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Effects of Semi Replacement of Dietary Olive Oil and Corn Oil with Conjugated Linoleic Acid (CLA) on Broiler Performance, Serum Lipoprotein Levels, Fatty Acid Composition in Muscles and Meat Quality During Refrigerated Storage

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Abstract: The effects of semi replacement of dietary Olive Oil (OO) and Corn Oil (CO) with Conjugated Linoleic Acid (CLA) on performance parameters, carcass composition, lipid oxidation and fatty acid compositions of thigh and breast tissues and serum lipoprotein in broiler chickens were investigated. One-day-old (n=160) Ross 308 broilers were fed with one of the five diets containing 3 % CLA source (2.4 % CLA); 3 % OO; 3 % CO; 1.5 % OO+1.5 % CLA source (1.2 % CLA) and 1.5 % CO+ 1.5 % CLA source (1.2 % CLA). The highest weight gain was obtained among birds fed with 1.5 % OO + 1.5% CLA. Thiobarbituric Acid Reactive Substances (TBARS) were significantly influenced by the dietary CLA, olive oil and storage. The CLA supplementation adversely effected the fatty acid composition of the muscles by increasing Saturated Fatty Acids (SFA) and Polyunsaturated Fatty Acids (PUFA) proportions at the expense of Monounsaturated Fatty Acids (MUFA). However olive oil and semi replacement with CLA increased MUFA and caused a decrease in LDL-C without reducing HDL-C. The oxidative stability of muscle tissue was also improved by conjugated linoleic acid supplementation (3% CLA), olive oil and especially OO+CLA and this may favorably influence meat.

Key words: Conjugated linoleic acid, olive oil, TBARS, fatty acid composition, serum lipoprotein

INTRODUCTION

Conjugated Linoleic Acid (CLA) is a collective name referring to the positional and geometric (*cis*, *trans*) conjugated dienoic isomers of linoleic acid. CLA has been reported to have beneficial effects on human health, atherosclerosis, hypercholesterolemia and cancer (Pariza and Hargraves, 1985; Chin *et al*., 1992; Ip *et al*., 1991). Ruminant products, such as milk, beef, and cheese are major CLA sources in human foods (Chin *et al*., 1992) whereas poultry products contain relatively low CLA.

CLA was found to have a potent immune modulating activity characterized by increased blastogenesis and macrophage killing ability. Recently there has been a lot of interest in enriching egg, meat and dairy products for human consumption due to these biological properties of CLA (Aydın, 2005). Therefore feeding animals with synthetic CLA sources should be a good way to enrich foods in biologically effective CLAs.

Simpson and Peters (1987) and Simpson *et al*. (1988) reported that olive oil a good protection against lipid peroxidation because of its high content of monounsaturated fatty acid namely oleic acid which was reportedly resistant to lipid peroxidation and inhibits it perhaps by chelating free iron, and vitamin E. It was also reported that serum cholesterol level was decreased by olive oil which provided a protective effect against the development of atherosclerosis (Gökçe *et al*., 2000).

Although current investigations have proven that CLA can be used successfully in poultry diets as energy source or antioxidants (Bölükbaşi and Erhan, 2005; Bölükbaşi, 2006; Schmid *et al*., 2006), this present experiment was aimed to investigate the effects of semi mixtures of corn oil and olive oil with CLA on broiler performance, serum lipoprotein levels, fatty acid composition in thigh and breast muscles and Thiobarbituric Acid Reactive Substances (TBARS) of thigh and breast tissues to determine lipid oxidation during storage at 4 °C.

MATERIALS AND METHODS

A total of 160 one day old male chickens with a weight 37.28-37.83 g of a commercial strain (Ross 308) were randomly allocated to five groups equally (n=32) and each treatment was replicated four times. Birds were given a starter diet from 1 to 21 days and a finisher diet from 22 to 42 d (Table 1). The five dietary treatments consisted of

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Table 1: Composition of experimental feeds

Ingredients and analyses Ingredients	Starter (%)	Finisher (%)
Yellow corn	53.44	60.90
Soybean meal	33.00	23.69
Fish meal	3.50	3.50
Sunflower meal	2.5	4.00
Limestone	1.0	1.0
Dicalcium phosphate	1.0	1.0
Vitamin premix[2]	0.65	0.65
Mineral premix[3]	0.15	0.15
Salt	0.36	0.36
DL-methionine	0.10	0.60
Lysine	1.30	1.15
Supplemental oil[1]	3	3
Total	100	100
Calculated analyses		
Crude protein	22.5	21
ME, kcal kg^{-1}	3070	3220

