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Fatty Acids Profiles in Meat of Broiler Chicks Fed Diet Containing Corn Oil Switched to Fish Oil at Different Weeks of Age

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Abstract: The aim of present study was to determine the optimal period of fish oil inclusion in diet to access suitable n-3 fatty acids content in broiler meat. A total of 600 one-day old chicks were randomly allocated to six groups and reared for seven weeks. The control group fed a diet containing 5% corn oil and in the other five experimental groups, fish oil was substituted corn oil from 2, 3, 4, 5 and 6th weeks of age. The addition of fish oil to diet resulted in significantly increased the concentration of n-3 fatty acid ($P<0.01$) in the form of linolenic acid (LNA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in the breast and thigh of chicks. Saturated fatty acid (SFA) significantly increased by consumption of fish oil in the diet ($P<0.05$) and monounsaturated fatty acid (MUFA) were significantly decreased ($P<0.05$). Duration of consumption of diet containing fish oil had no significant effect on concentration of n-3 fatty acid in meat. According to these results, addition of fish oil to broiler chicken diet at two final weeks of age could increase n-3 fatty acid level in meat.

Key words: Broiler chicks · Fatty acid profile · Fish Oil · Breast · Thigh · Consumption duration

INTRODUCTION

Lipids are commonly added to broiler diets as an economic means of producing energy-rich formulations. The composition of fatty acids in the broiler meat can be changed by including vegetable oil, fish oil or animal fat to diet [1], because a difference of fatty acids profile and a reduction in endogenous produced fatty acids occurs. Fish oils contain high levels of n-3 fatty acid named linolenic acid (C18:3n-3, LNA) that converted in the body to other n-3 fatty acids such as eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA), which has shown to reduce the risk of cardio-vascular diseases in humans [2]. Various studies have been conducted on the inclusion of fish oil in the diet of broilers [3-5], pigs [6, 7] and beef [8] in order to manipulate the lipid composition of the meat. In mentioned studies, fish oil was found to have had a positive effect on the fatty acid profile.

In the literature, minimum duration time of feeding diet containing fish oil to broiler chicks to have meat rich in beneficial fatty acids is not clear. Therefore, the objectives of this study were to determine the minimum duration time in which poultry meat can be enriched with polyunsaturated n-3 fatty acids supplied from fish oil.

MATERIAL AND METHODS

Animals, Diets and Experimental Design: Six hundreds 1-d-old broiler chickens of Ross 308 strain were obtained from a commercial hatchery and raised in 24 pens with 25 chicks per pen at Poultry Research Station of Baft-IAU (Kerman, Iran). On day 7 of age, the number of birds per pen and the average live weight per pen were balanced. Four pens were assigned to each of six treatments at a completely randomized design. Throughout the study, feed and water were provided for *ad libitum* consumption. Starter (1-10 days), grower (11-28 days) and finisher (29-49 days) diets were formulated based on corn-soybean meal presented in Table 1. Experimental diets were formulated to be isoenergetic and isonitrogenous. Broiler chicks were fed a corn oil-based control diet and the resulting chicks were fed a similar diet, but then as shown in Figure 1 switched to a fish oil-based diet at 2, 3,

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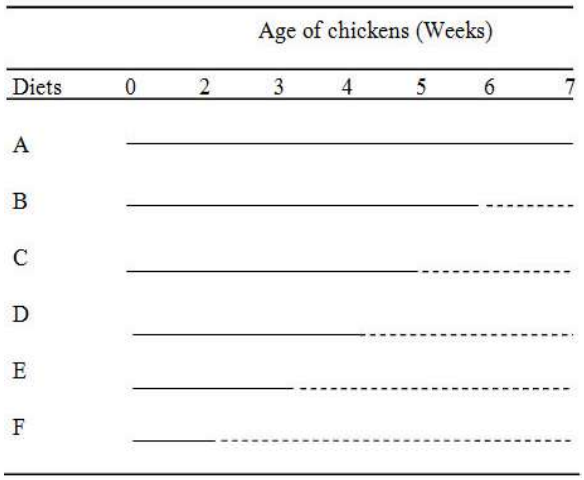

Fig. 1: Duration of corn oil (solid line) and fish oil (dash line) addition to diets.

