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Conjugated Linoleic Acid in Rumen Fluid and Milk Fat, and Methane Emission of Lactating Goats Fed a Soybean Oil-based Diet Supplemented with Sodium Bicarbonate and Monensin*

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ABSTRACT : A metabolic study was conducted with four ruminally-cannulated lactating goats (Saanen, 29 weeks lactation, 65± 5 kg) in a 4×4 Latin square design with 4 dietary treatments. The goats were fed a basal mixed diet consisting of 80% concentrate and 20% chopped rye grass hay (DM basis, CON). The goats were also fed the CON diet supplemented with soybean oil at a 5% level of the concentrate (SO), the SO diet supplemented with 0.5% of sodium bicarbonate (SO-B) or the SO-B diet supplemented with 30 ppm monensin (SO-BM). The goats were housed in individual pen and the study was conducted for 8 weeks. An increased molar proportion of propionate (C_3) was observed at 1 h ($p<0.003$) and 6 h ($p<0.029$) post-feeding from all the supplemented diets. Calculated methane emission was markedly decreased prior to morning feeding ($p<0.01$), and at 1 h ($p<0.05$) and 6 h post-feeding ($p<0.05$) in goats fed the supplemented diets. All the supplements increased ($p<0.0001$) *cis*9, *trans*11-CLA content in rumen fluid. Concentrations of both *cis*9, *trans*11-CLA ($p<0.0001$) and *trans*10, *cis*12-CLA ($p<0.026$) were also increased in the milk fat of lactating goats fed the supplemented diets. The SO-B and SO-BM diets further increased CLA content in goat milk compared to the SO diet. All supplements increased unsaturated (UFA, $p<0.002$), monounsaturated (MUFA, $p<0.002$) and polyunsaturated fatty acids ($p<0.014$) and reduced SFA to UFA ratio ($p<0.023$). The concentration of MUFA was even greater ($p<0.002$) for SO-BM than for the SO-B diet. In conclusion, feeding soybean oil (5% of concentrate) to lactating goats was a useful way to improve milk fat and to improve fatty acid profile in the milk by increasing potentially healthy fatty acids such as CLA. Supplementation of sodium bicarbonate or sodium bicarbonate with monensin to the soybean oil-based diet increased CLA content further in goat milk. Supplementation of soybean oil may be an effective method to reduce methane emission in lactating goats. (**Key Words :** Lactating Goat, Soybean Oil, Monensin, CLA, Milk, Methane Emission)

INTRODUCTION

Health-conscious consumers are demanding milk with a higher proportion of healthy fat. An improvement in the nutritional value of goat milk is of importance to human health. Dietary manipulation with lipid could improve goat milk composition and may indeed change fatty acid composition in milk fat (Chilliard, 1982), and thus is one way to alter the fatty acid profile of milk fat. Since conjugated linoleic acid (CLA) was identified as an anti-mutagenic substance (Ha et al., 1987), it has been shown to be a potent anti-carcinogen in several cell culture and animal models (Kritchevsky, 2000). Other benefits attributed to CLA consumption include effects on body composition, the immune system, atherosclerosis and bone health (MacDonald, 2000). Various attempts to increase CLA content in ruminant products have included the application of lipid source. Supplementation of linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) in the form of oil or oil seeds to the diet has been shown to enhance the CLA content of rumen fluid (Dhiman et al., 2000; Wang et al., 2003; Choi and Song, 2005), milk of dairy cattle (Dhiman et al., 2000; Loor et al., 2005), and fat tissues of sheep (Choi et al., 2006) and beef cattle (Wang et al., 2006). Feeding a moderate dose of plant oil to lactating goats was

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a useful way to increase milk fat and CLA content in milk (Bouattour et al., 2008; Bernard et al., 2009).

Meanwhile, ionophoric antibiotics such as monensin have been found to interfere with the bio-hydrogenation of *cis*-9, *cis*-12 $C_{18:2}$ with an accumulation of intermediate products including CLA (Fellner et al., 1997). Increased CLA concentration in milk fat of dairy cattle (Sauer et al., 1998) and beef fat (Wang et al., 2006) were also reported when the diet was supplemented with monensin, but its effect on CLA enrichment in milk fat has been controversial (Chouinard et al., 1998; Dhiman et al., 1999). In addition, sodium bicarbonate, having buffering activity (Erdman, 1988), may also influence CLA formation in the rumen since the bio-hydrogenation is affected by ruminal pH (Wang et al., 2003).

Most previous experiments with lactating goats have examined the CLA in milk fat in response to plant oil supplementation of the diet. Therefore, the objective of the current study was to examine the combined effect of soybean oil, sodium bicarbonate and monensin in the diet on fermentation characteristics in the rumen, and CLA content in rumen fluid and milk fat of the lactating goat. Methane emission from the rumen was also estimated since $C_{18:2}$ supplementation reduces fermentation rate (Prins et al., 1972) and depresses CH_4 generation in the rumen (Broudiscou et al., 1990).

