



Original Research Article

On growth performance, nutrient digestibility, blood T lymphocyte subsets, and cardiac antioxidant status of broilers



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ABSTRACT

Different lipid sources differ in the fatty acid profiles and differently affect growth performance as well as immune function of broilers. The influences of different dietary lipid sources on growth performance, nutrient digestibility, blood T lymphocyte population, and cardiac antioxidant status were investigated of broilers. A total of 360 one-day-old male broilers (BW = 44 ± 3 g) were randomized into 3 treatment groups, consisting of 6 replicates with 20 birds in each group. Broilers received standard diets supplemented with 5% (wt/wt) of lard (LD, as a control diet), sesame oil (SO), or flaxseed oil (FO). Broilers in both SO and FO treatment groups had lower ($P < 0.05$) feed conversion ratios from 22 to 42 d and during the overall phase compared to those in LD treatment group. Meanwhile, the apparent total tract nutrient digestibility of crude fat in SO and FO treatment groups was higher than that in LD treatment group. Both FO and SO treatments decreased ($P < 0.05$) abdominal fat percentage compared to LD treatment. Total triglycerides and total cholesterol in chicken blood were decreased ($P < 0.05$) by SO and FO treatments compared to LD treatment. Feeding broilers with FO and SO led to a decrease ($P < 0.05$) in blood CD4⁺ T lymphocyte count and in CD4⁺:CD8⁺ ratio compared to LD treatment. Sesame oil and FO treatments increased cardiac glutathione peroxidase ($P < 0.05$) compared to LD treatment. It is concluded that addition of 5% SO and FO to the standard corn-soybean meal diet improved feed efficiency, increased the activities of cardiac glutathione peroxidase, and affected the T lymphocytes ratio of fast growing broilers.

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1. Introduction

Dietary fatty acids are one of the primary energy sources and exert important physiological functions for fast-growing broilers, and the absorbed fat type has been reported to modify the fatty acid composition of chicken as well (Hulbert et al., 2005; Bautista-Ortega et al., 2009; Gonzalez et al., 2011; Sridhar et al., 2017).

Sesame oil (SO) is an edible vegetable oil, and derived from sesame seed. As a dietary source of n-6 polyunsaturated fatty acid (PUFA), SO contains approximately 41% linoleic acid (LA). Flaxseed oil (FO) is a source of n-3 PUFA and enriched in α -linoleic acid (ALA), while lard (LD) is a source of saturated fatty acid. Flaxseed oil is a common supplement of layer diet for producing n-3 PUFA enriched eggs. Linoleic acid and ALA are both substrates for Δ -6 desaturase (James et al., 2000). Both n-3 and n-6 PUFA share universal enzymes during the biosynthesis of long-chain fatty acids (LCFA); therefore, competition between n-3 and n-6 PUFA for the Δ -6 desaturase enzyme system influences elongation and desaturation of 18-carbon LCFA. This affects tissue incorporation of the long-chained n-3 PUFA (James et al., 2000). High level of dietary n-6 PUFA with a high n-6: n-3 PUFA ratio has been suggested to adversely affect health (Simopoulos, 2011). However, studies on the effect of n-3 PUFA enriched oil source (such as SO) on broilers are limited.

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Effector T lymphocytes consisting of CD8⁺ killer T cells and CD4⁺ helper T cells are major players in the inflammatory response. n-3 PUFA exhibits its anti-inflammatory effect by shifting the Th1 response (characterized by T cell proliferation and pro-inflammatory cytokine secretion) to a Th2 response, thus increases B cell activation and antibody production (Fritsche et al., 1999; Sijben et al., 2001; Zhan et al., 2009). However, Wang et al. (2011) reported that the circulating CD4⁺ population of broilers and the CD4⁺: CD8⁺ ratio could be increased by a high ratio of n-6: n-3 PUFA in the feed. Furthermore, increasing PUFA in diets of broiler chickens has also been associated with lipid oxidation (Chen et al., 2012). Increasing dietary PUFA supplementation has also been associated with lipid oxidation and alteration of animal performance and health (López-Ferrer et al., 2001a, b; Venkatraman et al., 1994), but the effects of different lipid sources on the cardiac antioxidant status of broilers are not well studied. Moreover, most of the research has been conducted during inflammation in response to immune stress. Reports on the effects of dietary lipids on immune response under physiological conditions are rare. The response of cytokines and T lymphocytes to supplementation with different dietary lipid sources may behave differently between challenged and unchallenged animals.

Therefore, the objective of the present study was to investigate the effects of different dietary oil sources on growth performance, nutrient digestibility, blood T lymphocyte subset, and cardiac antioxidant status of broilers.

