ISSN 1819-1878

Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2018.1.8

Research Article
Interaction Between Nitrate and Sunflower Oil on Feed Intake, Rumen Methane Production and Microbial Population in Goats

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Abstract

Background and Objective: Dietary manipulation can decrease rumen CH_4 production by competing for hydrogen utilization or inhibiting methanogenesis. The combination of ingredients, such as sunflower oil and nitrate may reduce CH_4 production and increasing efficiency of N utilization in meat goats fed rice straw. This study was aimed to study the interaction between nitrate and sunflower oil on feed intake, rumen production and microbial population in goats. **Materials and Methods:** Eight male rumen-fistulated 50% Anglo-nubian and 50%, Thai native goats with an initial body weight of 28.8±5 kg (Mean±SD) were used in this study. Goats were randomly assigned according to a 2×2 factorial arrangement in a 4×4 Latin square design to investigate the effect of the potassium nitrate (2 and 3% KNO_3 concentrate) and sunflower oil (3 and 6% Sunflower oil concentrate) levels on feed intake, rumen production and microbial population. **Results:** There was no interaction between the levels of potassium nitrate (KNO_3) and sunflower oil on any parameters. Voluntary feed intake, nutrient digestibility and nitrogen utilization were not influenced by the SFO and KNO_3 levels. An increased SFO level significantly increased ($p<0.05$) NH_3-N, propionate and CH_4 production, but an increased SFO level significantly decreased ($p<0.05$) the copies of *R. albus*, *P. bryantii* and *P. ruminicola* per millilitre of rumen fluid. An increased KNO_3 level significantly increased ($p<0.05$) propionate in the rumen but significantly decreased ($p<0.05$) the C_3: C_4 ratio and CH_4 production. Copies of Archaea *mcrA* were significantly increased ($p<0.05$) with increasing KNO_3 levels. **Conclusion:** This study discovered the feeding the combination between nitrate and sunflower oil can be beneficial to reduce CH_4 production by goats fed rice straw. So that this study would be pointed out that ruminant fed low quality roughages can be improved by combining nitrate and sunflower oil.

Key words: Sunflower oil, nitrate, nutrient digestibility, rumen microorganisms, meat goat, inhibiting methanogenesis

Citation: J. Khotsakdee, C. Yuangklang, S. Boonanuntanasar, S. Paengkoum and P. Paengkoum, 2018. Interaction between nitrate and sunflower oil on feed intake, rumen methane production and microbial population in goats. Asian J. Anim. Sci., 12: 1-8.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Methane (CH_4) emission from enteric fermentation of ruminants is responsible for global warming and CH_4 generated by livestock contributes up to 40% of greenhouse gas (GHG) emissions[1]. The CH_4 is also a key factor that indicates inefficient energy utilization. Thus, there have been many attempts to decrease CH_4 production from ruminants, including CH_4 vaccination, dietary manipulation and dietary management. Dietary manipulation that can decrease organic matter (OM) fermentation in the rumen may help mitigate CH_4 emission from ruminants[2]. Counteracting methanogenesis or competing for Hydrogen (H) utilization from CH_4 production may decrease CH_4 emission.

Fats and oils are supplemented in ruminant diets to enhance energy density and to manipulate ruminal fermentation. The use of oils, such as soybean oil (SO), sunflower oil (SFO) and fish oil (FO), modifies the biohydrogenation process, thereby managing rumen fermentation end products. The SFO a high polyunsaturated fat, has been demonstrated to reduce CH_4 production[3]. Non-protein nitrogen is generally used to increase nitrogen (N) in ruminant diets due to it is inexpensive and high contents of urea, nitrate (NO_3), nitrite. In addition, supplemental NO_3 has been reported to reduce CH_4 production. The mode of action of NO_3 induced decreases in CH_4 production may be associated with nitrate reduction during the fermentation process[4-7]. NO_3 is an alternative electron acceptor that can be modified to ammonia (NH_3) via nitrite reduction[8,9], which supplies N for microbial growth[10-14].

Based on the papers above, the combination of CH_4 and SFO could be potentially inhibited CH_4 production and then improved rumen fermentation and product. Therefore, the objectives of current study were to study the effect of SFO and NO_3 level in concentrate on feed intake, nutrient digestibility and rumen microorganisms of meat goats fed rice straw as the main roughage source.

