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The effects of dietary lipid sources on hepatic histopathological features and serum biochemical indices of broiler chickens

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Abstract

This study was conducted to evaluate the effects of dietary lipid sources on histological characteristics of liver and biochemical serum parameters. In a completely randomized design, 240 one-day-old broiler chicks were assigned to 5 treatments, 4 replicates and 12 chicks per each. Liver samples were taken at days 28 and 42 of age. Chickens fed control diet had no lesions in liver at days 28 and 42 of age. Except for chicks fed control diet and those fed olive oil, in the liver of other birds lipid droplet accumulation or steatosis observed. Broilers fed corn oil revealed lipid droplet accumulation in both sampling times. While these symptoms were not observed in the chicks fed fish oil or olive oil. The chicks fed diet containing tallow had apparent characteristic as sinusoid dilation, hyperemia and intense hepatic degeneration at day 28 of age. There were no significant differences among treatments for the serum total protein, albumin and globulin of chicks fed control diet or diet containing different oil sources at day 28 and 42 of age. The highest means of AST and ALT were for chicks fed diets containing corn oil and tallow and the lowest one was for those fed diet containing fish oil and olive oil. In conclusion, our result confirmed that fish oil as a source of n-3 poly unsaturated fatty acids was much more beneficial than n-6 poly unsaturated fatty acids or saturated fatty acids to keep the chicks in the healthy state.

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Introduction

Liver plays an important role in energy homeostasis, as it converts excessive dietary glucose into fatty acids (FA) which is exported as triglyceride (Clement *et al*., 2002; Rasooly *et al*., 2007; Cooper *et al*., 2008). Under normal situation, *de novo* lipogenesis allocates minimally to the lipid pool in the liver (Diraison and Beylot, 1998). When multifactorial agents cause that hepatic natural metabolism disturbs, one of the case that could be occur is fat accumulation or steatosis (abnormal retention of lipids within a cell). The factors leading to hepatic lipid accumulation are encompassing increased FA influx, hastened FA synthesis, and altered FA oxidation and triglyceride secretion insufficient to prevent lipid accumulation (Jourdan *et al*., 2009). in studies on mice, Degrace *et al*. (2004) accentuated that although increased fatty acid oxidation were associated with increased hepatic lipogenesis, but it might be due to higher rates of hepatic lipogenesis than rates of FA oxidation resulting in elevated lipid accumulation. In generally, substantial sources cause hepatic steatosis include peripheral fats stored in adipose tissue that flow to the liver by way of the plasma non-esterified fatty acid (NEFA) pool, fatty acids made *de novo* lipogenesis, dietary fatty acids spill over into the plasma NEFA pool and through the absorption of intestinally derived chylomicron (Miles *et al*., 2004; Havel and Hamilton, 2004).

Serum Aspartate transaminase (AST) and Alanine transaminase (ALT) are a significant index to assessment liver function. In addition, total protein and albumin concentrations are indicator of synthetic function of liver (Yap *et al*., 2002; Goldwasser and Feldman, 1997). Fatty acids as essential nutrients have a wide range of biological functions (Simopoulos, 1991). The association between the dietary fatty acids, membrane fluidity, membrane integrity and metabolic pathways in animals are obvious. Poly unsaturated fatty acids (PUFAs), mainly n-3 and n-6 fatty acids are very essential for growth and development and also for the regulation of the cellular functions in animals (Simpoulous, 1999; Zamaria, 2004). In an observation, Cabre *et al*. (1993) suggested PUFAs ameliorate mucosal damage and therefore exhibit anti-inflammatory properties. Similarly, Okita and Watanabe (1998) showed that intense liver diseases are accompanied with lower concentrations of n-3 and n-6 PUFA fatty acids and long chain PUFAs deficiency are often associated with liver cirrhosis. It has been found that n-3 PUFAs play importantly protective roles in the liver, cardiovascular system and kidney (Koletzko and Goulet, 2010; Fessett *et al*., 2010).