2. Materials and methods

2.1. Birds, housing, and experimental design

The Animal Care and Use Committee of the Henan Agricultural University approved the experimental protocol. A total of 360 one-day-old male (Cobb 500) broilers (BW = 44 ± 3 g) were obtained for a 6-week period trial. The broilers were placed in an enclosed fan-ventilated rearing house with suspended cages (2 m × 1.5 m) and provided with continuous light and free access to water and feed. This was achieved by equipping each cage with automated feeder lines and nipple waterer lines on both sides of the feeder. The room temperature was set to 33 °C for the first 3 days and was gradually reduced to 24 °C during the experimental period with a relative humidity maintained at 60%. The control diet was formulated using 5% of lard, and 2 experimental diets were formulated, in which LD was replaced equivalently by SO and FO, respectively. Lard was obtained from Yinghui Feed (Zhenzhou, China), while FO and SO were obtained from Wanlifu Bio-Technology Company (Inner Mongolia, China). The acid value and peroxide value were 2.15 mg KOH/g and 5.08 mmol/kg in LD, 1.87 mg KOH/g and 4.75 mmol/kg in FO, 1.36 mg KOH/g and 3.48 mmol/kg in SO.

Broilers were tested in 6 replicates (cages) with 20 birds each cage. Two nutritional phases, including a starter phase (1 to 21 d) and a finisher phase (22 to 42 d), were designed for this experiment (Table 1). All diets were formulated with equal contents of calories and nitrogen that met the nutritional requirements of broilers (NRC, 1994) and were supplied in mash form. The fatty acid composition of experimental diets is shown in Table 2.

2.2. Sampling and analysis

All diets were evaluated for composition of dry matter (DM), gross energy (GE), crude protein (CP), crude fat (CF), calcium (Ca), and phosphorus (P) according to the approved procedures of the AOAC (2003).

Feed intake (FI) of broilers was recorded on a weekly basis, and spilled feed from each cage was collected and weighed daily to calculate the feed consumption. The broilers were weighed weekly (via the cage) to estimate both body weight gain (BWG) and feed conversion rate (FCR). At the end of the experiment, blood samples were collected from 36 broilers (2 birds per cage and 12 birds per treatment) via bleeding from the wing vein. These broilers were individually weighed and then slaughtered via cervical dislocation. The breast, leg, heart, liver, abdominal fat, spleen, and bursa of Fabricius were harvested and weighed to calculate the relative organ weights (% of live BW). The blood samples were subjected to centrifugation at 3,000 × g for 15 min at 4 °C (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) to separate the serum, which were stored below −20 °C until further analysis.

2.3. Apparent total tract nutrient digestibility

The apparent total tract nutrient digestibility (ATTD) was determined on 42 d by adding 0.2% chromium oxide (Cr₂O₃) to the diets as an indigestible marker and by measuring fresh excreta samples collected during the 4 d preceding feeding the chromium diet. Each sample was placed in an aluminum foil cup and was dried for 72 h at 60 °C and ground through a 1-mm sieve. The ATTD of DM, crude fat, Ca, and P were calculated via the chromium oxide concentration of feces relative to feed using the index method described by Kong and Adeola (2014).

2.4. Blood metabolites

Serum samples were thawed and concentrations of total protein, glucose, albumin, and globulin were determined using an automatic biochemical analyzer (RA-1000 Bayer, NY, USA) according to the procedures of the manufacturer. The concentrations of cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides in serum were measured via reagent kits (Wako Pure Chemical Industries Ltd., Tokyo, Japan), following the manufacturer's instructions.

2.5. Lymphocyte subsets assay

Lymphocytes were obtained following the method detailed in Han et al. (2007) and Chen et al. (2012) and lymphocytes were further assayed via flow cytometry (CellQuest program; Becton Dickinson, Franklin Lakes, NJ, USA).

2.6. Determination of fatty acid profiles

The fatty acid content of the feed was determined via combination of gas chromatography and mass spectrometry (Agilent HP6890 and Agilent HP 5973) according to the manufacturer's instructions.

2.7. Heart antioxidant status analysis

The enzymatic activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured and the contents of hydroxyl radical (HO) and malondialdehyde (MDA) were obtained using relevant reagent kits (SOD, A001-1; GSH-Px, A005; HO, A018; MDA, A003-1 Nanjing, Jiangsu, China), following the manufacturer's instructions.

Table 1
Dietary composition (as-fed basis).

