

The effect of conjugated linoleic acid on the fatty acid composition of different tissues and yolk lipids in pigeons

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Abstract

Dietary conjugated linoleic acid (CLA) was reported to increase the levels of saturated fatty acids (SFA), to decrease monounsaturated fatty acids (MUFA) in egg yolk, liver and adipose tissue in chicken and quail. Hence, the objective of this study was to determine the effects of dietary CLA on the fatty acid composition of liver, breast muscle, abdominal adipose tissue and egg yolk lipids in pigeons. Eight established breeding pairs per group were fed either a commercially pelleted pigeon diet mixed with 0.5% safflower oil (SFO) or 0.5% CLA for 12 weeks. For fatty acid analyses, four eggs per group were collected at the 10th week of feeding. At the end of the 12th week of the CLA feeding, four pigeons per treatment were killed and liver, breast muscle and abdominal fat pads were obtained at necropsy. For fatty acid analyses, fats from liver, breast muscle, abdominal adipose tissue and egg yolks were extracted with chloroform : methanol (2 : 1, v/v). Dietary CLA had no effect on the fatty acid composition of the liver compared to the control. However, dietary CLA caused a significant increase in the level of SFA and a decrease in the level of MUFA in breast muscle, adipose tissue and egg yolk. Breast muscle, adipose tissue and egg yolk from the CLA-fed pigeons had 1.8-, 1.6-, and 1.5-fold increases in the ratio of C16:0/C16:1(n-7), respectively. Also the ratio of C18:0/C18:1(n-9) in breast muscle, adipose tissue and egg yolk from the CLA group increased 1.4-, 2.2- and 1.9-fold, respectively, compared to the control. Relative CLA (%) in the liver, breast muscle, adipose tissue and egg yolk from the CLA group was 0.71, 1.30, 2.14 and 1.22%, respectively. Control egg yolk contained only 0.06% CLA. This study showed that CLA altered the fatty acid composition of the related tissues and egg yolk when supplemented in the diet of pigeons.

Keywords: Conjugated linoleic acid, egg and tissue fatty acid compositions, pigeon
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Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometrical isomers of linoleic acid (C18:2 c-9, c-12) and has received much attention because of the many biological activities shown in animal models. CLA was first isolated from grilled ground beef (Ha *et al.*, 1987) and found to be a potent anti-carcinogen in several animal models (Ha *et al.*, 1990; Ip *et al.*, 1991; Ip *et al.*, 1994). Some researchers reported that chicks fed CLA had significantly less growth suppression than control fed chicks when they were induced by endotoxin injection (Cook *et al.*, 1993; Miller *et al.*, 1994). Dietary CLA was shown to affect body composition in several animal species by acting on fatty acid metabolism. Recently, it was shown that CLA reduced body fat and increased lean body mass in rats (Park *et al.*, 1997).

Dietary fats were shown to change the fatty acid composition of egg yolk, adipose tissue and liver of laying hens (Sim *et al.*, 1972). CLA was incorporated into milk fat, tissue lipids and foetal and neonatal tissues when dams were fed CLA (Chin *et al.*, 1994). It was also reported that CLA feeding caused significant changes in the fatty acid composition of the aorta, liver and plasma of rabbits (Lee, 1996). In the studies conducted on chickens, dietary CLA caused a significant increase in the level of SFA (mainly C16:0 and C18:0) and a decrease in the level of MUFA (mainly C16:1 n-7 and C18:1 n-9) in egg yolk (Chamruspollert & Sell, 1999; Aydin *et al.*, 2001). Another study conducted on quail showed that dietary CLA significantly altered the fatty acid composition of egg yolk and liver (Aydin & Cook, 2004). It was shown that dietary CLA caused higher levels of saturated fatty acids (SFA) and lower levels of monounsaturated fatty acids (MUFA) by inhibiting stearyl-CoA desaturase activity (Lee *et al.*, 1998). Nutritional studies showed that CLA isomers were readily incorporated into animal tissues depending on its level in the diet (Ip *et al.*, 1994; Banni *et al.*, 1995; Kramer *et al.*, 1998). It was also shown that CLA could

be incorporated into the lipids of liver, muscle, adipose tissue and egg yolks from laying hens fed CLA (Aydin *et al.*, 2001; Aydin & Cook, 2005).