In the literature, there was limited information concerning the effects of different lipid sources on hepatic histology and levels of proteins and liver enzymes in serum. Also, in the studies in human and laboratory animals, more focuses was done on the roles of n-3 fatty acid than n-6 and n-9 or saturated fatty acids (SFAs). Therefore, the objective of the current study was to evaluate and compare the effects of fish oil, corn oil, olive oil and tallow, respectively, as dietary sources of n-3, n-6, n-9 and SFA on hepatic histopathology characteristics and serum biochemical parameters levels in broiler chicks.

Materials and methods

Animal and diets

A total of 240 one-day-old male broiler chicks (Ross 308) was purchased from a commercial hatchery and used in a 42 days feeding trial. In a completely randomized design, chicks were divided into 5 treatments (control and four lipid sources) with 4 replicates and 12 chicks per each. Treatments included of free lipid diet as control group and 4 different levels of dietary lipids comprised of fish oil as a n-3 fatty acid source, corn oil as a n-6 fatty acid source, olive oil as a n-9 fatty acid source and tallow as a saturated fatty acid which were added to diets as 1.5, 3 and 4% in the starter, grower and finisher, respectively. Control diet had no supplemental dietary lipid and energy content was supplied by including pure starch (Tables 1, and 2). Throughout the study, chicks accessed to feed and water *ad libitum*. Lighting schedule were 23L/1D while the temperature was gradually reduced 3 °C from initially 32 °C in each week.

Blood and liver sampling

At days 28 and 42 of age, 8 chicks were randomly selected from each treatment (2 chicks per replicate) after an overnight fast, weighed and blood samples were obtained from wing veins and then killed by cervical dislocation. Blood samples were allowed to clot at 4 °C for 2 h and then centrifuged at 1,500 × *g* for 15 min and serum was separated and stored at -20 °C until analyses. Liver was immediately dissected out and placed in 10% neutral buffered formalin for further processing.

Lipid histology

After 72 h fixation, the sections of liver were stabilized in paraffin and sliced by a microtome with 6 µm thickness. Slices (at least 6 slices per section fixed on a microscope slide) were stained by hematoxylin and eosin stain and then mounted by entellan rapid mounting medium. Slides were evaluated under optic microscope. Changes were classified as not detected (ND), mild (+), moderate (++) and severe (+++).

Measurement of serum parameters

Serum AST and ALT were determined using an automatic biochemical analyzer (Hitachi/Boehringer Mannheim 911Analysis system, GmBH, Germany) following the methods described by Godkar (1994). Serum albumin and globulin was determined as described by Johnson *et al*. (1999). Serum total protein was determined according to the method of Young and Friedman (2001) using commercial kit (Bionik Reagent Packs) and an automatic biochemical analyzer (BT 1500, Italy).

Statistical analysis

All analyses were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute. Significant differences at $P < 0.05$ statistical level compared by Duncan's multiple range tests.

Results and discussion

Figure 1 and Table 3 show histological status of liver of chicks fed control diet or diets containing different oil sources. Except for chicks fed control diet and those fed olive oil, in the liver of other birds lipid droplet accumulation or steatosis observed. Chickens fed control diet had no lesions in liver at days 28 and 42 of age. Broilers fed corn oil revealed lipid droplet accumulation in both sampling times. While this symptoms was not observed in chicks fed fish oil or olive oil and also was detected in control group at day 42 of age. In agreement to our result, researchers indicated that increase in the n-6:n-3 ratio which favors lipid synthesis over lipid oxidation and secretion could lead to hepatic lipid accumulation (Badry *et al*., 2007). Also, in a study the addition of fish oil as a source of n-3 fatty acid to diet has been shown to ameliorate CLA-induced steatosis by increasing adiponectin levels (Ide, 2005). Swelling of hepatocyte and intense hepatic degeneration were observed in those fed diet containing fish oil at day 28 of age; while, predominant symptoms were not observed at day 42 of age.

