Asian-Aust. J. Anim. Sci.
Vol. 21, No. 12 : 1728 - 1735
December 2008

www.ajas.info

Effects of Monensin and Fish Oil on Conjugated Linoleic Acid Production by Rumen Microbes in Holstein Cows Fed Diets Supplemented with Soybean Oil and Sodium Bicarbonate*

Jin, G. L., S. H. Choi, H. G. Lee[1], Y. J. Kim[2] and M. K. Song**
Department of Animal Science, Chungbuk National University, Cheongju, 361-763, Korea

ABSTRACT : The present study was conducted with four ruminally canulated Holstein cows to observe the effects of monensin or fish oil on diet fermentation and production of conjugated linoleic acids (CLAs) in the rumen when fed diets supplemented with soybean oil and sodium bicarbonate. Cows of the control treatment were fed a basal diet (CON) consisting of 60% commercial concentrate and 40% chopped rye grass hay. Cows of other treatments were fed the same diet as CON, but the concentrate was supplemented with 7% of soybean oil and 0.5% of sodium bicarbonate (SO-B), SO-B supplemented with monensin (30 ppm, SO-BM) or concentrate supplemented with 6.3% of soybean oil, 0.5% of sodium-bicarbonate, 30 ppm of monensin and 0.7% of fish oil (SO-BMF). Dry matter (DM) intake of the cows was significantly ($p<0.011$) reduced by feeding the SO-BMF diet compared to the other diets which did not differ in DM intake. Whole tract digestibility of major dietary components was significantly ($p<0.004$-0.027) higher for SO-BMF than the other supplement-containing diets. Dietary supplements did not clearly affect rumen pH and ammonia concentrations compared to the CON diet. Significantly reduced ($p<0.05$) total VFA concentration was obtained by the addition of fish oil to the diet (SO-BMF) compared to other diets. No differences, however, were obtained in major VFA proportions as well as in total VFA between the supplemented diets. The SO-BM diet increased ($p<0.01$-0.05) the concentrations of *trans*-11 $C_{18:1}$ and linoleic acid in rumen fluid. Total CLA concentration was also increased by the feeding of SO-B and SO-BM diets during early fermentation times (up to 3 h) post-feeding. *Cis*-9, *trans*-11 CLA concentration in rumen fluid was highest ($p<0.05$) for SO-B up to 1 h while the highest ($p<0.01$) value for SO-BM occurred at 3 h post-feeding. An increased *trans*-10, *cis*-12 CLA concentration was obtained from the SO-B and SO-BM diets at 1 and 3 h post feeding compared to the other diets. Supplementation of oils with monensin and sodium bicarbonate increased ($p<0.05$) the proportions of $C_{18:1}$ and CLA in the plasma of cows, but the effect of monensin and/or fish oil was limited to *trans*-10, *cis*-12 CLA.
(**Key Words :** Monensin, Fish Oil, Soybean Oil, Buffer, CLA, Fatty Acid Composition, Biohydrogenation, Rumen Microbes, Plasma, Holstein Cows)

INTRODUCTION

There has been an increasing interest in natural nutrients of ruminant products that have health benefits for humans, and one of them is conjugated linoleic acid (CLA). CLA has several important physiological functions, such as anti-carcinogenic (Ha et al., 1987; Albright et al., 2005) and anti-atherogenic (Lee et al., 1994) effects, immune-modulation (Whigham et al., 2002), and reduction of body fat (Cook et al., 1993). These fatty acids are generated naturally in the rumen or mammary gland of ruminants.

Various attempts to increase CLA content in ruminant products have included the application of lipid source. High levels of linoleic and linolenic acids in the form of oil seeds or oils are known to enhance the formation of CLA in the rumen fluid (Wang et al., 2002), milk (Dhiman et al., 2000; Loor et al., 2005) or meat (Choi et al., 2006; Wang et al., 2006).

Because the ruminal environment is subject to change by dietary manipulation it is possible that changes in pH of rumen fluid will have an effect on CLA formation (Wang et

* This work was supported by a grant (Code 20050401034701) from BioGreen 21 Program, Rural Development Administration, Korea.
** Corresponding Author: Man K. Song. Tel: +82-43-261-2545, Fax: +82-43-273-2240, E-mail: mksong@cbnu.ac.kr
[1] Department of Bio-Resources and PNU-Special Animal Biotechnology Center, College of Natural Resources & Life Science, Pusan National University, Kyung Nam, 627-702, Korea.
[2] Department of Food and Biotechnology, Korea University, Chochiwon, Chungnam, 339-700, Korea.
Received February 12, 2008; Accepted June 5, 2008

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

Items	Treatments[1]			
	CON	SO-B	SO-BM	SO-BMF
Ingredients of concentrate				
Commercial concentrate (%)	100.00	92.50	92.50	92.50
Soybean oil (SO, %)	-	7.00	7.00	6.30
Sodium bicarbonate (B, %)	-	0.50	0.50	0.50
Monensin (M, ppm)	-	-	30.00	30.00
Fish oil (F, %)	-	-	-	0.70
Nutrient composition of total diets (%) [2]				
Dry matter	88.95	89.72	89.58	89.77
Organic matter	90.78	90.63	90.82	90.90
Crude protein	20.14	18.84	18.07	18.24
Ether extract	7.79	15.65	15.62	15.77
Neutral detergent fiber	40.74	38.29	38.21	38.23
Crude ash	9.22	9.37	9.08	9.10

[1] CON = Fed commercial concentrate and forage without supplements; SO-B = Supplemented with soybean oil (7%) and sodium bicarbonate (0.5%) to concentrate; SO-BM = Supplemented with monensin (30 ppm) to SO-B diet; SO-BMF = Supplemented with soybean oil (6.3%), sodium bicarbonate (0.5%), monensin (30 ppm) and fish oil (0.7%).

