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Influence of Fatty Acid Composition of Soybean Oil Vs. Beef Tallow on Egg Yolk Fatty Acid Profiles of Laying Hens

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Abstract: We investigated the effect of dietary soybean oil (SBO) vs. beef tallow (TAL) on egg yolk fatty acid composition in laying hens. Four laying hens (Hi-Sex brown chickens), 40 weeks of age, were given experimental diet and recorded data between day 7 and 42. The hens were fed cassava starch-soybean meal with 10% SBO or TAL. Fatty acid composition was determined for (a) soybean oil and beef tallow samples, (b) yolk lipids and (c) 5 yolks per hen on day 32 and 35. Feed intake of SBO treatment trended to be depressed (as low as 95.32 g/h/d) compared with the TAL treatment (105.55 g/h/d), albeit the difference was not significant ($p>0.05$). SFAs accumulation in egg yolks was between 1,141.84-1,509.42 mg/yolk mass, irrespective of SFA intake (i.e., 2,099.56 mg/h/d on the SBO diet or 8,878.16 mg/h/d on the TAL diet). The main residue of SFAs from the intake (stearic acid; 18:0) results in an increase in oleic acid (18:1n-9) or omega-9 fatty acid accumulation in the egg yolk. The linoleic acid (18:2n-6) in the yolk (i.e., 1,337.28 mg/yolk mass in the SBO treatment vs. 312.39 mg/yolk mass in TAL treatment) is directly related to dietary consumption ($p<0.05$) (i.e., 6,872.47 mg/h/d in the SBO diet and 1,010.40 mg/h/d in the TAL diet). The addition of TAL in diet strongly affects oleic acid (47.03% of 18:1n:9) accumulation in the yolk, while the addition of SBO to the diet resulted in PUFA (39.08%) accumulation in the yolk (mainly as omega-6 PUFAs).

Key words: Soybean oil, beef tallow, fatty acid composition, egg yolk, laying hens

INTRODUCTION

Dietary lipids are mainly triacylglycerols, which are hydrolyzed to monoacylglycerol and fatty acids in the gut, then re-esterified in the intestinal mucosa (Murray *et al*., 2003). These are then packed with protein and secreted into the lymphatic system and from thence to the blood stream for transport to yolk follicle formation. The yolk comprises 31-33% lipids, including triacylglycerols, phospholipids, free cholesterol and minor lipids (e.g., cerebroside). The fatty acid content in yolks is mainly medium to long chains, having 14-22 carbon atoms (Cherian, 2013). Differences in fatty acid composition of plant seed oil and animal tallow have been reported in the literature. Soybean oil mainly comprises unsaturated fatty acids (UFAs). UFAs induce lower fecal energy losses, so higher metabolizable energy than animal fats. Relatedly, it has been demonstrated that increased hormone levels (viz., triiodothyronine and thyroxine) are needed for improvement of growth performance of broiler chickens (Pruempornchai, 2009). High metabolizable energy from dietary soybean oil depresses feed consumption; due to high level of *de novo* heat production during metabolism in the tropics. In addition, a high level of soybean oil also reduces laying performance. Beef tallow has lower metabolizable energy values than vegetable oil (Pruempornchai, 2009); the latter having higher contents of stearic acid (18:0) and palmitic acid (16:0) long-chain, saturated fatty acids (SFAs). Stearic acid (18:0) is catalyzed in the liver by the desaturase enzyme ($\Delta 9$ -desaturase) to form oleic acid (18:1n-9) (omega-9 polyunsaturated fatty acids (PUFAs) and palmitic acid; in turn to form palmitoic acid (16:1n-7), an alternative precursor when essential fatty acids are deficient (Leonard *et al*., 2004; Marzouki and Coniglio, 1982; Engster *et al*., 1977). Soybean oil has high amounts of long-chain UFAs (essential fatty acids, especially linoleic acid) (18:2n-6), which are a source of omega-6 polyunsaturated fatty acids (PUFAs). The latter can be converted to important longer fatty acids (i.e., arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3)), by alternating the elongation and desaturation steps. PUFAs (rich in soybean oil) are essential for the synthesis of the hormones needed for growth (Casado *et al*., 2013; Guillou *et al*., 2010). The current study evaluated the fatty acid composition of egg yolks as well as the laying performance of hens fed different fatty acid compositions from soybean oil and beef tallow.

