Asian-Aust. J. Anim. Sci.
Vol. 24, No. 9 : 1249 - 1255
September 2011

www.ajas.info
http://dx.doi.org/10.5713/ajas.2011.11109

Influence of Dietary Conjugated Linoleic Acid and Its Combination with Flaxseed Oil or Fish Oil on Saturated Fatty Acid and n-3 to n-6 Fatty Acid Ratio in Broiler Chicken Meat

D. Shin[1,2], G. Kakani[1], A. Karimi[3], Y. M. Cho[4], S. W. Kim[4], Y. G. Ko[4], K. S. Shim[5] and J. H. Park*

[1] Department of Poultry Science, 2472 TAMU, Texas A&M University, College Station, TX 77843-2472, USA

ABSTRACT : This study examined the effect of CLA, flaxseed oil and fish oil and their combination forms on crude fat of liver and fatty acid profiles of liver, breast and thigh meat in broiler chicks. A total of 72, 1-day-old Cobb broilers were assigned to 6 groups, and fed an experimental diet supplemented with 5 different fat sources; conjugated linoleic acid (2% CLA), flaxseed oil (2% FXO), fish oil (2% FHO), CLA and flaxseed oil combination (1:1; 2% CXO), and CLA and fish oil combination (1:1; 2% CHO). Eight birds per treatment were processed, and liver, breast and thigh samples were investigated at 21 d of age. As a result of this study, most fatty acids of liver, breast and thigh meat were influenced by fat sources supplemented in the diet ($p<0.05$). CLA addition resulted in an increase of crude fat and saturated fatty acid (SFA) content but a concomitant decrease in n-3 to n-6 fatty acid ratio was observed in liver ($p<0.05$). Moreover, the same trends of SFA and n-3 to n-6 fatty acid ratio were also observed in breast and thigh meats of birds fed CLA alone. In the CXO-fed group or CHO-fed group, n-3 and n-3 to n-6 fatty acid ratio in both breast and thigh meat increased compared with CLA group, while SFA content decreased ($p<0.05$). FHO fed-groups had the lowest proportion of n-6 fatty acid in both breast and thigh meats compared to other fat source treatments ($p<0.05$). In conclusion, the increased levels of crude fat and SFA in liver and meats obtained by feeding CLA could be reduced by its combination with FXO or FHO. In addition, the combination of CLA and FXO or FHO fed to broiler chicks could increase the n-3 to n-6 fatty acid ratio of their meat along with the deposition of CLA. (**Key Words :** Broiler Chicken, Conjugated Linoleic Acid, Fish Oil, Flaxseed Oil, Omega Fatty Acid)

INTRODUCTION

It has been shown that consumers prefer poultry meat and its products for several reasons. The most important being the health point - lower fat level and better fatty acid composition as compared to beef and pork along with economical justification. Unlike other animal fats, around two third of poultry fat is composed of unsaturated fatty acids, and they are belonged to omega-3 (n-3) and omega-6 (n-6) fatty acids. In poultry, it is common that dietary fat has a great influence on fatty acid profiles of poultry meat (Crespo and Esteve-Garcia, 2001; Krejcí-Treu et al., 2010). Therefore, employing nutritional strategies including changing dietary fatty acid profile have a great potential in converting poultry meat from a simple animal protein source to a valuable functional product.

Polyunsaturated fatty acids (PUFA) intake including n-3 and n-6 fatty acids, has beneficial effects on both animal and consumer health. However, some limited studies imply that excessive intake of n-6 relative to n-3 fatty acids causes an increase in pathogenesis (Simopoulos, 2003). Since consuming higher amount of n-6 fatty acids has been closely associated with negative health impact, attempts have been made to manipulate the fatty acid profile in edible meat using several fat sources. Among them, flaxseed oil and fish oil are two valuable fat sources which not only increase n-3 fatty acid content but also decrease SFA content in meat (Huang et al., 1990; Olomu and

* Corresponding Author : Jae Hong Park. Animal Genetic Resources Station, National Institute of Animal Science, RDA, Namwon 590-832, Korea. Tel: +82-63-620-3529, Fax: +82-63-620-3590, E-mail: razorbacks@korea.kr

[2] Swine Science and Technology Center, Gyeongnam National University of Science and Technology, Jinju 660-758, Korea.

[3] Department of Animal Science, Faculty of Agricultural Science, University of Kurdistan, 416, Sanandaj, Iran.

[4] Animal Genetic Resources Station, National Institute of Animal Science, RDA, Namwon 590-832, Korea.

