Effects of the replacement of corn oil with linseed oil on fatty acid composition and the expression of lipogenic genes in broiler chickens

W. Chen[1], R. Zhao[1,3], B.X. Yan[1], J.S. Zhang[1], Y.Q. Huang[1], Z.X. Wang[1], Y.M. Guo[2]

[1]College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, P.R. China
[2]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, P.R. China
[3]Henan Province Xinxiang City Livestock Husbandry and Veterinary Management Bureau, Xinxiang, P.R. China

ABSTRACT: The effect of dietary 18:2n-6/18:3n-3 ratio (by the replacement of corn oil with linseed oil) on n-3 polyunsaturated fatty acids (PUFA) enrichment in breast muscle of broiler chickens and the expression of lipogenic genes were investigated. Broiler chickens were fed *ad libitum* with diets containing 5% corn oil (CO), 3.75% corn oil + 1.25% linseed oil (CL1), 2.5% corn oil + 2.5% linseed oil (CL2), and 5% linseed oil (LO) based on the basic diets, respectively. Dietary 18:2n-6/18:3n-3 ratio did not affect 42-day body weight and 0–42-day feed conversion efficiency (feed/gain, $P > 0.05$) of broiler chickens, however, 5% linseed oil significantly increased 0–21-day feed conversion efficiency (feed/gain, $P < 0.05$) and decreased breast muscle weight (by 16%, $P < 0.05$) of broiler chickens. With the decrease of dietary 18:2n-6/18:3n-3 ratio, the enrichment of total n-3 PUFA, 18:3n-3, 20:5n-3, and 22:5n-3 increased linearly ($P < 0.01$), while the enrichment of total n-6 PUFA and 18:2n-6 decreased linearly ($P < 0.01$) in breast muscle of broiler chickens. Dietary corn oil increased the enrichment proportion of 20:4n-6 in a dosage-independent manner. Replacing 1.5% corn oil with linseed oil increased the enrichment proportion of 22:6n-3 ($P < 0.05$), but continuing to increase dietary linseed oil could not further elevate its deposition. Real-time quantitative RT-PCR was used to determine the expression of the mRNA levels of related genes. Dietary PUFA had insignificant effect on the expressions of *LPIN2*, WD, and tetratricopeptide repeats 1 (*WDTC1*) and ∆-6 fatty acid desaturase (*FADS2*) in both breast muscle and abdominal fat. The effect of dietary PUFA on the expression of *LPIN1* gene showed clear tissue dependence. Equivalent adding of corn oil and linseed oil could up-regulate the mRNA level of *LPIN1* in abdominal fat ($P < 0.01$). This study demonstrated that decreasing dietary 18:2n-6/18:3n-3 ratio promoted the deposition of desirable n-3 long chain PUFA in the edible tissue and influenced the expression of *LPIN1* in a tissue-dependent manner.

Keywords: birds; performance; essential fatty acid; gene expression

INTRODUCTION

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were found to be benefit for human health (Brenna et al. 2009). Recent literature provides clues that the ratio of dietary n-6/n-3 PUFA also affects human health. It was suggested that the ratio of n-6 to n-3 PUFA should be approximately 3 : 1, even to 1 : 1 (Kim et al. 2007). Because modern human diet is rich in n-6 PUFA, the ratio of human n-6 and n-3 PUFA is imbalanced seriously, which can be as high as

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15 : 1. It was found that the human dietary imbalance between n-6 and n-3 PUFA has led to the development of an adverse cardiovascular and metabolic disease (Toshimitsu et al. 2007), and contributed to the pathogenesis of non-alcoholic fatty liver disease. Therefore, interest has turned to the intake of specific fatty acids, notably n-6 and n-3 PUFA and their ratios, rather than total fat intake (Leitzmann et al. 2004) in recent years.

Dietary fatty acids are absorbed by monogastric animals and deposited in their tissues, n-6 and n-3 PUFAs are competitively metabolized by the same pathway (Luo et al. 2009). So, there is a considerable potential for the manipulation of the fatty acid profiles of poultry tissue by dietary means, thus to increase the supply of n-3 PUFA (especially EPA and DHA), and to regulate n-6/n-3 PUFA ratio to a level which is suitable for human consumption. It seems that both the type and the ratio of dietary oils affect the deposition of fatty acid in most animals (including chickens). So it is interesting to explore ways for increasing the conversion efficiency from alpha-linolenic acid (ALA, 18:3n-3) to EPA and DHA, and seeking suitable n-6/n-3 PUFA ratio in chickens.

Apart from being an important macronutrient, dietary fat has recently gained much prominence for its role in regulating gene expression in poultry (Royan et al. 2011; Zhang et al. 2011; Jing et al. 2013). *LPIN1*, *LPIN2*, *WDTC1*/*ADIPOSE*, and Δ-6 fatty acid desaturase (*FADS2*) were reported as lipid metabolism genes in human and mouse. *LPIN1* and *LPIN2* were the members of Lipin protein family, one novel family of Mg^{2+}-dependent phosphatidic acid phosphatases with bifunctional proteins that dephosphorylate phosphatidic acid to produce diacylglycerol and act in the nucleus to regulate gene expression in human and mouse (Reue and Zhang 2008). *WDTC1* is an evolutionarily conserved gene isolated from naturally occurring obese *Drosophila* homozygous for an adipose mutation, the homologous gene in mouse appears to inhibit the fat formation in a dosage-sensitive manner (Hader et al. 2003; Suh et al. 2007). *FADS2* gene is one member of the fatty acid desaturase gene family, a component of lipid metabolic pathway that converts human essential fatty acids including linoleic acid (LNA, 18:2n-6) and ALA (18:3n-3) into long chain (LC)-PUFA among species. It was reported that n-3 PUFA could reduce the *FADS2* expression in muscle of bull (Herdmann et al. 2010). The homologous genes for *LPIN1* (Wang et al. 2012), *LPIN2*, *WDTC1*/*ADIPOSE* (Chen et al. 2010), and *FADS2* in chickens have been reported by our lab recently.

