

Effect of feeding hens trout by-product meal or tuna fish oil on production parameters and yolk fatty acid profile[□]

Efecto de la alimentación de gallinas con ensilaje de vísceras de trucha o aceite de atún en parámetros productivos y perfil de ácidos grasos en yema de huevo

Efeito da alimentação de galinhas com silagem de vísceras de truta ou óleo de atum nos parâmetros produtivos e o perfil de ácidos graxos na gema de ovo

Gonzalo J Díaz^{1*}, MV, PhD; Amparo Cortés¹, MV; Sandra M Cepeda¹

¹Laboratorio de Toxicología y Nutrición Aviar, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, D.C., Colombia.

(Recibido: 18 noviembre, 2010; aceptado: 23 agosto, 2011)

Summary

Objective: a study was conducted to evaluate two sources of omega-3 (n-3) fatty acid enrichment, namely a trout by-product meal (TBPM) and tuna fish oil (TFO), as potential sources for egg yolk n-3 enrichment. **Methods:** Seventy-27 week old commercial layers were assigned to each of seven dietary treatments, as follows. Group 1: control; group 2: 5% TBPM; group 3: 10% TBPM; group 4: 15% TBPM; group 5: 20% TBPM; group 6: 2% TFO; and group 7: 3% TFO. The experimental diets were fed for 28 days. **Results:** no effect on production parameters was caused by the experimental diets. All diets containing TBPM or TFO significantly ($p < 0.05$) increased n-3 egg yolk fatty acid content. Dietary levels of 5-20% TBPM increased egg yolk n-3 content between 1.97 and 3.54 times compared with control eggs. TFO levels of 2% and 3% increased the n-3 egg yolk content 3.37 and 4.13 times, respectively, compared with control eggs. The n-6/n-3 ratio in egg yolk lipids was significantly ($p < 0.05$) decreased by the experimental diets. The n-6/n-3 ratio in control eggs was 16.79 compared with ratios ranging from 4.34 to 8.11 in enriched eggs. **Conclusions:** the results showed that both TBPM and TFO are good sources of n-3 fatty acid enrichment in eggs. Further studies are required in order to determine the effects of TBPM and TFO inclusion on the organoleptic quality of eggs.

Key words: hens, n-3 fatty acids, omega-3 eggs, trout by-product meal, tuna fish oil.

□ To cite this article: Díaz GJ, Cortés A, Cepeda SM. Effect of feeding hens trout by-product meal or tuna fish oil on production parameters and yolk fatty acid profile. Rev Colomb Cienc Pecu 2011; 24:609-616

* Corresponding author: Gonzalo J Díaz. Laboratorio de Toxicología, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia. E-mail: gjdiazg@unal.edu.co

Resumen

Objetivo: se realizó un estudio para evaluar dos potenciales fuentes de enriquecimiento de ácidos grasos omega-3 (n-3) en yema de huevo: ensilaje de vísceras de trucha (TBPM) y aceite de atún (TFO). **Métodos:** un total de 70 gallinas de postura de 27 semanas se asignaron a siete tratamientos dietarios, así: grupo 1: control; grupo 2: 5% TBPM; grupo 3: 10% TBPM; grupo 4: 15% TBPM; grupo 5: 20% TBPM; grupo 6: 2% TFO y grupo 7: 3% TFO. Las dietas experimentales se suministraron durante 28 días. **Resultados:** no se encontraron efectos sobre los parámetros productivos a causa de las dietas experimentales, pero todas las dietas suplementadas con TBPM o TFO causaron un aumento significativo ($p < 0.05$) en el contenido de n-3 de la yema. La suplementación de 5-20% de TBPM causó un incremento de n-3 de 1.97 a 3.54 veces, comparado con el contenido de n-3 de los huevos control. La suplementación de 2% y 3% de TFO incrementó en contenido de n-3 en 3.37 y 4.13 veces, respectivamente. La relación n-6/n-3 en los lípidos de la yema aumentó significativamente en las dietas experimentales siendo esta relación de 16.79 en los huevos control y de 4.34-8.11 en los huevos de gallinas suplementadas con las fuentes de n-3. **Conclusiones:** los resultados del presente estudio demuestran que tanto el TBPM como el TFO constituyen fuentes apropiadas de enriquecimiento de huevos con ácidos grasos n-3. Se requieren más estudios para determinar los efectos de estas materias primas en la calidad organoléptica del huevo.