MATERIALS AND METHODS

Animals and treatments: Eight male rumen-cannulated Anglo-Nubian×Thai native crossbred goats with a mean body weight (BW) of 28.8±5 kg were used. This study had the following two factors: factor A was SFO level (3 and 6% in concentrate diet) and factor B was potassium KNO_3 (3 and 6% in concentrate diet). Treatment combinations were as follows: 3% SFO+3% KNO_3 (T1); 3% SFO+6% KNO_3 (T2); 6% SFO+3% KNO_3 (T3) and 6% SFO+6% KNO_3 (T4). The composition of dietary treatments and rice straw used was shown in Table 1. The trial had a 2×2 factorial arrangement in a 4×4 Latin

Table 1: Ingredient and chemical composition of diet in the experiment (g kg^{-1} DM)

	3% SFO[B]		6% SFO[C]		
	2% KNO_3[D]	3% KNO_3[E]	2% KNO_3[D]	3% KNO_3[E]	Rice straw
Ingredient (%) dry matter					
Cassava chip	37.50	38.60	33.50	35.60	-
Soybean meal	14.00	14.00	14.00	14.00	-
Corn distillers dried grains	14.00	12.00	15.00	12.00	-
Rice bran	10.00	10.00	10.00	10.00	-
Wheat bran	10.00	10.00	10.00	10.00	-
Molasses	7.00	7.00	7.00	7.00	-
Potassium nitrate	2.00	3.00	2.00	3.00	-
Urea	0.20	0.10	0.20	0.10	
sunflower oil	3.00	3.00	6.00	6.00	-
Mineral and vitamin mixture[A]	2.30	2.30	2.30	2.30	-
Total	100.00	100.00	100.00	100.00	
Dry matter	92.35	93.15	92.2	92.15	91.80
	Dry matter basis (%)				
Chemical composition					
Ash	7.14	7.41	6.12	6.14	12.30
Crude protein	15.22	15.29	15.33	15.37	3.30
Ether extract	3.12	3.17	6.25	6.15	1.05
Neutral detergent fiber	43.25	45.26	38.27	38.26	75.12
Acid detergent fiber	17.65	18.25	16.3	15.65	53.50
Acid detergent lignin	17.95	18.75	7.95	6.89	12.07
Hemicellulose	25.30	26.51	21.97	22.61	21.62
Cellulose	9.06	8.86	8.35	8.76	41.43

[A]Mineral and vitamin mix: Provided per kg of concentrate including Vitamin A: 5000 IU, Vitamin D_3: 2,200 IU, Vitamin E: 15 IU, Ca: 8.5 g, P: 6 g, K: 9.5 g, Mg: 2.4 g, Na: 2.1 g, Cl: 3.4 g, S: 3.0 g, Co: 0.16 mg, Cu: 100 mg, I: 1.3 mg, Mn: 64 mg, Zn: 64 mg, Fe: 64 mg, Se: 0.45 mg, [B]3% SFO: 3% of sunflower oil in diets, [C]6% SFO: 6% of sunflower oil in diets, [D]2% KNO_3: 2% of potassium nitrate in concentrate diets, [E]3% KNO_3: 3% of potassium nitrate in concentrate diets

Table 2: PCR primers for real-time PCR assay

Target	Forward (F)/revere (R)	Primer sequence (5'-3')	Annealing temperature (°C)	References
Total protozoa	F	CTTGCCCTCYAATCGTWCT	55	Sylvester *et al.*[20]
	R	GCTTTCGWTGGTAGTGTATT		
Total bacterial	F	CGGCAACGAGCGCAACCC	60	Denman and McSweeney[21]
	R	CCATTGTAGCACGTGTGTAGCC		
Total fungi	F	GAGGAAGTAAAGTCGTAACAAGGTTTC	60	Denman and McSweeney[21]
	R	CAAATTCACAAAGGGTAGGATGATT		
Fibrobacter succinogenes	F	GTTCGGAATTACTGGGCGTAAA	60	Denman and McSweeney[21]
	R	CGCCTGCCCCTGAACTATC		
Ruminococcus flavefaciens	F	CGAACGGAGATAATTTGAGTTTACTTAGG	60	Denman and McSweeney[21]
	R	CGGTCTCTGTATGTTATGAGGTATTACC		
Ruminococcus albus	F	CCC TAA AAG CAG TCT TAG TTCG	60	Koike and Kobayashi[22]
	R	CCTCCTTGCGGTTAGAACA		
Prevotella bryantii	F	AGTCGAGCGGTAAGATTG	68	Tajima *et al.*[23]
	R	CAAAGCGTTTCTCTCACT		
Prevotella ruminicola	F	GGTTATCTTGAGTGAGTT	53	Tajima *et al.*[23]
	R	CTGATGGCAACTAAAGAA		
Selenomonas ruminantium	F	TGCTAATACCGAATGTTG	57	Tajima *et al.*[23]
	R	TCCTGCACTCAAGAAAGA		
Methanogens	F	TTC GGT GGA TCD CAR AGR GC	56	Denman *et al.*[24]
	R	GBA RGT CGW AWC CGT AGA ATC C		