[5] Department of Animal Biotechnology, Chonbuk National University, Jeonju 561-756, Korea.

Received April 19, 2011; Accepted June 29, 2011

Baracos, 1991).

Conjugated linoleic acid (CLA) is another fatty acid source which due to its reported antimutigenic and potent anticaricnogenic effects often has been referred to as a functional food product with body fat reducing potential (Belury, 2002; Larsen et al., 2003). However, its related health benefits claims in some human and mouse studies are inconsistent (Wahle et al., 2004; Tricon et al., 2005; Kelley et al., 2009).

Though the effects of various fat sources on animal body fat deposition have been widely investigated, few studies on co-supplementation of CLA and other fat sources in broiler chickens have been reported yet. Therefore, the objective of this study was to investigate the influence of supplementation of CLA individually or in combination with n-3 sources on fatty acid profile in liver, breast and thigh meat in broiler chickens, and to provide the basic data to produce the chicken meat with enriching n-3 fatty acids, lower n-6 fatty acids and SFA contents.

MATERIALS AND METHODS

Animal model and sample preparation

A total of 72, 1 day old male Cobb 500 (41g±0.01) was raised up to 21 days of age. Six different experimental diets were prepared based on corn-soybean meal; i) basal diet (no oil addition, BAS); ii) 2% CLA (a mix of 50% c9t11+50% t10c12, CLA); iii) 2% flaxseed oil (FXO); iv) 2% menhaden fish oil (FHO); v) 2% combination of CLA and FXO (1:1, CXO); and vi) 2% combination of CLA and FHO (1:1, CHO) (Table 1). All chicks were benefited from day light (average 14 h/d), and feed and water were continuously available to chicks. A total of eight birds from each treatment were processed, and liver, breast and thigh samples were collected and stored at -80°C until analyzed.

Table 1. Formulation and chemical composition of basal diets

Ingredients (%)	Starter diet (1-21 days)
Corn	58.81
Soybean meal	34.81
Biophos[1]	1.67
Limestone	1.52
Oil[2]	2.00
Salt	0.51
Vitamin premix[3]	0.25
DL-methionine	0.20
Choline 60	0.10
Coban 60[4]	0.08
Mineral premix[5]	0.05
Calculated chemical composition	
Crude protein (%)	22.0
ME (kcal/kg)	3,200
Calcium (%)	0.95
Available phosphorous (%)	0.47
Methionine	0.53
Methionine+cystine (%)	0.90
Lysine (%)	1.18
Threonine (%)	0.82
Sodium (%)	0.22

[1] Biophos: 15.9% Ca, 21.2% P (Marshall Minerals Inc., Marshall, TX).

[2] Oil, alone or in combination, depending on the treatment, was from the following sources: conjugated linoleic acid (c9t11 and t10c12, 1:1 ratio, Luta-CLA® 60, BASF, Florham Park, NJ), flaxseed oil (Pizzey's Milling Co. Gurnee, IL), menhaden fish oil (Virginia Prime Silver™, Omega Protein Inc., Hammond, LA).

[3] Vitamin premix (kg): vitamin A 14,000,000 IU, vitamin D_3 5,000,000 IU, vitamin E 60,000 IU, vitamin B_{12} 24 mg, riboflavin 12,000 mg, niacin 80,000 mg, d-pantohenic acid 20,500 mg, K 2,700 mg, folic acid 1,800 mg, vitamin B_6 5,000 mg, thiamine 4,000 mg, d-biotin 150 mg (Sanderson, DSM Nutritional Products, Inc. Parsippany, NJ).

[4] Coban 60: 132 g/kg monensin (Elanco Animal Health Division of Eli Lilly & Co., Indianapolis, IN).

[5] Mineral premix (kg): Ca 1.20%, Mn 30.0%, Zn 21.0%, Cu 8,500 mg, I 2,100 mg, Se 500 mg, Mo 1,670 mg (Tyson Poultry 606 Premix, Tyson Foods, Springdale, AR).

Crude fat content and fatty acid profile determination

The crude fat content of liver, breast and thigh samples was measured using the CEM Smart Track system, a microwave drying and nuclear magnetic resonance system (CEM Co., Matthews, NC, USA). Around 3 g of the ground sample was used, and the crude fat content was reported as a percent of fat.

