ผลของการใช้เมล็ดเฟล็กซีดร่วมกับน้ำมันปลาในอาหารต่อสมรรถนะการผลิตของไก่ไข่ กรดไขมันในไข่แดงและปริมาณคอเลสเตอรอลในไข่แดงและในซีรั่มของไก่ไข่

Effects of Dietary Addition of Flaxseed and Fish Oil on Laying Performance, Egg Yolk Fatty Acids and Cholesterol in Yolk and Serum of Laying Hens

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Abstracts

This trial was conducted to investigate the effect of dietary addition of flaxseed and fish oil on productive performance, egg yolk fatty acid composition, and on cholesterol levels in yolk and blood serum of laying hens as well as sensory evaluation of eggs. In total, 200 laying hens were divided into 5 groups. Ten birds were randomly assigned to each treatment (5 cages) and each treatment was replicated 4 times. The birds were fed a standard layer diet (17% CP; 2800 Kcal/ME/kg diet). The diets contained 0-0, 10-1.5, 15-1.5, 10-3.0 and 15-3.0% combinations of flax seed to fish oil in FS_0FO_0, $FS_{10}FO_{1.5}$, $FS_{15}FO_{1.5}$, $FS_{10}FO_{3.0}$ and $FS_{15}FO_{3.0}$ treatments, respectively. When compared to the controls, only $FS_{15}FO_{3.0}$ hens consumed more feed per day ($P<0.01$) ; thus, feed conversion ratio of the controls was better ($P<0.01$) than those of the $FS_{15}FO_{3.0}$ groups. There were no significant differences in egg weight, egg mass and egg production among treatment groups. The level of MUFA of the yolk decreased progressively when dietary FS and FO increased ($P<0.01$). The addition of FS and FO significantly decreased the level of η-6 FAs, but the level of η-3 FAs (LNA, EPA, DPA and DHA) increased linearly and significantly with increasing FS and FO levels in diets ($P<0.01$). Consequently, the ratios of PUFA:SFA in the treated groups were significantly higher than those of the controls whereas the ratios of $\Sigma\eta$-6: $\Sigma\eta$-3 were significantly lower ($P<0.01$). The yolk cholesterol significantly decreased progressively with the increasing levels of dietary FS and FO when compared to the control ($P<0.01$). Apart from $FS_{10}FO_{1.5}$ hens, it was shown that serum cholesterol level significantly decreased by increasing FS and FO in diets ($P<0.05$). There were no significant difference in sensory evaluation of eggs among groups ($P>0.05$).