Hence, the objective of this study was to determine the effect of incorporating CLA in the diet on the fatty acid composition of the liver, muscle, abdominal adipose tissue, and egg yolk of pigeons.

Materials and Methods

CLA was synthesized from linoleic acid (99% pure; NU Check Prep., Elysian, MN) by alkali isomerization and analysed for purity by a capillary gas chromatography (GC), as previously described (Ha *et al.*, 1990; Chin *et al.*, 1992). Purity of CLA was determined to exceed 95% as total CLA isomers. Prepared CLA was stored under an argon atmosphere at -20 °C until used.

The pigeon house was divided into two sections and eight established breeding pairs per treatment were fed either a commercially pelleted pigeon diet (containing 150 g crude protein/kg and 12.13 MJ metabolisable energy/kg) mixed with 0.5% safflower oil (SFO, control) or 0.5% synthetically prepared CLA (contained 95% CLA) in the form of free fatty acid for 12 weeks. The experimental diets were mixed weekly and birds were fed fresh feed daily.

Four eggs from each group were collected for two consecutive days during the 10th week of the feeding study. Eggs were broken and the yolks and albumen of the eggs were separated. The last traces of albumen were removed by rolling the egg yolk on filter paper. Egg yolks separated from albumens were kept in vials at -20 °C until analysis for fatty acid content. At the end of the 12th week of the CLA feeding period, four pigeons per treatment were chosen at random and killed by means of carbon dioxide exposure. Livers, pectoralis (breast muscle) and abdominal fat pads from sacrificed pigeons were obtained at necropsy and their weights were recorded. Tissue samples were frozen at -20 °C until analysed for fatty acid composition. Fat from all tissues was extracted with chloroform : methanol (2 : 1 v/v) according to the method of Folch *et al.* (1957). Fatty acid methyl esters (FAME) were prepared by reaction with 4% HCL in methanol for 20 min at 60 °C and the composition of the FAME was determined by GC. Briefly, a Hewlett-Packard 5890 series II GC was fitted with a flame-ionization detector and 3396A integrator. A supercovax-10 fused silica capillary column (60 m X 0.32 mm i.d., 0.25 µm film thickness) was used. Oven temperature was programmed from 50 to 200 °C, increasing 20 °C per min., held for 50 min and increasing 10 °C per min to a final temperature of 230 °C, where it was held for 20 min. Heptadecanoic acid (Sigma Chemical Co., St Louis, MO) was used as an internal standard. The FAME were identified by comparison of retention times with methylated fatty acid standards (Sigma Chemical Co., St Louis, MO and Nu-Chek Prep., Elysian, MN) and expressed as percentage of total FAME (Chin *et al.*, 1992).

Differences in the fatty acid compositions of egg and tissues, with diet as main effect, were analysed statistically by using a t-test (SPSS software 10.0; Chicago, IL), with differences being considered significant at $P < 0.05$.

Results

Table 1 represents the effects of dietary CLA on the fatty acid profile of the liver in the pigeons. Feeding CLA to the pigeons did not cause a significant change in the fatty acid composition of the liver when compared to the control group ($P > 0.05$). The relative level of CLA (as % of fatty acid methyl esters) in the liver was 0.71%, although CLA was not detectable in the liver of pigeons fed the SFO diet.

The effect of feeding CLA on the fatty acid composition of the pigeon breast muscle is shown in Table 2. The relative level of CLA (%) in the breast muscle was 1.3%. In contrast, CLA was not detectable in the breast muscle of pigeons fed the SFO diet. Pigeons fed CLA had increased levels of (as % of fatty acid methyl esters) total SFA and decreased levels of total MUFA in the breast muscle when compared to the SFO group ($P < 0.05$). A higher concentration of C14:0, C16:0 and a lower concentration of C18:1(n-9) were found in the breast muscles of the CLA-fed pigeons when compared to the SFO group ($P < 0.001$). However, the relative level (%) of C18:0 in the breast muscles of the pigeons fed CLA was similar to the control group. In the present study, when CLA was added to the pigeon diet, the proportion of C18:1(n-9) decreased significantly from 27.6 to 21.3% of the total fatty acids in the breast muscle. In contrast, polyunsaturated fatty acids (PUFA) in the breast muscle of the pigeons fed the CLA diet did not differ significantly from those supplemented with the SFO diet.