The fatty acid profile of the liver, breast and thigh samples was determined by extracting the total lipid using a method described by Folch et al. (1957). The fatty acid composition from the extracted lipid samples was determined using the fatty acid methyl ester (FAME) methodology as previously described by Smith et al. (2002). The composition of the FAME was determined using a gas chromatography (GC, Varian Chrompack, CP-3800 Gas Chromatograph) fixed with a CP-8200 Auto-Sampler (Varian Chromatography System, Walnut Creek, CA), a WCOT Fused Silica Capillary Column (100 m×0.25 mm i.d., CP-7420) and a flame ionization detector (FID). The temperature was initially programmed to 185°C (hold for 32 min) followed by a gradual increase rate of 20°C/min to reach a final temperature of 235°C (hold for 15.50 min). The split ratio was 100, injector and detector temperature was 270°C and Helium (He) was used as a carrier. Each fatty acid profile was identified and expressed as percentage (%) of total known FAME.

Statistical analysis

Statistical analysis of the data was done using the General Linear Model (GLM) procedure of SAS program

Table 2. Crude fat content of broiler liver, breast and thigh fed with five different fat sources

	BAS[1]	CLA	FXO	FHO	CXO	CHO	SEM[2]
Liver	4.97[bc]	9.52[a]	4.21[c]	4.82[bc]	4.38[c]	6.66[b]	0.37
Breast	0.85	0.69	0.83	0.89	0.68	0.79	0.05
Thigh	5.68	5.92	7.67	6.61	6.02	6.77	0.33

[1] Treatments: BAS = Basal diet (no fat source); CLA = 2% conjugated linoleic acid FXO = 2% flaxseed oil; FHO = 2% fish oil; CXO = 1% CLA+1% flaxseed oil; CHO = 1% CLA+1% fish oil.
[2] Standard error of the means.
[a,b,c] Mean values within a row followed by the different letter are significantly different (p<0.05).

(Version 6.12, Cary, NC, 2004). Statistical differences are expressed at p<0.05.

RESULTS

Dietary oil effects on crude fat content in liver

The effects of different dietary fat sources on crude fat content and fatty acid profile of broiler liver, breast and thigh meat summarized in Table 2 to 5. As shown in Table 2, addition of CLA to the basal diet had significant increasing effects on liver crude fat content (p<0.05). However, other fat sources did not have significant effects on liver crude fat content. In addition, crude fat content of breast and thigh meat had not significantly influenced by the addition of CLA, FXO, FHO or their combination forms with CLA.

Dietary oil effects on fatty acid profile in liver

Most fatty acid profiles in liver were significantly affected by dietary fat sources (Table 3). CLA-fed group showed significantly higher SFA content than that of BAS

Table 3. Fatty acids composition of broiler liver fed with five different fat sources

	BAS[1]	CLA	FXO	FHO	CXO	CHO	SEM[2]
C14:0	0.38[d]	0.99[a]	0.27[d]	0.56[b]	0.51[bc]	0.43[bc]	0.04
C16:0	23.72[c]	38.27[a]	20.24[d]	23.73[c]	28.75[b]	25.97[c]	0.94
C16:1	4.57[a]	3.21[bc]	2.69[cd]	3.82[ab]	3.35[bc]	2.16[d]	0.16
C18:0	15.73[d]	21.21[a]	17.45[cd]	16.19[d]	18.97[bc]	20.42[ab]	0.40
C18:1c9	35.31[a]	22.39[cd]	25.37[bc]	28.81[b]	24.84[bc]	18.41[d]	0.94
C18:2	10.27[bc]	8.10[c]	12.72[a]	9.92[bc]	11.63[ab]	13.57[a]	0.40
C18:3	0.27[c]	0.22[c]	1.87[a]	0.50[bc]	1.23[ab]	0.61[bc]	0.14
c9t11	-	0.82[a]	-	-	0.38[c]	0.63[b]	0.05
t10c12	-	0.40[a]	-	-	0.17[b]	0.30[ab]	0.03
C20:3	1.11[b]	0.53[d]	1.61[a]	0.95[bc]	1.05[bc]	0.71[cd]	0.07
C20:4	6.05[a]	1.70[c]	5.87[a]	3.90[b]	3.09[b]	3.31[b]	0.28
C22:0	-	-	0.57	-	0.25	-	0.11
C22:1	-	-	0.63	0.22	0.30	0.32	0.06
C20:5	0.27[c]	0.37[c]	3.49[a]	3.08[a]	1.99[b]	3.00[a]	0.22
C22:5	0.14[c]	0.13[c]	1.39[ab]	1.30[b]	1.02[b]	1.98[a]	0.11
C22:6	0.50[d]	0.37[d]	4.75[b]	6.43[a]	2.03[c]	7.10[a]	0.43
SFA[3]	39.83[c]	60.47[a]	38.28[c]	40.47[c]	48.49[b]	46.82[b]	1.19
MUFA[4]	40.00[a]	25.60[cd]	28.53[bc]	32.66[b]	28.46[bc]	20.77[d]	1.07
PUFA[5]	12.83[d]	12.61[d]	31.70[a]	25.63[bc]	22.44[c]	31.17[ab]	1.36
n-3[6]	0.89[c]	1.06[c]	11.49[a]	10.86[a]	6.26[b]	12.69[a]	0.78
n-6[7]	17.42[ab]	10.33[c]	20.21[a]	14.77[b]	15.78[b]	17.59[ab]	0.65
n-3/n-6	0.05[d]	0.10[d]	0.57[b]	0.74[a]	0.39[c]	0.72[a]	0.04