square design. Animals were fed twice daily at 08.00 am and 17.00 pm. All goats were fed *ad libitum* with rice straw water and mineral salt block. Prior to the experiment, goats were dewormed by means of *Ivomectin* (IVOMEC F plus, Bangkok, Thailand) and injected with vitamins A (500,000 I.U.), D3 (75,000 I.U.) and E (50 I.U.) (Biotecnocem, Dallas, USA). Goats were individually housed in pens (60×120×90 cm). The study consisted of 4 periods of 28 days each consisting of 21 days of adjustment followed by 7 days for measurements. During the last 7 days, rumen fluid and blood were collected. Body weights were measured at the last day of each period. The experimental protocol was complied with the Thailand Code for the Care and Use of Animals for Scientific Purposes with the Project code SUT 4/2558 Certificate U1-02632-2559. The experiment was conducted at Suranaree University of Technology Farm during August to December, 2016.

Data collection and sampling procedures: Rice straw and concentrate samples were collected daily during the collection period and were combined prior to analyses. Composite samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for contents of dry matter, ether extract, ash, crude protein AOAC[15], neutral detergent fiber (NDF) and acid detergent fiber (ADF) Goering and Van Soest[16]. The rumen fluid samples were collected at 0, 3 and 6 h post-feeding. Rumen fluid was immediately measured for pH (HANNA instrument HI 8424 microcomputer). The filtrates were divided into two portions. The first portion was used for ammonia nitrogen (NH_3-N) analyses using the micro Kjeldahl method and Volatile fatty acids (VFA) was measured by gas chromatography (GC) analysis[17]. The second portion of rumen fluid and digesta were used for DNA extraction for use in real-time PCR using primers provided in Table 2. A blood sample was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 5,000×g for 10 min and stored at -20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker[18]. The calculation of ruminal CH_4 production was based on VFA proportions according to Moss *et al.*[19] as follows:

$$CH_4 \text{ production} = 0.45 \text{ (acetate)} - 0.275 \text{ (propionate)} + 0.4 \text{ (butyrate)}$$

Statistical analysis: All data obtained from the experiment were subjected to ANOVA for 4×4 Latin square design with a 2×2 factorial arrangement of treatments consisting of two levels of SFO and two levels of KNO_3 of the Statistical Analysis System Institute (SAS)[25]. Treatment means were compared by Duncan's New Multiple Range Test (DMRT) Steel and Torrie[26]. Differences among means with $p<0.05$ were accepted as statistically significant differences. The variation is given as standard error of the least square means (SEM).

The model was:

$$Y_{ijk} = \mu + \rho i + \alpha j + \beta k + \alpha\beta jk + \varepsilon ijk$$

Where:

Y_{ijk} = Dependent variable

μ = Overall mean

ρi = Effect of period
αj = Effect of factor A (SFO and KNO_3)
βk = Effect of factor B (level of SFO and KNO_3)
$\alpha\beta jk$ = Interaction AB between SFO and KNO_3
εijk = Experimental error for ijk on the observation

RESULTS AND DISCUSSION

Voluntary feed intake and apparent digestibility of nutrients: When expressed as g day^{-1}, BW (%) and g kg^{-1} $BW^{0.75}$, feed intake was not significantly different according to levels of SFO and KNO_3 and there was also no interaction between the level of SFO and level of KNO_3 with regard to feed intake. Li *et al.*[12] observed no difference in feed intake in lactating goats when the diets were supplemented with safflower oil or linseed oil. Pal *et al.*[27] reported that NO_3 fed to sheep at 2% of the concentrate mixture does not reduce feed intake, which is low for exhibiting any adverse effect on intake. Other studies[6] have shown that 2.6% NO_3 and 4% KNO_3. Nolan *et al.*[28] in sheep diets do not affect Dry matter intake (DMI). The apparent digestibilities of dry matter (DDM), OM, ether extract (EE), NDF and ADF were not significantly different according to levels of SFO and KNO_3 and there was also no interaction between the level of SFO and level of KNO_3 with regard to the DDM of nutrients (Table 3). Mewara *et al.*[29] also reported that digestibility of DM, Crud protein (CP) and fibre components is unaffected by added oil but that EE is increased by the addition of SFO to the concentrate mixture.

