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The Influence of Different Levels of Dietary Fish Oil on the Performance, Carcass Traits and Blood Parameters of Broiler Chickens

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Abstract: This study was conducted to determine effects of different levels of Fish Oil (FO) on performance, carcass and blood parameters on male broiler chickens. Male broiler wee fed isonitrogenous and isoenergetic diets contain 0, 1, 2 and 3% fish oil levels (treatment of 1, 2, 3 and 4, respectively) ad libitum in both starter (11-21 days) and growth periods. The diets met the requirements recommended by the national research council. The final week (42 to 49 days) removed fish oil from diet. At 42 and 49 days of age, blood samples were randomly collected from the wing vein from three birds per pen. The end of both 42 and 49 days also, before slaughtering, the final body weight and after that, weight of selected organs (breast, thighs, liver, heart, gizzard, spleen and abdominal fat) were recorded individually and presented as a percentage of live weight. The results from this research showed that increasing different levels of fish oil had significant effects on male broiler performance (final weight, weight gain and feed convention ratio) ($p<0.01$), spleen weight ($p<0.05$), whereas other parameters of performance (feed intake) and carcass did not show significant differences. In serum biochemical parameters, the birds fed 3% FO (T4) indicated highest High-Dencity Lipoproteins (HDL) and lowest Low-Dencity Lipoproteins (LDL) concentrations than control treatment in 42 days ($p<0.01$). The level highest of glucose (G) observed in T3 (2% FO) in 42 days old ($p<0.05$). Total protein (P) and albumin (A) concentration in T4 and globulin (GL) concentration in T3 were lowest. With increasing FO levels, cholesterol (CHOL), triglyceride (TG) and Very Low-Dencity Lipoproteins (VLDL) levels showed non significant decrease in collected samples of 42 days. But, decrease of cholesterol level after one week withdrawal of FO from diet, was significant ($p<0.05$).

Key words: Fish oil, performance, carcass traits, blood, broiler

INTRODUCTION

Nowadays, Fish Oil (FO) is recognized such as an far benefit fat origin for researchers, nutrition scientists and animal producer because, fish oil is contains of the long chain omega-3 polyunsaturated fatty acids that as being an important factor in the diet of human and animals. the researches on poultry indicated that with increase of unsaturation degree of fat, the performance increased due to improvement in fat digestibility (Pinchasov and Nir,1992; Crespo and Esteve Garcia, 2001). However, it was detected that by feeding of diet containing fish oil, the high performance is achieved, because fish oil reduce the catabolic response induced by immune stimulation and improving specific immunity (Chin *et al*., 1994). Studies with broilers (Zollitsch, 1997; Sanz *et al*., 2000a; Özdogan and Aksit, 2003) have reported that less abdominal fat accumulation in broilers fed the fats rich in LCn-3 PUFA such as linseed and rapeseed (vegetable origins) and fish oil (marin origin) than in those fed saturated or omega-6 PUFA fats (Pinchasov and Nir, 1992; Sanz *et al*., 2000b). Moreover, fish lipids improve animal health and produce healthy food. As some of those improvements have been searched the effect of omega-3 fatty acids on the health of human and beings animals (Manilla *et al*., 1999; Crespo and Esteve-Garcia, 2003; Abas *et al*., 2004; Alparslan and Ozdogan, 2006). On the other hand, the adding fish oil to diets entails several organoleptic problems that adversely affect the meats acceptability (Miller and Robisch, 1969; Lopez-Ferrer *et al*., 1999a, b, 2001; Özpinar *et al*., 2002; Alparslan and Ozdogan, 2006). The influence of fish oil on sensory characteristics and growth performance in broilers have been widely investigated in the studies

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reviewed above. Therefore, the purpose of the present study was to evaluate the performance, carcass traits and biochemical parameters, before (42 days) and after (49 days) withdrawal of FO from diet.

MATERIALS AND METHODS

Animals and diets: Six hundred one-day-old Ross-308 type broiler chicks of both sexes were obtained from a commercial hatchery. The chicks were fed a commercial feed starter from 1-10 days old. Two hundred fourty chicks (male) were sexed one day before start of trial (9 days) and in 10 days old were individually weighed, randomly assigned to cages (15 birds per pen) in 16 floor pens.

