Influence of different sources of fishmeal on the egg yolk fatty acids profile and plasma lipids profile of layers

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Summary

This trial was conducted to find out the influence of the adding marine and River Nile fishmeal in conventional layers feed at two different rates 2 and 1% for each meal on egg yolk fatty acids profile and plasma lipids profile of layers. Hundred laying hens - (Hisex breed), 20 weeks old, were used in this study. Feeding of fishmeal to laying hens for two months showed significant effect on egg yolk content of poly unsaturated fatty acids. Fish meal had significantly reduced plasma total lipids, triglycerides, cholesterol and low density lipoproteins, while high density lipoprotein was raised.

Introduction

The egg is one of the best and least-expensive sources of high-quality protein and contains a balanced distribution of most vitamins and minerals in relation to its low calorie content. However, one large egg contains 220 mg cholesterol, making it one of the major sources of dietary cholesterol for humans. Dietary manipulation of the n-3 fatty acid content of laying hen rations results in the production of eggs containing substantial amount of n-3 fatty acids, whereas, attempts to modify whole-egg cholesterol content to meet demands of health conscious consumers have been largely unsuccessful (Mary E.Van elswyk, 1997). These n-3 fatty acids-enriched eggs may serve as an alternative to fish for these fatty acids and may have health benefits despite their unaltered cholesterol concentration. In fact moderate lowering of plasma triglycerides concentrations was observed in human subjects consuming eggs enriched in EPA and DHA from hens fed fish oil. Eggs high in alpha-linolenic acid have been produced by feeding hens ground Flaxseed. Although less pronounced than for alpha-linolenic acid, the DHA

concentration is also significantly increased in these eggs (Ferrier *et al*., 1995).

Over the past twenty years many studies, and clinical investigations revealed that omega-3 (PUFA), particularly Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA), were found at great amounts in fish oil (Al-Daraji, *et al.*, 2010). Fish omega-3 FAs (namely EPA and DHA) possess a lot of properties that can explain their positive impact on cardiovascular events seen both in epidemiological and interventional studies. EPA and DHA differ in their ability to promote various effects of omega-3 FAs supplementation. Obviously, the two occur always together in natural sources – fish meals and fish oil. However, as highly purified EPA and DHA became available, evidence documenting individual effects of EPA and DHA has been accumulated (Vrablik, *et al.*, 2009).

In general, fish oils are rich sources of omega-3FA 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 and Ozdogan, 2005). Many studies have examined the effects of dietary LC-PUFA, supplied as fish oil or fish meal on the FA composition of the broiler carcass (Nash, *et al.,* 1995; Scaife, *et al.,* 1994 and Lopez-Ferrer, 2001). Marine sources of long chain omega-3 fatty acids, offer the benefit of direct incorporation of long chain omega-3 fatty acids into eggs, which are metabolically more important than ALA for humans (Simopoulos, 2000).

Marketing trends in egg industry have centered on supplying the consumers with a low cholesterol product. The fatty acids composition of egg yolk is readily altered by dietary manipulation. Recent evidence has suggested that incorporation of specific fatty acids (omega-3 fatty acids) into egg yolk may positively affect human plasma lipids, thus providing egg product of improved health quality (Hargis, Van Elswyk and Hagris (1991). The purpose of this study is to investigate into the effect of supplementing layers diet with two levels of Marine and River Nile fishmeal (2 and 1% each), on egg yolk fatty acids profile and plasma lipids profile of layers.

Materials and Methods

The experiment was held in the Veterinary Research Institute **(**VRI**)** at Soba, from January to March 2010. The duration of the experiment was 8 weeks.

Seven, full wire cages were made, each cage was (2 x 1.5 x 1 meter), and the capacity of each cage was 20 birds. The cages were placed in an open poultry house.

