High ω-3 Fatty Acid Eggs Produced by Laying Hens Fed with Sardine Oil

Umar Santoso*[1], M. Nur Cahyanto[1], Dwi Sulistiawati[2], Zuprizal[3] and Hari Eko Irianto[4]

[1]Fac, of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia.
[2]Fac, of Agriculture, Tadulako University, Palu, Central Sulawesi.
[3]Fac, of Animal Science, Gadjah Mada University, Yogyakarta
[4]Institute for Fishery Research & Development, Dept. of Agriculture of the Republic of Indonesia.
*To whom correspondence should be addressed.

ABSTRACT

The objective of the present research was to examine the effects of feeding laying hens with sardine oil on the ω-3 fatty acids levels of the egg yolk. Results of the study show that changes in the composition of the diet influenced the fatty acid composition of the yolk lipid. Diets with sardine oil from 2 to 8% significantly increased ω-3 PUFAs content of the yolk. The increase of ω-3 PUFAs was predominated by docosahexaenoic acid (DHA), followed by eicosapentaenoic acid (EPA), and α-linolenic acid (ALA). In term of egg weight basis, the increase of ω-3 PUFAs in egg due to sardine oil consumption was from 8.17mg/egg in diet without sardine oil to 102.27 mg/egg in diet with 2% sardine oil, and 232.63mg/egg in diet with 8% sardine oil. ω-6 PUFAs especially arachidonic acid tended to decrease with the increasing intake of sardine oil.

Diets with sardine oil significantly (P<0.05) decreased the cholesterol content of the yolk compared to that without sardine oil. Cholesterol content of the eggs produced by laying hens fed with 2% sardine oil was 191.73 mg/egg significantly lower than that of egg produced by laying hens fed without sardine oil. The cholesterol content of 179.74mg/egg was produced by hens fed with diet containing 8% sardine oil, this is lower than that of eggs produced by the commercial feed of 208.09mg/egg.

INTRODUCTION

Recently, functional foods have been gaining special concern since people are now obsessed with health, that it is believed to be associated with the food they consume. Foods are consumed not only for their nutritional but also for their health or medicinal benefits.

ω-3 Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linolenic acid (ALA) are recognized as functional compounds. These fatty acids are now gaining special attention as they are considered to be beneficial in decreasing plasma triacyl glycerols and cholesterol, and lowering the risk of atherosclerosis (Meydani et al., 1991). Epidemical studies suggest that consumption of fish oil that are rich in ω-3 PUFAs may protect against coronary heart diseases (Kobatake et al., 1983; Herold & Kinsella, 1986; Chee et al., 1990; Nardini et al., 1995). Result of some studies indicate that DHA is essential for normal functional development of the retina and brain, particularly in premature infants. Emerging scientific evidences suggest that these acids, especially DHA may be a dietary essential in growing infants as it is naturally found in human milk. Because ω-3 fatty acids are essential in growth and development throughout the life cycle, the should be included in the diets of all humans (Simopoulus, 1991). Thus, recent recommendations pertaining to the consumption of dietary fats have emphasized the importance of consuming higher levels of these fatty acids (Simopoulus, 1991; Chee et al., 1990).

In addition to increasing consumption of fish and fish oil products, intake of ω-3 fatty acids can be enhanced through the consumption of commonly occurring non-fish foods containing elevated levels of these fatty acids. Egg is an important food as a source of protein with high nutritional value, but the fat that concentrates in the yolk is relatively poor in ω-3 fatty acids (Noble, 1987). However, the lipid composition of the egg yolk can be modified by changes in the composition

egg yolk can be modified by changes in the composition of the poultry diet (Leskanich & Noble, 1997). Some investigations suggest that increasing dietary levels of mono- and polyunsaturated fatty acids have large effects on the unsaturated fatty acids of the yolk, feeding of long chain ω-3 PUFAs increases in their levels in yolk lipid (Caston & Leeson, 1990; Marshall et al., 1994, Oh et al., 1994).