Key words: Cholesterol, fish oil, flaxseed, laying hen, yolk fatty acids

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บทคัดย่อ

การศึกษาครั้งนี้มุ่งที่จะศึกษาผลของการใช้เมล็ดเฟล็กซีด และน้ำมันปลาในอาหารไก่ไข่ต่อสมรรถนะการผลิตของไก่ไข่ กรดไขมันในไข่แดง และปริมาณคอเลสเตอรอลในไข่แดงและในซีรั่มของไก่ไข่ รวมทั้งการตรวจสอบกลิ่นและรสชาติในไข่ โดยใช้ไก่ไข่จำนวน 200 ตัวแบ่งออกเป็น 5 กลุ่ม ๆ ละ 4 ซ้ำ ๆ ละ 10 ตัว (5 กรง) และใช้สูตรอาหาร 5 สูตร ที่มีระดับโปรตีน 17 เปอร์เซ็นต์ และมีพลังงาน 2800 กิโลแคลอรี่ ต่อกิโลกรัมอาหาร สูตรอาหารที่ 1, 2, 3, 4, และ 5 ประกอบไปด้วยเมล็ดเฟล็กซีดและน้ำมันปลาที่ระดับ 0–0, 10–1.5, 10–3.0, 15–1.5 และ 15–3.0 % ตามลำดับ ไก่ไข่กลุ่มที่ 5 กินอาหารในปริมาณมากกว่ากลุ่มควบคุม ($P<0.01$) ดังนั้นไก่ไข่กลุ่มควบคุมจึงมีประสิทธิภาพการเปลี่ยนอาหารเป็นไข่ดีกว่ากลุ่มที่ 5 อย่างมีนัยสำคัญยิ่งทางสถิติ ($P<0.01$) แต่ไม่พบความแตกต่างกันทางสถิติของน้ำหนักไข่ มวลไข่ และผลผลิตไข่ของไก่ไข่ทุกกลุ่ม ปริมาณกรดไขมันไม่อิ่มตัวพันธะคู่เดียวในไข่แดงลดลงเมื่อมีการเพิ่มเมล็ดเฟล็กซีดและน้ำมันปลาในสูตรอาหาร การใช้เมล็ดเฟล็กซีดและน้ำมันปลาในสูตรอาหารมีผลทำให้ปริมาณกรดไขมันโอเมก้า–6 ในไข่แดงลดลง แต่ขณะเดียวกันมีผลทำให้ปริมาณกรดไขมันโอเมก้า–3 ในไข่แดงเพิ่มขึ้นตามปริมาณเมล็ดเฟล็กซีดและน้ำมันปลาที่เพิ่มขึ้นในสูตรอาหาร ($P<0.01$) และมีผลทำให้สัดส่วนของกรดไขมันไม่อิ่มตัวต่อกรดไขมันมันอิ่มตัวเพิ่มขึ้นในขณะที่สัดส่วนของกรดไขมันโอเมก้า–6 ต่อ กรดไขมันโอเมก้า–3 ลดลง ($P<0.01$) นอกจากนี้ยังพบว่าปริมาณเมล็ดเฟล็กซีดและน้ำมันปลาที่ใช้ในสูตรอาหารมีผลทำให้ปริมาณคอเลสเตอรอลในไข่แดงลดลง อย่างมีนัยสำคัญยิ่งทางสถิติ ($P<0.01$) และปริมาณคอเลสเตอรอลในซีรั่มยกเว้นกลุ่มที่ 2 ลดลงต่ำกว่ากลุ่มควบคุม ($P<0.05$) และไม่พบความแตกต่างทางสถิติของกลิ่นที่ตกค้างในไข่ของไก่ไข่ทุกกลุ่มทดลอง

คำสำคัญ: กรดไขมันในไข่แดง คอเลสเตอรอล แม่ไก่ไข่ เฟล็กซีด น้ำมันปลา

Introduction

Consumers are increasingly interested in functional foods. One category of functional foods of great interest are products containing omega–3 fatty acids (n–3 PUFA). Consumption of n–3 PUFA has been reported to reduce the risk of atheroselerosis and stroke and to promote infant growth (Clandinin et al., 1989; Ferrier, 1992). Different feeds, such as flaxseed, safflower oil, perilla oils, marine algae, fish, fish oil, and vegetable oil have been added to chicken feeds to increase the n–3 PUFA content in the egg yolk (Jiang et al., 1991; Herber and Van Elswyk, 1996; Kim et al., 1997;Van Elswyk, 1997; Chae et al., 1998). The nutritional manipulation of the diets of laying hens to include sources of n–3 PUFA promotes the deposition of these nutrients into egg yolk.

Currently marketed n–3 enriched eggs are produced by including seeds of flax or chia and marine products such as algae, fish oil or meal in the hens' diet. Both seeds are rich in α–linolenie acid, while the marine sources contain the long chain n–3 acids, docosahexaenoie (DHA) and eicosapentaenoic acid (EPA). Number of reports evaluating the effects of terestrial sources of n–3 fatty acids (FAs) in laying diets on yolk FA composition reveald that the sources rich in linolenic acid (LNA) could increase levels of LNA in yolk and slightly increases long chain n– 3 FAs, especially as docosahexanoie acid (DHA) (Caston and Lesson, 1990; Cherian and Sim, 1991) by elongating and desaturating LNA by Δ–6 and

Δ-9 enzymes (Simopoulos, 1999). However, the increase in those long chain n-3 FAs in yolk would not occur proportionally to that of LNA since the conversion of LNA to long-chain derivatives and deposition them in peripheral tissues is limited and not sufficient to give nutritionally valuable modified products (Caston and Leeson, 1990 ; Cherian and Sim, 1991).

Fishy odor and/or flavor have occasionally been reported in eggs from hens fed marine products or flaxseed (Van Elswyk et al. 1995; Scheideler et al.1997). The flavor is thought to arise through oxidation of polyunsaturated fatty acids (Jiang and Sim 1992; Van Elswyk et al. 1995; Cherian et al. 1996); Leskanich and Noble (1997), however, suggested that the fishy odor arised from both lipid and non-lipid substances present in the feed.

