

## Effect of Addition of Fish Oil on the Performance Parameters of Laying Hens and the Fatty Acid Composition of Their Egg Yolk

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## Abstract

Fish oil (FO) has been reported to have a wide range of health-beneficial effects, including anti carcinogenic, anti atherogenic, anti-diabetic, and immune stimulatory effects. The aim of this study is undertaken to examine or to evaluate the effect of addition of FO on the performance parameters of laying hens and the fatty acid composition of their egg yolk. Ten months old single comb white leghorn (SCWL) layers were fed diets containing 3.0% FO or olein oil in a total of two experiments. Hen day egg production percentage, egg weight were determined daily for three weeks. The fatty acids composition was determined of the end of experiment (after three weeks of feeding the experiment feed) sixty hens was the total of the experiment birds (30 hens fed olein, 30 hens fed fish oil) were allocated to two dietary treatments (3% FO, 3% olein) with 3 replicates. The results of the study showed that hens fed 3% FO had increase egg, production, and egg weight (P < 0.05) than the hens fed 3% olein oil. Concentration of unsaturated fatty acids in egg yolk lipids of hen fed fish oil was increased significantly (P < 0.05), as a result of monounsaturated fatty acids and polyunsaturated fatty acids increased as FO added (P < 0.05). It can be concluded from the present experiments that addition of FO to hen's diet increased the amount of oleic and linoleic acids throw weeks of the experiment and that this increase is accompanied by egg size, and egg production.

#### **Keywords**

Fish Oil, Fatty Acids, Labeo Coubie, Egg Yolk, Laing Hens

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## **1. Introduction**

Cardiovascular disease (CVD) is one of the leading causes of death in men and women of all ethnic groups. Consuming saturated dietary fats and n-6 polyunsaturated fatty acids (PUFA) increase the risk of CVD, whereas consumption of n-3 PUFA may reduce CVD incidence (Simopoulos, 1997). Humans historically consumed a diet with an n-6:n-3 ratio of 1:1 to 4:1 (Eaton et al., 1996; Simopoulos, 2006). However, the typical ratio in developed countries is now greater than

10:1 (Azain, 2004). Major sources of n-6 PUFA are vegetable oils such as corn, sunflower, and soybean oil, whereas n-3 PUFA sources include fish such as salmon, trout, and tuna and plant sources such as flaxseed (Schmitz and Ecker, 2008). The US Food and Drug Administration (2004) gave qualified health claim status to n-3 PUFA. It was stated that "supportive but not conclusive research shows that consumption of EPA and DHA may reduce the risk of coronary heart disease." Omega-3 PUFA-enriched poultry meat has the potential to help meat consumers increase their

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n-3 PUFA intake. A minimum level of 300 mg of n-3 PUFA per 100 g of meat is needed to label the product n-3 PUFA-enriched (CFIA, 2003) .

In general, fish oils are rich sources of omega-3 FA and poor sources of omega-6, and the contents of linoleic acid (LA) are also low. The FA profile of the different oils varies with the time of year, the Processing method and the predominant fish species from which they were extracted (Alparsan. 2005). Fish oil has several positive effects, such as the physiological or metabolic effects on the performance parameters of broiler chickens. The role of omega-3 FA on the health of humans and animals (Pike, 1999), the effect of animal products that contain omega-3 on human health (Lopez- Ferrer, 1999, 2001), and a comparison of the effects of unsaturated and saturated diets on performance (Alparsan, 2005).

The present study was undertaken to examine or to evaluate the effect of addition of FO on the performance parameters of laying hens and the fatty acid composition of their egg yolk.

## 2. Materials and Methods

The Experiment was approved by Sudan University of Science and Technology, College of Agricultural Studies, Animal Production Department from: 16 / 7 / 2010 - 21 /8 /2010.

#### 2.1. Diets

Two dietary treatments were compared. Treatments consisted of layer ratio (Table1) based on corn and ground nut cake meal with fish oil or olein oil added at a level of 3%.

 Table 1. Composition and calculation of nutrition content of the experimental diets.

