Feeding Laying Hens Seal Blubber Oil: Effects on Egg Yolk Incorporation, Stereospecific Distribution of Omega-3 Fatty Acids, and Sensory Aspects

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ABSTRACT Seventy-two 26-wk-old Single Comb White Leghorn laying hens were randomly assigned to 36 cages (2 per cage) in a 3-orthogonal 4×4 latin square, with the fourth row suppressed, to assess the effect of feeding refined seal blubber oil (SBO, containing 22.2% omega-3 fatty acids) on the fatty acid composition and position in the egg yolk lipids. The experiment was conducted over a period of 9 wk. Eggs were collected and numbered, and the weights were recorded for each week and cage. Eggs collected at wk 5 and 9 were used for total lipid, lipid class, fatty acid, and positional analyses. Sensory evaluation was carried out on eggs collected at wk 6 and 7. Feeding SBO at 1.25% led to an increase ($P < 0.0001$) in the long-chain omega-3 polyunsaturated fatty acids (LCn3PUFA) and a concomitant decrease ($P <$ 0.0001) in arachidonic acid (ARA) in the egg yolk lipids. Yet this amount of SBO in the diet had no effect ($P > 0.1$) on the sensory attributes of the egg and on production parameters such as egg weight, number of eggs laid, and feed intake ($P > 0.05$). When feeding SBO in amounts higher than 1.25% proportionately, a plateau effect of the LCn3PUFA content of the eggs was observed. This appears to be because the PUFA content in the sn-2 position of the phospholipids cannot exceed a certain amount. When this amount is reached, the LCn3PUFA will be increasingly stored in triglycerides. The results presented here clearly indicate how eggs can be produced with optimized composition of LCn3PUFA without affecting ($P > 0.1$) the sensory properties of the eggs. The procedures elaborated herein provide directly applicable consequences for the food industry.

(*Key words*: egg yolk lipid, omega-3 fatty acid, phospholipid, seal blubber oil, triglyceride)

INTRODUCTION

The physiologically important long-chain omega-3 polyunsaturated fatty acids (LCn3PUFA) eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) are elongation metabolites of the essential α-linolenic acid (αLNA). However, the conversion of αLNA to LCn3PUFA is rather inefficient in the human as it is assumed that only 0.5 to 10% of the αLNA is actually converted to EPA (Indu and Ghafoorunissa, 1992; Hornstra, 2003). The LCn3PUFA are mainly found in marine species such as fatty fish or marine mammals but are underrepresented in the traditional Western diet. According to current knowledge, LCn3PUFA play an important role in the prevention and treatment of coronary artery disease (Wahlqvist, 1998), hypertension (Howe, 1997), diabetes (Krishna Mohan and Das, 2001), arthritis, and other inflammatory (Babcock et al., 2000) and (auto)-immune disorders (Wesley Alexander, 1998; Kelley and Rudolph, 2000; Kelley, 2001), as well as cancer (Rose and Connolly, 1999; Aronson et al., 2001), and are essential for normal growth and development (Anderson et al., 1990), especially of the brain and retina. It is assumed that the optimal ratio of omega-6 to omega-3 fatty acids (n6FA/n3FA) in the human nutrition is around 3/1 (Health and Welfare Canada, 1990; Sugano, 1996), but studies estimate that the actual ratio of n6FA/n3FA in industrialized countries ranges between 10/1 and 15/1 or higher (British Nutrition Foundation, 1992; Simopoulos, 2000).

Abbreviation Key: ARA = arachidonic acid; CH = cholesterol; DG = diacylglycerol; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; n3FA = omega-3 fatty acids; n6FA = omega-6 fatty acids; αLNA = α-linolenic acid; LCn3PUFA = long-chain omega-3 polyunsaturated fatty acid; MUFA = monounsaturated fatty acids; PC = phosphatidlycholine; PE = phosphatidylethanolamine; PL = phospholipids; SBO = seal blubber oil; SFA = saturated fatty acids; TG = triglyceride.

The incorporation of LCn3PUFA into eggs has turned out to be a successful strategy to alter this ratio toward the desired value. Sources of n3FA such as fish oils (Oh et al., 1991; Gonzalez-Esquerra and Leeson, 2000; Shimizu et al., 2001), fish meal (Nash et al., 1995, 1996), marine algae (Herber and Van Elswyk, 1996), plant seeds (Scheideler and Froning, 1996; Ayerza and Coates, 2000), or a combination of several of the above (Baucells et al., 2000) can be used as supplements in layer diets. However, supplementation with fishmeal or fish oil can exert a negative influence on the sensory properties of the egg (Nash et al., 1996), whereas supplementation with plant seeds result in much lower concentrations of LCn3PUFA in the egg (Scheideler and Froning, 1996; Ayerza and Coates, 2000).

Recently Shimizu et al. (2001) demonstrated that dietary LCn3PUFA accumulate preferably in the phospholipid (PL) fraction of the egg by efficiently replacing arachidonic acid (ARA), even after moderate supplementation of n3FA. This mechanism might possibly explain why, when fish oils are fed in excess of about 1.5% in the diet, the amount of LCn3PUFA in the egg is not altered to any great extent. An in-depth investigation of the distribution of fatty acids (FA) not only among the lipid classes of the egg yolk lipids, but also among the positions on the glycerol backbone within the main glycerolipids (triglycerides, TG; phosphatidylcholine, PC; phosphatidylethanolamine, PE), is needed in order to gain fundamental knowledge of the interactions between alterations in the FA profile and sensory properties of the egg. Furthermore, the positional distribution of FA on the glycerol backbone of TG is fundamentally different in seal blubber oil (SBO) than in fish oil (Ackman and Ratnayake, 1989). Whereas most of the PUFA of fish oil are located in the sn-2 position,[2] they are found in sn-1 and sn-3 in marine mammal fat such as the seal fat. This feature of SBO might have consequences on the enteral absorption process (Yang et al., 1989), which is believed to be a reason for the low incidence of cardiovascular disease among the Inuit of Greenland (Ackman and Ratnayake, 1989). Thus, the focus of this work was on the analysis of the positional distribution of FA in the lipid classes of egg yolk and on accompanying sensory evaluations.