Corn oil is enriched with LNA (18:2n-6), while linseed oil is enriched with ALA (18:3n-3). Till now, a certain amount of reports on the effects of diet rich in n-3 PUFA, especially 18:3 ALA (by the replacement of corn oil with linseed oil), on the enrichment of fatty acid in broiler chickens have been found, studies about its effects on the expression of lipid related genes have been focused on human and mice while few data have been found on broilers. Our objective was to evaluate the effects of dietary corn oil replaced by linseed oil (with different ratio of 18:2n-6/18:3n-3 PUFA) on the growth performances, carcass traits, fatty acid profiles, and gene expressions of *LPIN1*, *LPIN2*, *WDTC1*, and *FADS2* in chickens. The study should help reveal the mechanism of fatty acid deposition in poultry meat by diets.

MATERIAL AND METHODS

Birds, housing, and experimental design. The 240 female Cobb-500 broiler chickens at 1 day of age (body weight (BW) = 46 ± 4 g) were randomly allotted into four treatments (CO, CL1, CL2, and LO) with six replicates. The birds were raised in 24 cages (10 individuals per cage) under 24-hour light, standard temperature (33–35°C the first week, then decreasing by 2–3°C per week), humidity, and ventilation. Feed and water were provided *ad libitum*. The diets were formulated according to the broilers' requirements recommended by the NRC (1994) criteria (Table 1). CO, CL1, CL2, and LO diets contained 5% corn oil, 3.75% corn oil + 1.25% linseed oil, 2.5% corn oil + 2.5% linseed oil, and 5% linseed oil, respectively (Table 1). The BW of each broiler chicken and food consumption (per cage broilers) were recorded weekly. The fatty acid compositions of finisher diets were analyzed and listed in Table 2. The fatty acid composition of corn and linseed oil is presented in Table 3. The study was carried out according to the Henan Agricultural University Animal Care and Use Committee Guidelines.

Measurements and sampling. At day 42, 12 broiler chickens were randomly selected from each treatment (2 birds per replicate), weighed individually, and slaughtered. The heart, liver, breast muscle, leg muscle, and abdominal fat of broiler chickens were then rapidly removed and

Table 1. Ingredients and nutrient levels of the experimental diets (air-dry basis)

Ingredients/composition	Starter[1] (%)	Finisher (%)
Ingredients		
Corn	52.30	57.44
Soybean meal (CP 48%)	35.41	30.30
Corn gluten meal (CP 60%)	–	0.5
Variable oil[2]	5	5
Fish meal	3	3
Dicalcium phosphate	1.55	1.26
Limestone	1.26	1.20
Salt	0.35	0.35
DL-Methionine (98%)	0.14	0.05
L-Lysine-HCl (78%)	0.03	–
Choline chloride (60%)	0.26	0.20
Vitamin premix[3]	0.20	0.20
Trace mineral premix[4]	0.50	0.50
Total	100	100
Composition[5]		
ME (MJ/kg)	12.61	13.03
CP	21.5	20.0
Crude fat	7.65	7.77
Lysine	1.15	1.05
Met + Cys	0.90	0.72
Ca	1.00	0.92
Available P	0.45	0.40
Analytical composition (%)		
CP	21.40	19.88
Crude fat	7.60	7.71
Met + Cys	0.88	0.73
Ca	0.98	0.90
Available P	0.44	0.41

ME = metabolizable energy, CP = crude protein, Met = methionine, Cys = cystine

[1]starter diets, provided during weeks 0–3; finisher diets, provided during weeks 4–6

[2]5% variable oil, for CO, CL1, CL2, and LO treatments was 5% corn oil, 3.75% corn oil + 1.25% linseed oil, 2.5% corn oil + 2.5% linseed oil, and 5% linseed oil, respectively

[3]provided per kg of diet: vitamin A 1500 IU, vitamin D_3 200 IU, vitamin E 10 mg, vitamin K_3 0.5 mg, thiamin 1.8 mg, riboflavin 3.6 mg, vitamin B_6 3.5 mg, vitamin B_{12} 25 μg, niacin 35 mg, folic acid 0.55 mg, biotin 0.2 mg, pantothenic acid 10 mg

[4]provided per kg of diet: 40 mg Zn (as $ZnSO_4 \cdot 7H_2O$), 60 mg Mn (as $MnSO_4 \cdot 5H_2O$), 80 mg Fe (as $FeSO_4 \cdot 7H_2O$), 10 mg Cu (as $CuSO_4 \cdot 5H_2O$), 0.35 mg I (as KI), 0.15 mg Se (as $Na_2SeO_3 \cdot 5H_2O$)

[5]nutritional composition was the calculated value

weighed. About 50 g breast muscle of each chicken was stored at –20°C for fatty acid analysis. About 10 g breast muscle (*pectoralis major*) and abdominal fat tissues were snap-frozen in liquid nitrogen and transferred to –80°C for RNA extraction, respectively.