MATERIALS AND METHODS

Animals and diets

The metabolic study was conducted with four ruminally-cannulated lactating goats (Saanen, 29 weeks lactation, 65±5 kg) in a 4×4 Latin square design with 4 dietary treatments. The goats were fed a basal mixed diet consisting of 80% concentrate and 20% chopped rye grass hay (DM basis, CON). The goats were also fed the CON diet but supplemented with soybean oil at a 5% level of the concentrate (SO), the SO diet supplemented with 0.5% of sodium bicarbonate (SO-B) or the SO-B diet supplemented with 30 ppm monensin (SO-BM). Experimental diets were prepared at 5 day intervals. Cr_2O_3 as external marker was dosed (0.1% of diet, DM basis) to individual goats through the rumen cannula twice daily during feeding throughout the whole experimental period for the examination of whole tract digestibility. Concentrate constituents and nutrient composition of the total diet are shown in Table 1. Fatty acid composition of soybean oil and the basal diet are shown in Table 2.

Feeding management

The lactating goats were housed in individual pen and fed 1.6 kg (DM basis) of the mixed diets twice daily (08:00 and 18:00) in equal amounts, and were allowed free access to water and a trace-mineralized salt block. The feeding level of the basal diet (CON) was based on nutrient requirements for lactating goats (AFRC, 1993). The study was conducted for 8 weeks in total, with 10 days for diet adaptation and 4 days for sampling in each experimental period.

Measurements and analysis

Feed residues were collected daily at 30 min prior to morning feeding (08:00) to estimate feed intake and digestibility. Lactating goats were machine- milked twice daily (08:00 and 18:00). Milk yield was recorded daily during sampling days of each experimental period and

Table 1. Ingredients of concentrate and nutrient composition of the total diet (DM basis)

	Treatments[1]			
	CON	SO	SO-B	SO-BM
Ingredients of concentrate				
Commercial concentrate (%)	100.00	95.00	94.50	94.50
Soybean oil (SO, %)	-	5.00	5.00	5.00
Sodium bicarbonate (B, %)	-	-	0.50	0.50
Monensin (M, ppm)	-	-	-	30.00
Nutrient composition of total diet (%)[2]				
Dry matter	90.64	91.46	91.54	91.92
Crude protein	14.61	14.01	13.97	14.08
Ether extract	4.65	8.07	8.06	8.23
Neural detergent fiber	34.05	33.29	33.09	33.13
Crude ash	7.57	7.40	7.59	7.32

[1] CON = Commercial concentrate without supplements; SO = Supplemented with soybean oil (5%) to the concentrate; SO-B = Supplemented with soybean oil (5%) and sodium bicarbonate (0.5%) to the concentrate; SO-BM = Supplemented with soybean oil (5%) and sodium bicarbonate (0.5%), monensin (30 ppm) to the concentrate.

[2] Mixed diet of concentrate and forage.

Table 2. Fatty acid composition of feed and soybean oil

Fatty acids	Feeds and soybean oil (% of total fatty acid)		
	Concentrate	Ryegrass hay	Soybean oil
Dodecanoic acid ($C_{12:0}$)	11.88	2.71	0.33
Myristic acid ($C_{14:0}$)	4.67	2.02	0.10
Pentadecanoic acid ($C_{15:0}$)	0.09	0.68	0.03
Palmitic acid ($C_{16:0}$)	19.19	25.71	13.06
Palmitolic acid ($C_{16:1}$)	0.91	2.27	0.09
Margric acid ($C_{17:0}$)	0.18	0.51	0.11
Stearic acid ($C_{18:0}$)	4.40	3.58	4.71
Oleic acid ($C_{18:1}$)	23.66	7.87	20.19
Linoleic acid ($C_{18:2}$)	27.46	20.64	51.71
Lenolenic acid ($C_{18:3}$)	0.80	0.43	0.30
Arachidonic acid ($C_{20:0}$)	0.41	1.81	0.37
Eicosenoic acid ($C_{20:1}$)	1.59	25.12	8.27
Eicosatrienoic acid ($C_{20:3}$)	0.31	2.01	0.34
Arachidonic acid ($C_{20:4}$)	0.62	0.59	ND
Henecosanoic acid ($C_{21:0}$)	0.07	0.09	0.05
Behenic acid ($C_{22:0}$)	0.06	0.13	0.07
Docosenic acid ($C_{22:1}$)	0.04	0.08	ND
Docosadienoic acid ($C_{22:2}$)	0.19	0.32	0.05
Tricosanoic acid ($C_{23:0}$)	0.35	0.31	0.08
Tetracosanoic acid ($C_{24:0}$)	0.46	1.00	0.10
Sum of other fatty acids	2.64	2.10	0.04

ND = Not detected.