Hundred laying hens (Hisex breed), 20 weeks old, obtained from the Animal Production Research Centre (APRC) at Kuku, were used in this study. The birds were divided into five groups, 20 birds per treatment per cage. The diets were formulated to meet the nutrients requirement of laying hens according to the recommendations of the National Research Council (NRC, 1994). Four formulae of diets were prepared by inclusion of, marine fishmeal (2%, 1%) (Group B and C resp.) and river Nile fishmeal (2%, 1%) (Group D and E resp.). Twenty kilogrammes of marine fish were brought from Port Sudan, and twenty kilos of river fish were brought from a local fish market. Viscera and heads were removed, and then they were minced and sun-dried in an open yard. After that they were crushed down using electrical crusher. Each hen group received its experimental diet from day one. Drinking system contained two tanks for each cage, the tanks were cleaned, and the water was changed twice daily. Twenty eggs from each group; were collected randomly throughout the eighth week for egg yolk lipids profile. Egg was broken, the yolk was separated. Each two yolks were pooled together and placed into a glass container and stored at -20˚C until analysis. Three ml of blood were collected from seven birds of each group weekly, the blood was taken using a three ml syringe, and received into EDTA coated vials, the samples were centrifuged at 3000rpm, and plasma was transferred into plane vials. Plasma samples were stored at -20˚C until analysis. Lipids were extracted in chloroform-methanol (2:1 v/v) according to the method of Floch; *et al.*, (1957). Methyl esters of the lipid extract were prepared according to Wang; *et al.*, (2000). The analysis was performed

using (2010, Shimadzu, Japan) gas chromatograph, fitted with Flame ionization detector (FID). Separation of fatty acids was achieved using DB-WAX column, serial number (US6551263 H), of 0.25um film thickness, 30 meter length and 0.25 mm inner diameter. Fatty acids methyl esters were identified by comparison of retention times with standards, and expressed as percentage of methyl esters. **Table (3) and (4)**, shows the accumulative effect of feeding 2%and 1% marine fishmeal and

river Nile fishmeal on egg yolk fatty acids content. The plasma lipids profile were determined using commercial kits by Unicam 8625 Spectrophotometer following the instructions of the manufacture, **Table (5)**, shows the accumulative effect of feeding 2% and 1% marine fishmeal and river Nile fishmeal on plasma lipids profile.
Statistical analysis was done using Analytical Software, (2008). Statistics (9). User's Manual. Analytical Software, Tallahassee, FL.

Table 1. Experimental and control diet composition (%)

Diet/Feed ingredient	A (Control)	B	C	D	E
Sorghum	62.0	67.4	67.8	66.5	67.5
Ground nut cake	19.0	11.4	12.0	12.0	12.0
Wheat bran	3.5	3.4	3.4	3.7	3.7
Layer concentrate	5	5	5	5	5
Lime stone	10	10	10	10	10
Salt (Nacl)	0.125	0.125	0.125	0.125	0.125
Lysine Hcl (75%)	0.1	0.1	0.1	0.1	0.1
Dl-Methionine	0.1	0.1	0.1	0.1	0.1
Mycofix (Antimycotoxin)	0.1	0.1	0.1	0.1	0.1
Biotronic (Acidifier)	0.1	0.1	0.1	0.1	0.1
Marine fishmeal	0	2	1	0	0
River Nile fishmeal	0	0	0	2	1
Total	100	100	100	100	100

Table 2. Calculated chemical composition (%) of experimental and control diet.

Diet/ Nutrient	A	B	C	D	E
Crude Protein	18	17.75	17.5	18.2	18.0
Ether extract	5	3.5	3	3.1	3.0
Crude Fiber	4	4	4.1	3.3	3.3
Calcium	4.1	4	4	4	4
Total Phosphorous	0.31	0.64	0.62	0.64	0.62
Methionine+cystine	0.73	0.72	0.72	0.72	0.72
ME (Kcal/kg)	2800	2750	2753	2750	2753

Results

The accumulative effect of consuming different supplementary fishmeal sources in layers diet resulted in no significant difference in egg yolk saturated fatty acids content compared with the control group **(Table 3)**. On the other hand, groups (E), (C), and (D), showed a significant ($P<0.05$) high levels of mono unsaturated fatty acids (MUSFA), compared to the control group **(Table 4)**.