The experimental design consisted of four dietary treatments: control: Basal diet; Basal diet containing 1% fish oil (FO); Basal diet containing 2% FO; Basal diet containing 3% FO. A 4-phase feeding program was utilized in which the starter diet was fed for 3 week (1-10 and 11-21 days of age), the grower for 3 week and the finisher for 1 week (after withdrawal fish oil from diet). The four dietary treatments were split among the 16 floor pens in a complete design. The birds were given access to water and diets *ad libitum*. The composition and calculated nutrients contents of experimental diets are shown in Table 1.

Data collection

Dietary intake and body weight gain: The chicks were weighed individually every week. Data on weekly food intake, food conversion ratio (FCR) (food intake/ weight gain) were recorded in each replicate group. After blood samples were taken, the same broilers were slaughtered at 42 and 49 days (three samples per pen), before and after to exert of withdrawal of FO from diet, respectively, to determine of carcass yield, weight of abdominal fat, breast, thighs, liver and spleen.

Serum biochemistry: After the 6 and 7 week rearing period, 3 of the 15 broilers from each pen that had been randomly selected for serum biochemical analyses. The samples of blood were taken in non-heparinised tubes by puncturing the brachial vein. Serum was collected after 8-10 h using a standard procedure and stored at -20°C. Serum for analysis was obtained by centrifugation at

Table 1: Composition and calculated nutrients of starter, growth diets and withdrawal diets fed to chicks (%)

		Experimental Diet[1]								
		Starter diets (11-21 day)				Growth diets (22-42 day)				
Feedstuffs	Starter diet (1-10 day)	T1	T2	T3	T4	T1	T2	T3	T4	Withdrawal plan diet[2]
Yellow corn	63.83	58.35	55.68	52.15	49.25	51.81	50.86	47.97	45.07	66.70
Soybean meal	29.96	31.95	32.94	32.31	32.87	29.56	29.65	30.21	30.77	25.62
Wheat	-	-	-	-	-	6.99	1.20	1.20	1.20	2.82
Wheat bran	-	1.64	0.33	4.00	4.00	-	-	-	-	-
Fish meal	3.28	3.00	3.00	3.00	3.00	-	-	-	-	-
Soybean oil	-	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil	-	-	1.00	2.00	3.00	-	1.00	2.00	3.00	-
Inert	-	-	1.96	1.40	2.81	-	1.05	2.40	3.75	-
Oyster shell	0.76	0.78	0.71	0.77	0.76	1.18	1.19	1.18	1.18	1.15
Dicalcium phosphate	1.12	1.18	1.20	1.18	1.19	1.07	1.06	1.07	1.07	0.89
Salt	0.22	0.22	0.22	0.22	0.38	0.12	0.12	0.12	0.12	0.11
Sodium bicarbonate	0.12	0.23	0.22	0.23	-	0.28	0.28	0.27	0.27	0.20
Lysine hydrochloride	-	0.02	0.01	0.01	-	0.11	0.11	0.10	0.09	-
DL-Methionine	0.12	0.13	0.13	0.13	0.14	0.70	0.80	0.80	0.80	-
Vitamin/mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit E	-	-	0.10	0.10	0.10	-	0.10	0.10	0.10	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrient content										
ME (kcal kg^{-1})	2900	2950	2950	2950	2950	3000	3000	3000	3000	3070
Crude protein	20.71	21.20	21.20	21.20	21.20	18.75	18.75	18.75	18.75	17.26
Calcium	0.81	0.83	0.83	0.83	0.83	0.84	0.84	0.84	0.84	0.76
Available P	0.40	0.41	0.41	0.41	0.41	0.33	0.33	0.33	0.33	0.28
Lysine	1.13	1.18	1.18	1.18	1.18	1.05	1.05	1.05	1.05	0.87
Methionine	0.48	0.49	0.49	0.49	0.49	0.36	0.36	0.36	0.36	0.28
Methionine +cystine	0.81	0.83	0.83	0.83	0.83	0.68	0.68	0.68	0.68	0.57

[1]T1 = 0% FO, T2 = 1% FO, T3 = 2% FO and T4 = 3% FO.[2]oil remove for one week before slaughter (to decreased of unacceptable odors). [3]For each kg of the diets; vitamin A, 9,000,000 IU; vitamin D3, 2,000,000 IU; vitamin B1, 1,800 mg; vitamin B2, 6,600 mg; vitamin B3, 10,000 mg; vitamin B6, 3,000 mg; vitamin B12,15 mg; vitamin E, 18,000 mg; vitamin K3, 2,000 mg; vitamin B9, 1,000 mg; vitamin B5, 30,000 mg; folic acid, 21 mg; nicotinic acid, 65 mg; biotin, 14 mg; choline chloride, 500,000 mg; Mn, 100,000 mg; Zn, 85,000 mg; Fe, 50,000 mg; Cu, 10,000 mg; I, 1,000 mg; Se, 200 mg