[1]Conjugated Linoleic Acid (CLA) 3 %, Olive Oil (OO) 3 %, Corn Oil (CO) 3 %, OO 1.5 %+1.5 % CLA, CO 1.5 %+CLA 1.5%, [2]Provides per kg of diet: vitamin A 10000 IU; vitamin D3 5000 IU; vitamin E 15 IU; thiamine 2 mg; riboflavin 5 mg; niacin 33 mg; pantothenic acid 3.17 mg; pyridoxine 908 mg; folic acid 1 mg; biotin 30 mg, [3]Provides per kg of diet: Mn, 60 mg; Zn, 50 mg; Fe, 40 mg; Cu, 13 mg; I 1 mg

diets containing 3 % CLA source (2.4 % CLA), 3 % olive oil, 3 % corn oil, 1.5 % olive oil+1.5 % CLA source (1.2 % CLA), 1.5 % corn oil+1.5 % CLA source (1.2 % CLA). The CLA source contained 80 % CLA (CLA mix contained 39 % c9-t11, 39 % c10-t12, 2 % palmitic acid, 3 % stearic acid, 13 % oleic acid, 0.5 % linoleic acid and 2 % other CLA isomers, Pharmanutrients, Lake Bluff, IL 60044).

Body weights were measured at the beginning and end of the one period of 42 d and feed intake was measured over these periods. At 42 d of age, ten birds per treatment were slaughtered by neck cutting. Blood samples were obtained from the brachial veins and serum samples were separated by low-speed centrifugation (1500 g for 15 min at 20°C) to determine lipoprotein profiles. The analysis of Total Cholesterol (TC), Trigly ceride (TG) and High Density Lipoprotein Cholesterol (HDL-C) in serum were measured using commercially available kits (Sigma Diagnostics, Taufkirchen, Germany). Low Density Lipoprotein Cholesterol (LDL-C) levels were estimated by Friedawald equation (Friedawald *et al*., 1972).

Each bird was weighed live, slaughtered and allowed to bleed for 180 s, which was previously determined by Yalçın *et al*. (1999) to be sufficient time for bleeding. The birds were scalded at 50-52 °C for approximately 30 s and placed in a rotary drum plucker for 30 s to remove feathers. Carcasses were then plucked, the bird then processed by removing the head, neck, shanks and feet. The liver, lung and heart were dissected from the viscera. All of the above components were weighed individually, and then breast, wing and thigh samples were weighed and carcass yield was calculated. Breast and thigh meats were stored-20 °C until lipid analysis. Also, breast and thigh samples were stored 4 °C for TBARS analysis. TBARS were determined at 1, 3 and 7 d. (Bölükbaşı, 2006).

TBARS analyses: TBARS were determined on meat samples as described earlier (Cherian *et al*., 1996). Tissue samples (2 g) were weighed into test tubes each with 18 mL of 3.86% perchloric acid; samples were homogenized with a polytron for 15 s at a high speed. Fifty microliters of Butylated Hydroxy Anisole (BHA) (4.5 % BHA in ethanol) was added to the sample prior to homogenization. The homogenate was filtered through a Whatman #1 filter paper. Filtrate (2 mL) was mixed 2 mL of 20 mM TBA (thiobarbuturic acid) in distilled water and incubated in a boiling water bath for 30 min. After cooling, the absorbance of filtrate was determined at 531 nm against a blank containing 2 mL of 3.86 % perchloric acid and 2 mL of 20 mM TBA solution. The TBARS values were expressed as milligrams of Malonaldehyde (MA) per kilogram of tissue.

Fatty acid analysis: A representative sample of 4 g of muscle was taken and lipids were extracted by the method of Folch *et al*. (1957). Fatty acids of the lipids were methylated by adding 10 mL of anhydrous 3N HCL-methanol to 180 to 200 μg of lipid and heating this mixture for 40 min at 60°C (Chin *et al*., 1992). Analysis of fatty acid composition was performed with a gas chromatography (HP 6890) using a Supelcowax-10 fused silica capillary column (30 m×0.32 mm inner diameter, 0.25 μm film thickness). Over temperature was programmed at 100 °C for 2 min after injection and then was ramped up to 180 °C at 20°C min^{-1} and to 230°C at 2°C min^{-1} the temperature of 230°C was maintained for 15 min. temperature of the injector and flame ionization detector were 220 and 250°C, respectively. Helium was used as the carrier gas.

