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Research Article

Effect of Dietary Fatty Acid Pattern on Growth Performance, Carcass Characteristics, Fatty Acid Profile, and Serum Biochemistry Parameters in Broiler Chickens

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Abstract

This study was undertaken to investigate the effect of dietary fat supplementation with linseed oil and/or pig lard on performance, carcass characteristics, fatty acid profile and serum biochemistry parameters in broiler chickens. In the study, 240 one-day-old Cobb 500 broiler chickens were randomly distributed to one control and three experimental dietary groups: diet supplemented with pig lard; diet supplemented with linseed oil, and; diet supplemented with a 1:1 ratio of lard to linseed oil. The fat sources influenced serum biochemistry parameters and fatty acid profiles of drumsticks with thighs, but had no influence on growth performance or carcass characteristics. With dietary linseed oil, eicosapentaenoic acid and docosahexaenoic acid increased ($P<0.05$) in meat from drumsticks with thighs. Supplementation with pig lard significantly ($P<0.05$) increased the saturated and monounsaturated fatty acids in drumsticks with thighs. In conclusion, dietary incorporation of linseed oil and pig lard during starter, grower and finisher phases can enrich broiler chickens meat with n-3 PUFA. This study has clearly shown that linseed oil in broiler nutrition provided the best n-6/n-3 ratio.

Keywords: *Broiler chickens, Carcass characteristics, n-3 PUFA rich oils, Lard*

Etlik Piliçlerde Diyetteki Yağ Asitlerinin Büyüme Performansı, Karkas Özellikleri, Yağ Asidi Profili ve Serum Biyokimyasal Parametreleri Üzerine Etkisi

Öz

Bu çalışma broiler tavuklarda diyette keten tohumu yağı ve/ya domuz yağı ilavesinin performans, karkas özellikleri, yağ asidi profili ve serum biyokimyasal parametreleri üzerine etkisini araştırmak amacıyla yapılmıştır. Çalışmada 240 adet bir günlük Cobb 500 broiler tavuk rastgele olarak kontrol ile domuz yağı katkılı diyet, keten tohumu yağı katkılı diyet ve 1:1 oranında domuz yağı ve keten tohumu katkılı diyet olmak üzere üç deneysel diyet grubuna ayrıştırılmıştır. Yağ katkıları kalçalı butta yağ asidi profilini ve serum biyokimyasal parametrelerini etkilerken büyüme performansı ve karkas özellikleri üzerine etki etmedi. Keten tohumu yağı kalçalı but etinde eikosapentaenoik asit ve dokosahekzaenoik asit miktarını artırırdı ($P<0.05$). Domuz yağı katkısı kalçalı but etinde anlamlı derecede doymuş ve tekli doymamış yağ asidi miktarını artırdı ($P<0.05$). Sonuç olarak, başlangıç, büyüme ve bitirme evrelerinde diyete keten tohumu yağı ve domuz yağı ilavesi broiler tavuklarda etteki çoklu doymamış yağ asidi miktarını artırır. Bu çalışma, broiler yeminde keten tohumu yağının en iyi n-6/n-3 oranını sağladığını göstermiştir.

Anahtar sözcükler: *Etlik piliç, Karkas özellikleri, n-3 çoklu doymamış yağ asidinden zengin yağ, Domuz yağı*

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INTRODUCTION

The success of the poultry industry depends on enhancing growth performance and carcass characteristics, reducing fat deposition of growing broiler chickens and improving the products offered to consumers. Nutrition plays a strong role in growing broiler chickens [1]. Poultry meat is considered healthier, with a relatively lower fat content compared with other animal meat [2,3]. Fats from animal (beef tallow, pig lard, fish oil etc.) or plant origins (sunflower oil, linseed oil, corn oil, coconut oil etc.) are added to commercial broiler chicken feeds as a source of fatty acids and a source of energy. Supplementation and manipulation of the fatty acid composition is implemented for nutritional purposes and human health. Previous studies in broiler chickens have shown different relationships between the fatty acid contents of diets and tissues, especially for breast and thigh meat [1,4-7]. The concentration of n-3 PUFA in broiler chicken tissues depends mainly on the fatty acid composition of the diet [1]. The n-3 PUFA fatty acids decrease proinflammatory eicosanoids and inflammatory biomarkers in broiler chickens [8]. Plant oils (e.g., linseed oil) are rich in a-linolenic acid (ALA), which is the metabolic precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [9]. There is a potential to enrich the human diet with n-3 PUFA by modifying poultry feeding practices to satisfy human health, as the ratio of dietary oils and fats in the animals' diets affects the deposition of fatty acids in broiler meat. Recent studies have shown that dietary imbalance of the n-6/n-3 PUFA ratio can affect human health, especially with high n-6/n-3 PUFA ratios [1,10]. Supplementation of linseed oil at up to 4.5% in broiler diet increased the conversion of ALA to EPA and DHA in breast meat. Similarly, the total n-3 PUFA, including EPA and DHA in meat, were significantly increased as a result of decreases in the n-6/n-3 PUFA ratio or the addition of animal fats [11,12].