[1] Treatments: BAS = basal diet (no fat source); CLA = 2% conjugated linoleic acid FXO = 2% flaxseed oil; FHO = 2% fish oil; CXO = 1% CLA+1% flaxseed oil; CHO = 1% CLA+1% fish oil.
[2] Standard error of the means.
[3] Saturated fatty acid = C14:0+C16:0+C18:0+C22:0. [4] Monounsaturated fatty acid = C18:1+C22:1.
[5] Polyunsaturated fatty acid = C18:2+C18:3+c9t11+t10c12+C20:3+C20:4+C20:5+C22:5+C22:6.
[6] Omega-3 fatty acid = C18:3+C20:5+C22:5+C22:6. [7] Omega-6 fatty acid = C18:2+C20:3+C20:4.
[a,b,c,d] Mean values within a row followed by the different letter are significantly different (p<0.05).

or other fat groups (p<0.05). Although FXO-fed group or FHO-fed group had no significant effects on the SFA contents in liver compared to BAS group, the combination of CLA with FXO or FHO (CXO or CHO) had an alleviation effect on increasing the SFA contents in liver. MUFA contents were decreased in all of the fat-fed groups than those of BAS group (p<0.05). PUFA contents of birds fed with FXO, FHO, CXO and CHO were significantly higher than the CLA-fed group and BAS group (p<0.05). Results also showed that n-3 and n-6 fatty acid content of liver significantly affected by the addition of different fat sources to the diet (p<0.05). Birds fed with FHO and CHO had the highest n-3 to n-6 fatty acid ratio among the treatments (p<0.05), but CLA-fed group as single supplement did not have significant effects on n-3 to n-6 fatty ratio in liver compared to the BAS group.

Dietary oil effects on fatty acid profile in breast meat

A significant effect of dietary fat sources on fatty acid composition in breast meat was observed (Table 4). Results indicated that addition of CLA to the diet had significantly increased the SFA and n-6 fatty acids, while n-3 fatty acids and n-3 to n-6 fatty acid ratio were decreased as compared to other fat sources (p<0.05). Similar to the results of liver, CXO-fed group or CHO-fed group had an alleviation effect on increasing the SFA contents in breast meat. MUFA contents were decreased in all of the fat-added groups than those of BAS group (p<0.05). PUFA contents were increased by adding all of fat sources than the BAS group (p<0.05). The n-3 fatty acid contents of birds fed with FXO, FHO, CXO and CHO were higher than the CLA-fed group and BAS group (p<0.05). However, n-6 fatty acid contents were significantly increased in CLA-fed groups than BAS group and the other fat groups (p<0.05). The n-3 to n-6 fatty

Table 4. Fatty acids composition of broiler breast fed with five different fat sources