Feed Ingredients	Percentage
Corn	53.00 %
Ground Nut Meal	20.00 %
Wheat Bran	9.67 %
Lime stone	8.10 %
* Layer Concentrate Hendrix	5.00 %
Di calcium Phosphate	0.10 %
Salt	0.20 %
Choline	0.05 %
Anti toxins	0.20 %
Organic acid	0.20 %
Lysine	0.31 %
Meth	0.17 %
Fish oil or olein oil	3.00 %
Total	100 %

\*Protein 40%, ME 2,000 kcal/kg, Fat 3%, Fibre 2%, Lysine 6%, Methionine 2.8%, M+C 3.3%, Calcium 6%, total phosphorus 5.8%, Vit. A 240.000 IU/kg, Vit. D3 60.000 IU/kg, Vit. E 800 mg/kg, Vit. K3 40 mg/kg, Vit. B1 Thiamin 30 mg/kg, Vit. B2 100 mg/kg, Vit. B3 147, Vit. B12 400 mg/kg, Vit. B6 50 mg/kg, Vit. C 3.000 mg/kg, Niacine 700 mg/kg, Folic 10 mg/kg, Choline Chloride 8.000, Manganese 1.860 mg/kg, Copper 156 mg/kg, Iron 1.000 mg/kg, Zinc 1.080 mg/kg, Selenium 5 mg/kg, Iodine 20 mg/kg, Cobalt 20 mg/kg, Sodium 1.6% (Supplier Hendrix Co. Khartoum, Sudan).

## 2.2. Birds

Ten month old (60) layer hens single comb white leghorn (SCWL) was the total of the experiment (30 hens fed fish oil, 30 hens fed olein oil as treatments). Each treatment was randomly assigned to three pens each pen containing ten hens. Test diets, in a mach form, and water were offered on ad libitum basis light was provided for 17 hr per day. The housing was designed on the bases of the opened system (traditional battery); the dimensions of the cages were 3.0 meters length and 1.5 meter width.

#### 2.3. Oil Extraction from Egg Yolk

Egg yolk samples was separated and dried under specific condition (50°C) for 24 hours. Oil was extracted by Soxhlet apparatus using petroleum ether 40-60°C and determined according to the AOCS Official Method (1998). The oil content was determined as a percentage of the yolk sample weight (w/w). The samples were analyzed in triplicate then mean and standard deviation were calculated.

 Table 2. Hen day egg production (%) of layer fed corn-ground nut seed cake base with fish oil or olein oil.

Weeks Treatment	1	2	3
Fish oil diet	91.3a	91.7a	92.1a
Olein oil diet	64.5b	65.4b	68.8b
±MSE	0.43	0.13	0.24

Means with the same letter(s) are not significantly difference (P<0.05)  $\pm$ MSE: Means standard error.

The extracted oil was stored in  $(4^{\circ}C)$  in a dark glass bottle under nitrogen blanket for further analysis of fatty acids composition by gas chromatography (GC).

The fatty acid compositions of oil extracted from dry egg yolk were determined by GC. The egg yolk oil was converted to their corresponding methyl esters according to the AOCS Official Methods (1998). GC analysis of the fatty acids methyl esters FAME was performed using a Shimadzu 2101, gas chromatograph equipped with DB 23 capillary column from Agilent length 30 m I.D 0.25mm., film thickness of 0.25 um, polyamide coated with fused silica. C17 was used as internal standard. The column temperature program start from 90 °C hold 5 minute then until 208 °C with rate of 7 C/minute hold at this temperature for 12 minute. Both injector and detector temperatures were set at 260°C. The detector was FID. The carrier gas was hydrogen at a flow rate of 1.0 mL/min. The peaks of fatty acids were identified by comparing the retention times with those of a mixture of standard fatty acids methyl esters (Sigma Chemicals Co., Deisenhofen, Germany). Each fatty acid methyl ester sample was analyzed in duplicate.

## 2.4. Statistical Analysis

The analyses were performed with three replicates. The mean values and standard deviation (mean  $\pm$  SD) were calculated and tested using the Student t-test (P<0.05). Statistical Graphics System version 4.0 (Statgraphics® 1985–1989).