100ml of milk was sampled at each milking for 2 consecutive days throughout the trial. The am and pm milk samples were then combined for each goat on every sampling day. The collected milk samples were freeze dried and then subjected to analyses of fat, protein and lactose content and fatty acid composition of fat.

Ruminal contents were collected on 2 consecutive days in each period through the rumen fistula prior to morning feeding, and at 1, 3 and 6 h post-feeding, and were strained through 4 layers of cheese cloth to collect the rumen fluid at each sampling time. The pH of rumen fluid was measured instantly, and 5 ml rumen fluid was collected for ammonia and volatile fatty acid (VFA) analysis. All rumen fluid samples collected were kept frozen at -20°C until analyzed. Approximately 50 g of fresh feces was also collected at 6 h post-feeding after the collection of ruminal contents for two consecutive days in each experimental period.

Proximal analysis of the diets and feces, and of Cr in feces followed the methods of AOAC (1995). Neutral detergent fiber (NDF) content was estimated by the method of Van Soest et al. (1991). Ammonia-N concentration was determined by the method of Fawcett and Scott (1960) using a spectrophotometer (DU-650). Rumen fluid (4 ml) was mixed with 1 ml 25% phosphoric acid and 0.5 ml pivalic acid solution (1% w/v) as an internal standard for VFA analysis. The mixed solution was centrifuged at 15,000×g for 15 min. and the supernatant was used to determine the concentration and composition of VFA using a gas chromatograph (GC, HP5890 seriesII, Hewlett Packard Co.) equipped with a flame ionization detector (FID). Methane production was estimated from the molar proportion of major VFAs by the following equation of Moss et al. (2000).

$$CH_4 \text{ (mmoles/100 mmoles rumen fluid)} = 0.45C_2\text{-}0.275C_3\text{+}0.40C_4$$

An aliquot of goat milk was freeze dried and contents of fat and protein were determined by the method of AOAC (1995). Dried milk was defatted using Soxhlet apparatus. Five grams of the defatted milk powder was dissolved in 100 ml of distilled water, deproteinized with 10% sulfosalicylic acid (Whiting et al., 1971), and centrifuged (20,000×g, 20 min). The supernatant was filtered with a millipore syringe driven filter unit (Hydrophilic PTEE 0.45 μm) and then milk lactose content was measured by a High-performance Liquid Chromatograph (HPLC Acme9000, Yonglin Co., Korea) equipped with an ELS detector. A column (Luna 5μ NH_2 100A, 250×4.6 mm) was used, and effluent of acetonitrile: water (80:20) was chosen as the

mobile phase at a flow rate of 0.7 ml/min.

Fatty acid analyses

For the measurement of CLA production in rumen fluid, lipids in culture solution were extracted using Folch's solution (Folch et al., 1957), and methylation of fatty acid followed the method of Lepage and Roy (1986) prior to injecting into a GC (Agilent 6890N) equipped with HP chemistation software for peak integration. A 100 m fused silica capillary column (Supelco SP^{TM}-2560, 0.25 mm i.d. USA) was used with high purity (99.999%) He as carrier gas at a flow rate of 45 ml/min. Injector and detector temperatures were 240 and 250°C, respectively. The split ratio to the FID detector was 1:100. The oven temperature was maintained at 140°C for 2 min, increased to 240°C at a rate of 4°C/min, and maintained at 240°C for 40 min. Lipids in milk powder for fatty acid analysis were extracted using Folch's solution. Preparation of FA methyl esters of milk fat followed the method of ISO 15884 (2002) prior to injecting into a GC (Agilent 6890N). Capillary column, carrier gas and split ratio to the FID detector were the same as for lipids in rumen fluid. Injector and detector temperatures were 270 and 280°C, respectively. The oven temperature was multi-step programmed as follows: initial oven temperature was maintained at 40°C for 2 min, increased to 130°C at a rate of 10°C/min, and held for 1 min, then increased at a rate of 6.5°C/min to 170°C, followed by a rate of 2.75°C/min to 215°C and held there for 12 min, then increased at 40°C/min to 230°C and held for 10 min, and finally increased to 240°C and maintained for 5 min. *Cis*9,*trans*11-CLA and *trans*10,*cis*12-CLA isomers (Sigma, USA) were used to identify and quantify each CLA isomer. Other FA standards were obtained from Supelco Co. (18919, USA). Tridecanoic acid ($C_{13:0}$) was used as an internal standard and all CLA isomers and other FAs in rumen fluid were quantified using FA standards.

