

Supporting Information for

Feeding Laying Hens Stearidonic Acid-Enriched Soybean Oil, as Compared to Flaxseed Oil, More Efficiently Enriches Eggs with Very Long-Chain n-3 Polyunsaturated Fatty Acids

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Supplemental Table 1. Body weight (BW) change, daily feed consumption, egg production, egg weights, and feed conversion efficiency of hens fed diets containing 5% supplemental soybean oil (CON), stearidonic acid-enriched soybean oil (SDA), flaxseed oil (FLAX), or fish oil (FISH) for 28^{1,2}.

Diet	Supplemental lipid source	BW change ³	Daily feed consumption	Hen-d egg production ⁴	Average egg weight ⁵	Feed conversion efficiency
		(g)	(g)	(%)	(g)	(g feed/g egg mass)
1	CON	-5.4	96.3	88.78	61.1	1.78
2	SDA	-10.1	94.3	89.73	61.0	1.73
3	FLAX	22.4	97.7	91.33	60.6	1.77
4	FISH	29.2	98.2	91.96	60.1	1.78
	SEM	20.6	2.9	2.69	0.9	0.04
	<i>P</i> -value	0.41	0.74	0.82	0.84	0.76

¹Values are least square means of 7 hens each for Diets 1 and 3, and of 8 hens each for Diets 2 and 4.

²Each dietary group averaged 90% hen-day egg production and 61.15 g egg weights in the 5 d immediately prior to the initiation of the experiment.

³The initial body weights ($\pm$ SD) of hens fed diets 1-4 were: 1680.3 $\pm$ 189.4 g, 1629.6 $\pm$ 114.7 g, 1605.1 $\pm$ 152.7 g, and 1593.6 $\pm$ 86.4 g, respectively.

⁴Hen-d egg production was calculated as (100 x number of eggs laid)/(number of hens x d).

⁵Values are the means of 174, 201, 179, and 206 eggs from hens fed Diets 1, 2, 3, and 4, respectively.

Supplemental Table 2. Weights and relative percentages of eggs and egg components of hens fed diets containing 5% supplemental soybean oil (CON), stearidonic acid-enriched soybean oil (SDA), flaxseed oil (FLAX), or fish oil (FISH) for 28 d.

		Day 28 ²					
Item	Day 0 ¹	CON	SDA	FLAX	FISH	SEM	<i>P</i> -value ³
Weight (g)							
Egg	61.13	61.39	60.42	60.17	60.63	1.21	0.88
Yolk	15.05	15.38	14.94	15.11	15.18	0.39	0.74
Albumen	40.48	40.30	39.99	39.63	39.93	0.94	0.90
Shell	5.60	5.71	5.50	5.43	5.51	0.12	0.77
(%)							
Yolk	24.63	25.08	24.71	25.12	25.05	0.41	0.79
Albumen	66.20	65.63	66.18	65.83	65.84	0.43	0.85
Shell	9.22	9.29	9.11	9.05	9.11	0.22	0.87

¹Mean of 30 eggs (one egg from each hen in each diet).

²Values are the least square means of one egg from each of 7 hens per diet (CON and FLAX diets) or one egg from each of 8 hens per diet (SDA and FISH diets).

³Diet effect on d 28.

Supplemental Table 3. Total and relative cholesterol, crude fat, and crude protein contents of egg yolks of hens fed diets containing 5% supplemental soybean oil (CON), stearidonic acid-enriched soybean oil (SDA), flaxseed oil (FLAX), or fish oil (FISH) for 28 d¹.

Item	Day 0 ¹	Day 28 ²				SEM	P-value ³
		CON	SDA	FLAX	FISH		
(mg/g yolk, wet basis)							
Cholesterol	10.00	10.56	11.13	11.14	10.98	0.19	0.20
Crude fat	274.6	273.0	274.1	274.1	274.1	2.78	0.99
Crude protein	167.1	170.1	169.7	169.5	169.4	1.68	0.70
(mg/yolk, wet basis)							
Cholesterol	150	163	167	168	166	4.9	0.24
Crude fat	4,132	4,197	4,162	4,140	4,101	122	0.86
Crude protein	2,514	2,615	2,572	2,562	2,532	65	0.40
(% of yolk DM)							
Cholesterol	1.92	2.08	2.10	2.14	2.13	0.039	0.06
Crude fat	52.96	52.11	52.33	52.56	52.38	0.456	0.94
Crude protein	32.00	32.48	32.33	32.51	32.43	0.349	0.88

¹Mean of 30 eggs (one egg from each hen in each diet).

²Values are the least square means of one egg from each of 7 hens per diet (CON and FLAX diets) or one egg from each of 8 hens per diet (SDA and FISH diets).

³Diet effect on d 28.

Supplemental Table 4. Total and relative liver and abdominal fat pad weights from hens fed diets containing 5% supplemental soybean oil (CON), stearidonic acid-enriched soybean oil (SDA), flaxseed oil (FLAX), or fish oil (FISH) for 28 d^{1,2}.