Item	Starter (1 to 21 d)			Finisher (22 to 42 d)		
	LD	SO	FO	LD	SO	FO
Ingredients, g/kg						
Corn	552.0	552.0	552.0	601.0	605.0	605.0
Soybean meal (CP 48%)	350.9	350.9	350.9	310.0	297.0	297.0
Corn gluten meal (CP 60%)	—	—	—	—	9.0	9.0
Sesame oil	—	50.0	—	—	50.0	—
Flaxseed oil	—	—	50.0	—	—	50.0
Lard	50.0	—	—	50.0	—	—
Ca(H ₂ PO ₄) ₂	15.5	15.5	15.5	12.6	12.6	12.6
Limestone	12.6	12.6	12.6	11.8	11.9	11.9
NaCl	2.0	2.0	2.0	2.0	2.0	2.0
DL-methionine	1.4	1.4	1.4	0.5	0.5	0.5
L-lysine-HCl	3.0	3.0	3.0	0.1	—	—
Choline chloride	2.6	2.6	2.6	2.0	2.0	2.0
Vitamin premix ¹	5.0	5.0	5.0	5.0	5.0	5.0
Trace mineral premix ²	5.0	5.0	5.0	5.0	5.0	5.0
Analytical composition, % ³						
ME, MJ/kg	12.89	12.61	12.65	13.09	13.03	13.07
Crude protein	21.50	21.60	22.00	19.89	19.90	20.00
Crude fat	8.90	8.81	8.58	9.00	9.00	9.20
Ca	1.04	1.12	1.07	1.02	0.99	1.01
Lys	1.15	1.16	1.15	1.00	1.00	1.02
Met + Cys	0.91	0.87	0.88	0.78	0.72	0.73
Available P	0.45	0.50	0.47	0.46	0.42	0.44

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard.

¹ Provided per kg of diet: 10,000 IU vitamin A, 2,500 IU vitamin D₃, 50 mg vitamin E, 2 mg vitamin K₃, 2 mg thiamin, 10 mg riboflavin, 1 mg vitamin B₆, 5 µg vitamin B₁₂, 50 mg niacin, 2 mg folic acid, 25 µg biotin, and 10 mg pantothenic acid.

² Provided per kg of diet: 40 mg Zn (as ZnSO₄·7H₂O), 60 mg Mn (as MnSO₄·5H₂O), 80 mg Fe (as FeSO₄·7H₂O), 10 mg Cu (as CuSO₄·5H₂O), 0.2 mg I (as KI), and 0.25 mg Se (as Na₂SeO₃·5H₂O).

³ The value of ME was calculated and others were measured.

Table 2
Analyzed fatty acid composition (mg/g diet).¹

Item	Starter (1 to 21 d)			Finisher (22 to 42 d)		
	LD	SO	FO	LD	SO	FO
C14:0	1.94	0.61	0.82	0.63	0.14	0.23
C15:0	0.05	0.06	0.08	0.04	0.03	0.06
C16:0	23.67	13.96	14.58	23.07	13.33	10.45
C17:0	—	1.95	3.53	1.92	2.36	3.03
C18:0	10.6	3.19	5.24	14.13	2.25	2.52
C16:1n-7	11.34	8.4	10.46	8.57	11.19	8.23
C18:1n-9	28.84	22.47	21.93	30.62	21.88	23.57
C20:1n-9	0.16	0.41	1.29	0.24	0.62	0.79
C18:2n-6	7.85	27.58	5.17	6.00	27.52	9.90
C20:2n-6	0.7	1.39	1.97	0.68	0.98	1.61
C20:3n-6	0.5	1.22	1.12	0.81	0.96	1.18
C20:4n-6	0.21	0.95	1.25	0.31	0.95	1.21
C18:3n-3	0.81	4.59	19.46	0.5	4.98	25.74
C20:5n-3	0.7	0.35	0.08	0.84	0.47	0.17
C22:5n-3	0.62	0.01	0.5	0.29	0.32	0.06
C22:6n-3	0.11	0.07	0.45	0.47	0.8	0.73
Total SFA ¹	36.26	19.77	24.25	39.79	18.11	16.29
Total MUFA ¹	40.34	31.28	33.68	39.43	33.69	32.59
n-3 PUFA	2.24	5.02	20.49	2.10	6.57	26.70
n-6 PUFA	9.26	31.14	9.51	7.80	30.41	13.90
Total PUFA ¹	11.50	36.16	30.00	9.90	36.98	40.60

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

¹ Means of identified fatty acids of the respective group.

2.8. Statistical analysis

All data were analyzed via the GLM procedures of SAS (SAS Inst. Inc., Cary, NC), using each replicate cage as an experimental unit. Differences among all treatments were separated via the Tukey–Kramer test and $P < 0.05$ indicated significance. The variability of all data was expressed as the SE.

