The effect of omega-3 enriched meat production on lipid peroxidation, antioxidative status, immune response and tibia bone characteristics in Japanese quail

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ABSTRACT: The objective of the present study was to research the effects of different supplemented dietary sources of n-3 polyunsaturated fatty acids (n-3 PUFA) to produce n-3 enriched meat on growth performance, meat quality, serum antioxidative status, immune response and tibia bone characteristics in Japanese quail. Three hundred 1-day-old Japanese quail chicks were randomly weighed and divided into five dietary treatments containing 0% oil (C, negative control), 2% vegetable oil (VO, positive control), 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1% linseed oil +1% fish oil (LO+FO). Body weight and feed consumption were recorded. Fatty acid profile, cholesterol content, chemical composition and physical characteristics of breast meat were determined. Serum thiobarbituric acid-reactive substances (TBARS), glutathione peroxidase (GSH-Px) activity and total antioxidant capacity were measured. No negative effects were detected in live weight, feed consumption and physical characteristics of meat. The incorporation of n-3 PUFA in the meat proved to be successful when different sources of n-3 PUFA were used. The supplementation of n-3 PUFA caused a significant decrease in TBARS values and a significant increase in both the GSH-Px activity and total antioxidant capacity. Interestingly, the inclusion of n-3 PUFA in quail diets enhanced the antibody titre and bone morphological characteristics. Therefore, it can be concluded that the inclusion of n-3 PUFA in diets at moderate levels increased the n-3PUFA content in meat, improved the antioxidative status, reduced lipid peroxidation, enhanced the antibody response and bone morphological characteristics and did not have any negative influence on physical characteristics of meat and growth performance in Japanese quail.

Keywords: fish oil; linseed oil; meat; antioxidant

Omega-3 polyunsaturated fatty acids (n-3 PUFA) play an important role in human nutrition since they help to reduce the incidence of lifestyle diseases such as coronary artery diseases, hypertension and diabetes, as well as some autoimmune and inflammatory diseases such as arthritis and dermatitis (Simopoulos, 2000). These diseases are an urgent problem in countries of the Middle East and North Africa, due to the dominance of animal fats and partially hydrogenated vegetable oils in foods of the population of these countries. In addition, it has been shown that the consumption of long-chain n-3 PUFA [i.e. eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3)] ensures vital components for the retina and for the membrane phospholipids of the brain (Rymer and Givens, 2005).

Chicken meat is low in fat and cholesterol and is usually considered healthier than other animal protein sources, especially red meats of mammalian origin. In recent years, dietary supplements such as n-3 PUFA have been tested in an attempt to further decrease fat and cholesterol contents of poultry meat (Ayerza et al., 2002). Novel alternative strategies to produce meat with lower cholesterol and saturated fatty acid contents have focused on

dietary manipulations to modify the fatty acid (FA) composition of meat. Moreover, the enrichment of poultry meat and eggs with n-3 PUFA is a successful strategy to ensure an adequate supply of n-3 PUFA to a greater portion of the population. Production of such meats is feasible and could be realized by adding common sources of n-3 PUFA (i.e. fish oil – FO, linseed oil – LO or marine algae, etc.) to the diet. It has been shown that the content of n-3 PUFA, particularly of α-linolenic acid (ALA, 18:3n-3), in poultry meat can readily be improved by increasing the levels of n-3 PUFA in poultry diets through the incorporation of vegetable oils (López-Ferrer et al., 2001b; Schneiderova et al., 2007; Zelenka et al., 2008) or oily fish by-products (López-Ferrer et al., 2001a; Cortinas et al., 2004). However, some authors reported the unpleasant flavour of fish in the meat of broilers fed 1.5% to 2.5% of FO (Hardin et al., 1964). Therefore, the inclusion of low dietary levels of FO together with another source of ALA (e.g. LO) has been suggested to obtain an acceptable product with the increased ratio of n-3:n-6 fatty acids.

Several studies confirmed many positive effects of dietary n-3 PUFA including immune response, lipid peroxidation, and antioxidative properties (Yuan and Kitts, 2002; Ebeid et al., 2008). Some reports demonstrated that dietary n-3 PUFA could modulate a wide range of immune responses in poultry (Simopoulos, 2000; Wang et al., 2000; Ebeid et al., 2008). Recently, Ebeid et al. (2008) indicated that dietary FO levels below 3.5% increased the antibody titre in laying hens. This response is similar to the observations of Yuming et al. (2004), who proved that the antibody levels were higher in hens fed oils rich in n-3 PUFA (FO or LO) than in hens fed maize oil rich in n-6 PUFA. Some studies also elucidated that a moderate intake of n-3 PUFA could enhance the antioxidative properties including the activity of glutathione peroxidase (GSH-Px) in experimental animals (Marja et al., 1984; Ebeid et al., 2008) and reduce serum lipid peroxidation (Crespo and Esteve-Garcia, 2001; Yuan and Kitts, 2002; Ebeid et al., 2008).