As shown in Figure 2 and summarized in Table 3, the birds fed diet containing corn oil revealed sinusoid dilation and intense hepatic degeneration at day 28 of age, as well as, focal infiltration of mono nuclear cells and intense hepatic degeneration at day 42 of age. Hyperemia and intense hepatic degeneration were detected in broilers fed diet containing olive oil at day 28 of age, but interestingly, they displayed none hepatic diagnostic symptom at day 42 of age. The cause of degenerative changes in fast growing broiler chickens is a prolonged state of hypoxia (Olkowski *et al*., 2005). Under situation of continuous and high demand for oxygen, the liver tissue may respond with regressive lesions such as paranchymatous, vacuolar and fatty degeneration, and necrosis of hepatocyte (Madej *et al*., 2007). In this study, we observed hepatic lesions in sampling of day 28 of age much more than day 42 of age. These observations may be related to inherent predisposing of these birds to hypoxia. The liver of the birds had steatosis also was involved in hepatic degeneration at day 28 of age. This result was consistent with McLean and Dutton (1995). They also reported that hepatocyte degeneration could be due to impaired lipid transport

rather than increased lipid biosynthesis.

As shown in Figure 3 and summarized in Table 4, the chicks fed diet containing tallow had apparent characteristic as sinusoid dilation, hyperemia and intense hepatic degeneration at day 28 of age, but there was only focal infiltration of mono nuclear cells in their liver at day 42 of age. Metabolism differences between poly unsaturated fatty acids and olive oil as dietary n-9 source and tallow as dietary saturated fatty acid could be cause to hyperemia in the liver of birds fed olive oil and tallow. As it has been reported dilation of arterioles due to increased blood to the tissue causes hyperemia (Unal, 2012).

Table 1. Ingredients and nutrient compositions of experimental diets in grower (day 11 to day 28).

Ingredients (%)	Control	Fish oil	Corn oil	Olive oil	Tallow
Corn	51.70	55.44	55.44	55.44	55.44
Soybean Meal(46% CP)	27.40	31.70	31.70	31.70	31.70
Starch	8.70	0.00	0.00	0.00	0.74
Fish oil	0.00	3.00	0.00	0.00	0.00
Corn oil	0.00	0.00	3.00	0.00	0.00
Olive oil	0.00	0.00	0.00	3.00	0.00
Tallow	0.00	0.00	0.00	0.00	3.00
Corn Gluten	8.00	4.00	4.00	4.00	4.00
Calcium Carbonate	1.10	1.10	1.10	1.10	1.10
Dicalcium Phosphate	1.75	1.75	1.75	1.75	1.75
Sodium Chloride	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.28	0.28	0.28	0.28	0.28
Vitamin and Mineral Premix[1]	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.25	0.25	0.25	0.25	0.25
Zeolite	0.00	1.60	1.60	1.60	0.90
Nutrient Compositions (%)					
AME (kcal/kg)	3,050	3,050	3,050	3,050	3,050
Crude protein	21.01	21.00	21.00	21.00	21.00
Crude Fat	3.91	7.18	7.18	7.18	7.18
Lysine	1.15	1.24	1.24	1.24	1.24
Methionine	0.62	0.60	0.60	0.60	0.60
Methionine plus Cystine	0.98	0.95	0.95	0.95	0.95
Fatty acids (%)					
C18:1 n-9	1.03	1.99	1.99	3.32	2.14
C18:2 n-6	2.05	2.25	3.81	2.46	2.31
C18:3 n-3	0.09	0.15	0.13	0.12	0.13
(n-6)/(n-3)	22.77	15.00	29.30	20.05	17.76

1- premix supplying composition was as follows (amounts in 10 g): 0.5 g Mg, 0.3 g S, 1.0 g Na, 1.6 g Cl, 6.0 mg Cu, 0.2 mg I, 45.0 mg Fe, 59.0 mg Mn, 0.2 mg Se, 29.0 mg Zn, 4000 IU Vitamin A Palmitate, 1000 IU Cholecalciferol, 50 IU Vitamin E acetate, 0.5 mg Menadione sodium bisulfite, 0.2 mg biotin, 10 µg cyanocobalamin, 2 mg folic acid, 30 mg nicotinic acid, 16 mg calcium pantothenate, 7 mg pyridoxine - Hcl , 6.0 mg riboflavin, and 6.0 mg thiamin Hcl.