MATERIALS AND METHODS

Animals and experimental diets: A total of 4 laying hens (Hi-sex brown chickens), 40 weeks of age, were put in individual cages (30 x 40 x 40 cm) at the Poultry Research Unit, Department of Animal Science, Faculty of Agriculture, Khon Kaen University. The hens were adjusted pre-experimentally, by feeding them a

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commercial diet on day 0 to 7. Between day 7 and 42, two hens per group were fed a dietary treatment (i.e., a cassava starch-soybean meal diet) with 10% soybean oil or beef tallow. Experimental data were recorded between day 14 and 42 (a total of 28 days). The dietary treatments were defined according to the source of lipid added; either 10% as soybean oil (SBO) or as beef tallow (TAL). All of the hens were fed and watered *ad libitum* with 17 h of light per day at room temperature. Ten percent of fat, either 10% soybean oil (SBO diet) or 10% beef tallow (TAL diet) on a semi-purified basal diet of cassava starch-soybean meal-were formulated as experimental diets. The composition of nutrients (protein, metabolizable energy, vitamins and minerals) was calculated for laying hens as per the NRC (1994) recommendation (Table 1).

Data collection: Body weight was measured every 2 weeks. Feed intake, egg production and egg weight were recorded every day from day 14 to 42. The feed intake for egg production was calculated according to the average value between day 14 and 42 of the experiment. On day 32 and 35 of the experiment, 2 eggs per treatment were gathered (total 8), weighed then the yolks were separated for lipid extraction and calculation of total fat composition as per Folch *et al.* (1957) and Christie (1993). The yolks (100 mg) were homogenized for ~5 minutes with 10 ml chloroform to methanol (2:1; v/v), filtered (No. 41 filter paper) and rinsed (2 x or until dissolved) with chloroform to methanol (2:1; v/v). To this, 5 ml of distilled water and 4 ml of 0.88% NaCl were added. The mixture was kept at room temperature until separated into 2 layers. The top layer was suctioned off and the remaining lipid solution in the beaker evaporated, using nitrogen under 40°C. The residue in the beaker contained the extracted fat. Soybean oil, beef tallow and extracted fat from sampling day 32 and 35 and 5 fresh eggs per hen (taken between day 36 and 40) underwent fatty acid composition analysis at the ALS Laboratory Co. Ltd., Thailand, with GC analysis using the AOAC method (AOAC, 2012).

Statistical analyses: The data on productive performance and fatty acid composition of the yolks were analyzed using an ANOVA for a completely randomized design (CRD) using SAS software (Steel and Torrie, 1980; SAS Institute, 2003). Significant differences among means were compared using the Fisher's least significant difference (LSD) procedure.

RESULTS AND DISCUSSION

Feed intake of soybean oil treatment trended downward (as low as 95.32 g/h/d) when compared with beef tallow treatment (105.55 g/h/d), although a significant difference was not found ($p>0.05$). According to Pruempornchai (2009), the high metabolizable energy from SBO depresses feed consumption by chickens. Body weight at day 42 of hens fed the TAL diet was slightly greater than those fed the SBO diet (1,778 vs. 1,619 g/h, respectively). Total egg production between day 14 and 42 followed the daily feed intake: 23 eggs/period for the SBO treatment vs. 26 for the TAL treatment (Table 3). SBO contains mainly UFAs (83.90%) comprising: 52.70% linoleic acid (18:2n-6), 24.50% oleic acid (18:1n-9) and 6.27% α-linolenic acid (18:3n-3) (Table 2). Grobas *et al.* (2001) reported similar results, as soybean oil is rich in linloleic acid (52.90% of 18:2n-6). It was also found to be rich in omega-6 fatty acids (~52%). The TAL was found to comprise high proportions of SFAs (75.70%), mainly 36.80% stearic acid (18:0) and 29.30% palmitic acid (16:0) and had less UFAs (19.0%) than the SBO.

Table 1: Composition and nutrient content of experimental diets (kg/100 kg)

Ingredient	SBO diet*	TAL diet**
Cassava starch	25.00	25.00
Soybean meal (44%)	41.00	41.00
Rice hulls	10.80	10.80
Soybean oil	10.00	-
Beef tallow	-	10.00
DL-methionine[1]	0.40	0.40
Di-calcium phosphate (P18)[2]	4.00	4.00
$CaCO_3$	7.50	7.50
Salt	0.30	0.30
Premix (vitamins, minerals)[3]	1.00	1.00
Total	100.00	100.00
Calculated analysis (%)		
ME poult, kcal/kg	2750	2678
Crude protein	18.32	18.32
Crude fat	11.56	11.56
Ash	6.54	6.54
Calcium	3.85	3.85
Total phosphorus	0.92	0.92
Available phosphorus	0.61	0.61
Lysine	1.07	1.07
Methionine	0.64	0.64
Methionine+Cystine	0.90	0.90