Moreover, dietary lipids play an important role in the development, growth, and modelling of long bones. Numerous animal studies have shown a positive effect of n-3 PUFA on bone mineral density, bone mineral content and bone strength. Liu et al. (2003) found out significantly higher bone mineral density and bone strength in quails fed a FO-supplemented diet (high in n-3 PUFA) compared to a soybean oil diet group (high in n-6 PUFA). Similarly, FO-supplemented rats had significantly higher bone mineral density in the distal femur and proximal tibia than a maize oil-supplemented group (high in n-6 PUFA; Sun et al., 2003). However, other studies have shown no significant effect of a high n-3 PUFA diet on bone mineral density, bone mineral content or bone morphological characteristics (Baird et al., 2008).

The main source of long-chain n-3 PUFA is seafood, although its use is restricted due to odour constraints in the final product. On the other hand, whereas the inclusion of vegetable sources (e.g. LO or rapeseed oil) as alternative sources to n-3 PUFA involves a lesser degree of off-flavours or odours, it also results in diminished deposits of EPA and DHA while it increases the n-3 PUFA content in the form of ALA in the animal's tissues (López-Ferrer et al., 2001a,b; Zelenka et al., 2008). Thus, in order to maximize the n-3 PUFA enrichment of poultry meat without affecting sensory quality and to reduce the feeding costs, it may be beneficial to use a dietary mixture of LO+FO. Therefore, the objective of the present study was to research the effects of supplementation of different dietary sources of n-3 PUFA (LO, FO or mixture of LO+FO) to produce n-3 enriched meat in Japanese quail on growth performance, meat quality, meat lipid profile, serum lipid peroxidation, antioxidative status, immune response and tibia bone characteristics.

MATERIAL AND METHODS

Experimental design and dietary treatments

Three hundred 1-day-old Japanese quail (*Coturnix coturnix japonica*) chicks of egg-meat type were weighed and divided randomly into five dietary treatments. Each treatment (n = 60) was assigned randomly to 3 pens of 20 birds each. Birds were placed in separate temperature controlled brooder floor pens with 24 h of light on the Poultry Research Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt. Birds were fed *ad libitum* diets containing 0% oil (C, negative control), 2% vegetable oil (VO, positive control), 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1% linseed oil +1% fish oil (LO+FO) throughout the whole experiment which lasted for 42 days. Vegetable oil used in the present study was a mixture of cotton seed oil (50%) and sunflower oil (50%). Experimental diets were formulated to have the same metabolisable energy

Table 1. Composition, calculated and chemical analysis of experimental diets

Ingredients and composition	Experimental diet [1]				
	LO+FO	FO	LO	VO	C
Feed ingredients (%)					
Corn (yellow)	52.62	52.62	52.62	52.62	56.80
Soybean meal 44%	31.88	31.88	31.88	31.88	30.50
Protein concentrate[2]	10.00	10.00	10.00	10.00	10.00
Bone meal	1.50	1.50	1.50	1.50	1.50
Limestone	1.40	1.40	1.40	1.40	0.90
NaCl	0.30	0.30	0.30	0.30	0.20
Vitamin-mineral premix[3]	0.30	0.30	0.30	0.30	0.10
Vegetable oil	0.00	0.00	0.00	2.00	0.00
Linseed oil	1.00	0.00	2.00	0.00	0.00
Fish oil	1.00	2.00	0.00	0.00	0.00
Fatty acid composition of experimental diets (as % of total fatty acids)[4]					
n-3 PUFA	41.01	41.00	40.00	5.43	2.48
n-6 PUFA	22.18	16.35	23. 46	53.00	16.50
SFA	14.35	16.26	13.00	15.55	43.28
MUFA	22.91	23.36	22.10	24.16	45.63
PUFA	62.17	58.28	63.35	56.11	18.00
Calculated analysis[5]					
ME (MJ/kg diet)	12.14	12.14	12.14	12.14	12.14
Crude protein	24.00	24.00	24.00	24.00	24.00
Calcium (%)	1.70	1.70	1.70	1.70	1.50
Phosphorus (%)	0.43	0.43	0.43	0.43	0.40
Methionine (%)	0.43	0.43	0.43	0.43	0.43
Lysine (%)	0.86	0.86	0.86	0.86	0.86
Chemical analysis on dry matter basis[6]					
Dry matter	85.40	85.60	85.50	85.30	86.00
Crude protein	24.42	24.54	24.64	24.45	24.36
Ether Extract	4.17	4.09	4.05	4.24	2.17
Crude fiber	4.35	4.37	4.22	4.14	4.46
Ash	9.70	9.75	9.60	9.70	9.52

