The effect of diet that contained fish oil on performance, serum parameters, the immune system and the fatty acid composition of meat in broilers.

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(Received 28 July 2008, Accepted 17 March 2009)

Abstract: A study with a duration of 42 days was conducted to evaluate the influence of fish oil inclusion on performance parameters,serum lipid content, antibody responses to sheep red blood cells (SRBC) and the composition of antigen and meat fatty acids in broilers.Two hundred and sixteen 1-day-old broiler chicks from a commercial hybrid (Cobb 500) were allocated randomly to four groups, which received feed supplemented with 1.5%, 3.0% and 6% fish oil or feed that was not supplemented (control group). The differences among the groups were significant with regards to their performance, so that a low level of fish oil (1.5%) led to higher feed intakes and an improvement in the efficiency of feeding in comparison to the control group ($p<0.01$). The results of the omega-3 fatty acid evaluation indicated significant differences among groups ($p<0.01$) and the birds in the 6% fish oil-fed group had the highest level of n-3 fatty acid in their meat. The n-6:n-3 ratio of polyunsaturated fatty acids was lower in the fish oil-fed groups compared to the control group ($p<0.01$). Broilers that were fed with diets rich in omega-3 fatty acid had higher levels of anti-SRBC titers and lower levels of serum cholesterol and triglycerides than those fed with the control diet ($p< 0.05$).

Keywords: Performance, immune response, broiler meat, omega-3 fatty acid, serum lipid content.

Introduction

Feeding poultry with diets that contain fat can confer several economic advantages by providing increased energy levels and fatty acid composition (Newman *et al*., 2002). If the poultry is expected to show high performances, their high energy and protein needs should be provided through their feed. Providing their high energy needs requires the use of different fat sources (López-Ferrer *et al*., 2001; Sanz *et al*., 2000). If diets with similar energy and protein are compared, chickens fed with rations that contain oil showed better performances than birds fed diets without the inclusion of oil (Moura, 2005).

It has been demonstrated that the fatty acid (FA) composition of broiler meat can be altered by the type of the fatty acid (FA) content in their diet (Yau *et al*., 1991). Dietary long-chain n-3 polyunsaturated fatty acids (LC-PUFA; n-3), eicosapentanoiec acid (EPA; 20:5) and docosahexanoeic acid (DHA; 22:6) have been reported prevent cardiovascular disease, improve the immune response, and reduce the serum cholesterol concentration (Simopoulous, 1991). The differential action of n-6 PUFA as a pro-inflammatory factor and n-3 PUFA as an anti-inflammatory factor in animals and humans has been reported due to the effects of an increase in the concentration of n-3 fatty acid and a consequent decrease in the n-6:n-3 ratio in poultry meat (Calder, 2001). Newman *et al*., (2002) showed that dietary PUFA reduced plasma triglycerides (TG) and

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cholesterol (CHL) in broiler chickens when compared to groups fed with saturated FA. Omega-3 FA suppresses the synthesis of triglycerides and apolipoprotein B, increases the removal of very low density lipoprotein (VLDL) by peripheral tissues or the liver, and increases the excretion of bile in feces (Leaf and Weber, 1988), which can also reduce the serum concentrations of cholesterol and triglycerides.

In general, fish oils are rich sources of omega-3 FA and poor sources of omega-6, and the contents of linoleic acid (LA) are also low. The FA profile of the different oils varies with the time of year, the processing method and the predominant fish species from which they were extracted (Alparsan *et al*., 2005). Many studies have examined the effects of dietary LC-PUFA, supplied as fish oil or fish meal, on the FA composition of the broiler carcass (Nash *et al*., 1995 and Scaife *et al*., 1994; Lopez-Ferrer *et al*., 1999, 2001).

Fish oil has several positive effects, such as the physiological or metabolic effects of due to LC-PIFA on the performance parameters of broiler chickens. The role of omega-3 FA on the health of humans and animals (Pike, 1999), the effect of animal products that contain omega-3 on human health (López-Ferrer *et al*., 1999, 2001), and a comparison of the effects of unsaturated and saturated diets on performance (Alparsan *et al*., 2005) are all topics of intense further research.