The objectives of this study were to determine the effect of dietary combination of flax seed (FS) and fish oil (FO) in various proportions on laying performance, egg fatty acid composition, egg yolk and serum cholesterol of hens as well as on egg sensory attributes.

Materials and Methods

Animals and Experimental Design

Two hundred commercial Isa Brown laying hens, 25 wk of age, were used in this study. The hens were housed two to a cage (30x45x43 cm tall at the front, and 38 cm. tall at the back), in an environmentally controlled room at the Quality Meat Co., Ltd. farm in Lopburi. Sets of ten birds in 5 cages were randomly assigned to each treatment and each treatment was replicated 4 times in a Completely Randomized Design (CRD) experiment. The birds were fed a standard layer diet (17% CP ; 2800 Kcal/ME/kg diet) supplemented with 0-0, 10-1.5, 15-1.5, 10-3.0 and 15-3.0% combinations of flax seed to fish oil in FS_0FO_0, $FS_{10}FO_{1.5}$, $FS_{15}FO_{1.5}$, $FS_{10}FO_{3.0}$ and $FS_{15}FO_{3.0}$ treatments, respectively (table 1). Feed and water were provided *ad libitum*. Diet were fed for 8 weeks. Egg production was recorded throughout the trial, with all of the eggs collected and counted daily. Feed intake (g/hen/day) and egg weight (g/egg) were calculated by dividing total feed consumed in each week by days of week and total egg weight by number of eggs, respectively. Feed conversion and egg production were calculated as weights of feed consumed/kg, egg production and hen-day production in each week, respectively. All measurements were calculated for each replicate in groups. Mortality was determined for treatments.

Laboratory Analysis

At the end of the experiment, two eggs per replicate were randomly collected for yolk cholesterol and fatty acid analyses. The cholesterol levels were determined by the colorimetric method of Zlatkis et al. (1953) as modified by Siriloaphaisan (2004) and the levels of fatty acid were determined by gas chromatography (A.O.A.C.,1990). Besides, in order to determine the serum cholesterol levels, a total of 40 blood samples (2 samples from each group) were taken by puncturing the wing vein of hens and serum from each blood sample was collected. The serum cholesterol levels were determined spectro-photometrically by using commercial kits.

Sensory evaluation

Sensory evaluation was performed by a test panel. The eggs were randomly selected from those collected, from each treatment group, during the last week of the trial. All of the eggs were prepared in the same manner, 2 h before the test began. Eggs from each treatment were placed in water at room temperature, brought to boiling, then taken from the pot and placed under cold running water for 10 min. The eggs were peeled, cut longitudinally and placed in separate containers.

Eight untrained adult panelists (4 males and 4 females) performed the test. Each panelist individually received five plates, each containing half an egg randomly selected from each container. Each plate was marked with an A, B, C, D or E. These corresponed to five diets, FS_0FO_0, $FS_{10}FO_{1.5}$, $FS_{15}FO_{1.5}$, $FS_{10}FO_{3.0}$ and $FS_{15}FO_{3.0}$, respectively.

Statistical Analysis

Data were statistically analysed by one-way analysis of variance. The differences between groups were determined by Duncan's New Multiple Range test. All statistical analyses were performed using SAS statistical programme (SAS, 1998).

Results and Discussio

Production parameter

The effect of including FS and FO in diets on various production aspects, egg yolk fatty acid composition, blood and egg yolk cholesterol levels and sensory evaluation are given in tables 2, 3 and 4. Apart from the $FS_{15}FO_{3.0}$ group, there were no significant differences in feed intake of treated hens when compared to the controls. Feed intake values noticeably increased in $FS_{15}FO_{1.5}$ and $FS_{15}FO_{3.0}$ groups but only that of $FS_{15}FO_{3.0}$ was significantly different ($P<0.05$) when compared to the control. It might be caused by the lower true metabolizable energy of FS the than formulated value. This observation was supported by the results of Caston et al.(1994) and Novak and Scheideler (2001). Moreover, it has been hypothesized that anti-nutritional factors contained in FS may impair the digestion and absorption of energy yielding nutrients (Gonzalez-Esquerra and leeson, 2000; Rodriguez et al., 2001). Oritiz et al., (2001) concluded that deleterious compounds in FS interact with the other

