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Effect of the 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*

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ABSTRACT : The present study was conducted with twenty-four Korean native (Hanwoo) steers to observe the effect of mixed oil and monensin supplementation and duration of feeding on c9,t11-CLA content in plasma and fat tissues. The steers were randomly assigned to three groups of eight animals each according to body weight. Hanwoo steers in the control group were fed the commercial concentrate for the late fattening stage. The other groups of steers were fed the same diet as control steers, but the concentrate was supplemented with high-$C_{18:2}$ oil mixture (soybean oil, sunflower oil, safflower oil) and fish oil at 6% level of concentrate (DM basis), and monensin (20 ppm). The second and third group of steers was fed the oil mixture supplemented diet with monensin for the last 10 weeks and 20 weeks, respectively, prior to being slaughtered. The oil mixture consisted of 45% soybean oil, 20% sunflower oil, 20% safflower oil and 15% fish oil. Average daily gain ($p<0.049$) and feed efficiency ($p<0.018$) of the steers fed the diet supplemented with oil mixture and monensin (OM-M) for 20 weeks were higher than those of the other groups of steers. Dressing percent, fat thickness and *longissimus* muscle area were not affected by the OM-M supplementation and duration of its feeding. The OM-M supplementation increased the content of total-cholesterol ($p<0.0001$-0.0007) and HDL-cholesterol ($p<0.0001$) in the plasma of steers compared to the control diet. The steers fed the OM-M diet had a higher proportion of c9,t11-CLA in plasma ($p<0.048$-0.044) than the control steers. Feeding the OM-M diet for 20 weeks increased the proportion of CLA in intramuscular ($p<0.015$), intermuscular ($p<0.039$) and subcutaneous ($p<0.001$) fat tissues compared with both steers fed the control diet and the OM-M diet for 10 weeks. Increased ($p<0.007$) proportion of total unsaturated fatty acids in steers fed the OM-M diet for 20 weeks compared to those in control steers was related to the increased ($p<0.001$) $C_{18:2}$ and decreased ($p<0.001$) $C_{18:0}$ proportions in subcutaneous tissue. (**Key Words :** $C_{18:2}$-rich Oils, Monensin, Fish Oil, c9,t11-CLA, Fat Tissue, Hanwoo Steer)

INTRODUCTION

Numerous attempts have been made to increase the CLA contents in ruminant products due to the beneficial health effects such as in cancer (Ha et al., 1987), atherosclerosis (Lee et al., 1994), and immunity (Michal et al., 1992) from it. Attempts have been made to increase the CLA production by ruminant animals by grazing on pasture (Timmen and Patton, 1988; Zegarska et al., 1996), feeding the large quantities of plant oils (Tesfa et al., 1991; Kelly et al., 1998) or oil seeds (Stanton et al., 1997; Lawless et al., 1998), and manipulating the ratio of forage to concentrate in the diet (Jiang et al., 1996; Wang et al., 2002a). An increased CLA content in tissues of lamb was found by feeding the $C_{18:2}$-rich plant oil supplemented diet (Mir et al., 2000; Ivan et al., 2001). However, some results in ruminant fat are often not in effect. Supplementation of extruded full fat soybean (Madron et al., 2002) and soybean oil (Beaulieu et al., 2000, 2002) did not greatly changed the CLA content of body fat in steers.

Based on the previous *in vitro* (Wang et al., 2003) and metabolic studies (Wang and Song, 2002b), it could be postulated that ruminal pH, and addition of monensin and fish oil to the diet containing $C_{18:2}$-rich plant oil altered the ruminal fermentation and production of hydrogenation intermediates by microbes. It was found that monensin inhibited the bio-hydrogenation of $C_{18:2}$ with lowered $C_{18:0}$ accumulation and fish oil supplementation increased the

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Table 1. Composition (% of total fatty acids) of major fatty acids of oils

Oils	Palmitic acid ($C_{16:0}$)	Stearic acid ($C_{18:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)	Linolenic acid ($C_{18:3}$)	DHA ($C_{22:6}$)
Soybean oil	12.3	6.1	23.8	51.0	5.4	-
Sunflower oil	8.7	2.6	31.1	51.6	0.7	-
Safflower oil	9.4	3.3	17.8	62.5	0.4	-
Fish oil [1]	23.6	6.3	28.2	3.2	1.0	8.0

[1] Anchovy based mixed fish oil.