*SBO diet is 10% soybean oil contained in diet.
**TAL diet is 10% beef tallow contained in diet.
[1]DL-Methionine is 99% methionine
[2]Di-calcium phosphate is Ca 24 and P18%
[3]Premix is produced by Inteqcgroup Co., Ltd. Thailand and comprises vitamin A 4,800,000 IU, vitamin D3 800,000 IU, vitamin E 3,200 IU, vitamin K_3 0.80 g, vitamin B_1 0.32 g, vitamin B_2 1.60 g, pantothenic acid 2.00 g, niacin 6.00 g, folic acid 0.20 g, vitamin B_6 0.40 g, choline 48.00 g, biotin 0.006 g, vitamin B_{12} 0.002 g, copper 2.40 g, ferrous 16 g, Zinc 20.00 g, selenium 0.04 g, manganese 24.00 g, iodine 0.32 g, cobalt 0.08 g and a carrier added to 1 kg

The calculation of daily intake of UFAs and their accumulation in the yolks of laying hens is shown in Table 4. The total of daily fatty acids intake for egg production were not significantly different ($p>0.05$) between the SBO diet and the TAL diet (13,040.74 and 12,518.90 mg/h/d, respectively). The accumulation of total fatty acids (TFAs) in the yolk was 4,039.24 (SBO treatment) vs. 4,091.19 (TAL treatment) mg/yolk mass, which is between 30.90-32.70% of TFA intake. Respective saturated fatty acid (SFA) accumulation in egg yolks was not statistically different ($p>0.05$) (i.e.,

Table 2: Fatty acid composition in fat sources and egg yolks (% fat)

	Fat source[1]		Egg yolk from			
Item	SBO	TAL	SBO diet	TAL diet	p-value	SEM
C14:0 Myristic acid	0.08	4.39	0.20[b]	1.17[a]	<0.0001	0.023
C14:1 Myristoleic acid	0.00	0.15	0.00[b]	0.20[a]	<0.0001	0.006
C16:0 Palmitic acid	10.80	29.30	19.52[b]	24.87[a]	0.0009	0.433
C16:1 Palmitoleic acid	0.09	0.83	0.77[b]	3.17[a]	<0.0001	0.426
C17:0 Heptadecanoic acid	0.10	2.38	0.28[b]	1.04[a]	<0.0001	0.017
C18:0 Stearic acid	4.07	36.80	7.66[a]	7.87[a]	0.4258	0.173
C18:1n9c Oleic acid	24.50	17.00	30.38[b]	47.03[a]	0.0003	0.986
C18:2n6c Linoleic acid	52.70	0.69	32.53[a]	7.50[b]	<0.0001	0.451
C18:3n3 8-Linolenic acid	6.27	0.20	2.06[a]	0.34[b]	<0.0001	0.036
C20:4n6 Arachidonic acid	0.00	0.03	1.96[a]	1.34[b]	0.0200	0.118
C22:6n3 Docosahexaenoic acid	0.00	0.01	1.39[a]	0.89[b]	0.0020	0.049
Saturated fatty acid	16.10	75.70	27.93[b]	36.27[a]	0.0002	0.432
Unsaturated fatty acid	83.90	19.00	70.43[a]	61.33[b]	0.0061	1.214
Mono-unsaturated fatty acid	24.90	18.00	31.35[b]	50.77[a]	0.0002	1.023
Poly-unsaturated fatty acid	59.00	1.02	39.08[a]	10.53[b]	<0.0001	0.323
Omega-3 fatty acids	6.27	0.22	3.52[a]	1.24[b]	<0.0001	0.029
Omega-6 fatty acids	52.70	0.80	35.55[a]	9.32[b]	<0.0001	0.340
Omega-9 fatty acids	24.80	17.10	30.60[b]	47.42[a]	0.0003	0.986

[1]Fat source: SBO = soybean oil and TAL = beef tallow. [a,b]Means within rows with no common superscript differ significantly (p<0.05)
SEM: Standard error of means

Table 3: Production performance of laying hens between day 14 and 42: the experimental period

