Animal Nutrition 4 (2018) 44–51

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Changing dietary n-6:n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens

Doaa Ibrahim [a, *], Rania El-Sayed [a], Safaa I. Khater [b], Enas N. Said [c], Shefaa A.M. El-Mandrawy [d]

[a] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt
[c] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt
[d] Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt

ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form
25 July 2017
Accepted 5 August 2017
Available online 18 August 2017

Keywords:
Broiler
n-6:n-3 PUFA ratio
Performance
Antioxidant status
Meat
Immunity

ABSTRACT

Typical formulated broiler diets are deficient in n-3 poly-unsaturated fatty acids (PUFA) due to widening n-6:n-3 PUFA ratio which could greatly affect performance, immune system of birds and, more importantly, meat quality. This study was conducted to evaluate the effect of modifying dietary n-6:n-3 PUFA ratio from plant and animal oil sources on performance, behavior, cytokine mRNA expression, antioxidative status and meat fatty acid profile of broiler chickens. Birds ($n = 420$) were fed 7 diets enriched with different dietary oil sources and ratios as follows: sunflower oil in control diet (C); fish oil (FO); 1:1 ratio of sunflower oil to FO (C1FO1); 3:1 ratio of sunflower oil to fish oil (C3FO1); linseed oil (LO); 1:1 ratio of sunflower oil to linseed oil (C1LO1); 3:1 ratio of sunflower oil to linseed oil (C3LO1), resulting in dietary n-6:n-3 ratios of approximately 40:1, 1.5:1, 4:1, 8:1, 1:1, 2.5:1 and 5:1, respectively. The best final body weight, feed conversion ratio as well as protein efficiency ratio of broilers were recorded in the C1FO1 and C1LO1 groups. Compared with the control group, the dressing percentage and breast and thigh yield were highest in the C1FO1 and C1LO1 groups. Narrowing the dietary n-6:n-3 ratio increased ($P < 0.05$) n-3 PUFA content of breast meat. Moreover, the breast meat contents of eicosapentaenoic acid and docosahexaenoic acid increased ($P < 0.05$) with increasing dietary FO whereas α-linolenic acid content was higher with LO supplementation. Also, enriching the diets with n-3 PUFA from FO and LO clearly decreased ($P < 0.05$) serum total cholesterol, triglycerides and very low-density lipoproteins and enhanced antioxidative status. The feeding frequency was decreased ($P < 0.05$) in the C1FO1 and C1LO1 groups. Likewise, n-3 PUFA-enriched diets enhanced the frequency of preening, wing flapping and flightiness. Animal oil source addition, compared to plant oil, to broiler diets enhanced the relative mRNA expression of interferon gamma, interleukin-1 beta, interleukin-2 and interleukin-6 genes, especially at low n-6:n-3 ratios. This study has clearly shown that narrowing n-6:n-3 ratio through the addition of FO or LO improved performance and immune response of broilers and resulted in healthy chicken meat, enriched with long chain n-3 PUFA.

* Corresponding author.
E-mail address: doibrahim@vet.zu.edu.eg (D. Ibrahim).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

1. Introduction

The success of the modern poultry industry depends on enhancing growth performance, reducing fat deposition of growing chicks and improving the products offered to consumers. Nutrition plays a strong role in growing chickens, and early ingestion behavior generates feed experience that affects the bird's overall performance (Hale and Green, 1988). Recently,

http://dx.doi.org/10.1016/j.aninu.2017.08.003

significant effort has been made to produce poultry products enriched with n-3 poly-unsaturated fatty acid (n-3 PUFA) (Pietras and Orczewska-Dudek, 2013), and modify the potential of the bird's immune response (Swiatkiewicz et al., 2015). The concentration of n-3 PUFA in animal tissues depends mainly on the fatty acid composition of the diet (Bou et al., 2005). The omega-3 fatty acids can decrease the concentrations of C-reactive protein, proinflammatory eicosanoids, cytokines, chemokines and other inflammatory biomarkers (Schwab and Serhan, 2006). It is known that fish oil is an excellent source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (members of the n-3 family), which are precursors of the lipid mediators of inflammation and have anti-inflammatory and immunomodulatory functions (Calder, 2010). On the other hand, vegetable oils (e.g., linseed oil) are rich in α-linolenic acid (ALA), which is the metabolic precursor of EPA and DHA (Kouba and Mourot, 2011). Less than 20% of the world's population consume about 250 mg/day of n-3 PUFA from marine sources (Micha et al., 2014). So, there is a need to make n-3 PUFA available for a greater part of the remaining 80% of the world population. Recent studies have shown that dietary imbalance of n-6:n-3 PUFA ratio can affect human health, especially with high n-6:n-3 PUFA ratio in our modern diets, as it can lead to increased production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) and thus excessively augment inflammation (Simopoulos, 2002). It was recommended that n-6:n-3 PUFA ratio should be nearly 3:1 to 1:1 (Kim et al., 2007). In addition, human conversion of ALA to EPA is low, and to DHA is even lower (Burdge and Calder, 2005). Thus, there is a potential to enrich the human diet with n-3 PUFA by modifying poultry feeding practices to satisfy the human requirements, as both type and ratio of dietary oils affect the deposition of fatty acids in broiler meat. Therefore, the aim of the current study was to improve broiler performance and health, through consumption of specific fatty acids, particularly at the right n-6:n-3 PUFA ratio. This will produce meat that is beneficial to human consumers.

2. Materials and methods

The protocol for animal experiments was approved by the animal care and use committee at Faculty of Veterinary Medicine, Zagazig University.

2.1. Experimental birds and management

A total of 420 day-old Ross 308 broiler chickens were obtained from a commercial hatchery. On arrival, they were weighed and randomly assigned to 7 groups, each consisting of 5 replicates of 12 birds each. Birds were reared in a naturally ventilated open house with sawdust as litter, at a density 10 birds/m^2. Pens were equipped with semi-automatic tube feeders and bell drinkers.

2.2. Experimental diets and design

The birds were fed a basal diet formulated according to Ross 308 broiler nutrition specification. The nutrient composition of the basal diet is shown in Table 1. Seven dietary treatments were prepared using different oil sources (plant and animal) as follows: sunflower oil (C); fish oil (FO); sunflower oil and fish oil at a ratio of 1:1 (C1FO1); sunflower oil and fish oil at a ratio of 3:1 (C3FO1); linseed oil (LO); sunflower oil and linseed oil at a ratio of 1:1 (C1LO1); sunflower oil and linseed oil at a ratio of 3:1 (C3LO1), resulting in dietary n-6:n-3 ratios of approximately 40:1, 1.5:1, 4:1, 8:1, 1:1, 2.5:1 and 5:1, respectively. The different types of oils used in the experiments and their inclusion rates (%) are listed in Table 2. The fatty acid composition of experimental diets is shown in Table 3. The diets were prepared weekly and kept at 4 °C to prevent oxidative rancidity.

Table 1
Ingredients and nutrient composition of the basal diet (% of dry-matter basis).

Item	Starter diet (1 to 21 d)	Grower diet (22 to 42 d)
Ingredients, %		
Corn, ground	54.4	60
Soybean meal (48%)	37.2	31.8
Oil	4	4.5
Calcium carbonate	1.3	1.2
Calcium dibasic phosphate	1.5	1.25
NaCl (common salt)	0.5	0.3
L-lysine (78%)	0.24	0.21
DL-methionine (98%)	0.26	0.24
Vitamin and mineral premix[1]	0.6	0.5
Calculated composition, %		
ME, kcal/kg	3,113	3,212
Protein	22.62	20.50
Ether extract	6.30	7.00
Calcium	1.16	1.00
Avail. P	0.54	0.46
Lysine	1.41	1.24
Methionine	0.58	0.53

[1] Provided per kilogram of diet: 12 MIU vitamin A; 4 MIU vitamin D_3; 28 mg vitamin E (DL-α-tocopherol acetate); 3 mg Vitamin K; 2.0 mg menadione; 2 mg thiamine; 4.0 mg riboflavin; 50 mg niacin; 6 mg pyridoxine; 0.015 mg cobalamin; 15.0 mg pantothenic; acid, 6.0 mg folic acid, 0.16 mg biotin, 0.625 mg ethoxyquin, 500 mg $CaCO_3$; 80 mg Fe; 80 mg Zn; 110 mg Mn; 10 mg Cu; 0.7 mg I; 0.3 mg Se (as Na_2SeO_3); antioxidant 0.5 g.