Fish oil is abundantly available as a byproduct from fish meal and fish canning industry. There are many fish industries in Indonesia, one of them is the prominent sardine canning at Muncar, Banyuwangi, East Java. The factory processes approximately 30,000 tons marine fishes - chiefly sardine, annually. Sardine oil, the byproduct of the factory, is now unutilized commercially yet and the waste is potential to bear ecological problem. Sardine oil is potential as a source of ω-3 PUFAs (Chee et al., 1990), therefore, it is necessary to develop the utilization of the byproduct such as to enrich non-fish foods with ω-3 PUFAs so as to maximize its potential use.

The objective of the present research was to examine the effects of feeding laying hens with sardine oil on the ω-3 fatty acids levels of the egg yolk.

MATERIALS AND METHODS

Laying hens and rations

Laying hens of *Lohmann* MB 402 aged 28 weeks were purchased from PT Multibreeder Adirama, Indonesia. Ninety eight hens were selected and used for the experiments. Sardine oil was obtained from fish meal factory of PT Fishindo, Muncar, Banyuwangi, and palm oil was from PT Intiboga Sejahtera, Jakarta, Indonesia. Commercial feed was a product of *PT. Comfeed.*

The animals were divided into seven groups and the experiments were conducted in triplicate with two animals in each cage. Each group of animals were fed diet with different levels of sardine oil, *i.e.,* the different ratio of fish and palm oils. The diets were formulated according to NRC (1994) to meet the requirement for laying hens. Group 1 (SP-0/8) was given diet containing 0% sardine oil and 8% palm oil, group 2 (SP-2/6) was with diet containing 2% sardine oil and 6% palm oil, group 3 (SP-4/4) was with diet of 4% sardine oil and 4% palm oil, group 4 (SP-6/2) was with diet of 6% sardine oil and 2% palm oil, group 5 (SP-8/0) was with diet of 8% fish oil and 0% palm oil, group 6 (Control) was with diet of no addition of oil, and group 7 (F-Com) was with a commercial feed. The composition of the diets given to each group of animal are presented in Table 1, and the fatty acid composition of the diets are shown in Table 2. Laying hens were fed *ad libitum* and maintained in the cage for three months.

Table 1. **Composition of diets***

Material (%)	SP-0/8	SP-2/6	SP-4/4	SP-6/2	SP-8/0	Control	F-Com.
Ground corn	41.00	41.00	41.00	41.00	41.00	67.00	40.00
Soybean meal	27.50	27.50	27.50	27.50	27.50	17.00	
Rice bran	13.00	13.00	13.00	13.00	13.00		30.00
Grits ($CaCO_3$)	9.50	9.50	9.50	9.50	9.50	9.00	..
Fish meal	-	-	-	-	-	6.25	-
Commercial diet	-	-	-	-			30.00
Topmix	0.25	0.25	0.25	0.25	0.25	0.25	-
Salt (NaCl)	0.50	0.50	0.50	0.50	0.50	0.25	..
DL-metionine	0.25	0.25	0.25	0.25	0.25	0.25	-
Sardine oil	**0.00**	**2.00**	**4.00**	**6.00**	**8.00**	-	-
Palm oil	**8.00**	**6.00**	**4.00**	**2.00**	**0.00**	-	-
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrent contents#							
ME (Kcal/kg)	2863	2863	2863	2863	2863	2800	27500
Crude protein (%)	16.67	16.67	16.67	16.67	16.67	16.67	16.49
Crude fat (%)	11.79	11.79	11.79	11.19	11.79	2.95	7.10
Crude fiber (%)	5.43	5.43	5.43	5.43	5.43	2.95	8.31
Ca_{Total} (%)	3.67	3.67	3.67	3.67	3.67	3.91	3.63
P_{Total} (%)	1.09	1.09	1.09	1.09	1.09	1.08	0.97

*NRC (1994)

#Results of analysis in our laboratory.

