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Production of Omega-3-Enriched Meat through Feeding Broilers with Ultrasonicated Flaxseed Oil Nanoemulsions: Performance, Serum Composition, Physicochemical Properties and Oxidative Stability

Research Article

F. Abbasi[1], F. Samadi[1*], S.M. Jafari[2], S. Ramezanpour[3] and M. Shams Shargh[4]

[1] Department of Animal and Poultry Physiology, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
[2] Department of Food Materials and Process Design Engineering, Faculty of Food Science and Technology, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
[3] Department of Plant Breeding and Biotechnology, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
[4] Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran

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*Correspondence E-mail: f.samadi@gau.ac.ir

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ABSTRACT

This study aimed to produce omega-3-emriched broiler meat by using ultrasonicated flaxseed oil nanoemulsions coated with whey protein-sodium alginate. Three hundred 1-d-old (Ross, 308) male broiler chicks were allocated randomly to 5 experimental treatments with 4 replicate pens. Experimental treatments included basal diet (BD), basal diet plus flaxseed oil (BD+FO, 1 mL/kg/BW), basal diet plus ultrasonicated flaxseed oil nanoemulsions (BD+FON, 1 mL/kg/BW), basal diet plus flaxseed oil and vitamin E (BD+FO+E, 1 mL/kg/BW and 200 mg/kg diet vitamin E) and basal diet plus ultrasonicated flaxseed oil nanoemulsions and vitamin E (BD+FON+E, 1 mL/kg/BW and 200 mg/kg diet vitamin E). Results showed a better feed conversation ratio for birds received dietary treatments on d 42 of grower phase and overall (d 1 to 42), compared with those received BD ($P \leq 0.05$). Birds treated with dietary treatments (except BD+FO in the case of very low-density lipoprotein cholesterol (VLDL-c)) showed ($P \leq 0.05$) lower levels of serum triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-c) and VLDL-c, and LDL-c/HDL-c ratio and greater amounts of serum high-density lipoprotein cholesterol (HDL-c) than the control. Meat quality parameters including oxidative stability, pH, water holding capacity, moisture and color (L*, a*, and b*) values were affected by dietary treatments, showing positive effect. Using ultrasonicated flaxseed oil nanoemulsions resulted in lower saturated fatty acids in breast meat, higher omega-3 fatty acids in both breast and thigh meat and a lower ratio of n-6 to n-3 in both breast and thigh meat ($P<0.05$). In general, current study showed that using ultrasound waves to produce flaxseed oil nanoemulsions has potential to improve performance, blood serum lipid profile, oxidative stability and omega-6/omega-3 ratio in broiler meat and consequently might reduce the risk of lifestyle related diseases in human.

KEY WORDS broilers, flaxseed oil nanoemulsions, meat quality, omega-6/omega-3, ultrasonication.

INTRODUCTION

Changes in human lifestyle over the past century, particularly in the terms of dietary fat intake have become a major concern for nutritionist (Ayerza *et al.* 2002). Proper amounts of omega fatty acids are essential for normal growth and development. Today's, excessive amounts of omega-6 fatty acids in the Western diets, promote many

lifestyle-related dangerous diseases including cancers, heart coronary diseases, hypertension, diabetes, arthritis, inflammatory and some autoimmune disorders (Simopoulos, 2002). In contrast, omega-3 fatty acids play a vital role in the prevention and treatment of nominated diseases (Simopoulos, 2000), by reducing blood serum levels of low-density lipoprotein cholesterol (LDL-c), and total cholesterol and also increasing high-density lipoprotein cholesterol (HDL-c) (Long *et al.* 2018). Hence, there is a global trend toward enriching of human foods by omega-3 poly unsaturated fatty acids (PUFAs). One of the effective ways is supplementing animal diets by oils rich in n-3 PUFA, including fish and flaxseed oils (Lopez-Ferrer *et al.* 2001; Betti *et al.* 2009).