Fatty acid content. Lipids were extracted from the diet (3 g) and breast muscle (20 g) samples using chloroform/methanol (1 : 1 vol/vol) by a modified method of Folch et al. (1957). Fatty acid methyl esters were prepared for gas chromatography determination using KOH/methanol (Luo et al. 2009).

The 6890N-5973 gas chromatograph-mass spectrometer (Agilent Technologies Inc., Wilmington, USA) equipped with a quadrupole mass spectrometer detector, a hydrogen flame ionization detector, and a quartz capillary chromatographic column HP-5MS (30 m × 0.25 mm × 0.25 μm) was used in this experiment. The injector and quadrupole mass spectrometer detector were kept at 250°C and 150°C, respectively. Nitrogen was used as carrier gas with a flow rate of 1.0 ml/min, split ratio was 1 : 10. The column was programmed as follows: 60°C for 3 min, increase to 260°C (4°C/min), and held constant for 50 min. The fatty acids were identified by comparing the area of the peaks with those of known standards (Sigma Chemical Co., St. Louis, USA).

RNA isolation and cDNA synthesis. Total RNAs were extracted from *pectoralis major* and abdominal fat tissues of broiler chickens by using RNAiso™ Plus (TaKaRa Biotechnology, Dalian, China). Total RNA concentration and purity were determined using a NanoVue™ Plus spectrophotometer (GE Healthcare, Freiburg, Germany). The 2 μg total RNA was used to synthesize the cDNA using random hexamer primers with PrimeScript® RT reagent kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions. The synthesized first strand cDNA was stored at –20°C for use.

Real-time quantitative RT-PCR (qRT-PCR). Taqman qRT-PCR was used to determine the expression of *LPIN1* (GenBank accession No. HM473175), *LPIN2* (GenBank accession No. JN012098), *WDTC1* (GenBank accession No. XM_417728), and *FADS2* (GenBank accession No. EF636888.3). The *18S rRNA* (GenBank accession No. FM164514.1), *β-actin* (*ACTB*, GenBank accession No. L08165), and *GAPDH* (GenBank accession No. NM_204305.1) were used as the internal controls. Primer sequences are listed in Table 4. The primers/probes for qRT-PCR were

Table 2. Fatty acid composition (each fatty acid/total fatty acid; %) in finisher diets (measured value)[1]

Items	CO[2]	CL1	CL2	LO
14:0	0.42	0.39	0.43	0.42
16:0	18.02	18.95	17.34	16.05
17:0	2.67	2.57	2.7	3.22
18:0	2.6	2.64	2.58	2.75
SFA[3]	23.71	24.55	23.05	22.94
16:1	0.35	0.33	0.38	0.35
18:1	37.07	36.27	35.98	35.97
20:1	2.24	2.15	2.3	2.14
MUFA[4]	39.66	38.75	38.66	38.46
18:2 LNA	18.76	18.59	16.06	14.7
20:2	1.43	1.56	1.64	1.72
20:3	2.46	2.15	2.48	2.4
20:4 ARA	3.7	3.56	4.97	4.37
n-6 PUFA[5]	26.35	25.86	25.15	23.19
18:3 ALA	0.3	1.22	3.57	5.46
20:5 EPA	1.57	1.49	1.41	1.55
22:5 DPA	2.17	2.03	2.09	2.12
22:6 DHA	6.24	6.11	6.08	6.28
n-3 PUFA[6]	10.28	10.85	13.15	15.41
PUFA[7]	36.63	36.71	38.30	38.6
Total fatty acid	100	100.01	100.01	100
18:2n-6/18:3n-3	62.53	15.24	4.49	2.69
n-6/n-3 PUFA[8]	2.56	2.38	1.91	1.50

[1]considering the oil was added to the diets in the same pattern in starter and finisher diets, here only the fatty acid compositions of finisher diets were presented

[2]CO = 5% corn oil group, CL1 = 3.75% corn oil + 1.25% linseed oil group, CL2 = 2.5% corn oil + 2.5% linseed oil group, LO = 5% linseed oil group; n = 6

[3]Σ14:0 + 16:0 + 17:0 + 18:0; [4]Σ16:1 + 18:1 + 20:1; [5]Σ18:2 + 20:2 + 20:3 + 20:4; [6]Σ18:3 + 20:5 + 22:5 + 22:6; [7]Σn-3 PUFA + n-6 PUFA; [8]n-6 PUFA/n-3 PUFA

optimized according to the reference Gangisetty and Reddy (2009). The standard curve for each gene was constructed with purified PCR products generated using the specific primers of objective genes and reference genes. Purified PCR products were quantified using NanoVue™ Plus spectrophotometer (GE Healthcare) and serially diluted tenfold to span the range of predicted Ct values that would be obtained from experimental samples. The qRT-PCR was performed using the 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA), in a total volume of 25 µl with 1 µl cDNA, 12.5 µl TaqMan Universal PCR Master mix (TaKaRa), 260nM of each specific forward and reverse primer, and 250nM TaqMan™ fluorescent probe. Each sample (including no template control) had three replications. For the unstable expression of *18S rRNA*, *ACTB*, and *GAPDH* genes among treatments, finally, we normalized gene expression based upon the constant amount of RNA and cDNA amplified. This method has been proposed to be the most reliable standardization of quantitative measurement of mRNA expression (Bustin 2002; Lkhagvadorj et al. 2010). For each gene, the relative mRNA level = average copy number for each treatment/the copy number for the treatment with the lowest average value among treatments (including breast muscle and abdominal fat tissues). The mRNA level for the treatment with the lowest average value was normalized to 1.