	BAS[1]	CLA	FXO	FHO	CXO	CHO	SEM[2]
C14:0	0.46[b]	0.48[b]	0.41[b]	0.90[a]	0.41[b]	0.50[b]	0.03
C16:0	23.07[a]	23.31[a]	20.08[b]	22.20[a]	22.35[a]	22.51[a]	0.30
C16:1	4.96[a]	1.63[d]	2.94[c]	3.72[b]	2.28[cd]	2.08[d]	0.18
C18:0	9.82[c]	15.23[a]	10.48[c]	11.01[c]	13.28[b]	13.27[b]	0.32
C18:1c9	33.02[a]	19.70[d]	26.57[b]	24.83[bc]	22.75[c]	18.86[d]	0.75
C18:1c11	3.61[a]	2.01c	2.59[b]	3.31[a]	2.12[bc]	2.24[bc]	0.11
C18:2	14.65[d]	20.97[a]	16.70[c]	13.47[d]	18.27[b]	16.45[c]	0.41
C18:3	0.42[d]	0.70[cd]	4.06[a]	0.35[d]	3.25[b]	1.10[c]	0.23
c9t11	-	2.10[a]	-	-	0.97[b]	1.20[b]	0.12
t10c12	-	1.43[a]	-	-	0.70[b]	0.83[b]	0.08
C20:1	0.54	0.42	0.45	0.48	0.41	0.42	0.02
C20:2	0.38	0.51	0.48	0.47	0.43	0.53	0.02
C20:3	1.04	0.77	1.04	0.93	0.91	0.79	0.04
C20:4	2.71[b]	2.16[b]	3.48[a]	2.64[b]	2.61[b]	2.57[b]	0.12
C22:0	-	0.17	-	-	0.19	-	0.02
C22:1	-	0.45[a]	0.35[ab]	0.22[b]	0.40[ab]	0.33[ab]	0.02
C20:5	0.18[d]	0.56[d]	2.24[bc]	2.67[b]	1.97[c]	3.37[a]	0.18
C22:5	0.23[c]	0.39[c]	2.18[a]	2.43[a]	1.43[b]	2.69[a]	0.16
C22:6	0.37[c]	0.71[c]	2.45[b]	5.42[a]	1.30[c]	5.48[a]	0.34
SFA[3]	33.35[c]	39.07[a]	30.82[d]	34.11[c]	36.16[b]	36.28[b]	0.47
MUFA[4]	42.80[a]	23.82[d]	32.81[b]	32.54[b]	27.96[c]	23.93[d]	1.01
PUFA[5]	19.85[d]	30.26[bc]	32.56[ab]	28.35[c]	31.85[ab]	34.99[a]	0.80
n-3[6]	1.13[d]	2.18[d]	10.93[b]	10.84[b]	7.96[c]	12.64[a]	0.68
n-6[7]	18.40[cd]	23.91[a]	21.22[b]	17.04[d]	21.79[b]	19.80[bc]	0.43
n-3/n-6	0.06[d]	0.09[d]	0.52[b]	0.63[a]	0.37[c]	0.63[a]	0.04

[1] Treatments: BAS = basal diet (no fat source) CLA = 2% conjugated linoleic acid; FXO = 2% flaxseed oil; FHO = 2% fish oil; CXO = 1% CLA+1% flaxseed oil; CHO = 1% CLA+1% fish oil.
[2] Standard error of the means.
[3] Saturated fatty acid = C14:0+C16:0+C18:0+C22:0. [4] Monounsaturated fatty acid = C16:1+C18:1c9+C18:1c11+C20:1+C22:1.
[5] Polyunsaturated fatty acid = C18:2+C18:3+c9t11+t10c12+C20:2+C20:3+C20:4+C20:5+C22:5+C22:6.
[6] Omega-3 fatty acid = C18:3+C20:5+C22:5+C22:6. [7] Omega-6 fatty acid = C18:2+C20:3+C20:4.
[a,b,c,d] Mean values within a row followed by the different letter are significantly different (p<0.05).

acid ratio in breast was the highest in FHO-fed group and CHO-fed group, and it was the lowest in CLA fed-group (p<0.05).

Dietary oil effects on fatty acid profile in thigh meat

Fatty acid compositions of broiler thigh meat are presented in Table 5. CLA-fed group showed significantly higher SFA content than that of BAS or other fat groups (p<0.05). MUFA contents were decreased in CLA-fed group than BAS group or other fat group (p<0.05). PUFA contents of birds fed with fat sources were significantly higher than the BAS group (p<0.05). All fat sources significantly increased n-3 fatty acid of thigh meat, but CLA had lowest and FXO had highest improving effects on n-3 fatty acid content of thigh meat (p<0.05). The n3/n-6 fatty acid ratio was the highest in FXO-fed group compared to other groups (p<0.05).

DISCUSSION

The main aim of the present study was to investigate the influence of dietary CLA, flaxseed oil and fish oil, and their combination on the deposition of CLA and other essential fatty acid, particularly, SFA and n-3 to n-6 fatty acids ratio in chicken meat. As a heterogeneous group of positional and geometric isomers of linoleic acid, CLA exist mainly in meat and dairy products of ruminants. Previous research suggests that diet rich in CLA significantly reduces the cardiovascular diseases, carcinogenesis, and modulate the immune response and insulin resistance in animals and

Table 5. Fatty acids composition of broiler thigh fed with five different fat sources