[2] Mixed diet of concentrate with forage (chopped rye grass hay).

al., 2002). Wang and Song (2003) reported that formation of *cis*-9, *trans*-11 CLA by rumen bacteria increased with the increment of pH when ground oilseeds were incubated *in vitro*. In addition, ionophoric antibiotics have been known to inhibit the ruminal hydrogenation of unsaturated fatty acids. Monensin, for example, inhibited the growth of *Butyrivibrio fibrisolvens*, a very active Gram positive bacterium in hydrogenation, and resulted in an increased proportion of *cis*9, *trans*11-CLA with decreased proportion of stearic acid when incubated with linoleic acid ($C_{18:2}$) *in vitro* (Fellner et al., 1997). Unsaturated fatty acids were accumulated by presence of salinomycin in the rumen fluid and duodenal digesta of sheep (Kobayashi et al., 1992). Wang et al. (2006) also observed an increased proportion of the *cis*9, *trans*11-CLA from Korean native steers when the concentrate was supplemented with monensin.

Table 2. Fatty acid composition of oils used in the current study

Fatty acids	Oils (% of total fatty acid)	
	Soybean oil [1]	Mixed fish oil
Myristic acid ($C_{14:0}$)	5.8	5.7
Pentadecanoic acid ($C_{15:0}$)	ND	1.2
Palmitic acid ($C_{16:0}$)	9.9	21.6
Palmitoleic acid ($C_{16:1}$)	0.1	9.5
Margaric acid ($C_{17:0}$)	ND	2.1
Stearic acid ($C_{18:0}$)	3.3	6.4
Oleic acid ($C_{18:1}$)	17.7	28.2
Linoleic acid ($C_{18:2}$)	62.1	3.3
Linolenic acid ($C_{18:3}$)	0.4	1.1
Arachidonic acid ($C_{20:0}$)	0.3	0.5
Eicosenoic acid ($C_{20:1}$)	0.1	5.3
Eicosapentaenoic acid ($C_{20:5}$)	0.3	3.3
Behenic acid ($C_{22:0}$)	ND	2.0
Erucic acid ($C_{22:1}$)	ND	1.7
Docosahexaenoic acid ($C_{22:6}$)	ND	8.1

[1] ND = Not detected.

Meanwhile, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are rich in fish oil, might be resistant to ruminal hydrogenation *in vitro* and thus could increase CLA contents (Ashes et al., 1992). Wang et al. (2005) also observed *in vitro* an increased proportion of *cis*-9, *trans*-11 CLA from the supplementation of fish oil.

However, although each of these dietary manipulations had some effect on CLA production in the rumen and ruminant products, the effect was relatively small. The current *in vivo* study, therefore, was conducted to determine the effects of monensin and fish oil on the formation of CLA isomers (*cis*-9, *trans*-11 and *cis*-10, *trans*-12 $C_{18:2}$) and fermentation characteristics in the rumen of Holstein cows when fed a diet supplemented with $C_{18:2}$-rich soybean oil and sodium bicarbonate.

MATERIALS AND METHODS

Animals and diets

The metabolic study was conducted as a 4×4 Latin square design with 4 ruminally cannulated non-lactating Holstein cows (640±37 kg) for 4 dietary treatments. The cows were fed a basal diet consisting of 60% commercial concentrate and 40% chopped rye grass hay (DM basis) without any supplements (CON). The cows were also fed the same basal diet as CON cows, but the concentrate was supplemented with 7% of soybean oil and 0.5% of sodium-bicarbonate (SO-B), SO-B diet supplemented with monensin (30 ppm of concentrate, SO-BM) or SO-B diet supplemented with 6.3% soybean oil, 0.5% sodium bicarbonate, 30 ppm monensin and 0.7% fish oil (SO-BMF, Table 1). Experimental diets were prepared at 3 day intervals. Concentrate constituents and nutrient composition of the total diets are shown in Table 1, and fatty acid

Table 3. Supplementation effects of monensin and fish oil on nutrient intake (kg/day) by Holstein cows when fed soybean oil with buffer

Items	Treatments[1]				SEM[2]	Pr<F[3]
	CON	SO-B	SO-BM	SO-BMF		
Daily intakes (kg)						
Dry matter intake	12.46[a]	11.64[b]	11.63[b]	8.50[c]	0.188	<0.001
Organic matter	11.31[a]	10.55[b]	10.57[ab]	7.73[c]	0.086	<0.001
Crude protein	11.39[a]	10.64[b]	10.65[b]	8.42[c]	0.177	<0.001
Ether extract	2.06[a]	1.79[b]	1.65[c]	1.30[d]	0.035	<0.001
Neutral detergent fiber	1.09[b]	1.53[a]	1.48[a]	1.13[b]	0.031	<0.001
Whole tract digestibility (%)						
Dry matter	61.16[b]	55.77[b]	52.47[b]	75.70[a]	3.756	0.004
Organic matter	57.78[b]	59.13[b]	56.10[b]	78.46[a]	3.658	0.004
Crude protein	60.46[b]	60.46[ab]	54.92[b]	56.32[a]	5.687	0.027
Ether extract	65.07	77.42	72.92	88.48	6.580	0.140
Neutral detergent fiber	44.68[b]	53.32[b]	54.46[b]	76.60[a]	5.691	0.011

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

compositions of soybean oil and fish oil are shown in Table 2.