Statistical analysis

The results were analyzed as a 4×4 Latin square design using the general linear procedure of SAS (1985) with a model that included diet effect (main effect), and row and column effects (already known source of variation). Significances were compared by S-N-K Test (Steel and Torrie, 1980) with the following statistical model:

$$y_{ijk} = \mu+\alpha_i+\tau_j+\beta_k+\varepsilon_{ijk}$$

Where:

y_{ijk} : observation value in row i, column k, treatment j

μ : overall mean

α_i : i^{th} row effect

τ_j : j^{th} treatment effect(main effect)

β_k : k^{th} column effect

ε_{ijk} : random error ~ NID(0, σ^2)

RESULTS

Intake and whole tract digestibility

The daily intakes of dry matter (DM), crude protein, organic matter and NDF by lactating goats were slightly reduced by feeding the supplemented diets of soybean oil (SO), soybean oil with sodium bicarbonate (SO-B) and soybean oil with sodium bicarbonate and monensin (SO-BM) compared to the CON diet, but whole tract digestibility of these constituents was not different among treatments except that lipid digestibility was higher

Table 3. Daily intake and whole tract digestibility of the diets by lactating goats as influenced by soybean oil supplementation with sodium bicarbonate or with sodium bicarbonate and monensin

	Treatments[1]				SEM[2]	Pr<F[3]
	CON	SO	SO-B	SO-BM		
Daily intake (kg)						
Dry matter	1.60	1.54	1.46	1.50	0.135	0.791
Organic matter	1.48	1.43	1.34	1.38	0.067	0.597
Crude protein	0.23	0.24	0.20	0.21	0.021	0.635
Ether extract	0.07^{b}	0.12^{a}	0.13^{a}	0.12^{a}	0.026	0.006
Neutral detergent fiber	0.54	0.51	0.48	0.51	0.035	0.498
Whole tract digestibility (%)						
Dry matter	91.16	91.77	89.97	90.35	0.689	0.349
Organic matter	92.16	92.70	91.01	91.45	2.790	0.453
Crude protein	90.70	92.81	89.87	91.15	2.354	0.649
Ether extract	89.61^{b}	93.78^{a}	93.44^{a}	93.15^{a}	2.166	0.008
Neutral detergent fiber	86.28	84.30	84.47	85.21	3.264	0.456

[1] Referred to Table1. Means in the same row with different superscripts differ significantly.
[2] Standard error of the mean. [3] Probability levels.

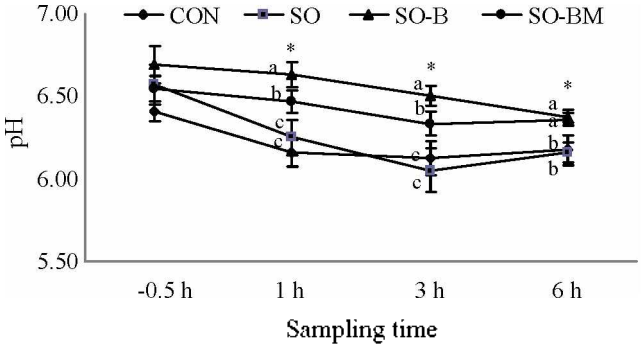

Figure 1. pH of rumen fluid in lactating goats when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin. CON = Commercial concentrate without supplements; SO = Supplemented with soybean oil (5%) to the concentrate; SO-B = Supplemented with soybean oil (5%) and sodium bicarbonate (0.5%) to the concentrate; SO-BM, supplemented with soybean oil (5%) and sodium bicarbonate (0.5%), monensin (30 ppm) to the concentrate. Means at the same sampling time with different superscripts differ significantly. * $p<0.05$.

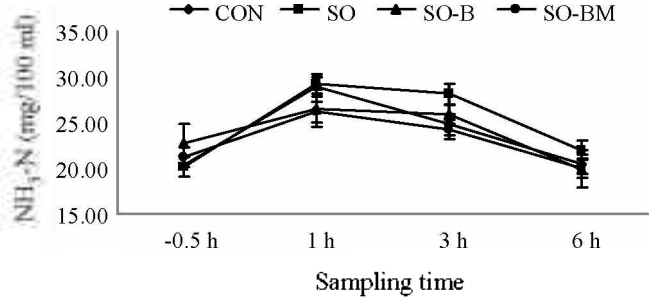

Figure 2. Ammonia-N concentration in rumen fluid of lactating goats when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin. CON, commercial concentrate without supplements; SO, supplemented with soybean oil (5%) to the concentrate; SO-B, supplemented with soybean oil (5%) and sodium bicarbonate (0.5%) to the concentrate; SO-BM, supplemented with soybean oil (5%) and sodium bicarbonate (0.5%), monensin (30 ppm) to the concentrate.

($p<0.008$) for SO, SO-B and SO-BM diets than for the CON diet (Table 3).