Infiltration of mononuclear cells are originated from cellular immunity process were observed in chicks fed corn oil at day 42 of age. In a study, James *et al.* (2000) reported that eicosanoids derived from n-6 poly unsaturated fatty acids promote inflammation. Also, in another study, it was observed small inflammatory cell infiltrated mononuclear cells to respond to degenerative hepatocytes in rats (Hinton *et al.*, 2003). The n-6 poly unsaturated fatty acids have inflammatory effect on cells induced degeneration and consequently, stimulated immunity response and this process was maintained to end of experiment. It has been shown that n-3 poly unsaturated fatty acids can protect hepatocytes inhibiting liver cell peroxidation *via* anti-oxidation and anti-inflammatory mechanisms (Richard *et al.*,

2008; Calder, 2009). The n-3 poly unsaturated fatty acids promoted liver regeneration through protecting the structure of the sinusoidal endothelial cells in rats (Yu-Dong *et al.*, 2012). Possibly, in day 28 of age, synergism between hypoxia and lesser levels of oils have created hepatic lesions. In regard to potential ability of liver to regenerate by hyperplasia of the residual lobes and mitosis of the hepatocyte and combination to functions of n-3 and n-9 fatty acids, the hepatoprotective effects observed at day 42 of age. The mean values of serum metabolites at days 28 and 42 of age are given in Tables 4 and 5, respectively. There were no significant differences among the indices of serum total protein, albumin and globulin of chicks fed control diet or diet containing different oil sources at day 28 and 42 of age. Numerically, broilers fed diets containing fish oil and olive oil had a decrease trend for each of mentioned parameters at days 28 and 42 of age, respectively; while, control diet showed elevated serum total protein, albumin and globulin levels in both periods compared with other groups. This finding was inconsistence with the previous study (Yu-Dong *et al.*, 2012), which reported that total protein and albumin levels were higher in rats fed diet containing n-3 poly unsaturated fatty acids. Also in another study (Roy *et al.*, 2007), dietary supplementation of n-3 polyunsaturated fatty acids or n-6 polyunsaturated fatty acids for 30 days lead to 18-35% enhancement of plasma protein concentration in mice.

Table 2. Ingredients and nutrient composition of experimental diets in finisher (day 29 to day 42).

Ingredients (%)	Control	Fish oil	Corn oil	Olive oil	Tallow
Corn	56.50	59.90	59.90	59.90	59.90
Soybean Meal(46% CP)	19.00	29.20	29.20	29.20	29.20
Starch	9.86	0.00	0.00	0.00	1.00
Fish oil	0.00	4.00	0.00	0.00	0.00
Corn oil	0.00	0.00	4.00	0.00	0.00
Olive oil	0.00	0.00	0.00	4.00	0.00
Tallow	0.00	0.00	0.00	0.00	4.00
Corn Gluten	10.60	1.90	1.90	1.90	1.90
Calcium Carbonate	1.03	1.04	1.04	1.04	1.04
Dicalcium Phosphate	1.70	1.60	1.60	1.60	1.60
Sodium Chloride	0.35	0.33	0.33	0.33	0.33
DL-methionine	0.19	0.26	0.26	0.26	0.26
Vitamin and Mineral Premix[1]	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.41	0.15	0.15	0.15	0.15
Zeolite	0.00	1.10	1.10	1.10	0.12
Nutrient Compositions (%)					
AME (kcal/kg)	3,150	3,150	3,150	3,150	3,150
Crude protein	19.00	19.00	19.00	19.00	19.00
Crude Fat	3.94	8.30	8.30	8.30	8.30
Lysine	1.09	1.09	1.09	1.09	1.09
Methionine	0.53	0.55	0.55	0.55	0.55
Methionine plus Cystine	0.86	0.86	0.86	0.86	0.86
Fatty acids (%)					
C18:1 n-9	1.05	2.34	2.33	4.11	2.53
C18:2 n-6	2.09	2.34	4.41	2.63	2.41
C18:3 n-3	0.07	0.16	0.13	0.12	0.13
(n-6)/(n-3)	29.85	14.62	33.92	21.91	18.53