Flaxseed oil contains 50-60 percent omega-3 PUFA in the form of alpha-linolenic acid (Betti *et al.* 2009). One of the major concerns associated with oils rich in PUFAs is their high susceptibility to oxidation (Frankel, 1993). Moreover, flaxseed oils contain much lower amounts of tocopherols as antioxidant, making these oils even more susceptible to oxidation (Olejnik *et al.* 1997). Lipid oxidation results in the loss of nutritional and sensory values as well as production of potentially toxic compounds that threaten meat quality and its shelf life (Cortinas *et al.* 2005). In addition, enriched broiler meat by n-3 PUFA is more susceptible to oxidation (Rymer and Givens, 2005). Hence, it is urgent to protect these sensible oils to make them more stable during handling, processing and storage (Augustin *et al.* 2006).

A recent investigation has shown that the use of oil compounds in the form of encapsulated particles help its storage time and also improves meat quality and consequently shelf life through expanding the oxidative stability (Rymer *et al.* 2010). Moreover, encapsulation has potential to improve bioavailability and controlled release of sensitive compounds (Cheong *et al.* 2016). Nanoemulsion is a potential tool to enhance the stability and bioavailability and also controlled release of bioactive compounds under the desired conditions (Jafari, 2017). Recently, ultrasound-assisted emulsification (UAE) has attracted considerable attention for the production of nanoemulsions because of its capability to produce small droplets at good energy efficiency and particularly for efficient nutrient delivery system (Abbas *et al.* 2014). UAE improves the stability and particularly bioavailability of bioactive compounds in digestive system (Mahfoudhi *et al.* 2016; Walia *et al.* 2017). Bioactive compounds with sub-micron particle size have a large surface area allowing a rapid penetration through the intestinal mucosa layer (Mahfoudhi *et al.* 2016). Therefore, we aimed to study the effects of ultrasonicated flaxseed oil nanoemulsions on growth performance, serum lipid profiles and broiler meat quality particularly in the term of omega-6/omega-3 ratio.

MATERIALS AND METHODS

The experiment was approved by the Ethics Committee of Animal Science Faculty of Gorgan University of Agricultural Science and Natural Resources.

Fabrication of ultrasonic nanoemulsion

Nanoemulsion coated with whey protein /sodium alginate (WP/SA) were prepared based on Fioramonti *et al.* (2015) method. To prepare primary emulsion, WP was dispersed in deionized water and then stirred for 2 h at room temperature. SA was scattered in primary emulsion and solution stirred at 70 ˚C for 35 min to produce secondary emulsion. The pH value of all dispersions was adjusted to 7.0, using HCl and NaOH (0.1 mol L^{-1}). Primary emulsions were produced by mixing oil phase with aqueous phase in 1:4 ratio (oil phase: aqueous phase), using a 20 kHz, 400 W ultrasound (Hielscher, Germany) for 15 min.

Birds and experimental design

A total of 300 male day-old chicks (Ross 308) were allocated randomly to 1 of 5 dietary treatments with 4 replicate pens per treatment in a completely randomized design. The dietary treatments included a basal diet (BD), basal diet plus flaxseed oil (BD+FO, 1 mL/kg of body weight), basal diet plus flaxseed oil nanoemulsions (BD+FON, 1 mL/kg of body weight), basal diet plus flaxseed oil and vitamin E (BD+FO+E, 1 mL/kg of body weight and 200 mg/kg diet vitamin E) and basal diet plus flaxseed oil nanoemulsions and vitamin E (BD+FON+E, 1 mL/kg of body weight and 200 mg/kg diet vitamin E).

All essential nutrients contained in the basal diet of starter phase from d 1 to 21 and grower phase from d 22 to 42 (Table 1) met the nutrients requirements of broiler chicks recommended by the NRC (1994). The birds were provided *ad libitum* access to the experimental diets and water. A 23L:1D lighting program was applied for the entire study. The treatments were delivered via oral gavage using a 1-mL syringe.

Total lipids were extracted from basal diet and flaxseed oil following homogenization in chloroform/methanol (2:1, v/v) as described by Folch *et al.* (1957). Fatty acids were methylated to their respective fatty acids methyl esters (FAMEs) as described by Eratte *et al.* (2017), with some modifications. The fatty acid profiles were measured on a gas chromatograph (GC-4600, Unicam, Germany) equipped with a flame ionization detector and a split injector (Table 2).