3. Results

3.1. Growth performance and apparent total tract nutrient digestibility

Both BWG and FI were not influenced by dietary treatments throughout the entire experimental period, whereas FCR was lower ($P < 0.05$) in both SO and FO treatments than in LD treatment both from 22 to 42 d and overall phase (Table 3). No difference was found in ATTD of Ca, P, and CP on 42 d among treatments (Table 3), while CF digestibility was increased ($P < 0.05$) in both SO and FO treatments compared to LD treatment group (Table 3).

3.2. Relative organ weight

Dietary inclusion of FO treatment led to a lower ($P < 0.05$) breast weight compared to SO and LD treatments, while LD increased ($P < 0.05$) the abdominal fat percentage compared to other treatments (Table 4). The relative weights of leg, heart, liver, spleen, and bursa of Fabricius remained unaffected by dietary treatments.

3.3. Blood metabolites

The total cholesterol concentration in the blood was decreased ($P < 0.05$) in both FO and SO treatments compared to LD treatment (Table 5). Triglyceride content was lower ($P < 0.05$) in FO diet than in both SO and LD diets, and FO treatment had the lowest value ($P < 0.05$) among all treatments.

3.4. Lymphocyte subsets

Broilers that received FO and SO diets had a lower ($P < 0.05$) concentration of blood CD4⁺ and lower ($P < 0.05$) CD4⁺: CD8⁺ ratio compared to LD treatment (Table 6).

Table 3Effects of different lipid sources on growth performance and nutrient digestibility in broilers.¹

Item	LD	SO	FO	SE	P-value
Growth performance					
Starter (1 to 21 d)					
BWG, g	487	503	500	24	0.90
FI, g	824	823	812	34	0.96
FCR	1.708	1.637	1.626	0.040	0.31
Finisher (22 to 42 d)					
BWG, g	1,678	1,763	1,752	39	0.27
FI, g	3,105	2,956	2,977	74	0.33
FCR	1.855 ^a	1.677 ^b	1.698 ^b	0.037	<0.01
Overall (1 to 42 d)					
BWG, g	2,176	2,266	2,253	61	0.54
FI, g	3,918	3,778	3,789	93	0.52
FCR	1.808 ^a	1.668 ^b	1.681 ^b	0.034	0.02
Total tract nutrient digestibility on d 42					
Ca	42.52	43.24	40.92	1.94	0.88
P	52.91	52.50	56.90	2.81	0.49
Crude fat	80.63 ^b	85.53 ^a	84.43 ^a	1.35	0.06
Crude protein	52.29	55.42	54.51	2.12	0.56

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard; BWG = body weight gain per broiler; FI = feed intake per broiler; FCR = feed conversion ratio.

^{a,b}Means in the same row with different superscripts indicate significant difference ($P < 0.05$).¹ $n = 360$ (120 broilers \times 3 treatments) with 20 broilers per cage and 6 cages per treatment.**Table 4**Effects of different lipid sources on relative organ weight (%) in broilers.¹

Item	LD	SO	FO	SE	P-value
Breast	20.23 ^a	20.44 ^a	17.56 ^b	0.50	<0.01
Leg	14.19	14.40	14.35	0.46	0.95
Heart	0.37	0.37	0.41	0.02	0.32
Liver	2.00	1.96	1.97	0.04	0.73
Abdominal fat	2.43 ^a	1.49 ^b	1.66 ^b	0.12	<0.01
Spleen	0.120	0.157	0.158	0.013	0.10
Bursa of Fabricius	0.205	0.175	0.193	0.018	0.52

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard.

^{a,b}Means in the same row with different superscripts indicate significant difference ($P < 0.05$).¹ $n = 36$ (12 broilers \times 3 treatments) with 2 broilers per cage and 6 cages per treatment.**Table 5**Effects of different lipid sources on blood characteristics in broilers.¹

Item	LD	SO	FO	SE	P-value
Total protein, mg/mL	30.83	31.52	31.85	1.12	0.84
Albumin, mg/mL	15.75	17.13	15.95	0.44	0.11
Globulin, mg/mL	12.29	12.57	13.05	0.31	0.26
Total cholesterol, μ mol/mL	3.47 ^a	2.98 ^b	2.59 ^b	0.18	0.03
Triglyceride, μ mol/mL	0.43 ^a	0.29 ^b	0.19 ^c	0.02	0.01
LDLC, μ mol/mL	2.02	2.00	1.80	0.31	0.51
HDLc, μ mol/mL	0.88	0.91	0.77	0.11	0.35
Glucose, μ mol/mL	12.29	12.57	13.05	0.31	0.27

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard; LDLc = low density lipoprotein cholesterol; HDLC = high density lipoprotein cholesterol.

