Ovarian follicular development, lipid peroxidation, antioxidative status and immune response in laying hens fed fish oil-supplemented diets to produce n-3-enriched eggs*

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The objective of the present study was to research the effect of feeding laying hens fish oil-supplemented diets to produce n-3-enriched eggs on their ovarian follicular development, serum lipid peroxidation, antioxidative status and immune response. A total of 105 white Bovens hens at 24 weeks of age were housed in cages in an open-sided building under a 16 h light : 8 h dark lighting schedule. Birds were randomly divided into five treatments and were fed, ad libitum, diets containing 0% (control), 1.25%, 2.5%, 3.5% or 5.0% fish oil from 24 to 36 weeks of age. Egg production and weight were recorded. By weeks 35 and 36 of age 15 eggs were taken at random from each treatment to determine the yolk lipid profile and cholesterol content. At the end of the experimental period, 10 females from each treatment were randomly chosen, anaesthetised and killed by decapitation. Ovary and oviduct samples were immediately weighted and ovarian follicles were classified. Serum thiobarbituric acid-reactive substance (TBARS), hepatic TBARS and hepatic glutathione peroxidase (GSH-Px) activity were measured. No clear trend was observed concerning egg production and egg yolk cholesterol. As dietary fish oil levels increased, n-3-polyunsaturated fatty acids (n-3 PUFA) increased, whereas n-6 PUFA tended to decrease in yolk lipids. No negative effects were detected in ovary and oviduct weights, expressed in both absolute terms and relative to body weight. The numbers and total weights of large yellow follicles (LYF) in the ovary were not significantly affected by fish oil supplementation. Low levels (1.25% to 2.5%) of fish oil reduced both plasma and hepatic TBARS and enhanced GSH-Px activity. It is also interesting to note that inclusion of 2.5% fish oil in laying hen diets enhanced the antibody titre in laying hens. Therefore, it could be concluded that inclusion of fish oil in laying hen diets at moderate levels increased the n-3 fatty acids content in eggs, improved antioxidative status, enhanced the antibody response and did not have a negative influence on the different reproductive morphology parameters in laying hens.

Keywords: antioxidative systems, immunity, n-3 PUFA, ovaries

Introduction

n-3-polyunsaturated fatty acids (n-3 PUFA) play an important role in human nutrition since they help to reduce the incidence of such lifestyle diseases as coronary artery diseases, hypertension and diabetes, as well as certain inflammatory diseases such as arthritis and dermatitis (Simopoulos, 2000). These diseases are an increasing problem in countries of the Middle East and North Africa, due to the dominance of animal fats and partially hydrogenated vegetable oils in the meals of the people of these countries.

Enrichment of n-3 PUFA in the eggs of laying hens is a successful strategy to ensure an adequate supply of n-3 PUFA for the greater population. Production of such eggs is feasible and could be realised by adding common sources of n-3 PUFA (i.e. fish oil, marine algae or linseed, etc.) to the layers diet (Baucells et al., 2000). By supplementing hen diets with 3% to 7% fish oil (Farrell, 1994). Furthermore, in many countries the consumption of marine products is low; hence, the benefit that could be derived from a diet rich in n-3 PUFA does not reach the majority of the population. Therefore, fish oils are common feed ingredients used in layers diet to increase egg-yolk n-3 PUFA (Shimizu et al., 2001).
Contradictory results have been reported concerning the possible effects of such n-3-enriched diets on layer performance. According to Schreiner et al. (2004), egg production was not significantly affected because of including fish oil in commercial layer diets. However, Cortinas et al. (2003) summarised that egg production was significantly higher when laying hens were fed diets containing 4% fish oil or flaxseed oil. Contrarily, other researchers have reported impaired production parameters in hens fed fish products, and particularly reduced egg weight (Pappas et al., 2005).

n-3 PUFA are more prone to oxidation because of their double bonds and may confer this undesirable trait to the n-3-enriched product. Furthermore, the highly polyunsaturated long-chain fatty acids (i.e. eicosapentaenoic acid (EPA), 20:5(n-3) and docosahexaenoic acid (DHA), 22:6(n-3)) of fish oils are extremely susceptible to lipid peroxidation, even in the presence of added dietary antioxidants (Gonzalez et al., 1992). The data evaluating the effect of fish oil n-3 PUFA on oxidative stress and lipid peroxidation markers in vivo vary with both animal and clinical studies reporting increased oxidation (Ibrahim et al., 1997), no effect on oxidation (Nenseter et al., 1992) and even some indication of decreased susceptibility to induced oxidation (Yuan and Kitts, 2002).