Table 1 Diet compositions and calculated analyses of the basal diets

Ingredients (%)	Starter (0 to 21 d)	Grower (22 to 42 d)
Corn	56.00	62.26
Soybean meal	37.42	31.29
Soybean oil	2.82	3.13
Dicalcium phosphate	1.41	1.05
Calcium carbonate	1.28	1.38
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL-methionine	0.15	0.07
Salt	0.42	0.32
Calculated analysis[3]		
Metabolizable energy (kcal/kg)	2950.00	3050.00
Crude protein (%)	21.22	19.06
Calcium (%)	0.92	0.86
Available phosphorus (%)	0.41	0.33
Sodium (%)	0.18	0.14
Lysine (%)	1.15	1.00
Methionine + cysteine (%)	0.83	0.69
Methionine (%)	0.48	0.37
Theronine (%)	0.81	0.72

[1] Premix supplied per kg diet: vitamin A: 3600000 IU; vitamin D_3: 800000 IU; vitamin E: 9000 IU; vitamin K_3: 1600 mg; vitamin B_1: 720 mg; vitamin B_2: 3300 mg; vitamin B_3: 4000 mg; vitamin B_5: 15000 mg; vitamin B_6: 150 mg; vitamin B_9: 500 mg; vitamin B_{12}: 600 mg and Biotin: 2000 mg.
[2] Premix supplied per kg diet: Mn: 50000 mg; Fe: 25000 mg; Zn: 50000 mg; Cu: 5000 mg; Iodine: 500 mg and Choline chloride: 134000 mg.
[3] Claculated composition was according to NRC (1994).

Table 2 Major fatty acid profiles of basal diet and flaxseed oil (% of total fatty acids)

Fatty acids[1]	Basal diet	Flaxseed oil
C14:0	0.11	ND
C16:0	14.56	7.05
C17:0	0.07	ND
C18:0	4.30	4.23
Total SFA	**19.04**	**11.28**
C16:1	0.17	ND
C18:1n-9	16.90	26.85
Total MUFA	**17.07**	**26.85**
C18:2n-6	58.04	21.49
C18:3n-3	5.80	40.37
Total PUFA	**63.84**	**61.86**
Σ PUFA n-3	5.80	40.37
Σ PUFA n-6	58.04	21.49
PUFA/SFA	3.35	5.48
MUFA/PUFA	0.26	0.43
PUFAn-6/n-3	**10.00**	**0.53**

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids and PUFA: poly unsaturated fatty acids.
ND: not detected.

Helium was the bearer gas, and operated with the initial flow rate of 0.95 mL/min. Peaks were determined using retention times created for standard FAME mixture.

Sampling and Processing

On d 21 and 42, birds were fasted for 12 h and broilers and feeders were then weighed to determine the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). The FCR was calculated as FI divided by body weight. Dead birds were weighed for adjustment of feed utilization.

On d 42, two birds (closest to the average body weight for each pen) were selected for blood samples. Blood samples (5 mL) were taken by cardiac puncture into a 10-mL anticoagulant-free vacutainer tube and then centrifuged at 3000 × g for 10 min at 4 ℃ to obtain serum. Serum samples were stored at −80 ℃ for further analysis. Blood serum concentrations of triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL-c) were measured, using an automatic biochemical analyzer.

The birds that were used on d 42 for blood sampling were also used to meat quality analysis.

The muscles of breast and thigh were removed and half stored in a freezer (-20 ˚C) for one month. Meat color, including lightness (L*), redness (a*), and yellowness (b*) values was measured at slaughter time and one month later, using the CIE (1978).

The pH values of meat samples were measured at slaughtering time and one month after frozen storage by homogenizing 10 g of meat sample with 50 mL of distilled water. The homogenates were filtered, and the pH values of each samples was measured, using a pH meter at room temperature (Trout *et al*. 1992).

