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Effect of Coconut Oil and Sunflower Oil Ratio on Ruminal Fermentation, Rumen Microorganisms, N-balance and Digestibility in Cattle

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Abstract: Four, rumen-fistulated Holstein-Friesian steers were randomly assigned to four treatments according to a 4×4 Latin square design to study effects of coconut oil and sunflower oil ratio on rumen fermentation, rumen microorganisms and methane concentration in the rumen. The dietary treatments were ratios of coconut oil and sunflower oil at 100:0, 75:25, 50:50 and 25:75 for treatment 1-4, respectively. Steers were fed concentrate at 0.5% of BW (DM) and urea-treated rice straw was given *ad libitum*. The results were found that coconut oil and sunflower oil ratio did not affect feed intake and rumen microbial population except for total viable bacteria in which 75:25 ratio was the highest. Dietary treatments had affected nutrient digestibility and rumen fermentation especially 50:50 ratio. Methane concentration was linearly decreased when sunflower oil proportion increased. Nitrogen balance and microbial protein synthesis were similar among treatments, although microbial nitrogen supply tended to have a quadratic response to oil ratios. It is concluded that combined supplementation of coconut oil and sunflower oil could be beneficial to improve the rumen ecosystem and potential productivity in ruminants.

Key words: Coconut oil, sunflower oil, rumen fermentation, microorganisms, methane, cattle

INTRODUCTION

Rumen fermentation is of prime importance with the formation of fermentation end-products such as volatile fatty acids and NH_3-N. It has been clearly shown that rumen fermentation result in the major supply of amino acid and energy for ruminants (Kebreab *et al*., 2008). Fat is an important energy component in the diet of ruminants and fat supplementation has become a common practice to increase the energy density of the diet (Bauman *et al*., 2003). However, high level of fat in ruminant diets may adversely affect microbial fermentation, hence general recommendation is that total dietary fat should not exceed 60-70 g kg^{-1} of dietary dry matter (Jenkins, 1993; NRC, 2001). Ruminal methane production represents energy loss to the host animal (Holter and Young, 1992) and it has become clear that methane plays an important role in global warming contributing 15% of all green house gases. Methane production by livestock represents 2% of total methane production (Moss *et al*., 2000). Various options such as chemical feed additives and manipulation of feed and feeding can be taken to reduce methane emission in livestock (Tamminga *et al*., 2007). Dietary fats have been identified as efficient means of decreasing ruminal methanogenesis (Jouany, 1994). In this context, several fats rich in medium-chain saturated fatty acids (C8:0-C14:0) were found to inhibit methane production in rumen fluid (Dohme *et al*., 2001; Soliva *et al*., 2003). Coconut (*Cocos nucifera*) oil rich in medium-chain saturated fatty acids was found to be equally or more effective against ruminal methanogenesis (Machmuller *et al*., 2000; Machmuller, 2006) than long chain fatty acids. However, sunflower (*Helianthus annus*) oil rich in unsaturated fatty acid can reduce methane production by reducing rumen ciliated protozoa (Ivan *et al*., 2001; McGinn *et al*., 2004), an alternative metabolic H acceptor (Johnson and Johnson, 1995). However, mixtures of medium-chain saturated fatty acid and unsaturated fatty acid sources on rumen fermentation have not been investigated. Therefore, the objective of this study was to investigate the effects of coconut oil (medium-chain saturated fatty acid source) and sunflower oil (unsaturated fatty acid source) mixed in different ratios on rumen fermentation, rumen microorganism, microbial protein synthesis andmethane concentration in dairy cattle steers fed on urea-treated rice straw.