[1]quail dietary treatments include negative control (C, 0% oil), positive control [VO, 2% vegetable oil which is a mixture of cotton seed oil (50%) and sunflower oil (50%)], 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1%LO+1% FO

[2]protein concentrate (CP 63.48%; ME 2.89 Mcal/kg)

[3]vitamin and mineral premix provided per kilogram of diet: vitamin A 12 000 IU, cholecalciferol 2000 IU, vitamin E 50 mg, vitamin K_3 5 mg, vitamin B_1 1.5 mg, vitamin B_2 6 mg, vitamin B_6 5 mg, vitamin B_{12} 30 µg, pantothenate 10 mg, folic acid 0.75 mg, biotin 0.08 mg, Mn 40 mg, Zn 60 mg, Fe, 50 mg, Cu 5 mg, Se 0.15 mg, Co 0.1 mg, I 0.4 mg

[4]n-3 = ω-3 fatty acids; n-6 = ω-6 fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; FA composition expressed as percentage of total FA

[5]according to NRC (1994)

[6]according to the method of AOAC (2000)

(ME, Table 1). The diets were formulated to meet the recommendations of the National Research Council for Japanese quail (NRC, 1994).

Growth Performance

Feed intake and body weight were measured weekly and feed conversion (feed/gain) was calculated.

Meat chemical and physical characteristics and meat fatty acid analysis

At the end of the experimental period (42 days of age), 10 birds from each treatment were randomly chosen, weighed and sacrificed. Ten breast meat samples were taken from each treatment to determine the chemical and physical characteristics of meat and to carry out the analysis of meat fatty acids.

Diets and meat samples were chemically analysed for the determination of crude protein, ether extract and ash content according to the methods of AOAC (2000). The pH value of meat was determined using a pH meter according to the method of Du and Ahn (2002). Meat tenderness and water-holding capacity were measured according to Wierbicki and Deatherage (1958) and Dawson et al. (1987), respectively. Meat cholesterol content was assayed according to Richmond (1973), using the commercial kits produced by Biodiagonistic, Egypt.

Fatty acid contents of feeds and meat were determined by gas chromatography according to the method described by Radwan (1978). In brief, lipids were extracted from fresh meat with chloroform/methanol at a ratio of 2:1(v/v). The fatty acid methyl esters were analysed using a GC-4 CM Shimadzu gas chromatograph (PFE) equipped with a flame ionisation detector. An analytical glass column (3 m × 3 mm internal diameter) was packed with 5% diethyl glycol succillate chromosorb on 80/100 mesh. The chromatography conditions were as follows: 180°C column temperature, 270°C injector temperature and 270°C detector temperature. Gas flow rates (ml/min) were as follows: nitrogen 30, hydrogen 1, air 0.5 and chart speed was 0.5 mm per min. A standard mixture of methyl esters was analysed under identical conditions prior to running the samples. The retention times of the unknown sample of methyl esters were compared with those of the standard. The concentration of methyl esters was calculated by the triangulation method (Radwan, 1978).

Antibody titre against Newcastle disease virus

Birds were vaccinated with Newcastle disease "La-Sota" vaccine (Intervet International B.V., Boxmeer, Holland) at 35 days of age for immunological examination. Blood samples (10 samples from each treatment) were collected 7 days after vaccination and antibody titre against Newcastle disease virus (NDV) was detected by a hemagglutination-inhibition test according to Alexander et al. (1983).

Lipid peroxidation and antioxidative status

Lipid peroxidation in the blood plasma was measured in the form of thiobarbituric acid reactive substances (TBARS) as described by Richard et al. (1992). TBARS, in particular malondialdehyde (MDA), are products of the oxidative degradation of PUFA, and they are used as an index of oxidative stress. The activity of the antioxidative enzyme GSH-Px and total antioxidant capacity in the blood plasma were measured according to Paglia and Valentine (1967) and Koracevic et al. (2001), respectively, using kits produced by Biodiagnostic, Egypt.

Bone measurements

The length of the right tibia of each bird was measured. Prior to breaking, each bone was marked at midpoint, and outside diameters (in mm) were measured perpendicularly and parallelly to the direction of the applied force using a calliper. The breaking strength of the tibia bone was determined using the Instron Universal Testing Machine (Model Ioll, Instron, Canton, USA) according to Wilson and Ruszler (1998). Following the bone breaking, the bone wall thickness was measured at the midpoint mark using a micrometer. To determine the tibia ash content, bones were oven-dried at 105°C for 24 h and ashed in a muffle furnace at 600°C for 14 h according to the procedure of Liu et al. (2003). The percentage ash was determined relative to dry weight of the tibia.