Dietary fat	SBO diet	TAL diet	p-value	SEM
Body weight, g/h				
Initial	1764	1649	0.5351	109.501
Final	1619	1778	0.3470	91.916
Daily feed intake, g/h/d	95.32	105.55	0.4705	8.198
Average egg weight, g/egg	64.20	61.83	0.5986	2.721
Feed intake for egg production, g/egg	112.83	113.52	0.9753	14.038
Egg mass, g/d	54.88	57.29	0.4842	2.005
Total egg, eggs/hen/28 d	23	26	0.1679	1.000

SEM: Standard error of means
The effect of period was not significant (p>0.05)

1,141.84-1,509.42 mg/yolk mass); even though SFA intake was 2,099.56 mg/h/d in the SBO diet and 8,878.16 mg/h/d in the TAL diet. Moreover, the main residuum of SFA intake [especially of stearic acid (18:0)] was SFA, which can be desaturated to form oleic acid (18:1n-9) or omega-9 fatty acid. Whereas the omega-3 and omega-6 fatty acids were found less in the yolks of hens fed TAL. The stearic acid (18:0) contains in yolk between 310.62-326.29 mg/yolk mass although obtaining from the diets were increased from 530.76 mg/h/d (SBO diet) to 4,249.49 mg/h/d (TAL diet). The oleic acid (18:1n-9) from the diet were increasingly accumulated in the yolk when the hens were fed TAL. The UFAs accumulation in yolk of the hen fed SBO diet daily intake had trended to higher than the yolk of the hen fed TAL diet variable to the difference of UFAs intake (2,891.81 mg/yolk mass of SBO treatment vs. 2,554.64 mg/yolk mass of TAL treatment), although the significant difference did not found (p>0.05). The addition of SBO in diet was highly contained the PUFAs accumulation in yolk especially the omega-3, -6 PUFAs. The PUFAs accumulations were contained 1,604.22 mg/yolk mass of SBO treatment from 7,694.04 mg/h/d intake and 437.27 mg/yolk mass of TAL treatment from 1,159.28 mg/h/d intake. The linoleic acid (18:2n-6) in yolk were susceptible to linoleic acid (18:2n-6) consumption from the diets (6,872.47 mg/h/d of SBO diet and 1,010.40 mg/h/d of TAL diet). They were accumulated in yolk as 1,337.28 mg/yolk mass of SBO treatment and 312.39 mg/yolk mass of TAL treatment, respectively. The importance role of linoleic acid (18:2n-6) intake may be the precursor in hormones synthesis, immune synthesis function and increased accumulation of omega-3, -6 PUFAs. Linoleic acid (18:2n-6) in the SBO diet of the current study was sufficiency for layer production and *de novo* biosynthesis to form α-linolenic acid (18:3n-3), arachidonic acid (20:4n-6) and docohexaenoic acid (22:6n-3) in yolk as 84.73, 79.25 and 56.28 mg/yolk mass, respectively.

Fatty acid composition in egg yolks as a percentage of total fat (Table 2) showed that the egg yolks of hens fed the SBO diet was mainly (32.53%) linoleic acid (18:2n-6) the main source of omega-6 PUFAs (n-6 PUFAs)-which is significantly different from the yolk of hens fed the TAL diet (p<0.05). Furthermore, the yolks of hens fed SBO trended to have more alpha-linolenic acid (18:3n-3), arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) than the yolks of hens fed the TAL diet (i.e., 2.06% of 18:3n-3, 1.96% of 20:4n-6 and 1.39% of 22:6n-3, respectively). The UFAs and n-6 PUFAs in the yolks of hens fed SBO were significant different (p<0.05) than those from hens fed the TAL treatment (i.e., 70.43% UFAs and 35.55% n-6 PUFAs, respectively). The linoleic acid (18:2n-6) from SBO may be preferentially deposited in the yolk (Sim *et al*., 1973). Increased accumulation of n-6 and n-3 PUFAs may occur because linoleic acid (18:2n-6) is important to endogenously synthesized PUFA products, especially arachidonic acid (20:4n-6) (Oliveira *et al*., 2010). Linoleic acid (18:2n-6) can be metabolized by desaturation and elongation to form

Table 4: Daily fatty acid (FA) intake for egg production and FA accumulation in yolk