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MATERIALS AND METHODS

Animals, diets and experimental designs: Four, rumen fistulated 75% crossbred Holstein-Friesian steers, with body weight 350±30 kg were used in the experiment. Animals were randomly assigned according to a 4×4 Latin Square design to receive four different treatments with different coconut oil to sunflower oil ratio in 50 g kg^{-1} fat concentrate. The dietary treatments were as follows: 100% of coconut oil, 75% of coconut oil and 25% of sunflower oil, 50% of coconut oil and 50% of sunflower oil and 25% of coconut oil and 75% of sunflower oil. Both kinds of oils were mixed in concentrate and fatty acids composition is given in Table 1. All concentrate mixtures contained similar ingredients as; cassava (*Manihot esculenta*) chip, rice (*Oryza sativa*) bran, palm kernel (*Elaeis* sp.) meal, cassava hay, urea, molasses, salt, sulphur and premix (minerals and vitamins mixed, each kg contains: Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g) at 630, 80, 70, 100, 25, 20, 05, 03 and 17 g kg^{-1} (DM), respectively. The concentrate mixed diets were formulated to be at 130 g kg^{-1} Crude Protein (CP) and 79% Total Digestible Nutrient (TDN). Steers were housed in individual pens and individually fed concentrate at 0.5% of BW (DM), twice daily at 700 and 1500. Therefore, steers received coconut oil and sunflower oil mixtures approximately at 90 g/hd/day. All animals were fed *ad libitum* with water and mineral salt-block. Urea-treated rice straw (Wanapat, 1990) was given *ad libitum.* During the preliminary period, cows received a control diet containing tallow as an oil source in the concentrate with urea-treated rice straw as a roughage. The animals were then fed one-half of the control diet and one-half of the respective experimental diet for 3 days during a transitional feeding period. Feed intake of concentrate and roughage were measured separately and refusals recorded. The experiment was run in four periods, each experimental period lasted for 4 weeks and the first 3 weeks as a period for DM feed intakes measurements while during the last week all steers were taken to metabolism crates for total fecal and urine collections and for subsequent evaluation of nutrient digestibility. Rumen fluid and gas were sampled at 0, 2, 4 and 6 h after morning feeding of 6 and 7th of last week of each period, respectively andpool before analyses.

Sample collection and chemical analysis: Urea-treated rice straw and concentrate were sampled daily during the collection period and were composited by period prior to chemical analyses. Fecal and urine samples were collected by total collection technique on metabolism crates during the last 7 days of each period during which feces and urine were sampled on each day (5% of urine and 20% of feces) and pooled before further analysis. Feeds and fecal samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and were analysed using the standard methods of AOAC (1995) for DM (ID 967.03), ash (ID 942.05) and ADF (ID 973.18). Neutral detergent fiber in samples was estimated according to Van Soest *et al.* (1991) with the addition of α-amylase but without sodium sulphite and the results were calculated with residual ash. Total N in samples of feeds, refusals and faeces was determined according to AOAC (1991) (ID 984.13). Rumen fluid and jugular blood samples were collected at 0, 2, 4 and 6 h post morning feeding on the last day of each period. Approximately 200 mL of rumen fluid was taken from the middle part of the rumen by using a 60 mL hand syringe at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH temperature meter (HANNA, instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into three portions; first portion was used for NH_3-N and volatile fatty acids analyses (HPLC, Instruments by controller water model 600E; water model 484 UV detector; column novapak C_{18}; column size 3.9×300 mm; mobile phase 10 mM H_2PO_4 [pH 2.5]) according to Samuel *et al.* (1997). Second portion was for total direct count of bacteria, protozoa and fungal zoospores using the methods of Galyean (1989) by a Sedgewick-Rafter chamber and add cover slide (SPI® supplies, Chaina). The last portion was taken to the laboratory immediately for culturing and identification of bacteria groups using the roll-tube technique (Hungate, 1969). Ruminal bacteria were cultured in separate medium including complete medium for total viable bacteria,

Table 1: Fatty acids composition of oils used in experiment (g/100 g)

Fatty acids	Coconut oil	Sunflower oil
C10:0	5.47	-
C12:0	39.87	-
C14:0	16.62	0.06
C16:0	10.19	6.07
C16:1	0.25	0.08
C18:0	8.67	4.62
C18:1 n-9	5.34	27.33
C18:2 n-6	12.73	60.47
C18:3 n-6	-	0.01
C18:3 n-3	-	0.12
C20:1 n-9	-	0.14
C20:5 n-3	0.44	0.69
C22:5 n-3	-	0.21
C22:6 n-3	0.39	0.13
ΣSFA	80.82	10.75
ΣMUFA	5.59	27.55
ΣPUFA	13.56	61.63
ΣSFA/ΣUSFA	4.22	0.12

