

## ORIGINAL ARTICLE

# Influence of dietary fish oil supplementation on humoral immune response and some selected biochemical parameters of broiler chickens

Navid Hosseini-Mansoub<sup>1</sup>, Yadollah Bahrami<sup>2</sup>

<sup>1</sup>Young Researchers Club, Maragheh Branch, Islamic Azad University, Maragheh, Iran

<sup>2</sup>Young Researchers Club, Khorasgan Branch, Islamic Azad University, Isfahan, Iran

Received: 5<sup>th</sup> January 2011

Revised: 27<sup>th</sup> April 2011

Published online: 22<sup>nd</sup> August 2011

---

### Abstract

The objective of this experiment was to evaluate the influence of fish oil (FO) supplementation in the diet of broiler chickens on the humoral immune response as well as some blood parameters. Two hundred and sixteen one day old broiler chickens were divided into four dietary groups 0, 1, 2, or 4% FO with 3 replicates of 18 birds. Four chicks randomly selected and marked from each replicate were immunized intramuscularly with 0.2 ml of 5% sheep red blood cells (SRBC) as a non-infectious antigen, at the ages of 15 and 35 days and blood samples were taken 7 days after each immunization. The highest BW was observed in the 2% FO dietary group ( $T_3$ ), followed by  $T_2$  ( $P<0.01$ ). The serum cholesterol and triglyceride levels significantly decreased in the FO groups at the age of 42 days ( $P<0.01$ ). In addition, the inclusion of FO in broiler diets significantly increased the blood glucose (G) level and decreased the total protein (TP), albumin (A) and globulin (GL) concentrations. Fish oil-treated birds had significantly more serum antibody (predominantly immunoglobulin M, IgM) to SRBC than the control group. The highest response to primary and secondary injections of SRBC after 7 days, were detected for group 4 (4% FO), followed by 2% FO group ( $P<0.05$ ). The results indicate that the addition of 2 % FO to broiler chick's diet may stimulate the development of the immune response and improve blood indices, while 4% level was not recommended because of probable off-flavours in the product.

**Key words:** fish oil; broiler; performance; blood parameters; immune system

---

✉ Navid Hosseini-Mansoub, Young Researchers Club, Maragheh Branch, Islamic Azad University, Maragheh, Iran

✉ [n.h.mansoub@gmail.com](mailto:n.h.mansoub@gmail.com)

---

## INTRODUCTION

Fish oil is the most easily available and commonly used omega-3 source used in the diet to provide energy for fowls while its supplementation has been shown to enhance production performance and immune responses and safety in the

poultry (Hulan et al. 1988, Fritsche et al. 1992, López-Ferrer et al. 1999, Schreiner et al. 2005, Farhoomand and Chekani-Azar 2009). Fish oil contains unsaturated fatty acids with long omega-3 chains (LC-n-3 PUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) which all improve health-related factors in humans and animals. The role of these fatty acids in decreasing cholesterol and triglycerides levels has been proven (Newman et al. 1998).

Numerous studies indicate that dietary fish oil lowers the level of total cholesterol, low density lipoprotein (the major carrier of cholesterol in plasma) and triglycerides (Crespo and Esteve-Garcia 2003). Also, it has been shown that oils rich in polyunsaturated fatty acids can modify abdominal fat deposits in broiler chickens. Tests were performed on groups fed on dietary fish oil to assess protection for the fowls against antigens and help the body in the fight against infection and it was found that the weight of the spleen was higher in these birds. The role of the spleen was enhanced resulting in a boosted immune system response to sheep red blood cells (SRBC) in bird immunization (Torki et al. 2000, Kidd 2004).

Researchers have reported that a diet rich in polyunsaturated fatty acid increases the amount of serum glucose because of a decline in insulin secretion (Grill and Qvigstad 2000, Storlien et al. 2000). Holness et al. (2004) demonstrated that omega-3 enrichment of diets can effect a rapid lowering of insulin secretion from the islets of langerhans and raise the plasma glucose concentration. Hence, one of the key reasons why omega 3 fish oil has such a powerful effect on fat and carbohydrate metabolism is that the levels of insulin secretion can be changed to FO or the omega-3 LC PUFAs thereof.

In a study on dietary protein in broilers (Touchburn et al. 1981, Tuncer et al. 1987, Olomu and Baracos 1991) the authors observed a decline in total protein, albumin and globulin concentrations of the brood birds. They reported that a decrease the protein density could be due to an increase in the lipid/protein ratio. Interestingly, early observations have suggested that diet enrichment with marine sources rich in LC n-3 PUFAs is a more effective method for n-3 enrichment of broiler meat López-Ferrer et al. (1999). The main factor for omega-3 enrichment of bird diet is the resulting readiness of the body against any risks, stress and diseases affecting bird performance, because the levels of deposited n-3 FAs can be reused in birds for maintenance

of the immune system and lipid oxidation (Kidd 2004, Farhoomand and Chekani-Azar 2009).

Therefore, the aim of this study is to evaluate the effect of different levels of fish oil on production performance, blood parameters and also the immune system response in male broiler chickens.