^{a,b,c} Means in the same row with different superscripts differ ($P < 0.05$).¹ $n = 36$ (12 broilers \times 3 treatments) with 2 broilers per cage and 6 cages per treatment.

3.5. Cardiac antioxidant status

Broilers that received the SO diet had higher HO and MDA concentration as well as higher GSH-Px activity ($P < 0.05$) in the heart compared to those received FO and LD treatments, and LD treatment showed the lowest GSH-Px activity. There was no difference of SOD concentration among treatments ($P > 0.05$; Table 7).

Table 6Effects of different lipid sources on blood lymphocyte subsets in broilers.¹

Item ²	LD	SO	FO	SE	P-value
CD3 ⁺	34.01	37.05	35.03	3.02	0.54
CD4 ⁺	14.36 ^a	6.62 ^b	7.77 ^b	2.44	0.05
CD8 ⁺	13.41	12.99	11.22	2.51	0.17
CD4 ⁺ :CD8 ⁺	1.07 ^a	0.51 ^b	0.69 ^b	0.21	0.02

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard.

^{a,b}Means in the same row with different superscripts indicate significant difference ($P < 0.05$).¹ $n = 36$ (12 broilers \times 3 treatments), with 2 broilers per cage and 6 cages per treatment.² Lymphocyte subpopulation: cluster of differentiation of antigen positive T cells 3, 4, and 8 (CD3⁺, CD4⁺, and CD8⁺, respectively).**Table 7**Effects of different lipid sources on antioxidant status of broilers.¹

Item	LD	SO	FO	SE	P-value
HO, U/mg protein	742 ^b	995 ^a	603 ^b	60	<0.01
MDA, nmol/mg protein	3.76 ^b	10.06 ^a	4.74 ^b	0.74	<0.01
SOD, U/mg protein	84.21	99.51	83.16	5.68	0.18
GSH-Px, U/mg protein	453 ^c	616 ^a	534 ^b	21	<0.01

SO = 5% sesame oil; FO = 5% flaxseed oil; LD = 5% lard. HO = Hydroxyl radical; MDA = malondialdehyde; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase.

^{a,b}Means in the same row with different superscripts indicate significant difference ($P < 0.05$).¹ $n = 36$ (12 broilers \times 3 treatments) with 2 broilers per cage and 6 cages per treatment.

4. Discussion

4.1. Growth performance and nutrient digestibility

In this study, we found that a PUFA enriched diet (with either SO or FO) could improve the feed efficiency of broilers, while no impacts were observed on BWG and FI throughout the entire experiment. Similarly, Crespo and Esteve-Garcia (2001) reported that broilers receiving sunflower oil riched n-6 PUFA and FO riched n-3 PUFA presented better values for feed efficiency, and both of which were better than those of broilers receiving tallow. In contrast to these studies, López-Ferrer et al. (2001a, b) reported that the broilers' BWG was improved by 4% for FO or fish oil treatments compared to feed supplemented with tallow. Several studies reported no difference in the observed growth performance parameters of broilers when different fat sources were used (Zolitsch et al., 1997; Sanz et al., 2000; Pesti et al., 2002; Ghazalah et al., 2008; Yan et al., 2015). This partly agrees with the results of our previous study (Chen et al., 2012), in which we reported that the dietary inclusion of n-6 and n-3 PUFA improved BWG and feed conversion, while changing the ratio of n-3 to n-6 PUFA in a short term has no significant influence on broilers' growth performance. This discrepancy between different studies may be due to the utilized lipid sources and the level of PUFA in the diet, and could also be attributed to animal species.

Furthermore, in the present study, dietary supplementations with SO and FO enhanced the ATTD of crude fat, which may also be one of the reasons that led to an improvement in feed efficiency. The digestibility of the fat contained in the feed is influenced by the composition of fatty acids. Celebi and Utlu (2004) reported that unsaturated fats yield higher metabolizable energy compared to saturated fats. Animal fats have been reported to have lower digestibility than vegetable oils due to higher content of saturated fatty acids (Ketels and De Groot, 1989).

4.2. Relative organ weight

In our study, the difference in lipid sources did not significantly change the relative weights of the majority of organs (breast, leg, heart, liver, spleen, and bursa of Fabricius) but the abdominal fat. Feeding broilers with unsaturated fatty lipids (SO and FO) led to lower relative abdominal fat weight. Several studies showed that replacing saturated animal fats (e.g., tallow) with vegetable oils, such as sunflower oil, soybean oil, or linseed oil, that are rich in PUFA could reduce abdominal fat deposition in broilers (Newman et al., 2002; Wongsuthavas et al., 2007). A study reported by Crespo and Esteve-Garcia (2003) also suggested that reduced abdominal fat in broilers fed dietary vegetable oil is an overall consequence of elevated lipid oxidation, which prevents the simultaneous increase of endogenous fatty acid synthesis (Crespo and Esteve-Garcia, 2003).