Feeding management

The cows were housed in metabolism crates, and were fed 13 kg (DM basis) of the mixed diets of concentrate and forage twice daily (08:00 and 18:00) in equal amounts, and were allowed free access to water and mineral blocks. Between experimental periods, the cows were allowed free walking in outdoor grounds for 7 days. The study was conducted for 12 weeks in total, with 10 days for diet adaptation and 6 days for the sampling in each experimental period.

Measurements and analysis

Feed residues were collected before morning feeding (08:00) daily to estimate feed intake and digestibility. Ruminal contents were collected on three days during each period from three sites in the rumen immediately before morning feeding and at 1, 3, 6 and 9 h post- feeding, and were squeezed to collect the rumen fluid at each sampling time. The pH of rumen fluid was measured instantly, and after being strained through 4 layers of cheese cloth 5 ml rumen fluid was collected for ammonia and volatile fatty acid (VFA) analysis. All samples collected were kept frozen at -20°C until analyzed. Feces were also collected for three days after the collection of ruminal contents to estimate whole tract digestibility of the major dietary components.

Blood was collected from the jugular vein with a vacutainer (Becton Dickinson) containing sodium heparin from cows at 4 h after feeding on the same days as fecal collection. Blood samples were centrifuged at 3,000×g for 10 min., and plasma was transferred to 30 ml screw-cap tubes and kept frozen at -70°C until analyzed.

Proximal analyses of the diets and feces followed the methods of AOAC (1995). NDF content was estimated by the method of Van Soest et al. (1991). Ammonia 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. 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 gas chromatography (GC, HP 5890II, Hewlett Packard Co.). Lipid was extracted from rumen fluid and plasma using a mixture of organic solvents (one part hexane, three parts isopropanol, one part acetone) purchased from Fisher Co. (Fair Lawn, NJ, USA). The suspensions were then centrifuged at 1,000×*g* for 3 min at 20°C. The solvent (top) layer was removed and flushed with nitrogen gas until dried. Methylation of the fatty acids followed the method of Lepage and Roy (1986) prior to injecting into the GC. A fused silica capillary column (100 m×0.25 mm, i.d.×0.20 μm thickness, Supelco, SP™-2560; USA) was used. *Cis*-9, *trans*-11 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. (18920, 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 obtained were subjected to least squares analysis of variance according to the general linear models procedure of SAS (1985) and significances were compared by S-N-K Test (Steel and Torrie, 1980).

RESULTS

The dry matter intake of the cows was significantly ($p<0.001$) reduced by feeding the SO-BMF diet compared to the other diets; thus, daily intakes of major nutrients were reduced by the SO-BMF diet (Table 3). Whole tract

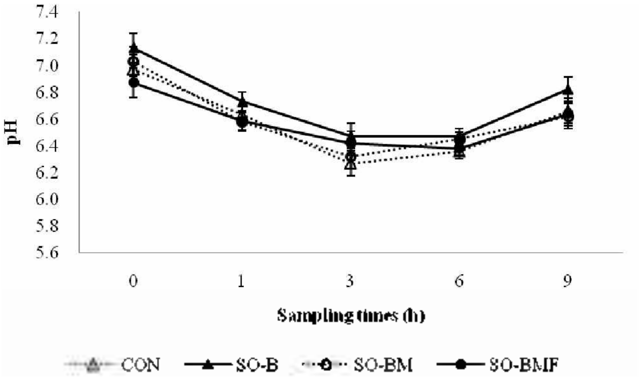

Figure 1. pH of rumen fluid in Holstein cows when fed soybean oil with buffer.

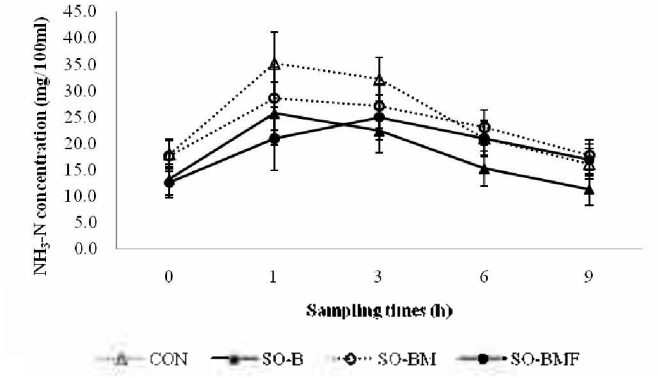

Figure 2. Ammonia-N concentration (mg/100 ml) in rumen fluid of Holstein cows when fed soybean oil with buffer.

