplements, but excess oil was detrimental to fibre digestion and productions. Oils may act as hydrogen sinks but medium chain length oils appeared to act directly on methanogens and reduced numbers of ciliate protozoa. In contrast, Johnson et al. (2002; 2008) found no responses to diets containing 2.3, 4.0 and 5.6% fat (cottonseed and canola) fed to lactating cows. So, the present results were similar to those found by Johnson et al. (2002; 2008).

Concerning the interaction effect of both NPN and oil supplement on intake, the higher intake was found for diets containing 4% nitrate. The highest and lowest DM intake were found for diet containing 4% nitrate plus 4.5% oil (3.36% BW) and 1.5% urea plus 6.0% oil (2.83% BW). However, the best level of CP, NDF and ADF intakes seemed to be diets containing 4% nitrate plus 1.5% oil (554 g CP, 3290 g NDF and 2329 g ADF per day). As explained above, nitrate was more important than from urea due to its low rate of reduction to ammonia and suitable level of oil supplement enhanced fibre digestion.

3.2. Effect of non-protein nitrogen sources on methane emissions

Effect of NPN source on methane emissions was shown in table 4. Results show that nitrate significantly reduced methane emissions by 22 and 24% for total methane emissions (117 vs 147 L/day) and for methane emissions rate (22 vs 29 L/kg DMI or 37 vs 49 L/kg NDFi – neutral detergent fibre intake) compared with urea. Normally, methane emissions increased with the level of intake (Giger-Reverdin et al., 2000). However, in this case, diet supplemented with nitrate had higher intake emitted lower methane. So, this illustrated the strong effect of nitrate on methane emissions.

Table 3. Effect of NPN sources and oil levels on feed intake

Variables	Dry mater		Protein *(g/day)*	NDF *(g/d)*	ADF *(g/d)*
	(kg/day)	*(%BW)*			
NPN sources					
Urea (1.5%)	5.04 ± 0.28	2.98 ± 0.20	507.31 ± 31.20	2997.50 ± 167.10	2116.30 ± 121.4
Nitrate (4%)	5.42 ± 0.23	3.18 ± 0.19	534.09 ± 24.40	3183.10 ± 130.70	2251.10 ± 94.90
p-value	*> 0.001*	*0.002*	*0.004*	*> 0.001*	*> 0.001*
Oil levels					
1.5%	5.35 ± 0.24	3.20 ± 0.15	539.95 ± 19.83	3193.40 ± 124.2	2258.60 ± 90.20
3.0%	5.11 ± 0.14	2.92 ± 0.09	512.06 ± 15.14	3044.00 ± 83.40	2150.10 ± 60.60
4.5%	5.31 ± 0.34	3.24 ± 0.19	527.53 ± 30.63	3126.90 ± 178.7	2210.20 ± 129.8
6.0%	5.15 ± 0.39	2.95 ± 0.22	506.80 ± 38.00	3015.80 ± 214.6	2129.60 ± 155.9
p-value	*ns*	*ns*	*ns*	*ns*	*ns*
Interactions					
U1.5 O1.5	5.15 ± 0.15	3.08 ± 0.09	525.87 ± 15.25	3096.90 ± 81.70	2188.50 ± 59.30
U1.5 O3.0	5.07 ± 0.15	2.88 ± 0.09	512.54 ± 15.82	3025.50 ± 84.70	2136.70 ± 61.60
U1.5 O4.5	5.07 ± 0.27	3.11 ± 0.17	509.10 ± 28.30	3007.00 ± 151.8	2123.20 ± 110.2
U1.5 O6.0	4.93 ± 0.42	2.83 ± 0.24	489.70 ± 44.00	2903.10 ± 235.5	2047.70 ± 171.1
N4.0 O1.5	5.55 ± 0.12	3.31 ± 0.07	554.03 ± 12.52	3289.90 ± 67.10	2328.60 ± 48.70
N4.0 O3.0	5.16 ± 0.14	2.95 ± 0.08	511.58 ± 16.86	3062.50 ± 90.30	2163.50 ± 65.60
N4.0 O4.5	5.55 ± 0.20	3.36 ± 0.13	545.96 ± 21.10	3246.70 ± 113.0	2297.30 ± 82.10
N4.0 O6.0	5.38 ± 0.22	3.07 ± 0.13	523.92 ± 23.15	3128.60 ± 124.0	2211.50 ± 90.00
p-value	*0.001*	*0.001*	*0.009*	*0.002*	*0.002*

Note: U1.5 is 1.5% urea level (on DM basic); N4.0 is 4.0% calcium nitrate level (on DM basic); O1.5, O3.0, O4.5 and O6.0 are 1.5%, 3.0%, 4.5% and 6.0% oil level (on DM basic)