4.3. Blood metabolites

A high-fat diet is a significant risk factor for the development of cardiovascular diseases due to the induction of hyperlipidemia, atherosclerosis, and disturbed lipid metabolism (Onody et al., 2003). Our findings indicated that higher dietary PUFA content (SO and FO) decreased the concentration of total cholesterol and triglyceride in serum. This result agrees with the findings of Crespo and Esteve-Garcia (2003) and Fébel et al. (2008), who also reported lower plasma cholesterol and triglyceride levels in broilers receiving diets with supplemented FO or sunflower oil compared to those receiving lard or tallow. The decrease of blood cholesterol via FO and SO supplementation may be attributed to a decrease of both free and esterified cholesterol. It is well established that ALA-enriched FO led to a higher cholesterol secretion in the bile, thus resulting in an increase in both cholesterol synthesis and turnover (Morise et al., 2004). Similar to cholesterol, a high level of triacylglycerol in the blood could damage vascular endothelial cells and thus lead to cardiac diseases (Suprijana et al., 1997). We furthermore found that the serum triglyceride level was lower in FO (n-3 PUFA) treatment than in SO (n-6 PUFA) treatment.

4.4. Lymphocyte subsets

Fatty acids have been shown to regulate both innate and adaptive immune responses by influencing the expression of co-stimulatory molecules and cytokines. Naive T lymphocytes are the main targets of n-3 PUFA (Jang et al., 2013) and several studies have established that n-3 PUFA effectively prevented inflammation via suppressing the activation of naive CD4⁺ T cells in response to various antigens (Pompos and Fritsche, 2002; Anderson and Fritsche, 2004; Zhang et al., 2006; Jang et al., 2013). In the current study, we found the blood CD4⁺ T cell population and the CD4⁺: CD8⁺ ratio were decreased by FO (n-3 PUFA enriched) and SO (n-6 PUFA enriched) treatments, which is in partial agreement with our previous study (Chen et al., 2012). It has been reported that lower CD4⁺: CD8⁺ ratios indicate enhanced cytotoxic activity by immune cells (Al-Khalifa et al., 2012). With respect to CD4⁺ T cells, n-3 PUFA inhibited CD4⁺ T cell activation at least to some extent by changing the organization of the lipid raft formation via n-3 PUFA accumulation (Calder, 2017; Erf, 2004; Kim et al., 2008; Hou et al., 2012). Since lipid rafts are important platforms for the clustering of different receptors and signaling molecules, disruption of the lipid raft could potentially inhibit signal transduction events that are required for T cell activation. Therefore, our result indicated that n-3 or n-6 PUFA addition can modulate the innate immune function by decreasing activation of the blood T lymphocyte subsets.

4.5. Cardiac antioxidant status

Differences in dietary lipid components have previously been suggested to influence antioxidant enzyme activity (Bhattacharya et al., 2003; Chen et al., 2012, 2015). In the current study, GSH-Px was increased via administration of a PUFA-enriched diet in broilers of both SO and FO groups compared to LD group. No difference in concentration of SOD was found among treatments. Interestingly, broilers receiving SO had higher HO and MDA concentrations in their hearts compared to those receiving FO and LD. Glutathione peroxidase and SOD have been reported to work in conjunction with reduced glutathione to scavenge free radicals such as H₂O₂ and lipid peroxides. Substantial evidence indicates that PUFA are capable of increasing GSH-Px activity (Demoz et al., 1992; Venkatraman et al., 1994; Bhattacharya et al., 2003). In addition, Chen et al. (2012) indicated that GSH-Px, GSH-ST, and SOD concentrations could be increased via enriching the diet with n-3 PUFA, while MDA should be decreased. This partly agrees with the findings of the current study. Fébel et al. (2008) reported that a PUFA-enriched diet resulted in an increase in the MDA level in the liver of broilers. Similarly, fish oil or linseed oil also increased MDA levels in the liver of hens compared to palm oil (Cherian et al., 1996). It is possible that both types and ratios of oil affect the fatty acid metabolism of broilers and use different biochemical pathway. However, this needs further investigation in a future study.

5. Conclusion

In summary, the results of present study can be concluded that modulation of the dietary PUFA content by adding vegetable oil (SO and FO) rather than LD improved the feed efficiency of broilers, but BWG and FI were not affected. Meanwhile, it modulates the innate immune function by decreasing the blood CD4⁺ T cell population and the CD4⁺: CD8⁺ ratio. Moreover, this can also improve lipid metabolism by reducing the serum concentrations of cholesterol and triglycerides.