	BAS[1]	CLA	FXO	FHO	CXO	CHO	SEM[2]
C14:0	0.57^{c}	0.87^{b}	0.60^{c}	1.44^{a}	0.65^{c}	0.97^{b}	0.05
C14:1	0.15^{b}	0.06^{e}	0.12^{c}	0.23^{a}	0.09^{d}	0.08d^{e}	0.01
C16:0	24.19^{c}	28.44^{a}	21.50^{d}	23.59^{c}	26.02^{b}	28.21^{a}	0.42
C16:1	6.18^{a}	2.29^{d}	4.79^{b}	6.43^{a}	3.23^{c}	3.52^{c}	0.24
C18:0	8.54^{c}	14.13^{a}	7.67^{c}	7.94^{c}	12.16^{b}	11.73^{b}	0.4
C18:1c9	34.97^{a}	22.11^{d}	32.38^{b}	31.95^{b}	25.82^{c}	24.88^{c}	0.73
C18:1c11	2.80^{a}	1.44^{c}	2.27^{b}	2.58^{ab}	1.70^{c}	1.70^{c}	0.09
C18:2	14.89^{bc}	16.82^{a}	16.74^{a}	14.02^{c}	15.99^{ab}	15.27^{bc}	0.23
C18:3	0.65^{e}	1.10^{d}	6.91^{a}	0.80^{de}	4.42^{b}	2.25^{c}	0.35
c9t11	-	3.48^{a}	-	-	1.15^{b}	1.59^{b}	0.22
t10c12	-	2.31^{a}	-	-	0.73^{c}	1.05^{b}	0.15
C20:1	0.37^{abc}	0.30^{c}	0.19^{d}	0.31^{bc}	0.41^{ab}	0.45^{a}	0.02
C20:2	0.21^{a}	0.16^{ab}	0.14^{b}	0.13^{b}	0.15^{b}	0.15^{b}	0.01
C20:3	0.45^{a}	0.24^{bc}	0.29^{bc}	0.27^{bc}	0.31^{b}	0.21^{c}	0.02
C20:4	2.22^{a}	1.38^{b}	1.33^{b}	1.37^{b}	1.77^{ab}	1.27^{b}	0.10
C22:0	-	0.08	0.05	-	0.07	0.04	0.01
C22:1	-	-	0.10^{c}	0.17^{a}	0.11^{c}	0.15^{b}	0.01
C20:5	0.10^{d}	0.21^{d}	0.72^{c}	1.51^{a}	0.71^{c}	0.97^{b}	0.07
C22:5	0.17^{c}	0.18^{c}	0.70^{b}	1.02^{a}	0.71^{b}	0.77^{ab}	0.06
C22:6	0.28^{d}	0.30^{d}	0.72^{c}	2.05^{a}	0.56^{cd}	1.23^{b}	0.1
SFA[3]	33.30^{d}	43.51^{a}	29.78^{e}	32.97^{d}	38.88^{c}	40.93^{b}	0.77
MUFA[4]	44.41^{a}	26.19^{d}	39.83^{b}	41.69^{b}	31.33^{c}	30.78^{c}	1.03
PUFA[5]	18.96^{c}	26.18^{ab}	27.48^{a}	21.16^{c}	26.51^{ab}	24.76^{b}	0.53
n-3[6]	1.20^{e}	1.78^{d}	9.05^{a}	5.38^{c}	6.41^{b}	5.22^{c}	0.41
n-6[7]	17.55^{a}	18.44^{a}	18.36^{a}	15.67^{b}	18.07^{a}	16.76^{ab}	0.28
n-3/n-6	0.07^{e}	0.10^{d}	0.49^{a}	0.34^{b}	0.36^{b}	0.31^{c}	0.02

[1] Treatments: BAS = basal diet (no fat source) CLA = 2% conjugated linoleic acid; FXO = 2% flaxseed oil; FHO = 2% fish oil; CXO = 1% CLA+1% flaxseed oil; CHO = 1% CLA+1% fish oil.
[2] Standard error of the means.
[3] Saturated fatty acid = C14:0+C16:0+C18:0+C22:0. [4] Monounsaturated fatty acid = C16:1+C18:1c9+C18:1c11+C20:1+C22:1.
[5] Polyunsaturated fatty acid = C18:2+C18:3+c9t11+t10c12+C20:2+C20:3+C20:4+C20:5+C22:5+C22:6.
[6] Omega-3 fatty acid = C18:3+C20:5+C22:5+C22:6. [7] Omega-6 fatty acid = C18:2+C20:3+C20:4.
[a,b,c,d] Mean values within a row followed by the different letter are significantly different (p<0.05).