Table 2. Chemical composition of diet (%, DM basis)

Components	Concentrate	Rice straw
Crude protein	13.03	3.92
Ether extracts	4.25	3.15
Neutral detergent fiber	35.95	80.96
Ash	6.43	10.73
Ca [1]	0.75	0.16
P [1]	0.36	0.25

[1] Chemical compositions of Ca and P in concentrate were given by the feed mill company and those in rice straw were cited from Korean feeding standard of Korean native cattle (RDA, 2002).

c9,t11-CLA proportion when $C_{18:2}$-rich safflower oil was incubated (Wang et al., 2005). Monensin and fish meal supplementation increased CLA content in milk fat as reported by Dhiman et al. (1999). But those effects have not been examined with Korean native (Hanwoo) steers. The effect of duration of feeding the $C_{18:2}$-rich oil diet supplemented with monensin and fish oil on CLA content in beef fat was not examined either.

Therefore, the present feeding trial was conducted to examine the feeding effect of diet supplemented with $C_{18:2}$-rich oils, fish oil and monensin, and duration of feeding on CLA contents in plasma and fat tissues of Hanwoo steers.

MATERIALS AND METHODS

Animals, diets and feeding management

Twenty-four Hanwoo steers (20 months old, 567±18 kg body weight) were divided into three groups of eight animals each according to body weight. The steers were castrated at 10 months old. Hanwoo steers in control group were fed the commercially produced concentrate for the late fattening stage. The other groups of steers were fed the same diet as control steers, but the concentrate was supplemented with high-$C_{18:2}$ oil mixture (soybean oil, sunflower oil, safflower oil) and fish oil at 6% level of concentrate (DM basis), and monensin (20 ppm). The second and third group of steer were fed the diets supplemented with oil mixture and monensin (OM-M) for last 10 weeks and 20 weeks, respectively, prior to being slaughtered. The oil mixture consisted of 45% soybean oil, 20% sunflower oil, 20% safflower oil and 15% fish oil. The oil mixture with monensin was again mixed with the concentrate daily prior to feeding.

Steers were raised in the 5×8 m pen by 4 heads and fed rice straw *ad libitum*, and were allowed a free access to water and mineral block. At the beginning stage of experiment the steers of control were fed 8.5 kg the concentrate (DM basis) per head daily, and thereafter, intake level of concentrate was increased by 0.15 kg/month as the age progressed. Hanwoo steers of control group met the daily nutrient requirement of the Korean Feeding Standard for Korean Cattle (2002). Steers of the OM-M feeding groups were fed the same concentrate to control steers but were fed 13% less than those of control for 10 weeks and 20 weeks prior to being slaughtered in order to make energy intake levels be similar to those of control. All the steers were fed the concentrate twice daily in an equal volume. The experiment was conducted in Korean Livestock Improvement Center, NACF. The total lipid contents of diets for control and supplemented groups were 4.03 and 9.91% (DM basis), respectively. The major fatty acid compositions of oils were presented in Table 1 and the chemical compositions of concentrate and rice straw were presented in Table 2.

Measurements and analysis

The Hanwoo steers were weighed at 4 weeks interval as well as at the beginning and the end of the experimental period. The leftover of concentrate was recorded at three days interval to measure intake. Random grab samples of the concentrate and rice straw were taken monthly, and the samples were ground through a 1 mm screen for the proximal analyses by the method of AOAC (1995). Neutral detergent fiber was analyzed by the method of Goering and Van Soest (1970).