## MATERIAL AND METHODS

### Chickens, housing and experimental design

216 male Ross308 broilers were selected at random from 400 birds of both sexes, were sexed and weighed at one day old before starting the trial. They were then allocated to 12 pens (18 birds per pen) and fed a commercial diet for 7 days and diet with added fish oil (0, 1, 2 and 4% FO, T<sub>1</sub>–T<sub>4</sub> respectively) for a subsequent 5 weeks. All diets were formulated using the user-friendly feed formulation (UFFDA) program (AFF16.ZIP, Stand-alone program for Windows) according to NRC (1994) guidelines. The composition and calculated nutrient contents of the experimental diets are shown in Table 1.

The mean BW, using the pen as the experimental unit at the beginning of the experiment (on day 1), was not significantly different ( $P>0.05$ ). The 3-phase feeding program consisted of periods of primary (second week), growth (weeks 3 and 4) and final (weeks 5 and 6). The food and also water were available to the birds *ad libitum*. The fish oil (*Clupeonella* oil from fish of the Caspian Sea) was obtained from Iranian sources (Mehregan Khazer Co., Bander Abbass, Iran) and was stored at 4 °C, in dry and dark places before being mixed with the other ingredients.

### Performance recording and sample collection

The birds were weighed every week and data on weekly food intake and food intake/weight gain were recorded in each replicate group up to 42 days to determine mean body weight (BW). After blood sampling and performance recording, the male broilers were slaughtered.

To assess the systemic antibody response, four chicks were randomly selected and marked from each replicate and were immunized intramuscularly with 0.2 ml of 5% sheep red blood cells (SRBC) as a non-infectious antigen, at the ages of 15 and 35 days, and blood samples used to assess the immune system response to SRBC, were taken 7 days after each immunization.

**Table 1.** Diet components for different study groups and chemical composite analysis

	Starter diet (1–14)				Grower diet (14–35)				Final diet (35–42)			
	T <sub>1</sub> <sup>2</sup>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Yellow corn	56.9	56.5	55.25	50.2	63	62.5	62.5	56.47	66	66	66	61
Soybean meal	33.5	30.3	33.5	36.20	28.17	27.9	27.2	29.20	22.6	22.6	22.25	24.5
Corn gluten	2.9	3	3	1.94	1.77	2.07	2.54	2	2.7	2.7	2.7	2.1
Inert	0	0	0	1.64	0.4	0.37	0.6	2.65	1.67	1.17	1.02	2.85
Fish oil	0	1.5	3	6	0	1.5	3	6	0	1.5	3	6
Oyster shell	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Dicalcium phosphate	2	2	2	2	1.7	1.7	1.7	1.7	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin/mineral premix <sup>1</sup>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03	0.09	0.09	0.09	0.09
L-Lysine	0.0	0.0	0.0	0.0	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04
Animal fat	2.75	1.2	0.0	0.02	3	0.5	0.5	0.02	3.5	0.5	0.5	0.02
Vitamin E	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.01	0.10	0.10	0.01	0.01
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated nutrient content												
Crude fat	0.06	0.07	0.07	0.07	0.06	0.07	0.07	0.08	0.07	0.08	0.08	0.08
Dry matter	89.03	89.97	88.80	89.00	89.00	89.07	89.57	88.57	88.00	87.77	88.47	87.80
Moisture	10.97	11.03	11.20	11.00	11.00	10.93	10.43	11.43	12.00	12.23	11.53	12.20
AME <sub>n</sub> (MJ)	3000				3050				3100			
Protein (%)	21.56				19.06				17.56			
Calcium	0.81				0.83				0.84			
Available P	0.40				0.41				0.32			
Lysine	1.19				1.18				1.04			
Methionine	0.48				0.49				0.33			
Methionine + cystine	0.81				0.73				0.65			

<sup>1</sup> For each kg of the diets; vitamin A 9,000,000 IU; vitamin D<sub>3</sub> 2,000,000 IU; vitamin B<sub>1</sub> 1,800 mg; vitamin B<sub>2</sub> 6,600 mg; vitamin B<sub>3</sub> 10,000 mg; vitamin B<sub>5</sub> 3,000 mg; vitamin B<sub>12</sub> 15 mg; vitamin E 18,000 mg; vitamin K<sub>3</sub> 2,000 mg; vitamin B<sub>9</sub> 1,000 mg; vitamin B<sub>6</sub> 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

<sup>2</sup> T<sub>0</sub> = 0% FO, T<sub>1</sub> = 1% FO, T<sub>2</sub> = 2% FO and T<sub>3</sub> = 4% FO

The last blood sampling was performed prior to slaughter. Similarly, some blood parameters such as triglycerides, cholesterol, glucose (G), total protein (TP), albumin (A) and globulin (GL) concentrations were evaluated in blood samples at the ages of 21 and 42 days which were taken from the wing vein by injection into acuum tubes and were collected in non-heparined tubes by puncturing the brachial vein for a duration of 4–5 h. All samples were kept at room temperature for 2 hours and then at 4 °C overnight. Blood samples were centrifuged for 10 min at 580 × g, and serum was isolated and stored at –80 °C.