2.3. Growth performance and carcass traits

The body weight, body weight gain, and feed intake of all broiler chickens were recorded weekly and feed conversion ratio (FCR), protein efficiency ratio (PER) and overall performance were calculated. Five birds from each group were selected at the end of the experiment, fasted overnight, weighed and then sacrificed to obtain weight of the dressed carcass, breast, thigh, and abdominal fat yields, expressed as a percentage of body weight. Samples were stored at −20 °C until analysis. Five samples from the breast and thigh muscles, from each experimental group were used for analysis of intramuscular fat and determined by extraction with petroleum ether in a Soxhlet apparatus (Horwitz, 2002).

2.4. Tissue fatty acid analysis and cholesterol

The experimental diets and homogenized freeze-dried breast meat were analyzed for fatty acid composition. For this purpose, total lipids were extracted from homogenized muscle tissue, using a solvent mixture of chloroform and methanol (2:1, vol/vol), which is suitable for quantitative extraction of lipids according to the method of Folch et al. (1957). The fatty acid methyl esters were prepared as described by Ichihara and Fukubayashi (2010) for gas chromatography (GC). The total cholesterol in breast and thigh was determined enzymatically and measured by GC using the method of Allain et al. (1974).

2.5. Determination of lipid parameters and oxidative status

At the end of the experimental period, blood samples were collected from 5 birds per group into tubes without anticoagulant. The separated serum was used for determination of total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C),

Table 2
The inclusion rate (%) of different oils in starter and grower diets in different experimental groups.

Oil types	Starter diets							Grower diets						
	C	FO	C1FO1	C3FO1	LO	C1LO1	C3LO1	C	FO	C1FO1	C3FO1	LO	C1LO1	C3LO1
Sunflower oil	4	–	2	3	–	2	3	4.5	–	2.25	3.375	–	2.25	3.375
Fish oil	–	4	2	1	–	–	–	–	4.5	2.25	1.25	–		
Linseed oil	–		–	–	4	2	1	–	–			4.5	2.25	1.25
Total	4	4	4	4	4	4	4	4.5	4.5	4.5	4.5	4.5	4.5	4.5

C = control diet supplemented with sunflower oil; FO = control diet supplemented with fish oil; C1FO1 = control diet supplemented with 1:1 ratio of sunflower oil to fish oil; C3FO1 = control diet supplemented with 3:1 ratio of sunflower oil to fish oil; LO = control diet supplemented with linseed oil; C1LO1 = control diet supplemented with 1:1 ratio of sunflower oil to linseed oil; C3LO1 = control diet supplemented with 3:1 ratio of sunflower oil to linseed oil.

Table 3
Fatty acid composition (% of total fatty acids) in the starter and grower diets (measured value).

Item	Starter diets							Grower diets						
	C	FO	C1FO1	C3FO1	LO	C1LO1	C3LO1	C	FO	C1FO1	C3FO1	LO	C1LO1	C3LO1
Dietary n-6:n-3	40:1	1.5:1	4:1	8:1	1:1	2.5:1	5:1	40:1	1.5:1	4:1	8:1	1:1	2.5:1	5:1
Fatty acids														
C18:2 n6	57.42	23.36	26.99	40.36	48.84	42.25	49.80	57.38	22.66	26.37	39.98	48.71	41.90	49.64
C18:3 n3	1.33	3.48	35.13	2.41	1.86	18.23	9.76	1.23	3.43	35.88	2.33	1.78	18.53	9.87
C20:5 n3, EPA	0.00	0.50	0.25	0.13	0.00	0.00	0.00	0.00	0.51	0.26	0.13	0.00	0.00	0.00
C22:6 n3, DHA	0.04	9.29	4.67	2.36	0.03	0.03	0.04	0.03	9.53	4.78	2.41	0.02	0.03	0.03
SFA[1]	14.44	14.23	14.40	14.47	12.49	13.54	14.04	14.56	14.20	14.37	14.48	12.41	13.49	14.02
MUFA[2]	26.55	39.49	33.02	29.76	24.88	25.73	2:44	26.65	39.93	33.27	29.99	24.93	25.80	26.22
PUFA[3]	58.94	44.27	51.59	55.22	62.56	60.79	59.82	58.79	45.95	52.33	55.60	62.68	60.73	59.75
PUFA n-6	57.46	24.24	40.83	49.10	27.35	42.45	49.92	57.42	23.56	40.45	48.96	26.73	42.09	49.75
PUFA n-3	1.48	20.02	10.76	6.12	35.21	18.35	9.90	1.41	22.45	11.93	6.68	36.31	18.84	10.12
EPA + DHA	0.04	9.79	4.92	2.48	0.03	0.03	0.04	0.03	10.04	5.04	2.54	0.02	0.03	0.03
n-6:n-3 PUFA ratio	38.93	1.21	3.79	8.02	0.78	2.31	5.04	40.59	1.05	3.39	7.33	0.74	2.23	4.92

EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
[1] SFA = C8:0 + C11:0 + C12:0 + C13:0 + C14:0 + C16:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0.
[2] MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1 + C122:1 + C24:1.
[3] PUFA = C18:2 + C18:3 + C18:3 + C18:4 + C20:2 + C20.3 + C20.4 + C20:5 + C22:2.

low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein (VLDL) concentrations colorimetrically using triglyceride (TR0100), total cholesterol (MAK043) and HDL, LDL/VLDL (MAK045), malonaldehyde (MDA) (MAK085) and glutathione S-transferase (GSH-ST) (CS0410) kits from Sigma–Aldrich, following the manufacturer's instructions.

2.6. Behavioral observations

Birds used in this study were observed as scan samples for 3 h/week per group and number of birds expressed as a percentage of total observed birds. The following behavioral parameters were observed and measured throughout the experiment; ingestive behavior (feeding and drinking); time use, including idling, walking, crouching, huddling, litter pecking, preening, wing flapping and leg stretching behavior.

2.7. Gene expression analysis by real-time PCR

At the end of the experiment, 3 birds were randomly selected from each group, marked and immunized intramuscularly with 0.2 mL of 5% sheep red blood cells before slaughter after 24 h. Total RNA was extracted from 30 mg of splenic tissue using Qiagen RNA extraction kits (Cat, No. 74104). Total RNA purity was measured using NanoDrop_ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The total RNA was reverse-transcribed into cDNA, using QIAGEN Long Range 2 Step RT-PCR Kit, following the manufacturer's instructions. One microliter of total cDNA was mixed with 12.5 μL of 2 × SYBR_Green PCR mixed with ROX from Bio-Rad, 5.5 μL of RNAse-free water and 0.5 μL of each forward and reverse primers for the measured genes. The β-actin gene was used as a control for normalization. The up- and downstream primer sequences and accession number of interferon gamma (*IFN-γ*), interleukin-1 beta (*IL-1β*), interleukin-2 (*IL-2*), interleukin-6 (*IL-6*) and β-actin genes are listed in Table 4.

2.8. Statistical analysis

The analysis of variance of the obtained data was performed in PASW statistics 18 (SPSS Inc., USA), using general linear model. *Post hoc* comparisons were applied, whenever appropriate, using Duncan's test. Statistical significance was considered at $P \leq 0.05$. Before carrying out the statistical analysis of gene expression, fold change was calculated using the $(2-\Delta\Delta Ct)$ method to quantify mRNA levels according to Livak and Schmittgen (2001).

Table 4
Gene-specific primer sequences for real-time PCR.

Genes	Primer sequences	GeneBank accession No.
IFN-γ	F: 5′GTGAAGAAGGTGAAAGATATCATGGA3′ R: 5′GCTTTGCGCTGGATTCTCA3′	Y07922
IL-1β	F: 5′GCTCTACATGTCGTGTGTGATGAG3′ R: 5′TGTCGATGTCCCGCATGA3′	AJ245728
IL-2	F: 5′TTGGAAAATATCAAGAACAAGATTCATC3′ R: 5′TCCCAGGTAACACTGCAGAGTTT3′	AJ009800
IL-6	F: 5′GCTCGCCGGCTTCGA3′ R: 5′GGTAGGTCTGAAAGGCGAACAG3′	AJ250838
β-actin	F: 5′AATGAGAGGTTCAGGTGCCC3′ R: 5′ATCACAGGGGTGTGGGTGTT3′	NW001486319

IFN-γ = interferon gamma; *IL-1β* = interleukin 1 beta; *IL-2* = interleukin 2; *IL-6* = interleukin 6.