1- premix supplying composition was as follows (amounts in 10 g): 0.5 g Mg, 0.3 g S, 1.0 g Na, 1.6 g Cl, 6.0 mg Cu, 0.2 mg I, 45.0 mg Fe, 59.0 mg Mn, 0.2 mg Se, 29.0 mg Zn, 4000 IU Vitamin A Palmitate, 1000 IU Cholecalciferol, 50 IU Vitamin E acetate, 0.5 mg Menadione sodium bisulfite, 0.2 mg biotin, 10 µg cyanocobalamin, 2 mg folic acid, 30 mg nicotinic acid, 16 mg calcium pantothenate, 7 mg pyridoxine - Hcl , 6.0
mg riboflavin, and 6.0 mg thiamin Hcl.

Table 3. Hepatic histopathology of broiler chicks fed diets containing different lipid sources.

Signs	Control		Fish oil		Corn oil		Olive oil		Tallow	
	Day of age									
	28	42	28	42	28	42	28	42	28	42
Steatosis	ND	+++	+++	+	+++	+++	++	ND	+++	+
Swelling	ND	ND	+++	ND	ND	ND	ND	ND	ND	ND
Sinusoid dilation	ND	ND	ND	ND	+++	ND	ND	ND	+++	ND
Hyperemia	ND	ND	ND	ND	ND	ND	+++	ND	+++	ND
Infiltration of mononuclear cells	ND	ND	ND	++	ND	+++	ND	ND	ND	+++
hepatic degeneration	ND	ND	+++	ND	+++	+++	+++	ND	+++	+

Table 4. The effect of diets containing dietary different lipid sources on total protein (TP), albumin (ALB), globulin (GLB), AST and ALT at day 28 of age.

	Control	Fish oil	Corn oil	Olive oil	Tallow	SEM
TP (g/dL)	3.66	2.76	3.16	3.26	3.06	0.406
ALB (g/dL)	1.96	1.30	1.33	1.46	1.36	0.290
GLB (g/dL)	1.70	1.46	1.83	1.80	1.70	0.165
AST (IU/L)	234^{b}	226^{b}	258^{a}	227^{b}	262^{a}	13.5
ALT (IU/L)	6.3^{b}	4.1^{b}	15.3^{a}	5.4^{b}	6.3^{b}	2.36

Table 5. Influence of dietary lipids sources on total protein (TP), albumin (ALB), globulin (GLB), AST and ALT at day 42 of age.

	Control	Fish oil	Corn oil	Olive oil	Tallow	SEM
TP (g/dL)	3.56	3.53	3.20	3.10	3.46	0.2
ALB (g/dL)	1.53	1.50	1.40	1.36	1.56	0.077
GLB (g/dL)	2.03	2.03	1.80	1.73	1.90	0.173
AST (IU/L)	342^{b}	349^{b}	447^{a}	360^{b}	322^{b}	28
ALT (IU/L)	8.2^{b}	6.9^{c}	10.3^{a}	7.5^{bc}	8^{b}	0.91

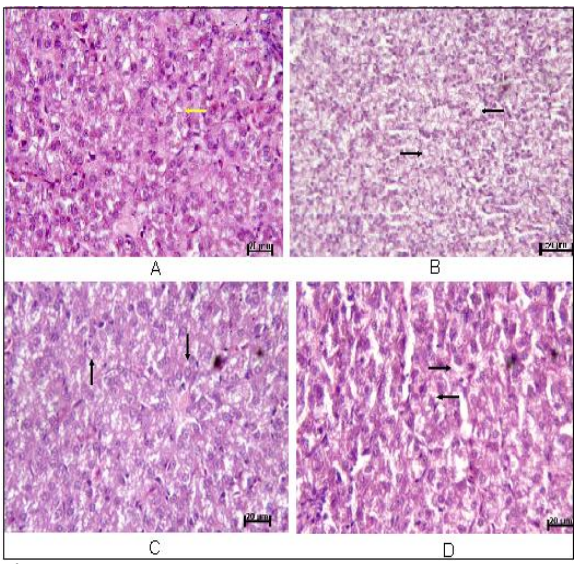

Fig. 1. Steatosis in the liver of chicks fed A, control diet; B, fish oil; C, corn oil and D, tallow.