The use of highly refined SBO provides an excellent basis for a nutritionally high value product, because, if properly refined, it will contain only trace amounts of environmental contaminants polychlorinated biphenyls (PCB), dioxins, dioxin-like PCB, furans), heavy metals (Pb, As, Cd, Hg), membrane components (sterols, PL), and flavor-intensive compounds (amines). The origin of SBO is the thick subcutaneous fat layer, which serves as a temperature "blanket" as well as depot fat for these animals. It contains almost entirely TG and is extremely low in cholesterol (CH), which is still an important health issue in the market place. Contrary to public perceptions, the North Atlantic Seal is by far not an endangered species, and the stocks have increased dramatically over the past 20 yr, after environmental activists achieved a ban on the traditional seal hunt. The latest count by the Fisheries and Oceans Canada of the harp seal population puts the number at around 5.2 million. Traditionally, the herd was maintained, by an annual hunt, at around 1.8 million animals. Today, the seal hunt in Atlantic Canada is strictly controlled with a limit of 350,000 adult seals per year. Although this amount is not even close enough to control the seal population, it accounts for an annual production of approximately 16,000 tonnes of blubber. Commercially, SBO products are mostly prepared as nutritional supplements in form of gelatin capsules but are also used as animal feed. The approximate costs for such high quality SBO range between US$2 and 3/kg. Information on seal hunt and oil production is presented in detail by Ackman (1997) and by the Fisheries and Ocean Canada (2003).

MATERIALS AND METHODS

Experimental Diet

A basal diet consisting of 30% corn, 25% wheat, 27% soybean, 1% cellulose, and 12% of a vitamin-mineral premix was prepared in a 475-kg batch. The SBO in amounts ranging from 0 to 5% was added to the 4 experimental diets. Tallow was added as control fat so that the amount of total fat supplement was 5% for all diets. The diets were isocaloric (isoenergic) and isonutritive (isonitrogenous) and differed only in the lipid composition. The tallow was stabilized with 0.01% t-butylhydroxytoluene. The SBO was stabilized by the addition of a mixture of α, β, γ, and δ d, l tocopherols at 0.35% by weight of the oil. The fat sources were mixed into the basal diet by spraying the fat into a revolving feed blender filled with 20 kg of the basal diet. The composition of the experimental diets is given in Table 1. The FA profiles of the supplemented lipid sources are presented in Table 2, as are, for comparison, the profiles of olive and fish oils (Mediterranean flounder). As these oils are connected with health benefits in the Mediterranean diet, this comparison seems useful for a better understanding of the context of this study.

Feeding Regimen

Seventy-two 26-wk-old Single Comb White Leghorn laying hens were randomly assigned to 36 cages (2 per cage) and to 4 dietary treatments in a 3-orthogonal 4 × 4 latin square with the fourth row suppressed. The study was conducted over a 9-wk period so that the experiment was terminated at 35 wk of age (peak egg production). Feed and water were provided ad libitum. Birds were provided with programmed lighting (14L:10D) and continuous ventilation.

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[2]Binding site of the fatty acid on the glycerol molecule presented in the stereospecific numbering (sn) nomenclature (IUPAC-IUB, 1967).

TABLE 1. Diet composition

Diet	1	2	3	4
		(g/100 g)		
Ingredient[1]				
Ground yellow corn	30.00	30.00	30.00	30.00
Ground wheat	25.00	25.00	25.00	25.00
Ground soybean	27.00	27.00	27.00	27.00
Cellulose	1.00	1.00	1.00	1.00
Vitamin-mineral premix	12.00	12.00	12.00	12.00
SBO	0.00	1.25	2.50	5.00
Tallow	5.00	3.75	2.50	0.00
Calculated composition				
Protein (%)	18.00	18.00	18.00	18.00
ME (kJ/kg)	11.82	11.82	11.82	11.82
Calcium (%)	4.00	4.00	4.00	4.00
Phosphorous (available)	0.40	0.40	0.40	0.40
Analysis				
Fat	8.25	8.14	8.16	8.22
Fatty acid (% of total fatty acids)				
C18:2n6 (LA)	14.21	14.32	14.21	15.15
C18:3n3 (αLNA)	1.38	1.27	1.24	1.13
C20:4n6 (ARA)	ND[2]	ND	0.08	0.23
C20:5n3 (EPA)	ND	1.46	2.25	4.44
C22:5n3 (DPA)	ND	1.01	1.52	2.98
C22:6n3 (DHA)	ND	1.86	2.82	5.47
Ratio of n6/n3 fatty acids	8.3	2.4	1.7	1.0
Calculated IV	54.2	75.4	86.7	119.3

[1]SBO = seal blubber oil, LA = linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; IV = iodine value, calculated from fatty acid profile.

[2]ND = not detected.

TABLE 2. Fatty acid composition of the fats used in the feeding trial and of other selected oils[1]

	SBO	Tallow	Olive oil[2]	Fish[3]
Fatty acid[4]		Fatty acid (weight %)		
Saturated				
C14:0	4.86	4.21	ND[5]	2.87
C16:0	9.04	28.91	13.70	13.98
C18:0	1.29	22.61	2.50	4.89
Others	0.24	2.78	0.90	3.58
Subtotal	15.43	58.51	16.20	25.32
Monounsaturated				
C16:1n7	17.48	2.63	1.20	8.54
C18:1n9	27.01	35.28	71.10	16.80
C20:1n9	13.98	0.10	ND	6.25
Others	3.85	0.67	ND	4.71
Subtotal	62.32	38.68	72.30	36.30
Polyunsaturated				
C18:2n6 (LA)	1.96	0.88	10.00	2.64
C18:3n3 (αLNA)	0.47	0.91	0.60	0.39
C18:4n3	1.23	0.52	ND	1.03
C20:4n6 (ARA)	0.34	ND	ND	1.30
C20:5n3 (EPA)	6.24	ND	ND	14.78
C22:5n3 (DPA)	3.99	ND	ND	4.11
C22:6n3 (DHA)	7.42	ND	ND	6.91
Others	0.47	0.31	ND	4.52
Subtotal	22.12	2.62	10.60	35.68
Total	99.87	99.81	99.10	97.30

[1]SBO (seal blubber oil) and tallow added to the diets as described in Table 1. Data for olive and fish oils were added for comparison.

[2]Data adapted from White (1992).

[3]Data adapted from Ackman (1992); species = *Passera* (flounder) of the Mediterranean Sea.

[4]LA = linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[5]ND = not detected.

Parameters of Production Performance

Eggs were collected and numbered, and the weights were recorded for each week and cage. Feed intake and feed conversion ratio (kg of feed/kg of egg) were also recorded on a weekly basis. Body weights of the hens were taken at beginning and end of the feeding trial.

Lipid Analyses

Eggs for lipid analyses were collected at wk 5 and 9 of the feeding trial. Three eggs of each replicate were selected at random. The eggs were weighed, and yolks were separated from the white and weighed as well. The yolks were homogenized by using an IKA Ultra Turrax T18.[3] Approximately 5 g of yolk was transferred into an Erlenmeyer flask and weighed to the nearest 0.1 mg.