3. Results and discussion

3.1. Growth performance

The effects of n-6:n-3 PUFA ratio on growth performance parameters are shown in Table 5. The broilers in C1FO1 and C1LO1 groups had the highest ($P < 0.05$) final body weight (FBW), followed by broilers in C3FO1 group, then C3LO1, FO and LO groups, whereas the C group recorded the lowest FBW at d 42. The best values for FCR and PER were noted in the group fed C1FO1 with (1.5:1) n-6:n-3 ratio followed by C1LO1 and FO groups when compared to other groups. These results are consistent with Qi et al. (2010) who found that by narrowing dietary n-6:n-3 ratio from 30:1 to 5:1, the FCR was improved. Also, broiler chicks fed 1.5% and 3% FO supplemented diets exhibited higher weight gain than a control group without fish oil and a 6% fish oil group (Chekani-Azar et al., 2010). In addition, at 6 weeks of age, heavier broiler weight was recorded in groups fed 2.5% flaxseed oil when compared with a standard control diet containing 2.5% tallow (Carragher et al., 2016). Wang et al. (2011) argued that the effect of n-3 PUFA in broiler diet was dependent on their dietary level since low levels of dietary fish oil are more effective than high levels in improving performance and feed gain. The improvement in productive performance of broilers in response to decrease in dietary n-6:n-3 PUFA ratio, especially with FO group containing n-6:n-3 ratio of 4:1 then in LO group of 2.5:1 may be due to increased diet digestibility, which stimulates growth and feed efficiency (Moura, 2005; Saleh et al., 2009). This phenomenon may be explained by the role of n-3 PUFA in activation of bile, which enhances fat digestion in the intestine, thus increasing the efficiency of feed digestion and absorption (Jameel and Sahib, 2014). On the other hand, a combination of different dietary fat sources in the bird's diet may enhance nutrient utilization regardless of metabolized energy content and may lessen the feed passage rate in the digestive tract, permitting better nutrient absorption and utilization (Latshaw, 2008; Firman et al., 2010). Our results on growth performance confirmed that the groups fed FO and LO with n-6:n-3 ratios of 4:1 and 2.5:1 respectively, attained the best FBW together with an improvement in feed utilization and the effect of decreasing n-6:n-3 ratio to (4:1) from animal source was more prominent on broiler performance.

3.2. Carcass characteristics and muscle cholesterol

Table 6 summarizes the effects of different sources of n-6:n-3 PUFA ratio on carcass, breast and thigh yields, and abdominal fat at the end of the experiment. Broilers fed dietary C1FO1 and C3FO1 and C1LO1 significantly had the highest ($P < 0.05$) carcass and breast yield followed by groups supplemented by FO, C3LO1 and LO when compared with C group. Broilers fed (4:1) and (2.5:1) n-6:n-3 ratios from C1FO1 and C1LO1 groups had higher thigh yield than the other groups. This result is similar to that of Chashnidel et al. (2010), who showed that the inclusion of FO in broiler diet significantly improved dressed weight compared to the control group. The reduction in abdominal fat was more prominent in groups supplemented with (4:1) and (2.5:1) n-6:n-3 ratios in C1FO1 and C1LO1 groups, respectively, followed by the groups supplemented with dietary FO or LO when compared with C group. Marine omega-3 fatty acids have been found to be involved in the suppression of lipogenic genes in liver (Kaur and Sinclair, 2010). Furthermore, Ferrini et al. (2010) showed that linseed oil reduces abdominal fat deposition by promoting fatty acid β-oxidation, rather than suppressing fatty acid biosynthesis. Chen et al. (2012) found that enriching diet with n-3 PUFA improved Lipin-1 (*LPIN1*) gene expression in the abdominal fat of chicken. Triglyceride synthesis can be regulated by *LPIN1* as it controls DNA-bound transcription factors to regulate gene transcription (Schweitzer et al., 2015).

The concentrations of cholesterol in breast and thigh muscles are shown in Table 6. Breast muscle exhibited lower values of cholesterol than the thigh. Moreover, increasing the level of dietary FO and LO in breast and thigh muscles reduced the cholesterol concentrations and this reduction was more prominent with dietary inclusion of FO. El-Katcha et al. (2014) stated that feeding of broiler on 1:5 of n-3:n-6 PUFA ratio reduced the cholesterol content of breast meat.

3.3. Antioxidant status and serum lipid profile

Serum concentrations of MDA, GSH-ST and lipid profile are shown in Table 7. The concentration of MDA was significantly decreased ($P < 0.05$) in broiler groups fed diet supplemented with FO and LO when compared with C group. Narrowing the n-6:n-3 PUFA ratios especially in FO groups was associated with a significant increase ($P < 0.05$) in GSH-ST values. Our findings are similar to those of Chen et al. (2012) who indicated that the concentrations of cardiac glutathione peroxidase (GSH-Px), GSH-ST and superoxide dismutase (SOD) were increased in an n-3 PUFA rich group, and the MDA and HO hydroxyl radical were reduced. Similarly, Bhattacharya et al. (2003) provided compelling evidence that n-3 PUFA scavenge H_2O_2 and lipid peroxides and thus can enhance the activities of the hepatic antioxidant enzymes, SOD, GSH-Px and GSH-ST. On the other hand, it could be deduced that increasing the FO and LO ratios were accompanied by increasing n-3 PUFA level in broiler diet and significantly decreased ($P < 0.05$) serum total cholesterol, triglycerides concentration, VLDL and increased serum high-density lipoprotein levels (HDL). The present results are in accordance with Calder (2001) and Saleh et al. (2009) who reported that increasing

Table 5
Effect of dietary n-6:n-3 PUFA ratios from different oil sources on overall broiler performance over 42 days.[1]

Item	Experimental groups (n-6:n-3 ratio)						
	C (40:1)	FO (1.5:1)	C1FO1 (4:1)	C3FO1 (8:1)	LO (1:1)	C1LO1 (2.5:1)	C3LO1 (5:1)
BW, g/bird	2,222 ± 0.93^{e}	2,427 ± 0.97^{c}	2,489 ± 0.75^{a}	2,473 ± 1.05^{b}	2,376 ± 1.44^{d}	2,486 ± 0.86^{a}	2,430 ± 1.28^{c}
BWG, g/bird	2,176 ± 0.60^{e}	2,381 ± 0.89^{c}	2,443 ± 1.24^{a}	2,426 ± 1.29^{b}	2,330 ± 1.52^{d}	2,440 ± 1.36^{a}	2,384 ± 1.44^{c}
FI, g/bird	3,928 ± 5.56^{e}	3,982 ± 2.91^{c}	3,764 ± 4.44^{d}	4,105 ± 3.49^{a}	4,112 ± 3.66^{a}	4,078 ± 0.60^{b}	4,115 ± 7.10^{a}
FCR	1.80 ± 0.00^{a}	1.67 ± 0.00^{e}	1.54 ± 0.00^{f}	1.69 ± 0.00^{d}	1.77 ± 0.00^{b}	1.67 ± 0.00^{e}	1.73 ± 0.00^{c}
PER	2.65 ± 0.00^{f}	2.87 ± 0.00^{b}	3.11 ± 0.00^{a}	2.84 ± 0.00^{c}	2.71 ± 0.00^{e}	2.87 ± 0.00^{b}	2.78 ± 0.00^{d}

C = control diet supplemented with sunflower oil; FO = control diet supplemented with fish oil; C1FO1 = control diet supplemented with 1:1 ratio of sunflower oil to fish oil; C3FO1 = control diet supplemented with 3:1 ratio of sunflower oil to fish oil; LO = control diet supplemented with linseed oil; C1LO1 = control diet supplemented with 1:1 ratio of sunflower oil to linseed oil; C3LO1 = control diet supplemented with 3:1 ratio of sunflower oil to linseed oil; BW = body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio; PER = protein efficiency ratio.