On 4 and 10 weeks of feeding the OM-M diet, 20 ml blood was collected from jugular vein of each steer with vacutainer (Becton Dickinson) containing sodium heparin. Samples were stored in ice box and centrifuged immediately at 3,000 rpm for 10 min, and then the supernatant (plasma) was transferred into 30 ml screw-cap tubes and were kept frozen at -70°C until analyzed. After thawed, 150-500 μl supernatant plasma was transferred into a Spotchem plasma sample tube. Reagent strip (ARKRAY, Inc. Japan) was used to determine the contents of total-cholesterol (T-C) and HDL-cholesterol (HDL-C) in plasma by the Spotchem Analyzer (SP-4410, KAK Corp.). Lipids were extracted from the residual plasma and followed by methylation. The plasma was homogenized in chloroform/

Table 3. Effect of oil mixture and monensin (OM-M) supplementation and duration of feeding on growth performance and carcass traits of Hanwoo steers

Items	Control	OM-M [1]		SEM [2]	Pr>F [3]
		10 weeks	20 weeks		
Growth performances :					
Initial BW (kg)	566.6	568.7	566.8	4.906	0.939
Final BW (kg)	652.9	646.5	657.6	8.402	0.649
Concentrate DM intake (kg)	10.1	9.81	9.62	-	-
ADG (kg)	0.48[b]	0.43[b]	0.62[a]	0.051	0.049
DMI/ADG	21.6[a]	25.1[a]	16.5[b]	1.925	0.018
Carcass traits :					
Dressing percent (%)	67.04	66.42	66.82	0.842	0.765
Fat thickness (mm)	13.08	15.23	15.49	1.286	0.319
Longissimus muscle area (cm^2)	83.44	86.74	89.25	1.564	0.278

[1] Feeding duration of diet supplemented with oil mixture and monensin.
[2] Standard error of the means. [3] Probability level.
[a, b] Means in the same row with different superscripts differ.

Table 4. Effect of oil mixture and monensin (OM-M) supplementation and duration of feeding on total- and HDL-cholesterol (mg/100 ml) in plasma of Hanwoo steers

Items	Control	OM-M [1]	SEM	Pr>F
	---------- 4 weeks ----------			
T-C (T) [2]	145.31	182.56	6.701	0.001
HDL-C (H) [3]	89.44	102.63	6.782	0.190
H/T	0.61	0.56	0.028	0.201
	---------- 10 weeks ----------			
T-C	119.00	235.25	18.84	0.0007
HDL-C	54.50	128.25	9.967	0.0001
H/T	0.44	0.55	0.034	0.034

[1] Feeding duration of diet supplemented with oil mixture and monensin.
[2] Total cholesterol. [3] High density lipoprotein-cholesterol.
[a, b] Means in the same row with different superscripts differ.

methanol solution (Folch et al., 1957) using a homogenizer (PT-MR3100, Switzerland). The chloroform from measured aliquots of the extract was evaporated on the Dri-Bath (Type l6500, USA) at 50°C under the nitrogen stream. Methylation of the lipids followed the method of Lepage and Roy (1986) prior to injecting into the gas chromatograph (GC, HP 5890II, Hewlett Packard Co.). A fused silica capillary column (100 m×0.25 mm, i.d. ×0.20 μm thickness, Supelco, SP^{TM}-2560, USA) was used. The injector and detector temperature was maintained at 250°C, respectively. The initial column temperature was 175°C (held for 30 min), and then increased by 15°C/min to 220°C (held for 40 min). Ultra pure helium was used as the carrier gas.

After the 20 week experiment in total was terminated all the steers (25 month of age) were slaughtered. Samples of intramuscular (*longissimus dorsi*), intermuscular and subcutaneous fat tissues were taken and ground, and mixed well to take sub-sample for the FA analyses. The samples were freeze dried and ground. The process of lipid extraction, methylation and fatty acid analyses of fat tissues were same as for the analyses of plasma fatty acids.

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's Test (Steel and Torrie, 1980).

RESULTS

Performance and carcass characteristics

Average daily gain (ADG) of Hanwoo steers was higher ($p<0.049$) for 20 week feeding of diet supplemented with OM-M than for the other groups of steers, and improved feed efficiency was also observed from 20 week feeding group of steers (Table 3). No difference in ADG was found between control and 10 week feeding group of steers. The OM-M supplementation and duration of feeding the OM-M diet did not affect the dressing percent, fat thickness and *longissimus* muscle area among treatments although fat thickness and *longissimus* muscle area were slightly greater in steers fed the diet supplemented with OM-M than in control steers (Table 3).