The positive effect on the nutritional value of chicken meat, achieved by replacing or supplementing fat with n-3 rich linseed oil and other plant oils, has been documented [13-15]. Little information is, however, available on the effects of replacing the saturated fatty acid (SFA) in rendered animal fat with pig lard. Thus, this study was undertaken to investigate the effect of dietary supplementation of linseed oil (n-3 PUFA rich) and/or pig lard (moderate SFA and MUFA) on broiler chicken growth performance, carcass characteristics, and fatty acid profile of drumsticks with thighs, and serum biochemistry parameters.

MATERIAL and METHODS

Animal Ethics

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade.

Housing and Trial Duration

A total of 240 one-day-old broiler chickens of both sexes and the same origin (Cobb 500) were used in this study during a 42-day period. Birds were randomly assigned to one of four dietary treatments (control and three experimental groups), each having 6 replicates (10 birds in each replicate). Birds were placed in an environmentally controlled room (stocking density 6 birds/m^2) with 5 cm thick sawdust. The temperature in the room was 32°C from days 1 to 5, and then gradually lowered to 22°C on day 21. This temperature was maintained until the end of the study. Humidity was 45-50% RH. The lighting of the rooms was 24 h. Water and feed were supplied *ad libitum* throughout the study.

Experimental Diets

From the start of the trial, each group of broilers was fed with one of four different diets, which comprised the same basal diet, but differed only in additive supplementation (lard and linseed oil) *(Table 1)*.

Basal diet was formulated to meet the maintenance and growth requirements of the animals used in the study. Broiler chickens were fed from day 1 to day 42 in three phases with three nutritionally different concentrated feed mixtures, namely, starter (up to 10 days), grower (11-21 days), and finisher (22-42 days) mixtures. The broilers in the control group were given a diet without lard and linseed oil. The other three treatment groups were given the same diet as fed to the control group (C group) but were supplemented with lard and/or linseed oil. Commercially prepared linseed oil (Granum^R, Serbia) and lard were added to the feed for the experimental groups *(Table 1)*.

Feedstuff Composition

The ingredients and chemical composition (calculated analyses) of the basal diets are listed in *Table 2*. All components of the diet were analyzed for moisture [16], crude protein [17], total lipids [18], ash [19], crude fiber [20], calcium [21], and phosphorus [22]. The content of nitrogen-free matter was determined by the formula:

BEM = 100 - (% moisture + % ash + % cellulose + % protein + % fat).

Performance and Carcass Characteristics of Broilers

Growth performance of broiler chickens was evaluated by recording body weight, weight gain, and feed conversion ratio during the 42-day experimental period. Weight gains of broiler chickens were recorded on a pen basis, the uneaten feed was discarded, and fresh feed replaced in feeders at the end of each day. Feed conversion ratio (FCR) was calculated as the amount of feed consumed per unit of body weight gain.

At the end of the study, animals were transported to the slaughterhouse and then individually weighed, electrically

Table 1. *Three broiler chicken diets supplemented with different sources of fat (%)*

Fat	Diets											
	Starter				Grower				Finisher			
	C	L	LO	L+LO	C	L	LO	L+LO	C	L	LO	L+LO
Lard	-	1		0.50	-	2.50	-	1.25	-	5		2.50
Linseed oil	-	-	1	0.50	-	-	2.50	1.25	-		5	2.50
Total	-	1	1	1	-	2.50	2.50	2.50	-	5	5	5

C- *Control diet without supplemented fat;* ***L-*** *Experimental diet supplemented with lard;* ***LO-*** *experimental diet supplemented with linseed oil;* ***L+LO-*** *experimental diet supplemented with 1:1 ratio of lard to linseed oil*