### Serological and statistical analysis

A direct haemagglutination assay was performed to measure the total antibody (IgM and IgG) response to SRBC in serum. Serum samples were incubated at 56 °C for 30 min to inactivate the complement. To determine anti-SRBC IgG and IgM antibodies, serum samples were treated with 0.2 M 2-mercaptoethanol (2-ME) for 30 min at 37 °C. This treatment inactivates IgM, and as a result, hem agglutination observed after treatment with 2-ME is due mostly to the presence of IgG antibodies. The difference between total antibody and IgG titres determines the IgM

titre. Other serum parameters were determined via enzyme methods using a commercial kit (Kone Specific, Kone Instruments Corp., Japan) by autoanalyzer (Autoanalyzer, ALCYON-300, Abbott, South, North, USA).

All data (pen means) were analysed using a completely randomised design and were subjected to ANOVA using the GLM procedures of SAS software (SAS Institute, 2001). Means were compared by the least significant difference (LSD) procedure of the same statistical package and then Statistical significance was considered at a  $P$  of  $<0.05$ .

## RESULTS

### Performance

The effects on performance of diet supplementation with FO are shown in Table 2. The addition of fish oil to diet significantly affected body weight in the 5<sup>th</sup> and 6<sup>th</sup> week ( $P<0.01$ ). Body weights increased up to 2% fish oil supplement but the final weight was significantly decreased in group 4 (broilers fed from 4% fish oil).

**Table 2.** Effect of fish oil on mean body weight (means of nine observations per treatment) in different weeks (g)

Treatment	Day 1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
T <sub>1</sub> <sup>1</sup>	45.43	122.15	339.75	740.25	1205.25	1656.10 <sup>a</sup>	2101.05 <sup>ab</sup>
T <sub>2</sub>	45.19	126.05	355.80	735.70	1210.50	1735.55 <sup>ab</sup>	2220.80 <sup>a</sup>
T <sub>3</sub>	45.71	125.00	345.23	758.55	1210.31	1710.80 <sup>ab</sup>	2248.25 <sup>ab</sup>
T <sub>4</sub>	45.43	117.04	347.35	700.60	1202.85	1696.50 <sup>c</sup>	2190.50 <sup>b</sup>
SE <sup>2</sup>	0.14	1.30	4.41	8.20	13.17	18.35	15.50
$P$ value <sup>3</sup>	ns	ns	ns	ns	ns	**	**

<sup>a-b</sup> Values in the same row and variable with no common superscript differ significantly

<sup>1</sup> T<sub>1</sub> = 0% FO, T<sub>2</sub> = 1% FO, T<sub>3</sub> = 2% FO and T<sub>4</sub> = 4% FO

<sup>2</sup> Standard errors

<sup>3</sup> ns = Not significant, \*\*  $P<0.01$

### Blood parameters

Data presented in Table 3 shows the effects of diet supplementation with fish oil on serum biochemical values and the immune system response of broilers at 21 and 42 days of age. Dietary fish oils altered the selected blood parameters and specific immune system response in broiler chicks. Blood amounts of cholesterol and triglycerides were decreased after a 35-d trial period. At the age of 21 days, a significant decline was observed in triglyceride content ( $P<0.01$ ). In samples also taken at this time, glucose (G) content was increased ( $P<0.05$ ) and the amounts of total protein (TP), albumin (A) and globulin (GL) concentrations showed a decline, (but the albumin value was not significant). On day 42, A, TP and GL values decreased mainly in comparison to the results observed on day 21; however, GL content declined significantly.

### Immune system responses to SRBC

Evaluation of the anti-SRBC antibody titre (Anti-SRBC-AT, Table 3, Fig. 1) in two responses – the primary (21 d) and secondary (42 d) – showed that inclusion of increasing amounts of fish oil in the diet improved the immune system response significantly ( $P<0.05$ ). The highest response to primary and secondary injections of sheep red blood cells after 6 days, was observed in T4 (4% FO), followed by 2% FO group ( $P<0.05$ ). Fish oil-treated birds had significantly more serum antibody (predominantly immunoglobulin M [IgM]) to SRBC than the birds that were not treated with fish oil. However, treatment with fish oil did not significantly enhance the serum IgG antibody responses to SRBC (Fig. 2).

**Table 3.** Effects of fish oil on some blood parameters (mg dL<sup>-1</sup>) immune system response (antibody titre) – means of eight observations per treatment