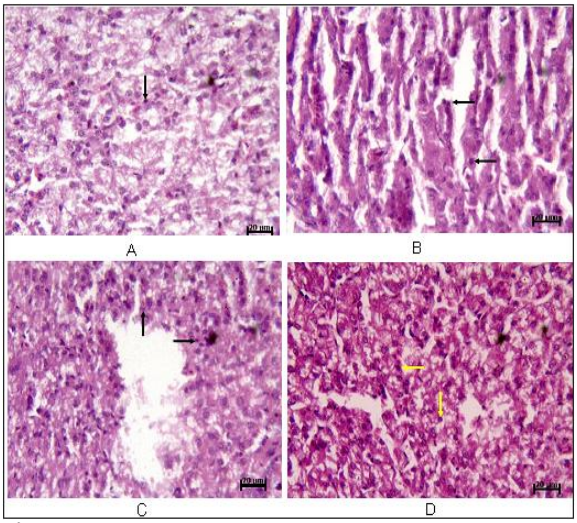

Fig. 2. Degeneration in the liver of chicks fed diet containing A, fish oil; B, corn oil at day 28 of age; C, corn oil at day 42 of age and D, olive oil.

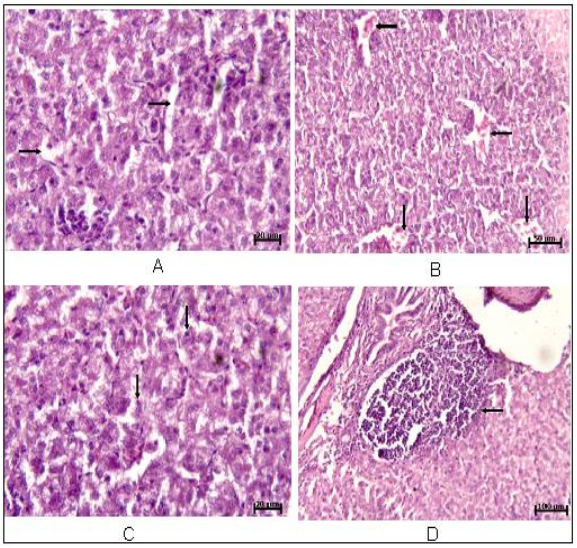

Fig. 3. Histology of liver of chicks fed diet containing tallow: image A indicate the sinusoid dilation, B hyperemia, C, hepatic degeneration and D, infiltration of mononuclear cells.

There were significant differences for AST and ALT levels of chicks fed diet containing different lipid sources (Tables 4 and 5), respectively. The highest mean of AST and ALT was for chicks fed diets containing corn oil and tallow and the lowest one was for those fed diet containing fish oil and olive oil. Liver transaminases (AST and ALT) are useful biomarkers of liver injury in a patient with some degree of intact liver function. There was no information regarding the effect of lipid type on serum AST and ALT of broilers. The comparison between the results of AST and ALT in Tables 4 and 5 with the findings in histological images (Figures 1-3), it was clearly revealed that the addition of tallow or corn oil to diet resulted in injury of the liver and increase in the levels of AST and ALT. In contrast, the addition of fish oil to diet of chicks resulted in decrease of AST and ALT levels, because the integrity of cell membrane of liver remained and cells did not disrupt.

In conclusion, our result confirmed that fish oil as a source of n-3 poly unsaturated fatty acids was much more beneficial than n-6 poly unsaturated fatty acids or saturated fatty acids to keep the chicks in the healthy state.

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