Table 1: Ingredients and chemical composition of experimental rations. a

Finisher	Starter	Grower	Finisher
Corn	38.85	49.04	63.86
Soybean meal (44%)	36.51	31.38	27.4
Wheat	15.0	10.5	---
Corn oil or fish oil	5.0	5.0	5.0
Oyster shell	1.38	1.43	1.3
Dicalcium phosphate	1.66	1.15	1
Vitamin premix[b]	0.6	0.6	0.6
Mineral premix[c]	0.6	0.6	0.6
Common salt	0.2	0.2	0.2
DL-Methionine	0.2	0.1	0.04
Ration components			
ME, kcal/kg	2900	3000	3100
Crud protein (%)	21.5	19.5	17.5
Calcium (%)	1.0	0.9	0.8
Available P (%)	0.45	0.35	0.3
Lysine (%)	1.1	1	0.85
Methionine (%)	0.5	0.38	0.32

[a] On an as-fed basis

[b] Vitamin content added to diet provided amounts of: vitamin A 7040 IU, vitamin D_3 2000 IU, vitamin E 8.8 mg, vitamin K_3 76.1 mg, vitamin B_1 2.1 mg, vitamin B_2 2.3 mg, vitamin B3 4.6 mg (calcium pantotenate), vitamin B_5 28 mg (niacin), vitamin B_6 97.1 mg, vitamin B_9 38 mg (folic acid), vitamin B_{12} 8 mg, vitamin H_2 12 mg (biotin) and Cholin chloride 320 mg per kg of diet.

[c] Mineral content added to diet provided amounts of manganese 60 mg, iron 60 mg, zinc 74.51 mg, copper 8.4 mg, iodine 69 mg and selenium 16 mg per kg diet.

4, 5 or 6 weeks of age, respectively. Lighting schedule were 23L/1D while the temperature was gradually reduced 3°C from initially 32°C in each week to reach 24°C.

Growth Performance: Daily feed intake and weight of chickens was measured at the experiment with a digital scale with preciseness of 2 g. According to total feed intake and weight gain, total feed conversion coefficient was determined.

Sample Collection: On day 49 of age, a total of 24 birds (8 per treatment; two per pen which their weights were about treatment mean) was randomly selected, individually weighed, stunned, killed by cervical dislocation and plucked in slaughterhouse. From each sample, one thigh and half a breast were taken after separating skin and bones, then using a mincing machine, they were minced for harmony. For analysis of fatty acid compositions, samples (10 g) from thigh and breast were collected separately and stored in plastic plates in temperature of -20°C until start of analysis.

Analysis: Extraction of fatty acids of samples of oil, food and meat was made through Metcalf *et al* (9) and detected by gas chromatography system model Unicam 4600 (Unicom company, NY, USA).

Statistical Analysis: The chicken (8 determinations per diet) was the experimental unit for organ weight and fatty acid profile. All values were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 (SAS Institute Inc. Cary, NC). When the *F*-test for treatments was significant at $P<0.05$ in the ANOVA table, means were compared for significant differences using the Tukey test of SAS.

RESULTS

Effect on Performance Parameters: Effects of experimental diets on performance parameters are in Table 2. Feeding broilers with diets containing fish oil at different duration periods has no effect ($P > 0.05$) on body weight. There were no significant differences among treatments for average daily gain, daily feed intake and feed conversion ratio at total period.

Effect on Fatty Acids Profile of Breast: The effect of experimental groups on breast fatty acid profile is in Table 3. The differences among treatments on concentration of $C_{16:0,}$ $C_{16:1}$ $C_{18:0}$ $C_{18:1}$ $C_{18:3}$ and total saturated fatty acids (SFA) and poly unsaturated fatty acids (PUFA) of breast was not significant ($P>0.05$), while on the concentration of $C_{14:0}$, $C_{18:2,}$ arachidonic acid (AA), EPA, DPA, DHA, total mono unsaturated fatty acids, total n-3 and n-6 fatty acids and n-6 to n-3 ratio was significant ($P<0.01$).