Table 1 Composition of experimental diets

Ingredient, %	C	$FS_{10}FO_{1.5}$	$FS_{15}FO_{1.5}$	$FS_{10}FO_{3.0}$	$FS_{15}FO_{3.0}$
Corn	46.28	44.78	43.70	44.14	36.90
Sol. rice bran	8.00	8.00	8.18	8.88	14.75
Soybean meal	23.37	18.36	15.84	18.16	14.68
Flaxseed	–	10.00	15.00	10.00	15.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Rice bran oil	6.39	1.52	–	–	–
Fish oil	–	1.50	1.50	3.00	3.00
Methionine (MHA)	0.27	0.25	0.24	0.25	0.25
Limestone	10.82	10.78	10.76	10.79	10.77
Sodium bicarb.	0.3	0.3	0.3	0.3	0.3
DCP21	1.08	1.00	0.97	0.97	0.83
Salt	0.24	0.26	0.26	0.26	0.27
Premix[1]	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated composition					
Protein	17.00	17.00	17.00	17.00	17.00
Crude fat	8.35	8.25	8.34	8.21	9.51
Crude fiber	3.99	4.52	4.79	4.60	5.37
ME Mcal/kg	2.80	2.80	2.80	2.80	2.80

[1] A standard vitamin and mineral premix provided the following per kilogram of rations: vitamin A,14,440 IU; cholecalciferol 2,220 IU ; vitamin E 22.2 IU; vitamin K 3.3 mg; vitamin B_1 2.2 mg; vitamin B_2 6.7 mg; nicotinic acid 38.9 mg; pantothenic acid 15.6 mg; vitamin B_6 6.7 mg; vitamin B_{12}, 0.028 mg; folic acid 1.1 mg; biotin 0.147 mg; manganese 50 mg; iodine 0.333 mg; zinc 88.9 mg; iron 66.7 mg; copper 8.9 mg; selenium 0.15 mg; and antioxidant (BHT) 111.2 mg.

dietary ingredients in flax-based diets resulting in decreased dietary AME_n. Thus, it is possible that antinutritional factors in the flax diet caused the increase of feed intake. Hen-day production appeared to be unaffected by the level of FS and FO in the diet. Hen-day egg production in all groups were higher than 96%. It could be concluded that the use of FS and FO at any percentage in diets never led to poorer performance of the layers and was in agreement with the findings of Baucells et al. (2001) Caston and Leeson, (1990), Jiang et al. (1991), Hulan (1989) and Sari et al. (2001). This with the higher feed intake, the feed efficiency values in the $FS_{15}FO_{3.0}$ group was found to be poorer than in the control. There were no significant differences on egg mass between treated and the control groups ; however, the control hens had the highest egg mass (61.78 g/hen/d). With respect to average egg weight, there was a nonsignificant trend that the treated hens laid smaller eggs than the controls. The highest egg weight was found to be 63.63 g in the control group whereas, the lightest one was 61.99 in the $FS_{10}FO_{3.0}$ group. Its possible causes could be due to the lower serum lipids of the birds fed high amounts of n-3 FA, thus limiting the available lipids for yolk formation or to the changes in circulating oestradiol as a consequence of n-3 FA intake. This observation was supported by serveral similar studies (Caston and Leeson, 1990; Jiang et al., 1991; Sari et al., 2001). Still, there was no significant difference in egg weight among groups. During the experimental period, no mortality and no damaged eggs were observed.

Table 2. The effects of addition of flaxseed and fish oil to layers' diets on production parameter

Diets	Feed intake g/hen/d	Feed efficiency kg feed/kg egg	Egg weight g/egg	Egg mass (g/Hen/d)	Egg production % (Hen-day)
Control	94.46^{B}	1.53^{B}	63.63	61.78	97.10
$FS_{10}FO_{1.5}$	94.78^{B}	1.56^{B}	63.30	60.94	96.25
$FS_{15}FO_{1.5}$	98.00^{B}	1.62^{BA}	62.77	60.62	96.56
$FS_{10}FO_{3.0}$	95.89^{B}	1.61^{BA}	61.99	59.62	96.12
$FS_{15}FO_{3.0}$	103.11^{A}	1.71^{A}	62.71	60.30	96.16
SEM	0.51	0.01	0.27	0.39	0.40
Significance	**	*	N.S.	N.S.	N.S.

$^{A-B}$ Means within a column with no common superscripts differ significantly, *P<0.05 ;
**P<0.01 ; N.S. : Not Significant.
SEM : Pooled Standard Error of the Means.