[a,b,c,d,e] Within a row, different superscript letters denote significant difference ($P < 0.05$).

[1] Values are means ± standard error.

Table 6
Effect of dietary n-6:n-3 PUFA ratios from different oil sources on carcass traits and total lipid and cholesterol content of breast and thigh at 42 days.[1]

Item	Experimental groups (n-6:n-3 ratio)						
	C (40:1)	FO (1.5:1)	C1FO1 (4:1)	C3FO1 (8:1)	LO (1:1)	C1LO1 (2.5:1)	C3LO1 (5:1)
Dressing yield, %	77.33 ± 0.13^{d}	77.76 ± 0.07^{bc}	78.34 ± 0.06^{a}	78.33 ± 0.06^{a}	77.58 ± 0.04^{c}	77.92 ± 0.03^{b}	77.72 ± 0.07^{bc}
Breast yield, %	33.56 ± 0.06^{d}	33.76 ± 0.05^{c}	35.32 ± 0.09^{a}	34.47 ± 0.05^{b}	33.66 ± 0.05^{cd}	34.54 ± 0.05^{b}	33.73 ± 0.04^{cd}
Thigh yield, %	28.42 ± 0.09^{b}	28.66 ± 0.08^{b}	29.25 ± 0.08^{a}	28.56 ± 0.09^{b}	28.70 ± 0.2^{b}	29.32 ± 0.09^{a}	28.40 ± 0.08^{b}
Abdominal fat, %	1.78 ± 0.06^{a}	1.57 ± 0.05^{b}	1.36 ± 0.02^{c}	1.52 ± 0.05^{b}	1.52 ± 0.01^{b}	1.40 ± 0.01^{c}	1.56 ± 0.04^{b}
Breast IMF, %	1.65 ± 0.01^{a}	1.35 ± 0.02^{e}	1.41 ± 0.01^{cd}	1.45 ± 0.00^{b}	1.37 ± 0.01^{e}	1.40 ± 0.01^{d}	1.44 ± 0.01^{bc}
Thigh IMF, %	2.42 ± 0.01^{a}	2.17 ± 0.02^{b}	2.34 ± 0.01^{a}	2.35 ± 0.01^{a}	2.18 ± 0.03^{b}	2.35 ± 0.02^{a}	2.35 ± 0.01^{a}
Thigh cholesterol, mg/100 mg	68.33 ± 0.36^{a}	59.16 ± 0.48^{b}	59.22 ± 0.62^{b}	67.19 ± 0.56^{a}	59.57 ± 0.35^{b}	60.22 ± 0.23^{b}	68.34 ± 0.50^{a}
Brest cholesterol, mg/100 mg	61.20 ± 0.30^{a}	52.16c ± 0.48^{c}	52.57 ± 0.37^{c}	56.49 ± 0.55^{b}	52.30 ± 0.65^{c}	53.01 ± 0.47^{c}	60.78 ± 0.33^{a}

C = control diet supplemented with sunflower oil; FO = control diet supplemented with fish oil; C1FO1 = control diet supplemented with 1:1 ratio of sunflower oil to fish oil; C3FO1 = control diet supplemented with 3:1 ratio of sunflower oil to fish oil; LO = control diet supplemented with linseed oil; C1LO1 = control diet supplemented with 1:1 ratio of sunflower oil to linseed oil; C3LO1 = control diet supplemented with 3:1 ratio of sunflower oil to linseed oil; IMF = intramuscular fat.
[a,b,c,d] Within a row, different superscript letters denote significant difference ($P < 0.05$).
[1] Values are means ± standard error.

Table 7
Effects of dietary n-6:n-3 PUFA ratios from different sources on serum lipid profile and antioxidative status of broiler chicks at 42 days.[1]

Item	Experimental groups (n-6:n-3 ratio)						
	C (40:1)	FO (1.5:1)	C1FO1 (4:1)	C3FO1 (8:1)	LO (1:1)	C1LO1 (2.5:1)	C3LO1 (5:1)
MDA, mmol/mg	6.97 ± 0.71^{a}	3.51 ± 0.12^{b}	3.12 ± 0.06^{b}	3.60 ± 0.12^{b}	3.52 ± 0.08^{b}	3.90 ± 0.10^{b}	3.60 ± 0.16^{b}
GSH-ST, IU/mg	46.12 ± 0.20^{e}	82.54 ± 0.49^{a}	76.06 ± 0.32^{b}	66.13 ± 0.38^{c}	75.48 ± 0.38^{b}	64.45 ± 0.32^{d}	63.93 ± 0.21
Total cholesterol, mg/dL	130.40 ± 0.93^{a}	83.40 ± 0.81^{d}	123.20 ± 0.66^{c}	130.60 ± 0.75^{a}	124.40 ± 0.68^{c}	127.8 ± 0.80^{b}	130.4 ± 0.51^{a}
Triglycerides, mg/dL	79.00 ± 0.45^{a}	51.4 ± 0.81^{d}	53.6 ± 0.93^{c}	54.00 ± 0.89^{c}	66.2 ± 0.66^{b}	66.2 ± 0.73^{b}	80.6 ± 0.6^{a}
HDL-C, mg/dL	50.40 ± 0.86^{c}	58.80 ± 0.87^{a}	56.40 ± 0.92^{ab}	49.2 ± 0.6^{c}	54.80 ± 0.84^{b}	54.80 ± 0.90^{b}	47.00 ± 0.8^{c}
LDL-C, mg/dL	74.2 ± 0.26^{a}	14.32 ± 0.70^{c}	56.08 ± 0.95^{b}	70.6 ± 0.94^{a}	56.36 ± 0.60^{b}	59.76 ± 0.62^{b}	73.88 ± 0.53^{a}
VLDL, mg/dL	15.8 ± 0.16^{a}	10.28 ± 0.06^{a}	10.72 ± 0.41^{ab}	10.8 ± 0.12^{ab}	13.24 ± 0.1^{bc}	13.24 ± 0.25^{bc}	14.52 ± 0.45^{c}

C = control diet supplemented with sunflower oil; FO = control diet supplemented with fish oil; C1FO1 = control diet supplemented with 1:1 ratio of sunflower oil to fish oil; C3FO1 = control diet supplemented with 3:1 ratio of sunflower oil to fish oil; LO = control diet supplemented with linseed oil; C1LO1 = control diet supplemented with 1:1 ratio of sunflower oil to linseed oil; C3LO1 = control diet supplemented with 3:1 ratio of sunflower oil to linseed oil; MDA = malonaldehyde; GSH-ST = glutathione S-transferase; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL = very low-density lipoprotein.
[a,b,c,d,e] Within a row, different superscript letters denote significant difference ($P < 0.05$).
[1] Values are means ± standard error.

dietary omega-3 fatty acids in broiler diet reduced plasma triglycerides and cholesterol. In addition, VLDL levels were reduced in broilers fed FO (4.5%) (Chashnidel et al., 2010). This reduction may be related to the role of omega-3 fatty acid in suppression of triglycerides and apolipoprotein synthesis B, higher elimination of VLDL by peripheral tissues of the liver and higher excretion of bile via feces (Leaf and Weber, 1988) which can also reduce the serum concentrations of cholesterol and triglycerides.

3.4. Fatty acid composition of breast muscle

The fatty acid composition of breast muscle in relation to dietary oil treatment is illustrated in Table 8. The most prominent result is that decreasing the ratio of n-6:n-3 PUFA, especially in FO and LO groups significantly decreased the levels of saturated fatty acids (SFA) These results agree with El-Katcha et al. (2014) who reported that deposition of SFA, mono-saturated fatty acids and n-6 PUFA in broiler meat increased in groups with wide n-6:n-3 PUFA ratios and this was mainly due to a higher concentration of palmitic and stearic acids. The high concentration of PUFA in the LO supplemented diet and C group was reflected in its significantly greater incorporation into muscle compared with FO groups. The proportion of breast muscle PUFA in C group were mainly due to n-6 PUFA linoleic acid (LA, C18:2 n-6) and in LO and FO mainly due to n-3 PUFA (ALA and EPA + DHA, respectively). Additionally, reducing n-6:n-3 PUFA ratio by increasing dietary n-3 PUFA modifies the meat fatty acid profile near a higher level of long-chain PUFA (Qi et al., 2010).