Ascensão (2010) found nitrate diets produced less methane (expressed by g/kg of DMI) than urea diet (P > 0.001). Methane production (g/day) of bulls fed nitrate diets was 41.6% lower than that from bulls fed urea diets (P > 0.001). Methane production (% gross energy intake - GEI) was 5.6% for urea diet and 3.1% for nitrate diets, resulting in a production of less 41.1% with nitrate diet compared with urea diet (P > 0.001). According to Leng (2008), nitrate reduction in anaerobic systems occurred by two distinct pathways: dissimilatory nitrate reduction to ammonia and assimilatory nitrate reduction to ammonia. And NO_3 had a higher affinity for H_2 than CO_2 and, when it is present, H_2 was first used in the reduction of NO_3 to NO_2 and NO_2 to NH_3 thereby reducing the production of methane from CO_2. In fact, 1 mol of nitrate would produce 1 mol of ammonia and reduce methane production by 1 mol. As a consequence, nitrate diet strongly reduced methane emissions compared with urea in our study.

3.3. Effect of oil levels on methane emissions

Effect of oil levels on methane emissions was shown in table 5. Results showed that cattle fed the diets supplemented sunflower oil at levels of 3.0% and 6.0% seems to have lowest level and intensity of methane emission. However, the differences was not statistically different (p > 0.05).

Table 4. Main statistics of methane emissions by different NPN supplement

NPN source	Total methane emission (l/day)	Methane emission rate	
		(l/kg DMI)	(l/kg NDFi)
Urea (1.5%)	147.15 ± 23.12	29.14 ± 3.96	48.99 ± 6.39
Nitrate (4%)	116.85 ± 6.87	21.60 ± 1.53	36.77 ± 2.67
p-value	> 0.001	> 0.001	> 0.001

Table 5. Main statistics of methane emissions by oil supplement

Oil level	Total methane emissions	Methane emission rate	
	(l/day)	(l/kg DMI)	(l/kg NDFi)
1.5%	144.80 ± 42.00	27.37 ± 8.91	45.75 ± 14.60
3.0%	124.48 ± 4.36	24.35 ± 0.90	40.91 ± 1.46
4.5%	136.51 ± 19.09	25.93 ± 4.65	43.94 ± 7.57
6.0%	123.98 ± 9.27	24.16 ± 2.15	41.23 ± 3.37
p-value	ns	ns	ns

According to Machmuller et al (2000), oils may be acted as hydrogen sinks an can reduce methane emission but too much oil was detrimental to fibre digestion and productions. But in this experiment, the different levels of oil supplementation from 1.5 to 6% did not affect level of methane emission (table 5) and also did not affect nutrient intake (table 3). Therefore, in further research should consider higher level of sunflower oil supplementation.

3.4. Interaction effect of NPN & oil on methane emissions

With regard to the best combination of NPN and oil supplement in diets, data were analysed for all combination to provide values of total and rate of methane emissions. Data in Table 6 showed that total methane emissions ranged from 119 l/day (4% nitrate + 6.0% oil diet) to 184 l/day (1.5% urea + 1.5% oil diet). However, the lowest methane emissions rate, expressed by l/kg DMI and l/kg NDFi), was found with the diet containing 4% nitrate + 1.5% oil (19 l/kg DMI and 32 l/kg NDFi). As a consequence, this combination seemed to be the best one in terms of methane reduction (Table 3).

Table 6. Main statistics of methane emissions by non-protein nitrogen and oil supplement interaction

Interactions	Total methane emissions (l/day)	Methane emissions rate	
		(l/kg DMI)	(l/kg NDFi)
U1.5 O1.5	183.97 ± 5.01	35.71 ± 0.04	59.40 ± 0.05
U1.5 O3.0	127.32 ± 3.69	25.13 ± 0.02	42.08 ± 0.04
U1.5 O4.5	153.92 ± 7.58	30.38 ± 0.14	51.19 ± 0.07
U1.5 O6.0	129.06 ± 10.2	26.22 ± 0.18	44.46 ± 0.09
N4.0 O1.5	105.60 ± 2.27	19.04 ± 0.00	32.10 ± 0.03
N4.0 O3.0	121.64 ± 3.05	23.57 ± 0.53	39.73 ± 1.13
N4.0 O4.5	119.11 ± 4.14	21.48 ± 0.04	36.69 ± 0.00
N4.0 O6.0	118.90 ± 4.77	22.11 ± 0.03	38.00 ± 0.02
p-value	*> 0.001*	*> 0.001*	*> 0.001*