Rumen fermentation, methane estimation and CLA production in rumen fluid

Supplementation of sodium bicarbonate (SO-B) or sodium bicarbonate with monensin (SO-BM) to the soybean

Table 4. Total concentration and proportions of major VFA in rumen fluid of lactating goats when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin

Time	Treatments[1]				SEM[2]	Pr<F[3]
	CON	SO	SO-B	SO-BM		
30 min prior to feeding						
Total VFA (mmole/100 ml)	31.71	33.80	38.59	31.74	3.148	0.102
Molar proportion (mmoles/100 mmoles)						
Acetic acid (C_2)	48.28	47.66	46.59	45.42	1.507	0.099
Propionic acid (C_3)	19.82^{b}	27.22^{a}	26.45^{a}	31.89^{a}	2.029	0.003
Butyric acid	23.86	17.64	20.39	20.51	2.785	0.077
C_2/C_3	2.44^{a}	1.81^{b}	1.77^{b}	1.47^{b}	0.263	0.003
1 h post feeding						
Total VFA (mmole/100 ml)	44.25	56.31	44.20	49.54	4.733	0.059
Molar proportion (mmoles/100 mmoles)						
Acetic acid (C_2)	46.55	44.17	45.27	45.95	4.032	0.876
Propionic acid (C_3)	24.38	28.81	29.56	28.55	2.479	0.129
Butyric acid	22.34	15.58	18.82	19.16	2.524	0.058
C_2/C_3	1.92	1.57	1.54	1.65	0.204	0.176
3 h post feeding						
Total VFA (mmole/100 ml)	44.82	56.27	48.12	57.77	5.067	0.072
Molar proportion (mmoles/100 mmoles)						
Acetic acid (C_2)	47.84	47.74	45.48	44.55	2.711	0.393
Propionic acid (C_3)	25.84	32.69	29.79	32.20	4.883	0.336
Butyric acid	20.75	14.00	18.90	19.30	4.203	0.251
C_2/C_3	1.86	1.50	1.53	1.44	0.270	0.291
6 h post feeding						
Total VFA (mmole/100 ml)	41.83	54.84	50.69	59.93	4.512	0.063
Molar proportion (mmoles/100 mmoles)						
Acetic acid (C_2)	48.15	46.97	45.80	44.33	2.187	0.204
Propionic acid (C_3)	24.51^{b}	30.98^{ab}	29.29^{ab}	34.15^{a}	3.137	0.029
Butyric acid	21.37^{a}	16.31^{b}	19.10^{ab}	18.15^{ab}	1.778	0.020
C_2/C_3	1.97^{a}	1.55^{ab}	1.56^{ab}	1.41^{b}	0.212	0.029

[1] Refer to Table1. [2] Standard error of the mean. [3] Probability levels.

oil (SO) added diet increased ($p<0.05$) ruminal pH at 1 h-6 h post-feeding compared to CON and SO diets (Figure 1), but ammonia concentration in rumen fluid was not influenced by the supplements although it was slightly increased on the SO diet at 3 h and 6 h post-feeding compared to the other diets (Figure 2). Feeding SO, SO-B and SO-BM diets slightly increased total VFA concentration in the rumen fluid of lactating goats for all sampling times compare to the CON diet (Table 4). Increased molar proportion of propionate (C_3) was observed at 1 h ($p<0.003$) and 6 h ($p<0.029$) post-feeding from the SO, SO-B and SO-BM diets without difference among supplemented diets. While molar proportion of acetate (C_2) was not influenced by the diets, that of butyrate (C_4) was lower ($p<0.020$) for the SO diet at the 6 h sampling time than for the CON diet. The C_2/C_3 ratios were lower at 1 h ($p<0.003$) and 6 h ($p<0.029$) post-feeding for all the supplemented diets than for the CON diet. Calculated molar proportion of methane based on major VFAs was markedly decreased prior to morning feeding ($p<0.01$), and 1 h ($p<0.05$) and 6 h post-feeding ($p<0.05$) for goats fed all supplemented diets (Figure 3).

Feeding the SO, SO-B or SO-BM diet increased ($p<0.0001$) *cis*9, *trans*11-CLA concentration in rumen fluid from 1 h to 6 h post-feeding compared to the CON diet and increased *trans*10, *cis*12-CLA at 3 h post-feeding (Table 5). Thus, the sum of both CLA isomers was greatly increased ($p<0.0001$-$p<0.0006$) from 1 h to 6 h post-feeding by feeding the supplemented diets compared to the CON diet. Concentrations of *cis*9, *trans*11-CLA ($p<0.0001$) and the sum of both CLAs ($p<0.0006$) were even greater in the rumen fluid of lactating goats fed the SO-B and SO-BM diets at 1 h post-feeding than the SO diet.