Statistical analyzes: Data were subjected one-way and two-way Analysis of Variance (ANOVA) by using the statistical package SPSS for Windows (1999), version 10.0. Significant means were compared with a multiple comparison test (Duncan) at α = 0.01 and 0.05 levels (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Although there were no significant differences between initial weights of all groups, final weights differed significantly ($p<0.05$). The highest value was determined form 50% replacement of dietary OO with CLA with respect to final body weights (Table 2). Adversely to our findings, Badinga *et al*., (2003) reported that broilers fed a CLA-supplemented diet had less body weight gain and feed intake than broilers fed with the diet containing corn oil.

Table 2: Influence of dietary Conjugated Linoleic Acid (CLA), olive oil and corn oil on performance of broilers with 42 d of age

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion
CLA	37.39	2352.95^{bc}	2315.56^{b}	3940.78^{ab}	1.70^{a}
OO	37.68	2384.52^{b}	2346.84^{b}	3993.58^{ab}	1.70^{a}
CO	37.33	2318.25^{c}	2280.92^{c}	3803.83^{b}	1.66^{b}
OO+CLA	37.83	2462.55^{a}	2424.72^{a}	4125.27^{a}	1.70^{a}
CO+CLA	37.28	2394.32^{b}	2357.04^{b}	4090.57^{a}	1.73^{a}
SEM	0.3	23.195	23.36	39.79	0.013
P	NS	*	*	*	*

*: p<0.05 ^{a,b,c}: Column means with no common superscript differ significantly, CLA: Conjugated Linoleic Acid, OO: Olive Oil, CO: Corn Oil

Table 3: Effect of CLA, olive oil and corn oil on carcass characteristics of broilers with age of 42 d

	CLA	OO	CO	OO+CLA	CO+CLA	SEM	*P*
Hot carcass (g)	1821^{ab}	1820.5^{ab}	1787^{bc}	1865^{a}	1737^{c}	10.55	*
Liver (g)	45^{c}	48.5^{ab}	51^{a}	49.5^{a}	47.5^{ab}	0.88	*
Heart (g)	15^{ab}	14.5^{abc}	15.5^{a}	12.5^{bc}	12^{c}	0.38	*
Wing (g)	175.5^{c}	191.5^{a}	185^{a}	191^{a}	180.5^{b}	0.79	**
Thigh (g)	620^{a}	608^{a}	545.5^{bc}	565.5^{b}	534^{c}	3.07	**
Breast (g)	655.7^{a}	644.5^{a}	598^{bc}	609.5^{b}	574^{c}	4.34	**
Carcass yield (%)	*77^{a}*	*76^{a}*	*77^{a}*	*76^{a}*	*73^{b}*	*0.24*	*

*: p<0.05 **: p<0.01 NS: Not significant ^{a,b,c} :Means without the same superscript within a row differ significantly, CLA: Conjugated Linoleic Acid, OO: Olive Oil, CO: Corn Oil

Table 4: Effect of dietary Conjugated Linoleic Acid (CLA), olive oil, corn oil and days of refrigeration on the Thiobarbituric Acid Reactive Substance (TBARS) of thigh and breast tissues of birds with age of 42 d

	TBARS (mg MDA kg^{-1} meat^{1})									
	Thigh					*Breast*				
Duration of refrigeration	CLA	OO	CO	OO+CLA	CO+CLA	CLA	OO	CO	OO+CLA	CO+CLA
1 d	0.10^{h}	0.09^{h}	0.24^{e}	0.01^{i}	0.19^{f}	0.11^{g}	0.07^{i}	0.09^{h}	0.07^{i}	0.17^{f}
3 d	0.11^{h}	0.14^{g}	0.25^{e}	0.01^{i}	0.17^{f}	0.13^{g}	0.12^{g}	0.15^{f}	0.08^{g}	0.17^{f}
7 d	1.96^{b}	1.30^{c}	4.29^{a}	1.02^{d}	1.92^{b}	1.99^{b}	1.25^{d}	4.45^{a}	0.99^{e}	1.76^{c}
SEM			0.003						0.002	
Diet			**						**	
Day			**						**	
Diet x Day			**						**	

**: p<0.01 NS: Not significant [1]: MDA: mg malondialdehyde $^{a-j}$: Means with no common superscript differ significantly, CLA: Conjugated Linoleic Acid, OO: Olive Oil, CO: Corn Oil

The results on the hot carcass weight, liver, heart, wing weight, leg weight, breast weight and hot carcass yields are shown in Table 3. As shown, birds fed CO+CLA had lowest hot carcass weight, heart, leg, breast weight and carcass yield. The group fed the 3 % CLA diet had lower (p<0.01) liver and wing weight than others groups (Table 3).