5000 rpm for 10 min. The concentrations of triglycerides, cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and glucose in serums were determined by using commercial kit (Kone commercial kit, Japan), in Autoanalyser (ALCYON-300 autoanalyser, American).

Statistical analysis: The data were subjected to ANOVA using the General Linear Model procedure of SAS Institute. Means showing significant differences in the ANOVA were separated using the Duncan's multiple range procedure. The pooled SEM were calculated by taking the square root of the ANOVA mean squares error term divided by the harmonic mean sample size.

RESULTS AND DISCUSSION

Performance parameters: The effects of different levels of FO added in broiler diets on the performance values are shown in Table 2. Increasing inclusion level of FO from 0 to 3% in broiler diets significantly ($p<0.05$) affected weight gain, feed conversion ratio in all periods. However, in comparision with the control diet, the level of fish oil did not significantly affect feed intake. In the all experiment period, the birds fed 3% FO (T4) were showed the highest live weight gain and final weight and the lowest FCR ($p<0.01$) than other treatments. However, the results of T4 birds had no significant differences with values of observed in the birds fed 2% FO (T3).

The results of this experiment is in agreement with the findings of Crespo and Esteve-Garcia (2001, 2003) and Lopez-Ferrer *et al.* (1999a b, 2001). The researchers (Pinchasov and Nir, 1992; Zolisch *et al.*, 1997) have reported that with increase of unsaturation degree of fats/oils was increased it's digestibility. Huang were observed that the inclusion of fish oil in poultry diets had no effect on consumption of feed. On the other hand, adding of FO in poultry diets significantly increased ($p<0.05$) the weight gain, final weight and FCR (Farell, 1995). Furthermore, according to the experiment of Farell (1995), withdrawal of FO from (for one week) before slaughtering, had no effect on the performance results.

Carcass traits: The carcass yield values of broiler chicks are shown in Table 3. At investigation of the results of related to the carcass traits, significant differences were not found in weight of the carcass yield, abdominal fat, thighs, breast, liver, gizzard and heart among the treatments (Table 3), whereas the relative weight of spleen was heavier ($p<0.05$) in the groups fed fish oil compared to the control. This results agree with the findings of Zolisch (1997) and Lopez-Ferrer *et al.* (2001). According to the results obtained in the present research, diets rich in PUFA couse of depress abdominal fat deposition, because, the LC n-3 PUFA content in diet reduces fat accretion in chickens that reported by Pinchasove and Nir (1992).

Table 2: Effect of fish oil on Final weight, feed intake, weight gain, feed conversion ratio (FCR) of treatment groups [1,2]

Groups	Starter period (11-21 day)	Growth period (22-42 day)	Withdrawal period (42-49 day)
Weight gain (g/bird/d)			
T1	40.67^{c}	68.00^{d}	76.02^{c}
T2	42.27^{b}	71.42^{c}	77.67^{b}
T3	46.07^{a}	76.37^{b}	86.00^{a}
T4	45.62^{a}	77.65^{a}	86.80^{a}
SEM	0.275	0.263	0.526
Significance	**	**	**
Feed intake (g/bird/d)			
T1	63.55^{a}	133.9^{a}	169.5^{a}
T2	63.80^{a}	134.1^{a}	170.8^{a}
T3	63.42^{a}	133.9^{a}	169.9^{a}
T4	63.55^{a}	133.9^{a}	169.5^{a}
SEM	0.119	0.081	0.054
Significance	NS	NS	NS
FCR (g:g)			
T1	1.55^{a}	1.95^{a}	2.24^{a}
T2	1.50^{b}	1.87^{b}	2.19^{b}
T3	1.38^{c}	1.75^{c}	1.98^{c}
T4	1.37^{c}	1.72^{d}	1.95^{c}
SEM	0.007	0.008	0.012
Significance	**	**	**
Final weight (kg/bird)			
T1	0.669^{c}	2.12^{c}	2.63^{c}
T2	0.697^{b}	2.19^{b}	2.73^{b}
T3	0.731^{a}	2.33^{a}	2.93^{a}
T4	0.728^{a}	2.35^{a}	2.96^{a}
SEM[1]	0.003	0.011	0.011
Significance	**	**	**