Group (B), which was fed 2% marine fishmeal, showed significant high levels of omega-3 fatty acids compared to all groups. Group (B), showed significant ($P<0.05$) high levels of the total (PUSFA), compared to group (D), while group (C), (D) and (E); showed no significant difference between them. The control group showed a significant ($P<0.05$) low levels of the total unsaturated fatty acids (USFA), compared to group (B), (D) and (E), while group (C), which was fed 1 % marine fishmeal; showed the lowest levels of the total unsaturated fatty acids (USFA), among all experimental groups as well as the control group (A). Group (B), which was supplemented, by 2% marine fishmeal; showed a significant ($P<0.01$) high level of

Docosahexaenoic acid (DHA), Docosapentaenoic acid (DPA) and Eicosapentaenoic acid (EPA), compared to all groups. These three very long chain poly unsaturated omega-3 fatty acids were not detected at all in the other groups. All groups deposited a significant ($P<0.01$) high level of linoleic acid (omega-6 FA), into their egg compared to the control group. Group (B), (C), (D), (E) and the control group (A), showed no significant different levels of Linolenic acid between them. The control group (A), deposited a significant ($p<0.05$) low levels of Oleic acid (omega-9 FA), compared to group (C), (D) and (E), on the other hand, the control group (A), showed a significant less levels of palmatic acid, compared to group (C) and (E). The control group (A), showed a significant ($P<0.01$) less levels of Stearic acid, compared to all other groups.

Group (B), which was supplemented by 2% marine fishmeal, showed a significant (P<0.05) high stearic acid levels compared to group (C), which was fed 1% marine fishmeal, **tables (3) and (4)**.

Table (3) The effect of feeding different diet types on egg yolk fatty acids content %.

Groups	Palmitic %	Stearic %	Oleic %	Linoleic %	Linolenic %	Arachidonic %	EPA %	DPA %	DHA %
Control (A)	21. 771 ± 4.9994^{B}	1.0222 ± 1.3269 ^{C}	35.11± 8.251^{B}	0.00 ± 1.2633 ^{D}	0.00 ± 0.8327^{B}	0.00 ± 0.1587^{B}	0.00 ± 0.071^{B}	0.00 ± 0.024^{B}	0.00 ± 0.032^{B}
Marine fish 2% (B)	30.766 ± 4.9994^{AB}	8.3796 ± 1.3269 ^{A}	47.94± 8.25^{AB}	9.1662 ± 1.2633^{BC}	0.4316 ± 0.8327^{B}	0.1758 ± 0.1587^{AB}	1.9416 ± 0.0716^{A}	0.677 ± 0.^{024A}	0.517± 0.032^{A}
Marine fish 1% (C)	33.286 ± 4.9994^{A}	5.6320 ± 1.3269^{B}	52.13± 8.251^{A}	8.9106 ± 1.2633^{BC}	0.00 ± 0.8327^{B}	0.0492 ± 0.1587^{B}	0.00 ± 0.0716^{B}	0.00 ± 0.024^{B}	0.00 ± 0.032^{B}
River fish 2% (D)	30.219 ± 4.9994^{AB}	7.6516 ± 1.3269^{AB}	52.72± 8.251^{A}	9.2680 ± 1.2633^{BC}	0.0488 ± 0.8327^{B}	0.1636 ± 0.1587^{AB}	0.00 ± 0.0716^{B}	0.00 ± 0.024^{B}	0.00 ± 0.032^{B}
River fish 1 % (E)	32.442 ± 4.9994^{A}	5.7918 ± 1.326^{AB}	54.11± 8.251^{A}	7.6490 ± 1.2633^{C}	0.00 ± 0.8327^{B}	0.00 ± 0.1587^{B}	0.00 ± 0.071^{B}	0.00 ± 0.02^{B}	0.00 ± 0.03^{B}

Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at (P<0.05).