Statistical analysis. Data was analyzed using the SAS software (Statistical Analysis System, Version 8.0, 2000). General linear model I was used to analyze carcass traits including breast

Table 3. Sequences of primers and probes

Gene	Sequences of primer and probe	Product length (bp)
LPIN1 (HM473175)	F: 5'GAGATGGATAACAATCAGGAG3' R: 5'GAAGATACACTGCTGTCCAG3' Probe: 5'CTGTCTACGTCCCCCATCCTGTCT3'	140
LPIN2 (JN012098)	F: 5'CCGGAATCAACCAAGATAAGC3' R: 5'ATCAGAGCTGAGGATGACTC3' Probe: 5'CTGGAACATCCCAGAACAGCTACC3'	105
WDTC1 (XM_417728)	F: 5'GGAGACTTGTTGGCCTCTG3' R: 5'GAATGCGGCAAGAACTTGAC3' Probe: 5'CCATTGTCTGGGATCCTCTGCACC3'	131
FADS2 (EF636888.3)	F: 5'ATTGGACACCTTAAGGGTGC3' R: 5'AGCATGTTCACATCTGGGTC3' Probe: 5'CCATCGTCACTTCCAACATCACGC3'	104
18S rRNA (FM165414.1)	F: 5'GATCCATTGGAGGGCAAGTC3' R: 5'CAGCTCGATCCCAAGATCC3' Probe: 5'CAGCAGCCGCGGTAATTCCAGC3'	113
ACTB (L08165)	F: 5'AGAGAGAAGATGACACAGATC3' R: 5'GTCCATCACAATACCAGTGG3' Probe: 5'CCTTCAACACCCCAGCCATGTATG3'	117
GAPDH (NM_204305.1)	F: 5'TTGTCAGCAATGCATCGTGC3' R: 5'TGATGGCATGGACAGTGGTC3' Probe: 5'CCACCAACTGCCTGGCACCCTT3'	108

muscle weight, leg muscle weight, heart weight, liver weight, and abdominal fat weight, where BW at day 42 was taken as a covariate to analyze the effect of dietary lipids on these carcass traits. Other data was analyzed by the general linear model II. The LSD method was used for multiple comparisons among the four groups. $P \leq 0.05$ was considered significant.

Model I :

$$y_i = \mu + G_i + b(W_i - \overline{W})e_i$$

Model II:

$$y_i = \mu + G_i + e_i$$

where:

y_i = observation value of the traits
μ = population mean
G_i = treatment effect (i = 1, 4)
b = regression coefficient for body weight
W_i = individual body weight
$\overline{W}$ = average body weight
e_i = random error

RESULTS

Fatty acid composition of diets. The fatty acid compositions of the finisher experiment diets are shown in Table 2. The n-3 PUFA (especially for ALA, 18:3n-3) content was obviously differ-

Table 4. Fatty acid composition of corn oil and linseed oil[1]

Items	C14:0	C16:0	C18:0	C18:1	C18:2LNA	C18:3ALA	C20:4ARA	C20:5EPA	C22:6DHA	n-6/n-3 PUFA[2]
Linseed oil		6.4	4.5	21.0	17.4	50.6	0.1			0.35
Corn oil		12.2	2.2	27.5	57.0	0.9				63.33

[1]fatty acid composition of corn oil and linseed oil was determined by gas chromatography of fatty acid methyl esters according to GB/T 17377-1998

[2]n-3 PUFA = Σ18:3 + 20:5 + 22:6; n-6 PUFA = Σ18:2 + 20:4; n-6/n-3 PUFA = n-6 PUFA/n-3 PUFA

Table 5. Effects of dietary oil lipids on growth traits of broiler chickens

Items	CO[1]	CL1	CL2	LO	RMSE	*P*-value
42-day body weight (kg)	2.32	2.31	2.31	2.25	0.12	0.786
0–21-day body gain (g)	585ab	636[a]	616[a]	556[b]	48	0.073
0–21-day feed/gain (g/g)	1.42[ab]	1.35[b]	1.39[b]	1.46[a]	0.05	0.019
21–42-day feed/gain (g/g)	1.80	1.83	1.81	1.77	0.08	0.657
0–42-day feed/gain (g/g)	1.70	1.70	1.70	1.69	0.06	0.991

RMSE = root mean-square error

[1]CO = 5% corn oil group, CL1 = 3.75% corn oil + 1.25% linseed oil group, CL2 = 2.5% corn oil + 2.5% linseed oil group, LO = 5% linseed oil group; $n = 30$

[a,b]within the same row, means with different letter represent $P \leq 0.05$, means with the same letter or no letter represent $P > 0.05$

ent among the four treatments. The replacement of corn oil with linseed oil increased n-3 PUFA (especially for ALA, 18:3n-3) content while decreased n-6 PUFA (especially for LNA, 18:2n-6), 18:2n-6/18:3n-3 ratio, and n-6/n-3 PUFA ratio in diets. ALA (18:3n-3) levels in CL1, CL2, and LO treatments were 4.06, 11.9, and 18.3 times of those in CO treatment, respectively. LNA (18:2n-6) levels in CL1, CL2, and LO treatments were 99, 85.6, and 78.4% of those in CO group, respectively. Dietary 18:2n-6/18:3n-3 ratios were 62.53, 15.24, 4.49, and 2.69, while dietary n-6/n-3 PUFA ratios were 2.56, 2.38, 1.91, and 1.50 in CO, CL1, CL2, and LO groups, respectively (Table 2).