humans (Wahle et al., 2004; Bhattacharya et al., 2006). Moreover, CLA has been reported to induce a reduction of body fat in mice, chicks, and pigs (Cook et al., 1997; Park et al., 1997; Thiel-Cooper et al., 2001). Although its physiological role and body fat reduction potential of CLA have been reported widely, the effect on lipid metabolism is controversial. However, studies performed by Kelley et al. (2004) and Wang and Jones (2004), *trans* 10, *cis* 12 CLA isomer was responsible for some negative effects including the increment of insulin resistance, liver weight, triacylglycerol content and cholesterol content in mouse. We also confirmed fat increase of liver in CLA-fed birds compared with BAS or other fat source groups, and identified that the combination of CLA and flaxseed oil or fish oil prevented an increase of liver fat induced by CLA alone. This finding suggests that feeding of CLA to birds may trigger fatty liver due to result from deficiency of n-3 fatty acids.

The effect of CLA supplement on the fatty acid composition in meats has been extensively studied (Szymczyk et al., 2001; Joo et al., 2002; Badinga et al., 2003), and it has been suggested that CLA causes an increase in SFA but decreases MUFA content in broiler chicken meat fed with CLA only (Suksombat et al., 2007; Buccioni et al., 2009). Changes in SFA and MUFA composition are considered to be due to the inhibitory effect of CLA on the Δ9-desaturase (stearoyl-coenzyme A desaturase), synthesizing oleic acid (OA) in the broiler chicken liver. Similar trends were observed in both breast and thigh samples in our study, and it seems that CLA supplement *via* diets dose-dependently reduce the mRNA transcription of the Δ9-desaturase as described in a previous study that was conducted in layers (Shang et al., 2004). Therefore, it seems that SFA reduction of observed in our study is possibly due to the inhibition of Δ9-desaturase by the addition of CLA.

Dietary n-3 fatty acid ratio, relative to n-6 fatty acid recommended not to be less than 0.1 for ideal nutritive value in human (FAO/WHO, 1994; Gerster, 1998), but it was lower for both the BAS and CLA groups in our study (0.06 and 0.09 in breast meat, 0.07 and 0.10 in thigh meat, respectively). On the other hand, FXO and FHO fed-birds showed the higher value than these groups, and the ratio also increased in CXO and CHO groups. It can thus be suggested that these combinations to broiler chicks is a natural feeding practice that is able to increase the n-3 to n-6 fatty acid ratio of their meat.

Fish oil, source of the n-3 fatty acids contains LNA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that improve health-related factors in humans and animals. Hulan et al. (1988) and Huang et al. (1990) demonstrated that chickens fed a diet containing high level of fish meal or fish oil had considerable amounts of n-3 fatty acids, namely EPA and DHA, accumulated in the meat. Olomu and Baracos (1991) reported that flaxseed oil supplementation resulted in increased desaturation and elongation products (EPA, docosapentaenoic acid (DPA), and DHA) of LNA in chicken meat. These results may be attributed to the fact that feeding of fish oil or flaxseed oil to broiler may have induced an increase in the amount of n-3 fatty acids and decreases n-6 fatty acids in chicken meats. As expected, the present study also indicate that the observed changes in omega fatty acid composition of meats depended on the addition of dietary flaxseed oil and fish oil, and the similar pattern was found in the ratio of n-3 to n-6 fatty acids. In conclusion, dietary CLA caused an increase of liver fat and SFA content of meats in broiler chicks. However, this adverse effect of CLA feeding in meat production could alleviate in terms of combination with CLA and flaxseed and fish oils. Therefore, these combinations of diets when fed to broiler chickens can increase the n-3 to n-6 fatty acid ratio of their meat along with the deposition of CLA.

REFERENCES

Badinga, L., K. T. Selberg, A. C. Dinges, C. W. Comer and R. D. Miles. 2003. Dietary conjugated linoleic acid alters hepatic lipid content and fatty acid composition in broiler chickens. Poult. Sci. 82:111-116.

Belury, M. A. 2002. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu. Rev. Nutr. 22:505-531.

Bhattacharya, A., J. Banu, M. Rahman, J. Causey and G. Fernandes. 2006. Biological effects of conjugated linoleic acid in health and disease. J. Nutr. Biochem. 17:789-810.

Buccioni, A., M. Antongiovanni, M. Mele, M. Gualtieri and S. Minieri. 2009. Effect of oleic and conjugated linoleic acid in the diet of broiler chickens on the live growth performances, carcass traits and meat fatty acid profile. Ital. J. Anim. Sci. 8:603-614.

Cook, M. E., D. L. Jerome and M. Pariza. 1997. Broilers fed conjugated linoleic acid had enhanced bone ash. Poult. Sci. 76 (Suppl 1):S41.