Table 4. The accumulative effect of feeding different diet types on egg yolk content (%) of fatty

Group	∑ Saturated %	∑ Unsaturated %	C	∑ Poly unsaturated %	∑ Omega3 %
Control (A)	41.396 ± 1.7728^{A}	58.517 ± 1.5430^{B}	35.11± 1.7728^{A}	0.00 ± 1.5119^{D}	0.00 ± 0.6172^{D}
Marine fishmeal 2% (B)	39.145 ± 1.7728^{AB}	60.855 ± 1.5430^{AB}	47.945 ± 1.7728^{D}	12.910 ± 1.5119^{B}	3.5682 ± 0.6172^{B}
Marine fishmeal 1% (C)	40.296 ± 1.7728^{AB}	52.384 ± 1.5430^{C}	52.138 ± 1.7728^{BC}	8.9106 ± 1.5119^{C}	0.00 ± 0.6172^{D}
River fishmeal 2% (D)	37.871 ± 1.7728^{AB}	62.345 ± 1.5430^{A}	52.720 ± 1.7728^{BC}	9.6200 ± 1.5119^{C}	0.0813 ± 0.6172^{D}
River fishmeal 1% (E)	38.234 ± 1.7728^{AB}	61.763 ± 1.5430^{AB}	54.114 ± 1.7728^{B}	7.6490 ± 1.5119^{C}	0.00 ± 0.6172^{D}

Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at (P<0.05).∑SFA (Palmitic-Stearic), ∑MUSFA (Oleic), ∑PUSFA (Linoleic-linolenic-Archidonic-EPA-DPA-DHA). ∑ Omega-3 FA (Linolenic-EPA-DPA-DHA).

The control group (A), showed a significant (P<0.01) high plasma triglycerides concentration compared to all treated groups. There was no significant difference between group (B) and (D), while group (B), showed a significant (P<0.01) low triglycerides compared to group (E).

The control groups (A), showed significant (P<0.01) high cholesterol concentration compared to all treated groups. Group (B) which supplemented by 2% marine fishmeal, recorded the lowest mean of plasma cholesterol 96.285±1.6620, **(Table 5)**.

The control group (A), showed a significant (P<0.01) low level of plasma high density lipoprotein (HDL) compared to all treated groups, Group (B), which was supplemented by 2% marine fishmeal, recorded the highest level of plasma HDL The control group (A), also showed a significant (P<0.01) high concentration of plasma low density lipoprotein (LDL) compared to all treated groups. There was no significant difference between group (C),

which was fed 1% marine fishmeal, and group (E), which was fed 1% river fishmeal, also there was no significant difference between group (B), which received 2% marine fishmeal and group (D), which received 2% river fishmeal. The control group (A), also showed significant ($P<0.01$) high plasma total lipids compared to group (B), (C), (D) and (E), **Table (5).**

Table 5. The accumulative effect of feeding 2% and 1% fish meal on plasma lipids (mg/dl) profile.

Group	Total lipids	Cholesterol	Triglycerides	LDL-Cholesterol	HDL-Cholesterol
Control (A)	592.7±10.247^{b}	124.28±1.7174^{b}	334.9±6.2518^{b}	49.143±1.3356^{b}	9.13±0.8646^{b}
Group (B)	539.9±6.7631^{b}	96.285±2.5633^{b}	219.13±5.6656^{b}	32.0872.4580^{b}	18.945±1.4837^{b}
Group (C)	551.2±6.8057^{b}	99.765±3.0666^{b}	227.61±9.6551^{a}	36.263±1.4418^{b}	17.542±1.7090^{b}
Group (D)	542.6±7.4450^{b}	96.565±2.3489^{b}	219.72±3.9346^{b}	31.328±2.5293^{b}	18.303±1.5120^{b}
Group (E)	552.2±8.5976^{b}	99.725±3.7516^{b}	232.00±4.2121^{b}	36.147±1.9124^{b}	18.155±1.1258^{b}

Data are means ± standard error. Means in the same columns followed by the same letters are not significantly different at ($P<0.05$).