Effects of dietary corn oil replaced by linseed oil on performance of broiler chickens. The effects of the replacement of dietary corn oil with linseed oil on growth traits of broiler chickens are shown in Table 5. The replacement of the dietary corn oil with linseed oil did not significantly affect 42-day body weight, 21–42-day and 0–42-day feed conversion (feed/gain, $P > 0.05$) of broiler chickens, but significantly affected 0–21-day feed conversion (feed/gain, $P < 0.05$) and weakly affected 0–21-day body gain ($P < 0.1$). The feed efficiency (feed/gain) and the body gain of CL1 and CL2 broilers in 0–21 days were significantly higher than those of LO broilers ($P < 0.05$) (Table 5). The effects of the replacement of dietary corn oil with linseed oil on carcass traits of broiler chickens are shown in Table 6. The replacement of dietary corn oil with linseed oil did not significantly affect the traits including heart weight, liver weight, leg muscle weight, and abdominal fat weight ($P > 0.05$). However, the breast muscle weight of LO broilers was by about 16% lower than that of animals in the other groups ($P < 0.05$) (Table 6).

Effects of the replacement of corn oil by linseed oil on fatty acid composition of breast muscle. Fatty acid compositions of total fat in breast muscle of broiler chickens are presented in Table 7. Corn oil replaced by linseed oil had no significant effect on the deposition of total saturated fatty acid (SFA) content in breast muscle. However, the 14:0 content in birds of CO group was significantly higher than that of animals in the other groups ($P < 0.01$), the 18:0 content in LO group was the most abundant ($P < 0.05$). The 16:0 was the major

Table 6. Effects of dietary oil lipids on carcass traits of broiler chickens

Group	CO[1]	CL1	CL2	LO	RMSE	*P*-value
Body weight (kg)	2.33	2.30	2.38	2.25	0.11	0.502
Breast muscle weight (g)	455[a]	458[a]	442[a]	388[b]	37	0.034
Leg muscle weight (g)	303	300	336	315	30	0.200
Heart weight (g)	7.8	8.2	8.8	9.2	1.2	0.272
Liver weight (g)	42.8	40.5	41.6	42.9	3.8	0.682
Abdominal fat weight (g)	29.1	34.2	38.5	34.9	8.1	0.279

RMSE = root mean-square error

[1]CO = 5% corn oil group, CL1 = 3.75% corn oil + 1.25% linseed oil group, CL2 = 2.5% corn oil + 2.5% linseed oil group, LO = 5% linseed oil group; $n = 6$

[a,b]within the same row, means with different letter represent $P \leq 0.05$, means with the same letter or no letter represent $P > 0.05$

SFA in breast muscle as that in diets, its proportion in breast muscle was not significantly changed by dietary fatty acid.

The replacement of dietary corn oil with linseed oil unchanged total monounsaturated fatty acid (MUFA) enrichment in breast muscle of broiler chickens. However, 20:1 proportion of CO group was lower than that of CL1 group ($P < 0.05$) in breast muscle, the differences among CL1, CL2, and LO groups were not significant (Table 7).

The replacement of corn oil with linseed oil had no significant effect on the total PUFA proportion, but it significantly changed the proportion of n-6 PUFA ($P < 0.01$), n-3 PUFA ($P < 0.01$), 18:2n-6/18:3n-3 ratio ($P < 0.01$), and n-6/n-3 PUFA ratio ($P < 0.01$) in breast muscles of broiler chickens. These items presented significant differences among the four treatments (Table 7). With the decrease of dietary 18:2n-6/18:3n-3 ratio by the replacement of corn oil with linseed oil, the enrichment of n-3 PUFA in breast muscle linearly increased ($P < 0.01$). Comparing with CO group, n-3 PUFA contents in breast muscle of CL1, CL2, and LO broilers were elevated by 50.1, 81.0, and 123.8%, respectively (Table 7). On the contrary, the content of n-6 PUFA ($P < 0.01$), 18:2n-6/18:3n-3 ratio ($P < 0.01$), and n-6/n-3 ratio ($P < 0.01$) in breast muscle of corresponding treatments linearly decreased (Table 7). The 18:2n-6/18:3n-3 ratios of CO, CL1, and CL2 groups were 32.84, 5.54, and 2.27 times of those in LO group, respectively. The n-6/n-3 PUFA ratios of CO, CL1, and CL2 groups

Table 7. Fatty acid composition (each fatty acid/total fatty acid; %) of breast muscle