Table 2: Carcass efficiency and performance parameters of broilers fed experimental diets.

Treatments	Body weight (g)	Daily weight gain (g)	Daily feed intake (g)	Feed efficiency
A	1869.9	37.35	77.70	2.09
B	1861.6	37.18	75.56	2.04
C	1912.6	38.22	76.56	2.01
D	1832.6	36.58	77.21	2.12
E	1943.2	38.84	76.44	1.99
F	2016.5	40.34	75.30	1.87
SEM	30.19	0.62	0.49	0.03
P value	0.58	0.58	0.74	0.35

*SEM: standard error of the means

[a,b] Means in the same column without superscripts are not differ (P >0.05). Treatments: A, chicks consumed corn oil as control and chicks in other groups fed fish oil instead of corn oil from 6 (B), 5 (C), 4 (D), 3 (E) and 2 (F) week of age.

Table 3: Fatty acids profile of breast (mg/g)

Treatments	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	AA	EPA
A	0.04[a]	1.35	0.14	0.49	1.45	1.20[a]	0.02	0.28[a]	0.003[b]
B	0.07[ab]	1.45	0.23	0.40	1.58	0.63[b]	0.02	0.06[c]	0.07[a]
C	0.09[a]	1.54	0.25	0.46	1.60	0.65[b]	0.03	0.07[c]	0.07[a]
D	0.07[ab]	1.44	0.22	0.46	1.50	0.63[b]	0.03	0.08[c]	0.07[a]
E	0.05[ab]	1.25	0.16	0.42	1.21	0.53[b]	0.02	0.11[bc]	0.06[a]
F	0.07[ab]	1.57	0.23	0.45	1.61	0.82[ab]	0.03	0.14[b]	0.06[a]
SEM	0.004	0.06	0.02	0.02	0.10	0.06	0.002	0.02	0.006
P value	0.04	0.73	0.41	0.82	0.87	0.01	0.38	0.0001	0.0001
Treatments	DPA	DHA	SFA	MUFA	N6	N3	PUFA	N6/N3	P/S
A	0.01[b]	0.02[b]	1.87	1.33	1.48[a]	0.06[b]	1.54	33.00[a]	0.80[a]
B	0.06[a]	0.28[a]	1.92	0.86	0.69[b]	0.44[a]	1.12	1.54[b]	0.58[b]
C	0.07[a]	0.30[a]	2.08	0.90	0.72[b]	0.47[a]	1.19	1.79[b]	0.57[b]
D	0.06[a]	0.26[a]	1.98	0.85	0.71[b]	0.41[a]	1.12	1.82[b]	0.57[b]
E	0.05[a]	0.27[a]	1.72	0.69	0.64[b]	0.39[a]	1.03	1.67[b]	0.61[b]
F	0.05[a]	0.26[a]	2.10	1.06	0.97[ab]	0.40[a]	1.36	2.44[b]	0.66[b]
SEM	0.004	0.02	0.08	0.07	0.08	0.03	0.07	2.81	0.02
P value	0.0001	0.0001	0.79	0.12	0.002	0.0001	0.27	0.0001	0.0001

*SEM: standard error of the means

[a,b,c] Means in the same column with different superscripts are differ (P <0.05).

Treatments: A, chicks consumed corn oil as control and chicks in other groups fed fish oil instead of corn oil from 6 (B), 5 (C), 4 (D), 3 (E) and 2 (F) week of age.

P/S, Polyunsaturated to saturated fatty acid ratio

Table 4: Fatty acids profile of thigh (mg/g)