SFA = Saturated Fatty Acids, MUFA = Monounsaturated Fatty Acids, PUFA = Polyunsaturated Fatty Acids, USFA = Unsaturated Fatty Acids

cellulose medium for cellulolytic bacteria, casein medium for proteolytic bacteria andstarch medium for amylolytic bacteria (Hobson, 1965). Blood sample (about 20 mL) was drawn from the jugular vein at the same time of rumen fluid sampling and separated by centrifugation at 500× g for 10 min and stored at -20°C until analysis of Blood Urea Nitrogen (BUN) according to the method of Crocker (1967). Gas was taken from ruminal atmosphere at dorsal sac area of rumen without opening of fistulae by 20 mL hand syringe with stainless tube at 0, 2, 4 and 6 h post morning feeding on the 27th day of each period. Rumen gas was immediately stored at -20°C prior methane concentration analyses according to the method of Soliva *et al.* (2005) andcalculated comparing to digested nutrients (Machmuller *et al.*, 2001). Urine samples were collected during the digestibility trial (day 21-28) of each period by acidified with 20% sulphuric acid to bring pH to <3; 10 L was subsampled anddiluted 3 times with tap water. These samples were stored at -20°C for purine derivatives determination. Purine derivatives were analyzed by High-Performance Liquid Chromatography (HPLC), as described by Chen *et al.* (1993). The supply of Microbial N (MN) was estimated by the urinary excretion of Purine Derivatives (PD) according to Chen and Gomes (1995):

$$Y = 0.85X + (0.385\ BW^{0.75})$$

$$MN\ (g\ day^{-1}) = 70X/(0.116\times0.83\times1000) = 0.727X$$

where, X and Y are respectively, the absorption and excretion of PD in mmol day^{-1}. The N content of purines was 70 mg $mmoL^{-1}$; the ratio of purine N to total N in mixed rumen microbes was 0.116 (Chen and Gomes, 1995). Mean endogenous contribution of urinary purine derivative excretion was 0.385 mmol kg^{-1} $BW^{0.75}$ (Verbic *et al.*, 1990), digestibility of microbial purines in the intestines was estimated at 0.83 (Chen and Gomes, 1995) andrecovery of absorbed purines as urinary purine derivatives was assumed to be 85% (Verbic *et al.*, 1990). Efficiency of Microbial Protein Synthesis (EMPS) was calculated using the following formula:

$$EMPS = \frac{\text{Microbial N (g day}^{-1})}{\text{DOMR}}$$

where, DOMR (digestible OM apparently fermented in the rumen) = DOMI (digestible OM intake)×0.65 (Agricultural Research Council, 1990).

Statistical analyses: All data obtained from the experiment were subjected to ANOVA according to a 4×4 Latin square design using the General Linear Models (GLM) procedure of the Statistical Analysis System program (SAS institute, 1996). Multiple comparisons among means were carried out by Duncan's New Multiple Range Test (DMRT) and using orthogonal polynomial for trend analysis. Unless otherwise stated the significance was measured at $p<0.05$.

RESULTS AND DISCUSSION

Effect on feed intake and nutrient digestibility: Chemical composition of Urea-Treated Rice Straw (UTS) and experimental diets fed in this study is shown in Table 2. The effect of coconut oil and sunflower oil ratio on feed intake and nutrient digestibility of dairy steers are shown in Table 3. Overall mean of feed intakes for the four diets in terms of total DM intake, UTS intake and EE intake (kg, BW%, g kg^{-1} $BW^{0.75}$) were similar for all dietary treatments ($p>0.05$), although CCO+SFO intake tended to be highest at 50:50 ratio. Whole tract digestibilitis of DM, OM and ADF quadratically responded with oil ratio ($p<0.05$). NDF digestibility tended to linearly increased ($p<0.07$) when proportion of sunflower oil increased while CP digestibility were not different among treatments ($p>0.05$). The 50:50 ratio of fats resulted in numerically the greatest DM, OM, NDF and ADF digestibilities.

Effect of rumen fermentation: Rumen ecology parameters were measured for pH, NH_3-N, volatile fatty acids and microbial population and are given in Table 4. Ruminal pH were different ($p<0.05$) among treatments and were in a high range (6.62-6.78). Ruminal pH quadratically ($p<0.5$) responded with oil ratio at which 75:25 ratio presented the lowest value (6.6). Coconut oil and sunflower oil ratio in concentrate did not affect NH_3-N, BUN and VFAs concentrations in the rumen ($p>0.05$). Rumen bacteria, protozoa and fungi zoospores by direct count technique and cellulolytic, amylolytic and proteolytic bacteria by roll-tube technique were not different ($p<0.05$) among treatments. However, at 75:25 ratio, total viable bacteria were lower at 100:0 ratio ($p<0.05$) while others were similar. Methane concentration in the rumen is shown in Table 5. Methane concentration linearly decreased with increasing proportion of sunflower oil up to the 50:50 ratio where methane concentration was at its lowest ($p<0.05$).