Statistical analysis

Before statistical analysis, all data were checked for normality and homogeneity of variance. The differences among treatments were statistically analysed by a one-way ANOVA test in a completely randomized design using Statistical Packages for the Social Sciences (SPSS®, 2001). Dietary treatment was considered as fixed effect, and pens were considered as random effects. Pen replications within

Table 2. Effect of different sources of n-3 PUFA on growth performance in Japanese quail to produce n-3 enriched meat

Characteristics	Treatment[1]					Significance
	C	VO	LO	FO	LO + FO	
Body weight (g/bird)						
Day 1	8.56 ± 0.18	8.25 ± 0.19	7.98 ± 0.15	8.26 ± 0.23	8.19 ± 0.22	NS
Day 7	24.83 ± 0.51	23.64 ± 0.52	24.91 ± 0.64	23.87 ± 0.82	24.49 ± 0.57	NS
Day 21	91.07 ± 1.87	92.86 ± 1.53	93.53 ± 1.92	96.09 ± 1.48	92.44 ± 1.84	NS
Day 42	182.28 ± 0.69	184.35 ± 1.46	185.09 ± 1.17	185.19 ± 1.38	184.08 ± 0.76	NS
Feed consumption (g/bird/day)						
Day 7	6.00 ± 0.007	5.99 ± 0.08	5.99 ± 0.06	6.02 ± 0.05	6.00 ± 0.02	NS
Day 21	16.12 ± 0.12	15.82 ± 0.10	15.81 ± 0.16	15.87 ± 0.08	15.94 ± 0.03	NS
Day 42	21.99 ± 0.43	22.01 ± 0.17	21.43 ± 0.11	21.91 ± 0.33	21.81 ± 0.12	NS
Feed conversion (feed/gain)						
Day 7	2.37 ± 0.14	2.52 ± 0.14	2.51 ± 0.13	2.79 ± 0.26	2.59 ± 0.17	NS
Day 21	3.57 ± 0.77	3.01 ± 0.39	3.04 ± 0.16	2.92 ± 0.16	2.57 ± 0.05	NS
Day 42	4.76 ± 0.17	4.41 ± 0.15	3.92 ± 0.42	4.42 ± 0.66	4.55 ± 0.46	NS

Values are means ± SE, and means in the same row without common superscripts are significantly different, NS = not significant

[1]quail dietary treatments include negative control (C, 0% oil), positive control 2% vegetable oil (VO), 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1% linseed oil + 1% fish oil (LO+FO)

dietary treatment served as the error term for the main effect of dietary treatment. Pen was an experimental unit. Significant differences among treatment means were compared using Duncan's new multiple-range test (Duncan, 1955). Differences were considered significant at $P \leq 0.05$.

RESULTS

Results concerning the effect of different dietary sources of n-3 PUFA on live weight, feed consumption and feed conversion are presented in Table 2. Dietary n-3 PUFA had no adverse effect on growth performance including final body weight, feed consumption and feed conversion in Japanese quail.

Data describing the effect of dietary n-3 PUFA treatments on the chemical and physical characteristics of meat are summarized in Table 3. No significant differences among treatments in the content of dry matter, crude protein and ash in meat were detected, while significant differences were observed in ether extract. The physical characteristics of meat were not significantly affected by supplemented dietary n-3 PUFA except for the water-holding capacity (Table 3). Insignificant differences among the experimental treatments were observed in meat tenderness (plasticity) and pH. It is interesting to note that the highest values of water-holding capacity were obtained with basal diet supplemented with a mixture of 1% FO + 1% LO followed by 2% FO, while the diets supplemented with 2% LO, 2% VO and C (0% oil) recorded the lowest values. As shown in Table 3, feeding 2% FO, 2% LO or a mixture of 1% FO +1% LO to birds resulted in a proportional increase in the total n-3 PUFA concentrations ($P \leq 0.01$) in meat lipids, as compared with the other treatments (C and VO). The highest n-3 PUFA concentration in meat was observed in treatments with 2% FO and with a mixture of 1% FO +1% LO. Dietary n-3 PUFA decreased saturated FA, monounsaturated fatty acids (MUFA) and total cholesterol in meat ($P \leq 0.001$). Quails fed a mixture of 1% FO +1% LO had the lowest cholesterol value in meat (113.31 mg/100 g meat), and those fed 2% VO showed the highest cholesterol value (160.03 mg/100 g meat).