Item	Day 0	Day 28				SEM	<i>P</i> -value ³
		CON	SDA	FLAX	FISH		
Weight (g)							
Body	1,584	1,675	1,620	1,627	1,623	53	0.86
Liver	30.1	30.4	27.9	28.1	29.5	1.3	0.48
Abdominal fat pad	68.1	82.5	77.8	87.3	82.6	7.6	0.84
(% of BW)							
Liver	1.93	1.82	1.72	1.73	1.82	0.06	0.47
Abdominal fat pad	4.28	4.81	4.78	5.38	5.06	0.36	0.61

¹Day 0 values are the least square means of 8 extra hens, which were euthanized on d 0 to provide baseline data. These hens had similar average egg production rates and egg weights as the 32 hens initially included in the feeding trial.

²Day 28 values are the least square means of 7 hens each per diet (CON and FLAX diets) or 8 hens each per diet (SDA and FISH diets).

³Diet effect on d 28.

Supplemental Table 5. Primers¹ used in Real-Time quantitative PCR.

Gene	ID	5' to 3'
Fatty acid desaturase 1	FADS1	F: CGTCATCAGCCACTACGCC R: GGTGCCAGTTCCCAATCAA
Fatty acid desaturase 2	FADS2	F: TGTCCTTGCGGAAAGTCAGC R: TACACGGGGATGAGCAGAGG
Fatty acid elongase 2	ELOV2	F: TTTGGCTGCCTCATGTTCCA R: TGTGACGGGGGTTTCCTTTG
Fatty acid elongase 5	ELOVL5	F: ATGCAGGGGCATTCTAGTGG R: CAGCTTCACCTCCACTGTGT
Fatty acid elongase 6	ELOVL6	F: TCCCTGAGCCTTGCTGTCTT R: GACTGCTTCAGGCCTTTGGT
Stearoyl-CoA desaturase 1	SCD1	F: CTGCGCACTTGCTACAGGAG R: TTCTTGGTCACCCGTGAGGT
Fatty acid synthase	FASN	F: CTTGAGTTGGCACAGTGGCT R: GATTCCCAGCGCCTTCCATT
Carnitine palmitoyltransferase 1a	CPT1a	F: AGGACCAAGGCTTCAGTGGT R: GGTCCACGCCCTCTAAGGTA
Acetyl CoA oxidase 1	ACOX1	F: CCGCTATAGTGCACTGAGGC R: ATACGCTGTTGCCAGAAGGG
HMG-CoA reductase	HMGCR	F: ATAGGGACTGTTGGGGGAGG R: CACCAGGGTTGTCTTGGCTT
Sterol response element binding protein 1	SREBP1	F: ACCGCTCATCCATCAACGAC R: TCAGGATCGCCGACTTGTG
Carbohydrate response element binding protein	ChREBP	F: GCAACCAGCTCTTCTGCAAC R: AGTCAAAGTGCTGCCTACCC
MAX-like protein X	MLX	F: GACGAGGACAGCGATTACCC R: GCCTTTCTTGATGGCGTTGC
Peroxisome proliferator-activated Receptor α	PPAR α	F: GCTGGGAAATGGTCCAGGAT R: CTACCTGGAGCTGTGGCAA
Beta-2-microglobulin	B2M	F: CGCTGATGAAGGATGGCGT R: GAACTCGGGATCCCACTTGAA
Beta actin	ACTB	F: CCCACACCCCTGTGATGAAA R: CCTGAGTCAAGCGCCAAAAG

¹F=forward; R=reverse.

Supplemental Table 6. Hepatic expression of lipid metabolism genes in hens fed diets containing 5% supplemental soybean oil (CON), stearidonic acid-enriched soybean oil (SDA), flaxseed oil (FLAX), or fish oil (FISH) for 28 d¹.

Gene	Day 0	Day 28				SEM	P-value ²
		CON	SDA	FLAX	FISH		
Fatty acid desaturases							
FADS1	98	100	83	92	98	7	0.31
FADS2	92	100	77	87	82	7	0.13
SCD1	111	100	104	101	92	10	0.84
Fatty acid elongases							
ELOVL2	115	100	108	110	86	13	0.53
ELOVL5	73	100	110	78	108	11	0.20
ELOVL6	88	100	90	84	100	12	0.72
Lipogenic enzyme							
FASN	96	100	89	80	72	18	0.69
Cholesterol synthesis enzyme							
HMGR	117	100	83	93	57	15	0.19
Fatty acid oxidation enzymes							
CPT1a	49	100	72	70	81	20	0.70
ACOX1	71	100	83	83	95	9	0.48
Lipogenic transcription factors							
SREBP1	116	100 ^a	91 ^a	90 ^{ab}	64 ^b	12	<0.05
MLX	85	100	96	86	102	8	0.49

¹Values are least square means of quadruplicate analyses of livers from 7 hens each for the CON and FLAX diets, and for 8 hens each for the SDA and FISH diets. Eight extra hens were euthanized on Day 0 to provide baseline data. Values are scaled to CON equal to 100.

²Diet effect on d 28.