Effect on nitrogen balance and microbial nitrogen supply. As shown in Table 6, Nitrogen intakes tended to linearly increase when proportion of SFO increased ($p<0.07$) but were not different among treatments (97.2-102.3 g day^{-1}). Nitrogen balance in terms of protein absorption and retention were similar across oil ratios ($p>0.05$). Efficiency of microbial nitrogen synthesis were similar among treatments ($p>0.05$) while microbial nitrogen supply tended to quadratically respond with oil ratios ($p<0.08$, respectively).

Table 2: Chemical composition of concentrate and Urea-Treated Rice Straw (UTS)

	Treatments (CCO:SFO)				
Feed composition	100:0	75:25	50:50	25:75	UTS
Dry matter (g kg^{-1})	888	889	889	887	492
Organic matter (g kg^{-1} DM)	979	982	982	986	974
Crude protein (g kg^{-1} DM)	131	133	134	129	70
Ether extracts (g kg^{-1} DM)	79	79	83	79	9
Neutral detergent fiber (g kg^{-1} DM)	231	200	217	220	682
Acid detergent fiber (g kg^{-1} DM)	101	89	97	91	457

CCO = Coconut Oil, SFO = Sunflower Oil, UTS = Urea-Treated Rice Straw

Table 3: Effect of coconut oil and sunflower oil ratio on daily feed intake and nutrient digestibility

	Dietary treatments (CCO:SFO)					Contrasts[1]		
Items	100:0	75:25	50:50	25:75	SEM	L	Q	C
Total DM intake								
kg	6.20	6.10	6.40	6.30	0.160	NS	NS	NS
BW (%)	1.80	1.90	1.90	1.90	0.060	NS	NS	NS
g kg^{-1} BW$^{0.75}$	77.10	76.90	81.20	78.80	1.570	NS	NS	NS
UTS intake								
kg	4.50	4.40	4.80	4.60	0.160	NS	NS	NS
BW (%)	1.30	1.30	1.40	1.40	0.040	NS	NS	NS
g kg^{-1} BW$^{0.75}$	57.10	56.20	60.00	59.20	1.650	NS	NS	NS
EE intake (g)	175.00	174.00	176.00	176.00	1.560	NS	NS	NS
CCO+SFO intake (g)	85.00	85.00	80.00	85.00	2.010	NS	NS	NS
Digestibility (%)								
Dry matter	0.59^{c}	0.62^{ab}	0.63^{a}	0.61^{bc}	0.005	NS	**	NS
Organic matter	0.64^{b}	0.68^{a}	0.68^{a}	0.66^{ab}	0.006	NS	**	NS
Crude protein	0.51	0.53	0.53	0.50	0.018	NS	NS	NS
Neutral detergent fiber	0.64^{b}	0.67^{ab}	0.70^{a}	0.67^{ab}	0.012	0.07	NS	NS
Acid detergent fiber	0.47^{b}	0.50^{b}	0.53^{a}	0.50^{b}	0.010	*	*	NS

CCO = Coconut Oil, SFO = Sunflower Oil, UTS = Urea-TreatedRrice Straw, ^{abc}Values on the same row with different superscripts differ ($p<0.05$), [1]L = Linear effect, Q = Quadratic effect, C = Cubic effect, SEM = Standard Error of the Means, *$p<0.05$, **$p<0.01$, NS = Non-Significant different

Table 4: Effect of coconut oil and sunflower oil ratio on rumen fermentation and microbial population of dairy steers