With respect to the effect of dietary n-3 PUFA oils on antioxidative properties including GSH-Px

Table 3. Effect of different sources of n-3 PUFA on the fatty acid profile of breast meat in Japanese quail to produce n-3 enriched meat

Characteristics	Treatment[1]					Significance
	C	VO	LO	FO	LO+FO	
Chemical composition of breast meat						
Dry matter (%)	25.16 ± 0.45	24.97 ± 0.23	25.74 ± 0.57	24.41 ± 0.37	25.52 ± 0.36	NS
Protein (%)	80.17 ± 0.36	79.88 ± 0.33	79.84 ± 0.61	79.38 ± 0.23	79.52 ± 0.16	NS
Ether extract (%)	13.48 ± 0.31	14.54 ± 0.32^{b}	16.73 ± 0.39^{a}	16.15 ± 0.19^{a}	15.87 ± 0.13^{a}	*
Ash (%)	6.05 ± 0.15	5.58 ± 0.14	6.13 ± 0.23	6.07 ± 0.28	6.03 ± 0.14	NS
Physical characteristics of breast meat						
Water holding capacity (cm^2)	4.85 ± 0.06^{c}	4.97 ± 0.13^{bc}	5.43 ± 0.29^{bc}	5.52 ± 0.21^{b}	6.77 ± 0.20^{a}	*
Tenderness (cm^2)	2.27 ± 0.15	2.46 ± 0.23	2.25 ± 0.05	2.19 ± 0.16	2.15 ± 0.12	NS
pH	6.30 ± 0.00	6.43 ± 0.02	6.37 ± 0.07	6.42 ± 0.03	6.38 ± 0.04	NS
Fatty acid profile and cholesterol of breast meat						
Total n-3 PUFA (%)	2.36 ± 0.21^{c}	5.46 ± 0.31^{b}	16.53 ± 0.29^{a}	15.69 ± 0.35^{a}	16.12 ± 0.22^{a}	**
Total n-6 PUFA (%)	10.25 ± 0.31^{c}	14.63 ± 0.23^{b}	19.64 ± 0.19^{a}	16.39 ± 0.41^{a}	16.57 ± 0.28^{a}	**
n-6/n-3 ratio	4.34 ± 0.16^{a}	2.56 ± 0.12^{b}	1.25 ± 0.18^{c}	1.12 ± 0.13^{c}	1.14 ± 0.13^{c}	*
Saturated FA (%)	42.03 ± 0.93^{a}	39.55 ± 0.57^{b}	30.74 ± 0.31^{c}	30.57 ± 0.25^{c}	31.48 ± 0.35^{c}	**
MUFA (%)	46.07 ± 0.78^{a}	41.73 ± 0.94^{b}	34.21 ± 0.25^{d}	38.69 ± 0.32^{c}	36.99 ± 0.54^{c}	**
PUFA (%)	12.29 ± 0.93^{c}	20.45 ± 0.57^{b}	36.26 ± 0.31^{a}	32.43 ± 0.25^{a}	33.52 ± 0.35^{a}	**
Meat cholesterol (mg/100 g)	159.01 ± 3.69^{a}	160.03 ± 4.25^{a}	120.99 ± 3.15^{b}	122.3 ± 3.55^{b}	113.31 ± 4.84^{b}	***

Values are means ± SE, and means in the same row without common superscripts are significantly different, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, NS = not significant

[1]quail dietary treatments include negative control (C, 0% oil), positive control (VO, 2% vegetable oil), 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1% linseed oil + 1% fish oil (LO+FO)

activity and total antioxidant capacity as well as lipid peroxidation index in the blood plasma (Figure 1A–C), it could be observed that dietary 2% LO, 2% FO and a mixture of 1% FO+1% LO significantly enhanced both GSH-Px activity and total antioxidant capacity in comparison with 2% VO and the negative control. Moreover, using TBARS as an index of lipid peroxidation, it could be observed in the present study that moderate levels of n-3 PUFA oils (2% LO, 2% FO, mixture of 1% FO + 1% LO) decreased TBARS in serum. It is noteworthy in the results of the present study that a moderate intake of n-3 PUFA can enhance the antioxidative properties and decrease lipid peroxidation in growing Japanese quail.

Results of immune response, represented graphically in Figure 1D, show that different sources of dietary n-3 PUFA had a positive effect on humoral immunity as measured by antibody titres against NDV when compared with VO and negative control diet ($P \leq 0.05$) at 42 days of age. The highest scores were attained by quails receiving treatments with n-3 PUFA oils (2% LO, 2% FO and a mixture of 1% FO + 1% LO).