Table 2. *Formulation and calculated contents of the basal diets for broilers*

Ingredients (%)	Starter (up to 10 days)		Grower (days 11-21)		Finisher (days 22-42)	
	C	E	C	E	C	E
Maize	50.85	49.85	44.15	41.65	44.95	39.95
Wheat	-	-	10.00	10.00	15.00	15.00
Soy grits	15.00	15.00	17.00	17.00	20.00	20.00
Soybean meal	12.40	12.40	1.00	1.00	1.00	1.00
Soybean cake	17.00	17.00	23.30	23.30	14.70	14.70
Monocalcium phosphate	1.20	1.20	1.00	1.00	0.90	0.90
Chalk	1.60	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Premix**	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Adsorbent	0.20	0.20	0.20	0.20	0.20	0.20
Parameter	**Calculated Values**					
Metabolic energy MJ/kg	12.69	12.71	13.01	13.03	13.11	13.13
Total ash	6.77	6.77	6.66	6.66	6.16	6.15
Total lipids	6.61	6.76	7.39	7.51	7.20	7.29
Crude fiber	3.89	3.89	3.97	3.97	3.44	3.44
Crude protein	22.24	22.22	21.14	21.13	19.62	19.62
Moisture	10.41	10.39	10.20	10.18	10.54	10.53
Lysine	1.50	1.49	1.42	1.42	1.17	1.17
Methionine+ cysteine	0.81	0.81	0.80	0.80	0.76	0.76
Tryptophan	0.31	0.31	0.29	0.29	0.27	0.27
Calcium	1.01	1.01	0.94	0.94	0.90	0.90
Phosphorus	0.59	0.59	0.56	0.56	0.54	0.54
NFE	50.08	49.96	50.63	50.55	53.04	52.97

*** Mineral- vitamin premix provided per kg of diet: Vit. A 12.999 IJ, Vit. D_3 4 950 IJ, Vit. E 75 mg, Vit. K_3 3 mg, Vit. B_1 3 mg, Vit. B_2 7.95 mg, Vit. B_6 4.05 mg, Vit. B_{12} 0.0195 mg, Vit. C 19.95 mg, Biotin 0.15 mg, Niacin 60 mg, Calcium pantothenate 15 mg, Folic acid 1.95 mg, Iodine 1.0005 mg, Selenium 0.3 mg, Choline chloride 399.9 mg, Iron 39.99 mg, Copper 15 mg, Manganese 99.9 mg, Zinc 99.9 mg, Methionine 2100 mg, Lysine 1200 mg*
C- *Control group;* ***E-*** *Experimental group (L, LO, L+LO)*

stunned and immediately slaughtered by severance of the jugular veins. Subsequently, animals were processed following standard industrial techniques, and hot carcass weight was recorded. During the first 24 h post-mortem, carcasses were stored in a ventilated cold room at 2°C, after which cold carcass weight was measured. After chilling, carcasses of broiler chickens from each group were separated into breast, drumsticks with thighs, wings, neck, and back with pelvis. The different carcass parts were weighed.

Meat Quality

At 24 h post-mortem, 6 drumsticks with thigh meat (i.e., muscle) samples from each experimental group were packed in polyethylene bags until analysis of moisture [23], lipid [24], protein [25], and ash [26]. The pH of drumsticks with thighs was determined using a digital pH meter (TESTO 205, Germany).

Fatty Acid Analysis

The fatty acid profiles of the diets are shown in *Table 3*. Total lipids for fatty acid determination were extracted from homogenized samples (diets and drumsticks with thighs) with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, GmbH, Idstein, Germany). After evaporation of solvent until dryness under a stream of nitrogen, total lipids were converted to fatty acid methyl esters (FAME) by trimethylsulfonium hydroxide. FAMEs were determined using a Shimadzu 2010 gas chromatograph equipped with flame ionization detector (FID) and cyanopropyl HP-88 capillary column (100 m x 0.25 mm x 0.20 mm) [27]. Temperature of the injector and detector were 250°C and 280°C, respectively. FAMEs were identified on the basis of relative retention time, compared with the relative retention times of the individual compounds in a standard mixture of fatty acid methyl esters, Supelco Component 37 FAME mix (Supelco, Bellefonte, USA). Quantification of fatty acids was determined relative to an internal standard, heneicosanoic acid, C21:0. The level of fatty acids (diets and drumsticks with thighs) is expressed as a percentage (%) of the total identified fatty acids. According to Fuchs et al.[28], indexes of lipid quality (atherogenic index (AI), index of thrombogenicity (TI), and hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH) were calculated using the following equations:

Table 3. *The fatty acid composition (%) of broiler chicken diets*

Fatty Acid	Diets											
	Control			L			LO			L+LO		
	I	II	III	I	II	III	I	II	III	I	II	III
C14:0	0.72	0.59	0.03	0.94	0.85	1.26	0.63	0.51	0.57	0.67	0.64	0.94
C16:0	11.05	11.72	10.62	14.13	15.00	15.65	10.30	9.27	8.29	10.74	12.16	10.18
C16:1	0.09	0.10	0.09	0.62	0.80	1.08	0.12	0.09	0.11	0.20	0.39	0.62
C17:0	0.08	0.09	0.08	0.14	0.13	0.20	0.09	0.07	0.06	0.09	0.10	0.14
C18:0	4.25	4.69	4.15	6.32	6.15	8.01	4.24	3.77	3.60	4.28	4.58	6.32
C18:1cis-9	24.63	24.72	26.74	32.87	31.58	32.60	24.84	24.79	26.93	25.36	27.60	27.22
C18:1cis-11	1.02	1.01	0.93	1.28	1.56	1.80	0.98	0.81	0.71	1.06	1.13	1.28
C18:2n-6	52.80	51.63	51.08	32.90	33.02	30.96	44.48	46.03	46.44	47.08	44.81	43.45
C20:0	0.42	0.46	0.44	0.35	0.39	0.31	0.40	0.34	0.33	0.39	0.37	0.35
C18:3n-6	0.04	0.04	0.05	0.15	0.09	1.09	0.11	0.13	0.31	0.30	0.12	0.15
C18:3n-3	3.71	3.79	3.51	7.04	7.26	5.92	11.06	11.85	10.14	7.51	5.90	6.09
C20:1	0.15	0.19	0.14	0.46	0.63	0.36	0.16	0.15	0.18	0.20	0.27	0.46
C20:2n-6	0.03	0.05	0.04	0.24	0.18	0.30	0.05	0.09	0.10	0.09	0.11	0.24
C22:0	0.69	0.63	0.50	0.38	0.33	-	0.50	0.49	0.51	0.50	0.04	0.38
C20:3n-6	0	0.00	1.31	1.74	1.57	-	1.34	1.33	1.30	1.09	1.48	1.74
C20:3n-3	0.07	0.03	0.04	0.08	0.06	0.06	0.03	0.03	0.02	0.04	0.06	0.08
C20:5n-3	0.09	0.07	0.08	0.07	0.06	0.10	0.19	0.08	0.11	0.08	0.09	0.07
C24:0	0.16	0.19	0.17	0.12	0.11	0.10	0.24	0.17	0.17	0.15	0.15	0.12
C22:6n-3	-	-	-	0.17	0.23	0.20	0.24	0.00	0.12	0.17	0.00	0.17
SFA	17.37	18.37	15.99	22.38	22.96	25.53	16.40	14.62	13.53	16.82	18.04	18.43
MUFA	25.89	26.02	27.90	35.23	34.57	35.84	26.10	25.84	27.93	26.82	29.39	29.58
PUFA	56.74	55.61	56.11	42.39	42.47	38.63	57.50	59.54	58.54	56.36	52.57	51.99
n-6	52.87	51.72	52.48	35.03	34.86	32.35	45.98	47.58	48.15	48.56	46.52	45.58
n-3	3.87	3.89	3.63	7.36	7.61	6.28	11.52	11.96	10.39	7.80	6.05	6.41
n-6/n-3*	13.66	13.30	14.46	4.76	4.58	5.15	3.99	3.98	4.63	6.23	7.69	7.11

C- *Control diet without supplemented fat;* ***L-*** *Experimental diet supplemented with lard;* ***LO-*** *Experimental diet supplemented with linseed oil;* ***L+LO-*** *Experimental diet supplemented with 1:1 ratio of lard to linseed oil;* ***I-*** *Starter diets;* ***II-*** *Grower diets;* ***III-*** *Finisher diets;* ******* *ratio*

AI = (C12:0 + 4xC14:0 + C16:0)/(Σn-3 PUFA + Σn-6 PUFA + ΣMUFA)

TI = (C14:0 + C16:0 + C18:0)/(0.5xΣMUFA + 0.5xΣn-6PUFA + 3xΣn-3PUFA + Σn-3PUFA/Σn-6PUFA)

HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:6n-3)/(C14:0+C16:0)

Serum Biochemistry Analyses

Blood samples were collected from the slaughtered birds in non heparinized tubes. The samples were centrifuged at 3000 rpm for 15 min, and the serum obtained was stored at -20°C until analysis. HDL-cholesterol and triglyceride were determined by the auto analyzer (CentroLIA LB 961, Berthold Technologies, Germany) using commercially available kits purchased from Accurex biomedical company.

Statistical Analyses

Statistical analysis of the results was conducted using software GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All parameters were described by means and standard error of means (SEM). One-way ANOVA with Tukey's post hoc test was performed to assess the significance of differences ($P<0.05$) among control and experimental groups.