Items	CO[1]	CL1	CL2	LO	RMSE	*P*-value
14:0	0.32[a]	0.24[b]	0.22[b]	0.24[b]	0.04	0.007
16:0	16.34	13.76	15.01	15.56	0.72	0.108
17:0	3.01	2.48	2.87	2.54	0.41	0.141
18:0	6.61[b]	6.22[b]	6.45[b]	7.02[a]	0.41	0.026
SFA[2]	26.29	22.70	24.55	25.35	0.86	0.168
16:1	2.32	1.93	2.02	2.11	0.25	0.195
18:1	25.60	26.05	25.92	26.62	1.86	0.890
20:1	1.41[b]	1.81[a]	1.85[a]	1.89[a]	0.20	0.011
MUFA[3]	28.74	28.87	29.12	30.57	1.67	0.404
18:2 LNA	24.35[a]	22.30[a]	18.06[b]	14.98[c]	1.51	0.000*
20:2	1.60	1.86	1.69	1.42	0.22	0.061
20:3	1.71	2.01	1.97	1.73	0.23	0.238
20:4 ARA	7.05[a]	7.08[a]	6.28[a]	4.42[b]	0.91	0.003
n-6 PUFA[4]	35.23[a]	32.74[b]	27.99[c]	21.88[d]	1.46	< 0.001*
18:3 ALA	0.50[d]	2.72[c]	5.37[b]	10.06[a]	1.07	< 0.001*
20:5 EPA	1.39[c]	2.02[b]	2.40[b]	3.10[a]	0.30	< 0.001*
22:5 DPA	1.15[b]	1.25[ab]	1.32[ab]	1.43[a]	0.20	0.020*
22:6 DHA	6.60[b]	8.43[a]	8.49[a]	7.09[ab]	0.93	0.04
n-3 PUFA[5]	9.76[d]	14.65[c]	17.67[b]	21.84[a]	0.85	< 0.001*
PUFA[6]	43.04	43.48	45.67	40.60	5.38	0.676
Total Fatty Acid	99.97	100.03	100.02	100		
18:2n-6/18:3n-3	48.61[a]	8.20[b]	3.37[c]	1.48[c]	2.28	< 0.01*
n-6 /n-3 PUFA[7]	3.62[a]	2.27[b]	1.58[c]	1.01[d]	0.25	< 0.001*

RMSE = root mean square error

[1]CO = 5% corn oil group, CL1 = 3.75% corn oil + 1.25% linseed oil group, CL2 = 2.5% corn oil + 2.5% linseed oil group, LO = 5% linseed oil group; $n = 6$

[2]Σ14:0 + 16:0 + 17:0 + 18:0; [3]Σ16:1 + 18:1 + 20:1; [4]Σ18:2 + 20:2 + 20:3 + 20:4; [5]Σ18:3 + 20:5 + 22:5 + 22:6; [6]Σn-3 PUFA + n-6 PUFA; [7]n-6 PUFA/n-3 PUFA

[a–d]within the same row, means with different letter represent $P \leq 0.05$, means with the same letter represent $P > 0.05$

*item linearly significant by One-Way ANOVA analysis with SPSS software (Version 13.0)

were 3.58, 2.24, and 1.56 times of those in LO group, respectively (Table 7).

For n-6 PUFA, LNA (18:2n-6) was the predominant PUFA in diets. Its deposition was abundant in breast muscle of broiler chickens, too (Table 7). The enrichment proportion of LNA (18:2n-6, $P < 0.01$) in breast muscle of broiler chickens linearly decreased with the increase of dietary linseed oil (enriching 18:3n-3). The content of LNA (18:2n-6) and arachidonic acid (ARA, 20:4n-6) in breast muscle of the LO group was significantly lower than that of other groups, and the LNA (18:2n-6) content of CL2 group was significantly lower than that of CO and CL1 groups (Table 7).

For n-3 PUFA, the enrichment of ALA (18:3n-3) in breast muscle of broiler chickens was dramatically elevated (linearly, $P < 0.01$) with the increase of dietary linseed oil (18:3n-3) content (Table 7). The enrichment of ALA (18:3n-3) in CL1, CL2, and LO groups was 5.44, 10.74, and 20.12 times of that in the CO group, respectively. The enrichment of EPA (20:5n-3, $P < 0.01$) and DPA (22:5n-3, $P < 0.05$) in breast muscle of broiler chickens was also linearly elevated with the increase of dietary linseed oil/18:3n-3 content, the contents of EPA (20:5n-3) and DPA in LO group were significantly higher than those in CO group ($P < 0.05$, Table 7). For the enrichment of DHA in breast muscle, the proportion of DHA in CO group was significantly lower than that in CL1 and CL2 groups, while there were not significant differences among other groups (Table 7).

Expression of lipid metabolism related genes in breast muscle and abdominal fat. In this study, it was found that the mRNA levels of three reference genes (*18S rRNA*, *ACTB,* and *GAPDH*) exhibited significant differences between breast muscle and abdominal fat, dietary oil significantly affected the expression of *18S rRNA*, *ACTB,* and *GAPDH* (Table 8). So the expression values of objective genes were normalized based upon the constant amount of RNA and cDNA amplified. The relative mRNA levels of *LPIN1*, *LPIN2*, *WDTC1,* and *FADS2* were presented in Table 8. The mRNA levels of *LPIN1* ($P < 0.01$), *LPIN2* ($P < 0.01$), and *FADS2* ($P < 0.01$) in abdominal fat of broiler chickens were markedly

Table 8. Effects of different dietary oil lipids on relative expression of genes in breast muscle and abdominal fat