One of the major results in the present study was that the n-3 PUFA supplementation of diets for growing Japanese quail appeared to improve the morphological characteristics of the tibia bone (Table 4). Tibia diameter, tibia bone wall thickness, tibia bone breaking strength and the percentage of tibia ash in quails fed 2% FO, 2% LO and a mixture of 1% FO + 1% LO were larger than in the negative control (0% oil). At 42 days of age, taking the bone breaking strength of the negative control

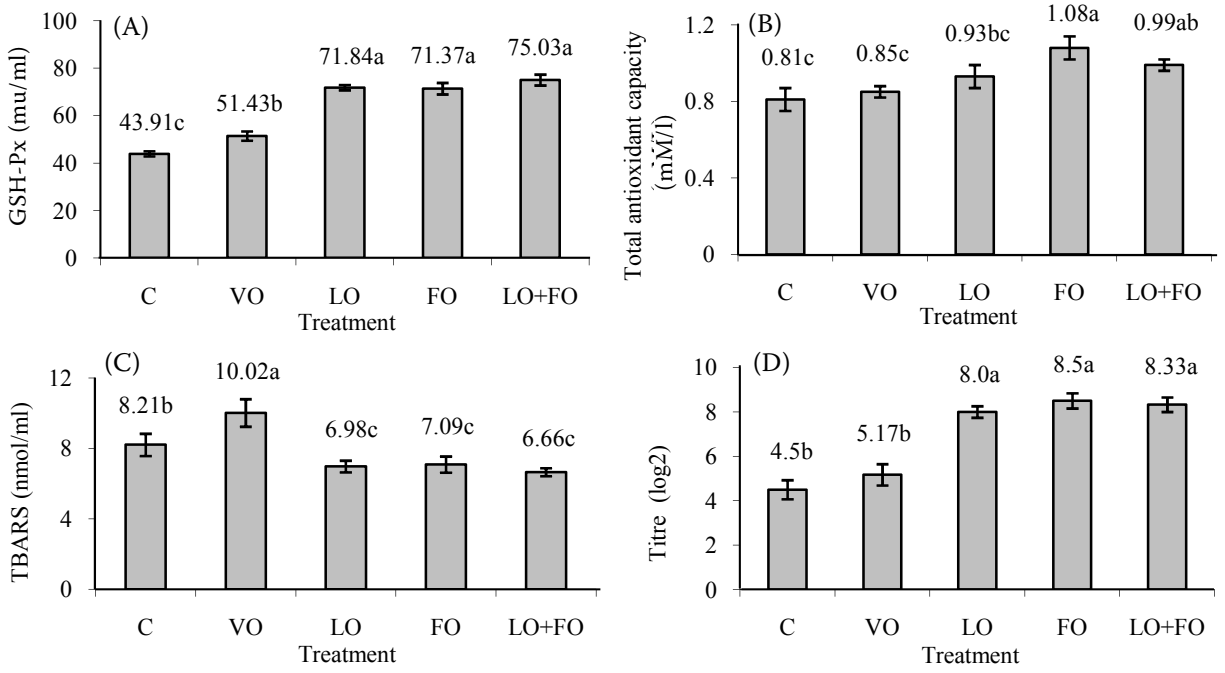

Figure 1. Effect of different sources of n-3 PUFA [negative control (C, 0% oil), positive control (VO, 2% vegetable oil), 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1% linseed oil +1% fish oil (LO+FO)] on (A) blood plasma GSH-Px, (B) blood plasma total antioxidant capacity, (C) blood plasma TBARS and (D) antibody titre at 42 day of age in Japanese quail to produce n-3 enriched meat

Values are expressed as means ± standard error; means with different letters differ from each other ($P \leq 0.05$)

treatment as a reference, birds receiving a mixture of 1% FO + 1% LO had the greater bone breaking strength ($P \leq 0.01$), being about 125% of the negative control.

DISCUSSION

Dietary n-3 PUFA had no adverse effects on the growth performance of Japanese quail chicks including live weight, feed consumption and feed conversion (Table 2). These results are in agreement with several previous studies which noted that FO in poultry diets had no effect on live weight and feed consumption (López-Ferrer et al., 2001a,b; Bou et al., 2005) compared to a control diet with no fat added. According to Bou et al. (2005), final body weight, feed intake, feed conversion, and mortality were not affected by dietary FO and LO. Gonzalez-Esquerra and Leeson (2000) stated that feeding FO and LO had no effects on final body weight at 49 days of age and weight gain of birds from 36 to 49 days. However, Hulan et al. (1988) reported that diets enriched with isoenergetic and isonitrogenous redfish meal and redfish oil led to lower feed consumption and body weights and poorer feed conversion efficiency than the control diet.