RESULTS

In the current study, the effects of differing broiler chickens' dietary fatty acid sources on meat from drumsticks with thighs, growth performance, carcass characteristics, fatty acid profile and serum biochemistry parameters (cholesterol and triglyceride levels) in broiler chickens were studied.

Table 4 shows body weight, weight gain, feed-conversion ratio and feed consumption of broilers receiving diets containing lard and/or linseed oil. The body weight did not differ across the treatment groups. However, numerically higher final body weight was observed in the group with added linseed oil than in the other groups. The weight gain for two periods (days 1-21 and days 1-42) was higher in the group with added linseed oil. Broiler chickens fed only with basal diet showed a higher feed-conversion ratio (from day 1 to day 42) than did those supplemented with linseed oil or/and pig lard.

The chemical composition (protein, lipids, moisture and ash), pH, and temperatures of the meat are shown in *Table 4*. Protein in meat from the control group was 17.79%, and it was 17.66% in meat from the group supplemented with lard, 17.11% in meat from the group supplemented with linseed oil and 17.84% in meat from the group supplemented with a 1:1 ratio of lard to linseed oil. The lipid content was the lowest in the lard group (4.24%), while in the control, linseed oil and lard plus linseed oil groups it was 4.63%, 4.74% and 4.76%, respectively. The moisture content in meat from the four groups was approximately the same and ranged from 77.21% (control) to 77.83% (lard).

Table 4. *Growth performance and carcass characteristics of broiler chickens in different diet groups*

Parameters		Groups				SEM	P value
		C	L	LO	L+LO		
BW		2452	2433	2551	2405	44.94	0.020
Weight gain (g)	Day 1-10	254.24	262.16	260.36	265.95	4.72	0.161
	Day 1-21	1034.60	1050.44	1061.24	1048.95	19.71	0.100
	Day 1-42	2354.60	2354.52	2486.57	2363.80	42.65	0.580
FCR	Day 1-10	1.35	1.27	1.30	1.33	0.01	0.0004
	Day 1-21	1.51	1.44	1.47	1.49	0.009	0.0002
	Day 1-42	1.86	1.77	1.79	1.81	0.01	0.0001
Feed consumption (g)	Day 1-10	343.22	332.94	338.46	353.71	3.60	0.14
	Day 1-21	1562.24	1512.63	1560.02	1562.93	8.21	0.0010
	Day 1-42	4379.55	4167.50	4450.96	4278.48	21.38	0.63
Meat characteristics	pH_{45min}	6.32	6.18	6.23	6.24	0.056	0.082
	pH_{24h}	6.07	6.02	6.03	5.95	0.032	0.170
	T_{45min} (°C)	34.27	33.01	33.23[c]	30.42	0.657	<0.0001
	Moisture (%)	77.21	77.83	77.78	77.75	0.189	0.438
	Lipid (%)	4.63	4.24	4.74	4.76	0.124	0.239
	Protein (%)	17.79	17.66	17.11	17.84	0.175	0.251
	Ash (%)	0.82[a]	0.84[a]	0.85	0.90[b]	0.009	0.012

Data are means and SEM (n=60 per group). Within a row, means with different letters significantly differ; [a,b] P<0.05; ***C****- Control diet without supplemented fat;* ***L****- Experimental diet supplemented with lard;* ***LO****- Experimental diet supplemented with linseed oil;* ***L+LO****- experimental diet supplemented with 1:1 ratio of lard to linseed oil;* ***BW****- The average body weight, g/live bird;* ***FCR****- Feed conversion ratio;* ***SEM:*** *Standard error of means*

Table 5. *Serum biochemistry parameters of broiler chickens fed different diets*

Parameters (mmol/L)	Day	Groups				SEM	P value
		C	L	LO	L+LO		
HDL-Cholesterol	Day 20	5.09[a]	2.80[b]	2.60[b]	2.90[b]	0.026	<0.0001
	Day 42	2.40	2.30	2.10	2.21	0.019	<0.0001
Triglyceride	Day 20	0.88	1.03	0.94	1.23	0.003	<0.0001
	Day 42	0.41	0.28	0.44	0.40	0.002	<0.0001

Data are means and SEM. Within a row, means with different letters significantly differ, [a,b] P<0.05; ***C-*** *Control diet without supplemented fat;* ***L-*** *Experimental diet supplemented with lard;* ***LO-*** *Experimental diet supplemented with linseed oil;* ***L+LO-*** *Experimental diet supplemented with 1:1 ratio of lard to linseed oil;* ***SEM:*** *Standard error of means*

Table 6. *Fatty acid profile (%) of meat from drumsticks with thighs of broilers fed different diets for 42 days*