Palabras clave: aceite de atún, ácidos grasos n-3, ensilaje de vísceras de trucha, gallinas de postura.

Resumo

Objetivo: Foi realizado um estudo para avaliar duas fontes potenciais de enriquecimento de ômega-3 (n-3) na gema de ovo: silagem de vísceras de truta (TBPM) e óleo de atum (TFO). **Métodos:** Um total de 70 galinhas poedeiras de 27 semanas, foram atribuídas a sete tratamentos dietéticos: grupo 1: controle, grupo 2: 5% TBPM, grupo 3: 10% TBPM, grupo 4: 15% TBPM, grupo 5: 20% TBPM, grupo 6: 2% TFO e grupo 7: TFO 3%. As dietas experimentais foram subministradas durante 28 dias. **Resultados:** Não foram encontradas diferenças nos parâmetros de produção por causa das dietas experimentais, mas todas as dietas suplementadas com TFO ou TBPM causaram um aumento significativo ($p < 0.05$) no conteúdo de n-3 da gema. A suplementação de 5-20% de TBPM causou um aumento de n-3 de 1.97 a 3.54 vezes, em comparação com o conteúdo de n-3 do controle. A suplementação de 2% e 3% de TFO aumentou o conteúdo de n-3 em 3.37 e 4.13 vezes, respectivamente. A relação n-6/n-3 nos lípidos da gema aumentou significativamente nas dietas experimentais, sendo esta relação de 16.79 nos ovos do controle e de 4.43 a 8.11 em ovos de galinha suplementados com fontes de n-3. **Conclusões:** Os resultados deste estudo demonstram que o TFO e o TBPM são fontes apropriadas de enriquecimento de ovos com n-3 ácidos graxos. Mais estudos são necessários para determinar os efeitos dessas matérias primas na qualidade organoléptica do ovo.

Palavras chave: ácidos graxos, avicultura, óleo de atum.

Introduction

The omega-3 (n-3) are a group of fatty acids with important implications for human health. Recommendations about the intake of dietary fat point out the importance of consuming higher levels of n-3 fatty acids (Leskanich and Noble, 1997; Kris-Etherton *et al.*, 2003). Current Western diets are not only deficient in n-3 fatty acids, but also have excessive amounts of omega-6 (n-6) fatty acids compared with the diet on which the human race evolved (Simopoulos, 2002).

The large amounts of dietary n-6 fatty acids and the high n-6/n-3 ratio commonly found in Western diets (10:1 to 25:1) promote the pathogenesis of

cardiovascular, inflammatory and autoimmune diseases, while increased levels of n-3 fatty acids (and a lower n-6/n-3 ratio) has protective effects (Simopoulos, 2002). One way of increasing the intake of n-3 fatty acids is through the intake of natural sources rich in these fatty acids such as oily fish, and flax seed. Another possibility is through the consumption of functional foods enriched in n-3 fatty acids such as enriched eggs (Lewis *et al.*, 2000).

Several studies have shown that it is possible to increase the amount of n-3 fatty acids (and decrease

the n-6/n-3 ratio) in the egg yolk by feeding hens diets enriched with sources of n-3 fatty acids such as menhaden oil, tuna fishmeal, linseed oil, and others (Leskanich and Noble, 1997; Baucells *et al.*, 2000; Gonzalez-Esquerria and Leeson, 2000; Lewis *et al.*, 2000; Howe *et al.*, 2002). These sources of n-3 fatty acids, however, are not readily available in such countries as Colombia. The aim the present study was to evaluate two sources of n-3 fatty acids available in the Colombian market, namely trout by-product meal (TBPM) and tuna fish oil (TFO), as potential sources for the enrichment of eggs with n-3 fatty acids.

Materials and methods

27-week old commercial layers of the Hy-Line Brown strain were assigned to each of seven dietary treatments that consisted of diets containing increasing levels of a source of n-3 fatty acids, as follows. Group 1: control; group 2: 5% TBPM; group 3: 10% TBPM; group 4: 15% TBPM; group 5: 20% TBPM; group 6: 2% TFO; and group 7: 3% TFO. Since there are several studies reporting the use of FO as a source of enrichment with n-3 fatty acids (Baucells *et al.*, 2000) it was decided to

use this ingredient in only two groups and to test four levels of TBPM, a novel source of n-3 fatty acids.