^{ab}: Values in the same row and variable with no common superscript differ significantly. *: $p<0.05$, **: $p<0.01$, NS: Not Significant, [1]Values are means of eight observations per treatment and their pooled SEM. [2]T1 = 0% FO, T2 = 1% FO, T3 = 2% FO and T4 = 3% FO

With the increase of FO in broiler diets, the relative weight of spleen significantly increased. There were no significant differences in relative weight of spleen with treatments containing fish oil (2, 3 and 4) at 42 and 49 days. In present experiment, the increase in relative weight of spleen with addition of FO to the diet is agrees with the reports. According to the results obtained in the research of Halver (2005), that was investigated effect of fish oils and meals on difference animals as broiler chickens, turkeys, ducks, pigs, rats and rabbits and reported that hypervitaminosis A (use of more FO) causative increase in relative weight of liver and spleen, abnormal growth, skin lesions, epithelial keratinization, hyperplasia. He, also observed that the use of fish oil in diet without or low vit E (α-Tocopherol) that is necessary for stopping per-oxidation of lipid, signs is followed by anemia, ascites, exophthalmia, poor growth, poor food conversion, epicarditis and ceroid deposits in spleen and liver.

Table 3: Effect of fish oil on Carcass yield parameters of broiler chicks (42 and 49 day)[1,2]

Variable	Carcass yield[3]	Abdominal fat[4]	Thighs[4]	Breast[4]	Heart[4]	Gizzard[4]	Spleen[4]	Liver[4]
In 42 days								
T1	67.80	3.04	17.87	26.40	0.380	1.27	0.080[b]	2.02
T2	67.61	3.02	18.12	26.89	0.440	1.32	0.102[a]	2.07
T3	66.21	3.20	18.82	26.38	0.452	1.26	0.105[a]	1.98
T4	66.70	3.25	18.52	26.50	0.441	1.14	0.115[a]	1.98
SEM	1.213	0.113	0.576	0.536	0.189	0.057	0.006	0.092
Significance	NS	NS	NS	NS	NS	NS	**	NS
In 49 days								
T1	68.60	2.59	20.15	27.52	0.401	1.32	0.105[b]	2.01
T2	68.25	2.72	19.13	27.50	0.424	1.44	0.116[a]	1.89
T3	67.84	2.82	19.06	27.98	0.426	1.11	0.117[a]	1.79
T4	69.62	3.13	19.89	28.29	0.350	1.18	0.120[a]	2.13
SEM	1.035	0.113	0.391	3.480	0024	0.036	0.003	0.204
Significance	NS	NS	NS	NS	NS	NS	*	NS

[ab]: Values in the same row and variable with no common superscript differ significantly . *: p<0.05, **: p<0.01, NS: Not Significant, [1]Values are means of 8 observations per treatment and their pooled SEM. [2]T1 = 0% FO, T2 = 1% FO, T3 = 2% FO and T4 = 3% FO [3] Carcass yield, without head, neck, or feet. [4]Percentage of carcass

Table 4: Effect of fish oil on blood parameters (mg dL^{-1}) of male broiler in 42 and 49 days

Variable	Triglyceride	Cholesterol	HDL	LDL	VLDL	Glucose	Total protein	Albomin	Globulin
In 42 days									
T1	58.75	138.38	15.62	12.80	11.75	82.25	3.87	2.08	1.78
T2	60.25	140.13	23.37	11.88	12.05	118.88	3.91	2.07	1.83
T3	53.62	142.00	22.75	11.30	10.72	137.25	3.50	1.86	1.63
T4	45.62	117.63	34.12	7.57	9.12	119.88	3.43	1.77	1.65
SEM	5.167	7.983	2.974	1.303	1.033	12.83	0.114	0.064	0.061
Significance	NS	NS	**	*	NS	*	**	**	*
In 49 days									
T1	60.80	108.60	15.40	6.48	12.16	106.80	3.72	2.06	1.66
T2	68.80	106.40	15.00	5.96	13.76	118.60	3.84	2.08	1.66
T3	60.20	99.60	15.00	4.88	12.04	120.00	3.82	2.06	1.76
T4	62.60	94.00	13.60	4.04	12.52	114.20	3.58	1.92	1.76
SEM	8.748	3.481	1.937	0.955	1.749	13.45	0.062	0.031	0.048
Significance	*	NS	NS	NS	NS	NS	*	**	NS