Table 3 The effects of addition of flaxseed and fish oil to layers' diets on fatty acid composition of the yolks (% of total fatty acid)

Fatty Acids	Control	$FS_{10}FO_{1.5}$	$FS_{15}FO_{1.5}$	$FS_{10}FO_{3.0}$	$FS_{15}FO_{3.0}$	SEM	Significance
Myristic	0.35^{C}	0.38^{C}	0.43^{C}	0.47^{BA}	0.49^{A}	0.00	**
Palmitic	27.89	6.81	27.84	27.98	27.99	0.13	N.S.
Palmitoleic	2.91^{A}	2.36^{B}	2.83^{A}	2.80^{A}	2.70^{A}	0.04	**
Stearic	8.95	8.73	8.73	8.81	8.95	0.08	N.S.
Oleic	40.06^{A}	31.90^{B}	27.44^{D}	29.74^{C}	26.58^{D}	0.30	**
Linoleic (LA)	14.18^{A}	13.18^{A}	12.99^{B}	12.31^{B}	12.24^{B}	0.17	**
Lunoleic (LNA)	0.33^{C}	5.71^{B}	8.31^{A}	5.78^{B}	8.20^{A}	0.10	**
Arachidonic (AA)	1.37^{A}	0.99^{B}	0.89^{CB}	0.86^{CB}	0.76^{C}	0.03	**
Eicosapentaenoic (EPA)	0.03^{C}	0.34^{B}	0.35^{B}	0.36^{BA}	0.41^{A}	0.01	**
Docosapentaenoic (DPA)	0.14^{C}	0.38^{B}	0.41^{B}	0.44^{BA}	0.48^{A}	0.01	**
Docosahexaenoic (DHA)	0.78^{D}	5.44^{C}	5.58^{C}	6.24^{B}	6.76^{A}	0.07	**
Σn-3	1.28^{E}	11.87^{D}	14.65^{B}	12.81^{C}	15.84^{A}	0.12	**
Σn-6	15.54^{A}	14.16^{B}	13.87^{B}	13.16^{B}	12.99^{B}	0.18	**
Σn-6: Σn-3	12.40^{A}	1.20^{B}	0.95^{B}	1.03^{B}	0.82^{B}	0.13	**
ΣSaturated FA (SFA)	37.52	36.40	37.50	37.83	38.06	0.17	N.S.
ΣMonounsaturated (MUFA)	44.48^{A}	36.01^{B}	132.21^{C}	134.57^{B}	131.30^{C}	0.31	**
ΣPolyunsaturated (PUFA)	16.83^{C}	26.03^{B}	28.52^{A}	25.97^{B}	28.83^{A}	0.21	**
ΣPUFA:ΣSFA	0.45^{C}	0.72^{B}	0.76^{A}	0.69^{B}	0.76^{A}	0.01	**

A-E Means within a row with no common superscripts are significantly different, *P<0.05 ;
**P<0.01; N.S. : Not Significant.
SEM : Pooled Standard Error of the Means