Incorporation of FO and LO in the diets significantly increased ($P < 0.05$) the n-3 PUFA in breast muscle, and these had a reverse effect on the n-6 PUFA, thus decreasing the dietary n-6:n-3 PUFA ratio. Moreover, as reflected by dietary composition, decreasing n-6:n-3 PUFA ratios increased the proportion of EPA and DHA concentration in breast meat by nearly 8- and 14-folds in FO, C1FO1 and 5.5 and one folds in LO, C1LO1, respectively when compared with the wide n-6:n-3 PUFA ratios in C group. In addition, the concentration of ALA in breast meat increased with decreasing n-6:n-3 PUFA ratios, which was more prominent in LO supplemented groups due to its higher composition in LO than FO. Supplementation of LO in broiler diet up to 4.5% increased the conversion of ALA to EPA and DHA in breast meat. Similarly, the total n-3 PUFA, including EPA and DHA of breast muscle were significantly increased as a result of decrease in n-6:n-3 PUFA ratios or the addition of tuna oil (Maroufyan et al., 2012; Morales-Barrera et al., 2013). Our results on breast muscle indicated that the best fatty acid profile in breast tissues with higher n-3 PUFA are the C1FO1 and C1LO1 groups.

3.5. Welfare conditions

Data related to behavioral observation are shown in Table 9. As behavior is a good indicator for the assessment of the well-being of broilers, our results revealed that feeding behavior was significantly increased in C3FO1, C3LO1 and LO compared to C and C1FO1 with the C1LO1 groups being intermediate. Although addition of FO in the diets did not increase the feed intake, the feed conversion rate was improved. Moreover, the probability of drinking tended to be higher in FO than in LO supplemented groups. Decrease in feed intake may be attributed to the unpleasant flavor of fish oil (Hardin et al., 1964). In addition, Symeon et al. (2010) stated that feeding

Table 8
Effect of dietary n-6:n-3 PUFA ratios from different oil sources on the fatty acid profile[1] of breast meat at 42 days.[2]

Fatty acids	Experimental groups (n-6:n-3 ratio)						
	C (40:1)	FO (1.5:1)	C1FO1 (4:1)	C3FO1 (8:1)	LO (1:1)	C1LO1 (2.5:1)	C3LO1 (5:1)
C8:0	0.05 ± 0.00^{a}	0.04 ± 0.01^{b}	0.03 ± 0.02^{b}	0.03 ± 0.00^{b}	0.02^{b} ± 0.00^{b}	0.03 ± 0.02^{b}	0.04 ± 0.00^{b}
C11:0	0.06 ± 0.01^{a}	0.02 ± 0.00^{c}	0.03 ± 0.01^{b}	0.05 ± 0.00^{a}	0.03^{b} ± 0.00^{b}	0.03 ± 0.01^{b}	0.04 ± 0.00^{b}
C12:0	0.07 ± 0.00^{c}	0.02 ± 0.00^{f}	0.04 ± 0.00^{e}	0.05 ± 0.00^{d}	0.11 ± 0.00^{a}	0.04 ± 0.00^{e}	0.08 ± 0.0^{bc}
C13:0	0.04 ± 0.00^{c}	0.00 ± 0.00^{f}	0.02 ± 0.00^{e}	0.03 ± 0.00^{d}	0.08 ± 0.00^{a}	0.02 ± 0.00^{e}	0.05 ± 0.00^{b}
C14:0	1.20 ± 0.03^{d}	1.49 ± 0.01^{b}	1.77 ± 0.04^{a}	1.34 ± 0.00^{c}	0.09 ± 0.01^{g}	1.77 ± 0.04^{a}	0.83 ± 0.02^{e}
C15:0	0.04 ± 0.00^{d}	0.23 ± 0.00^{a}	0.08 ± 0.00^{b}	0.07 ± 0.00^{c}	0.00 ± 0.00^{g}	0.08 ± 0.00^{b}	0.02 ± 0.00^{e}
C16:0	12.48 ± 0.26^{c}	9.12 ± 0.00^{g}	10.68 ± 0.03^{f}	11.23 ± 0.00^{e}	11.70 ± 0.10^{d}	10.68 ± 0.03^{f}	13.25 ± 0.00^{b}
C17:0	0.00 ± 0.00^{c}	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.01 ± 0.00^{b}	0.00 ± 0.00^{c}
C18:0	4.94 ± 0.09^{b}	3.35 ± 0.03^{d}	4.07 ± 0.03^{c}	5.54 ± 0.00^{a}	4.90 ± 0.00^{b}	4.07 ± 0.03^{c}	1.25 ± 0.00^{e}
C20:0	0.20 ± 0.00^{d}	0.85 ± 0.00^{a}	0.47 ± 0.04^{c}	0.64 ± 0.00^{b}	0.18 ± 0.03^{d}	0.47 ± 0.04^{c}	0.22 ± 0.00^{d}
C22:0	0.37 ± 0.00^{a}	0.10 ± 0.00^{c}	0.14 ± 0.03^{c}	0.15 ± 0.00^{c}	0.26 ± 0.03^{c}	0.33 ± 0.03^{ab}	0.31 ± 0.03^{ab}
C23:0	1.00 ± 0.00^{a}	0.24 ± 0.03^{d}	0.54 ± 0.07^{c}	0.32 ± 0.00^{d}	0.40 ± 0.10^{cd}	0.54 ± 0.07^{c}	0.80 ± 0.03^{b}
C24:0	0.02 ± 0.00^{b}	0.05 ± 0.00^{b}	0.64 ± 0.07^{a}	0.06 ± 0.00^{b}	0.08 ± 0.00^{b}	0.64 ± 0.07^{a}	0.07 ± 0.01^{b}
C16:1	0.09 ± 0.01^{e}	4.14 ± 0.04^{a}	1.47 ± 0.07^{b}	0.80 ± 0.00^{c}	0.23 ± 0.07^{d}	0.01 ± 0.00^{e}	0.01 ± 0.00^{e}
C17:1	0.12 ± 0.00^{a}	0.02 ± 0.00^{c}	0.04 ± 0.00^{bc}	0.09 ± 0.00^{a}	0.05 ± 0.03^{bc}	0.04 ± 0.00^{bc}	0.08 ± 0.01^{ab}
C18:1	20.67 ± 0.08^{e}	27.13 ± 0.10^{a}	23.76 ± 0.33^{c}	22.37 ± 0.18^{d}	20.87 ± 0.69^{e}	22.57 ± 0.26^{d}	25.45 ± 0.07^{b}
C20:1	0.84 ± 0.03^{c}	0.90 ± 0.00^{c}	0.68 ± 0.03^{d}	0.45 ± 0.01^{e}	2.00 ± 0.06^{a}	1.76 ± 0.06^{b}	1.80 ± 0.03^{b}
C22:1 n9	0.23 ± 0.03^{b}	0.12 ± 0.00^{c}	0.15 ± 0.04^{d}	0.19 ± 0.01^{bc}	0.50 ± 0.06^{a}	0.2 3 ± 0.01^{bc}	0.21 ± 0.00^{bc}
C24:1 n9	0.54 ± 0.03^{bc}	0.44 ± 0.03^{bc}	0.36 ± 0.07^{c}	0.58 ± 0.00^{b}	0.88 ± 0.09^{a}	0.58 ± 0.07^{c}	0.51 ± 0.00^{bc}
C18:2 n6	55.18 ± 0.20^{a}	20.27 ± 0.12^{e}	36.87 ± 0.27^{c}	44.29 ± 0.16^{b}	22.38 ± 0.29^{d}	36.21 ± 0.50^{c}	44.87 ± 0.48^{b}
C18:3 n6	0.02 ± 0.01^{d}	0.05 ± 0.00^{c}	0.03 ± 0.00^{d}	0.02 ± 0.00^{d}	0.13 ± 0.01^{b}	0.17 ± 0.00^{a}	0.11 ± 0.00^{b}
C18:3 n-3	1.20 ± 0.03^{e}	3.20 ± 0.00^{d}	2.03 ± 0.07^{de}	1.56 ± 0.00^{e}	30.87 ± 0.70^{a}	1.20 ± 0.70^{e}	8.99 ± 0.31^{c}
C18:4 n-3	0.00 ± 0.00^{d}	1.78 ± 0.02^{a}	0.82 ± 0.01^{b}	0.38 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
C20:2 n6	0.00 ± 0.00^{c}	0.53 ± 0.00^{a}	0.21 ± 0.03^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
C20.3 n3	0.00 ± 0.00^{d}	0.94 ± 0.00^{a}	0.32 ± 0.00^{b}	0.02 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
C20:4 n6	0.00 ± 0.00^{d}	0.88 ± 0.05^{a}	0.52 ± 0.00^{b}	0.18 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
C20:5 n3	0.11 ± 0.01^{e}	8.74 ± 0.07^{a}	5.32 ± 0.00^{b}	3.28 ± 0.11^{c}	1.27 ± 0.12^{d}	0.07 ± 0.01^{e}	0.06 ± 0.00^{e}
C22:2 n6	0.00 ± 0.00^{d}	0.57 ± 0.03^{a}	0.26 ± 0.00^{b}	0.10 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
C22:5 n3, EPA	0.15 ± 0.05^{e}	1.81 ± 0.10^{a}	1.19 ± 0.12^{b}	0.87 ± 0.04^{d}	0.72 ± 0.06^{c}	0.43 ± 0.11^{e}	0.19 ± 0.07^{e}
C22:6 n3, DHA	0.34 ± 0.03^{f}	12.73 ± 0.07^{a}	7.29 ± 0.20^{b}	5.12 ± 0.00^{c}	1.82 ± 0.13^{d}	1.05 ± 0.10^{e}	0.65 ± 0.05^{f}
SFA[3]	20.44 ± 0.23^{a}	15.53 ± 0.03^{f}	18.51 ± 0.10^{c}	19.52 ± 0.00^{b}	17.87 ± 0.24^{d}	17.46 ± 0.13^{d}	16.95 ± 0.07^{e}
MUFA[4]	22.61 ± 0.07^{e}	32.97 ± 0.06^{a}	26.63 ± 0.33^{c}	24.65 ± 0.20^{d}	24.96 ± 0.6 1^{d}	25.42 ± 0.16^{d}	28.18 ± 0.09^{b}
PUFA[5]	57.00 ± 0.20^{a}	51.51 ± 0.05^{c}	54.86 ± 0.23^{b}	55.83 ± 0.19^{b}	57.19 ± 0.76^{a}	57.12 ± 0.29^{a}	54.87 ± 0.15^{b}
n-3	1.82 ± 0.00^{f}	29.25 ± 0.12^{b}	17.00 ± 0.09^{d}	11.26 ± 0.11^{e}	34.81 ± 0.68^{a}	20.91 ± 0.79^{c}	10.01 ± 0.33^{e}
n-6	55.20 ± 0.21^{a}	21.77 ± 0.08^{e}	37.68 ± 0.27^{c}	44.59 ± 0.16^{b}	22.51 ± 0.29^{e}	36.37 ± 0.50^{d}	44.98 ± 0.48^{b}
EPA + DHA	0.49 ± 0.05^{g}	14.54 ± 0.09^{a}	8.48 ± 0.09^{b}	5.99 ± 0.03^{c}	2.54 ± 0.09^{d}	1.48 ± 0.17^{e}	0.84 ± 0.11^{f}
n-6:n-3	30.34 ± 0.12^{a}	0.74 ± 0.01^{f}	2.22 ± 0.02^{d}	3.96 ± 0.04^{c}	0.65 ± 0.02^{f}	1.75 ± 0.09^{e}	4.51 ± 0.20^{b}

EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
[a-g] Within a row, different superscript letters denote significant difference ($P < 0.05$).
[1] Fatty acid profile (% of total fatty acids).
[2] Values are means ± standard error.
[3] SFA = C8:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C22:0 + C23:0 + C24:0.
[4] MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1 + C122:1 + C24:1.
[5] PUFA = C18:2 + C18:3 + C18:3 + C18:4 + C20:2 + C20.3 + C20.4 + C20:5 + C22:2.

Table 9
Effect of dietary n-6:n-3 PUFA ratio from different oil sources on behavioral activities (over 42 days).[1]

Item	Experimental groups (n-6:n-3 ratio)						
	C (40:1)	FO (1.5:1)	C1FO1 (4:1)	C3FO1 (8:1)	LO (1:1)	C1LO1 (2.5:1)	C3LO1 (5:1)
Feeding	33.28 ± 0.48^{f}	37.61 ± 0.30^{e}	40.16 ± 0.16^{d}	69.61 ± 0.33^{a}	49.05 ± 0.30^{c}	41.00 ± 0.57^{d}	63.44 ± 0.90^{b}
Drinking	18.16 ± 0.33^{abc}	19.61 ± 0.81^{a}	19.39 ± 0.43^{ab}	18.44 ± 0.73^{abc}	17.11 ± 0.98^{bc}	18.33 ± 0.67^{abc}	16.11 ± 0.91^{c}
Idling	44.72 ± 0.05^{d}	46.44 ± 0.14^{c}	49.72 ± 0.82^{a}	48.55 ± 0.43^{a}	47.33 ± 0.67^{bc}	49.77 ± 0.29^{a}	47.44 ± 0.14^{bc}
Walking	52.83 ± 0.34^{b}	53.27 ± 0.24^{ab}	53.05 ± 0.39^{b}	52.33 ± 0.78^{b}	54.83 ± 0.53^{a}	54.00 ± 0.41^{ab}	52.89 ± 0.64^{b}
Crouching	19.00 ± 0.34^{a}	16.33 ± 0.78^{bc}	15.88 ± 0.74^{cd}	17.39 ± 0.87^{abc}	18.05 ± 0.24^{ab}	14.05 ± 0.31^{d}	18.16 ± 0.69^{ab}
Huddling	30.83 ± 1.05	31.33 ± 0.33	31.00 ± 1.00	32.33 ± 0.33	31.00 ± 1.00	30.33 ± 0.33	30.16 ± 0.83
Litter pecking	11.73 ± 0.46^{a}	9.90 ± 0.15^{b}	9.13 ± 0.37^{b}	12.00 ± 0.15^{a}	11.70 ± 0.49^{a}	8.93 ± 0.20^{b}	11.60 ± 0.40^{a}
Preening	20.96 ± 0.12^{b}	23.53 ± 0.58^{a}	23.53 ± 0.40^{a}	20.36 ± 0.61^{b}	22.76 ± 0.52^{a}	22.76 ± 0.52^{a}	19.63 ± 0.50^{b}
Wing shaking	16.40 ± 0.69^{c}	18.40 ± 0.40^{a}	18.53 ± 0.40^{a}	17.53 ± 0.40^{ab}	17.43 ± 0.44^{ab}	17.46 ± 0.69^{ab}	16.40 ± 0.64^{c}
Leg stretching	7.63 ± 0.26	8.73 ± 0.49	8.83 ± 0.57	8.36 ± 0.20	8.60 ± 0.37	8.56 ± 0.37	8.40 ± 0.11
Flying	8.36 ± 0.27^{c}	11.53 ± 0.34^{a}	11.33 ± 0.29^{a}	8.40 ± 0.35^{c}	11.77 ± 0.32^{a}	10.30 ± 0.43^{b}	8.17 ± 0.20^{c}

C = control diet supplemented with sunflower oil; FO = control diet supplemented with fish oil; C1FO1 = control diet supplemented with 1:1 ratio of sunflower oil to fish oil; C3FO1 = control diet supplemented with 3:1 ratio of sunflower oil to fish oil; LO = control diet supplemented with linseed oil; C1LO1 = control diet supplemented with 1:1 ratio of sunflower oil to linseed oil; C3LO1 = control diet supplemented with 3:1 ratio of sunflower oil to linseed oil.
[a-f] Within a row, different superscript letters denote significant difference ($P < 0.05$).
[1] Values are means ± standard error, represented as percentage.