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References

- Al-Khalifa H, Givens DI, Rymer C, Yaqoob P. Effect of n-3 fatty acids on immune function in broiler chickens. *Poult Sci* 2012;91:74–88.
- Anderson MJ, Fritsche KL. Dietary polyunsaturated fatty acids modulate in vivo, antigen-driven CD4⁺ T-cell proliferation in mice. *J Nutr* 2004;134:1978–83.
- AOAC. Official methods of analysis. 2003. Arlington, Virginia.
- Bautista-Ortega J, Goeger DE, Cherian G. Egg yolk omega-6 and omega-3 fatty acids modify tissue lipid components, antioxidant status, and ex vivo eicosanoid production in chick cardiac tissue. *Poult Sci* 2009;88:1167–75.
- 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;22:388–99.
- Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans* 2017;45:1105–15.
- Celebi S, Utlu N. Laying performance, serum lipoproteins, cholesterol and triglyceride of hens as influenced by dietary fat sources. *J Appl Anim Res* 2004;25:121–4.
- Chen W, Wang JP, Huang YQ. Effect 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;150:114–20.
- Chen W, Jiang YY, Wang JP, Yan BX, Huang YQ, Wang ZX. Effect of flaxseed on the fatty acid profile of egg yolk and antioxidant status of their neonatal offspring in Huoyan geese. *Animal* 2015 Nov;9:1749–55.
- Cherian G, Wolfe FW, Sim JS. Dietary oils and added tocopherols effects on egg or tissue tocopherols, fatty acids, and oxidative stability. *Poult Sci* 1996;75:423–31.