Table 1 Effect of feeding a commercial pelleted pigeon diet¹ supplemented with 0.5% safflower oil (SFO) or conjugated linoleic acid (CLA) on the liver fatty acid compositions² of pigeons

Fatty acids	% of Total fatty acids (Means ± s.e.)		P-value
	SFO	CLA ³	
C14:0	0.18 ± 0.02	0.20 ± 0.02	0.48
C16:0	19.7 ± 2.31	23.05 ± 2.53	0.367
C16:1(n-7)	1.18 ± 0.13	0.95 ± 0.13	0.243
C18:0	27.81 ± 3.92	23.32 ± 2.42	0.367
C18:1(n-9)	22.1 ± 2.98	19.46 ± 3.35	0.577
C18:2(n-6)	23.13 ± 3.08	24.63 ± 2.60	0.723
αC18:3(n-3)	0.12 ± 0.02	0.15 ± 0.02	0.425
C20:4(n-6)	5.78 ± 1.17	7.55 ± 0.88	0.271
ΣCLA	nd	0.71 ± 0.10	-
ΣSFA	47.69 ± 3.84	46.56 ± 0.38	0.779
ΣMUFA	23.29 ± 2.93	20.41 ± 3.38	0.544
Σ PUFA	29.03 ± 3.95	33.03 ± 3.41	0.472

¹Commercial pigeon diet containing 150 g crude protein/kg and 12.13 MJ metabolisable energy/kg

Diet SFO - 99.5% commercial pelleted diet plus 0.5% safflower oil

Diet CLA - 99.5% commercial pelleted diet plus 0.5% CLA (synthetically prepared and containing 95% pure CLA)

²Values are means ± s.e. (4 observations) expressed as percentage total fatty acid methyl esters

³CLA-95 was synthesized from linoleic acid (99% pure); nd - not detectable

ΣSFA - total saturated fatty acids; ΣMUFA - total monounsaturated fatty acids; ΣPUFA - total polyunsaturated fatty acids, including total CLA; ΣCLA - total conjugated linoleic acids

Table 2 Effect of feeding a commercially pelleted pigeon diet¹ supplemented with 0.5% safflower oil (SFO) or conjugated linoleic acid (CLA) on the breast muscle fatty acid composition² of pigeons

Fatty acids	% of Total fatty acids (Means ± s.e.)		P-value
	SFO	CLA ³	
C14:0	0.35 ± 0.01	0.42 ± 0.01	0.005
C16:0	19.0 ± 0.57	21.5 ± 0.28	0.009
C16:1(n-7)	3.8 ± 0.11	2.4 ± 0.11	0.012
C18:0	15.9 ± 0.50	17.2 ± 0.64	0.139
C18:1(n-9)	27.6 ± 0.86 ^a	21.3 ± 1.16	0.005
C18:2(n-6)	26.7 ± 1.31	28.0 ± 0.27	0.363
αC18:3(n-3)	0.35 ± 0.03	0.38 ± 0.02	0.659
C20:4(n-6)	6.3 ± 0.79	7.5 ± 0.58	0.254
ΣCLA	nd	1.30 ± 0.15	-
ΣSFA	35.25 ± 1.06	39.12 ± 0.74	0.024
ΣMUFA	31.40 ± 1.09	23.70 ± 1.21	0.003
Σ PUFA	33.35 ± 1.87	37.18 ± 0.61	0.10

¹Commercial pigeon diet containing 150 g crude protein/kg and 12.13 MJ metabolisable energy/kg

Diet SFO - 99.5% commercial pelleted diet plus 0.5% safflower oil

Diet CLA - 99.5% commercial pelleted diet plus 0.5% CLA (synthetically prepared and containing 95% pure CLA)

²Values are means ± s.e. (4 observations) expressed as percentage total fatty acid methyl esters

³CLA-95 was synthesized from linoleic acid (99% pure); nd - not detectable

ΣSFA - total saturated fatty acids; ΣMUFA - total monounsaturated fatty acids; ΣPUFA - total polyunsaturated fatty acids, including total CLA; ΣCLA - total conjugated linoleic acids

Table 3 Effect of feeding a commercially pelleted pigeon diet¹ supplemented with 0.5% safflower oil (SFO) or conjugated linoleic acid (CLA) on the adipose tissue fatty acid composition² of pigeons