digestibility of dry matter ($p<0.004$), crude protein ($p<0.027$), neutral detergent fiber ($p<0.011$) and organic matter ($p<0.004$) were significantly higher for SO-BMF than for other diets which were not influenced by the supplements (Table 3). Dietary supplements did not influence ruminal pH (Figure 1) but slightly lowered

Table 4. Total VFA concentration and individual VFA proportions in rumen fluid of Holstein cows when fed soybean oil with buffer

Items	Treatments[1]				SEM[2]	Pr<F[3]
	CON	SO-B	SO-BM	SO-BMF		
	---------------------- 0.1 h prior to feeding -------------------					
Total VFA (mmoles/100 ml)	124.1	101.6	121.4	91.0	12.352	0.163
Proportions of VFAs (mmoles/100 mmoles)						
Acetic acid (C_2)	43.50	46.84	42.36	41.72	1.565	0.120
Propionic acid(C_3)	31.73	31.70	30.33	31.93	2.374	0.964
Butyric acid	14.39	12.66	16.41	14.37	1.208	0.786
C_2/C_3	1.49	1.60	1.49	1.37	0.181	0.834
	---------------------- 1 h prior to feeding -------------------					
Total VFA (mmoles/100 ml)	140.3[a]	121.1[a]	133.9[a]	102.7[b]	5.256	0.041
Proportions of VFAs (mmoles/100 mmoles)						
Acetic acid (C_2)	43.61	45.03	41.32	41.85	1.390	0.239
Propionic acid(C_3)	31.79	31.92	31.53	33.60	2.594	0.940
Butyric acid	15.03	13.27	16.54	13.48	1.336	0.293
C_2/C_3	1.49	1.49	1.42	1.31	0.165	0.833
	---------------------- 3 h prior to feeding -------------------					
Total VFA (mmoles/100 ml)	129.9[a]	120.3[a]	126.2[a]	87.1[b]	5.584	0.001
Proportions of VFAs (mmoles/100 mmoles)						
Acetic acid	43.84	43.26	41.99	42.77	1.737	0.893
Propionic acid	29.03	31.33	29.65	26.23	3.119	0.710
Butyric acid	15.38	16.35	16.40	12.59	1.780	0.403
C_2/C_3	1.70	1.47	1.56	2.17	0.338	0.472
	---------------------- 6 h prior to feeding -------------------					
Total VFA (mmoles/100 ml)	138.8	124.3	131.2	103.1	14.524	0.149
Proportions of VFAs (mmoles/100 mmoles)						
Acetic acid	43.01	42.53	42.15	43.03	2.226	0.991
Propionic acid	30.58	30.48	30.73	29.10	3.646	0.788
Butyric acid	16.67	17.46	16.46	11.72	0.924	0.163
C_2/C_3	1.60	2.35	1.52	2.15	0.711	0.800
	---------------------- 9 h prior to feeding -------------------					
Total VFA (mmoles/100 ml)	108.2[a]	110.4[a]	111.8[a]	63.5[b]	10.325	0.042
Proportions of VFAs (mmoles/100 mmoles)						
Acetic acid	41.50	39.66	41.09	43.86	1.408	0.228
Propionic acid	33.24	35.72	32.35	32.18	2.343	0.695
Butyric acid	15.40	15.77	16.40	13.84	1.580	0.714
C_2/C_3	1.34	1.14	1.34	1.44	0.143	0.535

[1] Referred to Table 1. [2] Standard error of the mean. [3] Probability levels.

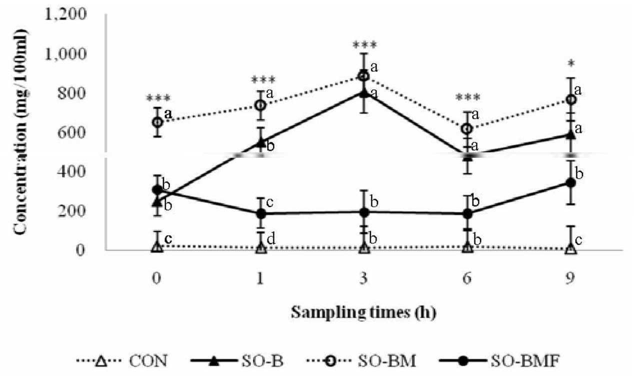

Figure 3. Additive effects of monensin or fish oil on stearic acid ($C_{18:0}$) content (mg/100 ml) in rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$; *** $p<0.005$.

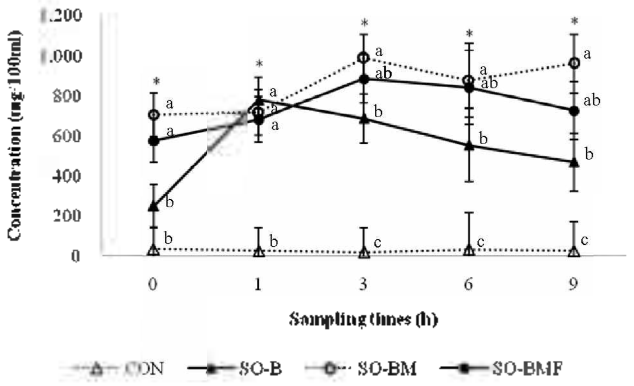

Figure 4. Additive effects of monensin or fish oil on oleic acid (*cis*9-$C_{18:1}$) content (mg/100 ml) in rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$.