The objective of this study was to investigate the effects of diet that contained 1.5%, 3% and 6% fish oil on the body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), mortality, blood factors, immune system and FA composition of the meat of broilers.

Materials and Methods

Birds and diets

Two hundred and sixteen Cobb 500 1-day- old male and female chicks were weighed individually. Eighteen chicks (nine males and nine females) were placed randomly into 12 floor plans. Birds were housed in deep litter pens (1×2 m^2). The birds were housed in an environmentally-controlled room and they had free access to feed (mash) and water *ad libitum*. The room temperature was set at 32°C on Day One, which was lowered in a stepwise manner to 23-24°C for rest of the experiment. There were four dietary treatments, which were carried out in triplicate. Birds were given the starter diet for the first two weeks (starter period), the grower diet between the third and fourth weeks, and the finisher diet on the fifth and sixth weeks of experimental period (42 days in total). The diets were formulated according to the recommendations of the National Research Council (NRC, 1994). Diets were calculated to be isocaloric and isonitrogenous. The fish oil was provided by Mehreghan Khazar (Rasht, Iran). The fish oil was used at levels of 1.5% (O_1 diet), 3% (O_2 diet) and 6% (O_3 diet; Table 1). The results were compared to the chicks that were fed with an diet that was not supplemented with fish oil (O_0 diet, the control group).

Table1: Ingredients and compositions of the basal diets from 0 to 6 weeks of age

Ingredients	(%)	Calculated nutrient content	
Corn	56.9	Dry matter (%)	89.03
Soybean meal	33.5	Crude fat (%)	6.1
Corn gluten meal	2.9	Crude protein (%)	21.56
Limestone	0	ME, kcal/kg	3000
Animal Fat	2.75	Calcium	1.02
Fish oil	0	Available P	0.45
oyster	1.1	Methionine + Cysteine	0.86
DCP	2	Lysine	1.15
Salt	0.3		
Vitamin premix[1]	0.25		
Vitamin mineral[2]	0.25		
Lysine-L	0.01		
Methionine-D-L	0.1		

[1]Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7mg Cu, 0.4 mg Co, 0.17 mg
Se, and 0.75 mg I.

[2] 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

Performance parameters and blood factors

Feed intake (FI) and the feed conversion ratio (FCR) were calculated in each pen on a weekly basis. The average body weight (BW), a daily body weight gain (BWG), and the FCR were determined over the overall experiment. Mortality was recorded a daily basis, and FI data were corrected for body weight of dead birds. Blood samples were taken randomly from the wing vein of three birds in each pen on the 27th and 41th day of age. The blood samples were centrifuged at 4000 rpm for five minutes, and the serum was collected for analysis. The concentration of TG and CHL were determined with the use of spectrophotometry by commercial enzymatic kits (Pars Azmun Commercial Kit, Iran).

Humeral immune response to SRBC

Nonpathogenic antigens of sheep red blood cells (SRBC) were used to monitor the immune response of chickens. The anti-SRBC titers for total antibodies were measured by a hemagglutination test, according to Boa-Amponsem *et al*. (2001).SRBC were washed three times in phosphate buffered solution (PBS) and diluted in PBS to 5% (vol/vol).Three chicks per replicate were immunized with 0.1 ml of a 0.5% suspension of SRBC via the brachial vein at the 21st and 35th day of age. Antiserum caused by SRBC was collected six days after the immune challenge in 27 and 42 days of age.