- Crespo N, Esteve-García E. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult Sci* 2001;80:71–8.
- Crespo N, Esteve-García E. Polyunsaturated fatty acids reduce insulin and very low density lipoprotein levels in broiler chickens. *Poult Sci* 2003;82:1134–9.
- Demoz A, Willumsen N, Berge RK. Eicosapentaenoic acid at hypotriglyceridemic dose enhances the hepatic antioxidant defense in mice. *Lipids* 1992;27:968–71.
- Erf GF. Cell-mediated immunity in poultry. *Poult Sci* 2004;83:580–90.
- Fébel H, Mézes M, Pálffy T, Hermá A, Gundel J, Lugasi A, et al. Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. *J Anim Physiol Anim Nutr* 2008;92:369–76.
- Fritsche KL, Byrge M, Feng C. Dietary omega-3 polyunsaturated fatty acids from fish oil reduce interleukin-12 and interferon-gamma production in mice. *Immunol Lett* 1999;65:167–73.
- Ghazalah AA, Abd-Elsamee MO, Ali AM. Influence of dietary energy and poultry fat on the response of broiler chicks to heat therm. *Int J Poult Sci* 2008;7:355–9.
- Gonzalez D, Mustacich DJ, Traber MG, Cherian G. Early feeding and dietary lipids affect broiler tissue fatty acids, vitamin E status, and cyclooxygenase-2 protein expression upon lipopolysaccharide challenge. *Poult Sci* 2011;90:2790–800.
- Han KN, Yang YX, Hahn TW, Kwon IK, Lohakare JD, Lee JK, et al. Effects of chitooligosaccharides supplementation on performance, nutrient digestibility, pork quality and immune response in growing-finishing pigs. *J Anim Feed Sci* 2007;16:607–20.
- Hou TY, Monk JM, Fan YY, Barhoumi R, Chen YQ, Rivera GM, et al. n-3 polyunsaturated fatty acids suppress phosphatidylinositol 4,5-bisphosphate-dependent actin remodelling during CD4+ T-cell activation. *Biochem J* 2012;443:27–37.
- Hulbert AJ, Turner N, Storlien LH, Else PL. Dietary fats and membrane function: implications for metabolism and disease. *Biol Rev Camb Philos Soc* 2005;80:155–69.
- James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 2000;71:343S–8S.
- Jang HY, Lim L, Lee SM, Park BH. Effects of n-3 PUFA on the CD4+ type 2 helper T-cell-mediated immune responses in Fat-1 mice. *Mol Nutr Food Res* 2013;58:365–75.
- Ketels E, De Groot G. Effect of ratio of unsaturated to saturated fatty acids of the dietary lipid fraction on utilization and metabolizable energy of added fats in young chicks. *Poult Sci* 1989;68:1506–12.
- Kim W, Fan YY, Barhoumi R, Smith R, McMurray DN, Chapkin RS. N-3 polyunsaturated fatty acids suppress the localization and activation of signaling proteins at the immunological synapse in murine CD4+ T cells by affecting lipid raft formation. *J Immunol* 2008;181:6236–43.
- Kong C, Adeola O. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. *Asian Austral J Anim Sci* 2014;27:917–25.
- López-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA. N-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influences on meat quality: fish oil. *Poult Sci* 2001a;80:741–752a.
- López-Ferrer S, Baucells MD, Barroeta AC, Galobart J, Grashorn MA. N-3 Enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil. *Poult Sci* 2001b;80:753–761b.
- Morise A, Sérougne C, Gripois D, Blouquit MF, Lutton C, Hermier D. Effects of dietary alpha linolenic acid on cholesterol metabolism in male and female hamsters of the LPN strain. *J Nutr Biochem* 2004;15:51–61.
- Newman RE, Bryden WL, Fleck E, Ashes JR, Buttemer WA, Storlien LH, et al. Dietary n-3 and n-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition. *Bri J Nutr* 2002;88:11–8.
- NRC. Nutrient requirements of poultry. 9th ed. Washington, DC: Natl. Acad. Press; 1994.
- Onody AM, Csonka C, Giricz Z, Ferdinandy P. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxidative formation in rat hearts. *Cardiovasc Res* 2003;58:663–70.
- Pesti GN, Bakalli RI, Qiao M, Sterling KG. A comparison of eight grades of fats as broiler feed ingredients. *Poult Sci* 2002;81:382–90.
- Pompos LJ, Fritsche KL. Antigen-driven murine CD4+ T lymphocyte proliferation and interleukin-2 production are diminished by dietary (n-3) polyunsaturated fatty acids. *J Nutr* 2002;132:3293–300.
- Sanz M, Flores A, Lopez-Bote CJ. The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. *Bri Poult Sci* 2000;41:61–8.
- Sijben JWC, Schrama JW, Pamentier HK, van der Poel JJ, Klasing KC. Effect of dietary polyunsaturated fatty acids on in vivo splenic cytokine mRNA expression in layer chicks immunized with *Salmonella typhimurium* Lipopolysaccharide. *Poult Sci* 2001;80:1164–70.
- Simopoulos AP. Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. *Mol Neurobiol* 2011;44:203–15.
- Kalakuntla Sridhar, Nagireddy Nalini K, Panda Arun K, Jatoth Narasimha, Thirunahari Raghunandan, Vangoor Ravinder R. Effect of dietary incorporation of n-3 polyunsaturated fatty acids rich oil sources on fatty acid profile, keeping quality and sensory attributes of broiler chicken meat. *Anim Nutr* 2017;3:386–91.
- Suprijana O, Terpstra AHM, Vanlith HA, Vantol A, Lemmens AG. Plasma lipids and apolipoproteins in rats fed diets with type of fat (fish oil, corn oil and fiber (pectin versus cellulose)) as variables. *Nutr Res* 1997;17:1187–97.
- Venkatraman JT, Chandrasekar B, Kim JD, Fernandes G. Effects of n-3 and n-6 fatty acids on the activities and expression of hepatic antioxidant enzymes in autoimmune-prone NZB×NZW F1 mice. *Lipids* 1994;29:561–8.
- Wang MZ, Ding LY, Wang JF, Wang HR, Yu LH. Effects of n-6: n-3 polyunsaturated fatty acid ratio on heterophil:lymphocyte ratio and T lymphocyte subsets in the peripheral blood of the Yangzhou gosling. *Poult Sci* 2011;90:824–9.
- Wongsuthavas S, Yuangklang C, Vasupen K, Mitchaothai J, Isrenau P, Wittayakun S, et al. Assessment of de-novo fatty acid synthesis in broiler chickens fed diets containing different mixtures of beef tallow and soybean oil. *Int J Poult Sci* 2007;6:800–6.
- Yan BX, Zhao R, Wang JP, Chen W, Huang YQ, Wang ZX, et al. Effect of different dietary oil sources on the growth performance, blood characteristics, fatty acid profiles, and expression of lipogenic genes in the liver of broiler chickens. *Czech J Anim Sci* 2015;60:487–97.
- Zhan ZP, Huang FR, Luo J, Dai JJ, Yan XH, Peng J. Duration of feeding linseed diet influences expression of inflammation-related genes and growth performance of growing-finishing barrows. *J Ani Sci* 2009;87:603–11.
- Zhang P, Kim W, Zhou L, Wang N, Ly LH, McMurray DN, et al. Dietary fish oil inhibits antigen-specific murine Th1 cell development by suppression of clonal expansion. *J Nutr* 2006;136:2391–8.
- Zolitsch W, Knaus W, Aichinger WF, Lettner F. Effect of different dietary fat sources on performance and carcass characteristics of broilers. *Anim Feed Sci Tech* 1997;66:63–73.