Lipid Extraction. Yolk lipids were extracted following the method of Bligh and Dyer (1959). Before extraction, 5 mL of H_2O was added to the yolk to obtain the required proportions of solvents as described by the authors of the method. After 2 extraction steps, in which the proportions of chloroform/methanol/water were kept at 1/2/0.8 and 2/2/1.8, respectively, the mixture was filtered through a Buchner funnel by applying a vacuum with a water pump. The organic layer was removed with a Pasteur pipette, and the solid residue was re-extracted with 10 mL of chloroform. The organic layers were combined into a preweighed round-bottom flask and evaporated on a Buechi Rotovapor R-220.[4] After the aqueous residues were dried in a desiccator filled with phosphorous-V-oxide, the lipid content of the yolks was measured gravimetrically.

FA Analyses. Extracts were diluted in 50 mL of n-heptane. Three milliters of the extract was transmethylated in 7% borontrifluoride/methanol after addition of C17 methyl ester and C23 methyl ester as internal standards. The reaction was performed in reaction tubes with Teflon-lined screw caps in a boiling water bath for 1 h. After the vials cooled, the organic layer was removed, dried over Na_2SO_4, and split-injected (ratio 1/25) into a Hewlett-Packard 5890 gas chromatograph[5] using a SUPELCOWAX 10 column[6] 30 m in length and with an inner diameter of 0.32 mm. Millennium 2.10 software[7] was used for data processing.

Positional Analyses. Approximately 50 mg of egg yolk lipid was streaked in a line of 5 cm on a Silicagel G thin layer plate[8] with 250-μm layer thickness. Neutral lipids (TG and CH) were separated using a mobile phase consisting of hexane/diethylether/acetic acid (85/15/1). Polar lipids (PC and PE) were separated with chloroform/methanol/water (65/35/4). The separated bands were visualized by spraying the plates with a 2% dichlorfluorescein solution in ethanol. Bands were scraped off the plates and mixed with 3 mL of n-heptane and 3 mL of 7% borontrifluoride/methanol. Transmethylation was performed as described above. For positional analyses, bands were scraped off the plates and re-extracted with chloroform (TG) or methanol (PC and PE). Positional analyses of PC and PE were performed following the protocol of Amate et al. (1999). The positional assessment of the sn-2 position of the TG as well as the preparation of the racemic diacylglycerol (DG) mixture, which is required for the stereospecific determination of the terminal positions in the TG, was performed by splitting the terminal FA with pancreatic lipase (EC 3.1.1.3) as described by Luddy et al. (1963). Direct assessment of the sn-1 position required the synthesis of an artificial PL from the racemic DG mixture, which was achieved by synthesizing PC according to Myher and Kuksis (1979). After the obtained extract was cleaned as described by those authors, the sn-1 position of the TG was determined following the procedure that was used for PC and PE (Amate et al., 1999). To check whether the pancreatic hydrolysis produced representative DG, which is a prerequisite for accurate positional determination (Brockerhoff, 1965), the method was compared with an alternative procedure using methyl magnesium bromide instead of pancreatic lipase (Yurkowsky and Brockerhoff, 1966).

Lipid Class Analyses. Main lipid classes of the egg yolk lipids (TG, PC, PE, CH) were separated on an Iatroscan/Chromarod system.[9] An amount of approximately 200 μg of lipid dissolved in n-heptane was applied on each plate by using a microsyringe. Samples were focused with acetone twice and developed as follows: 2 developments at 25 min each in hexane/diethylether/acetic acid (99/1/0.05, by volume) followed by a partial scan (setting on the Iatroscan: PPS 25), 1 development in hexane/diethylether/acetic acid (80/20/1, by volume) followed by a partial scan (PPS 25), and finally 2 developments at 35 min in chloroform/methanol/water (70/35/3.5, by volume) followed by a full scan. The system was calibrated with quantitative standards obtained from Sigma-Aldrich Canada[10] (product numbers: CH-C8667, TG-T7140, PE-P7943, PC-P7318).

Sensory Evaluation

A sensory panel was set up in a sensory lab, equipped with single cabins under red light to rule out any visual influence. For the sensory detection of any off-flavor in the egg it was important to provide the eggs to the panelists in a soft-boiled and unsalted state. The eggs were tempered in a water bath at 50°C, then placed in boiling water for precisely 3 min and 15 s, and then returned to the 50°C water bath. With this procedure, the eggs kept their soft-boiled condition for up to 1 h.

TABLE 3. The mean body weight, feed intake, egg production, egg weight, and feed conversion of laying hens fed the experimental diets

Item	Week of experiment									
	1	2	3	4	5	6	7	8	9	Mean
Mean body weight (g)										
Diet 1	1,570	—	—	—	—	—	—	—	1,607	—
Diet 2	1,582	—	—	—	—	—	—	—	1,664	—
Diet 3	1,563	—	—	—	—	—	—	1,669	—	—
Diet 4	1,542	—	—	—	—	—	—	—	1,662	—
Feed intake (g/d per bird)										
Diet 1	123	124	126	124	128	127	123	123	125	125
Diet 2	122	120	124	127	131	128	121	123	123	124
Diet 3	116	115	117*	113*	115	109*	109*	111*	113*	113
Diet 4	120	114	118	118	124	117	114	111*	118	117
Layer performance (eggs/wk per bird)										
Diet 1	6.56	6.67	6.72	6.61	6.28	6.33	6.22	6.17	6.33	6.43
Diet 2	6.67	6.50	6.78	6.78	6.61	6.22	6.28	6.44	6.61	6.54
Diet 3	6.72	6.78	6.89	6.83	6.56	6.28	6.28	6.28	6.11	6.53
Diet 4	7.00*	6.61	6.89	7.00	6.61	6.22	6.39	6.44	6.39	6.62
Mean egg weight (g)										
Diet 1	60	60	61	62	63	65	65	66	66	63
Diet 2	60	60	61	61	63	65	65	65	65	63
Diet 3	60	60	61	62	62	63	64	64	64	62
Diet 4	56*	58	58	59	59*	62	61	62*	62	60
Feed conversion (g feed/g egg)										
Diet 1	2.20	2.18	2.18	2.14	2.27	2.20	2.14	2.13	2.12	2.17
Diet 2	2.15	2.19	2.11	2.14	2.24	2.29	2.12	2.06	2.02	2.15
Diet 3	2.04	1.99	1.97*	1.88*	2.00	1.94	1.92	1.96	2.04	1.97
Diet 4	2.13	2.09	2.07	2.00	2.23	2.15	2.05	1.97	2.09	2.09

*Values differ from the control, diet 1 ($P < 0.05$).

Eight trained panelists were given 2 eggs, one from an experimental group and one from the control. In a pretest the panelists had to find a difference between the 2 eggs. After the pretest, the panelists were given 10 double samples of eggs. A difference ($P < 0.1$) was found when the panelist could recognize the SBO egg in at least 9 out of 10 samples.