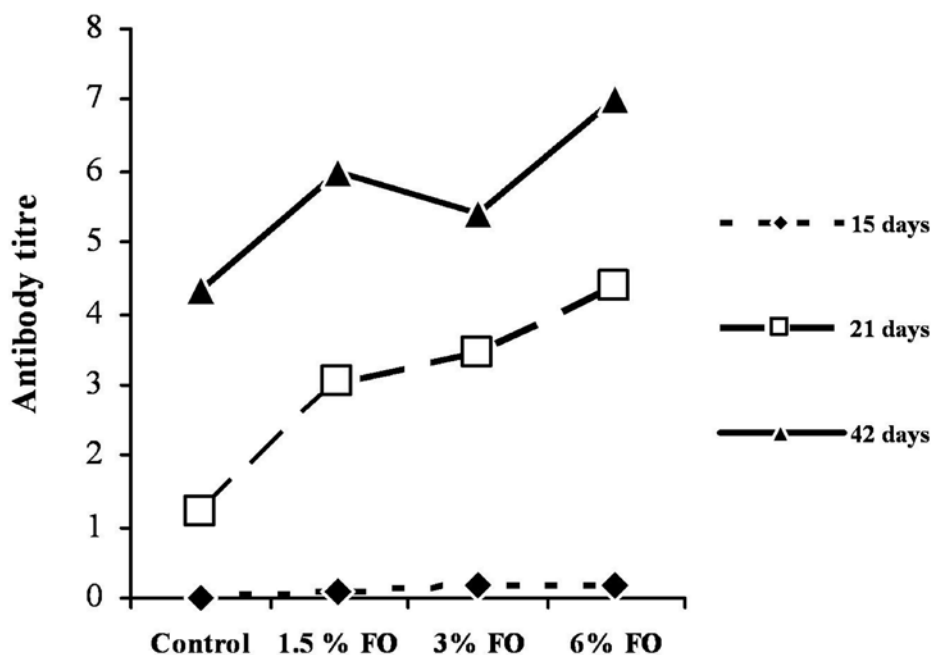
Variable (mmol/l)	TG	Chol	G	TP	A	GL	Anti-SRBC-AT
<b>In 21 d</b>							
T <sub>1</sub> <sup>1</sup>	112.31	114.74	88.02	3.79	2.02	1.78	1.22
T <sub>2</sub>	118.11	114.98	98.88	3.78	2.06	1.73	3.03
T <sub>3</sub>	104.52	113.58	120.05	3.70	2.06	1.69	3.43
T <sub>4</sub>	101.23	111.96	121.12	3.54	1.94	1.59	4.40
SE	1.21	0.55	2.51	0.114	0.064	0.061	0.46
P value <sup>3</sup>	**	ns	*	**	ns	*	*
<b>In 42 d</b>							
T <sub>1</sub>	111.53	114.60	110.75	3.85	2.10	1.72	4.33
T <sub>2</sub>	106.62	105.56	109.60	3.74	2.08	1.65	6.01
T <sub>3</sub>	113.62	104.52	123.10	3.65	1.90	1.66	5.42
T <sub>4</sub>	99.85	98.11	131.20	3.43	1.82	1.65	7.00
SE <sup>2</sup>	2.28	2.53	3.38	0.058	0.033	0.035	0.049
P value <sup>3</sup>	**	*	*	*	**	ns	*

TG = triglycerides, Chol = cholesterol, G = glucose, TP = total protein, A = albumin, GL = globulin, Anti-SRBC-AT = anti sheep red blood cells antibody titre

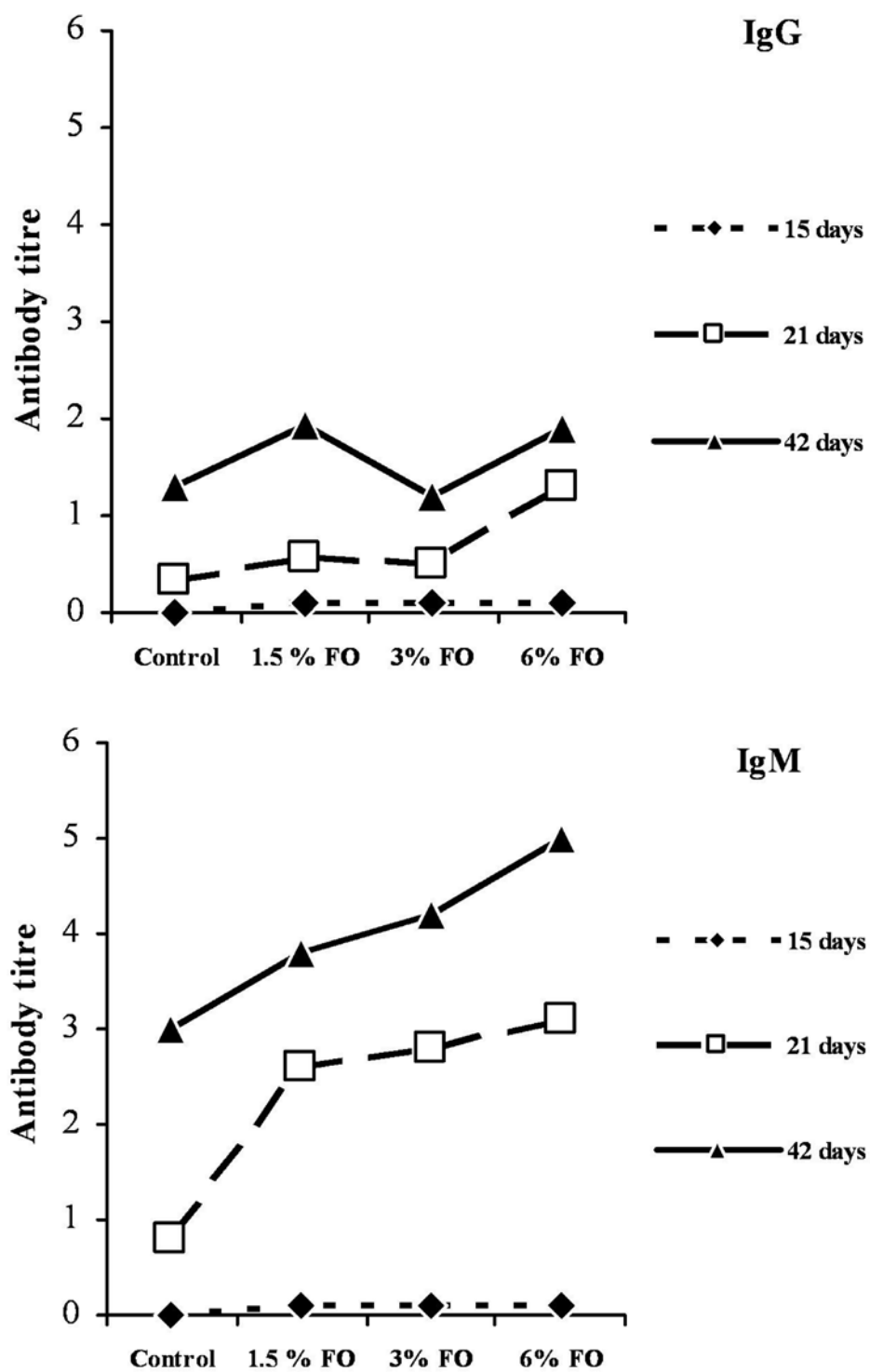
<sup>1</sup> T<sub>1</sub> = 0% FO, T<sub>2</sub> = 1% FO, T<sub>3</sub> = 2% FO and T<sub>4</sub> = 4% FO