Fatty acids	% of Total fatty acids (Means ± s.e.)		P-value
	SFO	CLA ³	
C14:0	0.7 ± 0.03	1.6 ± 0.06	< 0.001
C16:0	19.8 ± 0.25	24.1 ± 0.84	0.003
C16:1(n-7)	5.8 ± 0.48	4.5 ± 0.46	0.084
C18:0	8.9 ± 0.61	14.7 ± 1.48	0.008
C18:1(n-9)	43.0 ± 0.94	31.7 ± 0.55	< 0.001
C18:2(n-6)	21.2 ± 1.12	20.7 ± 0.65	0.693
αC18:3(n-3)	0.56 ± 0.05	0.49 ± 0.06	0.217
C20:4(n-6)	0.04 ± 0.00	0.07 ± 0.01	0.133
ΣCLA	nd	2.14 ± 0.37	-
ΣSFA	29.40 ± 0.66	40.40 ± 1.14	< 0.001
ΣMUFA	48.80 ± 1.28	36.20 ± 0.67	< 0.001
Σ PUFA	21.80 ± 1.17	23.40 ± 0.75	0.312

¹Commercial pigeon diet containing 150 g crude protein/kg and 12.13 MJ metabolisable energy/kg

Diet SFO - 99.5% commercial pelleted diet plus 0.5% safflower oil

Diet CLA - 99.5% commercial pelleted diet plus 0.5% CLA (synthetically prepared and containing 95% pure CLA)

²Values are means ± s.e. (4 observations) expressed as percentage total fatty acid methyl esters

³CLA-95 was synthesized from linoleic acid (99% pure); nd - not detectable

ΣSFA - total saturated fatty acids; ΣMUFA - total monounsaturated fatty acids; ΣPUFA - total polyunsaturated fatty acids, including total CLA; ΣCLA - total conjugated linoleic acids

Table 4 Effect of feeding a commercially pelleted pigeon diet¹ supplemented with 0.5% safflower oil (SFO) or conjugated linoleic acid (CLA) on pigeons' egg yolk fatty acid composition²

Fatty acids	% of Total fatty acids (Means ± s.e.)		P-value
	SFO	CLA ³	
C14:0	0.24 ± 0.02	0.34 ± 0.02	0.005
C16:0	21.0 ± 0.27	27.2 ± 0.36	< 0.001
C16:1(n-7)	1.9 ± 0.12	1.6 ± 0.17	0.191
C18:0	12.0 ± 0.19	16.9 ± 0.28	< 0.001
C18:1(n-9)	44.1 ± 0.52	32.5 ± 0.23	< 0.001
C18:2(n-6)	18.8 ± 0.65	18.3 ± 0.42	0.543
αC18:3(n-3)	0.28 ± 0.02	0.34 ± 0.33	0.236
C20:4(n-6)	1.62 ± 0.04	1.60 ± 0.07	0.246
ΣCLA	0.06 ± 0.01	1.22 ± 0.06	< 0.001
ΣSFA	33.24 ± 0.36	44.44 ± 0.33	< 0.001
ΣMUFA	46.00 ± 0.58	34.10 ± 0.30	< 0.001
Σ PUFA	20.76 ± 0.67	21.46 ± 0.45	0.443

¹Commercial pigeon diet contained 150 g crude protein/kg and 12.13 MJ metabolisable energy/kg

Diet SFO - 99.5% commercial pelleted diet plus 0.5% safflower oil

Diet CLA - 99.5% commercial pelleted diet plus 0.5% CLA (synthetically prepared and contained 95% pure CLA)

²Values are means ± s.e. (4 observations) expressed as percentage total fatty acid methyl esters

³CLA-95 was synthesized from linoleic acid (99% pure)

ΣSFA - total saturated fatty acids; ΣMUFA - total monounsaturated fatty acids; ΣPUFA - total polyunsaturated fatty acids, including total CLA; ΣCLA - total conjugated linoleic acids

Dietary CLA also caused significant changes in the fatty acid profile of adipose tissue of pigeons fed CLA compared to the SFO group (Table 3). The content of total CLA (%) in the abdominal adipose tissue was 2.14%. Similar to the fatty acid composition of the liver and breast muscle, CLA was not detectable in the abdominal adipose tissue of pigeons fed the SFO diet. Dietary CLA increased the levels of C14:0, C16:0 and C18:0 and decreased the level of C18:1(n-9) significantly in the abdominal adipose tissue compared to the control ($P < 0.05$). The ratio of MUFA/SFA was approximately 1.9-fold higher in the abdominal adipose tissue from the pigeons fed 0.5% SFO compared to those given the 0.5% CLA diet.