Table 2. **Fatty acid composition* of rations**

Fatty acid	SP-0/8	SP-2/6	SP-4/4	SP-6/2	SP-8/0	Control	F-Com.
C14:0	0.09	0.25	0.38	0.71	0.98	0.02	0.04
C16:0	3.72	3.51	3.13	2.96	2.63	0.40	1.50
C16:1	0.02	0.24	0.41	0.68	0.94	0.02	0.05
C18:0	0.44	0.46	0.47	0.49	0.53	0.80	0.63
C18:1	4.78	4.34	3.90	2.91	2.27	0.80	2.71
C18;2ω-6	2.37	2.31	2.35	2.17	1.96	0.82	1.85
C18;2ω-3	0.08	0.09	0.11	0.12	0.16	0.03	0.01
C20:0	0.06	0.07	0.07	0.03	0.08	0.00	0.05
C20:1	0.23	0.11	0.16	0.21	0.40	0.02	0.02
C20:4ω-6	0.00	0.04	0.09	0.40	0.19	0.01	0.08
C20:5ω-3	0.00	0.20	0.38	0.56	0.89	0.00	0.03
C22:6ω-3	**0.00**	**0.16**	**0.36**	**0.55**	**0.76**	**0.02**	**0.13**
SFA	4.30	4.28	4.04	4.19	4.22	1.21	2.23
USFA	7.49	7.51	7.75	7.60	7.57	1.72	4.87
MUFA	5.03	4.70	4.47	3.80	3.62	0.84	2.78
PUFA	2.45	2.81	3.28	3.80	3.96	0.88	2.09
ω-3 FA	0.08	0.45	0.84	1.24	1.81	0.05	0.16
ω-6 FA	2.37	2.36	2.43	2.56	2.15	0.83	1.93
ω-6/ω-3 ratio	29.7/1	5.2/1	2.9/1	2.1/1	1.2/1	16.6/1	12.1/1

*Analyzed by Gas Chromatograph of HITACHI 163GC completed with Fused Silica Cappilary Column of Carbowax (L : 30m, D: 0.25 cm), equipped with FID.

Eggs

The eggs taken for analysis were those produced at the 4th week every month.The eggs were analyzed for fatty acid composition and cholesterol content. Daily egg production (% hen day average) and the average weight of egg produced daily were also recorded.

Fatty acid analysis

Fat was extracted from the yolk with chloroform: methanol (2/1) according to Folch et al. (1957), and methylated with BF3-methanol. Methyl esters were analyzed by Gas Chromatograph of HITACHI 163 GC equipped with Flame Ionization Detector (FID) of Shimadzu C-RGA and Chromapac recorder. Column used was Fused Silica Capillary (L:30m, D:0,25Cm) filled with carbowax. The condition of HPLC analysis were, carrier gas of N_2 (30mL/min), ignition gas of H_2 (0.9kg/Cm^2), air (1.5kg/Cm^2), column temperature 200^0 C, injection temperature 250^{0}C, attenuation 7, recorder speed 4mm/min, and injection volume 0.1µL.

Cholesterol

Analysis of cholesterol was conducted by Leiberman-Burchard method. Cholesterol was extracted from the yolk with acetone:ethanol (1/1) at boiling water temperature. After centrifugation, supernatant was transferred into a test tube and the solvent was evaporated. To the precipitate was added chloroform and a mixture of acetic acid anhydride : sulfuric acid (30/1), after mixing thoroughly, the absorbance at 680nm was read on a spectrophotometer. Purified cholesterol was used to plot a standard curve.

RESULTS AND DISCUSSION

The eggs produced by laying hens fed with different levels of sardine oil were analyzed for fatty acid composition and cholesterol contents.

ω-3 PUFAs and ω-6 PUFAs

Fatty acid composition of the yolk lipid of each group of hens is presented in Table 3. Changes in the composition of the diet influenced the fatty acid composition of the yolk lipid. The increase of sardine oil of the diet significantly increased ω-3 PUFAs content of the yolk.

