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Full Length Research Paper

Effects of dietary conjugated linoleic acid, fish oil and soybean oil on body-fat deposition and serum lipid fractions in broiler chickens

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An experiment was conducted on broiler chickens to study the effects of dietary fats rich in conjugated linoleic acid (CLA), fish oil (n-3 rich oil), soybean oil (n-6 rich oil), polyunsaturated fatty acids (PUFAs) alone or in dual mixtures, as well as palm oil as a more saturated fat on tissue fat deposition and serum lipid concentrations of broiler chickens. The fat included in the experiment's diets is a dose of 7% for single fats and 3.5 + 3.5% for the dual mixtures. The conjugate linoleic acid (CLA) supplement used in this study was LUTA-CLA 60, containing 60% CLA, so that dietary inclusion of 7 and 3.5% LUTA-CLA 60 were supplied with 4.2 and 2.1% CLA, respectively. The chickens fed diets containing palm oil, soybean oil or fish oil as the only dietary fat deposited more fat in breast tissue compared with other groups ($P < 0.05$). The highest fat in thigh tissue was observed in birds fed 7% fish oil ($P < 0.05$). The CLA containing diets resulted in fatter liver tissue ($P < 0.05$). The diets containing 7% fish oil effectively decreased the lipid content of chicken's liver ($P < 0.05$). The fish oil and soybean oil as n-3 and n-6 rich sources, respectively demonstrated a comparable reduction in the serum cholesterol and low density lipoprotein (LDL) concentrations ($P < 0.05$), while the 7% CLA diet increased serum high density lipoprotein (HDL) level ($P < 0.05$). The results of this study showed that dietary fish oil, and CLA effectively increased fat content of meat and liver tissues, respectively; and the dietary soybean oil and fish oil were more effective on reducing serum undesired lipoproteins, as compared with CLA.

Key words: CLA, PUFA, body fat deposition, serum lipids and broiler chickens.

INTRODUCTION

Chicken has been used as a suitable model for lipid metabolism studies, because dietary modifications, especially dietary fat type can change the chicken body

composition. Broiler chickens are at risk of fatness and their liver synthesis and secrete large amount of triglyceride and lipoproteins (Griffin et al., 1991). CLA is a natural constituent of meat and dairy products from ruminants, originated from bacterial bio-hydrogenation in the rumen and its anti-lipogenic effects have been reported in different species (Ha et al., 1989). Fish oil is a rich source of n-3 PUFAs, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid which are well-known for their favorite effects on human health (Knapp, 1991). Soybean oil and palm oil are good causes of oils

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Abbreviations: CLA, Conjugated linoleic acid; PUFAs, polyunsaturated fatty acids; LDL, low density lipoprotein; HDL, high density lipoprotein.

rich in n-6 PUFAs and SFAs. The objective of the present study was to assess the effects of altering the dietary fat type with inclusion of CLA, fish oil, soybean oil or their dual mixtures as well as palm oil on breast, thigh and liver tissues fat deposition and serum lipid concentrations in broiler chickens.

MATERIALS AND METHODS

Animal management

A total of 560 Ross 308 male broiler chickens were used in this study. All chicks were fed on the same corn-soybean meal based starter diet for day 1 to 10 and then allocated to the experimental grower (day 11 to 28) and finisher (day 29 to 42) diets. Seven isocaloric and isonitrogenous diets were formulated and they contain: 7% soybean oil (7% SO), 7% LUTA-CLA 60 (CLA), 7% fish oil (FO), 3.5% LUTA-CLA 60+ 3.5% soybean oil (CLA + SO), 3.5% fish oil + 3.5% Soybean oil (FO + SO), 3.5% LUTA-CLA 60 + 3.5% fish oil (CLA + FO) or up to 12% palm oil (PO) (Table 1). The CLA supplement of LUTA-CLA 60 was prepared and supplied by BASF Company (Germany) and it contained 30% isomer 9c, 11t and 30% isomer 10t, and 12c of conjugated linoleic acid plus a large amount of oleic acid. However, the dietary inclusion of 7 and 3.5% LUTA-CLA 60 were supplied by 4.2 and 2.1% CLA isomers, respectively. The higher palm oil content in the latest diet was due to the lower metabolizable energy content of palm oil.