	------ Daily intake (mg/h/d) ------		-- FA in yolk[1] (mg/yolk mass) --		------ Statistic analysis ------	
Treatment	SBO diet	TAL diet	SBO diet	TAL diet	Daily intake	FA in yolk
Feed intake, g/egg	112.83[a]	113.52[a]	-	-	NS	-
Yolk mass, g/h/d	-	-	13.56[a]	14.16[a]	-	NS
Total fat, g/100 g yolk	-	-	32.82[a]	31.06[b]	-	*
C14:0 Myristic acid	10.43[b]	499.76[a]	8.07[b]	48.97[a]	**	**
C14:1 Myristoleic acid	0.00[b]	17.03[a]	0.11[b]	8.37[a]	**	**
C16:0 Palmitic acid	1408.40[b]	3517.13[a]	800.34[b]	1035.82[a]	*	*
C16:1 Palmitoleic acid	11.74[b]	95.81[a]	31.95[b]	132.47[a]	**	**
C17:0 Heptadecanoic acid	13.04[b]	271.94[a]	11.29[b]	42.97[a]	**	**
C18:0 Stearic acid	530.76[b]	4249.49[a]	310.62[a]	326.29[a]	**	NS
C18:1n9c Oleic acid	3194.98[a]	2363.14	1247.40[b]	1960.08[a]	NS	**
C18:2n6c Linoleic acid	6872.47[a]	1010.40[b]	1337.28[a]	312.39[b]	*	**
C18:3n3 8-Linolenic acid	817.65[a]	133.60[b]	84.73[a]	14.29[b]	*	**
C20:4n6 Arachidonic acid	0.00[b]	3.41[a]	79.25[a]	54.80[b]	**	**
C22:6n3 Docosahexaenoic acid	0.00[b]	1.14[a]	56.28[a]	36.84[b]	**	**
Total of fatty acids	13040.74[a]	12518.90	4039.24[a]	4091.19[a]	NS	NS
Saturated fatty acid	2099.56[b]	8878.16[a]	1141.84[a]	1509.42[a]	**	NS
Unsaturated fatty acid	10941.18[a]	3640.75[b]	2891.81[a]	2554.64[a]	*	NS
Mono-unsaturated fatty acid	3247.14[a]	2483.74	1287.42[a]	2115.88[b]	NS	**
Poly-unsaturated fatty acid	7694.04[a]	1159.28[b]	1604.22[a]	437.27[b]	*	**
Omega-3 fatty acids	817.65[a]	135.87[b]	143.69[a]	51.21[b]	*	**
Omega-6 fatty acids	6872.47[a]	1022.88[b]	1459.96[a]	386.94[b]	*	**
Omega-9 fatty acids	3234.10[a]	2379.80[a]	1256.36[b]	1976.31[a]	NS	**

[1]Fatty acid accumulation in egg yolk. [NS]Not significant. *Means within rows with no common superscript differ significantly ($p<0.05$)
**Means within rows with no common superscript differ highly significantly ($p<0.01$)

longer 20-carbon chain (arachidonic acid; 20:4n-6), a precursor of eicosanoids related to immunity and the inflammatory system. The SFAs were highly present in the egg yolk of hens fed the TAL diet (36.27%) than the SBO treatment (27.33%).

The yolks of hens fed TAL had mainly oleic acid (47.03% of 18:1n-9) and palmitic acid (24.87% of 16:0), although the hens received large amounts of stearic acid (18:0) from the TAL diet. The stearic acid (18:0), SFAs from the TAL diet, can be rapidly converted into oleic acid (18:1n-9) or desaturated ($\Delta 9$-desaturase) by enzymes facilitating induction of one double bond to form a monounsaturated fatty acid (MUFA) (n-9 PUFAs source) (Cherian, 2013; Leonard *et al*., 2004) in the liver microsomal desaturase system, which is accumulated in the yolk (Cherian, 2013; Murray *et al*., 2003; Pandey *et al*., 1998).

Conclusion: Dietary SBO trended to depress feed intake; ostensibly because SBO is mainly composed of UFAs, which has a higher metabolizable energy than TAL. Egg laying performance similarly reflected the fatty acid composition of the diets and SFA and UFA accumulation in yolks varied with the fatty acid profile of the diet. A diet containing 10% TAL was high in stearic acid (18:0), which is a source saturated fatty acids (SFAs). Oleic acid (18:1n-9) in the diet accumulated in the yolk when the hens were fed TAL. SFA accumulation in egg yolks was not significantly different although SFA intake was. A diet of 10% SBO was high in linoleic acid (18:2n-6) and resulted in enrichment of omega-6 fatty acids in the yolk. The latter is important to endogenous synthesis of PUFA products: n-6 PUFAs are converted to n-3 PUFAs and accumulate in the yolk. The addition of TAL to the diet strongly affects oleic acid (18:1n:9) accumulation in the yolk. Similarly, the addition of SBO to the diet strongly promoted PUFA accumulation in the yolk, especially omega-6 PUFAs.

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