Fatty acid content

On the 42nd day of age, one male and one female from each pen (six birds per treatment) were euthanized humanely by cervical dislocation. The carcass samples from each bird were mixed and packed in plastic bags (approximately 20 gram per bag) and immediately stored at -20°C for three months. The total lipids of diets and tissues were extracted following the method of Folch *et al.* (1957). The FA compositions of diet and tissue samples were determined by gas chromatography following the method of Metcalf *et al*. (1996). The lipid composition was determined by gas chromatography in a UNICAM system equipped with a BPX70 fused silica capillary column film (SGE capillary column: length 30 m, internal diameter 0.22 mm; 70% cyanopropyl polysilphenylene-siloxane stationary phase) and a flame ionization detector. The operating conditions of the gas chromatograph were as follows: the initial temperature was 160°C, which increased by 40°C/min to 180°C; after ten minutes, the temperature was increased at the rate of 20°C/min. The temperature of the injector was 240°C and the detector remained stable at 280°C. Each FA was identified in the form of a methyl ester by comparing the retention times with the internal standard (pentadecanoic acid, Merck) (Lopez-Bote *et al*., 2000).

Statistical analysis

Data were analyzed in a completely randomized design in triplicate, using the procedures described by the SAS Institute (1999). Significant differences among treatments were determined according to the General Linear Model (GLM) procedure. Means were compared by using Duncan's multiple-range test and significance was determined when the p-value was less the 0.05. The following model was used: $X_{ij} = \mu + \tau_j + \varepsilon_{ij}$ where: X_{ij} = is the observation of jth treatment on ith pen; μ = the overall means of the sampled observation; τ_j = is the effect of treatment; ε_{ij} = the experimental error component.

Results

The effects of different fish oil levels on broiler performance are summarized in Table 2. The results showed that the group with 1.5 % fish oil had the best values for FCR, BW and BWG; this level of supplementation increased the FI and therefore improved the BWG and FCR. A significant reduction in FI, BW and BWG were observed when the diet was supplemented with the highest level of fish oil (6%). Low levels of fish oil in the diet improved the productive parameters in broilers.

The antibody titer against SRBC (Table 3) was affected significantly by the dietary

treatments. The fish oil groups had higher antibody titers compared to control group. Based on the results of this study, the lowest antibody titers in the primary responses were found to be 4.17 in the control group, whereas the highest one was 5.00 in group O_3. The lowest antibody titers in the secondary responses were found to be 4.33 in the control group, while the highest titer was 7.00 in group O_2. In conclusion, antibody production against SRBC of chicks fed with a diet that contained a low level of fish oil was superior to those of chicks fed by a diet with high level of fish oil and the control group ($p<0.01$).

Broilers that were fed with fish oil had lower levels of CHL and TG concentrations compared with those fed with the control diet (Table 4).

The fish oil used in this study was a rich source of LC PUFA; n-3 and contained EPA (14.55 mg/g) and DHA (5.74 mg/g). The FA composition of the experimental diets is shown in Table 5.The FA composition of diet, particularly long-chain n-3 PUFA, was modified according to the level of fish oil added to the feed ($O_0<O_1<O_2<O_3$). The total n-3, PUFA and monounsaturated FA (MUFA) increased with increasing levels of fish oil in the diet, and the ratio of n-6:n-3 and saturated FA (SFA) also decreased.

The values of EPA, DHA, LNA and n-3 PUFA were significantly higher ($p<0.01$) in the meat of broilers fed with fish oil compared to the control group. The birds in the 6% fish oil fed group had the highest values of these parameters.

As showed in Table 6, the levels of LA and long-chain n-6 PUFA were not significantly in the meat of broilers fed with fish oil diets compared with the control diet. The decrease in the total level of MUFA and the total SFA content in meat was not statistically significant (Table 6).

Discussion

These findings were in agreement with previous results reported by Alparsan *et al*. (2005) and Koreleski and Swiatkiewiecz, (2006). Hulan *et al.* (1988) reported that 6% fish oil in the diet caused

Table 2: Performance parameters of chicks according to different amount of fish oil in diet (Day 1 to 42)

Variable	O_0	O_1	O_2	O_3	P	SEM
Feed intake, g per bird per day	99.73^{a}	99.94^{a}	98.11^{ab}	95.66^{b}	0.01	0.66
Weight Gain, g per bird per day	55.29^{b}	57.98^{a}	55.96^{b}	54.54^{b}	0.005	0.39
Feed efficiency, g:g	1.79^{a}	1.71^{b}	1.74^{b}	1.75^{a}	0.005	0.01
Final weight, kg per bird	2.32^{b}	2.43^{a}	2.355^{ab}	2.290^{b}	0.002	0.15
Mortality (%)	3.92	1.96	5.88	7.69	NS	0.38