Dietary n-3 PUFA are incorporated into cellular lipids where they exert essential effects on many biological systems, such as immune reactions, blood platelet aggregation and growth regulation of some cell types (De Pablo et al., 2002). In fact, the immunomodulatory functions of dietary n-3 PUFA have been widely studied in mammals, but research on poultry is limited. Moreover, in poultry, there are conflicting results on the effect of dietary lipids on immune functions. In general, published reports indicated that increasing the consumption of n-3 PUFA in diets has been shown to either increase or decrease immunological parameters of both humoral and cell-mediated immunity (Wang et al., 2000).

Therefore, the objective of the current study was to research the effects of different levels of supplemented dietary fish oil (0%, 1.25%, 2.5%, 3.5% or 5%) on ovarian follicular development, serum lipid peroxidation, antioxidant status and immune response in laying hens. In addition, the cholesterol, n-3 PUFA and n-6 PUFA levels in produced eggs were determined.

### Material and methods

A total of 105 white Bovens hens at 24 weeks of age with 83.12 ± 8.9% egg production were housed individually in laying cages in an open-sided building under a 16 h light: 8 h dark lighting schedule in the research farm, Faculty of Agriculture, Kafrelsheikh University, Egypt. Hens were randomly divided into five experimental treatments. Each treatment (21 hens) was further randomly divided into three replicates of seven hens (i.e. seven adjacent individually caged hens serving as a replicate). Birds were fed, ad libitum, diets containing 0% (control), 1.25%, 2.5%, 3.5% or 5.0% fish oil from 24 to 36 weeks of age. Experimental diets were formulated to have the same metabolisable energy (ME). The birds were fed a standard layer diet (17% crude protein; 2850 kcal; 3% available phosphorus; 7.15% calcium; 3% lysine; 1.8% methionine; 1200 IU vitamin A; 20 mg vitamin D; 2500 IU vitamin D; 25 mg vitamin B6; 10 mg vitamin B1 and 50 mg vitamin B12).

#### Table 1 Composition of experimental diets

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Control</th>
<th>1.25</th>
<th>2.5</th>
<th>3.5</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Feed ingredients (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize (yellow)</td>
<td>58.67</td>
<td>58.67</td>
<td>58.67</td>
<td>58.67</td>
<td>58.67</td>
</tr>
<tr>
<td>Soybean meal 44%</td>
<td>18.00</td>
<td>18.00</td>
<td>18.00</td>
<td>18.00</td>
<td>18.00</td>
</tr>
<tr>
<td>Layer concentrate†</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>7.50</td>
<td>7.50</td>
<td>7.50</td>
<td>7.50</td>
<td>7.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>α-methionine</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0.00</td>
<td>1.25</td>
<td>2.50</td>
<td>3.50</td>
<td>5.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>5.00</td>
<td>3.75</td>
<td>2.50</td>
<td>1.50</td>
<td>0.00</td>
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<tr>
<td>Fatty acids analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-3 PUFA†</td>
<td>1.01</td>
<td>1.35</td>
<td>2.63</td>
<td>3.76</td>
<td>5.52</td>
</tr>
<tr>
<td>SFA†</td>
<td>32.02</td>
<td>30.67</td>
<td>29.36</td>
<td>27.1</td>
<td>21.01</td>
</tr>
<tr>
<td>USFA†</td>
<td>67.98</td>
<td>69.33</td>
<td>70.64</td>
<td>72.9</td>
<td>78.98</td>
</tr>
</tbody>
</table>

†Layer concentrate provide per kg of diet containing the following: 50% crude protein; metabolisable energy, 2400 kcal; 3% available phosphorus; 7.15% calcium; 3% lysine; 1.8% methionine; 1200 IU vitamin A; 20 mg vitamin D; 2500 IU vitamin D; 25 mg vitamin B6; 10 mg vitamin B1 and 50 mg vitamin B12.