The TBARS values of thigh and breast samples in all groups increased significantly (p<0.05) at the end of the storage time. However, the usage of 1.5 % CLA+1.5 % OO in poultry diets prevented lipid oxidation at significant (p<0.01) level considering thigh and breast tissues (Table 4). This may be caused by the antioxidant effects of CLA (Ha *et al*., 1990; Ip *et al*., 1991).

Each of the fatty acid levels of both thigh and breast tissues differed significantly (p<0.01) based on the diet type. Total SFA and total PUFA values were different from each other in all groups considering thigh and breast tissues. SFA and PUFA values from CLA group were higher than other groups in thigh and breast. And a total MUFA value of OO group was higher than other groups (Table 5). Similarly high SFA values were reported from the CLA groups (Szmyczyk *et al*., 2001; Aletor *et al*., 2003; Sirri *et al*., 2003).

Myristic and palmitic acid content of thigh and breast tissue from the CLA group was significantly (p<0.01) greater than other groups. The ratio of stearic acid in thigh tissue from CLA and CLA+CO group was greater than those of OO, OO+CLA and CO group. However, the highest level of stearic acid in breast tissue was obtained from the diet contained 3% CLA and 3 % OO.

The proportions of MUFA changed significantly (p<0.01) between diet groups except for the CLA and CO in thigh tissue and except for the CO and CO+CLA in breast tissue. These results were in accordance with the findings of Szmyczyk *et al*. (2001) and Sirri *et al*. (2003). The semi replacement of CLA into olive oil (OO+CLA) decreased oleic acid content of the thigh tissues. However, the high levels of oleic acid concentrations determined from OO in thigh and OO+CLA in breast tissues may be caused by the high oleic acid concentration of olive oil. Similarly, Li and Watkins (1998)

Table 5: Influence of dietary Conjugated Linoleic Acid (CLA), olive oil, corn oil on fatty acid composition of thigh and breast tissues of birds with age of 42 d

	Thigh							*Breast*						
	CLA	OO	CO	OO+CLA	CO+CLA	SEM	P	CLA	OO	CO	OO+CLA	CO+CLA	SEM	P
Lipid Content[1]	2.98	3.1	3.3	3	3.1	0.002	NS	0.90	1.1	1.3	0.99	1.15	0.003	NS
Fatty acids (%)														
Myristic (14:0)	1.88^{b}	1.40^{e}	1.05^{e}	2.20^{a}	1.25^{d}	0.007	**	2.86^{a}	1.46^{d}	1.90^{d}	2.01^{c}	2.08^{b}	0.007	**
Palmitic (16:0)	21.00^{a}	17.06^{e}	17.35^{d}	18.22^{c}	19.59^{b}	0.13	**	20.12^{a}	17.06^{e}	16.86^{d}	17^{c}	17.76^{b}	0.012	**
Palmitooleic (16:1)	1.17^{e}	3.49^{a}	2.06^{b}	1.83^{c}	1.34^{d}	0.010	**	0.88^{e}	1.61^{a}	1.51^{b}	0.96^{d}	1.47^{c}	0.005	**
Stearic (18:0)	8.12^{a}	4.06^{e}	4.98^{d}	5.09^{c}	7.68^{b}	0.015	**	6.15^{a}	6.11^{a}	5.67^{b}	5.23^{d}	5.59^{c}	0.011	**
Oleic (18:1)	23.59^{c}	31.67^{a}	23.68^{d}	26.68^{b}	23.0^{e}	0.015	**	17.36^{d}	20.77^{b}	19.48^{e}	21.77^{a}	19.49^{e}	0.013	**
Linoleic (18:2)	29.46^{e}	34.08^{c}	35.70^{a}	35.14^{b}	33.72^{d}	0.016	**	30.42^{c}	28.96^{e}	29.26^{d}	31.98^{b}	34.54^{a}	0.009	**
Linolenic (18:3)	1.53^{a}	0.53^{e}	0.73^{d}	1.01^{c}	1.21^{b}	0.008	**	1.35^{a}	1.32^{ab}	1.27^{b}	1.33^{a}	1.28^{b}	0.007	*
Arachidonic (20:4)	1.00^{e}	1.78^{d}	6.22^{a}	2.07^{c}	3.03^{b}	0.008	**	2.85^{e}	8.87^{b}	9.34^{a}	4.30^{c}	3.82^{d}	0.012	**
*cis*9-*trans*11 CLA	6.72^{a}	ND	ND	3.21^{b}	3.02^{c}	0.004	**	4.90^{a}	ND	ND	1.96^{b}	1.93^{c}	0.004	**
*cis*12-*trans*10 CLA	5.46^{a}	ND	ND	2.58^{b}	2.45^{c}	0.011	**	3.87^{a}	ND	ND	1.55^{b}	1.51^{c}	0.005	**
SFA[2]	31.00^{a}	22.52^{e}	23.38^{d}	25.51^{c}	28.52^{b}	0.008	**	29.13^{a}	24.63^{c}	24.43^{d}	24.24^{e}	25.43^{b}	0.007	**
MUFA[3]	24.76^{c}	35.16^{a}	25.74^{c}	28.51^{b}	24.34^{d}	0.021	**	18.24^{d}	22.38^{b}	20.99^{c}	22.73^{a}	20.96^{c}	0.008	**
PUFA[4]	44.17^{a}	36.39^{d}	42.93^{c}	44.1^{a}	43.43^{b}	0.015	**	43.39^{a}	39.15^{e}	39.87^{d}	41.12^{c}	43.08^{b}	0.004	**