Fatty Acid	Groups				SEM	P value
	C	L	LO	L+LO		
C14:0	0.82	1.03	0.72	0.75	0.020	<0.0001
C15:0	0.08[a]	0.09[a]	0.07[b]	0.08[a]	0.002	0.002
C16:0	17.46	21.11	18.13	20.32	0.201	<0.0001
C16:1	2.04	2.98	3.30	3.13	0.112	0.0003
C17:0	0.16[a]	0.19[a]	0.12[b]	0.15[a]	0.004	<0.0001
C18:0	6.70	7.47	5.96	6.98	0.183	0.011
C18:1cis-9	28.44	37.44	32.25	34.41	0.262	<0.0001
C18:1cis-11	1.27	2.10	1.43	1.66	0.026	<0.0001
C18:2n-6	38.36[a]	23.90[b]	26.64[b]	25.12[b]	0.362	<0.0001
C20:0	0.15	0.13	0.15	0.13	0.005	0.368
C18:3n-6	0.21[a]	0.18[a]	0.14[b]	0.13[b]	0.006	<0.0001
C18:3n-3	2.47	1.21	8.40	4.71	0.098	<0.0001
C20:1	0.30[a]	0.50[b]	0.23[a]	0.35[a]	0.011	<0.0001
C20:2n-6	0.45[a]	0.35[b]	0.28[b]	0.32[b]	0.014	0.0002
C22:0	0.08[a]	0.07[a]	0.11[b]	0.07[a]	0.006	0.014
C20:3n-6	0.30[a]	0.23[b]	0.25[b]	0.31[a]	0.011	0.016
C20:3n-3	0.05	0.05	0.19	0.12	0.006	<0.0001
C20:5n-3	0.10	0.58	0.60	0.50	0.055	0.033
C22:5n-3	0.31[a]	0.25[a]	0.64[b]	0.50[b]	0.019	<0.0001
C22:6n-3	0.18	0.25	0.34	0.34	0.030	0.009
SFA	25.44	30.09	25.25	28.48	0.318	<0.0001
MUFA	32.05[a]	43.02[b]	37.20[a]	39.54[a]	0.330	<0.0001
PUFA	42.51	26.89	37.55	31.98	0.442	<0.0001
n-6	39.27[a]	24.66[b]	27.31[b]	25.87[b]	0.347	<0.0001
n-3	3.24	2.23	10.25	6.11	0.123	<0.0001
n-6/n-3*	12.18	11.24	2.67	4.25	0.255	<0.0001

Data are means and SEM. Within a row, means with the different letter significantly differ; [a,b] P<0.05; ***C-*** *Control diet without supplemented fat;* ***L-*** *Experimental diet supplemented with lard;* ***LO-*** *Experimental diet supplemented with linseed oil;* ***L+LO-*** *experimental diet supplemented with 1:1 ratio of lard to linseed oil* ***SEM:*** *Standard error of means;* * ratio

Ultimate meat pH measured 24 h post-mortem did not change markedly when lard or linseed oil were supplemented in the diet, although the pH tended to decrease when diet was supplemented with lard plus linseed oil (L+LO) *(Table 4)*. However, the pH of meat from drumsticks with thighs was not significantly different (P>0.05) from meat from the other dietary groups. Temperatures after 45 min were significantly decreased (P<0.05) in the lard plus linseed oil group compared to those in the control, lard, and the linseed oil groups.

Table 7. *Effect of different diets on atherogenic index (AI), index of thrombogenicity (TI), and hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH) of broiler chicken meat (42 d)*

Parameter	Groups				SEM	P value
	C	L	LO	L+LO		
AI	0.28[a]	0.36[b]	0.27[a]	0.32[b]	0.031	<0.0001
TI	0.13[a]	0.12[a]	0.10[b]	0.12[a]	0.009	0.034
HH	3.80[a]	1.40[b]	3.61[a]	2.87[b]	0.190	<0.0001

Data are means and SEM. Within a row, means with the different letter significantly differ, [a,b] P<0.05, C- Control diet without supplemented fat; ***L****- experimental diet supplemented with lard;* ***LO****- Experimental diet supplemented with linseed oil;* ***L+LO****- Experimental diet supplemented with 1:1 ratio of lard to linseed oil;* ***SEM:*** *Standard error of means*

The mean values of serum biochemistry parameters (HDL-cholesterol and triglyceride) of broiler chickens are shown in *Table 5*. Supplementation of lard or supplementation of lard plus linseed oil produced significant differences (P<0.05) in the mean concentration of HDL-cholesterol in the broilers' sera. At 42-days-old, cholesterol levels were lower in broilers fed diets with lard (L) or linseed oil (LO). There was a decrease in the triglyceride levels in the broilers fed diet supplemented with lard compared with the control group and other experimental groups (day 42).