Lipid susceptibility to oxidation was measured, using the thiobarbituric acid reactive substance (TBARS) test (Buege and Aust, 1978) at 0 and 30 d postmortem on both breast and thigh meat. Briefly, samples (5 g) from pooled breast meat and pooled thigh meat were homogenized with 25 mL of 0.375% TBA, 15% trichloroacetic acid (TCA), and 0.25 N HCl stock solutions. The mixture was heated in boiling water for 10 min, followed by cooling with running water. The mixture was centrifuged at 5500 rpm for 25 min using a centrifuge (Hitachi, Tokyo, Japan). Absorbance was read at 532 nm using a spectrophotometer. Thiobarbituric acid reactive substances was calculated from the standard curve and expressed as µg malondialdehyde (MDA)/mg of meat.

Water holding capacity (WHC) was measured by method described by (Bouton *et al.* 1971). In brief, one gram of minced meat was placed on a round plastic plate with small holes. The plate with meat sample on it was then fitted into a 2 mL plastic tube. This tube was centrifuged at 1500 × g for 4 min. After centrifugation, the remained water was measured by drying the samples in 70 ˚C over night. The moisture of meat samples were calculated according to the AOAC (1990). Determination of breast and thigh meat fatty acid profiles was performed according to AOAC (1990), using gas chromatography (GC) (GC-FID, Agilent -7820A) equipped with an automatic injector as described by Folch *et al*. (1957).

Statistical analysis

A completely randomized design was performed with 5 dietary treatments and 4 replicate pens per treatment. All analyses were performed using SAS Version 9.1 (SAS, 2003). When significant effects were found, comparisons among multiple means were made by Duncan's multiple range tests. Statistical significance was considered as $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance

Data on growth performance is presented in Table 3. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) did not differ between treatments on d 21. Similarly, ADG and ADFI did not differ in grower phase and through whole period of the experiment (d 1 to 42). However, FCR was better in birds fed by dietary treatments compared with those received basal diet on d 42 of phase 2 and overall (d 1 to 42) ($P \leq 0.05$). These results could be attributed to the positive effects of n-3 PUFA on improving blood serum lipid profile (Abudabos *et al.* 2013; Long *et al.* 2018). Moreover, our results agreed with Rymer *et al.* (2010) who reported no significant effect on feed intake of broilers fed by encapsulated fish oil. These authors stated enhanced oxidative stability for encapsulated oil, which results in better performance.

Serological indices

Effects of dietary treatments on serological indices are shown in Table 4. Birds treated with experimental treatments (except BD+FO in the case of VLDL-c) showed ($P \leq 0.05$) lower levels of serum triglyceride, total cholesterol, LDL-c and VLDL-c, and LDL-c to HDL-c ratio and a higher level of serum HDL-c compared with the control. Birds received BD+FON+E had lower ($P \leq 0.05$) levels of triglyceride, cholesterol, LDL-c, VLDL-c and LDL-c to HDL-c ratio and a higher level of HDL-c than other dietary treatments.

Flaxseed oil is rich in omega-3 poly unsaturated fatty acids (n-3 PUFAs) in the form of alpha-linolenic acid (Betti *et al.* 2009). It seems there is a link between the lower fat contents in blood serum of birds, especially triglyceride, cholesterol, LDL-c, and VLDL-c with higher concentrations of n-3 PUFA in dietary treatments containing flaxseed oil. Similarly, Bernstein *et al.* (2011) and Abudabos *et al.* (2013) reported that n-3 PUFA supplementation results in lower levels of blood serum triglyceride and cholesterol and a higher level of HDL-c. Moreover, it has been documented that n-3 PUFA, in particularly docosahexaenoic acid (DHA) decreases serum level of triglyceride by reducing hepatic VLDL-c synthesis, as precursor of LDL-c and HDL-c (Roche and Gibney, 2000; Mori *et al.* 2000). Current study showed an improvement of blood lipid profile by lowering LDL-c and increasing HDL-c levels in birds fed dietary treatments containing flaxseed oil especially in the form of nanoemulsion along with vitamin E as antioxidant agents. Due to that, LDL-c and HDL-c are considered as the main health indicators of animal products for human consumption; therefore improved serum profile in broilers treated by omega-3 sources (flaxseed oil) might reduce the risk of lifestyle disease such as cardiovascular diseases.

Physicochemical properties

Effects of dietary treatments on physicochemical properties of meat are depicted in Tables 5 and 6.