The effects of including FS and FO in diets on egg FA profiles are given in table 3. Incorporation of the dietary key fatty acids into the egg was as efficient as expected. Myristic acid percentage was lowest in yolk of the control but was highest in yolk of the $FS_{15}FO_{3.0}$ groups (P<0.01). Nevertheless, palmitic acid percentage was similar (P>0.05) among dietary treatments. Besides, total saturated fatty acid percentage (ΣSFA), calculated as the sum of myristic, palmitic and stearic acids, was not significantly different (P>0.05) among treatments. The level of monounsaturated fatty acids (MUFA, palmitoleic and oleic) decreased progressively as the dietary FS and FO increased (P<0.01). Oleic acid was significantly lower (p<0.01) in yolks of the treated than that in yolks of the control groups. Total MUFA percentage, calculated as the sum of palmitoleic and oleic acid percentage, was significantly lower (p<0.05) in the yolks of hens fed the treatment diets than that of hens fed the control diet. This substantiates previous report, by Garg et al. (1988), that PUFA could limit $\Delta-9$ desaturase activity, resulting a limited monounsaturated fatty acid formation. The observation is supported by several similar studies (Caston and Leeson, 1990; Jiang et al. 1991; Sari et al. 2001). Addition of FS and FO to the diets increased the percentage of total polyunsaturated fatty acids (PUFA) in yolks relative to the control diet ($p<0.01$). The level of PUFA increased linearly with increasing FS and FO levels. Total n-6 PUFA percentage, calculated as the sum of linoleic and arachidonic acid, and total n-3 PUFA percentage, calculated as the sum of linolenic acid (LNA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) were significantly linearly increased ($P<0.01$) with increasing dietary FS and FO levels. Among the PUFAs, the addition of FS and FO lowered the levels of n-6 PUFAs ($P<0.01$). Matthews and Van Holde (1990) suggested that LNA ($C_{18:3}$) and its metabolites decreased the production of arachidonic acid ($C_{20:4}$) and its metabolites or displacing $C_{20:4}$ in the phospholipids, or both. Jiang et al. (1991) also observed a negative relationship between $C_{18:3}$ and $C_{20:4}$ and between $C_{20:4}$ and longer-chain n-3 (LCn-3) PUFAs in total yolk lipids, triglycerides, phosphatidylcholine, and phosphatidylethanolamine. They reported that the enzymatic pathway for the synthesis of $C_{20:4}$ from $C_{18:3}$ was shared by the n-3 fatty acids, and $C_{18:3}$ inhibited the n-6 desaturase, thereby reducing the conversion of $C_{18:3}$ to $C_{20:4}$. They also showed that the level of n-3 FAs (LNA, EPA, DPA and DHA) increased linearly and significantly with increasing FS and FO levels in diets ($P<0.01$). In addition, increasing concentrations of FS proportionally increased deposition of yolk LNA and its derivatives (EPA, DPA and DHA). However, the efficiency of converting LNA to LCn-3 PUFA is limited (Scheideler and Froning 1996; Van Elswyk 1997), as the increases of deposited EPA, DPA and DHA in yolk of FS-fed hens are much lower than that of the LNA. Increasing the amount of dietary FO results in sequential increases in LCn-3 PUFA deposition in eggs. Marine sources of n-3 PUFA offer the benefit of direct incorporation of LCn-3 into eggs which are metabolically more important than LNA for humans (Simopoulus 2000). It was concluded that the FO diets were efficient in depositing the long chain $C_{22:6}$ fatty acid which was in agreement with a previous trial (Cherian et al. 1995 ; Sari et al. 2001).

General recommendations for reducing the risk of coronary heart disease (CHD) are consuming n-6: n-3 ratio of 5:1 to 4:1 in the diet (Okuyama et al., 1997; British Nutrition Foundation, 1999) Currently, the normal diets do not meet these recommendations, mainly due to the high n-6 fatty acid content of present diets. Feeding flaxseed or fish oil to layers significantly lowered the n-6: n-3 ratio of egg yolks as compared with egg yolks from the control group, which brought the n-6: n-3 ratio more in line with nutritional recommendations. Works related to this subject showed similar results (Ferrier et al., 1995; Scheideler and Froning, 1996; Sari et al., 2001)

The ratios of Σ6:Σ3 in yolks of the control, $FS_{10}FO_{1.5}$, $FS_{15}FO_{1.5}$, $FS_{10}FO_{3.0}$ and $FS_{15}FO_{3.0}$ groups were 12.40, 1.2, 0.95, 1.03 and 0.82, respectively. It was shown that the ratio ΣPUFA: ΣSFA in yolks increased when increasing inclusion of FS and FO in the diet as compared with the control. Several nutritional studies strongly supported a relationship between SFA and the risk of CHD, and hence there is a need to reduce consumption of SFA and increase consumption of PUFA. Flaxseed and fish oil could improve the ΣPUFA : ΣSFA ratio in egg yolks and brought it more in line with the 1:1 ratio that has been recommended (Canada Health and Welfare, 1990 ; American Heart Association, 1991). Hence, it can be concluded that yolks can be enriched in n-3 fatty acids by dietary means and the level of cholesterol in egg can be manipulated in the same way. Cholesterol in yolk and blood

The effects of diets on cholesterol levels in egg yolk and blood are given in table 4. The addition of FS and FO resulted in significant differences in egg yolk cholesterol levels. They were reduced cholesterol levels in treated groups as compared to the control (P<0.01). Some data from literature supported these results (Jiang et al., 1991; Sari et al., 2001). But, experiments of Caston and Leeson (1990) and Scheideler and Froning (1996) showed that egg