Statistical Analyses of the Data

One-way ANOVA was performed on the raw data, and statistical differences ($P < 0.1$ for sensory evaluation and $P < 0.05$ for all other analyses) among the dietary groups were determined by the Bonferroni t-test using SigmaStat software.[11]

RESULTS

Production Performance

Egg production, egg weight, feed intake, feed conversion ratio, and BW are presented in Table 3. No differences in BW among the dietary groups were found after 9 wk of feeding SBO. Reduced feed intake correlated with increasing amount of SBO in the diet was found

in wk 3 to 9 for diet 3 and in wk 8 for diet 4 ($P < 0.05$). There was no difference ($P > 0.05$) in the number of eggs laid, except for diet 4 during wk 1. A reduction ($P < 0.05$) of the egg weight, of about 5% between diets 1 and 4, was observed at wk 1, 5, and 8.

FA Composition

Results of the FA analyses are given in Table 4. The most obvious effect from feeding SBO is the marked increase ($P < 0.001$) of LCn3PUFA (EPA, DPA, DHA), which occurred mainly at the expense of ARA and to a lesser degree MUFA. This exchange of n6FA for n3FA is also reflected in the iodine value,[12] which increased ($P < 0.001$) by about 15% between the control eggs and the eggs of hens fed diet 4 (Table 4), whereas the iodine value of the diets increased more than 100% (54.2 for the control diet vs. 119.3 for diet 4; Table 1). It should be noted that the content of LCPUFA (n3 and n6) was lower at wk 9 than at wk 5, with significance ($P < 0.05$) for EPA for diet 3, DPA for diet 2, and DHA for diet 3. However, this effect was compensated by egg weight, which was higher at wk 9 than at wk 5 (Table 3). Hence, when the results were expressed as milligrams per egg, there was no difference in the absolute amount of LCPUFA of the eggs between the two egg collections.

FA Composition and Positional Distribution of FA in Egg Yolk TG, PC, and PE

The FA profiles of the main lipid classes, TG, PC, and PE are presented in Table 5. The positional distribution

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[11]SigmaStat Inc., Chicago, IL.
[12]The iodine value of a fat is the grams of halogen absorbed by 100 g of the fat and expressed as the weight of iodine. It is a measure for the grade of unsaturation of a fat (IUPAC, 1987).

TABLE 4. The effect of feeding different levels of seal blubber oil (SBO) to laying hens on the fatty acid profile of egg yolk lipids[1]

	Fatty acids[2]									
	Wk 5					Wk 9				
Fatty acid[3]	Diet 1[4]	Diet 2	Diet 3	Diet 4	P[5]	Diet 1	Diet 2	Diet 3	Diet 4	P
	— (wt %) —									
Saturated										
C14:0	0.49^c	0.48^c	0.54^b	0.64^a	***	0.51^b	0.51^b	0.54^b	0.62^a	***
C16:0	26.09^{ab}	25.50^b	25.82^{ab}	26.59^a	**	25.63	25.75	25.63	26.11	
C18:0	10.29^a	10.14^a	10.06^a	9.37^b	***	9.97^a	9.71^a	9.81^a	9.05^b	***
Subtotal	36.87	36.12	36.42	36.60		36.11	35.97	35.98	35.78	
Monounsaturated										
C16:1n7	2.76^d	3.23^c	3.67^b	5.16^a	***	2.71^c	3.39^b	3.68^b	5.08^a	***
C18:1n9	47.25^a	46.08^a	44.17^a	41.20^b	***	46.84^a	45.53^a	44.43^a	41.68^b	***
C20:1n9	0.25^d	0.41^c	0.56^b	1.12^a	***	0.28^d	0.44^c	0.55^b	1.07^a	***
Subtotal	50.26	49.72	48.49	47.48		49.83	49.36	48.66	47.83	
Polyunsaturated										
C18:2n6 (LA)	9.25^b	9.27^b	9.78^{ab}	10.11^a	**	10.21^{ab}	9.85^b	10.35^{ab}	11.05	**
C18:3n3 (αLNA)	0.33^b	0.38^{bc}	0.42^{ac}	0.43^a	***	0.34^c	0.38^{bc}	0.41^{ab}	0.43^a	***
C18:4n3	0.46^a	0.41^b	0.34^c	Trace^d	***	0.46^a	0.38^b	0.31^c	Trace^d	***
C20:4n6 (ARA)	1.60^a	0.91^b	0.78^c	0.55^d	***	1.66^a	0.89^b	0.77^c	0.61^d	***
C20:5n3 (EPA)	Trace^d	0.23^c	0.34^b	0.59^a	***	Trace^d	0.21^c	0.31^b	0.51^a	***
C22:5n3 (DPA)	Trace^d	0.21^c	0.28^b	0.42^a	***	Trace^d	0.17^c	0.23^b	0.33^a	***
C22:6n3 (DHA)	0.91^d	2.49^c	2.91^b	3.48^a	***	0.95^d	2.46^c	2.65^b	3.09^a	***
Subtotal	12.55	13.90	14.85	15.58		13.62	14.34	15.03	16.02	
Total	99.68	99.74	99.76	99.66		99.56	99.76	99.67	99.63	
n6/n3	6.13^a	2.74^b	2.48^b	2.17^c	***	6.78^a	2.98^b	2.65^b	2.50^b	***
Total lipid (%)	28.41^a	29.35^a	29.94^a	29.07^a		30.16^a	30.05^a	29.49^a	29.84^a	
Calculated IV	64.1^c	69.7^b	71.1^b	73.8^a	***	66.1^c	70.2^b	70.2^b	73.8^a	***
	— (mg/egg) —									
Saturated										
C14:0	16.3^b	15.7^b	17.3^b	22.0^a	***	18.8^b	19.4^b	19.8^b	23.0^a	**
C16:0	862.0	835.8	831.5	878.6		944.1	983.3	935.0	958.7	
C18:0	339.9^a	332.4^{ab}	323.6^{ab}	309.5^b	*	367.2	371.1	358.6	331.8	†
Subtotal	1,218.2	1,183.9	1,172.4	1,210.1		1,330.1	1,373.8	1,313.4	1,313.5	
Monounsaturated										
C16:1n7	91.1^c	106.1^{bc}	118.3^b	170.4^a	***	100.0^c	129.6^b	134.3^b	186.3^a	***
C18:ln9	1,559.1^a	1,510.7^{ab}	1,421.1^{bc}	1,360.2^c	***	1,726.9^a	1,737.0^a	1,622.1^{ab}	1,528.8^b	**
C20:ln9	8.0^d	13.0^c	17.3^b	35.4^a	***	10.2^d	16.2^c	19.7^b	36.6^a	***
Subtotal	1,658.2	1,629.8	1,556.7	1,566.3		1,837.1	1,882.8	1,776.1	1,751.7	
Polyunsaturated										
C18:2n6 (LA)	305.3	303.8	315.0	333.7		376.5	376.5	378.7	405.9	
C18:3n3 (αLNA)	10.8^c	12.4^{bc}	13.4^{ab}	14.3^a	***	12.6^b	14.6^{ab}	15.0^a	15.9^a	**
C18:4n3	15.1^a	13.5^a	11.0^b	2.0^c	***	16.8^a	14.4^b	11.4^c	2.3^d	***
C20:4n6 (ARA)	52.8^a	29.2^b	24.1^c	17.4^d	***	60.2^a	33.2^b	27.8^c	21.1^d	***
C20:5n3 (EPA)	Trace^d	7.3^c	10.4^b	18.8^a	***	Trace^d	7.9^c	11.1^b	17.7^a	***
C22:5n3 (DPA)	2.5^d	6.6^c	8.6^b	13.2^a	***	2.7^d	6.5^c	8.1^b	11.5^a	***
C22:6n3 (DHA)	30.1^d	79.9^c	90.3^b	110.3^a	***	34.4^c	91.6^b	94.7^{ab}	106.1^a	***
Subtotal	416.6	452.7	472.8	509.7		503.2	544.7	546.8	580.5	
Total	3,293.0	3,266.4	3,201.9	3,286.1		3,670.4	3,801.3	3,636.3	3,645.7	