Increasing the level of dietary linseed oil and lard in broiler chickens serum reduced the animals' blood cholesterol concentrations *(Table 5)*.

The fatty acid composition of meat (i.e., muscle) from drumsticks with thighs in relation to dietary oil and fat is illustrated in *Table 6*. It was observed that the fatty acid composition of meat from drumsticks with thighs reflected the fatty acid profile of the experimental diet. The major fatty acids detected in the meat from drumsticks with thighs of the lard (L) group and affected by the dietary fat were C14:0, C15:0, C18:0, C18:1n-9, C18:1n-11. The concentration of oleic acid (C18:1 n-9) in the meat from drumsticks with thighs of broilers fed the lard diet was significantly higher than those groups fed linseed oil (LO) or lard plus linseed oil (L+LO) diets. Broilers fed linseed oil (LO) had a significantly higher concentration of C18: 3n-3 (8.40%) compared with L (1.21%), C (2.47%) and L+LO (4.71%) groups. The concentration of C20:0 in meat from drumsticks with thighs did not differ significantly among the dietary treatments (P>0.05). Significantly higher concentrations of EPA, docosapentaenoic acid (DPA) and DHA were found in broiler chickens fed linseed oil than in the control group (P<0.05). The different dietary fat sources influenced (P<0.05) the mono unsaturated fatty acid (MUFA) deposition in the meat from drumsticks with thighs. Similarly, dietary replacement of lard had influence on SFA levels in the meat (drumsticks with thighs). A significant increase in the concentration of total SFA and MUFA was found in broiler chickens fed the lard diet compared with other groups. The PUFA were increased in meat from drumsticks with thighs of the control group (without any supplementation). In the meat from drumsticks with thighs, total n-6 PUFA content was lowered with lard supplementation compared with other groups. Dietary supplementation with linseed oil, a rich n-3 PUFA source, improved the total n-3 PUFA in the meat (LO<C<L+LO<L). The highest total n-3 PUFA deposition in the meat from drumsticks with thighs was recorded with linseed oil supplementation.

The n-6/n-3 ratio differed among sources of fat. Broiler chickens fed linseed oil had lower n-6 /n-3 (2.67) compared with animals fed control (12.18), lard (11.24) and lard plus linseed oil (4.25) diets.

There were significant treatment differences 5 observed in atherogenic index (AI), index of thrombogenicity (TI) and hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH) of meat from drumsticks with thighs in the present study *(Table 7)*. A lower thrombogenic index of meat from linseed oil-supplemented animals with the higher n-3 level has been reported [28]. In the current study, a significantly lower hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH) of meat from drumsticks with thighs was found in the lard group (P<0.05). Thus, a significantly higher 5 atherogenic index was found in in the lard group compared to the control group. Lower values of atherogenic index and index of thrombogenicity indicate a healthier ratio regarding the higher content of fatty acids that inhibit the aggregation of platelets and reduce the potential for coronary diseases. In contrast, a higher hypocholesterolemic/hypercholesterolemic fatty acid ratio indicates a more nutritionally suitable fatty acid profile [29].

DISCUSSION

Many studies indicated no difference in broilers' growth performance parameters when the animals were fed different fat sources [30-32]. Also, Andreotti et al.[33] demonstrated no effects on performance when broiler chickens were fed from days 21 to 49 with diets containing lard. The growth performance of broilers fed on n-3 PUFA-enriched diets (linseed oil) was not different from those fed on a control diet. These results are in agreement with several previous studies [34-36]. The weights of basic cuts of broiler carcasses (breast, drumsticks with thighs, wings, neck, and back with pelvis) did not significantly differ among the compared groups *(Table 4)*. Supplementation of linseed oil improved

the yield of carcass cuts, where the linseed oil group had higher weight of breast, drumsticks with thighs, wings, neck, and back with pelvis compared to other groups, but differences were not significant.

The chemical composition (protein, moisture, and fat) of meat from different groups of broiler chickens was not significantly different ($P>0.05$), but the ash content was significantly different ($P<0.05$). Zelenka et al.[37] noticed no variation in chemical composition of meat from drumsticks with thighs due to dietary incorporation of linseed oil in broiler chicken diet.