Table 3. **Fatty acid composition* of yolk lipid**

Fatty acid	SP-0/8	SP-2/6	SP-4/4	SP-6/2	SP-8/0	Control	F-Com.
C14:0	0.36	0.49	0.64	0.88	1.19	0.40	0.32
C16:0	23.21	23.64	24.15	24.44	24.89	23.97	23.31
C16:1	4.62	3.30	4.09	6.04	6.57	6.12	6.35
C18:0	6.36	6.24	6.45	6.34	6.94	7.35	6.27
C18:1	46.59	44.49	42.52	38.96	36.19	43.75	41.10
C18:2ω-6 (linoleic)	16.77	16.93	16.72	16.72	16.98	15.78	19.73
C18:3ω-3 (ALA)	0.14^a	0.26^b	0.32^c	0.36^c	0.46^d	0.13^a	0.25^b
C20:0	0.02	0.06	0.05	0.01	0.07	0.03	0.05
C20:1	0.16	0.28	0.41	0.29	0.36	0.12	0.18
C20:4ω-6(Arach.)	1.53	1.14	0.81	0.75	0.74	1.65	1.99
C20:5ω-3 (EPA)	ND^a	0.13^a	0.31^b	0.39^b	0.71^c	ND^a	ND^a
C22:6ω-3 (DHA)	0.23^a	3.04^c	3.73^d	4.82^e	4.9^e	0.69^b	0.46^{ab}
SFA	29.95	30.43	31.29	31.67	33.09	31.75	29.75
USFA	70.05	69.57	68.71	68.33	66.91	68.25	70.05
ω-3 FA	0.37^a	3.47^c	4.38^d	5.56^e	6.06^f	0.91^b	0.71^{ab}
ω-6 FA	18.45^b	18.08^{ab}	17.32^a	17.46^a	17.51^{ab}	17.44^{ab}	21.99^c
ω-6/ω-3 ratio	49.9/1	5.2/1	4.0/1	3.1/1	2.9/1	19.2/1	30.9/1

*Analyzed by Gas Chromatograph of HITACHI 163GC completed with Fused Silica Cappilary Column of Carbowax (L : 30m, D: 0.25 cm), equipped with FID.

Diets with sardine oil from 2 to 8% significantly increased ω-3 PUFAs content of the yolk, which were significantly different from that of diets without addition of fish oil. This result is in agreement with the investigation of Marshall et al (1994) that diet containing 1.5% menhaden oil increased ω-3 PUFAs content of the yolk compared to control.

Diet with 2% sardine oil (SP-2/6) elevated 0.37% ω-3 PUFAs if compared to diet without sardine oil (SP-0/8), and the elevation of 6.06% ω-3 PUFAs was reached by diet with 8% sardine oil (SO-8/0) (Table 3). The increase of ω-3 PUFAs was predominated by docosahexaenoic acid (DHA), followed by eicosapentaenoic acid (EPA), and α-linolenic acid (ALA).

Astuti (1997) used diet with the waste of sardine canning to increase the level of ω-3 PUFAs of eggs. She reported that diet with 5% of the sardine waste could elevate ω-3 PUFAs content of 1.77%. Caston and Leeson (1990) investigated that diet with flaxseed as a source of ω-3 PUFAs given to laying hens produced eggs with the increase of DHA of 0.22%. ω-3 PUFAs are essential for poultry, and the ω-3 PUFAs content of egg is dependent of ω-3 PUFAs intake (van Elweysk, 1997).

ω-6 PUFAs especially arachidonic acid tended to decrease with the increasing intake of sardine oil. The result is in accordance with the report of Caston and Leeston (1990) that the intake of ω-3 PUFAs from flaxseed decreased the arachidonic acid of the yolk. This might be associated with the inhibition of ω-6 biosynthesis by ω-3 PUFAs.

In term of egg weight basis, the increase of ω-3 PUFAs in egg due to sardine oil consumption was from 8.17mg/egg in diet without sardine oil to be 102.27 mg/egg in diet with 2% sardine oil, and 232.63mg/egg in diet with 8% sardine oil (Table 4). The increase ω-3 PUFAs content was similar with the report of Hargis and van Elswyk (1993) that diet with 3% menhaden oil elevated ω-3 PUFAs to be 235 mg/egg.