<sup>2</sup> Standard errors

<sup>3</sup> ns = Not significant, \* P<0.05, \*\* P<0.01



**Fig. 1.** Least squares means (LSM) of serum anti-SRBC antibody titre, as determined using a direct hem agglutination assay



**Fig. 2.** Least squares means (LSM) of serum anti-SRBC IgG and IgM antibody responses as determined by a direct hem agglutination assay and from the difference between total antibody and IgG titres

## DISCUSSION

### Growth performance

The growth performance of broilers fed from FO were improved probably because of the dietary fat composition; a long-chain n-3 FA that makes it possible to increase diet digestibility and to stimulate growth (Table 2). Dobrzański et al. (2002) reported an increased daily weight gain compared to the control group by adding 2% fish oil to the based diet. Results related to performance parameters in the present experiment are in agreement with the findings of Farhoomand and Chekani-Azar (2009), Newman et al. (1998) and López Ferrer et al. (1999) have reported that the digestibility of fat increases as the degree of unsaturation increases. Therefore, the good performance of FO-fed broilers may be related to the FA composition of the FO.

A low percentage (3–8 grams per kg or less than 1%) of auto oxidized fish oil (Koreleski and Świątkiewicz 2006) or ethyl ester fish oil or glyceryl ester fish oil (Schreiner 2005) leads to an improvement in performance with an increased food consumption rate and high percentage results (2% and 4%) in lower food consumption rate in the final period. Hence, the appropriate proportion of these fatty acids can be effective at different ages of the birds with regard to the appropriate digestion of fat.

Fish oil rich in n-3 FA has been shown to reduce the catabolic response induced by immune stimulation and may be effective in promoting growth (Rymer and Givens 2005). In this regard, the diet containing higher levels of unsaturated FA had an acceptable explanation for the resulting better performance compared to the control diet. However, this advantage is accompanied by sensory losses of the product because diet supplementation with fish oil can affect consumers acceptance probably because of off-flavors and organoleptic problems that might adversely affect meat quality. Hence, Farrell (1995) reported that the removal of FO from all the experiment diets for 1 wk before slaughtering can have a beneficial effect on meat flavor and acceptance without having an effect on the growth performance of the birds (Grashorn 1995). In addition, it is recommended that for n-3 enrichment of broiler meat, it is necessary to ensure the stability of the diet with antioxidants and fortifying vitamin E levels, e.g. 100 IU in broiler diets – relatively high levels can be used (Surai and Spark 2000, Koreleski and Świątkiewicz 2006). The recommended time

for withdrawal of FO from diets is usually after 42 days of age (Farhoomand and Chekani-Azar 2009). Because removing FO from the diet for one week cannot effect broiler performance, the present study was conducted for a 35 days trial period. Fish oil rich in long chain n-3 PUFA is highly susceptible to the oxidation process which may harm human health (Hamilton 1989) and vitamin E is a good defender against lipid oxidation hence, an amount of 1 g/kg of  $\alpha$ -Tocopherol was added to the diet.

From the performance results of the study here presented, it is concluded that, the optimum level of fish oil is 2%. Based on the data presented here, the 2% level of dietary FO was the most efficacious with respect to performance. These studies should provide more insights into the immunomodulatory activities of fish oil and can be used now or in the future for the development of new products with an immune-enhancing ability.

### Blood parameters

Diets containing omega-3 and omega-6 fatty acids decreased the plasma cholesterol and triglyceride levels compared to diets supplemented with saturated fatty acids (Newman et al. 1998). These differences may be due to alteration in the fluidity and composition of the plasma cell walls. By adding fish oil to the broiler diet, the amount of the triglyceride, cholesterol, total protein, albumin, and globulin were decreased and glucose was increased. These changes could be due to fish oil and the enrichment of diet with omega-3 fatty acids (Manilla et al. 1999).