Table 3 Effects of dietary treatments on growth performance of broilers

Treatments	Starter (d 1 to 21)			Grower (d 22 to 42)			Whole period (d 1 to 42)		
	ADG (g/d)	ADFI (g/d)	FCR	ADG (g/d)	ADFI (g/d)	FCR	ADG (g/d)	ADFI (g/d)	FCR
BD	25.7	39.7	1.54	91.3	157.2	1.72^{a}	58.5	98.4	1.68^{a}
BD + FO	25.9	39.5	1.52	97.3	156.8	1.61^{b}	61.6	98.1	1.59^{b}
BD + FON	26.2	39.4	1.50	98.3	156.0	1.58^{b}	62.2	97.7	1.57^{b}
BD + FO + E	26.5	39.5	1.49	98.1	156.3	1.59^{b}	62.3	97.9	1.57^{b}
BD + FON + E	27.0	39.3	1.45	98.8	155.5	1.57^{b}	62.8	97.4	1.55^{b}
SEM	0.26	0.65	0.01	6.63	7.85	0.04	0.67	1.65	0.05

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
ADG: average daily gain; ADFI: average daily feed intake and FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of dietary treatments on blood biochemical parameters of broilers at 42 d

Treatments	Blood biochemical parameters					
	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL-c^{2} (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)	LDL-c/HDL-c
BD	97.00^{a}	152.00^{a}	64.10^{c}	68.50^{a}	19.40^{a}	1.06^{a}
BD + FO	83.00^{b}	140.00^{b}	87.60^{b}	35.80^{b}	16.60^{ab}	0.40^{b}
BD + FON	68.00^{d}	128.50^{c}	103.60^{a}	11.30^{d}	13.60^{c}	0.10^{d}
BD + FO + E	77.00^{c}	137.00^{b}	100.70^{a}	20.90^{c}	15.40^{bc}	0.20^{c}
BD + FON + E	52.00^{e}	127.20^{c}	102.00^{a}	14.80^{c}	10.40^{c}	0.14^{d}
SEM	1.08	1.31	1.56	0.96	0.47	0.01

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol and VLDL-c: very low-density lipoprotein cholesterol.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of dietary treatments on malondialdehyde (MDA), pH, water holding capacity (WHC) and moisture of thigh and breast meats of broilers at slaughter time (d 42)

Treatments	MDA (µg/g)		pH		WHC (%)		Moisture (%)	
	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast
BD	0.20^{a}	0.19^{a}	5.71^{c}	5.84	53.72^{c}	51.35^{c}	75.00^{b}	74.25^{b}
BD + FO	0.16^{b}	0.17^{b}	5.84^{b}	5.85	56.30^{b}	53.87^{b}	77.10^{a}	76.17^{a}
BD + FON	0.15^{b}	0.13^{cd}	5.96^{ab}	5.95	59.30^{a}	56.90^{ab}	77.47^{a}	76.85^{a}
BD + FO + E	0.14^{b}	0.13^{cd}	5.89^{b}	5.86	59.25^{a}	56.72^{ab}	77.22^{a}	76.32^{a}
BD + FON + E	0.13^{b}	0.12^{d}	6.01^{a}	5.97	59.90^{a}	57.25^{a}	77.80^{a}	77.15^{a}
SEM	0.004	0.015	0.039	0.042	0.695	0.996	0.705	0.430

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of dietary treatments on malondialdehyde (MDA), pH, water-holding capacity (WHC) and moisture of thigh and breast meats of broilers after month frozen storage

Treatments	MDA (µg/g)		pH		WHC (%)		Moisture (%)	
	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast
BD	0.36^{a}	0.20^{a}	5.62^{b}	5.81^{b}	52.92^{b}	51.00^{b}	74.00^{b}	74.00^{b}
BD + FO	0.29^{b}	0.18^{b}	5.81^{a}	5.82^{b}	54.95^{a}	53.77^{a}	75.55^{a}	75.50^{a}
BD + FON	0.25^{bc}	0.15^{c}	5.94^{a}	5.94^{a}	57.37^{a}	54.47^{a}	77.00^{a}	75.90^{a}
BD + FO + E	0.24^{bc}	0.15^{c}	5.84^{a}	5.85^{b}	56.67^{a}	54.20^{a}	76.30^{a}	75.60^{a}
BD + FON + E	0.21^{c}	0.14^{c}	5.96^{a}	5.95^{a}	57.90^{a}	54.65^{a}	77.60^{a}	76.15^{a}
SEM	0.015	0.007	0.051	0.025	0.977	0.591	0.778	0.448