Nitrogen utilization: Data in Table 4 showed the N utilization of goats fed different levels of SFO and KNO_3. N intake, N excretion (urine and faeces), N absorption and N retention were not significantly different according to levels of SFO and KNO_3 ($p>0.05$) and there was also no interaction between the level of SFO and level of KNO_3 with regard to N utilization ($p>0.05$). These results contrast those reported by Doranalli and Mutsavangwa[30], who found that diets supplemented with SFO improve N retention in sheep compared to non-supplemented diets.

Ruminal fermentation end products: Ruminal pH, total volatile fatty acids (TVFAs), butyrate concentration and bacterial count were not significantly different ($p>0.05$) according to the level of SFO and KNO_3 or their interaction presented in Table 5. Alaboudi and Jones[31] observed that nitrate and nitrite quickly clear from rumen fluid within 3 h and that the molar proportion of VFAs quickly returns to the pre-nitrate levels. The increasing level of NO_3 had no influence on TVFAs. The proportion of acetate and the acetate (C_2)/propionate (C_3) ratio tended to increase and the proportion of C_3 tended to be less for the NO_3 diet[7-9-12]. NH_3-N concentration was decreased ($p<0.05$) with increasing levels of KNO_3. There was no interaction between the level of SFO and KNO_3 with regard to C_2 concentration. Increasing levels of SFO decreased ($p<0.05$) the C_3 concentration, while increasing levels of KNO_3 increased ($p<0.05$) the C_3 concentration. Increasing levels of SFO decreased ($p<0.05$) the C_3 concentration, while increasing levels of KNO_3 increased

Table 3: Effect of dietary treatment on feed intake and digestibility of nutrients in meat goats

	3% SFO		6% SFO			Contrast		
	2% KNO_3	3% KNO_3	2% KNO_3	3% KNO_3	SEM	SFO	KNO_3	SFO×KNO_3
Voluntary dry matter intake								
g day^{-1}	838.00	881.00	886.00	849.25	12.760	0.756	0.905	0.144
BW (%)	2.73	2.83	2.79	2.68	0.025	0.401	0.826	0.056
$BW^{0.75}$ (g kg^{-1})	64.34	66.83	66.34	63.58	0.550	0.579	0.903	0.053
Roughage intake								
g day^{-1}	492.75	518.50	521.75	499.50	7.660	0.750	0.911	0.144
BW (%)	1.60	1.66	1.64	1.57	0.015	0.395	0.837	0.051
$BW^{0.75}$ (g kg^{-1})	37.75	39.50	39.00	37.50	0.344	0.596	0.859	0.036
Concentrate intake								
g day^{-1}	345.25	362.50	364.25	349.75	5.102	0.765	0.895	0.146
BW (%)	1.125	1.165	1.152	1.102	0.011	0.429	0.819	0.057
$BW^{0.75}$ (g kg^{-1})	26.50	27.49	27.28	26.18	0.220	0.560	0.896	0.053
Apparent digestibility (%)								
Dry matter	74.46	75.23	75.76	75.35	0.305	0.266	0.770	0.351
Organic matter	76.99	77.99	78.26	77.88	0.292	0.267	0.719	0.331
Ether extract	67.87	69.20	56.62	54.31	3.220	0.665	0.940	0.783
NDF[A]	67.79	68.83	63.44	64.87	0.302	0.186	0.704	0.209
ADF[B]	59.48	58.69	56.55	55.65	0.565	0.991	0.892	0.962

3% SFO: 3% of sunflower oil in concentrate diets, 6% SFO: 6% of sunflower oil in concentrate diets, 2% KNO_3: 2% of potassium nitrate in concentrate diets, 3% KNO_3: 3% of potassium nitrate in concentrate diets, SFO: Effect of sunflower oil, KNO_3: Effect of KNO_3, SFO×KNO_3: Interaction between SFO×KNO_3, SEM: Standard error of the mean, [A]NDF: Neutral detergent fiber, [B]ADF: Acid detergent fiber