Gene	Tissue	CO[1]	CL1	CL2	LO	RMSE	*P*-value	Tissue[2] ($n = 24$)
LPIN1	breast muscle	1.00	1.18	1.47	1.57	0.62	0.440	1.30
	abdominal fat	2.26^{ab}	1.33^{a}	6.33^{c}	4.66^{bc}	1.83	0.001	3.53*
LPIN2	breast muscle	1.26	1.00	1.76	1.21	0.55	0.233	1.27
	abdominal fat	4.80	6.25	7.34	6.85	2.16	0.306	6.31*
WDTC1[3]	breast muscle	2.19	1.76	2.96	2.59	0.98	0.275	2.33*
	abdominal fat	1.00	1.25	1.27	1.13	0.32	0.517	1.77
FADS2[4]	breast muscle	1.14	1.00	1.30	1.50	0.41	0.224	1.23
	abdominal fat	3.64	4.16	4.39	4.69	1.94	0.850	4.22*
GAPDH	breast muscle	260.73^{ab}	212.01^{a}	388.26^{b}	137.06^{a}	81.50	0.002	235.767*
	abdominal fat	1.45	1.61	1.09	1.00	0.38	0.053	1.303
ACTB	breast muscle	1.41^{a}	1.00^{a}	1.62^{a}	2.67^{b}	0.53	0.000	1.691
	abdominal fat	2.37	3.39	3.36	2.57	1.13	0.356	2.946*
18S rRNA	breast muscle	1.95^{ab}	2.21^{ab}	1.64^{a}	2.88^{b}	0.55	0.015	2.232*
	abdominal fat	1.00	1.09	1.22	1.41	0.39	0.399	1.176

RMSE = root mean square error

[1]CO = 5% corn oil group, CL1 = 3.75% corn oil + 1.25% linseed oil group, CL2 = 2.5% corn oil + 2.5% linseed oil group, LO = 5% linseed oil group; $n = 6$

[2]comparison between the mRNA level of breast muscle and abdominal fat

[3]*WDTC1* = WD and tetratricopeptide repeats 1

[4]*FADS2* = Δ-6 fatty acid desaturase

[a–c]within four groups, means with different letter represent $P \leq 0.05$, with the same letter or no letter represent $P > 0.05$; for each gene, the relative mRNA level of each treatment was normalized by that with the lowest average copy number

*$P < 0.01$

higher than those in breast muscle, which were about 3, 5, and 3 times of those in breast muscle, respectively; while *WDTC1* had a higher expression level in breast muscle than that in abdominal fat ($P < 0.01$). Dietary corn oil replaced with linseed oil had no significant effect on the expression of *LPIN2*, *WDTC1,* and *FADS2* in both breast muscle and abdominal fat of broiler chickens, while the expression of *LPIN1* was significantly affected by dietary oil in abdominal fat ($P < 0.01$). The *LPIN1* mRNA level of the CL2 group in abdominal fat was the highest, being significantly higher than that in CO group ($P < 0.01$) and CL1 group ($P < 0.05$), and LO group value was significantly higher than that of CL1 group (Table 8).

DISCUSSION

In this study, we confirmed that the change of dietary 18:2n-6/18:3n-3 PUFA did not affect 42-day body weight, 0–42-day feed conversion efficiency (feed/gain), leg muscle weight, abdominal fat weight of broiler chickens as reported in previous studies (Martin et al. 2007; Qi et al. 2010; Benatmane et al. 2011). We observed that 5% linseed oil significantly decreased the breast muscle weight by about 16% (Table 6), it indicated that linseed oil as a single oil lipid provider in diets had negative effect on the development of breast muscle in broiler chickens, which might be related with that chickens of LO group had low body gain in the early growth stage (0–21 days, Table 5). This result was inconsistent with some reports that feeding a linseed diet had no significant effect on breast muscle weight (Martin et al. 2007; Qi et al. 2010). The inconsistence might be due to the differences among the trial designs of those researches. The relatively higher breast muscle weight in CO, CL1, and CL2 treatments may be related with that the diets containing corn oil were more beneficial to the breast muscle development of broiler chickens, or that the antinutritional factors in linseed oil had some negative effects on the breast muscle development of broilers (Bond et al. 1997). It was reported by Brue and Latshaw (1985) that daily heat production was lesser when corn oil was fed to broiler chickens.

The MUFA and SFA have double origins, directly from the diet and by *de novo* synthesis, while PUFA can only have a dietary origin (Villaverde et al. 2006; Ferrini et al. 2010). Our research indicated that varying the ratio of 18:2n-6/18:3n-3 in diets had a weak effect on 16 : 1 and 18 : 1 concentrations in tissues as observed by Qi et al. (2010). We found that increasing dietary 18:3n-3 in a low-dosage manner (replacing 1.5% dietary corn oil with linseed oil) could increase the deposition of 20 : 1 in breast muscle; however, further adding linseed oil in diets did not change 20 : 1 proportion in breast muscle of broiler chickens, which reflected that the deposition of 20 : 1 appeared to be tightly regulated by feedback inhibition of n-3 PUFA.

Our study confirmed that the fatty acid compositions of total n-3 PUFA (including ALA, 18:3n-3) and n-6 PUFA (including LNA, 18:2n-6) in poultry meat were affected by the dietary fat source in a dosage-dependence manner as Qi et al. (2010) reported. It may be related with that a competition existed between the n-3 and n-6 fatty acid families for metabolism with an excess of one causing a significant decrease in the conversion of the other (Schmitz and Ecker 2008).

Individuals eating diets containing large amounts of linoleic acid will deposit this component readily in the tissue and complex lipids where it will be elongated and desaturated to ARA. In this study, the ARA (20:4n-6) levels of treatments containing corn oil with different proportions (CO, CL1, and CL2, enriching with LNA) were higher than that of LO group in breast muscle, but the ARA (20:4n-6) levels were similar among CO, CL1, and CL2 groups, which suggested that dietary LNA (18:2n-3) could increase ARA deposition in meat in a dosage-independent manner. LO group had the lowest ARA (20:4n-6), which also reflected that high amounts of ALA (18:3n-3) could decrease the conversion of LNA (18:2n-6) to ARA (20:4n-6) (Coetzee and Hoffman 2003; He et al. 2009).