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The birds treated with dietary treatments showed a higher amount of pH in breast and thigh meat compared to birds fed basal diet (excluding breast meat at slaughter time and BD + FO and BD + FO + E treatments in the case of breast meat after one month frozen storage). Moreover, birds fed BD + FON + E showed higher pH values in breast and thigh meat than other treatments. Flaxseed oil is sensitive to oxidization because of its high contents of omega-3 PUFAs in the form of alpha-linolenic acid. This leads to a faster pH decline. In addition, encapsulating flaxseed oil may maintain its oxidative stability during storage (Rymer *et al.* 2010). Our results showed that inclusion of vitamin E results in a higher pH value on breast and thigh meat. Shen and Du (2005) commented that using dietary antioxidant results in higher values of pH due to inhibition of glycolysis and consequently lower acid accumulation. Generally, it is accepted that the greater pH of meat might have reflected different glycogen reserves pre-slaughter (Simitzis *et al.* 2008). Similarly, Yesilbag *et al.* (2011) reported that the dietary vitamin E at the 50 mg/kg level increased the pH in broiler meat.

The WHC is the capacity of muscle to keep the water. The reduction in the WHC results in poor meat quality. However, the WHC is affected by the rate of pH fall post mortem which leads protein denaturation and reduced WHC (Allen *et al.* 1997). Our results indicated that supplementation of basal diet with FO, FON, and vitamin E resulted in higher amount of WHC and moisture in breast and thigh meat compared to birds fed basal diet. WHC and moisture were greater in birds treated with BD + FON + E treatment. In general, meat with high pH value has a high WHC (Jang *et al.* 2008), which is consistent with our results. Variation in WHC at given pH is proposed to be partially due to variation in proteolysis and the resulting muscle cell shrinkage and mobilization of water to the extracellular space (Huff-Lonergan and Lonergan, 2005). It has been shown that inclusion of antioxidants to the diet reduces moisture loss in meat (Samadi *et al.* 2015). Our result was in agreement with those by Samadi *et al.* (2015) who reported that artichoke powder have antioxidant role which helps to preserve the integrity of membrane structure and reduces moisture outflow at the storage period.

Effects of dietary treatments on the color values (L*, a*, b*) of breast and thigh meat are presented in Tables 7 and 8. The lightness (except for thigh at slaughter time), redness (except for breast at one month after frozen storage) and yellowness of breast and thigh meat were affected by dietary treatments. A lower L* value was observed in breast meat of birds fed BD + FON and BD + FON + E at slaughtering time, but all dietary treatments resulted in lower L* in breast and thigh meat a month after frozen storage.

In the case of redness, only thigh meat was affected by dietary treatments a month after frozen storage. Dietary treatments affected the yellowness of thigh and breast meat at both 0 and 30 days after frozen storage. These results offered that lower L* values may be related to the high pH values (Jang *et al.* 2008).

Our result was in agreement with those reported by Samadi *et al.* (2015) who stated that dietary supplementation with antioxidant sources such as artichoke decreases L* values of thigh and breast meats in quails which could be attributed to the higher pH values of thigh and breast meats. Higher values of L* indicate lighter color, indicating that meat have low pH, whereas lower values indicate that meat are darker and have high pH (Barbut, 1997). Since, pH values are depended on the content of meat glycogen, nutrition is an important factor influencing muscle glycogen levels (Simitzis *et al.* 2008). Therefore, at the present study lower L* values of thigh and breast meat are partly attributed to the higher pH values of thigh and breast meat. The lower b* values and higher a* values are mainly related to three reasons. First, PUFA which plays a crucial role in the prevention of lipid peroxidation in tissues (when MDA content is reduced) (Jiang *et al.* 2014). Second, dietary supplementation with antioxidant sources can improve oxidative stability (Cui *et al.* 2018). Third, encapsulating flaxseed oil may help to maintain its oxidative stability during storage (Rymer *et al.* 2010).