Results of the present study indicated that the inclusion of FO and/or LO in quail diet did not adversely affect the chemical and physical characteristics of meat quality (Table 3). Similar findings were reported by Schiavone et al. (2004), who stated that the mean values for moisture, protein and lipid contents of breast meat of Muscovy duck were not significantly influenced by dietary FO. Suksombat et al. (2007) also indicated that moisture, crude protein and fat contents of carcass were not affected by the consumption of conjugated linoleic acid. As shown in Table 3, no significant differences could be observed among dietary treatments in meat tenderness and pH, however, a dietary mixture of 1% FO + 1% LO increased the water-holding capacity of meat. These observations are in harmony with López-Ferrer et al. (2001a,b) who postulated that different amounts (0%, 2%, or 4%) of LO and FO in diet did not result in any significant differences among treatments in meat quality parameters (juic-

Table 4. Tibia bone measurements at 42 days of age of Japanese quail supplemented with different sources of n-3 PUFA oils

Characteristics	Treatment[1]					Significance
	C	VO	LO	FO	LO+FO	
Tibia weight (g)	2.21 ± 0.068	2.34 ± 0.05	2.29 ± 0.034	2.32 ± 0.054	2.39 ± 0.044	NS
Tibia length (cm)	4.14 ± 0.04	4.33 ± 0.06	4.22 ± 0.06	4.31 ± .06	4.33 ± .06	NS
Diameter (mm)	2.32 ± 0.03^{b}	2.38 ± 0.03^{ab}	2.43 ± 0.02^{a}	2.47 ± 0.03^{a}	2.47 ± 0.03^{a}	*
Bone wall thickness (μm)	397.94 ± 44.29^{b}	417.50 ± 14.55^{b}	507.80 ± 4.77^{a}	512.70 ± 5.75^{a}	499.40 ± 4.09^{a}	**
Tibia breaking strength (kg/cm^{2})	6.89 ± 0.33^{b}	7.14 ± 0.23	9.37 ± 0.22^{a}	9.29 ± 0.31^{a}	8.64 ± 0.39^{a}	**
Ash (% dry weight)	35.67 ± 0.68^{b}	36.68 ± 0.80^{b}	41.46 ± 1.05^{a}	40.94 ± 0.84^{a}	40.14 ± 0.56^{a}	**

Values are means ± SE, and means in the same row without common superscripts are significantly different, *$P \leq 0.05$, **$P \leq 0.01$, NS = not significant

[1]quail dietary treatments include negative control (C, 0% oil), positive control (VO, 2% vegetable oil), 2% linseed oil (LO), 2% fish oil (FO) and a mixture of 1% linseed oil +1% fish oil (LO+FO)

iness, grilling losses, and toughness) of the breast samples of chicks.

The fatty acid profile of meat could be altered by adding fish products to the feed for broilers (López-Ferrer et al., 2001a; Cortinas et al., 2004) or vegetable oils (López-Ferrer et al., 2001b; Schneiderova et al., 2007; Zelenka et al., 2008). Similar findings were detected in the present study (Table 3). Generally, the concentrations of n-3 PUFA increased, whereas n-6 PUFA tended to decrease in meat lipids in response to dietary n-3 PUFA. The highest n-3 PUFA concentration in meat was observed in treatments with 2% FO and a mixture of 1% FO + 1% LO. The changes in meat n-3 PUFA shown here due to feeding FO and/or LO (Table 3) were in agreement with previous reports (López-Ferrer et al., 2001a,b; Cortinas et al., 2004) and these studies clearly established that n-3 PUFA-enriched diets increased the deposition of these fatty acids in muscle and adipose tissues. Cortinas et al. (2004) proved that the n-3 PUFA contents of broiler thigh and breast muscles were increased by dietary supplementation of n-3 PUFA (LO and FO). Therefore, the inclusion of low dietary levels of FO together with another source of ALA (i.e. LO) has been suggested to obtain an acceptable product with a decreased ratio between fatty acids (n-6:n-3). The results of the present study confirmed that the incorporation of n-3 PUFA in the meat was successful when a dietary mixture of 1% FO + 1% LO was applied.

Opposite to the aforementioned trend observed in meat PUFA, dietary n-3 PUFA decreased saturated FA, MUFA and total cholesterol in meat (Table 3). These results agree with those of other authors who observed an inverse relationship between the accumulation of total MUFA and PUFA, mainly as n-3 PUFA, and to a lesser extent in saturated FA deposition, as dietary FA were modified (López-Ferrer et al., 2001a,b; Cortinas et al., 2004). Cortinas et al. (2004) documented that the contents of saturated FA and MUFA in thigh and breast were decreased linearly as the inclusion of dietary n-3 PUFA increased.