## 3. Results and Discussion

## 3.1. The Effects of the Experimental Diets on Egg Production, Egg Weight, During the Experimental Period

The all performance of laying hens fed 3% FO showed significant (P < 0.05) difference in hen day egg production (Table 2) and egg weight (Table 3) among the treatment throughout the experiment compared to the performance of those fed 3% olein oil diets. In this study, average egg productivity from the beginning to end of the experiment was in the range of 64-92 % in two groups (Table 2) and there were statistical differences between the groups (P<0.05). The highest percentage in egg production was found in laying hens fed 3% FO supplementation. It was reported that oils added to the poultry rations to compensate energy requirements increased the weight of egg yolk besides the egg weight (Whitehead et al., 1991). Cetingül and Inal (2003) determined that addition of sunflower oil (1.5%) and hazelnut oil (1.5-3%) to the ration did not affect egg productivity. Balevi and Coşkun (2000) reported that supplementation of nine different kinds (sunflower, cotton, corn, flax, soybean, olive, fish, tallow and rendering oil) of oil sources at 2.5% concentration did not have significant effect on egg production parameters. In contrast to our results Jiang et al. (1991) reported that addition of flaxseed oil and sunflower oil to the ration at the same level did not affect this parameter.

 Table 3. Egg weight (g) of layers fed corn-ground nut seed cake base with

 Fish oil or olein oil.

Weeks Treatment	1	2	3
Fish oil diet	53.92a	56.38a	57.02a
Olein oil diet	51.53a	51.11b	51.19b
±MSE	0.84	0.91	0.82

Means with the same letter(s) are not significantly difference (P<0.05).  $\pm$ MSE: Means standard error .

It is known that oil supplementation to the ration creates positive effect on egg weight (Inalet al. 1994, Coşkun et al. 1996). Shafey et al. (1999) compared groups having rations with 2% soybean oil to one which have no oil supplementation, they found that, egg weight was increased from 53.7 g to 54.5 g. However, in other studies egg weights were not affected (p>0.05) by addition of different oil supplementations (Balevi and Coşkun 2000, Baucells et al. 2000). There is another study (Whitehead et al. 1991) comparing the effect of high and low levels of oil on egg weight in layers. In the group where there was a high level of oil supplementation the egg weight was found to be 56 g and 64 g at the week 22 and 32, respectively, while it was 55 g and 61 g in the group containing low level oil.

The effect of fat efficiency feed could be related to the degree of unsaturation, because some authors (ALao and Balnave, 1985; Pinchasov and Nir, 1992; Zolisch et al, 1997) have reported that digestibility of fat increases as the degree of unsaturation increases. The inclusion of fish oil in poultry diets has also been reported to have no effect on consumption of feed (Huang et al, 1990). This was observed in this study, where no effect on consumption of feed of laying hens fed added dietary 3% fish oil throw three weeks compared to other fed 3% olein oil. This is in agreement with the findings of Huang et al (1990), Newman etal, (1998), Crespo et al (2001, 2002) and Lopez Ferrer et al, (1991, 2001).

Table 4. Fatty acid composition of egg yolk obtained from laying hens fed corn-ground nut seed cake base with fish oil control and treatments.

*BW3	*BW2	*BW1	Fatty acid
0.44±0.4	0.46±0.3	0.45±0.2	C14:0
28.56±0.7	28.77±0.8	28.86±0.9	C16:0
3.90±0.1	4.39±0.1	4.21±0.1	C16:1
7.68±0.3	7.58±0.3	7.42±0.2	C18:0
47.46±0.7	47.12±0.6	47.06±0.7	C18:1
11.55±0.2	11.49±0.2	11.39±0.2	C18:2
0.34±0.3	0.34±0.3	0.31±0.4	C18:3
0.42±0.1	0.40±0.2	0.38±0.1	C20:0
36.7	36.9	37.1	TSFA
63.3	63.1	62.9	TUSFA

BW1=treatment (fish oil 3%) in the first week, BW2=treatment (fish oil 3%) in the second week, BW3=treatment (fish oil 3%) in the third week.

 Table 5. Fatty acid composition of egg yolk obtained from laying hens fed corn-ground nut seed cake base with olein oil.