At 42 days of age, following feed withdrawal overnight to permit gut clearance and before slaughtering, 5 ml of blood samples were harvested from the wing vein of two male chicks selected randomly per pen. Serum was harvested with centrifugation at 3000 rpm for 10 min and then the serum was frozen for future analysis of serum lipid and lipoprotein concentrations, and was stored at -20°C. On day 42, two male birds per cage were weighed alive, and then slaughtered after an overnight withdrawal period. Samples of Liver, breast and thigh muscles were collected and stored at -20°C until lipid analysis. Lipids content of liver, breast and thigh tissues were measured by the method of Folch et al. (1957). The serum lipoprotein fractions were measured using an autoanalyzer (HITACHI 902 automatic autoanalyzer).

Individual chicks were the experimental units for tissue and serum analysis data. Data sets of completely randomized design with seven treatments, and eight replicate (four replicate pens and two samples per pen), were compared across the treatments using the one-way analysis of variance (ANOVA) procedure. Significant means were then elucidated using the Duncan multiple range tests. All statistical tests were conducted at 95% confidence level using the SAS program (SAS, 9.1, 2002).

RESULTS

Table 2 shows the lipid content of breast, thigh and liver tissues of broiler chickens fed different dietary fats. For lipid content of breast tissue, the treatments formed two distinct groups so that the chickens fed PO, SO or FO diets had significantly higher breast tissue lipid than those fed on the other diets ($P < 0.05$). The highest lipid deposition in thigh tissue was observed in birds fed FO diet, such that the difference was significant when compared with the birds fed CLA, CLA + SO or FO + SO diets ($P < 0.05$).

The lipid content of breast tissue in chickens fed the CLA + FO diet was closer to those fed on CLA than FO

diet ($P < 0.05$), which suggest a dominant effect for dietary CLA in this respect. Combination of soybean oil with CLA or fish oil in diets resulted in less fat deposition in both breast and thigh tissues compared with the SO diet ($P < 0.05$).

The CLA containing diets resulted in more fat deposition in the liver of broiler chickens ($P < 0.05$), except for CLA + FO diet, which resulted in a comparable lipid deposition in liver to the PO and FO + SO diets. The FO and then the SO diets effectively decreased chickens liver lipid content ($P < 0.05$).

The serum lipid fractions of experimental birds is shown in Table 3. The serum triglyceride and very low density lipoprotein (VLDL) levels of birds fed FO diet were lower than those of birds fed one of the CLA containing diets (CLA, CLA + FO and CLA + SO) as well as the FO + SO diet ($P < 0.05$). The serum total cholesterol concentration of birds fed FO or SO diets were lower than other treatments ($P < 0.05$), except for the birds fed the CLA diet. The FO diet reduced the serum LDL concentration as compared with the other diets ($P < 0.05$) with the exception of SO diet.

There was no difference in serum composition between treatments containing soybean oil in combination with CLA or fish oil (CLA + SO vs. FO + SO). The serum glucose level of birds fed PO diet was higher than that of birds fed other experimental diets ($P < 0.05$) except for the 3.5% FO + 3.5% SO diet.

DISCUSSION

Breast and thigh tissues fat content

In this study, the CLA containing diets resulted in different changes in lipid contents of thigh and breast tissues. The dietary CLA effectively decreased fat deposition in broiler chickens meat, and especially breast tissue was more affected. This observation is in agreement with the report of Kawahara et al. (2009) who found that contents of total lipid and triglyceride in breast meat tended to decrease in the broilers fed 1 to 2% dietary CLA. On the contrary, Buccioni et al. (2009) couldn't show any changes in lipid content of muscles in broilers fed different dietary CLA levels. Javadi et al. (2007) even reported a higher lipid content in meat of broiler chickens fed 1% dietary CLA as compared with the control group.