Cholesterol and fatty acid composition in plasma

The OM-M supplemented diet increased T-C ($p<0.001$) in plasma of steers at 4 week feeding, and T-C ($p<0.0007$) and HDL-C ($p<0.0001$) at 10 week feeding compared to control diet (Table 4). Supplementation of OM-M did not affect the composition of most fatty acids in plasma except for the increased ($p<0.048$) cis9,trans11-CLA proportion at 4 week feeding compared to those of steers fed the control diet. While the proportion of oleic acid ($C_{18:1}$) was decreased ($p<0.004$) in plasma those of palmitoleic acid ($C_{16:1}$, $p<0.001$) and cis9,trans11-CLA ($p<0.044$) were increased by the supplementation of OM-M compared to those of steers fed the control diet for 10 week feeding (Table 5).

Table 5. Effect of oil mixture and monensin (OM-M) supplementation and duration of feeding on composition (%) of fatty acids in plasma of Hanwoo steers

Fatty acids	Control	OM-M [1]	SEM	Pr>F
	---------- 4 weeks ----------			
$C_{14:0}$	1.08	0.82	0.119	0.132
$C_{16:0}$	17.25	16.44	0.392	0.143
$C_{16:1}$	0.51	0.79	0.142	0.160
$C_{17:0}$	1.04	0.92	0.091	0.061
$C_{18:0}$	31.93	31.74	0.810	0.287
$C_{18:1n-9}$	15.86	14.73	0.958	0.960
$C_{18:2n-6}$	30.62	32.42	1.302	0.384
C9,t11-CLA	0.43	0.86	0.106	0.048
$C_{18:3n-3}$	0.81	0.75	0.109	0.736
Other fatty acids	0.47	0.53	-	-
SFA (S) [2)]	51.30	49.92	0.627	0.062
UFA (U) [3)]	48.24	49.53	0.627	0.062
U/S	0.94	0.99	0.035	0.064
	---------- 10 weeks ----------			
$C_{14:0}$	1.15	1.02	0.073	0.234
$C_{16:0}$	18.70	17.66	0.491	0.172
$C_{16:1}$	0.25	0.76	0.035	0.001
$C_{17:0}$	0.80	0.95	0.049	0.061
$C_{18:0}$	30.99	31.77	0.692	0.462
$C_{18:1n-9}$	17.98	14.12	0.832	0.004
$C_{18:2n-6}$	28.31	31.23	1.595	0.203
C9,t11-CLA	0.51	0.87	0.101	0.044
$C_{18:3n-3}$	0.61	0.73	0.147	0.933
Other fatty acids	0.83	0.80	-	-
SFA	51.64	51.40	0.973	0.910
UFA	47.75	47.78	0.973	0.910
U/S	0.92	0.93	0.036	0.844

[1] Feeding duration of diet supplemented with oil mixture and monensin.
[2] Saturated fatty acids. [3] Unsaturated fatty acids.
[a, b] Means in the same row with different superscripts differ.

Contents of CLA and other fatty acids in fat tissues

Feeding the OM-M supplemented diet of 20 weeks decreased ($p<0.031$) $C_{16:1}$ proportion but increased the proportions of $C_{17:0}$ ($p<0.0004$), cis9,trans11-CLA ($p<0.015$) and $C_{18:3}$ ($p<0.003$) in intramuscular fat (Table 6) compared to other group of steers. The OM-M supplemented diet increased the cis9,trans11-CLA proportions in intermuscular fat ($p<0.039$, Table 7) of the steers for both feeding durations and in subcutaneous fat ($p<0.001$, Table 8) for 20 week feeding compared to those of steers fed control diet. There was no difference in cis9,trans11-CLA proportion in intermuscular fat between the durations of feeding (Table 7), but enhanced ($p<0.001$) cis9,trans11-CLA proportion was observed from the subcutaneous fat of the steers fed OM-M supplemented diet for 20 weeks compared to those for10 weeks feeding (Table 8). While the feeding of OM-M diet for 20 weeks increased the proportions of $C_{18:2}$ ($p<0.001$) and unsaturated fatty acids (UFA, $p<0.009$), 10 week feeding the diet decreased the proportions of $C_{18:0}$ (0.001) and saturated fatty acids (SFA, $p<0.007$) in subcutaneous fat (Table 8).