*BW3	*BW2	*BW1	Fatty acid
0.42±0.2	0.45±0.2	0.43±0.2	C14:0
28.59±0.6	28.65±0.3	$28.08 \pm 0.4$	C16:0
3.69±0.2	$4.04{\pm}0.2$	3.76±0.1	C16:1
6.82±0.2	7.92±0.3	6.86±0.2	C18:0
48.27±0.2	47.70±0.3	48.57±0.4	C18:1
11.70±0.3	10.40±0.2	11.79±0.3	C18:2
0.14±0.1	0.45±0.2	0.15±0.1	C18:3
0.36±0.1	0.35±0.1	0.35±0.1	C20:0
36.7	36.9	37.1	TSFA
63.3	63.1	62.9	TUSFA

BW1=treatment (olein oil 3%) in the first week, BW2=treatment (olein oil 3%) in the second week, BW3=treatment (olein oil 3%) in the third week.

Fish or olein oil compared with the standard of Hi sex company standard performance.

#### **3.2. Fatty Acid Composition**

# The fatty acid composition of the egg yolk, as analyzed by GC.

The fatty acids used in this study fish oil (FO) (Labeo Coubie origin ) have high concentrations of linolenic acid (LNA, C18:3n-3) and long chain n-3 PUF (C:20) is given in (Table 4). These data are consistent with those obtained in other studies (Phetteplace and Watkins, 1990; Olomu and Barcosa, 1991; and Manilla et, 1999).

#### The inclusion of fish oil

This can be attributed to the high concentration of linoleic and linolenic acids in fish oil diet. Thus, the level of linoleic and linolenic acids has increased. The addition of fish oil in the diets significantly increased (P < 0.001) saturated fatty acids (SFA) (C:16 and C:18). Linoleic acid (LA) content of egg yolk increased (P < 0.001) within weeks, LA in first week was 11.39 and increased to, 11.49 and 11.55, within 2<sup>nd</sup>, and 3<sup>rd</sup> week, respectively.

The dietary fatty acid composition affected the yolk fatty acid profile. Raes et al. (2002) found that when diets containing only plant oil without CLA addition were considered, the yolk fat was relatively unsaturated. The amount of SFA did not vary greatly among these diets, yet no major differences were present in the 18 unsaturates, particularly 18:2 where it was decreased but not significantly. In contrast to the above research, the present study found no significant increases in C18:3, C16:0, and C18:0, C14:0 but found decreases in C18:2.

## 3.3. The Effects of Fish Oil on Fatty Acid Composition of Egg Yolk

The effects of fish oil diet and olein oil diet on fatty acid composition of egg yolk is summarized in (Tables 4 and 5). The results showed that all fatty acids of yolk lipids were significantly altered by dietary fish oil supplementation. As compared with each other, feeding 3% fish oil decreased of C18:3, C14:0, C16:0, and C18:0 in the egg yolk lipids (Table 4), whereas other fatty acids were decreased linearly and quadratically, except for C20:0 and PUFA, which were reduced linearly. Total saturated fatty acids (TSFA), were decreased, while total unsaturated fatty acids (TUFA), were increased (P<0.05), whereas the contents of monounsaturated fatty acids (C16:1, C18:1) were increased also (Table 4). The results of the study were similar to previous reports (Schafer et al., 2001; Cherian et al., 2002; Yang et al., 2002; Szymczyk and Pisulewski, 2003, Shang et al., 2004, 2005). The putative benefits of conjugated linoleic acids (CLA) to human health, food products rich in CLA have been extensively investigated in recent years. It has been found that egg yolk is a good carrier of fatty acids (Chamruspollert and Sell, 1999). Chamruspollert and Sell (1999) found that the CLA concentration of egg yolk lipids increased linearly with increasing dietary CLA. Other studies have achieved similar results (Ahn et al., 1999; Cherian et al., 2002; Szymczyk and Pisulewski, 2003; Shang et al., 2004). It has been found that the greater supplementation of dietary FO is deposited in yolk lipids. In this study, the total C18:3 in yolk lipids were changed (P < 0.05).

## 4. Conclusions

The performance of layer hens fed FO as measured by hen day egg production percentage and egg weight was improved in comparison to the birds fed the olein oil. The composition of eggs was altered by using FO supplements as one of the dietary energy sources. The content of linoleic, and linolenic in eggs was increased by increasing FO in the diet throw weeks of the experiment. The 3% level of dietary FO was the most efficacious with respect to performance.

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