The concentration of triglyceride was decreased in the broiler chicken group fed diet supplemented with lard (L) compared with the other groups. These results are in accordance with Ibrahim et al.[1], who reported that increasing dietary n-3 fatty acids in broiler chicken diet reduced cholesterol. El-Katcha et al.[6] stated that feeding broilers on a diet with a 1:5 ratio of n-3/n-6 PUFA reduced the cholesterol content of broiler chicken meat. The effect of dietary fatty acid profile on cholesterol levels of broiler chickens was also reported by Maraschiello et al.[38]. These authors found higher levels of cholesterol for broiler chickens fed lard than for those fed plant oils. Peebles et al.[39] concluded that added lard fed to broiler chickens in starter diets produced responses in serum low density lipoprotein cholesterol concentrations.

After breast meat, drumsticks with thighs is a very important cut in broiler chickens. As a result, many studies focused on the nutritional aspects of broiler chicken meat by assessment of drumsticks with thighs. Earlier studies showed clear correlations between dietary fatty acid composition and the fatty acid composition of chicken meat [1,4,13,40]. Previous studies reported that fatty acid composition of drumsticks with thighs reflected the differences in dietary fatty acid (from plants and animals) intake more than breast meat of broiler chickens [1,4,13,40]. This is related to the higher total lipid content in drumsticks with thighs. The fatty acid composition of meat from drumsticks with thighs is shown in *Table 6*. Broiler chickens fed lard presented higher values of SFA, mainly myristic, palmitic, and stearic acids (C14:0, C16:0, and C18:0), than those fed linseed oil. Also, broiler chickens fed with linseed oil had a substitution of these SFA (mainly stearic acid) with linoleic acid (18:2n-6). The deposition of linoleic (C18:2n-6) and a-linolenic (C18:3n-3) acids in drumsticks with thighs was more correlated with their content in the feed. Thus, broiler chickens fed linseed oil presented the lowest values of stearic acid in drumsticks with thighs, in accordance with Scaife et al.[40]. Rosa [41] used three types of plant and animal oil (linseed, soybean, and a mixture of linseed and fish) at inclusion levels of 1, 2 and 3% in broiler diets and observed that the composition of fatty acids in the rations influenced the fatty acid profile of drumsticks with thighs. In the present study, the proportion of EPA and DHA found in drumsticks with thighs were higher in broiler chickens fed linseed oil. The concept of synergism between animal fats and vegetable oils has been recognized for many years [42-44]. Animal fats such as lard are rich in long-chain saturated fatty acids. Most vegetable oil sources have a high content of unsaturated fatty acids. Sanz et al.[45] observed the effect of sunflower oil and a mixture of bovine tallow and swine fat on fatty acid profiles of broiler chicken meat. These results [44] were similar to ours in the current study. Comparison of fatty acid profiles in drumsticks with thighs evidences changes in the preferential localization of SFA, MUFA and PUFA according to the dietary fat and oil sources. Furthermore, it was well documented that oleic acid is the major fatty acid of drumsticks with thighs [40,46,47]. Nevertheless, the maximal MUFA contents were observed in drumsticks with thighs of lard-fed groups. Crespo and Esteve-Garcia [45] indicated the muscle from drumsticks with thighs of chickens fed with lard had the highest concentrations of linoleic acid. In the current study, broiler chickens fed with supplemented fat and oil (L+LO) showed similar high PUFA contents in muscle from drumsticks with thighs.

Broiler chickens fed lard ingested the highest amount of SFA, so the highest percentages of SFA were found in broilers fed lard and in broiler chickens fed lard plus linseed oil ($P<0.01$). Similar results were found in the study of Crespo and Esteve-Garcia [46]. In the current study, broiler chickens fed linseed oil had higher values of linoleic acid (C18:2 n-6) and other n-6 derivatives than those in the other treatments. These fatty acids replaced SFA and MUFA compared to the broilers fed lard. The same effect was observed with n-3 fatty acids in broilers fed linseed oil [48]. Despite the higher content of linoleic acid in the diet with linseed oil compared with the lard diet, n-6 derivatives were found in higher amounts in broilers in our control group. Supplementation with lard in broiler chicken diets caused increases in the n-6/n-3 fatty acid ratio in drumsticks with thighs compared with other groups. However, the most favorable n-6/n-3 fatty acid ratio in drumsticks with thighs was found in broiler chickens fed linseed oil (2.67). The proportion of total n-6/n-3, fulfilling the demands of health-conscious consumers, should be from 1 to 5, so the best oil supplement in broiler chickens diets in other studies were linseed oil, followed by the mixture of linseed oil and lard [1,4,13,39]. The dietary incorporation of linseed oil and pig lard during starter, grower and finisher phases can enrich broiler chickens meat with n-3 PUFA. This study has clearly shown that linseed oil in broiler nutrition provided the best n-6/n-3 ratio.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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