Table 4: Effect of dietary treatment on nitrogen balance

	3% SFO		6% SFO			Contrast		
	2% KNO_3	3% KNO_3	2% KNO_3	3% KNO_3	SEM	SFO	KNO_3	SFO×KNO_3
Nitrogen intake (g)	11.05	11.61	11.67	11.19	0.170	0.766	0.906	0.155
Nitrogen excretion (g)								
Fecal	4.62	4.61	4.61	4.63	0.090	0.957	0.978	0.935
Urine	3.22	3.00	3.25	3.24	0.127	0.608	0.663	0.683
Nitrogen absorption (g)	6.42	6.99	7.05	6.56	0.137	0.731	0.890	0.076
Nitrogen retention (g)	3.20	3.99	3.79	3.31	0.162	0.904	0.640	0.072
Nitrogen absorption (%)	58.27	60.09	60.40	58.62	0.652	0.805	0.988	0.193
Nitrogen retention (%)	29.11	34.15	32.43	29.54	1.165	0.786	0.652	0.114

3% SFO: 3% of sunflower oil in concentrate diets, 6% SFO: 6% of Sunflower oil in concentrate diets, 2% KNO_3: 2% of Potassium nitrate in concentrate diets, 3% KNO_3: 3% of Potassium nitrate in concentrate diets, SFO: Effect of Sunflower oil, KNO_3: Effect of KNO_3, SFOxKNO_3: Interaction between SFO x KNO_3, SEM: Standard error of the mean

Table 5: Effect of dietary treatment on rumen ecology and fermentation characteristics in meat goats

	3% SFO		6% SFO			Contrast		
	2% KNO_3	3% KNO_3	2% KNO_3	3% KNO_3	SEM	SFO	KNO_3	SFO×KNO_3
Ruminal pH	6.69	6.64	6.67	6.62	0.028	0.695	0.367	0.965
[A]NH_3-N (mg,%)	14.22	18.26	19.76	20.13	0.803	0.040	0.194	0.276
[B]BUN (mg,%)	14.19	16.46	17.80	18.38	0.756	0.092	0.363	0.588
[C]TVFA (mM L^{-1})	85.36	83.08	77.68	83.95	1.248	0.198	0.440	0.113
Acetate (%)	65.94	63.58	70.64	64.99	0.834	0.092	0.033	0.343
Propionate (%)	26.63	30.53	21.53	27.67	0.724	0.018	0.005	0.454
Butyrate (%)	7.42	7.13	7.82	7.32	0.428	0.735	0.654	0.908
Acetate: Propionate ratio	2.50	2.09	3.46	2.36	1.550	0.070	0.032	0.290
[D]CH_4 , mol/100 mol	25.32	23.06	29.00	24.56	0.508	0.026	0.006	0.305
Protozoa count (10^6 cells mL^{-1})	2.96	2.94	2.86	3.05	0.021	0.864	0.086	0.072
Bacteria count (10^{11} cells mL^{-1})	4.84	4.62	4.62	4.57	0.069	0.329	0.337	0.548

33% SFO: 3% of sunflower oil in concentrate diets, 6% SFO: 6% of sunflower oil in concentrate diets, 2% KNO_3: 2% of potassium nitrate in concentrate diets, 3% KNO_3: 3% of potassium nitrate in concentrate diets, SFO: Effect of Sunflower oil, KNO_3: Effect of KNO_3, SFO×KNO_3: Interaction between SFO×KNO_3, SEM: Standard error of the mean, [A]NH_3-N: Ammonia nitrogen (mg %), [B]BUN: Blood urea nitrogen (mg %), [C]TVFA: Total Volatile fatty acids (mM L^{-1}), [D]CH_4 = (0.45×acetate) (0.275×propionate)+(0.4×butyrate) according to Moss *et al.*[19]

($p<0.05$) the C_3 concentration. Van Zijderveld *et al.*[6] noted that there is no difference in TVFAs concentration and molar proportion of C_2 and C_3 in the rumen fluid. There was no interaction between the levels of SFO and KNO_3 with regard to C_3 concentration.