The enhancement of oxidative stability was supposed to be the main reason for meat color improvement. Myoglobin, one of the most important intracellular proteins responsible for meat color, is oxidized during the storage, which will cause the change of meat color from purplish red or purplish pink to cherry red and then discoloration (Cui *et al.* 2018).

Oxidative stability

Effects of dietary treatments on oxidative stability of meat are shown in Tables 5 and 6. Physiologically, birds are prone to lipid peroxidation due to their specific characteristics. The MDA, an end product of the lipid peroxidation, is an important marker of oxidative stress (Lu *et al.* 2010). In consistent with Long *et al.* (2018), our results showed that dietary treatments (except BD+FO in the case of thigh meat at slaughter time) were effective in decreasing the amounts of MDA in thigh and breast meats (Tables 4 and 5). It has been reported that supplementing n-3 PUFA results in a lower MDA production by activation the enzymatic and non-enzymatic antioxidant systems (Delles *et al.* 2014). Moreover, encapsulated flaxseed oil resulted in lower MDA production than non-encapsulated flaxseed oil and control, showing a better availability of n-3 PUFA to cells ($P \leq 0.05$).

Table 7 Effect of dietary treatments on color characteristics (International Commission on Illumination L*a*b*) of thigh and breast meats of broilers at slaughter time (d 42)

Treatments	L* (lightness)		a* (redness)		b* (yellowness)	
	Thigh	Breast	Thigh	Breast	Thigh	Breast
BD	65.54	60.43^{a}	12.30	14.35	11.15^{a}	11.65^{a}
BD + FO	64.32	59.45^{ab}	12.50	14.57	10.87^{a}	11.30^{ab}
BD + FON	63.56	58.64^{b}	12.86	14.75	10.04^{b}	10.74^{c}
BD + FO + E	64.11	59.00^{ab}	12.70	14.60	10.53^{ab}	10.98^{bc}
BD + FON + E	63.31	58.01^{b}	12.90	14.85	9.95^{b}	10.12^{d}
SEM	1.26	0.453	0.272	0.182	0.192	0.164

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effect of dietary treatments on color characteristics (International Commission on Illumination L*a*b*) of thigh and breast meats of broilers after one month frozen storage

Treatments	L* (lightness)		a* (redness)		b* (yellowness)	
	Thigh	Breast	Thigh	Breast	Thigh	Breast
BD	70.95^{a}	65.21^{a}	11.10^{c}	13.30	12.02^{a}	12.80^{a}
BD + FO	66.50^{b}	62.96^{b}	11.20^{bc}	13.50	11.77^{a}	12.72^{a}
BD + FON	65.60^{b}	61.43^{b}	11.60^{ab}	13.90	10.60^{b}	11.80^{b}
BD + FO + E	65.90^{b}	62.07^{b}	11.40^{abc}	13.77	10.80^{b}	12.35^{a}
BD + FON + E	64.07^{b}	60.97^{b}	11.76^{a}	14.05	10.57^{b}	11.72^{b}
SEM	1.196	0.815	0.145	0.226	0.177	0.149

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 9 Effect of dietary treatments on the profile of fatty acids in breast meat

Fatty acids	Treatments					
	BD[1]	BD + FO	BD + FON	BD + FO + E	BD + FON + E	SEM
SFA	33.49^{a}	32.99^{ab}	32.49^{b}	32.71^{b}	32.37^{b}	0.218
MUFA	34.11	34.08	33.81	34.05	33.71	0.330
PUFA	32.39	32.91	33.69	33.24	33.92	0.586
n-3	2.40^{e}	3.24^{d}	4.32^{b}	3.73^{c}	4.77^{a}	0.047
n-6	29.99	29.67	29.37	29.50	29.14	0.541
PUFA/SFA	0.96^{c}	0.99^{bc}	1.03^{a}	1.01^{ab}	1.04^{a}	0.011
MUFA/PUFA	1.05^{a}	1.03^{ab}	1.00^{dc}	1.02^{bc}	0.99^{d}	0.009
LA/ALA	16.92^{a}	14.19^{8b}	12.82^{d}	13.69^{c}	12.58^{d}	0.102
n-6/n-3	12.48^{a}	9.15^{b}	6.79^{d}	7.90^{c}	6.10^{e}	0.154