^{a,b}Value in the same row with no common superscript are significantly different ($P< 0.05$)

Table 3: Total anti-SRBC (1/log 2) titre

Treatment	27d of age[1]	42d of age[1]
O_0	1.22^{b}	4.33^{b}
O_1	3.53^{ab}	6.33^{ab}
O_2	2.66^{ab}	7.00^{a}
O_3	5.00^{a}	5.27^{ab}
Significance	0.01	0.001
SEM	0.43	0.15

[1]the values are means of triplicate per replicate determinations
^{a,b}Value in the same column with no common superscript are significantly different ($P< 0.05$).

Table 4: Lipid content of serum (mg/dl)

	CHL[1]		TG[1]	
Treatment	TCOL1	TCOL2	TG1	TG2
O_0	114.74^{a}	114.17^{a}	114.31^{a}	112.14^{a}
O_1	114.98^{ab}	103.09^{b}	113.16^{a}	100.73^{a}
O_2	113.58^{ab}	98.29^{b}	96.46^{b}	95.90^{ab}
O_3	111.96^{b}	95.38^{b}	93.45^{b}	92.31^{b}
Significance	0.01	0.01	0.001	0.01
SEM	0.55	2.53	1.21	2.28

[1]the values are means of triplicate per replicate determinations
^{a,b}Value in the same column with no common superscript are significantly different ($P< 0.05$).

T**able 5:** Fatty acid composition of diets (mg/g diet)[1,2]

Treatment	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C20:4	EPA	DHA	SFA[3]	MUFA	PUFA	n-3	n-6	n-6/n-3
O_0	1.39	32.16	1.13	15.98	25.44	24.85	1.8	trace	0.8	0	49.54	26.57	26.97	1.88	24.85	13.21
O_1	1.79	31.29	2.94	12.9	25.37	24.91	1.94	0.10	1.27	2.57	44.98	29.31	29.79	5.78	25.01	4.38
O_2	2.25	28.78	4.37	13.5	24.58	22.32	2.45	trace	1.64	3.11	44.54	28.95	32.69	7.20	22.32	3.23
O_3	3.38	27.40	5.84	7.96	25.85	19.58	2.2	0.11	2.95	7.85	38.75	31.69	32.85	12.99	19.67	1.61

[1]the values are means of triplicate determinations.
[2]fish oil was provided by Mehreghan Khazar, Rasht, Iran.
[3]SAT=saturated fatty acid, MUFA=Mono unsaturated fatty acid,PUFA=Poly unsaturated fatty acid

Table 6: Fatty acid composition of meat broiler lipids (g/ 100g of meat)

Treatment	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C20:4	EPA	DHA	SFA[3]	MUFA	PUFA	n-3	n-6	n-6/n-3
O_0	1.95	24.77	12.52	5.16	45.35	11.30	0.58^{b}	0.11	0.09^{c}	0.06^{c}	31.87^{a}	57.87	12.15	0.73^{c}	11.41	16.16^{a}
O_1	1.93	22.77	11.57	5.08	44.79	11.06	0.60^{ab}	0.13	0.59^{ab}	1.1^{ab}	29.87^{a}	56.39	13.49	2.30^{b}	11.19	4.97^{b}
O_2	1.40	22.40	11.50	5.02	42.58	12.91	1.37^{ab}	0.14	1.57^{ab}	2.95^{ab}	28.09^{a}	54.08	19.00	5.90^{b}	13.10	2.22^{b}
O_3	1.07	20.07	9.28	4.90	40.70	12.93	1.39^{ab}	0.18	1.80^{ab}	3.64^{ab}	26.05^{ab}	50.06	19.94	6.83^{a}	13.11	1.87^{b}
SEM	0.12	0.37	0.03	0.77	0.12	0.43	0.02	0.01	0.13	0.45	0.55	1.11	1.12	0.26	0.80	1.78
Significance	NS	NS	NS	NS	NS	**	**	NS	**	**	NS	NS	**	**	NS	**