	Dietary treatments (CCO:SFO)					Contrasts[1]		
Items	100:0	75:25	50:50	25:75	SEM	L	Q	C
Ruminal pH	6.7^{a}	6.6^{b}	6.7^{a}	6.8^{a}	0.02	*	*	NS
Ammonia nitrogen (mg dL^{-1})	6.6	7.0	7.5	8.0	0.82	NS	NS	NS
Blood urea nitrogen (mg dL^{-1})	9.5	7.5	8.1	8.2	0.89	NS	NS	NS
Total volatile fatty acid (mmol L^{-1})	102.1	102.1	101.9	103.6	7.30	NS	NS	NS
Acetic acid (%)	69.2	68.0	66.3	65.2	3.47	NS	NS	NS
Propionic acid (%)	21.7	23.5	24.7	24.6	2.69	NS	NS	NS
Butyric acid (%)	9.1	8.5	9.0	10.2	1.57	NS	NS	NS
Acetic acid to propionic acid ratio	3.3	2.9	2.8	2.7	0.54	NS	NS	NS
Microbial population								
Total direct count (cell mL^{-1})								
Bacteria (×10^{10})	3.8	4.4	4.0	4.2	0.59	NS	NS	NS
Protozoa (×10^{5})	7.0	7.7	8.1	8.9	0.59	NS	NS	NS
Fungi zoospores (×10^{7})	5.1	5.1	5.3	5.2	0.19	NS	NS	NS
Roll tube technique (CFU2 mL^{-1})								
Total viable bacteria (×10^{9})	3.1^{a}	4.4^{b}	4.0^{ab}	4.1^{ab}	0.36	NS	NS	NS
Cellulolytic bacteria (×10^{8})	3.7	4.1	4.9	4.0	0.52	NS	NS	NS
Amylolytic bacteria (×10^{7})	6.5	6.1	5.7	5.2	0.60	NS	NS	NS
Proteolytic bacteria (×10^{7})	6.5	5.7	5.8	6.1	0.61	NS	NS	NS

CCO = Coconut Oil, SFO = Sunflower Oil, UTS = Urea-Treated Rice Straw, ^{abc}Values on the same row with different superscripts differ ($p<0.05$), [1]L = Linear effect, Q = Quadratic effect, C = Cubic effect, [2]CFU = Colony Forming Unit, SEM = Standard Error of the Means, *$p<0.05$, NS = Non-Significant different

Based on the chemical composition of Urea-Treated Rice Straw (UTS), it contained 70 g kg^{-1} CP which was slightly lower than that reported by Wanapat (1999). This difference could be due to differences in variety of rice straw, fertilizer application level to rice straw etc. Concentrate diet contained similar concentration of DM, OM, CP, EE, NDF and ADF.

The data indicated that different proportions of coconut oil and sunflower oil in concentrate diet had no effect on feed intake of dairy steers. However, using combination of oil had positive effects on nutrient digestibility. In contrast, Palmquist and Jenkins (1980) suggested that unsaturated fatty acids had a greater influence on rumen fermentation than saturated fatty acid.

Table 5: Effect of coconut oil and sunflower oil ratio on methane concentration in the rumen

	Dietary treatments (CCO:SFO)					Contrasts[1]		
Items	100:0	75:25	50:50	25:75	SEM	L	Q	C
Methane concentration								
CH4 (mmol L^{-1})	17.2[a]	16.6[ab]	16.0[b]	16.3[ab]	0.30	*	NS	NS
CH4/DM intake (mmol kg^{-1})	2.9[a]	2.8[ab]	2.6[b]	2.6[ab]	0.09	*	NS	NS
CH4/NDF intake (mmol kg^{-1})	4.9	5.0	4.3	4.5	0.19	0.07	NS	NS
CH4/DM digested (mmol kg^{-1})	4.9[a]	4.6[ab]	4.1[b]	4.3[b]	0.14	*	NS	NS
CH4/NDF digested (mmol kg^{-1})	7.6[a]	7.5[a]	6.1[b]	6.8[ab]	0.26	*	NS	*

DM = Dry Matter, NDF = Neutral Detergent Fiber, ADF = Acid Ddetergent Fiber, [abc]Values on the same row with different superscripts differ ($p<0.05$), [1]L = Linear effect, Q = Quadratic effect, C = Cubic effect, SEM = Standard Error of the Means, *$p<0.05$, NS = Non-Significant different

Table 6: Effect of coconut oil and sunflower oil ratio on nitrogen balance and microbial protein synthesis in dairy steers

	Dietary treatments (CCO:SSO)					Contrasts[1]		
Items	100:0	75:25	50:50	25:75	SEM	L	Q	C
Nitrogen balance (g day^{-1})								
Nitrogen intake	97.2	97.4	102.3	100.7	1.71	0.07	NS	NS
Nitrogen absorption	49.2	51.5	54.1	50.1	1.74	NS	NS	NS
Nitrogen retention	5.2	6.2	6.5	5.7	0.65	NS	NS	NS
MNS (g N day^{-2})	63.3	71.7	67.1	58.6	8.59	NS	0.08	NS
EMPS (g N kg^{-1} of OMDR[3])	29.0	30.6	25.2	25.1	4.17	NS	NS	NS