A decrease in the cholesterol and triglycerides content in the serum of birds could result from the presence of polyunsaturated fatty acids from direct deposit from the diet (more likely) or conversion from precursors by *de novo* synthesis (desaturation and elongation) in the liver and tissue. But several factors influence the activities of desaturases and elongases (Cook 1981). Trans fats, saturated fatty acids, and cholesterol interfere with (essential fatty acid) EFA metabolism and promote inflammation, atherosclerosis and coronary heart disease (Lopez-Garcia et al. 2005).

Crespo and Esteve Garcia (2003) have reported that omega-3 fatty acids reduce the very low density lipoprotein (VLDL) levels in the blood, acting to lower the circulating free low density lipoprotein (LDL) concentration and also, to reduce the rate of triglyceride synthesis in the liver. Özdoğan and Aksit (2003) found that

marine and vegetable origins rich in LC-3 PUFA improve animal growth and product quality but a more important result could be lower blood high density lipoprotein (HDL) and LDL values and thus lesser Chol and TG content (Grundy 1991). Researchers have shown that low HDL and high LDL are values associated with atherosclerosis and coronary heart disease (Guyton and Hall 1996, Couderc and Machi 1999, Crespo and Esteve-Garcia 2003).

Diet supplementation with FO increased the blood glucose content (Table 3); the highest value was observed in the group 4% FO ( $T_4$ ). It has also been reported that a diet rich in polyunsaturated fatty acid increases the amount of serum glucose because of the decline in insulin secretion (Grill and Qvigstad 2000, Storlien et al. 2000). Mori et al. (1999) reported that feeding dietary fish and fish oil/meal to human and animals, decreased blood pressure, and the G value was higher ( $P < 0.05$ ). Long chain n-3 enrichment of a high-saturated fat diet exerts a rapid effect to lower insulin secretion from the islets of Langerhans and raising the plasma glucose concentration (Crespo and Esteve-Garcia 2003, Holness et al. 2004).

One of the key reason as to why omega 3 fish oil has such a powerful effect on fat and carbohydrate metabolism is that the secretion of insulin levels can be changed to FO or the omega-3 PUFAs thereof (Crespo and Esteve-Garcia 2003). Insulin is a hormone that reduces the use of fat for fuel while promoting fat storage in the presence of excess calories. It inhibits the action of hormone sensitive lipase, which is responsible for breaking down stored fat and preparing it for use as energy. In addition, insulin activates an enzyme, which, along with fatty acid synthesis, is responsible for converting carbohydrate into fat (Holness et al. 2004). Therefore, the drop in insulin levels when the diet is supplemented with fish oil, allows more fat to be used for energy with an accompanying rise in blood glucose.

The serum TP, A and GL concentrations of the birds fed dietary FO were significantly decreased in comparison with the birds of the control group (Table 3). Touchburn et al. (1981) in a study on glucose/insulin imbalance and dietary protein in broilers reported that diet supplementation with fats rich in PUFA, TP, A and GL levels of serum were lowered. Because fats for transmission in blood must be mixed with proteins in the form of complex compositions of hydrophile lipoprotein and the density of pure lipids is less from water, a decrease in the protein density could be due to an

increased lipid/protein ratio (Tuncer et al. 1987, Olomu and Baracos 1991).

### **Systemic antibody responses to SRBC**

Diet is one of the many factors that affect the immune system of fowls (Klasing 1988, Fritsche et al. 1992). Fish oil containing omega-3 polyunsaturated fatty acids (n-3 PUFAs), a predominant and useful content of oil, especially if be in long chain forms (C20:5n-3, EPA and C22:6n-3, DHA), improves the immune system of birds. Other marine sources of oil (plants or algae which grow in the sea) or of vegetable origin such as linseed and rapeseed oil, are rich in n-3 PUFA, but in lesser ratio than oils from sea fish. Torki et al. (2000) used diets containing identical amounts of protein and energy but using different oil sources such as cotton and fish, to evaluate the immune system of broilers. They reported that the groups fed diets containing 2.25% fish oil showed the highest response against SRBC compared to other groups with lower percentage of fish oil or cotton. It is concluded that the immune system improved, probably because of the effect of fish oil on eicosanoid (leukotriene) and interleukin levels (Kidd 2004).

The chickens fed fish sources rich in n-3 fatty acids did showed an increase in humoral immune activity in response to the injection of sheep red blood cells (SRBC) but stopped lymphocytic proliferation.

The mechanism of altered proliferation probably involves interference of n-3 fatty acids with the metabolism of arachidonic acid. Turnover of membrane phospholipids is early even in T cell activation (Resch et al. 1972). Liberated arachidonic acid can be metabolized to prostaglandin E2 ( $PGE_2$ ) and leukotriene B4 ( $LTB_4$ ) by monocytes. These two metabolites differ in their effect on lymphocyte proliferation (Goodwin et al. 1977).  $PGE_2$  inhibits lymphocyte proliferation, as demonstrated directly by the suppressive action of exogenously added  $PGE_2$  and indirectly by the enhancing effect of cyclooxygenase inhibitors, such as indomethacin (Resch et al. 1972, Goodwin et al. 1977).