Table 6. Effect of oil mixture and monensin (OM-M) supplementation and duration of feeding on fatty acid composition (%) in intramuscular fat of Hanwoo steers

Fatty acids	Control	OM-M [1]		SEM	Pr>F
		10 weeks	20 weeks		
$C_{14:0}$	2.71	2.64	2.90	0.172	0.563
$C_{16:0}$	29.39	27.70	28.27	0.597	0.689
$C_{16:1}$	4.66[a]	4.39[ab]	3.87[b]	0.198	0.031
$C_{17:0}$	0.66[b]	0.53[c]	1.11[a]	0.039	0.0004
$C_{18:0}$	11.71	12.06	11.31	0.390	0.412
$C_{18:1n-9}$	41.00	41.42	41.92	0.880	0.901
$C_{18:2n-6}$	8.91	9.47	9.09	0.878	0.902
C9,t11CLA	0.26[b]	0.36[b]	0.41[a]	0.011	0.039
$C_{18:3n-3}$	0.23[b]	0.25[b]	0.33[a]	0.020	0.003
Other fatty acids	0.47	1.18	0.79	-	-
SFA (S)	44.47	42.48	43.59	0.717	0.781
UFA (U)	55.06	55.89	55.62	0.717	0.781
U/S	1.24	1.31	1.28	0.028	0.607

[1] Feeding duration of diet supplemented with oil mixture and monensin.
[a, b, c] Means in the same row with different superscripts differ.

Table 7. Effect of oil mixture and monensin (OM-M) supplementation and duration of feeding on fatty acid composition (%) in intermuscular fat of Hanwoo steers

Fatty acids	Control	OM-M [1]		SEM	Pr>F
		10 weeks	20 weeks		
$C_{14:0}$	3.46	3.40	3.37	0.224	0.958
$C_{16:0}$	28.38	26.39	26.94	0.644	0.101
$C_{16:1}$	4.99	5.17	4.60	0.317	0.438
$C_{17:0}$	1.09	0.95	1.05	0.048	0.132
$C_{18:0}$	13.16	12.61	13.27	0.674	0.765
$C_{18:1n-9}$	44.65	47.48	46.66	0.947	0.117
$C_{18:2n-6}$	2.16	1.96	2.14	0.111	0.398
C9,t11-CLA	0.36[b]	0.41[a]	0.44[a]	0.020	0.039
$C_{18:3n-3}$	0.17	0.19	0.22	0.017	0.148
Other fatty acids	1.58	1.44	1.31	-	-
SFA (S)	46.09	43.35	44.63	1.134	0.233
UFA (U)	52.33	55.21	54.06	1.134	0.233
U/S	1.16	1.29	1.23	0.053	0.256

[1] Feeding duration of diet supplemented with oil mixture and monensin.
[a, b] Means in the same row with different superscripts differ.

DISCUSSION

Increased daily gain of Hanwoo steers by feeding the OM-M supplemented diet for 20 weeks would be due to the confound effect of increased energy intake from oil mixture and monensin. Addition of lipids (4%) to corn-based cattle finishing diets improved feed efficiency (Krehbiel et al., 1995). Vonghia et al. (1997) and Kott et al. (2003) also reported improved daily gain and feed efficiency for feedlot lambs fed the diet supplemented with safflower seed. In addition, monensin can increase the ratio of C_3 to C_2 (Russell, 1987) and improve energetic efficiency in beef cattle (Spears and Harvey, 1984). Increased daily gain from steers fed for 20 weeks compared to that of steers fed for 10

Table 8. Effect of oil mixture and monensin (OM-M) supplementation and duration of feeding on fatty acid composition (%) in subcutaneous fat of Hanwoo steers

Fatty acids	Control	OM-M [1]		SEM	Pr>F
		10 weeks	20 weeks		
$C_{14:0}$	4.04	3.57	3.55	0.193	0.152
$C_{16:0}$	28.78	27.58	27.35	0.568	0.186
$C_{16:1}$	6.41	6.18	7.54	0.476	0.120
$C_{17:0}$	1.04	0.91	0.94	0.038	0.066
$C_{18:0}$	8.81[a]	9.40[a]	6.79[b]	0.467	0.001
$C_{18:1n-9}$	45.99	48.06	47.87	0.882	0.207
$C_{18:2n-6}$	2.21[b]	1.95[b]	2.74[a]	0.129	0.001
C9,t11-CLA	0.41[b]	0.43[b]	0.59[a]	0.031	0.001
$C_{18:3n-3}$	0.21	0.29	0.24	0.021	0.197
Other fatty acids	2.40	1.63	2.39	-	-
SFA (S)	42.37[a]	41.46[a]	38.63[b]	0.822	0.007
UFA (U)	55.23[b]	56.91[b]	58.98[a]	0.822	0.007
U/S	1.30[b]	1.37[b]	1.53[a]	0.049	0.009

[1] Supplementation of mixed oil plus monensin.
[a, b] Means in the same row with different superscripts differ significantly.

weeks could be mainly due to the duration of feeding of OM-M diet.