Table 4. **ω-3-, ω-6 PUFAs and cholesterol content of eggs**

Description	SP-0/8	SP-2/6	SP-4/4	SP-6/2	SP-8/0	Control	F-Com.
ω-3 PUFAs (mg/egg)	$8.17^{a\#}$	102.27^{c}	132.95^{d}	156.64^{e}	232.63^{f}	17.14^{b}	16.78^{ab}
ω-6 PUFAs (mg/egg)	434.49^{b}	443.36^{ab}	410.88^{a}	399.53^{a}	388.35^{ab}	332.69^{ab}	548.8^{c}
EPA (mg/egg)	0^{a}	3.83^{a}	9.41^{b}	10.99^{b}	27.25^{c}	0^{a}	0^{a}
DHA (mg/egg)	5.08^{a}	89.59^{c}	113.22^{d}	135.79^{e}	188.11^{f}	10.76^{b}	10.87^{b}
Cholesterol (mg/g yolk)	13.07^{a}	13.13^{a}	12.52^{a}	12.53^{a}	12.58^{a}	12.12^{a}	13.09^{b}
Cholesterol (mg/egg)	201.46^{d}	191.73^{c}	187.38^{bc}	183.36^{bc}	179.74^{b}	166.6^{a}	208.09^{d}

*Means within the same row without a common superscript letter are significantly different at P<0.01.

Fatty acid composition

Table 3 shows that the profile of fatty acids of the egg yolk was predominated by oleic, palmitic, linoleic, stearic acids. The result shows that palmitic and stearic acids were constant with the increasing of sardine oil in the diets. Powrie (1977) suggested that palmitic and stearic acids are relatively constant in eggs, *i.e.*, approximately 30%. The composition of saturated and unsaturated fatty acids in eggs were relatively the same profiles, *i.e.*, the unsaturated have a higher proportion of 69-70% and the saturated was 30-33%.

Cholesterol

Cholesterol content of eggs is shown in Table 4. Diets with sardine oil significantly ($P<0.05$) decreased the cholesterol content of the yolk compared to that without sardine oil. This is associated with the ω-3 PUFAs content of the diets.

Cholesterol content of the eggs produced by laying hens fed with 2% sardine oil was 191.73 mg/egg (SP-2/6) significantly lower than that of egg produced by laying hens fed without sardine oil (SP-0/8). The cholesterol content of 179.74mg/egg was produced by hens fed with diet containing 8% sardine oil, this is lower than that of eggs produced by the commercial feed of 208.09mg/egg. The cholesterol content of hen eggs generally ranges between 200-250mg/egg depending on the diet composition (Griffin, 1992).

The decreasing effect of sardine oil on the cholesterol level in the egg yolk is associated with the inhibition effect of ω-3 PUFAs on the cholesterol biosynthesis. The advantages of fish oil diet on lowering cholesterol level of serum and organs have been well recognized (Herold et al., 1986; Podczasy et al., 1995; Cameron et al., 1995, Griffin, 1992).

The increasing intake of sardine oil influenced the weight of the yolk. The more sardine oil in the diets the lower weight of the yolk (data not shown). This is in agreement with the report of Jiang & Sim (1991) that cholesterol content correlates with the weight of the yolk.

Egg production

Egg production of laying hens fed diet with sardine oil was higher than that of those fed without sardine oil. Among those experimented, diet containing 2% sardine oil gave the highest egg production (89.9 %/ hen day average). The result of the present investigation is in agreement with the report of Farrel (1995) that diet containing ω-3 PUFAs performed a better egg production.

The average weight of eggs produced in the present research ranged from 59-64g. Diets with sardine oil tended to decrease the weight of eggs. However, egg weight of laying hens fed with sardine oil were not significantly different from that of laying hens fed without oil (Control) or with the commercial feed (F-Com.). It seemed that the size and weight of eggs are influenced by linoleic acid level of the ration (March & McMillan, 1990).

CONCLUSION

The results of this study show that laying hens fed diets containing sardine oil produced eggs with elevated level of total ω-3 PUFAs. The higher content of sardine oil of the diets, the higher level of ω-3 PUFAs, especially DHA and EPA of the yolk. Diet with 8% sardine oil produced eggs with 232.63mg/egg of total ω-3 PUFAs. However, the optimum egg production (% hen day average) was achieved with diet containing combination of 2% sardine oil and 6% palm oil. Diets with sardine oil also have advantages in decreasing cholesterol levels of the yolk.

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