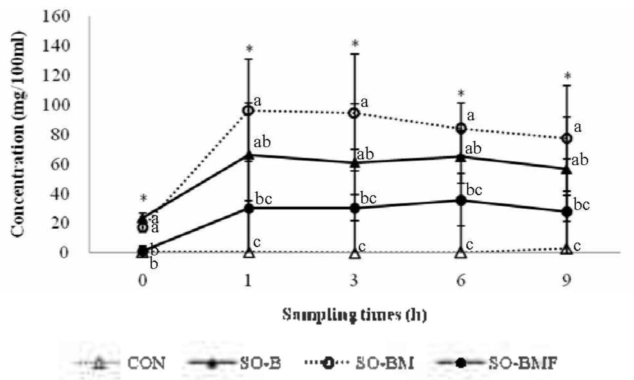

Figure 5. Additive effects of monensin or fish oil on vaccenic acid (*trans*11-$C_{18:1}$) content (mg/100 ml) in the rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$.

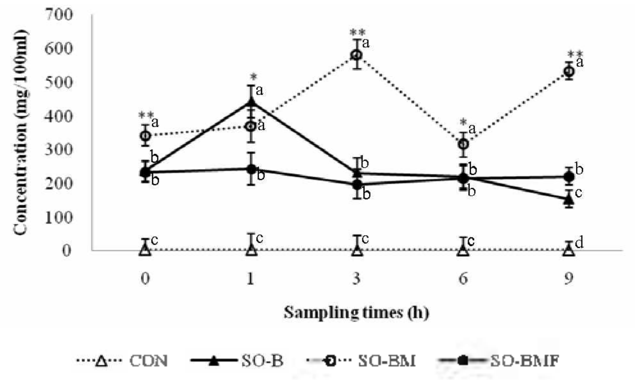

Figure 6. Additive effects of monensin or fish oil on linoleic acid ($C_{18:2}$) content (mg/100 ml) in rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$; ** $p<0.01$.

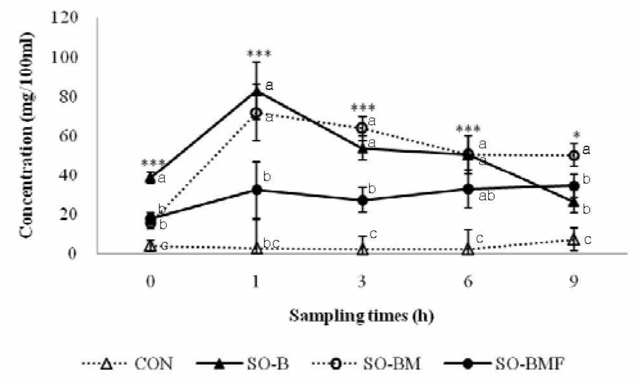

Figure 7. Additive effects of monensin or fish oil on total CLA content (mg/100 ml) in rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$; *** $p<0.005$.

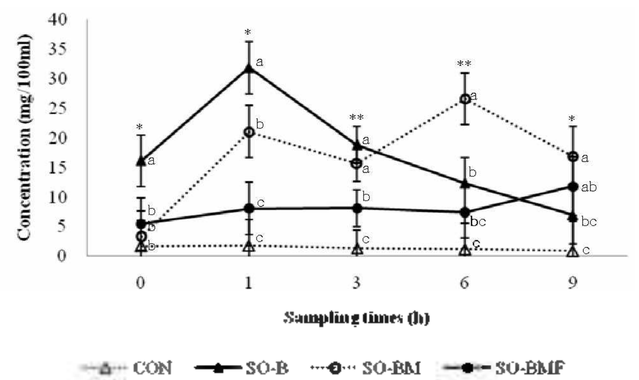

Figure 8. Additive effects of monensin or fish oil on *cis*-9, *trans*-11 CLA content (mg/100 ml) in rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$; ** $p<0.01$.

ammonia concentrations in rumen fluid at 1 and 3 h post feeding compared to the CON diet (Figure 2). Significantly reduced ($p<0.05$) total VFA concentration was obtained by the addition of fish oil (SO-BMF) compared to other diets at most sampling times post-feeding (Table 4). No differences, however, were obtained in major VFA proportions or in total VFA between the supplement combinations, although butyric acid proportion was slightly decreased by the SO-BMF diet (Table 4).

The SO-B and SO-BM diets increased ($p<0.001$-0.05) the ruminal concentrations of both $C_{18:0}$ (Figure 3) at all sampling times and $C_{18:1}$ (Figure 4) at 3, 6 and 9 h post-

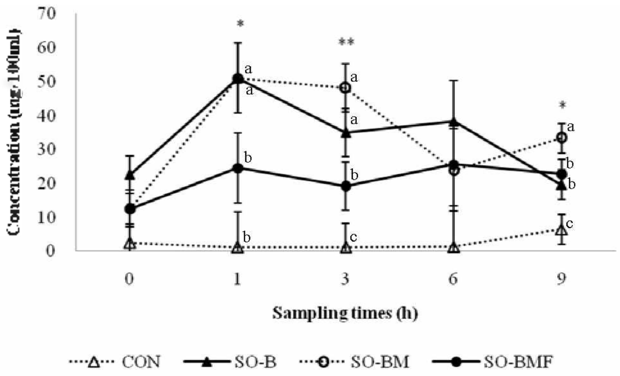

Figure 9. Additive effects of monensin or fish oil on *trans*-10, *cis*-12 CLA content (mg/100 ml) in rumen fluid of cows when fed soybean oil with buffer. * $p<0.05$; ** $p<0.01$.