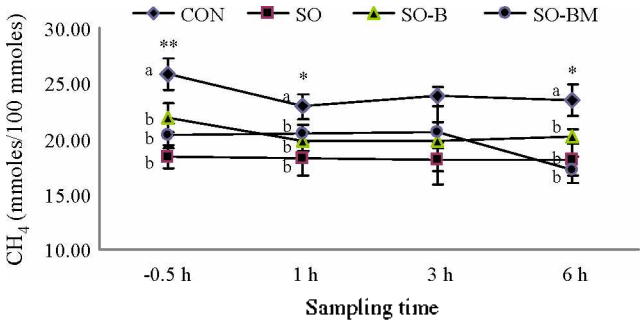

Figure 3. Estimation of methane emission (mmoles/100 mmoles VFA) from rumen fluid of lactating goats when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin. CON = Commercial concentrate without supplements; SO = Supplemented with soybean oil (5%) to the concentrate; SO-B = Supplemented with soybean oil (5%) and sodium bicarbonate (0.5%) to the concentrate; SO-BM = Supplemented with soybean oil (5%) and sodium bicarbonate (0.5%), monensin (30 ppm) to the concentrate. Means at the same sampling time with different superscripts differ significantly. * $p<0.05$; ** $p<0.01$.

Milk components, C_{18}-fatty acid profile and CLA content in milk fat

Milk yield, and contents of fat and lactose in goat milk were not influenced but milk fat was increased ($p<0.035$) by feeding of SO, SO-B or SO-BM diets compared to the CON diet (Table 6). Table 7 shows C_{18}-fatty acid profile in

Table 5. CLA production in rumen fluid of lactating goats when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin

	Treatments (mg/50 ml rumen fluid)[1]				SEM[2]	Pr<F[3]
	CON	SO	SO-B	SO-BM		
30 min prior to feeding						
*cis*9,*trans*11CLA	0.20^{b}	0.45^{ab}	0.29^{b}	0.72^{a}	0.158	0.026
*trans*10,*cis*12CLA	0.06	0.17	0.09	0.11	0.046	0.073
Sum of CLAs	0.26^{b}	0.62^{ab}	0.38^{b}	0.83^{a}	0.159	0.011
1 h post feeding						
*cis*9,*trans*11CLA	0.15^{c}	1.32^{b}	1.37^{b}	2.20^{a}	0.260	<0.0001
*trans*10,*cis*12CLA	0.10	0.53	0.46	0.58	0.349	0.272
Sum of CLAs	0.25^{c}	1.85^{b}	1.82^{b}	2.78^{a}	0.549	0.001
3 h post feeding						
*cis*9,*trans*11CLA	0.37^{c}	0.84^{b}	1.72^{a}	1.71^{a}	0.229	<0.0001
*trans*10,*cis*12CLA	0.06^{c}	0.39^{b}	0.38^{b}	1.05^{a}	0.097	0.013
Sum of CLAs	0.43^{c}	1.23^{b}	2.11^{a}	2.77^{a}	0.404	0.0002
6 h post feeding						
*cis*9,*trans*11CLA	0.35^{b}	1.25^{a}	1.21^{a}	1.27^{a}	0.162	<0.0001
*trans*10,*cis*12CLA	0.10	0.38	0.25	0.29	0.147	0.152
Sum of CLAs	0.45^{b}	1.63^{a}	1.35^{a}	1.56^{a}	0.228	0.0001

[1] Refer to Table1. [2] Standard error of the mean. [3] Probability levels.

Table 6. Yield and composition of lactating goat milk when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin

Items	Treatment[1]				SEM[2]	Pr<F[3]
	CON	SO	SO-B	SO-BM		
Milk yield (kg/d)	1.62	1.46	1.50	1.55	0.523	0.979
Milk DM (%)	11.32	12.20	12.13	12.25	0.550	0.106
Milk protein (%)	4.05	4.35	4.32	4.37	0.179	0.091
Milk fat (%)	3.17[b]	3.99[a]	4.00[a]	4.11[a]	0.366	0.035
Milk lactose (%)	3.81	3.93	3.82	3.78	0.261	0.579

[1] Refer to Table1. [2] Standard error of the mean. [3] Probability levels.

milk fat as influenced by the supplementation of soybean oil alone (SO) or with sodium bicarbonate (SO-B) or with sodium bicarbonate and monensin (SO-BM). Concentrations of stearic acid ($p<0.001$), elaidic acid ($p<0.020$), oleic acid ($p<0.046$), vaccenic acid ($t11$-$C_{18:1}$, $p<0.001$) and $C_{18:2}$ ($p<0.009$) were increased in the milk fat of goats fed the supplemented diets compared to those fed the CON diet. The highest concentration of $t11C_{18:1}$ was obtained from the SO-BM diet followed by the SO-B diet. As expected, concentrations of both *cis*9, *trans*11-CLA ($p<0.0001$) and *trans*10, *cis*12-CLA ($p<0.026$), and the sum of both CLAs ($p<0.001$) were also increased in the milk fat for all supplemented diets (Table 7).