For the preparation of the TBPM a total of 200 kg of trout inner guts was mixed with 15% molasses, 0.2% *Lactobacillus bulgaricus*, 0.1% potassium sorbate (as antifungal agent) and 50 ml/kg of crushed onion as antioxidant, as described before (Betancourt *et al.*, 2005). The mix was kept in tightly closed 50 kg plastic drums and mixed three times daily in order to homogenize the fermentation process. The fermentation lasted for 30 days until a complete liquefaction of the product was obtained. The experimental diets were formulated to contain or exceed the concentrations of required nutrients for laying hens and were isocaloric and isoproteic (Leeson and Summers, 2001). The ingredient composition and calculated analysis of the experimental diets is summarized in table 1. Fatty acid compositions of TBPM, TFO, and each experimental diet are presented in table 2. Proximate analysis of the TBPM revealed the following composition (g/kg): Dry matter, 384.0; ether extract, 353.0; crude protein, 204.0; ash, 66.0; crude fiber, 10.0; non-nitrogen extract (calculated), 186.0.

Table 1. Experimental diets composition (g/kg).

Ingredients	Diets ¹						
	1	2	3	4	5	6	7
Corn	391.0	458.1	435.9	431.4	401.5	527.8	484.2
Soybean meal	236.6	260.0	260.0	260.0	231.0	172.2	166.5
Full fat soybean	...	33.6	25.8	20.5	50.0	40.0	40.0
Wheat middlings	200.0	95.9	106.9	102.6	107.5	76.0	115.4
Rice meal	60.0	60.0
Palm oil	60.0	30.0	30.0	25.0
Dicalcium phosphate	14.7	7.1	6.4	6.4	7.1	8.9	8.4
Limestone	86.6	88.8	89.2	89.4	93.6	87.4	87.6
Salt	2.7	3.0	3.0	3.0	3.0	2.9	2.9
Vitamin-mineral premix ²	7.5	7.5	7.5	7.5	7.5	7.5	7.5
DL-methionine	0.9	2.2	2.2	2.1	2.0	1.6	1.6
Trout by-product meal	...	50.0	100.0	150.0	200.0
Tuna fish oil	20.0	30.0

¹Calculated analysis: ME, 2800 kcal/kg; CP, 18%; fat 8.44%; calcium, 3.7%; available phosphorus, 0.42%; methionine, 0.37%; and lysine, 0.97%.

²Provides per kilogram of diet; vitamin A, 8,000 IU (retinyl palmitate); cholecalciferol, 40 µg; vitamin E, 150 mg; riboflavin, 9.0 mg; biotin, 0.25 mg; pantothenic acid, 11.0 mg; vitamin B12, 13 mg; niacin, 26 mg; choline, 900 mg; vitamin K, 1.5 mg; folic acid, 1.5 mg; ethoxyquin, 125 mg; manganese, 55 mg; zinc, 50 mg; copper, 5 mg; and selenium, 0.1 mg.

Table 2. Fatty acid composition of the trout-by product meal (TBPM), tuna fish oil (TFO), and experimental diets (g/100 g fat).

Fatty acid	n-3 source		Experimental diets						
	TBPM	TFO	Control	5% TBPM	10% TBPM	15% TBPM	20% TBPM	2% TFO	3% TFO
C14:0	5.65	3.94	0.17	1.44	2.04	2.23	2.83	1.15	1.54
C15:0		1.09							
C16:0	20.7	24.0	13.8	26.1	25.6	24.8	24.0	16.5	17.4
C16:1	9.48	5.81	0.21	1.81	3.02	3.51	4.77	1.47	1.96
C18:0	5.58	7.79	2.04	4.01	4.26	4.34	4.64	3.50	3.68
C18:1 n-9	28.0	20.8	35.8	33.6	32.7	31.7	31.3	25.5	25.0
C18:2 n-6	12.2	1.70	44.3	28.3	25.7	25.7	23.1	37.7	33.6
C18:3 n-3	1.18	0.59	2.46	1.45	1.39	1.39	1.45	2.56	2.36
C18:3 n-6		0.61							
C20:0			0.36	0.27	0.30	0.32	0.28	0.39	0.35
C20:1 n-9	1.01	1.49	0.28	0.36	0.45	0.49	0.65	0.60	0.73
C20:2	0.52								
C20:3 n-6	1.22			0.20	0.37	0.42	0.58	0.51	0.62
C20:4 n-6	0.62	1.89							
C20:5 n-3	4.18	6.19	0.25	0.69	1.08	1.34	1.84	1.86	2.38
C22:0			0.22						
C23:0				0.34	0.41	0.56	0.43	0.36	0.42
C22:5 n-3	1.63	1.43		0.26	0.55	0.57	0.72	0.50	0.60
C22:6 n-3	8.14	22.7	0.05	1.22	2.10	2.58	3.39	7.46	9.33
SFA ¹	31.9	36.8	16.7	32.1	32.6	32.3	32.2	21.9	23.4
MUFA ¹	38.4	28.1	36.3	35.8	36.2	35.7	36.7	27.6	27.7
PUFA ¹	29.7	36.6	47.0	32.4	30.9	32.4	31.4	50.3	48.8
n-6	14.0	4.20	44.3	28.5	26.1	26.1	23.7	38.2	34.2
n-3	15.1	30.9	2.77	3.63	5.13	5.88	7.39	12.4	14.7
n-6/n-3	0.923	0.136	15.99	7.84	5.08	4.44	3.20	3.09	2.34