Treatments	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	AA	EPA
A	0.09[b]	4.22[ab]	0.80[b]	0.94[b]	6.11	4.66[a]	0.08[b]	0.36[a]	0.01[d]
B	025[b]	4.46[ab]	1.24[ab]	1.08[b]	5.82	2.03[bc]	0.12[ab]	0.11[c]	0.26[ab]
C	0.19[b]	3.08[b]	0.73[b]	0.78[b]	3.65	1.46[bc]	0.06[b]	0.11[c]	0.16[bc]
D	0.54[a]	7.26[a]	2.20[a]	1.92[a]	10.0	4.36[ab]	0.24[a]	0.19[bc]	0.39[a]
E	0.19[b]	3.64[ab]	0.73[b]	1.05[b]	4.58	2.42[abc]	0.10[b]	0.20[bc]	0.17[bc]
F	0.20[b]	4.51[ab]	1.02[b]	0.92[b]	6.04	3.23[abc]	0.12[ab]	0.22[b]	0.10[cd]
SEM	0.03	0.41	0.14	0.09	0.69	0.37	0.02	0.02	0.03
P value	0.0001	0.04	0.005	0.0006	0.12	0.05	0.01	0.0001	0.0001
Treatments	DPA	DHA	SFA	MUFA	N6	N3	PUFA	N6/N3	P/S
A	0.01[b]	0.02[b]	5.25[b]	5.46	5.02[a]	0.12[c]	5.14	45.86[a]	0.96[a]
B	0.13[a]	0.64[a]	5.79[b]	3.27	2.14[bc]	1.15[ab]	3.28	1.87[b]	0.58[b]
C	0.09[a]	0.50[a]	4.05[b]	2.19	1.57[c]	0.80[b]	2.37	1.93[b]	0.59[b]
D	0.16[a]	0.76[a]	9.71[a]	6.56	4.54[ab]	1.53[a]	6.07	2.83[b]	0.65[b]
E	0.10[a]	0.53[a]	4.88[b]	3.15	2.61[abc]	0.88[b]	3.49	2.93[b]	0.72[b]
F	0.09[b]	0.46[a]	5.62[b]	4.25	3.44[abc]	0.77[b]	4.22	4.32[b]	0.72[b]
SEM	0.01	0.06	0.51	0.48	0.38	0.10	0.41	3.47	0.03
P value	0.0004	0.0001	0.009	0.06	0.04	0.0001	0.09	0.0001	0.0001

*SEM: standard error of the means

[a,b,c] Means in the same column with different superscripts are differ (P <0.05).

Treatments: A, chicks consumed corn oil as control and chicks in other groups fed fish oil instead of corn oil from 6 (B), 5 (C), 4 (D), 3 (E) and 2 (F) week of age.

P/S, Polyunsaturated to saturated fatty acid ratio

The chickens fed oil had the greatest concentrations of 14:0, 15:0, 17:0, 20:ln-9, 20:5n-3, 22:5n-3 and 22:6n-3. Chickens fed fish oil had lower levels of 20:4n-6 compared with those levels in chickens fed corn oil. The group fed corn oil had the most 18:2n6, while chickens fed chicken fat had the greatest levels of 18:ln-9, 20:2n-6, 20: 3n-6, 20:4n-6 and 22:4n-6 in muscle tissues.

There were no differences among chicks fed fish oil for most fatty acids profile or their ratio at different duration of inclusion.

Effect on Fatty Acids Profile of Thigh: Effect of experimental groups on pattern of fatty acids of thigh has been shown in Table 4. The effect of treatments on concentration of all fatty acids and calculated parameters was significant. Concentration of $C_{18:3}$, EPA, DPA, DHA and total fatty acids of n-3 in thigh increased ($P<0.01$) with consumption of fish oil, so that the maximal concentration belonged to experimental group D that had consumed fish oil for 4 weeks. It was concluded that for increase in fatty acids of n-3 in breast, two weeks and in thigh, four weeks of fish oil consumption is needed.

Experimental groups showed a significant effect on concentration of saturated fatty acids of $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ ($P<0.01$) and SFA ($P<0.05$) in thigh, so that with consumption of fish oil the concentration of these fatty acids increased compared with corn oil. The highest concentration was observed in group D that had consumed fish oil for 4 weeks.

Experimental groups showed a significant effect on MUFA in thigh ($P<0.05$). With consumption of fish oil the concentration of MUFA decreased. Experimental groups showed a significant effect on fatty acids of $C_{18:2}$ and AA and total n-6 fatty acids in thigh ($P<0.05$). With increased consumption duration of fish oil in diet, the concentration of n-6 fatty acids in thigh decreased.