Table 4 shows the effects of dietary CLA on the fatty acid composition of pigeons' egg yolk. Feeding CLA in a low-fat diet significantly increased the SFA and decreased the MUFA in the egg yolk. In contrast, PUFA in the egg yolks of the CLA fed pigeons did not significantly differ from those supplemented with the SFO diet. The C14:0 content of the egg yolks from the CLA group was significantly higher than the control ($P < 0.01$). The ratios of C16:0/C16:1(n-7) and C18:0/C18:1(n-9) in the egg yolk from the CLA group increased about 1.5- and 1.9-fold, respectively, when compared to those fed the SFO diet. In the present study, hens fed CLA had yolks with a 23-fold increase in total CLA (as a percentage of fatty acid methyl esters) when compared to the yolks of hens fed the SFO diet (1.22% and 0.06%, respectively). In the present study, dietary CLA was shown to have no effect on the levels of C18:2(n-6) and C20:4(n-6) in the egg yolk.

Discussion

CLA is a group of naturally occurring fatty acids and was shown to inhibit the activity of liver stearoyl-CoA desaturase (Lee *et al.*, 1998; Park *et al.*, 2000), an enzyme that catalyzes the insertion of a double bond between the C9 and C10 atoms of C16:0 or C18:0 in the formation of C16:1(n-7) and C18:1(n-9), respectively. Therefore, CLA was shown to cause significant changes in the fatty acid composition by altering the level of MUFA and SFA in egg yolk (Chamruspollert & Sell, 1999; Du *et al.*, 1999; Jones *et al.*, 2000; Aydin *et al.*, 2001), milk (Kelly *et al.*, 1998) and breast muscle from Cobb 500 broilers (Sirri *et al.*, 2003).

In the present study, feeding CLA to pigeons did not cause a significant change in the fatty acid composition of the liver, the main site of *de novo* fatty acid synthesis, when compared to the control group. Similar to the findings of the present study, it was shown that a diet containing 0.5% CLA did not influence the levels of C16:1(n-7) and C18:1(n-9) significantly in liver from quail (Aydin & Cook, 2004). However, adding even 0.25% CLA to the diet of quail significantly altered the level of C16:0 in the liver. In the same study, diets containing 2% or more CLA caused alterations in the level of C18:1(n-9) of the liver (Aydin & Cook, 2004). It was also shown that when quail were fed a diet containing CLA, only the level of C16:0 in the liver increased significantly and not C18:0 (Aydin & Cook, 2004). It was reported that the use of 5% CLA in a diet significantly changed the fatty acid composition of the liver in broiler chickens (Badinga *et al.*, 2003). In another study conducted on single comb white leghorn laying hens, a diet containing 0.5% CLA significantly increased the levels of SFA (mainly C14:0, C16:0, and C18:0) and decreased the levels of MUFA (mainly C16:1, n-7 and C18:1, n-9) in the liver (Aydin & Cook, 2005). However, in the present study it was observed that dietary CLA had no effects on the liver fatty acid composition in the pigeon (Table 1).

In the present study, in contrast to the effects of CLA on the fatty acid composition of pigeon liver, dietary CLA increased the levels of C14:0 and C16:0 significantly in the breast muscle compared to the control group ($P < 0.01$) (Table 2). However, the relative level (%) of C18:0 in the breast muscle of pigeons fed CLA was similar to the control group. In a recent study conducted on chickens, it was shown that 2% CLA affected the levels of MUFA (mainly C16:1, n-7 and C18:1, n-9), but not the levels of SFA in breast fillet (Sirri *et al.*, 2003). In the present study, when CLA was incorporated into the pigeon diet, the level of C18:1(n-9) decreased from 27.6 to 21.3% of the total fatty acids in the breast muscle (Table 2). Pigeons fed CLA had increased levels of total SFA and decreased levels of total MUFA in the breast muscles as compared to the SFO group (Table 2). In contrast, the PUFA in the breast muscles of the pigeons fed the CLA diet did not differ significantly from those supplemented with the SFO diet.