Discussion

The significant high levels of DHA, DPA and EPA, which were deposited in the egg yolk of hens fed 2% marine fishmeal (group B) were also reported by (Gonzalez-Esquerra and Leeson, 2001). Menhadin oil, fishmeal and marine algae were frequently used by (Gonzalez-Esquerra and Leeson, 2001) to enrich egg yolk with EPA, DPA and DHA. In this study, similar results were obtained, when the layers fed 2% marine fishmeal (group B), they deposited high levels of DHA, DPA and EPA in their egg yolk.

Vertebrates lack delta-12 and delta-15 fatty acids desaturates responsible for converting oleic acid into linoleic acid and α-linolenic acid. Therefore, they are unable to biosynthesize poly unsaturated fatty acids *de novo* (Tinoco, 1982), and (Holman, 1986). So the long chain omega-3 fatty acids which were found in fish are actually obtained from marine algae. In group (D) layers which were fed 2% river fishmeal, there was no deposition of longer chain omega-3 fatty acids in their yolk, this could be probably to lack of algae or it could be that omega-3 fatty acids are available in trace amount undetectable by gas chromatograph analysis, also it could be due to difference between fish species that live in the sea and that live in river water, and may also be due to difference, in marine and fresh water algae constituent, perhaps marine algae are rich in long-chain omega-3 precursors than fresh water algae.

All four groups which fed fishmeal, showed a significant reduction of plasma total lipids, cholesterol, triglycerides and LDL-cholesterol concentrations; compared to the control group, while the HDL-cholesterol level was significantly increased, these findings agree with (AL-Sultan, 2005), who reported that feeding 1.5% and 3% of fish oil to laying hens resulted in low concentration of total lipids, cholesterol, triglycerides and LDL-cholesterol in plasma, while the concentration of HDL-cholesterol was significantly elevated. Simopoulos, (1991), studied the effect of dietary omega-3 fatty acids on factors and mechanisms involved in the development of inflammation, atherosclerosis and immune diseases; reported that; a reduction in LDL-cholesterol, triglycerides and an increase in levels of HDL-cholesterol. (Harris, and Connor, 1984), reported a reduction in LDL-cholesterol levels in normal subjects, fed diets containing fish oil as a source of omega-3 fatty acids, also there was low concentration of cholesterol and triglycerides. The same authors; justified the reduction of LDL-cholesterol in human subjects consuming diets rich in long chain omega-3 FA, from fish oil, or omega-6 FA, from vegetables oils may be due to a reduction in LDL-cholesterol synthesis, an increased fractional rate of catabolism of LDL, or combination of both.

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أثر مصادر مختلفة من مسحوق السمك على تكوين الاحماض الدهنية فى صفار البيض وتكوين الدهون في البلازما فى الدجاج البياض

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الملخص:

تم اجراء هذه التجربة لمعرفة اثر إضافة مسحوق السمك البحري والنيلي بمقدار 1 و 2% لكل على حدة في عليقة تقليدية للدجاج البياض على تركيبة الأحماض الدهنية في صفار البيض و تركيبة الدهون فى بلازما الدم في الدجاج البياض. استخدمت 100 دجاجة بياضة من سلالة (الهايسكس) عمر 20 اسبوع فى هذه الدراسة. أظهرت تغذية الدجاج البياض لمدة شهرين علي ان اضافة مسحوق السمك أثر معنويا علي محتوي صفار البيض من الاحماض الدهنية الغير مشبعة . كما أدت إضافة مسحوق السمك لعليقة الدجاج البياض الى انخفاض في مستوي كل من الدهون الكلية للبلازما, الجلسريدات الثلاثية, الكلسترول والبروتينات الدهنية منخفضة الكثافة, بينما ارتفعت البروتينات الدهنية عالية الكثافة.