[a–d]Means within each row with no common superscript differ ($P < 0.05$).

[1]Values are means of 9 independent replicates.

[2]C15:0, C20:0, C22:0, C14:1n5, and C18:3n6 were found in trace amounts (<0.1%). Other fatty acids found in amounts of <0.1% or 1 mg/egg are indicated as traces (Trace).

[3]LA = linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; IV = iodine value, calculated from fatty acid profile.

[4]Amount of SBO in diet: diet 1, 0%; diet 2, 1.25%; diet 3, 2.5%; diet 4, 5%.

[5]ANOVA level of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, †$P < 0.10$.

of FA on the glycerol molecule is given in Table 6 for TG, Table 7 for PC, and Table 8 for PE. The results of the positional analyses are given in mole-percentage because only this unit permits the direct comparison among the positions. Among the main FA, palmitic acid (C16:0) was found predominantly in position sn-1 of TG, whereas oleic acid (C18:1n9) was mainly in the sn-2 and sn-3 positions of TG. LCPUFA (ARA, EPA, DPA, DHA) were located almost entirely in the sn-2 positions of PC and PE. It should be noted that the relative amount of LCPUFA in the TG fraction increased when SBO was fed, which is shown in Table 9, where the horizontal distribution of LCPUFA between TG and PL (PC + PE) is presented. The results of the lipid class analyses are presented in this Table 9 as well. No differences in lipid class composition were found among the diets fed. In

TABLE 5. The effect of feeding different levels of seal blubber oil (SBO) to laying hens on the fatty acid profile of egg yolk triglyceride (TG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE)

Fatty acid[2]	Fatty acids (wt %)[1]											
	TG				PC				PE			
	Diet 1[3]	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
Saturated												
C14:0	0.61	0.59	0.64	0.74	0.25	0.26	0.25	0.32	0.45	0.47	0.43	0.45
C16:0	25.39	25.39	24.35	25.27	30.98	33.29	32.62	33.68	16.38	18.58	18.04	19.40
C18:0	7.23	7.40	7.77	7.40	14.06	12.40	12.23	11.41	28.33	26.44	26.19	25.88
Subtotal	33.23	33.38	32.75	33.41	45.29	45.95	45.10	45.40	45.16	45.49	44.66	45.73
Monounsaturated												
C16:1n7	3.30	4.06	4.46	6.07	1.16	1.52	1.55	2.17	0.49	0.52	0.52	0.69
C18:1n9	52.54	51.41	50.24	46.73	33.44	31.72	31.69	28.92	21.45	19.26	18.61	18.12
C20:1n9	0.31	0.48	0.65	1.21	0.14	0.22	0.27	0.65	0.14	0.30	0.39	0.87
Subtotal	56.16	55.94	55.34	54.01	34.74	33.46	33.51	31.73	22.07	20.09	19.52	19.68
Polyunsaturated												
C18:2n6 (LA)	8.92	8.97	9.83	10.56	13.43	11.93	11.98	11.97	10.23	6.78	7.13	6.77
C18:3n3 (αLNA)	0.40	0.40	0.51	0.50	0.19	0.13	0.12	Trace	0.13	0.10	Trace	Trace
C18:4n3	0.50	0.40	0.34	Trace	0.44	0.32	0.25	Trace	0.55	0.38	0.31	Trace
C20:4n6 (ARA)	0.18	0.13	0.13	0.11	3.30	1.68	1.48	1.36	14.0	37.8	46.5	74.78
C20:5n3 (EPA)	Trace	Trace	0.10	0.17	0.12	0.52	0.73	1.25	Trace	1.19	1.56	1.81
C22:5n3 (DPA)	Trace	Trace	0.13	0.18	Trace	0.28	0.30	0.47	0.50	0.81	1.03	1.17
C22:6n3 (DHA)	Trace	0.30	0.44	0.55	1.94	5.32	6.15	7.27	6.79	16.86	18.73	19.35
Subtotal	9.99	10.20	11.48	12.07	19.41	20.19	21.02	22.32	32.22	33.95	35.32	33.89
Total	99.38	99.52	99.58	99.49	99.44	99.59	99.63	99.45	99.46	99.53	99.50	99.29

[1]Values were means of duplicate analyses, obtained from a pool of 9 independent samples collected at wk 5 of the feeding trial. C15:0, C20:0, C22:0, C14:1n5, and C18:3n6 were found in trace amounts (<0.1%). Other fatty acids were found in amounts of <0.1% are indicated as traces (Trace).

[2]LA = linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[3]Amount of SBO in diet: diet 1, 0%; diet 2, 1.25%; diet 3, 2.5%; diet 4, 5%.

TABLE 6. The effect of feeding different levels of seal blubber oil (SBO) to laying hens on the stereospecific distribution of fatty acids in egg yolk triglyceride (TG)