Chickens have the ability to convert a small portion of dietary ALA (18:3n-3) to its derivatives. Conversion of ALA (18:3n-3) to EPA (20:5n-3) involves sequential Δ6-desaturation, chain elongation, and Δ5- desaturation. EPA is, in turn, converted to DPA (Olomu and Baracos 1991; Lopez-Ferrer et al. 1999; Coetzee and Hoffman 2003). As several reports (Haug et al. 2007; Ponte et al. 2008; Qi et al. 2010), our study (Table 7) showed that reducing dietary 18:2n-6/18:3n-3 ratio linearly increased the enrichment of EPA and DPA, which implied that dietary n-3 PUFAs gave rise to higher tissue levels of EPA and DPA in a dosage-dependent manner. It was reported that when the ratio of human dietary LNA and ALA was 4 : 1 or even lower, more ALA could convert to EPA (Indu 1992).

DHA is synthesized from DPA. In this study (Table 7), we observed that decreasing dietary 18:2n-6/18:3n-3 ratio (from 48.6 to 8.2) increased the enrichment proportion of DHA (22:6n-3), but further decreasing 18:2n-6/18:3n-3 ratio in diets could not increase the enrichment of DHA (22:6n-3). It indicated that DHA synthesis appeared to be tightly regulated by feedback inhibition as observed by Leitzmann et al. (2004). This also reflects that ALA conversion is severely constrained for DHA. It has been reported that increasing the availability of dietary ALA (18:3n-3) did not obligatorily enhance DHA synthesis and even might decrease tissue DHA concentrations (Leitzmann et al. 2004). This study showed that CL2 chickens had relatively high LC-PUFA content (especially DHA; Table 7) and breast muscle weight (Table 6). It seems that feeding diets containing equivalent corn oil and linseed oil would be a good choice for producing broiler chickens enriching with LC-PUFA.

PUFA including n-3 PUFA or its derivatives can directly act at the level of nucleus to influence the transcription activity of transcription factors and in turn the transcription of their target genes responding to diverse cellular processes including adipogenesis (Sampath and Ntambi 2005; Luo et al. 2009). So the expression levels of the genes involved in lipogenic metabolism were assessed in our study. We observed that increasing dietary n-3 PUFA increased the expression of chicken *LPIN1* in abdominal fat, but further increasing n-3PUFA in diet could not up-regulate the mRNA level of chicken *LPIN1*. It indicated that the expression of chicken *LPIN1* appeared to be tightly regulated by feedback inhibition in a tissue-specific manner. We also noticed that the mRNA level of *LPIN1* in abdominal fat of CL2 chickens was the highest among treatments, suggesting a co-regulation of enriching n-3PUFA diet with genes involved in LC-PUFA and cholesterol synthesis.

Several studies indicated the expressions of *LPIN1, LPIN2, FADS2,* and *WDTC1* were under the regulation of fatty acids in other species. Lai et al. (2008) found that the effect of human *WDTC1* variation on obesity had a strong interaction with MUFA intake. Martin et al. (2007) studied the expression of *LPIN1*, *LPIN2,* and *FADS2* in the same population. They found that comparing with a saturated fatty acid diet, *LPIN1* transcription in liver was negatively regulated by PUFA containing diets in wild-type mice whereas not in *PPARα-/-*mice, *FADS2* was negatively regulated by PUFA containing diets in both wild-type mice and *PPARα-/-*mice, while *LPIN2* was negatively regulated by several types of PUFA diets. *FADS2* was considered the rate-limiting enzyme in the formation of LC-PUFAs and could promote the conversion of ALA (18:3) to EPA (20:5) (Innis 2003; Nakamura and Nara 2004; Park et al. 2009; Glaser et al. 2010). Theil and Lauridsen (2007) found that the expression of hepatic *FADS2* gene in porcine was influenced by the n-6 to n-3 PUFA ratio. In this study, we failed to detect the change of chicken *LPIN2*, *FADS2,* and *WDTC1*, which may be related with that the expression changes of these genes induced by diet PUFAs may be tissue/genotype-specific, or oil type/dosage-dependent. More works need to be done for unveiling the molecular mechanism about the deposition of fatty acid.

CONCLUSION

Dietary 18:2n-6/18:3n-3 ratio did not affect the growth performances of broilers in 0–42 days. The deposition of desirable n-3 LC-PUFA, 18:2n-6/18:3n-3, and n-6/n-3 PUFA ratios in breast muscle were more favourable with a lower ratio of dietary 18:2n-6/18:3n-3, while comparing with LO diet, increasing the 18:2n-6/18:3n-3 ratio significantly increased the breast muscle weight of broilers. Our research helps offer a valid way to meet the demand of producer and consumer for meat that is nutritionally beneficial. The significant change of the expression of *LPIN1* implied that *LPIN1* may be involved in the regulation of PUFA content. A better study of *LPIN1* would help understanding the mechanism underlying the deposition of PUFA in poultry meat.

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Corresponding Author

Prof. YuMing Guo, Ph.D., China Agricultural University, College of Animal Science and Technology, Beijing, 100193, P.R. China
Phone: +420 227 010 389, e-mail: guoyum@cau.edu.cn