Experimental groups showed a significant effect on total fatty acids of PUFA ($P<0.05$). with increased consumption duration of fish oil in diet, the concentration of PUFA in thigh decreased due to reduction of fatty acids of n-6 despite the increase in fatty acids of n-3 and this resulted in significant reduction of polyunsaturated to saturated fatty acids ratio ($P<0.01$). Chicks in groups fed fish oil had lower ratio of n-6 to n-3 ($P<0.01$).

Concentration of fatty acids of LNA, EPA, DPA and DHA in fish oil used in trial was rather high and they are 1.31, 5.74, 3.5 and 14.55 (mg/g fish oil), respectively. Presence of these fatty acids in diet resulted in its increase in breast and thigh, so that with increased consumption duration, it increased as well.

DISCUSSION

The main purpose of present study was to examine the effect of supplementation of fish oil to diet on fatty acid composition in broiler tissues. We hypothesized that supplementation of fish oil to diet results in enrichment of n-3 fatty acids and change of fatty acid composition in broiler tissues. The results showed that supplementation of fish oil to diet enriched the meat with n-3 fatty acids.

Duration of fish oil consumption has no significant effect on performance parameters of broilers. Other researchers [10, 11] also did not observed significant difference in final weight in chickens fed with fish oil compared with basal diet. It has been shown that 4% fish oil does not have negative effect on feed consumption [1]. However, there are some other results indicating that high levels of Menhaden fish oil (8.2%) result in increased feed consumption compared with control group though such increase is less than in low percentages fish oil [12]. Among different groups of dietary feed efficiency in different weeks and starter, grower and finisher periods and the whole growth period of broiler there was no significant difference. The results obtained from the recent research is consistent with results of Alparslan *et al.* [13] and also with the results obtained from experiments of Abas *et al.* [14] that reported that different sources of fat do not have any effect on feed efficiency.

The result of this study was in agreement with the findings of Marion and Woodroof [15] who found that the fatty acid composition in broiler tissues was easily influenced by dietary lipid. Miller and Robisch [16] found that n-3 fatty acids were incorporated into the muscle tissues of broilers fed fish oil at 1.5 or 2.5% of the diet. The fatty acid compositions of the breast and thigh tissue in the present study were similar although the levels of 18:2n-6 were lower. This difference might be accounted for by the inclusion of wheat into the basal diet which is lower in linoleate compared with corn, which the latter was fed in the previous studies of Miller and Robisch [16].

Newman *et al.* [17] in a research that compared the effect of fish oil and cow fat in broiler reported that the diet containing fish oil increased the concentration of n-3 fatty acids while it reduced AA at the same time. In other words, with increase of fish oil, linoleic acid (along with decreased saturation and lengthening of chain) decreases to derivatives like AA.

In this study, fish oil supplementation to ration of chicks decreased n-6 to n-3 fatty acids ratio. This amount is not significant among fish oil consuming groups; thus

it can be concluded that consuming fish oil in the diet for two weeks is sufficient for reducing n-6 to n-3 fatty acids ratio.

The concentration of $C_{14:0}$, EPA, DPA, DHA and total n-3 fatty acids increased as consuming period of fish oil increased, but differences among them was not significant; hence it can be concluded that consumption of fish oil in the diet for two weeks is sufficient for increasing n-3 fatty acids. With consumption of fish oil, AA and $C_{18:2}$ decreased significantly and with increasing the length of consuming fish oil decreased even more. This indicated the reduction in n-6 fatty acids in fish oil consuming groups and in control group this amount is significantly higher than fish oil consuming groups and with consuming fish oil these concentrations decrease and the concentration of MUFA and PUFA to SFA ratio (P/S) also decrease.

Diet supplementation with fish oil results in increase in concentration of fatty acids of n-3 [1, 18]. N-6 to n-3 ratio decreases in both tissue samples with consumption of fish oil. This portion in fish oil used in the experiment is about 0.08. With respect to reduction of fatty acids of n-6 and increase of fatty acids of n-3 with consuming fish oil, high reduction of n-6 to n-3 ratio in carcass and its components is expected. The results are consistent with the results obtained in references [12, 18-20].