CH_4 production was increased ($p<0.05$) with increasing levels of SFO and increasing the levels of KNO_3 increased ($p<0.05$) CH_4 production. There was no interaction between the levels of SFO and KNO_3 with regard to CH_4 production. McGin *et al.*[32] demonstrated that SFO decreases protozoa populations and that the lowest protozoa population is found in a treatment containing 6% SFO and 2% KNO_3 ($p<0.05$). There was no influence of the levels of SFO and KNO_3 on protozoa population. Lipids have also been shown to inhibit methanogenesis, even in the absence of rumen protozoa, probably due to the toxicity of long chain fatty acids (LCFA) to methanogenic bacteria. These LCFA have the capacity to attract more H_2 atoms and, thus, may be more able to influence the H_2 balance in the rumen when large quantities are included in the diet compared to short fatty acids (SFA) Ellis *et al.*[33], Jouany *et al.*[34] showed that utilization of polyunsaturated fatty acids (PUFA) may decrease rumen methanogens.

Microbial population: There was no interaction between the level of SFO and level of KNO_3 with regard to real-time PCR parameters. Total fungi decreased ($p<0.05$) with increasing levels of KNO_3 and SFO did not affect total fungi as shown in Table 6. The level of KNO_3 did not influence real-time PCR parameters. Zhou *et al.*[35] reported that supplemental NO_3 does not significantly change the population of *Ruminococcus albus* and *Ruminococcus flavefaciens* because they adapt to NO_3. In contrast, NO_3 has been found to reduce cellulolytic bacteria, total bacteria, *R. flavefaciens*, *Butyrivibrio fibrisolvens* and *Fibrobacter sucinogenes*[36]. In the present study, *R. albus*, *Prevotella bryantii* and *Prevotella ruminicola* decreased ($p<0.05$) with increasing levels of SFO. This finding correlated with decreasing NDF and ADF

Table 6: Effect of dietary treatment on population of rumen microbial population using real-time PCR

	3% SFO		6% SFO			Contrast		
	2% KNO_3	3% KNO_3	2% KNO_3	3% KNO_3	SEM	SFO	KNO_3	SFO×KNO_3
Quantity real-time PCR, copies mL^{-1} of rumen content								
Total protozoa, ×10^6	4.34	4.19	4.37	4.60	0.206	0.593	0.920	0.652
Total bacteria, ×10^{11}	2.15	2.04	2.18	2.35	0.153	0.593	0.920	0.652
Total fungi, ×10^8	4.32	4.07	4.35	3.97	0.058	0.757	0.011	0.559
Ruminococcus succinogenes, ×10^8	5.80	6.04	5.86	5.98	0.150	0.997	0.558	0.840
Ruminococcus flavefaciens, ×10^9	3.67	2.98	3.53	3.16	0.238	0.978	0.278	0.736
Ruminococcus albus, ×10^8	2.86	2.59	1.91	1.92	0.091	<0.001	0.491	0.447
Prevotella bryantii, ×10^4	6.19	5.73	4.95	4.92	0.168	0.005	0.475	0.531
Prevotella ruminicola, ×10^7	9.34	8.91	7.47	6.74	0.393	0.016	0.467	0.850
Selenomonas ruminantium, ×10^4	3.35	3.77	3.45	3.36	0.240	0.964	0.532	0.850
Archaea *mcrA*, ×10^3	2.29	1.68	2.09	1.42	0.092	0.219	0.002	0.893

3% SFO: 3% of sunflower oil in concentrate diets, 6% SFO: 6% of sunflower oil in concentrate diets, 2% KNO_3: 2% of potassium nitrate in concentrate diets, 3% KNO_3: 3% of potassium nitrate in concentrate diets, SFO×KNO_3: Interaction between SFO×KNO_3, SEM: Standard error of the mean

digestibilities with 6% SFO as compared to 3% SFO regardless of KNO_3 level. It is well known that *R. albus* is a predominant fibre-degrading bacteria in the rumen. Moreover, polyunsaturated fatty acids have been reported to be toxic toward rumen microorganisms[37]. The present experiment was showed that addition of 6.0% SFO reduced total bacteria population when compared with 3.0% SFO.