[ab]: Values in the same row and variable with no common superscript differ significantly . *: p<0.05, **: p<0.01, NS: Not Significant, [1]Values are means of 8 observations per treatment and their pooled SEM. [2]T1 = 0% FO, T2 = 1% FO, T3 = 2% FO and T4 = 3% FO

Serum biochemistry: Data presented in Table 4 show the effects of dietary treatments on serum biochemical values of broilers at 42 and 49 days of age. There were no significant differences in cholesterol and triglyceride with treatment but the withdrawal of fish oil (49 days) caused significant decrease in the serum cholesterol. The birds of four treatment showed the lowest CHOL concentration in both the samples of blood taken before (42 days) and after (49 days) withdrawal FO from diets.

The High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) levels were significantly increased ($p<0.01$) and decreased ($p<0.05$), respectively in all of the treatments containing fish oil the serum samples in 42 days. Although, the HDL and LDL values was not significant in 49 days, but in serum of male broiler were decreased. The VLDL concentration in both the serum samples (42 and 49 days) were not significant. Chin *et al.* (1994), Manilla *et al.* (1999) and Crespo reported that omega-3 fatty acids present in fish oil reduce the VLDL levels in blood, with acting to lower the circulating free LDL concentration and also, reduce the rate of triglyceride synthesis in the liver. Significant depression in cholesterol and LDL and increase in HDL contents observed by Ozdogan and Aksit (2003). They used sunflower oil as vegetable oil rich of PUFA in their trail.

Marine and vegetable sources rich in LC -3 PUFA is necessary in improving animals health, producing of healthy foods, which is one of the reasons that the blood and products of animals had high HDL and low LDL values. Researchers showed that low HDL and high LDL are values associated with atheroschlorosis and coronary heart disease (Bachorik *et al.*, 1991; Guyton and Hall, 1996; Ozdogan and Aksit, 2003). Grundy (1991) also indicated that increasing in serum HDL is useful in decreasing the negative effect of high blood cholesterol.

When the effect of adding fish oil on the values of glucose was evaluated periodically (Table 4), a significant difference was seen at before withdrawal FO from diets ($p<0.05$). The lowest glucose (G) content was determined in the group control and the highest glucose concentration found in the groups fed 2% FO (T3). In the withdrawal period (finisher period), the removing fish oil from diet did not effect on the glucose (G) values. Researches on the male and animal have indicated that

increasing unsaturation of diet fat, increase G amount of blood couse of insulin secretion depress (Grill and Qvigstad, 2000; Storlien *et al*., 2000). Mori *et al*. (1999) reported that with feeding Dietary fish and fish oil / meal to human and animals, decreased blood pressure, TG and CHOL- LDL levels whereas the serum concentration of CHOL- HDL and G were higher ($p<0.05$).

With the increase of FO levels in experimental diets was observed significant decrease ($p<0.05$) in TP, AL and GL levels (Table 4). But, after one week withdrawal FO from diet, only GL concentration was not significant. The lowest Serum TP and AL levels was determined in T4 (in 42 and 49 days) and The lowest serum GL concentration was determined in T3 (in 42 days). Because, fats for transmission in blood must were mixed with proteins in the form of complex compositions of hydrophile lipoprotein and with consider to density of pure lipids is less from water. therefore, is decreasing the protein dencity because of increasing lipid/protein ratio (Tuncer, 1987). It is cleared that serum TP, AL and GL concentrations of the birds fed dietary FO were significantly decreased in compared to the birds of control group. Also, Touchburn with study of glucose/ insulin balance and dietary protein on broiler reported that with adding of fats rich in PUFA in diet of bird, serum TP, AL and GL levels were depressed.

Results from this experiment showed that broilers fed diets contain fish oil rich of Omega-3 were improved the performance and same blood traits. Although, with remove of FO from experimental diets, some of serum parameters did not affect, but significant decrease of cholesterol was indicated in 49 days. Furthermore, by considering the outcomes, in order to lower sensory losses and the economic advantage, the authors recommend using fish oil in diets of male broiler chickens about 2% levels.

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