**: p<0.01 $^{a-e}$ Means without same superscript within a row differ significantly at alpha 0.01 levels in thigh and breast, [1] lipid content (g/100 g of muscle), [2]Saturated fatty acids, [3] Monounsaturated fatty acids, [4] Polyunsaturated fatty acids, CLA: Conjugated Linoleic Acid, OO: Olive Oil, CO: Corn oil

Table 6: Influence of dietary conjugated linoleic acid (CLA), olive oil and corn oil on serum lipoprotein of birds with the age of 42 d

Parameters (mg dL^{-1})	CLA	OO	CO	OO+CLA	CO+CLA	SEM	P
Triglyceride	92^{c}	85^{c}	136^{a}	91^{c}	115^{b}	1.95	**
HDL-C	105^{a}	103^{a}	95^{b}	102^{a}	101^{a}	1.86	*
LDL-C	60.7^{a}	49.3^{d}	50.9^{c}	55.4^{b}	57.5^{ab}	0.83	**
Total cholesterol	160^{a}	152^{b}	145^{c}	154^{b}	156^{b}	2.89	*

*: p< 0.05; **: p<0.01 ^{a,b,c} Means without the same superscript within a row differ significantly, CLA: Conjugated Linoleic Acid, OO: Olive Oil, CO: Corn Oil, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol

speculated that CLA reduced the levels of oleic acid by inhibiting liver Ä9-desaturase activity. And they also reported that dietary CLA decreased the concentrations of palmitoleic and oleic acids as observed in the present study.

PUFA level was significantly (p<0.01) high in thigh and breast tissues from the diets with CLA compared to the groups without CLA. Similar findings were also reported early by Szymczyk *et al.* (2001) and Sirri *et al.* (2003). CLA was not detected in the tissue lipids of broilers fed diet without CLA in this study.

The greatest concentration of CLA, *cis*9-*trans*11 plus *trans*10-*cis*12 isomers, in thigh and breast tissue was obtained when the diet contained 3% CLA. Moreover accumulation of *cis*9-*trans*11 was higher than that of *trans*10-*cis*12 in the present study. The proportion of arachidonic acid in thigh and breast tissue lipids was decreased by dietary CLA. Present data were in accordance with the findings of Szmyczyk *et al.* (2001) and Sirri *et al.* (2003). Linolenic acid was highest in thigh of CLA group in the present study.

Triglyceride and LDL-C were decreased significantly and HDL-C was increased (p<0.01) by feeding with olive oil (Table 6). Moreover, olive oil with high MUFA (oleic acid) compared to the other oils with high level of PUFA did not reduce the HDL level which was reported very important in cholesterol metabolism (Gökçe *et al.*, 2000). Total cholesterol (TC) concentrations and LDL-C reached a maximum in broilers fed 3% CLA. HDL-C level was low in CO. Lee *et al.* (1994) and Nicolosi *et al.* (1997) reported that CLA was shown to decrease the levels of plasma triacylglycerol, VLDL and LDL in rabbits. Similarly, Szymczyk *et al.* (2001) reported that CLA increased TC and HDL- C, but reduced HDL-C: TC in plasma of broilers.

CONCLUSION

The data of the present study showed that feeding broilers with diet containing 3% CLA was an effective way to obtain CLA-enriched poultry meat. The CLA supplementation adversely effected the fatty acid composition of the muscles by increasing their SFA and PUFA proportions at the expense of MUFA percentage. However olive oil and semi replacement with CLA oil increased MUFA and caused a decrease LDL-C without reducing HDL-C. At the same time the oxidative stability of muscle tissue was also improved by conjugated linoleic acid supplementation (3% CLA), olive oil and especially OO+CLA and this may favorably influence meat shelf-life. Considering the data determined it may be suggested that the replacement of 50% of OO can be successfully used in poultry feeds.

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