No differences in most carcass characteristics among treatments in the present experiment are in agreement with the results reported by Vonghia et al. (1997) and Kott et al. (2003) in which they suggested safflower seed supplementation (5%) to diets did not affect these carcass measurements. The simultaneous increase in T-C and HDL-C levels in the plasma by OM-M supplementation in the present study indicates that the oil supplementation responded to the plasma cholesterol level in steers, and increased T-C was mostly due to the increased HDL-C. Because the beneficial effect of HDL-C on coronary heart disease (Rudel et al., 1998) its increase in plasma might be desirable.

The $C_{18:2}$ rich three different oils with fish oil were applied in the present study. Increased level of the c9,t11-CLA in plasma of steers fed the OM-M diet compared to those fed control diet (Table 5) may reflect the inhibition of bio-hydrogenation by monensin in the rumen as indicated by Bauman et al. (2003). Fellner et al. (1997) also found that the monensin inhibited bio-hydrogenation of UFA and increased ruminal CLA content *in vitro*. Fish oil may have similar effect to monensin in this aspect. Borsting et al. (1992) and Scollan et al. (1997) found the inhibition effect of fish oil in the ruminal hydrogenation. Wang et al. (2005) also observed lowered $C_{18:0}$ accumulation but increased c9,t11-CLA proportion when $C_{18:2}$-rich safflower oil was incubated fish oil supplementation.

A consistent increase in c9,t11-CLA proportion in the tissues examined was observed from the steers fed the OM-M supplemented diet for 20 weeks compared to other group of steers, indicating that OM-M supplementation is, to some degree, an effective method to enhance the CLA content of tissues. However, the increase in c9,t11-CLA level seemed to be limited. These results might be caused by various factors in the rumen and fat tissues. The CLA in the animal fat has been mainly originated from the rumen. One of the possible reasons for the smaller production of c9,t11-CLA than was expected in the present study might be the lowered ruminal pH although monensin and fish oil could contribute to its production in the rumen. The steers used in the present study were in the late fattening stage in which they were fed the concentrate almost *ad libitum* level. This could make the pH of rumen fluid lower, resulted in a limited bacterial isomerase activity (Wang et al., 2003) which is necessary for the production of CLA. Relatively higher levels of c9,t11-CLA have been observed at 6.1 and 6.9 of pH levels from *in vitro* study (Wang et al., 2002a). Lowered concentrate to roughage ratio also increased the ruminal pH and thus enhanced c9,t11-CLA proportion in the rumen (Wang et al., 2002b). The CLA in the animal fat can also be synthesized in the animal body through the process of desaturation of trans-9 octadecenoic acid (t9-$C_{18:1}$) which is another major intermediate of ruminal hydrogenation (Bauman et al., 2003). If the production of t9-$C_{18:1}$ in the rumen is reduced by the lowered ruminal pH CLA content in the animal fat could also be reduced.

Unlikely to the increased CLA content in milk from the supplementation of $C_{18:2}$ rich vegetable oils (Bauman et al., 2000), reduced CLA content might also be due to the wide distribution of CLA to the whole body where the fat is deposited although its content was not measured in many fat depots.

Based on the results of the present feeding trial with Hanwoo steers, supplementations of $C_{18:2}$-rich oils, fish oil and monensin to the diet could be one of the effective methods to increase the CLA content in fat tissues. But the degree of their supplementation effect may depend upon the duration of feeding and probably confounded action of the intake levels of high $C_{18:2}$ oil, fish oil and monensin. Further study relating to the intake level of oil, feeding stage and duration of feeding, however, is still required to maximize the production of CLA in Hanwoo steers.

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