The effect of such alteration in the diet is clearer when higher-than-normal levels of immunity are required; it changes the amount of resistance against infectious diseases to a significant extent (Klasing 1998). Because of the antioxidant role of vitamin E for stability of dietary fat, we were compelled to add vitamin E to the diets to attain competent results in our study. Sijben et al. (2002) in their investigation of



interactions of dietary polyunsaturated fatty acids and vitamin E with regard to vitamin E status, fat composition and antibody responsiveness in layer hens, reported that the antioxidant effect of vitamin E for protection from LC n-3 PUFAs of high susceptibility to lipid oxidation, can help to produce an appropriate deposit of EPA and DHA in the body by direct transfer from diet or liver conversion and therefore, a higher immune system response of the body against diseases will actually be achieved.

Fish oil-treated birds had significantly more serum antibody (predominantly immunoglobulin M [IgM]) to SRBC than the birds that were not treated with fish oil. The IgM response to cellular antigen, such as SRBC, was significantly higher in male broiler chickens (Ross308 strain) compared to the IgG response (Figs 1 and 2). The main reason for this phenomenon is not known, but it is conceivable that several parameters such as presence of vitamin E or chicken strain are involved in determining the efficacy of fish oil in the stimulation of the immune response, and as a result, the immune-enhancing effects of fish oils may not be generalized. In chickens, three classes of antibody molecules (immunoglobulin; Ig) have been identified. These are IgM, IgG (IgY), and IgA. Depending on the type and stage of a humoral immune response, different classes of antibodies predominate (Erf and Bottje 1996). The majority of antibodies during a primary immune response are of the class IgM. A switch from IgM to IgY or IgA can be observed towards the end of a primary immune response, but Ig class switching to IgY or IgA is most apparent during a second exposure to the same antigen. Different classes of antibodies have different functional abilities. To mention a few, IgM has the ability to easily agglutinate large antigens and to cause the precipitation of soluble antigens, thus greatly enhancing the immune system's ability to remove antigens through phagocytosis. IgA is found in secretions and functions at mucosal surfaces, and IgY can be transferred from the peripheral circulation of the hen into the egg (maternal antibody). Additionally, the findings support the notion that the immunomodulatory activities of fish oils in enhancing the antibody response are highly dependent on the antigen, immunization regimen, type and number of species of bacteria injected to birds, and the genetic background of the host (Fritsche et al. 1992, Korver and Klasing 1997, Torki et al. 2000).

In conclusion, this study provides evidence that the routine administration of fish oil to broiler

chickens, could enhance antibody responses to cellular antigens, such as SRBC. Dietary fish oil could increase the specific immunity of the body, tends to produce better growth accompanied by an alteration in the selected blood parameters in broiler chicks; so that cholesterol and triglycerides were decreased, glucose content increased significantly and the amounts of total protein albumin and globulin concentrations showed a decline at day 42. Based on the data presented in the current study, the 2% level of dietary FO was the most efficacious with respect to growth performance. These studies may provide more insights into the immunomodulatory activities of fish oil and can be used currently or in the future for the development of new products with an immune-enhancing ability.

## REFERENCES

- Cook HW (1981): The influence of trans acids on desaturation and elongation of fatty acids. *Lipids* 16: 920–926.
- Couderc R, Machi M (1999): Lipoprotein (a): risk factor for atherosclerotic vascular Disease important to take into account in practice. *Ann Biol Clin* 57: 157–167.
- Crespo N, Esteve-Garcia E (2003): Polyunsaturated fatty acids reduce insulin and very low density lipoprotein levels in broiler chickens. *Poult Sci* 82: 1134–1139.
- Dobrzański Z, Jamroz D, Bykowski P, Trziszka T (2002): Effect of fish oil on broiler performance and meat quality. *Electronic J Polish Agric Universities* 1: 43–51.
- Erf GF, Bottje WG (1996): Nutrition and immune function in chickens: Benefits of dietary vitamin E supplementation. In: *Proceedings of the Arkansas Nutrition Conference*, University of Arkansas, Fayetteville, AR, pp. 113–130.
- Farhoomand P, Chekani-Azar S (2009): Effects of graded levels of dietary fish oil on the yield and fatty acid composition of breast meat in broiler chickens. *J Appl Poult Res* 18: 508–513.
- Farrell DJ (1995): The enrichment of poultry products with the omega (n)-3 polyunsaturated fatty acids: a selected review. *Proc Australian Poult Sci Symp* 7: 16–21.
- Fritsche KL, Cassity NA, Huang SC (1992): Dietary (n-3) fatty acid and vitamin E interactions in rats: effects on vitamin E status, immune cell prostaglandin E production and primary antibody response. *J Nutr* 122: 1009–1018.