Table 4 The effect of addition of flaxseed and fish oil and layer diets on egg yolk and blood cholesterol level and flavor rating for eggs

Diets	Egg yolk cholesterol mg/g.	Blood cholesterol mg/100 ml	flavor
Control	13.38^{A}	161.50^{A}	1.75
$FS_{10}FO_{1.5}$	11.35^{B}	141.50^{BA}	1.75
$FS_{15}FO_{1.5}$	11.33^{B}	124.75^{BC}	2.00
$FS_{10}FO_{3.0}$	10.91^{B}	114.50^{BC}	2.38
$FS_{15}FO_{3.0}$	10.84^{B}	106.25^{C}	2.00
SEM	0.15	4.80	0.19
Significance	**	*	N.S.

$^{A-C}$ Means within a column with no common superscripts differ significantly,

*P<0.05 ; **P<0.01 ; N.S. : Not Significant.

SEM : Pooled Standard Error of the Means.

yolk cholesterol levels were not affected noticeably by FS level in the diet. A number of theories have been suggested to explain the mechanism whereby fish oil caused reduction in cholesterol, triglycerides and lipoproteins. These included the reduction of hepatic synthesis and secretion of triglycerides by decreasing activity of synthetic enzymes (Rustan et al., 1988), increased proximal beta oxidation, increasing in the expression of hepatic receptor for LDL (Shephered et al., 1980) and increasing LDL apo-B fraction catabolic rate (Femandez, 1992). Furthermore, the hypocholestrolemic effect of FO may be attributed to the oxidation of cholesterol to bile acids (Barbara et al., 1977), decreasing activity of esterifying enzymes (Rustan, 1988) or diverting lipids metabolism to phospholipids formation (Wong et al, 1985; Wong and March, 1988) the mechanism explaining the elevation in phospholipids observed in this study.

It was shown that blood cholesterol level decreased by increasing dietary FS and FO and this reduction was significant among the $FS_{15}FO_{1.5}$, $FS_{10}FO_{3.0}$ and $FS_{15}FO_{3.0}$ groups vs control groups ($P>0.05$). The lowest cholesterol level in blood was detected in the $FS_{15}FO_{3.0}$, 106.25 mg/100 ml, whereas the highest level was detected in the control group, 161.50 mg/100 ml. This result was in agreement with the results of Jiang and Sim (1992) who reported from in vitro and in vivo study with rats that n-3 FA reduced plasma triglycerides (TG), cholesterol and very low density lipoproteins (VLDL) by inhibiting hepatic secretion of TG and VLDL. Also, Harris (1989) demonstrated that the reductions resulted by inhibition of de novo FA synthesis, increased FA oxidation, and decreased TG synthesis and cholesterol formation.

Sensory Evaluations

Data from the taste panel indicated no significant difference ($P>0.05$) in flavor among treatments (Table 4). However, the flavor scores the $FS_{15}FO_{1.5}$, $FS_{10}FO_{3.0}$ and $FS_{15}FO_{3.0}$ were higher than control group. The sensory characteristics of the n-3 egg reported by other researchers, vary substantially depending on which supplement was fed. Huyghebaert (1995) reported that there were no differences in sensory characteristies for eggs laid by hens fed different dietary level (0, 3, 6 or 9%) and combinations of lard, soybean oil and FS oil. Herber-McNeil and Van Elswyk (1998) reported that consumer panelists found n-3 FA eggs enriched with marine algae were as acceptable as typical eggs. In addition, Scheideler et al. (1994) found that the overall acceptability of eggs from FS hens was similar to that of eggs from diets enriched with menhaden fish oil and did not greatly differ from that of regular eggs. According to the findings of Hammershoj (1995), the sensory evaluation of yolk differed by lowering grades for eggs from diets with FO, although they still were above the acceptability standard.

In conclusion, addition flaxseed and fish oil in laying diets could modify fatty acid profile and cholesterol of yolk. The level of n-3 increased proportionally as dietary flaxseed and fish oil increased. The yolk cholesterol decreased progressively with the increasing levels of dietary FS and FO. Apart from $FS_{10}FO_{1.5}$ hens, serum cholesterol level significantly decreased by inclusion of FS and FO in the diets. In addition, there was no adverse effect of dietary FS and FO on egg production, egg weight and egg mass among group. Only feed intake of the FS15FO3.0hens were higher than that of the control group; consequently, feed conversion of formers were poorer.

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