In the present study, similar to the fatty acid profile of the liver and breast muscle, CLA was not detectable in the abdominal adipose tissue of pigeons fed the SFO diet (Table 3). Pigeons fed CLA had increased levels (%) of total SFA and decreased levels of total MUFA in the adipose tissue. The ratio of MUFA/SFA was approximately 2-fold higher in the abdominal adipose tissue from the pigeons fed the SFO diet compared to those given the CLA diet. Dietary CLA increased the levels of C14:0, C16:0 and C18:0 and

decreased ($P < 0.01$) the level of C18:1(n-9) in the abdominal adipose tissue compared to the control. Similarly, in a study conducted in pigs, it was shown that CLA differentially modified the fatty acid composition in subcellular fractions of muscle and adipose tissue (Demaree *et al.*, 2002). In another study conducted in pigs, dietary CLA was shown to increase the levels of C14:0, C16:0, and C18:0 and decreased the level of C18:1(n-9) significantly in the adipose tissue (Corino *et al.*, 2003). However, in a study conducted on chickens, dietary CLA had no effect on the levels of C14:0 and C16:0 in the adipose tissue (Aydin & Cook, 2005). In the same study, CLA decreased the levels of C16:1(n-7) and C18:1(n-9) and increased the level of C18:0 significantly in the adipose tissue (Aydin & Cook, 2005).

As previously reported in chickens (Lee, 1996), dietary CLA caused similar changes in the fatty acid composition of pigeons' egg yolk. In the present study, feeding CLA in a low-fat diet significantly increased the level of SFA and decreased the level of MUFA in the egg yolk ($P < 0.05$). In contrast, the PUFA in the egg yolk of the CLA fed pigeons did not differ significantly from those supplemented with the SFO diet. The level of C14:0 of the egg yolk from the CLA group was significantly higher than the control ($P < 0.01$). The ratios of C16:0/C16:1(n-7) and C18:0/C18:1(n-9) in the egg yolk from the CLA group increased about 1.5- and 1.9-fold, respectively, compared to those fed the control diet. In another study conducted on chickens, the ratios of C16:0/C16:1(n-7) and C18:0/C18:1(n-9) in the egg yolk from the CLA fed group were reported to increase about 1- and 1.5-fold, respectively, when compared to those fed the control diet (Aydin *et al.*, 2001). It was also shown that adding olive oil (rich in C18:1, n-9) completely prevented the CLA-induced increase in the levels of C14:0, C16:0 and C18:0 and the CLA-induced decrease in C18:1(n-9) in egg yolk from chickens fed CLA (Aydin *et al.*, 2001). Although dietary CLA had no effect on the concentration of yolk C18:2(n-6), the level (%) of C20:4(n-6) in the yolk was decreased significantly in eggs from CLA-fed hens (Aydin *et al.*, 2001). In the present study dietary CLA did not significantly influence the levels of C18:2(n-6) and C20:4(n-6) of the pigeon egg yolks. Pigeons fed CLA had yolks with a 23-fold increase in total CLA (as a percentage of fatty acid methyl esters) when compared to yolks of hens fed the control diet (0.06% and 1.22%, respectively). Previously, it was shown that egg yolks from the CLA-fed chickens could be enriched 17-fold in total CLA (as a percentage of fatty acid methyl esters) when compared to egg yolks of hens fed the control (0.11% and 1.91%, respectively) (Aydin *et al.*, 2001). Since yolk fat has a crucial role as an energy source and essential nutrients in the avian embryo development, significant alteration in the fatty acid composition of egg yolk will have adverse effects on embryo survival, as shown by Aydin *et al.* (2001) and Aydin & Cook (2004).

Conclusion

CLA is a group of naturally occurring fatty acids which affects the fatty acid composition of the tissues and egg yolk by inhibiting the stearoyl-CoA desaturase enzyme (Park *et al.*, 2000). Dietary CLA influences the tissue and egg yolk fatty acid composition in a time and dose-dependent manner (Aydin & Cook, 2004). In the light of the previous experiments conducted in various species, it was suggested that dietary CLA influenced the fatty acid composition of different tissues and egg yolk differently in the pigeons.

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