Fatty acid[2]	Fatty acids (mol %)[1]											
	Diet 1[3]			Diet 2			Diet 3			Diet 4		
	Position			Position			Position			Position		
	sn-1	sn-2	sn-3	sn-1	sn-2	sn-3	sn-1	sn-2	sn-3	sn-1	sn-2	sn-3
Saturated												
C14:0	0.85	0.45	0.88	0.97	0.73	0.40	0.85	0.58	0.84	1.22	0.48	0.95
C16:0	57.07	4.91	19.67	55.75	16.30	9.07	59.04	10.78	8.03	63.36	4.47	12.78
C18:0	7.43	3.63	10.50	8.32	4.73	9.53	8.63	4.46	10.55	7.92	3.21	10.93
Subtotal	65.35	8.99	31.05	65.04	21.76	19.00	68.51	15.82	19.42	72.49	8.16	24.65
Monounsaturated												
C16:1n7	3.26	2.69	4.72	3.63	4.03	5.41	4.19	3.90	6.28	4.49	5.01	10.00
C18:1n9	28.43	63.94	59.50	26.95	52.69	68.94	25.06	55.61	64.58	20.98	55.48	58.88
C20:1n9	ND	0.17	0.67	0.34	0.25	0.67	0.27	0.34	1.11	0.42	0.53	2.27
Subtotal	31.69	66.79	64.89	30.91	56.97	75.02	29.52	59.85	71.97	25.89	61.02	71.16
Polyunsaturated												
C18:2n6 (LA)	1.79	22.30	2.14	3.00	19.12	4.24	1.16	21.87	5.89	1.38	28.23	1.36
C18:3n3 (αLNA)	0.28	0.60	0.30	0.31	0.70	0.17	0.24	0.79	0.48	0.24	0.91	0.32
C18:4n3	ND	0.40	1.08	0.14	0.27	0.78	0.12	0.30	0.57	ND	Trace	0.12
C20:4n6 (ARA)	ND	0.27	0.21	Trace	0.19	0.13	ND	0.20	0.16	ND	0.21	Trace
C20:5n3 (EPA)	ND	ND	ND	ND	Trace	Trace	ND	Trace	0.18	ND	0.11	0.36
C22:5n3 (DPA)	ND	Trace	Trace	ND	0.12	Trace	ND	0.19	0.13	ND	0.30	0.15
C22:6n3 (DHA)	ND	0.10	Trace	Trace	0.35	0.40	ND	0.43	0.68	ND	0.69	0.69
Subtotal	2.06	23.67	3.73	3.44	20.75	5.71	1.52	23.78	8.10	1.62	30.46	3.00
Total	99.11	99.46	99.66	99.39	99.49	99.73	99.55	99.46	99.49	100.00	99.64	98.81

[1]Values were means of duplicate analyses, obtained from a pool of nine independent samples collected at wk 5 of the feeding trial. C15:0, C20:0, C22:0, C14:1n5, and C18:3n6 were found in trace amounts (<0.1%). Other fatty acids were found in amounts of <0.1% are indicated as traces (Trace); ND = not detected.

[2]LA = Linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[3]Amount of SBO in diet: diet 1, 0%; diet 2, 1.25%; diet 3, 2.5%; diet 4, 5%.

TABLE 7. The effect of feeding different levels of seal blubber oil (SBO) to laying hens on the stereospecific distribution of fatty acids in egg yolk phosphatidylcholine (PC)

	Fatty acids (mol %)[1]							
	Diet 1[3]		Diet 2		Diet 3		Diet 4	
	Position		Position		Position		Position	
Fatty acid[2]	sn-1	sn-2	sn-1	sn-2	sn-1	sn-2	sn-1	sn-2
Saturated								
C14:0	0.49	0.29	0.49	0.15	0.49	0.15	0.61	0.21
C16:0	62.80	6.00	66.75	4.28	67.03	6.36	69.12	3.97
C18:0	27.20	2.04	23.72	1.46	23.53	2.24	20.56	1.24
Subtotal	90.49	8.32	90.96	5.90	91.05	8.75	90.28	5.42
Monounsaturated								
C16:1n7	1.43	1.01	1.88	1.36	1.84	1.38	2.68	1.99
C18:1n9	6.52	55.96	5.81	53.53	5.79	53.85	5.55	49.11
C20:1n9	0.12	Trace	0.19	0.22	0.21	0.27	0.57	0.62
Subtotal	8.06	56.97	7.87	55.11	7.84	55.50	8.80	51.72
Polyunsaturated								
C18:2n6 (LA)	0.78	24.47	0.62	22.66	0.70	21.90	0.46	22.56
C18:3n3 (αLNA)	Trace	0.25	Trace	0.22	ND[6]	0.17	Trace	0.14
C18:4n3	Trace	0.53	Trace	0.46	ND	0.42	ND	ND
C20:4n6 (ARA)	Trace	5.61	Trace	3.47	ND	2.50	ND	2.70
C20:5n3 (EPA)	ND	Trace	ND	1.08	ND	1.17	ND	2.46
C22:5n3 (DPA)	ND	0.17	ND	0.50	ND	0.43	ND	0.87
C22:6n3 (DHA)	ND	2.91	Trace	10.26	Trace	8.84	ND	13.70
Subtotal	0.78	33.94	0.62	38.65	0.70	35.43	0.46	42.43
Total	99.33	99.23	99.45	99.65	99.58	99.68	99.54	99.57

[1]Values were means of duplicate analyses, obtained from a pool of nine independent samples collected at wk 5 of the feeding trial. C15:0, C20:0, C22:0, C14:1n5, and C18:3n6 found in trace amounts (<0.1%). Other fatty acids found in amounts of <0.1% are indicated as traces (Trace); ND = not detected.

[2]LA = linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[3]Amount of SBO in diet: diet 1, 0%; diet 2, 1.25%; diet 3, 2.5%; diet 4, 5%.

diet 1 (control), only 8.8% of DHA was located in the TG fraction, whereas this amount increased to 16.2% when 5% SBO was fed (diet 4). The results of the positional distribution of FA in the sn-2 position of PC and PE clearly show the effective exchange of ARA and DHA after feeding the lower level (1.25%) of SBO.

Sensory Evaluation

Fifty percent of the panelists were able to distinguish ($P < 0.1$) between control eggs and the eggs from the group fed with 5% SBO (diet 4), whereas only 3 (diet 3, 2.5% SBO) and 1 (diet 2, 1.25% SBO) out of 8 panelists could find a difference between the eggs from those 2 diets and the control ($P < 0.1$). The effect of feeding SBO on the sensory attributes of the eggs followed a more linear trend than actual enrichment of LCn3PUFAs in the eggs (Figure 1).

DISCUSSION

Supplementing with SBO had only minor effects on layer performance. The slightly reduced mean egg weight ($P < 0.05$) in some cases might have resulted from the slightly reduced feed intake (in 6 out of 9 wk in diet 3, 2.5% SBO in diet; see Table 3). However, it might also have been due to the lower plasma TG in the SBO--fed groups, a result of the hypotriglyceridemic

effect of n3FA (Harris et al., 1983), as it has been observed in other studies (Van Elswyk, 1997; Gonzalez-Esquerra and Leeson, 2000). The lower ($P < 0.05$) feed intake in the group fed diet 3 vs. the control was reflected by the better feed conversion efficiency of this group ($P < 0.05$, wk 3 and 4). Except for 1 wk, no differences were found for diet 4 (5% SBO in diet) vs. the control (Table 3). This finding could not be explained conclusively, but errors in the statistical design were ruled out, because no row or block effects were observed for any of the data measured.