feeding compared to the CON and SO-BMF diets. The SO-BM diet also increased ($p<0.01$-0.05) the concentrations in rumen fluid of *trans*-11 $C_{18:1}$ (Figure 5) and $C_{18:2}$ (Figure 6) at most sampling times post-feeding. Total CLA concentration was also increased by the feeding of SO-B and SO-BM diets during early fermentation times (up to 3 h) post-feeding (Figure 7). *Cis*-9, *trans*-11 CLA concentration was highest ($p<0.05$) for SO-B up to 1 h while the highest ($p<0.01$) value for SO-BM occured at 3 h in rumen fluid (Figure 8). Increased *trans*-10, *cis*-12 CLA concentration in rumen fluid was obtained from SO-B and SO-BM diets (Figure 9) at 1 and 3 h post feeding compared to other diets.

Supplementation of oils with monensin and sodium bicarbonate increased ($p<0.005$) the proportions of $C_{18:1}$, total CLA and both isomers of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in the plasma of cows (Table 5), but the effect of monensin and/or fish oil was greater for *trans*-10, *cis*-12 CLA than for *cis*-9, *trans*-11 CLA. The supplement also slightly increased the proportions of unsaturated fatty acid and polyunsaturated fatty acid in the plasma.

DISCUSSION

The present study was focused to maximize CLA production in the rumen with the combination of various dietary factors. Soybean oil has been useful as a source of $C_{18:2}$ which is one of the two major precursors of CLA (Wang et al., 2002), and sodium bicarbonate acts for pH control in rumen fluid (Wang and Song, 2003). Supplementation of monensin (Fellner et al., 1997; Sauer et al., 1998; Wang et al., 2006) or fish oil (Ashes et al., 1992; Wang et al. (2005) has resulted in an increased ruminal CLA production.

Addition of supplements including oil to the diet decreased DM intake and the reduction was greater for the cattle fed the fish oil-supplemented diet (SO-BMF, Table 3), despite the addition level being only 0.7% (Table 1). The intake of SO-BMF by cows was only approximately 71% of the mean intake of other diets (Table 3). The reduced DM intake of SO-BMF might occur due to the sickening smell of fish oil as observed in Korean native steers fed a diet supplemented with fish oil (Wang et al., 2006), and affected many observations from total VFA production and whole tract digestibility of nutrients to CLA contents. Overall patterns of ruminal pH and ammonia-N concentration in the rumen after feeding looked normal, but the ruminal pH might not have responded much to the addition of sodium bicarbonate (0.5% of concentrate) since the total diet consisted of 60% concentrate and 40% forage and its range was 6.2 to 7.1 during the indicated sampling times for all the diets (Figure 1). Decreased ammonia concentration in rumen fluid from the supplemented diets (Figure 2) might be due to the oil reducing the rate of ruminal fermentation (Wang and Song, 2001). Lowered total VFA concentration in rumen fluid from SO-BMF could be due to the reduced

Table 5. Additive effect of monensin and fish oil on fatty acid composition (%) in the plasma of Hostein cows when fed soybean oil with buffer

Fatty acids	Treatments[1]				SEM[2]	Pr<F[3]
	CON	SO-B	SO-BM	SO-BMF		
$C_{18:0}$	26.31	27.19	27.06	26.38	4.870	0.372
$c9$-$C_{18:1}$	16.70^{b}	26.46^{a}	27.44^{a}	30.15^{a}	3.844	0.002
$t11$-$C_{18:1}$	3.45	3.38	5.04	2.69	3.367	0.631
$C_{18:2}$	2.66	11.46	10.64	13.78	1.494	0.105
Total CLA	0.65^{b}	1.19^{ab}	1.36^{a}	1.29^{a}	0.327	0.049
c9,t11-CLA	0.31^{b}	0.70^{a}	0.65^{a}	0.43^{ab}	0.201	0.038
t10,c12-CLA	0.34^{b}	0.49^{ab}	0.71^{a}	0.86^{a}	0.221	0.048
$C_{18:3}$	2.33	1.59	1.10	1.59	0.166	0.092
Others	45.24	28.73	27.36	24.12	4.284	0.163
SFA	61.65	50.06	46.99	35.75	0.178	0.132
UFA	38.35	49.94	53.01	64.25	0.159	0.269
MUFA	30.83	33.99	36.14	47.26	0.120	0.138
PUFA	7.52	15.95	16.87	16.99	0.121	0.243

[1] Referred to Table 1. [2] Standard error of the mean. [3] Probability levels.

feed intake in comparison with the other diets (Table 4). No differences in the proportions of major VFA indicated that oil supplementation at the level of 7% to the concentrate may not interfere with ruminal fermentation of the total diet. Another possible reason for no difference in VFA proportion compared to the CON diet may be, in part, due to the addition of sodium bicarbonate to the oil supplemented diets.