It seems that in the present study, CLA had a dominant effect over soybean oil so that the 2.1% CLA + 3.5% SO diet resulted in less fat deposition in both breast and thigh tissues, than 7% SO diet (and comparable to the 4.2% CLA diet). This dominant effect of CLA is absorbable over fish oil in breast tissue too. Previous studies have shown that CLA increases energy expenditure through increased oxygen consumption (Choi et al., 2004) and increased gene expression of uncoupling proteins, which both are indices for energy expenditure and less fat deposition (Choi et al., 2004).

Table 1. Ingredients and compositions of the experimental diets.

Ingredient	Starter		Grower (11 to 28 days of age)						Finisher (29 to 42 days of age)						
	(1-10days)	PO ²	SO	FO	CLA	FO+SO	CLA+SO	CLA+FO	PO	SO	FO	CLA	FO+SO	CLA+SO	CLA+FO
Corn (%)	60.23	48.46	53.99	53.99	55.8	53.99	54.13	54	52.62	57.98	57.98	59.5	57.98	59.06	58.92
Soy meal (%)	30.81	30.56	32.27	32.27	28.6	32.27	31.96	32.26	27.76	30.27	30.27	26.38	30.27	28.3	28.6
Fish Meal (%)	5.37	5	3	3	5	3	3.2	3.01	3	1	1	2.99	1	1.7	1.51
Soybean oil (%)	-	-	7	-	-	3.5	3.5	-	-	7	-	-	3.5	3.5	-
Fish oil	-	-	-	7	-	3.5	-	3.5	-	-	7	-	3.5	-	3.5
Palm oil (%)	-	12.6	-	-	-	-	-	-	12.92	-	-	-	-	-	-
CLA(%) ¹	-	-	-	-	7	-	3.5	3.5	-	-	-	7.4	-	3.5	3.5
Oyster shell (%)	1.41	1.34	1.42	1.42	1.33	1.42	1.41	1.42	1.3	1.39	1.39	1.3	1.39	1.35	1.36
DCP (%)	0.51	0.48	0.66	0.66	0.52	0.66	0.64	0.66	0.69	0.84	0.84	0.71	0.84	0.81	0.82
Salt (%)	0.25	0.29	0.32	0.32	0.28	0.32	0.31	0.32	0.32	0.35	0.35	0.32	0.35	0.34	0.34
Vit-Min P(%) ³	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
DL-Met (%)	0.26	0.23	0.25	0.25	0.23	0.25	0.25	0.25	0.17	0.18	0.18	0.16	0.18	0.18	0.18
L-Lys (%)	0.15	0.04	0.09	0.09	0.24	0.09	0.09	0.09	0.22	-	-	0.25	-	0.27	0.27
Analysis															
ME (Kcal/Kg)	2860	3175	3205	3211	3175	3208	3175	3175	3225	3235	3241	3225	3238	3225	3225
CP (%)	22.5	21	21	21	21	21	21	21	19	19	19	19	19	19	19
Crude Fat (%)	2.86	15	9.52	9.52	9.65	9.52	9.54	9.52	15.34	9.55	9.55	10.07	9.5	9.61	9.6
Linoleic a (%)	1.46	2.63	5.37	1.39	5.27	3.38	5.3	3.31	2.74	5.45	1.46	5.56	3.46	5.4	3.4
Ca (%)	0.95	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Ava P (%)	0.475	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.425	0.425	0.425	0.425	0.425	0.425	0.425
Na (%)	0.152	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Lys (%)	1.368	1.23	1.23	1.23	1.34	1.23	1.23	1.23	1.2	1.02	1.02	1.2	1.01	1.2	1.2
Met (%)	0.662	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.48	0.48	0.48	0.48	0.48	0.49	0.49
Met+Cys (%)	1.0355	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.8	0.8	0.8	0.8	0.8	0.8	0.8

¹CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2 and 2.1%, respectively. ²PO= Diet containing palm oil, SO=diet containing 7% soybean oil, FO= diet containing 7% fish oil, CLA = diet containing 4.2% CLA, CLA+SO = diet containing 2.1% CLA+3.5% soybean oil, CLA+FO = diet containing 2.1% CLA+3.5% fish oil, FO+SO = diet containing 3.5% fish oil+3.5% soybean oil. ³Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7mg Cu, 0.4 mg Co, 0.17mg Se, and 0.75 mg I. Vitamin premix provided per kg of ration with 6,000,000 IU vitamin A, 1,500,000 IU vitamin D3, 15,000 IU vitamin E, 2.5 mg vitamin K3, 0.02 mg vitamin B12, 3,000 mg riboflavin, 7000 mg pantothenic.