and drinking were highly and significantly related in birds. The comfort behavior patterns are an indicator of animal welfare (Jensen, 2002). The idling and crouching behavior were significantly increased in C1FO1 and C1LO1 in comparison with other groups. It was more prominent that increasing n-3 PUFA in the diet significantly ($P < 0.05$) increased the frequency of walking behavior. In addition, narrowing the n-6:n-3 PUFA ratio enhanced the activities of preening, wing shaking and flying especially in FO

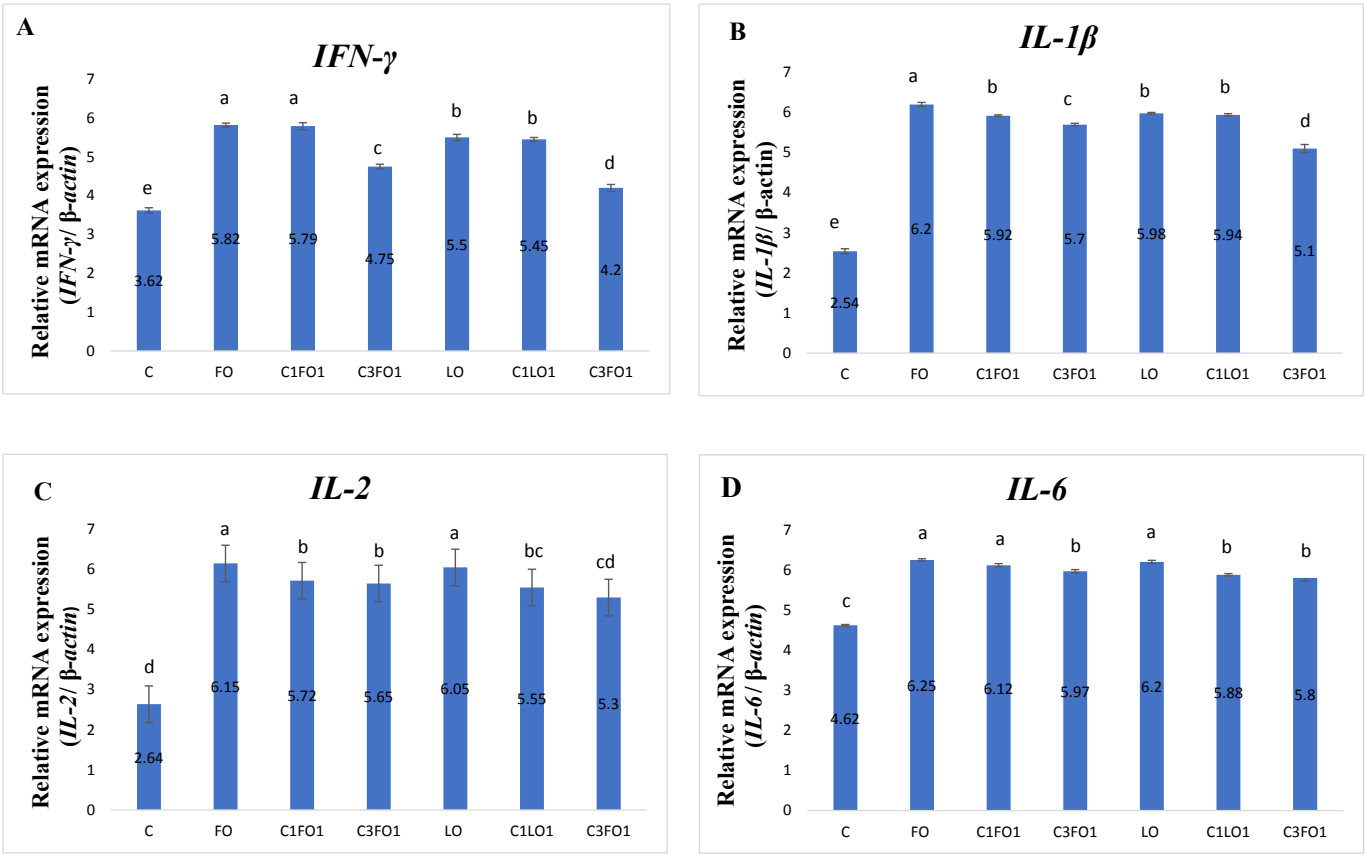

Fig. 1. Effect of different dietary n-3:n-6 ratios on the relative mRNA expression of cytokines genes (A) *IFN-γ*; (B) *IL-1β*; (C) *IL-2*; (D) *IL-6* in the spleen of broiler chickens at 42 days. *IFN-γ* = interferon gamma; *IL-1β* = interleukin 1 beta; *IL-2* = interleukin 2; *IL-6* = interleukin 6; C = control diet supplemented with sunflower oil; FO = control diet supplemented with fish oil; C1FO1 = control diet supplemented with 1:1 ratio of sunflower oil to fish oil; C3FO1 = control diet supplemented with 3:1 ratio of sunflower oil to fish oil; LO = control diet supplemented with linseed oil; C1LO1 = control diet supplemented with 1:1 ratio of sunflower oil to linseed oil; C3LO1 = control diet supplemented with 3:1 ratio of sunflower oil to linseed oil.

supplemented groups. These results may be attributed to higher dietary n-3 PUFA, which can modulate the metabolic function, pathophysiological processes thus affecting health, neuronal development and regulate immune and cardiovascular function (Stulnig, 2004).

3.6. Cytokine genes expression in spleen

Data on mRNA expression of cytokine genes 2 days post-challenge are presented in Fig. 1. Enrichment of broiler diets with n-3 PUFA significantly increased ($P < 0.05$) mRNA expression of interferon gamma (*IFN-γ*) and *IL-1β* genes, especially in FO groups with dietary 1.5:1 and 4:1 n-6:n-3 ratios and LO groups supplemented with (1:1) and (2.5:1) n-6:n-3 ratios when compared with other groups. The expression of the *IL-2* gene was increased by reducing n-6:n-3 PUFA, due to increasing dietary levels of FO and LO. The groups supplemented with (1.5:1), (4:1) n-6:n-3 PUFA ratios from dietary FO and (1:1) n-6:n-3 PUFA from dietary LO significantly increased ($P < 0.05$) the expression of *IL-6* gene followed by (8:1) n-6:n-3 PUFA ratio from dietary FO and (2.5:1) and (5:1) from dietary LO when compared with control. These results are supported by those obtained by Sadeghi et al. (2014) confirming that decreasing n-6:n-3 PUFA ratio by feeding broiler chicks on FO (2.15% or 3%) can alleviate *IFN-γ* gene expression and improve humoral response. In addition, decreasing n-3 PUFA in livestock feed increases pro-inflammatory cytokine levels, such as IL-1, IL-2, IL-6, and TNF-α, therefore extremely boost inflammatory response (Simopoulos, 2002). Eicosapentaenoic acid and DHA have a role in inflammatory gene expression by inhibiting the activation of the transcription factor, nuclear factor κB (Calder, 2010). The possible mechanisms by which dietary n-3 PUFA can modulate cytokine might be the decreased production of metabolites of n-6 PUFA, such as prostaglandin E2 (Trebble et al., 2003). Also, due to a competition between n-3 PUFA and n-6 PUFA for incorporation into the cell membrane phospholipids, thus changing the phospholipids composition of immune cell membranes. Prostaglandin E2 can inhibit T cell proliferation, Th1 cell, IL-2 and IFN-γ production (Goodwin and Webb, 1983; Betz and Fox, 1991). Our results suggest that enriching broiler diets with n-3 PUFA can modulate broiler immune response through their effects on cytokine expression. These results strongly relate to source and concentration of dietary oils, and the intake levels of EPA and DHA. Additionally, increasing dietary EPA and DHA contents in FO groups has a great effect on boosting immune response after challenge than increasing dietary ALA contents in LO groups.

4. Conclusions

The results of this study established that reducing too high n-6:n-3 PUFA ratio in broiler diets can improve their performance and immunity without any anomalies in behavior. Changes in dietary n-6:n-3 PUFA ratio clearly affected meat fatty acid composition. The beneficial effects of reducing n-6:n-3 PUFA ratio were more prominent in broiler groups supplemented with animal (FO) than

plant (LO) oil sources. Moreover, 4:1 and 2.5:1 n-6:n-3 PUFA ratios from FO and LO respectively, produced desirable effects on performance and immunity of birds. Finally, enriching human food with n-3 PUFA may confer health benefits on the human consumer.