The possibility of n-3 PUFA, ovary, antioxidative system and immunity in layers.
rates (ml/min) were as follows: nitrogen 30, hydrogen 1, air 0.5 and chart speed was 0.5 mm/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 were calculated by the triangulation method (Radwan, 1978).

Total antibody titres against sheep red blood cells (SRBC) were measured by the 12th week of dietary treatments. Humoral immunity is an organism’s antibody response to pathogens. Antibody response to a T-dependent (both T- and B-cells must recognise antigen) antigen was assessed by measuring serum antibody titre following a primary intramuscular injection of 0.5 ml of a 40% SRBC suspension (in phosphate-buffered saline) in the right pectoral muscle. Antiserum to SRBC was collected 7 days after challenge. Antibody titres to SRBC were measured using a micro-haemagglutination assay by the method of Wegmann and Smithies (1966).

At the end of the experimental period, 10 females from each treatment were randomly chosen, weighed, anaesthetised and killed by decapitation. Blood plasma and liver samples were taken and stored at −40°C for subsequent analysis. The abdominal cavity was opened and the ovary and oviduct were removed and weighed. The number and weight of normal large yellow follicles (LYF) (>10 mm diameter), and the number of small yellow follicles (SYF) (5 to 10 mm diameter), large white follicles (LWF) (3 to 5 mm diameter) and medium white follicles (MWF) (1 to 3 mm diameter) were recorded. Follicle size classifications were based on Renema et al. (1995). The stroma weight comprising the ovarian tissue remaining after the LYF were counted and removed.

Lipid peroxidation in the serum and liver was measured in the form of thiobarbituric acid-reactive substance (TBARS) according to Richard et al. (1992). Activity of the antioxidative enzyme glutathione peroxidase (GSH-Px) activity was determined by the method of Levander et al. (1983).

### Table 2 Effects of feeding graded levels of supplemental dietary fish oil on egg production, feed intake, egg weight and fatty acid composition of eggs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>0</th>
<th>1.25</th>
<th>2.5</th>
<th>3.5</th>
<th>5.0</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg production (%)</td>
<td>83.31 ± 9.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.39 ± 9.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.42 ± 11.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>81.39 ± 12.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.85 ± 8.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Feed intake (g per hen per day)</td>
<td>102.94 ± 12.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.97 ± 12.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.78 ± 12.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.60 ± 11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.96 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>53.71 ± 5.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.16 ± 6.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.97 ± 5.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.54 ± 4.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.07 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Total n-3 PUFA (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.87 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.17 ± 0.61&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.20 ± 0.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.27 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.88 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Total n-6 PUFA (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.24 ± 2.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.35 ± 1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.90 ± 2.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.33 ± 1.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.50 ± 2.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>*</td>
</tr>
<tr>
<td>Total n-9 FA (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.33 ± 2.29</td>
<td>35.92 ± 3.36</td>
<td>36.6 ± 2.66</td>
<td>36.95 ± 3.0</td>
<td>36.67 ± 2.37</td>
<td>NS</td>
</tr>
<tr>
<td>Saturated FA (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.84 ± 2.5</td>
<td>40.33 ± 2.45</td>
<td>40.18 ± 2.88</td>
<td>40.42 ± 2.63</td>
<td>41.45 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Unsaturated FA (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.15 ± 2.4</td>
<td>59.66 ± 2.6</td>
<td>59.81 ± 2.88</td>
<td>59.57 ± 2.46</td>
<td>58.54 ± 1.63</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/g yolk)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.53 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.48 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.82 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.42 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.23 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>*</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean values followed by different letters in the same row are significantly different (P<0.05).
<sup>b</sup>P < 0.05. NS – non-significant.
<sup>n</sup>Sample size.
<sup>*P</sup> = 0.05.