¹SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

The birds were allocated at random to one of the seven dietary treatments, with 10 individual birds per treatment. Hens were kept in individual wire cages with artificial light provided 14 h per day. Feed and water were provided *ad libitum* during 28 days.

Body weight was recorded at the beginning (27 weeks of age) and at weekly intervals until the end of the experiment (31 weeks of age). Egg production and egg weight was recorded daily and individually. Individual feed intake was measured at weekly intervals. At days 7, 14, 21 and 28 of the experiment, eight yolks from each dietary treatment were pooled into two 4-yolk replicates and analyzed to determine the fatty acid composition.

The samples were processed according to the methods described by Folch *et al.* (1957) for extraction of lipids and by Betancourt *et al.* (2005) for the determination of the fatty acid profile. In summary, to one gram of yolk 40 ml of chloroform:methanol 2:1 were added, and the

mix was homogenized and filtered to obtain 20 ml of extract to which 5 ml of water were added. The mix was homogenized again and centrifuged at 3.000 rpm for 10 minutes. The supernatant was removed and eliminated and 1 ml of the lower layer was transferred to a previously weighed test tube. The solvent was then evaporated under a gently stream of nitrogen and the dry film remaining was redissolved with methyl chloride to a concentration of 25 mg/ml. From this solution 20 µl were taken and added with 160 µl methylene chloride and 20 µl of Meth-Prep II transesterification reagent (Alltech Associates, Inc., Deefield, IL, USA). The mix was left at ambient temperature for 30 minutes and then 1 µl of the solution was injected into the gas chromatograph for the determination of the fatty acid methyl esters (FAMES).

The FAMES were separated using a 30 m x 0.32 mm inside diameter x 0.25 µm film thickness Omegawax™ 320 fused silica capillary GC column (Supelco, Inc., Bellefonte, PA, USA) and analyzed

with a Shimadzu GC-14A Gas Chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector. The separation was obtained with a temperature ramp (initial temperature 80°C, 10 °C/min to 190 °C, 20 min at 190 °C, 2 °C/min to 220 °C and 10 min at 220 °C) using helium as carrier gas. The injection was made in split mode with a split ratio of 1:50. Retention times were compared with known standards (Supelco, Inc., Bellefonte, PA, USA).

Statistical Analysis

Data (treatment means) were subjected to one-way ANOVA by using the General Linear Model procedure of SAS® (SAS Institute, 2008). When the F-test for treatments was significant at $p < 0.05$ in the ANOVA analysis, means were compared for significant differences using Duncan's multiple range test. Regression analysis was also conducted to determine the relationship between dietary and

egg yolk n-3, n-6 and n-6/n-3 fatty acids in order to obtain both the correlation coefficient and P value of the regression model.

Results

Table 3 summarizes the effect of the experimental diets on layer performance. In general, no effects on layer performance were caused by the experimental diets, although layers fed 20% TBPM had a significantly lower ($p < 0.05$) percent production during week 4 of the experiment compared with controls. However, the overall 28-day percent production of the hens fed 20% TBPM was not significantly different from that of the control group. No other significant differences in layer performance were observed during the experiment. No significant differences in body weight were recorded at any of the scheduled sampling times (data not shown).

Table 3. Effect of the experimental diets on layer performance.