PUFA decreased with consumption of fish oil in both tissues, but in breast the reduction was only numerical and was not significant statistically. Comparison of analysis of fish oil and corn oil for PUFA also confirms this issue that concentration of PUFA in corn oil is more than 10 times higher in fish oil. As observed, the minimal concentration of PUFA is in breast and its maximal concentration in carcass. The main reason for this difference may be related to the difference in concentrations of fat contained in their tissue since fat of breast is much less than fat of the carcass and thigh. These results contradict with the results obtained in references [12, 18, 20, 21].

Lopez-Ferrer *et al.* [1] reported that the concentration of SAT and MUFA in tissue have less dependence on contents of diet and concentration of PUFA in tissue is completely dependent on contents of diet. They maintained that this may be dependent on relations between conversion in liver, deposition on tissue and turnover from carbohydrates; moreover, the concentration of PUFA is resulted from direct deposition of diet fat. The concentration of EPA and DHA in both tissues was observed higher than their pre-makers such as LNA ($C_{18:3}$, n-3).

MUFA decreases with consumption of fish oil in both tissues. Cortinas *et al.* [21] showed that with increase in concentration of PUFA in diet, concentration of MUFA decreases in tissue. These results were also obtained by Schreiner *et al.* [18] and Dobrzaneki *et al.* [22]. In this study, however, despite reduction in PUFA concentration, the amount of MUFA also decreased and the results obtained by this study contradict with findings of Lopez-ferrer *et al.* [1] showed that with increased fish oil the concentration of MUFA increases in diet and tissue.

With respect to the results obtained from present study, addition of fish oil to the diet of broiler chickens results in change of fatty acid profile especially n-3 fatty acids and also results in reduction of n-6 to n-3 ratio in protein tissues of poultry. But it is not necessary to use fish oil from the beginning of rearing course; rather, consumption of fish oil in two final weeks of rearing is sufficient for getting the above results.

Fatty acid of LNA mainly is concentrated in dark meat (thigh) and LA (n-6 fatty acid) in light meat (breast) that is because of difference in lipid part existing in dark and light meat and subcutaneous fat. Phospholipids are the dominant fat of light meat while triglyceride is the dominant fat in dark meat and subcutaneous tissues [16].

There is a linear relation between concentration of EPA in diet and concentration of EPA in edible tissues of poultry meat. A considerable dispersion has been determined in reaction with diet containing low concentration of EPA [23].

Concentration of fatty acids of n-6 in both tissue samples decreased and concentration of fatty acids of n-3 in both tissues increased with consumption of fish oil. The cause of this difference is probably the concentration of fatty acids of n-3 and n-6 in fish oil and corn oil. Concentration of fatty acids of n-6 in fish oil is 1.82 mg/g that can be provided only from LA while concentration of n-6 in corn oil is 273.43 mg/g that is 150 times higher than the concentration of LA in fish oil and AA does not exist in fish oil and corn oil. But the concentration of n-3 in fish oil is provided from four kinds of fatty acids consisting LNA, EPA, DPA and DHA that contain 1.31, 5.74, 3.5 and 14.55 mg/g respectively and total up to 25.1 mg/g, however, in corn oil there exists only LNA with concentration of 2.71 mg/g.

CONCLUSIONS

The results of this study indicated that addition of corn oil to diet could increase n-6 fatty acid and fish oil results in increase of n-3 in meat of poultry. Our data indicate that dietary n-3 fatty acids addition to diet for two last weeks of production is sufficient and it could influence the fatty acid composition in chicken tissues. When fed fish oil treatment, chickens formed C20:5n-3 and the conversion of 18:2n-6 to 20:4n-6 was decreased. When the diet contained high levels of 18:2n-6 (corn oil treatment) formation of 20:4n-6 was increased. These conclusions are based solely on the fatty acid compositions of tissue lipids but the data would seem to indicate that dietary sources of n-6 and n-3 fatty acids influence flux through pathways generating long-chain PUFA in the fowl.

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