Crespo, N. and E. Esteve-Garcia. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. Poult. Sci. 80:71-78.

FAO/WHO. 1994. Fats and oil in human nutrition (report of a joint expert consultation), FAO Food and Nutrition No 57, Rome: WHO/FAO.

Folch, J., M. Less and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Gerster, H. 1998. Can adults adequately convert alpha-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid. Int. J. Vitam. Nutr. Res. 68:159-173.

Huang, Z. B., R. G. Ackman, W. M. N. Ratnayake and F. G.

Proudfoot. 1990. Effect of dietary fish oil on n-3 fatty acid levels in chicken eggs and thigh flesh. J. Agric. Food Chem. 38:743-747.

Hulan, H. W., R. G. Ackman, W. M. N. Ratnayake and F. G. Proudfoot. 1988. Omega-3 fatty acid levels and performance of broilers chickens fed redfish meal or redfish oil. Can. J. Anim. Sci. 68:533-547.

Joo, S. T., J. I. Lee, Y. L. Ha and G. B. Park. 2002. Effects of dietary conjugated linoleic acid on fatty acid composition, lipid oxidation, color, and water-holding capacity of pork loin. J. Anim. Sci. 80:108-112.

Kelley, D. S., M. Vemuri, Y. Adkins, S. H. Gill, D. Fedor and B. E. Mackey. 2009. Flaxseed oil (*ALA*) prevents trans-10, cis-12-conjugated linoleic acid-induced insulin resistance in mice. Br. J. Nutr. 101:701-708.

Krejcí-Treu, T., E. Straková, P. Suchý and I. Herzig. 2010. Effect of vegetable oil fortified feeds on the content of fatty acids in breast and thigh muscles in broiler chickens. Acta Vet. Brno. 79:S21-S28.

Larsen. T. M., S. Toubro and A. Astrup. 2003. Efficacy and safety of dietary supplements containing conjugated linoleic acid (CLA) for the treatment of obesity: evidence from animal and human studies. J. Lipid. Res. 44:2234-2241.

Olomu, J. M. and V. E. Baracos. 1991. Influence of dietary flaxseed oil on the performance, muscle protein deposition and fatty acid composition of broiler chicks. Poult. Sci. 70:1403-1411.

Park, Y., K. J. Alright, W. Liu, J. M. Storkson, M. E. Cook and M. W. Pariza. 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids 32:853-858.

SAS. 2004. SAS User Guide to Statistics (Version 9.12). SAS Inst. Inc., Cary, NC. USA.

Shang, X. G., F. L. Wang, D. F. Li, J. D. Yin and J. Y. Li. 2004. Effects of dietary conjugated linoleic acid on the productivity of laying hens and egg quality during refrigerated storage. Poult. Sci. 83:1688-1695.

Simopoulos, A. P. 2003. Importance of the ratio of omega-6/omega-3 essential fatty acids: Evolutionary aspects. World Rev. Nutr. Diet. 92:171-174.

Smith, S. B., T. S. Hively, G. M. Cortese, J. J. Han, K. Y. Chung, P. Castenada, C. D. Gilbert, V. L. Adams and H. J. Mersmann. 2002. Conjugated linoleic acid depresses the Δ9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. J. Anim. Sci. 80:2110-2115.

Suksombat, W., T. Boonmee and P. Lounglawan. 2007. Effects of various levels of conjugated linoleic acid supplementation on fatty acid content and carcass composition of broilers. Poult. Sci. 86:318-324.

Szymczyk, B., P. M. Pisulewsky, W. Szczurek and P. Hanczakowsky. 2001. Effects of conjugated linoleic acid on growth performance, feed conversion efficiency, and subsequent carcass quality in broiler chickens. Br. J. Nutr. 85:465-473.

Thiel-Cooper, R. L., F. C. Parrish, J. C. Jr., J. C. Sparks, B. R. Wiegand and R. C. Ewan. 2001. Conjugated linoleic acid changes swine performance and carcass composition. J. Anim. Sci. 79:1821-1828.

Tricon, S., G. C. Burdge, C. M. Williams, P. C. Calder and P. Yaqoob. 2005. The effects of conjugated linoleic acid on human health-related outcomes. Proc. Nutr. Soc. 64:171-182.

Wahle, K. W., S. D. Heys and D. Rotondo. 2004. Conjugated linoleic acids: are they beneficial or detrimental to health? Prog. Lipid. Res. 43:553-587.

Wang, Y. and P. J. Jones. 2004. Dietary conjugated linoleic acid and body composition. Am. J. Clin. Nutr. 79:1153S-1158S.