The differences among treatments were statistically analysed by one-way ANOVA in a completely randomised design using Statistical Packages for the Social Sciences (2001). The significant differences among means of treatments were compared by Duncan’s new multiple-range test. P = 0.05 was set as limit of significance.

### Results

Results concerning the effect of fish oil supplementation on hen-day egg production, egg weight, yolk cholesterol content, total n-3 PUFA and total n-6 PUFA are presented in Table 2. Supplemented dietary fish oil showed an effect on layer performance. Fish oil addition did not cause a decrease in egg production but no clear trend was observed. However, fish oil supplementation had a significantly negative effect on egg weight. The egg weight decreased concomitantly with the increase of fish oil supplementation. As shown in Table 2, dietary fish oil has also been found to decrease feed intake (P < 0.05). Data in the present study showed that incorporation of n-3 PUFA in the egg yolks has been successful by using dietary fish oil. Generally, the fatty acids composition of the yolks reflected the dietary fat levels (Table 2). Feeding fish oil to birds resulted in a proportional increment of the total n-3 PUFA concentrations (P < 0.05) in egg yolk lipids, whereas concentrations of the total n-6 PUFA decreased as dietary fish oil increased (P < 0.05). Egg yolk cholesterol content significantly differed due to dietary fish oil treatment; however, no clear trend was observed (Table 2). Hens fed 2.5% fish oil had the lowest egg cholesterol value (12.82 mg/g yolk) compared with control (14.53 mg/g yolk).

Most ovarian morphological parameters were not significantly affected by supplemented fish oil except for the number of SYF and F1 follicles. Results presented in Table 3 indicated that ovary and oviduct weights, expressed in either absolute terms or relative to body weight, were not significantly different.
significantly affected by dietary fish oil. The numbers and total weights of LYF in the ovary were not significantly affected by fish oil supplementation, while the weight of the F1 (largest) follicle was significantly heavier in the 2.5% fish oil treatment than in other treatments (Table 3) where the F1 (largest) follicle was significantly higher when laying hens were fed diets containing fish oil in commercial layer diets does not usually affect body weight gain, feed intake or egg production (Gonzalez-Esquerra and Leeson, 2000). In the current study, no clear specific trend was observed in hen-day egg production by using fish oil supplementation and this could be due to insufficient replicates. However, it could be noted that dietary fish oil did not cause a significant decrease in egg production. Baulcels et al. (2000) stated that the use of fish oil at any percentage (0% to 4%) in diets never led to poorer performance of the layers. On the other hand, Cortinas et al. (2003) reported that egg production was significantly higher when laying hens were fed diets containing 4% fish oil.

Fish oil supplementation had a significantly negative effect on egg weight. However, no significant differences were detected among control, 1.25% and 2.5% fish oil, whereas using dietary fish oil more than 2.5% resulted in concomitant reduction in egg weight (Table 2). This response was similar to observations by Gonzalez-Esquerra and Leeson (2000) who reported that egg weight decreased (\(P < 0.05\)) linearly with increasing menhaden oil (2%, 4% and 6%). Also, Pappas et al. (2005) established that inclusion of fish oil significantly reduced the weight of the egg that was laid, as well as of its component parts. These results were in agreement with some published work

Discussion

Virtually, commercial table eggs contain a high proportion of n-6 PUFA but are a poor source of n-3 fatty acids. The inclusion of n-3 PUFA into yolk lipids is feasible and achieved by feeding diets rich in n-3 PUFA to hens. Fish oils are common feed ingredients used to increase yolk n-3 PUFA in layers (Leskanich and Noble, 1997). Including fish oil in commercial layer diets does not usually affect body weight gain, feed intake or egg production (Gonzalez-Esquerra and Leeson, 2000). In the current study, no clear specific trend was observed in hen-day egg production by using fish oil supplementation and this could be due to insufficient replicates. However, it could be noted that dietary fish oil did not cause a significant decrease in egg production. Baulcels et al. (2000) stated that the use of fish oil at any percentage (0% to 4%) in diets never led to poorer performance of the layers. On the other hand, Cortinas et al. (2003) reported that egg production was significantly higher when laying hens were fed diets containing 4% fish oil.