[1]L = Linear effect, Q = Quadratic effect, C = Cubic effect, SEM = Standard Error of the Means, NS = Non-Significant different, [2]MNS = Microbial Nitrogen Supply, calculated according to Chen *et al.* (1993), [3]EMPS = Efficiency of Microbial Protein Synthesis, OMDR = Organic Matter Digestible in the Rumen (65% of organic matter digestible in total tract) according to Agricultural Research Council (1984)

Czerkawski *et al.* (1966) found that the inhibition of gram-positive bacteria growth was achieved with unsaturated fatty acid supplementation. However, the amount of sunflower oil at 50:50 ratio may have been too small to affect ruminal bacteria and/or modify the biohydrogenation pathway (Kepler *et al.*, 1966).

Ruminal pH were in a high range (6.62-6.78) and ruminal NH_3-N concentration ranged from 6.6-8.0 mg dL^{-1} which was relatively lower than those reported by Wanapat (1990) (15-30 mg dL^{-1}). Differences among treatments of DM, OM and fiber digestibilities were small and did not affect VFA concentration. Therefore different proportions of coconut oil and sunflower oil had no effect on volatile fatty acid concentration. Under this result, it was relatively low which could be on effect of oil. According to Galbraith and Miller (1973) who reported that long-chain fatty acids are toxic to some micro-organisms. Total viable bacteria, cellulolytic bacteria and proteolytic bacteria in this experiment were slightly higher than those reported by Khampa *et al.* (2004) who also studied in dairy steer. It could be major due to differences of diet which they used higher proportion of cassava chip andsome unlike of other experiment conditions.

Methane concentration was linearly decreased with proportion of sunflower oil to the 50:50 ratio. This result indicated that sunflower oil had a greater impact on methane concentration than coconut oil. It implies that unsaturated fatty acid particularly linoleic acid can depress methane production more than saturated fatty acid particularly medium-chain fatty acids. These results agree with the research of Dohme *et al.* (2001) who reported that methane release and methanogenic counts were suppressed by linoleic acid (C18:2) whereas palmitic acid (C16:0) and stearic acid (C18:0) showed no corresponding effects. Giger-Reverdin *et al.* (2003) reviewed and suggested that the addition of unsaturated fats might be of interest for decreasing methane production. Czerkawski *et al.* (1966) reported that the presence of long-chain polyunsaturated fatty acids inhibits methane production in the rumen through two ways: provision of an alternative metabolic H acceptor in reduction of CO_2 and direct toxic effects on ruminant microorganisms (Johnson and Johnson, 1995). However, rumen microbes in this study did not relate with methane concentration so that decrease of methane concentration could be from the provision of an alternative metabolic H acceptor to reduction CO_2 than direct toxic to rumen microbes.

On the other hand, Odongo *et al.* (2007) found that dietary supplementation with myristic acid reduced methane production in dairy cows. According to Soliva *et al.* (2004a) who found clear synergistic effect of mixtures of myristic and lauric acid on methanogenesis which was probably mediated by direct inhibitory effects of fatty acids on the methanogens. In addition, Soliva *et al.* (2004b) also found that myristic acid did not reduce methanogenesis although populations of archaea were decreased. However, such effects were not found under this study. Nitrogen balance was similar across oil ratios. These results indicate that the ratios of coconut oil to sunflower oil in concentrate did not affect nitrogen

metabolism and microbial protein synthesis in the rumen of dairy steers fed on 50 g kg^{-1} urea-treated rice straw as a roughage while microbial population in the rumen were similar across treatments. The absence of any effect oil on nitrogen balance could be due to a small of ether extract intake by animals.

CONCLUSION

Based on this result it could be concluded that coconut oil to sunflower oil ratio in concentrate mixtures did not affect feed intake, NH_3-N, BUN and VFA concentration andmicrobial population in dairy steers. However, nutrient digestibility, ruminal pH and methane concentration were responded quadratically to oil ratio and at 50:50 ratio, the results could reduce methane concentration without impact on rumen fermentation and ruminal microorganisms. However, further studies should be conducted to investigate the relationship between fatty acid compositions in feed, rumen fluid, rumen methane concentration and their effects on meat, milk yield and quality.

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