[a-c]Values in the same column with no common superscript differ significantly.
$P > 0.05$, $*P \leqslant 0.05$, $**P \leqslant 0.01$

adverse effects on the productive parameter in broilers. The manipulation of the PUFA to SFA ratio can result in a better absorption of these FA in intestine, and so the addition of PUFA to animal fat (as SFA) improves the apparent metabolic energy value in the fat mixture. The lower levels of performance parameters in the 6% fish oil group were likely to be caused by an increasing sensitivity to the fishy smell in the diet and a consequent decrease in FI. As has already been observed (Phettleplace and Watkins, 1992), the inclusion of fish oil in the diets did not cause any increase in the mortality rate compared with the control diet.

The level and sources of n-3 PUFA had effects on antibody production in chicks. The long chain FA n-3 PUFA (EPA and DHA) has the ability to increase the immune response (Calder, 2001). The results of this study were in accordance with the results of previous findings suggested that a low level of fish oil in the diet can cause an efficient improvement in broiler immunity and the antibody production of broilers (Torki *et al*., 2000; Kidd, 2004).

The results of blood factors in this study agreed with the results of Newman *et al*., (2002). Celebi and Utlu (2006) showed that the lipid content of serum reduced as the levels of dietary PUFA increased. However, in opposite results to our findings, Alparsan *et al*. (2005) showed that the level of serum CHL was not affected noticeably by dietary rich fish oil. The discrepancies between studies on the lipid content of serum may be attributed to the genetic, sex and dietary factors. In general, the best results for CHL and TG values were seen in the groups that contained fish oil.

As a consequence of adding fish oil to the diet, after a three month extended period of storage, the FA profile of lipids from the meat of broilers was significantly changed in relation to some of the FA

(Table 5). The FA composition of chick tissue generally reflected the FA profile of the diet.

A number of studies have examined the effects of dietary LC PUFA, such as those contained in fish oil or fish meal, on the FA composition of the broiler carcass (Phettleplace and Watkins 1992; Lopez-Ferrer *et al*., 1999, 2001). Supplementation of the basal diet with 1.5%, 3.0% and 6% fish oil caused 3, 14 and 60 folds increase in levels of LNA, EPA and DHA, respectively, in the meat of broilers compared to the control group. The increased deposition of n-3 PUFA in the birds that were fed with fish oil has been observed by Schreiner *et al*. (2005) and Cortinas *et al*. (2004).

LA and n-6 PUFA decreased with increasing level of fish oil in diet. This contradictory between fatty acid of tissue and diet could be explained by competing both LNA and LA for enzyme system responsible for their elongation and desaturation to form the long-chain metabolites.

The inclusion of fish oil in diets compared to the control diet significantly reduced the n-6: n-3 ratio of the meat of broilers ($p<0.01$). These findings are similar to the results reported by Gonzalez-Esquerra and Leeson (2000) and Schreiner *et al*. (2005). This effect resulted from existence of higher levels of n-3 and decreasing levels of n-6 in the broiler meat.

The concentration of MUFA found in meat decreased as the level of dietary n-3 PUFA increased. As suggested by Ayerza *et al*. (2002), the decrease in oleic and palmitoleic acids could be related to the inhibitory effect of PUFA against the activity of Δ9-desaturase, which prevents the formation of MUFA from their precursors. Δ-9 desaturase is the key enzyme needed to convert palmitic to palmitoleic acid and stearic to oleic acid. This interaction between MUFA and PUFA has also been reported in other animals (Ayerza *et al*., 2002).

In conclusion, the results of this study showed that the supplementation of feeds with fish oil at 1.5% improved performance parameters in broiler chickens. The supplementation of 3% fish oil in the diet improved the level of n-3 FA and the immune system, and decreased the levels of CHL and TG in the plasma of broilers. The highest level of fish oil (6%) in the diet had adverse effects on the performance parameters, which may be due to the unpalatable smell of fish in the feed.

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