The lowest contents of C18-fatty acids on the CON diet were certainly due to no addition of oil while monensin (30 ppm in concentrate) in the SO-BM diet caused slight increases in the content of these fatty acids (Figure 3 to Figure 6) in rumen fluid compared to other diets. These results indicate that monensin may stimulate some hydrogenation activity by rumen microbes although its action is limited to a certain stage of the fermentation process. However, Fellner et al. (1997) reported that ionophoric antibiotics inhibit the growth of *Butyrivibrio fibrisolvens* which is involved in ruminal hydrogenation. Kobayashi et al. (1992) reported that salinomycin supplementation resulted in accumulation of unsaturated fatty acids in rumen fluid and duodenal digesta of sheep.

The lowest contents of CLA from the CON diet among all diets were also due to the absence of supplemented oil (Table 7 to Table 9). The monensin effect on CLA production by rumen microbes has been controversial. Monensin increased the proportion of *cis*-9, *trans*11-CLA *in vitro* (Fellner et al., 1997) and *in vivo* (Sauer et al., 1998; Wang et al., 2006), whereas Dhiman et al. (1996) and Chouinard et al. (1998) observed no effect of monensin on CLA content in milk fat. Wang et al. (2005) found only a small effect of monensin (10 ppm, w/v) when incubated with safflower *in vitro*. In the present study, total CLA content in rumen fluid was not greatly affected by monensin. The effect of monensin might differ depending on the duration. A monensin effect (SO-BM) was not apparent in *cis*-9, *trans*-11 CLA content until 3h post-feeding compared to other diets, while *trans*-10, *cis*-12 CLA content was not influenced by monensin. The possibility of a time-dependent effect of monensin was suggested by Wang et al. (2005) who observed the highest *cis*-9, *trans*-11 CLA content from the longest incubation (12 h) *in vitro*.

Meanwhile, fish oil has been applied to enhance CLA production in rumen fluid. Ashes et al. (1992) postulated, based on an *in vitro* study, that EPA and DHA in fish oil might be resistant to ruminal biohydrogenation and thus could increase CLA content. In another *in vitro* study, Wang et al. (2005) observed an increased proportion of *cis*-9, *trans*-11 CLA from the supplementation of fish oil without interference in fermentation. Loor et al. (2005) reported that the fish oil effect on CLA production in the rumen may be related to reduced populations of ruminal bacteria associated not only with the final reduction step but with the overall process of biohydrogenation. They reported that sunflower oil with fish oil resulted in increased flows of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. However, in the present study, a fish oil effect (SO-BMF) on CLA production could not be properly evaluated nor compared among diets since feed intake by these cows was only approximately 71% of the mean intake of other diets (Table 3). Although fish oil helps to enhance ruminal CLA production, the mode of action still remains to be elucidated through further studies.

Unlike the concentration of fatty acid in rumen fluid, its profile in plasma was expressed as a proportion (%). Increased proportions of c9-$C_{18:1}$, CLA and UFA in the plasma of cows were mostly due to the soybean oil supplementation of the diet (Table 5). An effect of monensin and/or fish oil on the *trans*-10, *cis*-12 CLA was not big enough and these supplements did not even affect the *cis*-9, *trans*-11 CLA proportion in plasma. There is much evidence that dietary supplementation of C_{18}-rich plant oil enhances the CLA and UFA proportions. Bell and Kennelly (2000) observed increased *cis*-9, *trans*-11 CLA and UFA proportions in milk from cows fed a diet supplemented with plant oil. Supplementation of soybean oil also slightly increased CLA and UFA proportions in both plasma and subcutaneous fat of sheep (Choi et al., 2007). Feeding a diet supplemented with rolled flaxseed (15% of the concentrate) increased UFA proportion in milk compared to the control diet (Chung et al., 1996). Increased UFA by an oil-enriched diet is beneficial to health (Grummer, 1991). Although effects of monensin and fish oil are known to limit microbial action in the rumen, Wang et al. (2006) observed that supplementation of mixed oil ($C_{18:2}$-rich safflower oil and soybean oil) and fish oil with monensin to the diet slightly increased CLA and UFA proportions in both intramuscular- and subcutaneous fats in Korean native steers. This effect might be due to the transfer of increased CLA content in the rumen to the blood as influenced by monensin and/or fish oil.

Based on the results obtained from the present study, the supplements did not clearly affect the fermentation of diet in the rumen. Effects of monensin and fish oil in soybean oil supplemented diets on CLA production were small, and might appear differently depending on CLA isomers under the condition of the present study. Although it has some effect, fish oil may not be practical to apply for the enhancement of CLA production *in vivo* due to the intake problem.

REFERENCES

Albright, C. D., E. Klem, A. A. Shah and P. Gallagher. 2005. Breast cancer cell targeted oxidative stress: Enhancement of cancer uptake of conjugated linoleic acid, activation of p53, and inhibition of proliferation. Exp. Molec. Pathol. 79:118-125.

AOAC. 1995. Official methods of analysis (15th ed.). Association of Official Analytical Chemists, Washington, DC.

Ashes, J. R., B. D. Siebert, S. K. Gulati, A. Z. Cuthbertson and T. W. Scott. 1992. Incorporation of *n*-3 fatty acids of fish oil into tissue and serum lipids of ruminants. Lipids 27:629-631.

Bell, J. A. and J. J. Kennelly. 2000. Producing conjugated fatty acid enriched milk through practical dairy nutrition. Conference abstract:8th World Congress on Clinical Nutrition, Bankok, Thailand.