The reduced egg weight that was found for 3 of 9 wk was of no practical significance, because it only occurred in diet 4 (5% SBO in diet) vs. the control diet ($P < 0.05$), whereas for diet 2 (1.25% SBO in diet) vs. the control diet, no ($P < 0.05$) decrease of egg weight was observed. The mean egg weights in all dietary groups were within the limits of medium-size eggs according to the egg regulation of the Canadian Food Inspection Agency (CFIA, 2003). Indeed a slight decrease of egg weight might be an advantage for genotypes that have a propensity to lay larger eggs.

The substantial increase ($P < 0.0001$) of LCn3PUFA from 33 mg/egg (diet 1) to 142 mg/egg (diet 4) was satisfactory and required to produce eggs with nutritionally optimized content of LCn3PUFA. Supplementation with moderate amounts of SBO (1.25%) to the diet resulted in eggs with around 90 mg LCn3PUFA, repre-

SCHREINER ET AL.

TABLE 8. The effect of feeding different levels of seal blubber oil (SBO) to laying hens on the stereospecific distribution of fatty acids in egg yolk phosphatidylethanolamine (PE)

Fatty acid[2]	Fatty acids (mol %)[1]							
	Diet 1[3]		Diet 2		Diet 3		Diet 4	
	Position		Position		Position		Position	
	sn-1	sn-2	sn-1	sn-2	sn-1	sn-2	sn-1	sn-2
Saturated								
C14:0	1.46	0.39	1.07	Trace	1.14	Trace	1.22	0.15
C16:0	34.24	7.04	38.65	3.39	38.30	4.77	41.80	3.36
C18:0	51.18	4.34	52.59	3.28	52.87	5.39	49.32	2.93
Subtotal	86.87	11.77	92.31	6.67	92.31	10.17	92.34	6.44
Monounsaturated								
C16:1n7	0.62	0.69	0.40	0.76	0.34	0.69	0.48	1.11
C18:1n9	10.45	32.12	5.82	31.87	5.62	29.34	5.27	32.71
C20:1n9	0.20	0.24	0.20	0.35	0.26	0.41	0.68	1.01
Subtotal	11.26	33.05	6.42	32.97	6.22	30.44	6.43	34.83
Polyunsaturated								
C18:2n6 (LA)	0.26	17.48	0.30	14.00	0.35	12.41	0.17	13.04
C18:3n3 (αLNA)	Trace	0.22	Trace	0.16	ND	0.14	0.03	0.12
C18:4n3	Trace	0.95	Trace	0.70	ND	0.48	0.04	0.09
C20:4n6 (ARA)	Trace	23.93	Trace	13.86	Trace	11.21	ND	8.51
C20:5n3 (EPA)	ND	0.15	ND	2.10	ND	2.63	ND	3.03
C22:5n3 (DPA)	ND	0.79	ND	1.36	ND	1.62	ND	1.97
C22:6n3 (DHA)	ND	11.00	0.03	27.93	Trace	30.64	ND	31.49
Subtotal	0.26	54.51	0.34	60.10	0.35	59.13	0.23	58.25
Total	98.39	99.33	99.07	99.74	98.88	99.73	99.00	99.52

[1]Values were means of duplicate analyses, obtained from a pool of nine independent samples collected at wk five of the feeding trial. C15:0, C20:0, C22:0, C14:1n5, and C18:3n6 were found in trace amounts (<0.1%). Other fatty acids were found in amounts of <0.1% are indicated as traces (Trace); ND = not detected.

[2]LA = Linoleic acid; αLNA = α-linolenic acid; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[3]Amount of SBO in diet: diet 1, 0%; diet 2, 1.25%; diet 3, 2.5%; diet 4, 5%.

senting a 3-fold increase compared with the control. Interestingly, this level of dietary SBO had a negligible effect on the sensory properties of the eggs. The levels
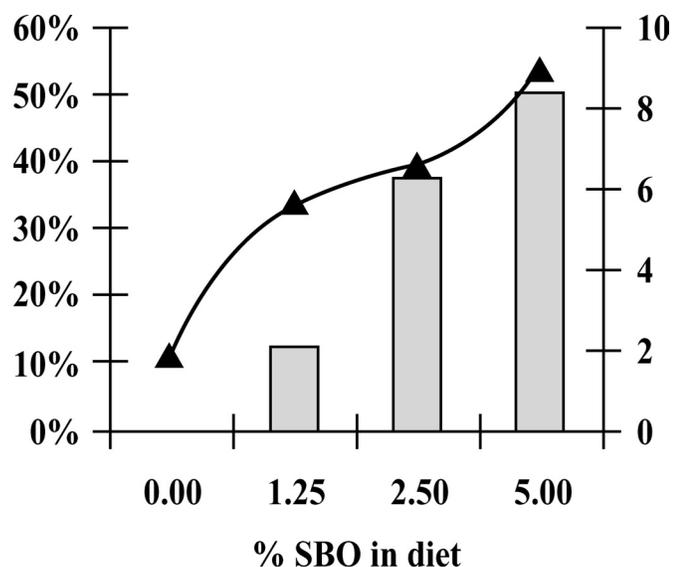

FIGURE 1. Levels of long-chain omega-3 polyunsaturated fatty acids (LCn3PUFA) versus sensory properties of eggs from hens fed different levels of seal blubber oil (SBO). Bars indicate the percentage of panelists who found a difference (*P < 0.1); filled triangles indicate milligrams of LCn3PUFA per gram of yolk. *Difference detected versus control (% SBO in diet).

of LCn3PUFA achieved in this study are comparable with the findings of other studies, in which fish oil was used as dietary supplement for laying hens (Baucells et al., 2000; Gonzalez-Esquerra and Leeson, 2000) but were considerably higher than the levels obtained when plant seeds rich in αLNA were fed (Scheideler and Froning, 1996; Ayerza and Coates, 2000).

The decrease of MUFA with increasing amounts of SBO in the diet can be accounted for in the reduction of C18:1n9. The other 2 MUFA that were detected in more than trace amounts (C16:1n7 and C20:1n9) showed a consistent increase when SBO was fed. This finding was not surprising because it reflected the composition of SBO, which contains remarkably high amounts of C16:1n7 (17.5%) and C20:1n9 (14.0%). Indeed a unique property of SBO is its content of MUFA, which is higher compared with most fish oils, such as salmon (Ackman, 1992; Kakela and Hyvarinen, 1998). This high content of MUFA (usually around 60% in SBO, 33% in salmon) is accompanied by a substantially lower content of SFA (29% in salmon, 14% in SBO).