- Goodwin JS, Bankhurst AD, Messner RP (1977): Suppression of human T cell mitogenesis by prostaglandin. *J Exp Med* 146: 1719–1734.
- Grashorn MA (1995): Instrumental methods for measuring meat quality features. In *Proceedings of the XII European Symposium on Quality Poultry Meat, Zaragoza, Spain, September 25–29*, pp. 489–495.
- Grill V, Qvigstad E (2000): Fatty acids and insulin secretion. *Br J Nutr* 83: 579–584.
- Grundy SM (1991): Multifactorial etiology of hypercholesterolemia. *Arterioscler Thromb* 11: 1619–1635.
- Guyton AC, Hall JE (1996): Metabolism and temperature regulation. In *Textbook of medical physiology*. WB Saunders Company, London, pp. 855–876.
- Hamilton RJ (1989): The chemistry of rancidity in foods. In Allen JC, Hamilton RJ (eds.): *Rancidity in foods*. Elsevier Publishers, London, UK, pp. 1–20.
- Holness MJ, Smith ND, Greenwood GK, Sugden MC (2004): Acute  $\omega$ -3 fatty acid enrichment selectively reverses high-saturated fat feeding-induced insulin hypersecretion but does not improve peripheral insulin resistance. *Diabetes* 53: S166–171.
- Hulan HW, Ackman RG, Ratnayake WMN, Proudfoot FG (1988): Omega-3 fatty acid levels and performance of broilers chickens fed redfish meal or redfish oil. *Can J Anim Sci* 68: 533–547.
- Kidd MT (2004): Nutritional modulation of immune function in broiler. *Poult Sci* 83: 650–657.
- Klasing KC (1988): Influence of acute feed deprivation or excess feed intake on immunocompetence of broiler chicks. *Poult Sci* 67: 626–634.
- Klasing KC (1998): Nutritional modulation of resistance to infectious diseases. *Poult Sci* 77: 1119–1125.
- Koreleski J, Świątkiewicz S (2006): The influence of dietary fish oil and vitamin E on the fatty acid profile and oxidative stability of frozen stored chicken breast meat. *J Anim Feed Sci* 15: 631–640.
- Korver DR, Klasing KC (1997): Dietary fish oil alters specific and inflammatory immune responses in chicks. *J Nutr* 127: 2039–2046.
- López-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA (1999): N-3 enrichment of chicken meat using fish oil: Alternative substitution with rapeseed and linseed oils. *Poult Sci* 78: 356–365.
- Lopez-Garcia E, Schultze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, Willett WC, Hu FB (2005): Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* 135: 562–566.
- Manilla H, Husveth AF, Nemeth K (1999): Effects of dietary fat origin on the performance of broiler dietary fat origin on the performance of broiler. *Acta Agr Kaposvar* 3: 47–57.
- Mori TA, Michael O, Michael JM (1999): Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. *Am J Clin Nutr* 70: 817–825.
- National Research Council (1994): *Nutrient requirements of poultry*. Natl Acad Press, Washington, DC.
- Newman RE, Downing JA, Bryden WL, Fleck E, Buttemer WA, Storlien LH (1998): Dietary polyunsaturated fatty acids of the n-3 and the n-6 series reduce abdominal fat in the chicken (*Gallus domesticus*). *Proc Nutr Soc Aust* 22: 54.
- Olomu JM, Baracos VE (1991): Influence of dietary flax- seed oil on the performance, muscle protein deposition, and fatty acid composition of broiler chicks. *Poult Sci* 70: 1403–1411.
- Özdoğan M, Aksit A (2003): Effects of feeds containing different fats on carcass and blood parameters of broilers. *J Appl Poult Res* 12: 251–256.
- Resch K, Gelfand EW, Hansen K, Feber E (1972): Lymphocyte activation. Rapid changes in the phospholipids metabolism of plasma membranes during stimulation. *Eur J Immunol* 2: 598–601.
- Rymer C, Givens DI (2005): N-3 fatty acid enrichment of edible tissue of poultry: A review. *Lipids* 40: 121–130.
- Schreiner M, Hulan HW, Razzazi-Fazeli E, Bohm J, Moreira R (2005): Effect of different source of dietary omega-3 fatty acid on general performance and fatty acid profiles of thigh, breast, liver and portal blood of broilers. *J Sci Food Agri* 85: 216–226.
- Sijben JW, Schrama JW, Nieuwland MG, Hovenier R, Beynen AC, Versteegen MW (2002): Interactions of dietary polyunsaturated fatty acids and vitamin E with regard to vitamin E status, fat composition and antibody responsiveness in layer hens. *Br Poult Sci* 432: 297–305.

- Storlien LH, Higgins JA, Thomas TC, Brown MA, Wang HQ, Huang XF, Else PL (2000): Diet composition and insulin action in animal models. *Br J Nutr* 83: 85–90.
- Surai PF, Spark NHC (2000): Tissue-specific fatty acid and tocopherol profiles in male chickens depending on dietary tuna oil and vitamin E provision. *Poult Sci* 79: 1132–1142.
- Torki M, Golian A, Arshami J, Tavakkoli J (2000): Effect of dietary fat source and fatty acid composition on immune responses of male growing broiler chicks. *Poult Sci* 79: 105.
- Touchburn S, Simon J, Leclercq B (1981): Evidence of glucose-insulin imbalance and effect of dietary protein and energy level in chickens selected for high abdominal fat content. *J Nutr* 111: 325–335.
- Tuncer SD, Asti R, Coskun B, Tekes MA, Erer H (1987): The effects of different energy sources on fattening performance, abdomen fat accumulation and liver fat in broiler I. The effects of fattening performance and abdomen fat accumulation. *J Vet Fac* 3: 25–40.