Acknowledgments

This research was financially supported by the present authors. All data made or analyzed during this study are available from the corresponding authors. All authors shared in designing, performing, and reading the research then approved the final manuscript.

References

Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4):470–5.
Betz M, Fox BS. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. J Immunol 1991;146:108–13.
Bhattacharya A, Lawrence RA, Krishnan A, Zaman K, Sun D, Fernandes G. Effect of dietary n-3 and n-6 oils with and without food restriction on activity of antioxidant enzymes and lipid peroxidation in livers of cyclophosphamide-treated autoimmune-prone NZB/W female mice. J Am Coll Nutr 2003;22(5):388–99.
Bou R, Guardiola F, Barroeta AC, Codony R. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. J Poult Sci 2005;84:1129–40.
Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev 2005;45(5):581–97.
Calder PC. Omega-3 fatty acids and inflammatory processes. Nutrients 2010 Mar 18;2(3):355–74.
Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 2001;36(9):1007–24.
Carragher JF, Mühlhäusler BS, Geier MS, House JD, Hughes RJ, Gibson RA. Effect of dietary ALA on growth rate, feed conversion ratio, mortality rate and breast meat omega-3 LCPUFA content in broiler chickens. Anim Prod Sci 2016;56(5):815–23.
Chashnidel Y, Moravej H, Towhidi A, Asadi F, Zeinodini S. Influence of different levels of n-3 supplemented (fish oil) diet on performance, carcass quality and fat status in broilers. Afr J Biotechnol 2010;9(5):687–91.
Chekani-Azar S, Hosseini-Mansoub N, Bahrami Y, Ahadi F, Lotfi A. Dietary fish oil improves performance and carcass characterizes of broilers immunized with sheep erythrocytes. Int J Acad Res 2010;1(2):94.
Chen W, Wang JP, Huang YQ. Effects of dietary n-6:n-3 polyunsaturated fatty acid ratio on cardiac antioxidative status, T-cell and cytokine mRNA expression in the thymus, and blood T lymphocyte subsets of broilers. Livest Sci 2012;150(1):114–20.
El-Katcha MI, El-Kholy ME, Soltan MA, EL-Gayar AH. Effect of dietary omega-3 to omega-6 ratio on growth performance, immune response, carcass traits and meat fatty acids profile of broiler chickens. J Poult Sci 2014;2(2):71–94.
Ferrini G, Manzanilla EG, Menoyo D, Esteve-Garcia E, Baucells MD, Barroeta AC. Effects of dietary n-3 fatty acids in fat metabolism and thyroid hormone levels when compared to dietary saturated fatty acids in chickens. Livest Sci 2010;131(2):287–91.
Firman JD, Leigh H, Kamyab A. Comparison of soybean oil with an animal/vegetable blend at four energy levels in broiler rations from hatch to market. Int J Poult Sci 2010;9(11):1027–30.
Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226(1):497–509.
Goodwin JS, Webb DR. Regulation of the immune response by prostaglandins. J Clin Immunol 1983;3:295–315.
Hale C, Green L. Effects of early ingestional experiences on the acquisition of appropriate food selection by young chicks. Anim Behav 1988;36(1):211–24.
Hardin JO, Milligan JL, Sidwell VD. The influence of solvent extracted fish meal and stabilized fish oil in broiler rations on performance and on the flavor of broiler meat. J Poult Sci 1964;43(4):858–60.
Horwitz W. Instructions for inserting: official methods of analysis of AOAC international. AOAC International; 2002.
Ichihara KI, Fukubayashi Y. Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res 2010;51(3):635–40.
Jameel YJ, Sahib AM. Study of some blood parameters of broilers fed on ration containing fish oil. J Biol Agric Healthc 2014 ISSN: 2224-3208.
Jensen P. The etiology of domestic animals: an introductory text. CABI; 2002.
Kaur G, Sinclair AJ. Regulation of gene expression in brain and liver by marine n-3 polyunsaturated fatty acids. Prog Nutr 2010;12(1):24–8.
Kim SC, Adesogan AT, Badinga L, Staples CR. Effects of dietary n-6:n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. J Anim Sci 2007;85(3):706–16.
Kouba M, Mourot J. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie 2011 Jan 31;93(1):13–7.
Latshaw JD. Daily energy intake of broiler chickens is altered by proximate nutrient content and form of the diet. J Poult Sci 2008;87(1):89–95.
Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. N Engl J Med 1988;318(9):549–57.
Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 2001;25(4):402–8.
Maroufyan E, Kasim A, Ebrahimi M, Loh TC, Hair-Bejo M, Soleimani AF. Dietary methionine and n-6:n-3 polyunsaturated fatty acid ratio reduce adverse effects of infectious bursal disease in broilers. J Poult Sci 2012;91(9):2173–82.
Micha R, Khatibzadeh S, Shi P, Fahimi S, Lim S, Andrews KG, et al. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. BMJ 2014;348:g2272.
Morales-Barrera JE, Gonzalez-Alcorta MJ, Castillo-Dominguez RM, Prado-Rebolledo OF, Hernandez-Velasco X, Menconi A, et al. Fatty acid deposition on broiler meat in chickens supplemented with tuna oil. Food Nutr Sci 2013;4(09):16.
Moura BH. Desempenho e composição da carcaça de frangos de cortealimentados com differentesníveisenergéticos com e semóleo [dissertação]. Belo Horizonte: Escola de Veterinária, UFMG. BRAZ J Poult Sci 2005;7:129–41.
Pietras MP, Orczewska-Dudek S. The effect of dietary Camelina Sativa oil on quality of broiler chicken meat/Wpływ Udziału Oleju Z Lnianki Siewnej (Camelina Sativa) W Dawkach Dla Kurcząt Rzeźnych Na Jakość Mięsa. Ann Anim Sci 2013;13(4):869–82.
Qi KK, Chen JL, Zhao GP, Zheng MQ, Wen J. Effect of dietary ω6/ω3 on growth performance, carcass traits, meat quality and fatty acid profiles of Beijing-you chicken. J Anim Physiol Anim Nutr 2010;94(4):474–85.
Sadeghi AA, Safaei A, Aminafshar M. The effects of dietary oil sources on performance, serum corticosterone level, antibody titers and IFN-γ gene expression in broiler chickens. Kafkas Univ Veteriner Fak Derg 2014;1(6):20.
Saleh H, Rahimi S, Karimi Torshizi MA. The effect of a diet that contained fish oil on performance, serum parameters, the immune system and the fatty acid composition of meat in broilers. Int J Vet Res 2009;3(2):69–75.
Schwab JM, Serhan CN. Lipoxins and new lipid mediators in the resolution of inflammation. Curr Opin Pharmacol 2006;6(4):414–20.
Schweitzer GG, Chen Z, Gan C, McCommis KS, Soufi N, Chrast R, et al. Liver-specific loss of lipin-1-mediated phosphatidic acid phosphatase activity does not mitigate intrahepatic TG accumulation in mice. J Lipid Res 2015;56(4):848–58.
Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002;56(8):365–79.
Stulnig TM. Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. Int Arch Allergy Immunol 2004;132(4):310–21.
Swiatkiewicz S, Arczewska-Wlosek A, Jozefiak D. The relationship between dietary fat sources and immune response in poultry and pigs: an updated review. Livest Prod Sci 2015;180:237–46.
Symeon GK, Zintilas C, Demiris N, Bizelis IA, Deligeorgis SG. Effects of oregano essential oil dietary supplementation on the feeding and drinking behavior as well as the activity of broilers. Int J Poult Sci 2010;9(4):401–5.
Trebble T, Arden NK, Stroud MA, Wootton SA, Burdge GC, Miles EA, et al. Inhibition of tumor necrosis factor-alpha and interleukin 6 productions by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. Br J Nutr 2003;90:405–41.
Wang YB, Yang XJ, Qin DK, Feng Y, Guo YM, Yao JH. Effects of eicosapentaenoic acid and docosahexaenoic acid on responses of LPS-stimulated intestinal B lymphocytes from broiler chickens studied in vitro. Eur Food Res Technol 2011;233(4):677.