The low incidence of cardiovascular disease among the natives of Greenland (Bang et al., 1971) seems to be related to the consumption of seal fat and meat rather to consumption of fish (Ackman and Ratnayake, 1989). This is not surprising because the Inuit are opportunistic hunters. They hunt, gather, and consume fatty fish in season (2 to 3 mo per year), but the basis of their diet

is seal blubber (fat) and seal meat with its 40% associated fat (Hulan, 2002). Investigations regarding the cardioprotective properties of SBO were focused on the positional distribution of FA in the TG of SBO (Ackman and Ratnayake, 1989), as well as on the unusually high content of DPA, which is a potent cardioprotective agent (Kanayasu-Toyoda et al., 1996). However, the low ratio of SFA/MUFA in SBO might be another conclusive explanation for the cardioprotective properties of SBO. In addition to the Greenland natives, low risks for cardiovascular diseases are encountered in Mediterranean countries as well (Buzina et al., 1991). The traditional food of these countries is characterized by a high content of olive oil and fish. Interestingly, a mixture of fish and olive oils mimics the FA composition of SBO very closely with regards to SFA and MUFA. The low ratio of SFA/MUFA must also be considered as favorable when SBO is used as a dietary supplement for poultry, as high amounts of SFA are not usually found in typical poultry feeds. From a physiological standpoint, the FA profile of the n3FA-enriched eggs appears to be favorable as clinical studies have clearly indicated that consumption of fish (Albert et al., 2002), or n3FA concentrates (GISSI Prevenzione Investigators, 1999) on a regular basis can reduce ($P > 0.05$) the risk of cardiovascular death.

The effect of feeding SBO on the lipid composition of the eggs was basically reflected by a decrease of ARA and a concomitant increase of LCn3PUFA with only minor changes in the overall degree of unsaturation, as reflected by the iodine value. This finding was of great importance because the ratio of n6FA/n3FA was mainly responsible for the biological response of essential FA (Holman, 1960). Given the fact that only slight changes in the iodine value occurred, it seems reasonable to assume that the oxidative stability of the eggs was not jeopardized to any great extent by feeding SBO.

The distribution of FA among TG, PC, and PE and the positional distribution of FA within these lipid classes were within the ranges that had been described by other authors (Privett et al., 1962; Holub and Kuksis, 1969; Christie and Moore, 1970; Gornall and Kuksis, 1971). The horizontal distribution data clearly shows that DHA, the most prominent LCn3PUFA in egg yolk, was evenly distributed between PC and PE, although the content of PC in egg yolk is much higher than the content of PE. To our knowledge, it has not been previously reported how FA profiles change within the single positions of the lipid classes when diets rich in n3FA are fed. However, Shimizu et al. (2001) reported that the exchange of n6FA and n3FA takes place in the PL rather than in the TG fraction. This observation was confirmed in the study reported here, and indeed the effects were more pronounced as the FA profiles were measured directly in the single positions. The most interesting result was that the profile of LCPUFA (ARA, EPA, DPA, DHA) showed marked changes in the sn-2 position of PC and PE. When 1.25% SBO was fed, there was an almost complete exchange of ARA and DHA in the sn-2 position of PC and PE (Tables 7 and 8). However, when SBO was fed in excess of 1.25%, the content of LCn3PUFA in the sn-2 position of PC and PE plateaued. Furthermore, it was noted that feeding more than 1.25% SBO in the diet caused an increase of LCn3PUFA in the TG fraction (Table 9). However, this increase was much smaller than the alteration of these FA in the PL fraction when 1.25% SBO was fed. This finding, in combination with the results of the sensory evaluation, leads to the conclusion that the addition of roughly 1.25% SBO to the layer diet must be regarded as optimum with maximal enrichment of LCn3PUFA and minimal changes in sensory properties. Therefore, for the commercial production of high omega-3 eggs, the addition of 1.25% SBO appears to be the most favorable from the standpoints of optimal FA content and sensory properties.

When 1.25% SBO was fed, the iodine value of the egg yolk lipids matched very closely the diets fed. When these 2 curves were plotted, an overlap was noted at around 1% SBO in the diet. Thus it would appear that in

TABLE 9. The effects of feeding different levels of seal blubber oil (SBO) to laying hens on the lipid class profile and horizontal distribution of fatty acids between triglyceride (TG) and phospholipids (PL) of the egg yolk lipid

Horizontal distribution of fatty acids[1] (mol %)	Diet 1[2]		Diet 2		Diet 3		Diet 4	
	TG	PL	TG	PL	TG	PL	TG	PL
20:4n6 (ARA)	10.4	89.6	12.5	87.5	16.0	84.0	15.0	85.0
20:5n3 (EPA)	34.1	65.9	20.0	80.0	28.8	71.2	31.4	68.6
22:5n3 (DPA)	33.1	66.9	40.9	59.1	52.9	47.1	50.1	49.9
22:6n3 (DHA)	8.8	91.2	11.6	88.4	16.3	83.7	16.2	83.8
Lipid classes[3] (wt %)	Wk 5	Wk 9	Wk 5	Wk 9	Wk 5	Wk 9	Wk 5	Wk 9
TG	69.7	68.4	69.6	69.9	70.7	70.2	70.3	69.7
PC	19.1	20.2	19.7	19.9	19.3	19.5	19.1	19.7
PE	6.7	7.4	6.2	6.2	5.6	6.2	6.2	6.5
CH	4.4	4.0	4.4	4.0	4.4	4.0	4.4	4.0

[1]ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[2]Amount of SBO in diet: diet 1, 0%; diet 2, 1.25%; diet 3, 2.5%; diet 4, 5%.

[3]None of the values differ ($P > 0.05$), between week or among the diets. PC = phosphatidylcholine, PE = phosphatidylethanolamine, CH = cholesterol.

order to achieve optimized incorporation of LCn3PUFA into egg yolk lipid with minimal influence on the sensory properties, the degree of saturation of the diet and that of the egg yolks of the eggs produced therefrom must match.

When more unsaturated fat is fed to laying hens, the hen has the capacity to saturate the fat, because the unsaturation of egg yolk lipids must be within certain limits for biological and reproductive reasons. Therefore, an excess of unsaturated fat in the diet will not affect the degree of unsaturation, or consequently the enrichment of LCn3PUFA, but will exert a negative effect on the sensory properties. Given this observation, an optimized feeding regimen can be developed by performing a simple pilot feeding trial and determining the iodine value of the diets and the eggs. Thus sensory evaluations, which normally require a broader experimental set-up and are more difficult to interpret, could be avoided.

Concluding Remarks

The results presented in this study clearly show how eggs can be produced with optimized composition of LCn3PUFA without affecting the sensory properties of the eggs. The interactions involving the alterations of the FA profile, sensory attributes, and feeding regimen can be explained not only by the distribution of FA in the different lipid classes of the egg yolk, as proposed by Shimidzu et al. (2001), but also by the distribution of FA on the glycerol backbone of the lipids. The procedures elaborated herein provide direct application to the food industry. Considering the studies of Farrell (1998) and Oh et al. (1991), separate evaluations of the physiological effects of eggs enriched with n3FA, especially those enriched by feeding SBO, have to be conducted to obtain conclusive information regarding the proposed health benefits of LCn3PUFA-enriched eggs.

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