Table 7. Comparison of energy loss from estimated and measured methane emissions

Variables	Total methane emissions (l/day)		Methane emissions rate (l/kg DMI)		Energy loss (%)	
	Actual	*Moe and Tyrel*	*Actual*	*Moe and Tyrel*	*Actual*	*Moe and Tyrel*
NPN sources						
Urea (1.5%)	147.15 ± 23.12	266.56 ± 10.10	29.14 ± 3.96	52.93 ± 1.10	6.81 ± 0.986	12.36 ± 0.28
Nitrate (4%)	116.85 ± 6.87	277.77 ± 7.90	21.60 ± 1.53	51.29 ± 0.80	5.09 ± 0.35	12.10 ± 0.28
p-value	*> 0.001*	*ns*	*> 0.001*	*ns*	*> 0.001*	*ns*
Oil levels						
1.5%	144.80 ± 42.00	278.39 ± 7.50	27.37 ± 8.91	52.08 ± 0.99	6.52 ± 2.10	12.42 ± 0.19
3.0%	124.48 ± 4.36	269.37 ± 5.04	24.35 ± 0.90	52.68 ± 0.55	5.76 ± 0.16	12.47 ± 0.14
4.5%	136.51 ± 19.09	274.37 ± 10.80	25.93 ± 4.65	51.78 ± 1.31	6.06 ± 1.06	12.11 ± 0.26
6.0%	123.98 ± 9.27	267.66 ± 12.97	24.16 ± 2.15	52.08 ± 1.65	5.59 ± 0.47	12.05 ± 0.33
p-value	*ns*	*ns*	*ns*	*ns*	*ns*	*0.001*
Interactions						
U1.5 O1.5	183.97 ± 5.01	272.56 ± 4.94	35.71 ± 0.04	52.91 ± 0.54	8.48 ± 0.014	12.26 ± 0.10
U1.5 O3.0	127.32 ± 3.69	268.25 ± 5.12	25.13 ± 0.02	52.96 ± 0.57	5.90 ± 0.01	12.49 ± 0.16
U1.5 O4.5	153.92 ± 7.58	267.13 ± 9.17	30.38 ± 0.14	52.75 ± 1.05	7.07 ± 0.03	11.94 ± 0.15
U1.5 O6.0	129.06 ± 10.22	260.85 ± 14.23	26.22 ± 0.18	53.08 ± 1.74	6.04 ± 0.03	11.88 ± 0.16
N4.0 O1.5	105.60 ± 2.27	284.22 ± 4.05	19.04 ± 0.00	51.25 ± 0.38	4.56 ± 0.00	12.57 ± 0.14
N4.0 O3.0	121.64 ± 3.05	270.49 ± 5.46	23.57 ± 0.53	52.41 ± 0.43	5.61 ± 0.07	12.45 ± 0.14
N4.0 O4.5	119.11 ± 4.14	281.61 ± 6.83	21.48 ± 0.04	50.80 ± 0.61	5.05 ± 0.01	12.29 ± 0.24
N4.0 O6.0	118.90 ± 4.77	274.48 ± 7.49	22.11 ± 0.03	51.07 ± 0.72	5.14 ± 0.01	12.22 ± 0.38
p-value	*> 0.001*	*ns*	*> 0.001*	*ns*	*> 0.001*	*ns*

3.5. Energy loss from estimated and measured methane emissions

Typically, about 6 to 10% of GEI by ruminants was converted to CH_4 and released via the breath (Brouwer, 1965). Johnson et al. (1993) found that the energy loss from methane varied from approximately 2 to 12% GEI depending on diet quality.

Estimation of energy loss from enteric methane emissions in the present study was presented in Table 7. Results showed that the energy loss due to methane emissions from the diet without supplement, as estimated by Moe and Tyrel equation (1979) varied around 12% of GEI. But the energy loss from diet supplemented with NPN and oil was strongly reduced by 33-62% (52% on average), lowest in diet containing 4% nitrate + 1.5% oil (only 4.56%, 62% reduction) and highest in diet containing 1.5% urea + 1.5% oil (8.5%, 33% reduction).

There were big differences between the level and intensity of methane emissions estimated by the equation of Moe and Tyrell (1979) and the corresponding values measured by the methods of Madsen et al. (2010). The estimated values by equation of Moe and Tyrell (1979) almost double the actual values measured by method of Madsen et al. (2010). The method of Madsen et al. (2010) is an accurate method to measure methane emissions which has been applied and improved by many studies (Huhtanen et al., 2015, Haque et al., 2014). Thus, the differences here can be because the equation of Moe and Tyrell (1979) only estimates the amount of methane emissions via the chemical compositions of the feeds. Therefore, it seems that the equation of Moe and Tyrell (1979) might not reflect the real values. However, this should be clarified by the further experiments.

4. CONCLUSIONS

The supplementation of nitrate significantly increased DM intake (by 8%) and reduced efficiently methane emissions (by 22-24%) compared with urea supplementation. Increasing oil levels in diets unlinearly decreased methane emissions . However, supplementation of both nitrate and sunflower oil in diets reduced methane emissions by 33-62% compared with methane emissions estimated by Moe and Tyrell equation. The best level of supplement combination for methane reduction was 4% nitrate + 1.5% oil. These findings are significant for cattle feeding for contributing to reduce seriousness of global warming.

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