Fish oil supplementation had a significantly negative effect on egg weight. However, no significant differences were detected among control, 1.25% and 2.5% fish oil, whereas using dietary fish oil more than 2.5% resulted in concomitant reduction in egg weight (Table 2). This response was similar to observations by Gonzalez-Esquerra and Leeson (2000) who reported that egg weight decreased (\(P < 0.05\)) linearly with increasing menhaden oil (2%, 4% and 6%). Also, Pappas et al. (2005) established that inclusion of fish oil significantly reduced the weight of the egg that was laid, as well as of its component parts. These results were in agreement with some published work
Effects of feeding graded levels of fish oil on (a) plasma thiobarbituric acid-reactive substance (TBARS) and yolk arachidonic acid (20:4n-6), (b) hepatic TBARS and glutathione peroxidase (GSH-Px) activity and (c) antibody titre. Values are expressed as mean ± standard deviation error; mean values with different letters are significantly different from each other (**P < 0.01).
content was significantly lower in 3% fish oil treatments. This phenomenon is probably due to the greater utilisation of Δ-6-desaturase in the n-3 fatty acid pathway with respect to the n-6 pathway, as this enzyme acts in both pathways. High concentrations of dietary n-3 fatty acids reduce the activity of the enzyme in the n-6 pathway and the conversion of linoleic into ARA. The decrease in ARA content could be important for human health, as this acid is a precursor of some proinflammatory eicosanoids (Simopoulos, 2000).

The egg cholesterol content significantly differed due to dietary fish oil treatment (Table 2). Our results indicated that no clear trend was observed in egg cholesterol content; however, hens fed 2.5% fish oil had the lowest egg cholesterol value. Lewis et al. (2000) noted that cholesterol content in the modified egg has also been reduced to 180 mg/egg, compared with the standard egg value of 210 mg/egg. Moreover, Hargis (1988) suggested that egg yolk cholesterol showed little or no variation in response to genetic, pharmacological or dietary manipulation. Furthermore, Schreiner et al. (2004) did not find any differences in the yolk cholesterol content in response to dietary n-3 PUFA.

Using supplemental levels of fish oil (1.25% and 2.5%) significantly decreased (P ≤ 0.05) lipid peroxidation in both blood serum and liver compared with control (Figure 1a and b). This may refer to the hypolipidemic effects of dietary fish oil, 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 produces lower abdominal fat deposition than saturated or monounsaturated fatty acids (Crespo and Esteve-Garcia, 2001). This in turn will reduce the free radicals attack on these lipids, and consequently reduce lipid peroxidation. Not surprising, the same trend was observed in hepatic GSH-Px activity (Figure 1b). Supplemented fish oil (more than 2.5%) significantly reduced the hepatic GSH-Px activity, but addition of 1.25% to 2.5% significantly enhanced the activity compared with the control. This decline in the GSH-Px activity in high levels of fish oil treatments could be explained by the presence of high levels of the free radicals, which induced oxidative stress in all the body due to high levels of n-3 PUFA. Taking into account the involvement of GSH-Px in the immune function, it is important to note that the antioxidative enzyme GSH-Pxs are 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 regulation of the biosynthesis of prostaglandins from their precursor, ARA; however, the precise nature of GSH-Px involvement in eicosanoid metabolism is not fully understood (Pappas et al., 2005). Interestingly, it is important to mention that fish oil can decrease the oxidation products generated by the inflammatory cells which accompany ischaemic reperfusion. Moreover, Constant (2004) concluded that when concentrated n-3 PUFA is fed to animals no peroxides are produced, and so no antioxidants are necessary. Therefore, it could be documented that enriching chicken eggs with n-3 PUFA using fish oil not more than 2.5% may enhance the antioxidative status and reduce lipid peroxidation, which probably resulted in improving the immune responsiveness in laying hens in the present study.