Moderate dietary levels of n-3 PUFA oils (2% LO, 2% FO and a mixture of 1% FO + 1% LO) significantly enhanced both the GSH-Px activity and total antioxidant capacity as well as decreased lipid peroxidation (TBARS values) in the serum of growing Japanese quail (Figure 1A–C). These results confirmed our previous results (Ebeid et al. 2008) that the application of not more than 2.5% of FO may enhance the antioxidative status in laying hens. Additionally, Marja et al. (1984) indicated that the plasma GSH-Px levels were higher in chicks receiving 4% PUFA in their diet as compared to those fed the same amount of butter fat or olive oil. This may be connected with the hypolipidemic effects of dietary n-3 PUFA, which are fairly consistent in lowering plasma triacylglycerols, with variable effects on total and low-density lipoprotein (LDL)

cholesterol concentrations (Yuan and Kitts, 2002). Additionally, n-3 PUFA produce the lower abdominal fat deposition than saturated or monounsaturated fatty acids (Crespo and Esteve-Garcia, 2001). This in turn will reduce the free radical attack on these lipids, and consequently it will reduce lipid peroxidation. From another point of view, taking into account the involvement of GSH-Px in the immune function, it is important to note that the antioxidative enzyme GSH-Px is likely to protect neutrophils from oxygen-derived radicals that are produced to kill ingested foreign organisms (Arthur, 2000). Furthermore, GSH-Px plays an important role in the regulation of biosynthesis of prostaglandins from their precursor, arachidonic acid (ARA, 20:4; n-6). However, the precise nature of GSH-Px involvement in eicosanoid metabolism has not been fully understood yet (Pappas et al., 2005). Interestingly, it is important to mention that FO can decrease the oxidation products generated by the inflammatory cells which accompany ischaemic reperfusion. Moreover, Constant (2004) concluded that when concentrated n-3 PUFA are fed to animals, no peroxides are produced, and so no antioxidants are necessary. Therefore, it could be stated that the enrichment of poultry meat with n-3 PUFA using a mixture of 1% FO + 1% LO might enhance the antioxidative status and reduce lipid peroxidation, which probably resulted in improvement of the immune responsiveness in growing Japanese quail in the present study.

The present study demonstrated that different sources of dietary n-3 PUFA affected positively the immune response ($P \leq 0.05$) in growing Japanese quail (Figure 1D) and this result supported those concerning the antioxidative parameters discussed above. This response is similar to observations of Ebeid et al. (2008), who postulated that the inclusion of FO in laying hen diets at moderate levels enhanced the antibody response in laying hens. Similarly, Yuming et al. (2004) proved that the antibody levels were higher in hens fed oils rich in n-3 PUFA (FO or LO) than in hens fed maize oil rich in n-6 PUFA. In this respect, several studies have demonstrated that dietary n-3 PUFA can modulate a wide range of immune response in poultry. Apparently, diets enriched with FO rich in long-chain n-3 PUFA (EPA and DHA) have anti-inflammatory properties (Wang et al., 2000), increase delayed-type hypersensitivity (Korver and Klasing, 1997), increase antibody responses (Ebeid et al., 2008) and decrease lymphocyte proliferation (Fritsche et al., 1991). Further experiments by Wang et al. (2000) demonstrated that diets enriched with FO suppressed prostaglandin E_2 (PGE_2) production derived from ARA. Therefore, it can be assumed that the addition of anti-inflammatory lipids, such as FO and LO, has also been shown to be beneficial to birds' health. Furthermore, taking into account our results in lipid peroxidation and antioxidative properties, it can be concluded that mixed sources of dietary n-3 PUFA at moderate levels (2%) may enhance the immune responsiveness.

An interesting finding in the present study was the improvement of bone morphological characteristics in growing Japanese quail due to dietary n-3 PUFA supplementation (Table 4). Several studies confirmed that dietary lipids could modulate the fatty acid profile in cartilage and bone tissues in growing chicks (Watkins et al., 1997). The mechanisms by which dietary fat influences the cartilage and bone metabolism and function are not completely clear. However, it is well established that some fatty acids and lipids have a direct and important role in facilitating the mineralization of bone tissues (Urist et al., 1997) and influencing the strength of bone (Liu et al. 2003). Furthermore, the dietary supplementation of n-3 PUFA suppresses PGE2 production, which consequently resulted in increasing bone formation rates in growing rats (Watkins et al., 2003). Dietary FO also inhibited osteoclastogenesis and loss of bone mass in rats (Sun et al., 2003).

Based on the data presented above, it can be concluded that the inclusion of mixed sources of n-3 PUFA in diets at moderate levels increased the n-3 PUFA content in meat, improved the antioxidative status, reduced lipid peroxidation, enhanced the antibody response and bone morphological characteristics and did not have any negative influence on meat physical characteristics and growth performance in Japanese quail.

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