When compared with the CON diet, all supplements increased the concentrations of saturated fatty acids (SFA, $p<0.038$), unsaturated fatty acids (UFA, $p<0.002$), monounsaturated fatty acids (MUFA, $p<0.002$) and polyunsaturated fatty acids ($p<0.014$) but reduced SFA to UFA ratio ($p<0.023$, Table 7). The concentration of MUFA was even greater ($p<0.002$) for the SO-BM than the SO-B diet.

DISCUSSION

The present study was conducted to examine CLA production in the rumen and in milk fat, and to evaluate methane production in the rumen of lactating goats when various dietary supplements were applied. Soybean oil added to the diet should increase energy density and has been useful as a source of $C_{18:2}$, which is one of the two major precursors of CLA. Sodium bicarbonate acted to control pH in rumen fluid (Schmidely et al., 2005), and monensin modified ruminal fermentation (Wang et al., 2005) and affected, to some extent, CLA production in the rumen (Wang et al., 2006).

Supplementation of a high level of fat or oil to the diet generally reduces intake and digestibility, depending upon level of addition. Reduction in DM intake has been widely

Table 7. C_{18} fatty acid profiles and CLA (mg/g milk powder, DM) concentration in milk fat of lactating goats when fed a soybean oil supplemented diet either with sodium bicarbonate or with sodium bicarbonate and monensin

	Treatments[1] (mg/g milk powder)				SEM[2]	Pr<F[3]
	CON	SO	SO-B	SO-BM		
$C_{18:0}$	9.31[b]	19.34[a]	18.33[a]	17.27[a]	2.697	0.001
$c9$-$C_{18:1}$	7.95[b]	26.50[a]	23.19[a]	20.26[a]	5.540	0.020
$t9$-$C_{18:1}$	13.06[b]	22.49[a]	29.63[a]	22.88[a]	3.764	0.046
$t11$-$C_{18:1}$	0.69[c]	1.36[b]	1.64[a]	1.69[a]	0.685	0.001
$C_{18:2}$	3.15[b]	6.64[a]	6.33[a]	6.85[a]	1.726	0.009
Sum of CLAs	1.22[c]	3.18[b]	3.59[a]	3.96[a]	0.870	0.001
$c9,t11$CLA	1.04[b]	2.95[a]	3.29[a]	3.74[a]	0.908	<0.0001
$t10,c12$CLA	0.074[c]	0.16[b]	0.29[a]	0.22[a]	0.042	0.026
$C_{18:3}$	0.15	0.19	0.25	0.21	0.067	0.655
others	48.73	56.61	58.42	55.73	9.560	0.123
SFA	48.95[b]	73.26[a]	67.26[a]	69.92[a]	6.420	0.038
UFA	36.00[c]	60.93[b]	81.02[a]	74.94[a]	4.523	0.002
MUFA	31.88[c]	47.26[b]	66.24[a]	58.98[a]	3.420	0.002
PUFA	4.13[b]	13.67[a]	14.78[a]	15.97[a]	2.072	0.014
SFA/UFA	1.36[a]	0.93[b]	0.84[b]	0.93[b]	0.107	0.023

[1] Refer to Table1. [2] Standard error of the mean. [3] Probability levels.

reported after the inclusion of high levels of oils to ruminant diets (Sutton et al., 1983; Sutter et al., 2000; Jordan et al., 2004). In the present study, however, DM intake and whole tract digestibility of most major dietary components, except for lipid, were not greatly affected by addition of soybean oil at 5% level (DM) to the concentrate (Table 3). This result might be due to the low level of oil addition.

Increased ruminal pH of lactating goats fed SO-B and SO-BM diets (Figure 1) was likely due to the addition of sodium bicarbonate (0.5% of concentrate) as reported by Hadjipanayiotou (1988). Schmidely et al. (2005) also found that soybean oil supplementation with sodium bicarbonate slightly increased rumen VFA concentrations and the molar proportion of acetate. In the present study, feeding of all supplemented diets slightly increased total VFA concentration and molar proportion of propionate in the rumen (Table 4). The increased propionate proportion was associated with decreased proportions of acetate and butyrate.

In the current study, the reduction in CH_4 output (Figure 3) as calculated by molar proportion of major VFAs at indicated sampling times of rumen fluid was more likely to be a result of supplementation of oils with sodium bicarbonate and monensin, and shifted molar concentration of VFA from acetate to propionate (Table 4). Such theoretical calculations have been confirmed by *in vitro* study where the end products were easily quantified (Moss et al., 2000). Acetate and butyrate promote methane production while propionate formation can be considered as a competitive pathway for hydrogen use in the rumen (Moss et al., 2000; Newbold et al., 2005). This result is consistent with the idea that propionate production and methane generation are competing for hydrogen in the rumen. Monensin depressed methane production by mixed rumen microbes *in vitro* (Van Nevel and Demeyer, 1992). In addition, reductions in CH_4 emissions in the rumen have been widely reported when a variety of plant oils were added to the diet (Cies'lak, 2003; Jordan et al., 2004) and the level of methane emission in ruminants is directly proportional to bio-hydrogenation of UFA (Plascencia et al., 1999). It was also found that the addition of soybean oil in the diet (6% of DMI) decreased 40% of CH_4 output without decreasing the intake (Jordan et al., 2006). Otherwise, Chen and Wolin (1979) reported that methane depression by oil supplementation might result from a shift in bacterial population from gram positive to gram negative organisms with a concurrent shift in fermentation from acetate to propionate.