Liver fat content

In the present study, the CLA containing diets increased liver fat accumulation, and the diet with

4.2% CLA was more effective than diets containing 2.1% CLA. The birds fed 7% fish oil (n-3 rich) as the only dietary fat source had a less fat accumulation in liver tissue as compared with the

birds fed diet with 7% soybean oil (n-6 rich). Mikkelsen et al. (1993) reported that more unsaturated fatty acids are more effective on the fatty acid synthesis inhibition; DHA (22:6n-3)

Table 2. The liver, breast and thigh tissues Fat content in broiler chickens fed different dietary fats.

Parameter	Tissue fat content (% of dry matter)		
	Liver	Breast	Thigh
PO ¹	3.34 ^b	2.46 ^a	3.2 ^{ab}
SO	2.85 ^c	2.02 ^a	3.38 ^a
FO	2.42 ^d	2.02 ^a	3.94 ^a
CLA	4.22 ^a	1.11 ^b	2.55 ^{bc}
CLA+SO	4.1 ^a	1.55 ^b	2.16 ^c
CLA+FO	3.86 ^{ab}	1.23 ^b	3.01 ^{ab}
FO+SO	3.16 ^{bc}	1.31 ^b	2.39 ^{bc}
SEM	0.16	0.06	0.13

^{a-d}Means with different superscripts within column differ significantly at $P < 0.05$. ¹CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2% and 2.1% respectively. 2PO= diet containing Palm oil, SO = diet containing 7% Soybean oil, FO= diet containing 7% Fish oil, CLA = diet containing 7% CLA, CLA+SO = diet containing 3.5% CLA+3.5% Soybean oil, CLA+FO = diet containing 3.5% CLA+3.5% Fish oil, FO+SO = diet containing 3.5% Fish oil+3.5% Soybean oil.

being more effective than EPA (20:5:n-3) or arachidonic (20:4n-6).

Serum parameters

The effect of n-3 rich fats, especially fish oil on serum triglyceride reduction has been previously reported in chicks (Akiba et al., 1995). In the study of Phetteplace and Watkins (1989), the chickens fed menhaden oil had lower plasma triacylglycerol levels compared with the values for those fed chicken fat; they concluded that a decrease in TG synthesis by the liver could result in lower amounts of TG in the VLDL + LDL fraction. We observed the same relationship between dietary fish oil and palm oil as a more saturated fat.

There are some reports which show that n-3 PUFAs may act in a similar way to fibrate drugs, which enhance the conversion rate of VLDL to LDL (Despres et al., 2004). These opinions are not in agreement with our observation for serum thyroglobulin (TG) and LDL levels, which can be attributed to the species differences because all the reports given in the foregoing are noted in humans.

The report which shows that the effect of 7% CLA diet on increasing serum TG concentration is higher than birds fed 7% FO, 7% SO or PO diets is in agreement with the observations of Du and Ahn (2003) in chicken and Du and Ahn (2002) in rat; but the majority of the previous reports in different species have shown a decreased serum or plasma TG following CLA administration (Bhattacharya et al., 2006; Baddini et al., 2009).