Diet	Week														
	1			2			3			4			1-4		
	FI	EM	FC	FI	EM	FC	FI	EM	FC	FI	EM	FC	FI	EM	FC
Control	115.5	420.1	1.9	125.6	428.9	2.1	132.5	447.5	2.0	125.2	451.1	1.9	123.8	1747.7	1.9
5% TBPM	122.1	429.8	2.0	114.5	403.9	2.0	118.6	430.0	1.9	119.0	438.6	1.9	118.6	1702.3	1.9
10% TBPM	120.2	433.7	1.9	114.8	444.0	1.8	106.1	447.1	1.8	104.2	448.5	1.8	116.8	1773.3	1.8
15% TBPM	123.6	421.2	2.0	117.9	428.2	1.9	120.2	414.6	2.0	118.6	419.8	2.0	120.3	1683.8	2.0
20% TBPM	121.9	417.2	2.1	103.9	418.9	1.9	119.0	430.9	1.9	114.2	405.4	1.9	117.3	1672.5	1.9
2% TFO	118.9	429.2	1.9	110.4	418.2	1.8	118.7	422.5	1.9	119.5	426.2	1.9	116.9	1696.2	1.9
3% TFO	119.8	431.6	1.9	101.0	420.1	1.9	120.5	427.5	1.9	117.6	395.8	2.0	117.8	1675.1	1.9
Pooled SD	11.6	39.7	0.2	21.5	47.8	0.2	18.5	42.5	0.2	15.4	47.6	0.2	10.7	133.6	0.2
P	0.8	0.9	0.7	0.2	0.7	0.13	0.13	0.5	0.49	0.12	0.08	0.19	0.8	0.55	0.34

FI: feed intake (g/hen/day), EM: egg mass (g/hen/week) and FC: feed conversion, feed intake:egg mass (g:g)

TBPM = trout by-product meal, TFO = tuna fish oil.

Data are mean \pm pooled S.D. of 10 observations per treatment.

Within a row, means with no common superscript differ significantly.

Table 3 (cont'd). Effect of the experimental diets on layer performance.

Diet	Week									
	1		2		3		4		1-4	
	EP	EW	EP	EW	EP	EW	EP	EW	EP	EW
Control	95.7	60.0	97.1	63.1	100.0	63.9	100.0 ^a	64.4	98.2	62.9
5% TBPM	97.1	61.4	95.7	60.7	97.1	63.1	97.1 ^{ab}	64.3	96.8	62.4
10% TBPM	98.6	61.9	98.6	64.5	100.0	63.9	100.0 ^a	64.1	99.3	63.6
15% TBPM	94.1	60.2	100.0	61.2	95.7	61.8	98.6 ^a	60.8	97.9	61.0
20% TBPM	95.7	59.6	97.1	61.5	97.1	63.3	91.4 ^b	63.4	95.3	61.9
2% TFO	100.0	61.3	97.1	61.5	97.1	62.2	97.1 ^{ab}	62.6	97.8	61.9
3% TFO	98.6	60.9	95.7	62.8	97.1	62.9	96.8 ^{ab}	60.8	96.8	61.5
Pooled SD	6.0	5.7	5.7	6.7	6.4	4.1	5.15	5.1	3.1	4.3
P	0.65	0.95	0.63	0.88	0.7	0.88	0.01	0.48	0.15	0.88

EP: egg production (%) and EW: egg weight (g)

TBPM = trout by-product meal, TFO = tuna fish oil.

Data are mean \pm pooled S.D. of 10 observations per treatment.

Within a row, means with no common superscript differ significantly.

No significant differences in the fatty acid profile were observed at days 7, 14, 21 or 28 of the experiment (data not shown), and therefore it was decided to pool the data corresponding to the four sampling times (Table 4). All diets containing TBPM or TFO caused a significant increase in n-3 egg yolk fatty acid content. Levels of n-3 fatty acids were especially high in the groups fed 20% TBPM and 3% TFO (3.65%, and 4.25%, respectively)

which corresponded to 3.5 and 4.1 times the levels of the control group (1.03%). The n-6/n-3 ratio was significantly lower in all egg yolks from hens receiving the dietary sources of n-3 fatty acids. The reduction in the n-6/n-3 ratio was significant ($p < 0.05$) in all dietary treatments and corresponded to a n-6/n-3 ratio close to 17 in control eggs, compared with ratios ranging from 4.34 to 8.11 in n-3 enriched eggs.

Table 4. Effect of the experimental diets on yolk lipid fatty acids.