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: poly unsaturated fatty acids; n-3: omega-3; n-6: omega-6; ALA: α-linolenic acid and LA: linoleic acid.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The birds treated with BD + FON + E showed a lower level of MDA in both breast and thigh meat than other treatments, indicating antioxidant role of vitamin E in protecting cell lipid membranes from peroxidation and oxidation (Narciso-Gaytan *et al.* 2011). Similarly, Rymer *et al.* (2010) and Armin *et al.* (2015) reported the same results for vitamin E in improving oxidative stability of omega-3-enriched broiler meat. The greater MDA values in thigh than breast meat could be attributed to the greater fat content of the thigh meat (Betti *et al.* 2009).

Fatty acids profile

Results of the fatty acids composition of breast and thigh meat are indicated in Tables 9 and 10. Birds received ultrasonicated flaxseed oil nanoemulsions had lower amount of breast saturated fatty acids, higher amount of breast and thigh omega-3 fatty acids and consequently lower n-6 to n-3 ratio.

Table 10 Effect of dietary treatments on the profile of fatty acid profile of thigh meat

Fatty acids	Treatment					
	BD	BD + FO	BD + FON	BD + FO + E	BD + FON + E	SEM
SFA	29.67	29.61	29.22	29.49	29.09	0.206
MUFA	40.56	40.44	40.28	40.33	40.18	0.181
PUFA	29.74	29.94	30.50	30.17	30.72	0.442
n-3	2.01^{d}	2.34^{dc}	3.18^{b}	2.63^{c}	3.59^{a}	0.127
n-6	27.72	27.60	27.32	27.54	27.12	0.338
PUFA/SFA	1.00^{c}	1.01^{c}	1.04^{ab}	1.02^{bc}	1.05^{a}	0.009
MUFA/PUFA	1.36	1.35	1.32	1.33	1.30	0.173
LA/ALA	15.95^{a}	13.45^{b}	10.20^{d}	12.30^{c}	9.11^{e}	0.276
n-6/n-3	13.78^{a}	11.81^{b}	8.59^{d}	10.46^{c}	7.54^{e}	0.256

BD: basal diet; BD + FO: basal diet plus 1 mL flaxseed oil per kg BW; BD + FON: basal diet plus 1 mL flaxseed oil nanoemulsions per kg BW; BD + FO + E: basal diet plus 1 mL flaxseed oil per kg BW and 200 mg vitamin E per kg diet and BD + FON + E: basal diet plus 1 mL per kg BW flaxseed oil nanoemulsions and 200 mg vitamin E per kg diet.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: poly unsaturated fatty acids; n-3: omega-3; n-6: omega-6; ALA: α-linolenic acid and LA: linoleic acid.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Our results agreed with Betti *et al.* (2009) who fed broilers flaxseed supplemented diet. This reduction in n-6 to n-3 ratio is a consequence of a significant increase in 3-omega fatty acids. It has been documented that bioactive compounds with sub-micron particle size have a large surface area allowing a rapid penetration through the intestinal mucosa layer (Mahfoudhi *et al.* 2016).

Ours results showed a better ratio of n-6 to n-3 in poultry meat received ultrasonicated flaxseed oil nanoemulsions than the control, which is in line with the ideal ratio of 3:1 to 6:1 in comparison to the ratio in Western diets ranging from 10:1 to 25:1 (Simopoulos, 2000). Therefore, our results strongly confirmed the efficiency of ultrasonicated flaxseed oil nanoemulsions in omega-3-enriched broiler meat.

CONCLUSION

To our knowledge, this is the first study using ultrasonicated flaxseed oil nanoemulsion to produce omega-3-enriched broiler meat. The results of the present study indicated that ultrasonicated flaxseed oil nanoemulsions has potential to improve growth performance, blood serum profile, oxidative stability, and specially to enrich broiler meat by 3-omega fatty acids and consequently the possible reduction in the risk of lifestyle related diseases.

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