The reason for the higher serum TG concentration in birds fed dietary CLA is not clear, but it could be attributed to the alterations in activities of enzymes involved in hepatic lipid metabolism. In the study of Du and Ahn (2003), dietary CLA caused a significant increase in liver fatty acid synthase (FA synthase) activity

and an increase (even though not significant) in acetyl-CoA carboxylase activity. FA synthase and acetyl-CoA carboxylase are the main enzymes regulating fatty acid synthesis. The higher FA synthase activity could be explained in part by the increased plasma TG levels. In the cultured adipose cells, FA synthase gene expression was not decreased by dietary CLA (Choi et al., 2000). These results show that dietary CLA decrease lipogenesis in mammary glands and adipose tissues but not in liver. This could be the explanation for ineffectiveness of CLA in decreasing fat deposition in birds (Du and Ahn 2002), because the liver is the main site of lipogenesis.

In the present study, the higher TG levels in the serum of birds fed on CLA containing diets indicates that the increase in hepatic steatosis by dietary CLA could be due to the action of CLA to change lipid metabolism in the liver. However, the ineffectiveness of CLA on serum total cholesterol in the present research was not surprising, because there are various previous reports on the decreasing (Zanini et al. 2006) or increasing (Szymczyk et al., 2001; Du and Ahn, 2003) effects of CLA on serum total cholesterol in broiler chickens.

Except for the one report on pigs (Stangl et al., 1999), almost the majority of the previous studies indicated a reducing effect of CLA on serum or plasma total cholesterol in different species (Bhattacharya et al., 2006; Baddini et al., 2009).

The increased serum HDL level in broiler chickens fed on CLA in this study is in agreement with previous reports in broilers (Du and Ahn., 2003), mice (Bhattacharya et al., 2006) and human (Smedman and Vessby., 2001). Although, in the research of Szymczyk et al. (2001) on broiler chickens, the level of 1% dietary CLA increased serum HDL concentrations, but the 1.5% CLA level decreased it. It seems that there is also an interaction between CLA and soybean oil effects on the serum LDL concentration; so that the combination of these two fat sources resulted in a significantly higher LDL level as

Table 3. Serum composition of birds fed on experimental diets containing different fat types.

Parameter	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Glucose (mg/dl)
PO ¹	40.2 ^{bc}	99.5 ^a	77.2 ^a	38.3 ^{ab}	8.0 ^{bc}	124.7 ^a
SO	40.2 ^{bc}	62.8 ^b	34.2 ^c	24.7 ^{bc}	8.0 ^{bc}	80.2 ^{bc}
FO	37.0 ^c	60.7 ^b	48.5 ^{bc}	21.8 ^c	7.1 ^c	82.3 ^{bc}
CLA	53.3 ^a	76.7 ^{ab}	75.2 ^a	41.7 ^a	10.7 ^a	72.2 ^c
CLA+SO	48.5 ^{ab}	90.8 ^a	51.5 ^{bc}	40.0 ^a	9.7 ^{ab}	85.7 ^{bc}
CLA+FO	44.5 ^a	92.2 ^a	63.5 ^{ab}	41.3 ^a	8.9 ^a	85.2 ^{bc}
FO+SO	44.0 ^a	94.3 ^a	65.0 ^{ab}	39.7 ^a	8.7 ^a	117.5 ^{ab}
SEM	1.8	3.5	2.2	1.8	0.4	4.6

^{a-c}Means with different superscripts within column differ significantly at $P < 0.05$. ¹CLA used in this experiment was CLA LUTA60 which contains 60% CLA, then 7% and 3.5% dietary inclusion of CLA will be equal to 4.2% and 2.1%, respectively. 2PO = diet containing Palm oil, SO=diet containing 7% Soybean oil, FO= diet containing 7% Fish oil, CLA = diet containing 7% CLA, CLA + SO = diet containing 3.5% CLA + 3.5% Soybean oil, CLA + FO = diet containing 3.5% CLA + 3.5% Fish oil, FO + SO = diet containing 3.5% Fish oil + 3.5% Soybean oil.

compared with the birds fed each fat separately. It seems that the effects of dietary CLA on glucose metabolism in human are inconsistent and probable effects of CLA on metabolic syndromes are still controversial (Bhattacharya et al., 2006).

The results of the present study showed that the dietary soybean oil and fish oil were more effective on serum lipoproteins reduction, than CLA; however, CLA more effectively increased serum HDL concentrations.

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