The increased content of CLA in rumen fluid of lactating goats fed the supplemented diets (Table 5) might be basically due to the soybean oil supplementation, and monensin could also contribute indirectly to CLA production in the rumen. Supplementation of plant oil to the diet has been shown to increase CLA production in rumen fluid (Dhiman et al., 2000; Wang et al., 2003). The monensin effect on CLA production by microbes, however, has been controversial. Monensin increased the proportion of *cis*-9,*trans*-11CLA *in vitro* (Fellner et al., 1997) and *in vivo* (Sauer et al., 1998; Wang et al., 2006) by interfering with the bio-hydrogenation of *cis*-9, *cis*-12 $C_{18:2}$, with an accumulation of intermediate products, including CLA (Fellner et al., 1997). In contrast, Wang et al. (2005) did not find any effect of monensin (10 ppm) on CLA production when incubated with safflower oil *in vitro*. Jin et al. (2008) also found a small effect of monensin on CLA production in the rumen of Holstein cows when fed a diet supplemented with $C_{18:2}$-rich soybean oil and sodium bicarbonate.

In the present study, content of milk fat was markedly increased and content of milk protein was slightly increased by supplementation of soybean oil at a 5% level of concentrate (Table 6). Increased milk fat content of lactating goats as influenced by plant oil supplementation was similar to the results reported by Daccord (1987). However, Chilliard et al. (2006) reported that the addition of poly-unsaturated fatty acid rich-plant oil to high-concentrate diets did not increase milk fat of lactating goats. Bauman and Griinari (2003) also reported that goat milk fat content was not decreased when vegetable oils were added to a low-forage diet. Chilliard and Bocquier (1993) found that, although fat supplementation to the diets of lactating cows and ewes often decreased milk protein content and associated coagulation properties, these negative effects were not observed in goats. Such differences in milk fat between species (lactating goat vs. dairy cow) could be related to differences in the metabolism of fatty acids in the rumen or in the mammary gland (Schmidely and Sauvant, 2001).

High levels of $C_{18:2}$ or $C_{18:3}$ in the form of oil seeds or oils are known to enhance the CLA content in milk fat of dairy cows (Loor et al., 2005; Shingfield et al., 2006; Bu et al., 2007; Chantaprasarn and Wanapat, 2008) and in meat (Choi et al., 2006; Wang et al., 2006). Bouattour et al. (2008) suggested that feeding a moderate dose of soybean oil to lactating goats would be a useful way to increase CLA content in milk, and to reduce the atherogenicity index without negative effects on intake, milk yield, and protein content. Gómez-Cortés et al. (2008) found that CLA content in milk of lactating sheep can be substantially increased (more than 3-fold) by adding high levels of soybean oil in the diet without any negative effects on animal performance. In the present study, the significant increase in concentration of *cis*9, *trans*11-CLA was certainly due to the supplemented soybean oil. An increase in ruminal pH by

addition of sodium bicarbonate might also stimulate the rate of lipolysis and CLA content in milk (Van Nevel and Demeyer, 1996; Kennelly et al., 1999; Khorasani and Kennelly, 2001) as ruminal pH modifies the extent of biohydrogenation of dietary polyunsaturated fatty acids in the rumen (Kalscheur et al., 1997). In addition, Alzahal et al. (2008) found monensin interacted with PUFA rich-soybean oil, and thus linearly increased *cis*9, *trans*11-CLA concentration in milk fat of lactating goats. In contrast, Dhiman et al. (1996) and Chouinard et al. (1998) observed no effect of monesin on CLA content in milk fat. In the current study, monensin supplemented with soybean oil and sodium bicarbonate slightly increased *cis*9, *trans*11-CLA content in milk fat compared to other oil treatments.

In conclusion, feeding a moderate dose of soybean oil (5% of concentrate) to lactating goats was a useful way to improve both milk fat and fatty acid profile in the milk by increasing potentially healthy fatty acids such as CLA without any detrimental effects on milk production. Supplementation of sodium bicarbonate or sodium bicarbonate with monensin to the soybean oil based diet increased CLA content. Supplementation of soybean oil may be an effective means to reduce methane emission in the lactating goat.

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