CONCLUSION

It can be concluded that there was no interaction between the levels of SFO and KNO_3 on any parameters. Voluntary feed intake, nutrient digestibility and nitrogen utilization were not influenced by the levels of SFO and KNO_3. Increasing SFO levels significantly increased ($p<0.05$) NH_3-N, propionate and CH_4 production but significantly decreased ($p<0.05$) copies of *R. albus*, *P. bryantii* and *P. ruminicola* per milliliter of rumen fluid. Increasing levels of KNO_3 significantly increased ($p<0.05$) propionate in the rumen but significantly decreased ($p<0.05$) the C_3:C_4 ratio and CH_4 production. Copies of Archae *mcrA* were significantly increased ($p<0.05$) with increasing KNO_3 levels. Thus, these findings suggested that SFO can be used as an energy source and that KNO_3 can be used as a CH_4 inhibitor in goat diets.

SIGNIFICANCE STATEMENT

This study discovers the feeding combination between nitrate and sunflower oil can be beneficial to reduce CH_4 production by goats fed rice straw. So that the study would be pointed out that ruminant fed low quality roughages can be improved by combining nitrate and sunflower oil.

ACKNOWLEDGMENTS

The authors would like to express their sincerest gratitude and appreciation to the Thailand Research Fund (TRF) via "The Royal Golden Jubilee Ph.D. Program" and Suranaree University of Technology for providing laboratories and facilities and financially supporting this research with grant number (RU3-303-55-01).

REFERENCES

1. Key, N. and G. Tallard, 2012. Mitigating methane emissions from livestock: A global analysis of sectoral policies. Climate Change, 112: 387-414.
2. Johnson, K.A. and D.E. Johnson, 1995. Methane emissions from cattle. J. Anim. Sci., 73: 2483-2492.
3. Bauman, D.E., B.A. Corl and D.G. Peterson, 2003. The Biology of Conjugated Linoleic Acids in Ruminants. In: Advances in Conjugated Linoleic Acid Research, Sebedio, J.L., W.W. Christie and R.O. Adlof (Eds.). Vol. 2, AOCS Press, Champaign, Illinois, pp: 146-173.
4. Takahashi, J. and B.A. Young, 1991. Prophylactic effect of L-cysteine on nitrate-induced alterations in respiratory exchange and metabolic rate in sheep. Anim. Feed Sci. Technol., 35: 105-113.
5. Sar, C., B. Mwenya, B. Santoso, K. Takaura and R. Morikawa *et al*., 2005. Effect of *Escherichia coli* W3110 on ruminal methanogenesis and nitrate/nitrite reduction *In vitro*. Anim. Feed Sci. Technol., 118: 295-306.
6. Van Zijderveld, S.M., W.J.J. Gerrits, J.A. Apajalahti, J.R. Newbold, J. Dijkstra, R.A. Leng and H.B. Perdok, 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci., 93: 5856-5866.