The current study demonstrated that the levels of dietary n-3 PUFA affected immunity in laying hens (Figure 1c). It is also interesting to note that with dietary fish oil levels below 3.5%, an increase in antibody titre against a non-replicating antigen, SRBC, in laying hens takes place as fish oil concentration of the diet increases. This response is similar to observations by Fritsche et al. (1991) who showed that 2% fish oil gave the highest antibody titre in birds challenged with SRBC. Moreover, Wang et al. (2000) reported similar results in which birds fed n-3 PUFA (fish oil diet) had a significantly higher antibody production than those fed linseed oil or animal fat at 8 weeks of age. Furthermore, studies have demonstrated that dietary PUFA can modulate a wide range of immune responses in poultry. Apparently, diets enriched with fish oil rich in long-chain n-3 PUFA (EPA and DHA) have anti-inflammatory properties (Surette et al., 1995), increase delayed-type hypersensitivity (Korver and Klasing, 1997), increase in antibody responses (Friedman and Sklan, 1995) and decrease in lymphocyte proliferation (Fritsche et al., 1991). Further experiments by Wang et al. (2000) demonstrated that diets enriched with fish oil suppress ARA-derived prostaglandin E2 (PGE2) production. Therefore, it could be assumed that addition of anti-inflammatory lipids, such as fish oil, has also been shown to be beneficial for birds’ health. In fact, the immunomodulatory functions of dietary PUFA have been widely studied in mammals; however, research on poultry is limited. Moreover, in poultry, there are conflicting results on the effect of dietary lipids on immune functions. Several studies indicated that increasing the consumption of n-3 PUFA in diets has been shown to either increase or decrease immunological parameters of both humoral and cell-mediated immunity (Wang et al., 2000). Suppression of the inflammatory response could be beneficial to poultry in minimising the catabolic effect from both pathogens and environmental immunogens (Korver and Klasing, 1997). Furthermore, it has been reported that feeding high levels of n-3 PUFA (5% to 7% fish oil in the diet) suppressed the cell-mediated immune response, as measured by in vitro lymphocyte proliferation in laying hens (Wang et al., 2000). Parmentier et al. (1997) showed that antibody responses to different antigens were decreased by dietary n-3 PUFA. In addition, consuming n-3 PUFA resulted in a reduction in the production of cytokines such as interleukin-1, interleukin-2 and tumour necrosis factor-α, which are important in cell-mediated and inflammatory immune responses (Endre et al., 1993). Likewise, Korver and Klasing (1997) reported that fish oil suppresses the release of interleukin-1 and tumour necrosis factor-α in chicken. Collectively, researchers observed that fish oil has also been shown to be immunosuppressive at high concentrations and immunostimulatory at lower concentration in chickens (Fritsche et al., 1991; Korver and Klasing, 1997; Wang et al., 2000).
Korver and Klasing (1997) declared that inclusion of low levels of n-3 PUFA (=2% fish oil) in broiler chick diets improved or did not change indices of the cell-mediated immune response. Similarly, Fritsche et al. (1991) have also shown that 2% fish oil gave the highest antibody titre in birds challenged with SRBC. Therefore, it is worth noting that the effect of n-3 PUFA on immune responses in our study appeared to be dependent on their dietary levels. Also, by taking into account our results in lipid peroxidation and antioxidative properties, it could be concluded that the optimum level of dietary fish oil may be not more than 2.5%.

Based on the data presented above, it could be concluded that inclusion of fish oil in laying hen diets at moderate levels increased the n-3 fatty acids content in eggs, improved antioxidative status, reduced lipid peroxidation and enhanced the antibody response in laying hens. Additionally, the current study confirmed that the dietary fish oil did not have a negative influence on different reproductive morphology parameters.

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