Choi, N. J., J. Y. Imm, S. J. Oh, B. C. Kim, H. J. Hwang and Y. J. Kim. 2005. Effect of pH and oxygen on conjugated linoleic acid (CLA) production by mixed rumen bacteria from cows fed high concentrate and high forage diets. Anim. Feed Sci. Technol. 123-124:643-653.

Choi, S. H., K. W. Lim, H. G. Lee, Y. J. Kim and M. K. Song. 2007. Supplementation effects of $C_{18:2}$ or $C_{18:3}$ rich oils on formations of CLA and TVA, and lipogenesis in adipose tissues of sheep. Asian-Aust. J. Anim. Sci. 20:1417-1423.

Chouinard, P. Y., L. Corneau, M. L. Kelly, J. M. Griinari and D. E. Bauman. 1998. Effect of dietary manipulation on milk conjugated linoleic acid concentrations. J. Dairy Sci. 81(Suppl. 1):233(Abstr.).

Chung, T. Y., M. K. Song, C. S. Moon, C. M. Kim, D. I. Kim and J. S. Sim. 1996. Effect of flaxseed supplementation on the nutrient utilizing efficiency, milk productivity and fatty acid composition of milk by lactating Holstein cows. Kor. J. Nutr. Feed. 20:443-452.

Cook, M. E., C. C. Miller, Y. Park and M. Pariza. 1993. Immune modulation by altered nutrient metabolism: Nutritional control of immune induced growth depression. Poult. Sci. 72:1301-1305.

Dhiman, T. R., G. R. Anand, L. D. Satter and M. W. Pariza. 1996. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 79(Suppl. 1):137(Abstr.).

Dhiman, T. R., L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright and M. X. Tolasa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83:1016-1027.

Fawcett, J. K. and J. E. Scott. 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. 13:156-163.

Fellner, V., F. D. Sauer and J. K. Kramer, 1997. Effect of nigericin, monensin, and tetranasin on biohydrogenation in continuous flow-through ruminal fermenters. J. Dairy Sci. 80:921-928.

Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244-3251.

Kobayashi, Y., M. Wakita and S. Hoshino. 1992. Effects of the ionophore salinomycin on nitrogen and long-chain fatty acid profiles of digesta in the rumen and the duodenum of sheep. Anim. Feed Sci. Technol. 36:67.

Lee, K. N., D. Kritchevsky and M. W. Pariza. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis 108:19-25.

Lepage, G. and C. C. Roy. 1986. Direct transesterification of all classes of lipid in a one-step reation. J. Lipid Res. 27:114-121.

Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard and M. Doreau. 2005. Intestinal flow and digestibility of *trans* fatty acids and conjugated linoleic acids (CLA) in dairy cows fed a high-concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. Anim. Feed Sci. Technol. 119:203-225.

SAS. 1985. SAS User's Guide: Statistical Analysis Systems Institute, Inc., Cary, NC.

Sauer, F. D., V. Fellner, R. Kinsman, J. K. G. Kramer, H. A. Jackson, A. J. Lee and S. Chen. 1998. Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet. J. Anim. Sci. 76:906-914.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. Mcgraw Hill Book Co., NY.

The British Nutrition Foundation 1992. Sources of unsaturated fatty acids in the diet. In: The report of the British Nutrition Foundation's task force. Unsaturated fatty acids: nutritional and physiological significance, London, UK: Chapman and Hall, pp. 6-11.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wang, J. H. and M. K. Song. 2001. Effect od sources and levels of carbohydrates on fermentation characteristics and hydrogenation of linoleic acid by rumen bacteria *in vitro*. Asian-Aust. J. Anim. Sci. 14:48-53.

Wang, J. H. and M. K. Song. 2003. pH affects the *in vitro* formation of cis-9, trans-11 CLA and trans-11 Octadecenoic acid by ruminal bacteria when incubated with oilseeds. Asian-Aust. J. Anim. Sci. 16:1743-1748.

Wang, J. H., M. K. Song, Y. S. Son and M. B. Chang. 2002. Effect of concentrate level on the formation of conjugated linoleic acid and *trans*-octadecenoic acid by ruminal bacteria when incubated with oilseed *in vitro*. Asian-Aust. J. Anim. Sci. 15:1115-1120.

Wang, J. H., S. H. Choi, C. G. Yan and M. K. Song. 2005. Effect of monensin and fish oil supplementation on *bio*hydrogenation and CLA production by rumen bacteria *in vitro* when incubated with safflower oil. Asian-Aust. J. Anim. Sci. 18:221-225.

Wang, J. H., S. H. Choi, K. W. Lim, K. H. Kim and M. K. Song. 2006. Effect of mixed oil and monensin supplementation, and feeding duration of supplements on c9,t11-CLA contents in plasma and fat tissues of Korean native (Hanwoo) steers. Asian-Aust. J. Anim. Sci. 19:1464-1469.

Whigham, L. D., A. Higbee, D. E. Bjorling, Y. H. Park, M. W. Pariza and M. E. Cook. 2002. Decreased antigen-induced eicosanoid release in conjugated linoleic acid-fed guinea pigs. Am. J. Physiol. Intergrative Comp. Physiol. 282(4):R1104-R1112.