7. Hulshof, R.B.A., A. Berndt, W.J.J. Gerrits, J. Dijkstra, S.M. van Zijderveld, J.R. Newbold and H.B. Perdok, 2012. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. J. Anim. Sci., 90: 2317-2323.
8. Nakamura, Y., J. Yoshida, R. Nakamura and H. Horie, 1975. Nitrate metabolism of microorganisms in the rumen of sheep fed high nitrate forages. Jap. J. Zootech. Sci., 47: 63-67.
9. Iwamoto, M., N. Asanuma and T. Hino, 2001. Effects of pH and electron donors on nitrate and nitrite reduction in ruminal microbiota. Anim. Sci. J., 72: 117-125.
10. Caver, L.A. and W.H. Pfander, 1974. Some metabolic aspects of urea and-or potassium nitrate utilization by sheep. J. Anim. Sci., 38: 410-417.
11. Sophea, I. and T.R. Preston, 2011. Effect of different levels of supplementary potassium nitrate replacing urea on growth rates and methane production in goats fed rice straw, mimosa foliage and water spinach. Livest. Res. Rural Dev., Vol. 23.
12. Li, L., J. Davis, J. Nolan and R. Hegarty, 2012. An initial investigation on rumen fermentation pattern and methane emission of sheep offered diets containing urea or nitrate as the nitrogen source. Anim. Prod. Sci., 52: 653-658.
13. Silivong, P., O. Xaykham, O. Aloun and T.R. Preston, 2012. Effect of potassium nitrate and urea on feed intake, digestibility, N balance and methane production of goats fed a basal diet of Gliricidia (*Gliricidia sepium*) and Mimosa (*Mimosa pigra*) foliages supplemented with molasses. Livest. Res. Rural Dev., Vol. 24.
14. Thanh, V.D., N. van Thu and T.R. Preston, 2012. Effect of potassium nitrate or urea as NPN sources associated with Mangosteen peel (*Garcinia mangostana*) on methane production, rumen parameters and growth performance of Phan Rang sheep in the Mekong Delta of Vietnam. Livest. Res. Rural Dev., Vol. 24.
15. AOAC., 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., Pages: 684.
16. Goering, H.K. and P.J. van Soest, 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and some Applications). Agriculture Handbook No. 379, US Department of Agriculture, Agricultural Research Service, USA., pp: 1-20.
17. Erwin, E.S., G.J. Marco and E.M. Emery, 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci., 44: 1768-1771.
18. Crocker, C.L., 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinization. Am. J. Med. Technol., 33: 361-365.
19. Moss, A.R., J.P. Jouany and J. Newbold, 2000. Methane production by ruminants: Its contribution to global warming. Annales Zootechnie, 49: 231-253.
20. Sylvester, J.T., S.K.R. Karnati, Z. Yu, M. Morrison and J.L. Firkins, 2004. Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. J. Nutr., 134: 3378-3384.
21. Denman, S.E. and C.S. McSweeney, 2006. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiol. Ecol., 58: 572-582.
22. Koike, S. and Y. Kobayashi, 2001. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol. Lett., 204: 361-366.
23. Tajima, K., R.I. Aminov, T. Nagamine, H. Matsui, M. Nakamura and Y. Benno, 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Applied Environ. Microbiol., 67: 2766-2774.
24. Denman, S.E., N.W. Tomkins and C.S. McSweeney, 2007. Quantitation and diversity analysis of ruminal methanogenic populations in response to the antimethanogenic compound bromochloromethane. FEMS Microbiol. Ecol., 62: 313-322.
25. SAS., 1998. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC., USA.
26. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 9780070609266, pp: 20-90.
27. Pal, K., A.K. Patra, A. Sahoo and N.M. Soren, 2015. Effects of nitrate and fumarate in tree leaves-based diets on nutrient utilization, rumen fermentation, microbial protein supply and blood profiles in sheep. Livest. Sci., 172: 5-15.
28. Nolan, J.V., R.S. Hegarty, J. Hegarty, I.R. Godwin and R. Woodgate, 2010. Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Anim. Prod. Sci., 50: 801-806.
29. Mewara, K.K., A. Kumar, P.V.R. Rao and D.P. Tiwari, 2008. Effect of soybean oil supplementation on nutrient utilization, milk yield and its quality in lactating crossbred cows. Indian J. Anim. Sci., 78: 751-757.
30. Doranalli, K. and T. Mutsvangwa, 2011. Feeding sunflower oil to partially defaunate the rumen increases nitrogen retention, urea-nitrogen recycling to the gastrointestinal tract and the anabolic use of recycled urea-nitrogen in growing lambs. Br. J. Nutr., 105: 1453-1464.
31. Alaboudi, A.R. and G.A. Jones, 1985. Effect of acclimation to high nitrate intakes on some rumen fermentation parameters in sheep. Can. J. Anim. Sci., 65: 841-849.

32. McGinn, S.M., K.A. Beauchemin, T. Coates and D. Colombatto, 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast and fumaric acid. J. Anim. Sci., 82: 3346-3356.
33. Ellis, J.L., J. Dijkstra, E. Kebreab, A. Bannink, N.E. Odongo, B.W. McBride and J. France, 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci., 146: 213-233.
34. Jouany, J.P., J. Gobert, B. Medina, G. Bertin and V. Julliand, 2008. Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet. J. Anim. Sci., 86: 339-347.
35. Zhou, Z.M., Z.T. Yu and Q.X. Meng, 2012. Effects of nitrate on methane production, fermentation and microbial populations in *in vitro* ruminal cultures. Bioresour. Technol., 103: 173-179.
36. Marais, J.P., J.J. Therion, R.I. Mackie, A. Kistner and C.D. Dennison, 1988. Effect of nitrate and its reduction products on the growth and activity of the rumen microbial population. Br. J. Nutr., 59: 301-313.
37. Zhang, C.M., Y.Q. Guo, Z.P. Yuan, Y.M. Wu, J.K. Wang, J.X. Liu and W.Y. Zhu, 2008. Effect of octadeca carbon fatty acids on microbial fermentation, methanogenesis and microbial flora *in vitro*. Anim. Feed Sci. Technol., 146: 259-269.