Fatty acid in yolk fat	Diets							P	SE
	Control	5% TBPM	10% TBPM	15% TBPM	20% TBPM	2% TFO	3% TFO		
C14:0	0.34 ^d	0.42 ^c	0.49 ^b	0.54 ^{ab}	0.58 ^a	0.41 ^c	0.43 ^c	0.000	0.016
C16:0	27.07 ^a	25.73 ^{cd}	26.04 ^{bc}	26.35 ^b	25.39 ^d	26.17 ^{bc}	26.52 ^b	0.000	0.113
C16:1	3.01 ^b	2.77 ^c	3.03 ^b	3.34 ^a	3.28 ^a	2.71 ^c	2.67 ^c	0.000	0.054
C18:0	7.36 ^a	6.52 ^{bc}	6.38 ^{cd}	6.18 ^d	6.42 ^{cd}	6.76 ^b	6.69 ^{bc}	0.000	0.078
C18:1 n-9	44.21 ^b	45.99 ^a	43.46 ^b	44.06 ^b	44.15 ^b	42.16 ^c	41.03 ^d	0.000	0.300
C18:2 n-6	15.67 ^c	15.46 ^c	16.58 ^b	15.41 ^c	15.50 ^c	17.47 ^a	17.60 ^a	0.000	0.184
C18:3 n-3	0.50 ^{bc}	0.46 ^c	0.54 ^b	0.50 ^{bc}	0.51 ^{bc}	0.73 ^a	0.73 ^a	0.000	0.022
C20:1 n-9	0.19 ^a	0.18 ^a	0.22 ^a	0.23 ^a	0.23 ^a	0.22 ^a	0.22 ^a	0.497	0.007
C20:3 n-6	1.13 ^a	0.92 ^b	0.92 ^b	0.76 ^{bcd}	0.81 ^{bc}	0.64 ^{cd}	0.59 ^d	0.000	0.039
C20:5 n-3	0.00 ^d	0.00 ^d	0.09 ^c	0.12 ^{bc}	0.24 ^a	0.16 ^{bc}	0.18 ^{ab}	0.000	0.018
C22:5 n-3	0.02 ^b	0.00 ^b	0.17 ^a	0.24 ^a	0.24 ^a	0.16 ^a	0.19 ^a	0.000	0.020
C22:6 n-3	0.48 ^d	1.50 ^c	2.12 ^b	2.28 ^b	2.16 ^b	2.43 ^b	3.15 ^a	0.000	0.162
SFA ¹	34.78 ^a	32.66 ^{de}	32.90 ^{ode}	33.07 ^{cd}	32.39 ^e	33.34 ^{bc}	33.63 ^b	0.000	0.502
MUFA ¹	47.42 ^{bc}	48.94 ^a	46.70 ^c	47.63 ^b	47.66 ^b	45.07 ^d	43.92 ^e	0.000	0.320
PUFA ¹	17.81 ^e	18.41 ^e	20.40 ^c	19.30 ^d	19.96 ^{cd}	21.59 ^b	22.45 ^a	0.000	0.301
n-6	16.79 ^c	16.37 ^{cd}	17.49 ^b	16.16 ^d	16.31 ^{cd}	18.12 ^a	18.20 ^a	0.000	0.168
n-3	1.03 ^f	2.03 ^e	2.90 ^d	3.14 ^{cd}	3.65 ^b	3.47 ^{bc}	4.25 ^a	0.000	0.198
n-6/n-3	16.72 ^a	8.11 ^b	6.06 ^c	5.21 ^{cd}	4.51 ^d	5.28 ^{cd}	4.34 ^d	0.000	0.800

Values are means of 4 observations per treatment analyzed in duplicate for each sampling time.

^{a-f}Values followed by different letters within rows are significantly different ($P < 0.05$).

TBPM = trout by-product meal, TFO = tuna fish oil.

¹SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Data are mean \pm S.D. of four observations per treatment.

Figure 1 shows the regression analysis of the n-3, and n-6 fatty acid content, and the n-6/n-3 ratio in the diet vs. their corresponding values in egg yolk. A linear relationship was observed for the n-3

content ($r^2 = 0.689$, $P = 0.0208$), and for the n-6/n-3 ratio ($r^2 = 0.968$, $P = 0.0001$). However, the n-6 dietary content was not linearly related to the n-6 egg yolk content ($r^2 = 0.127$, $P = 0.4318$).

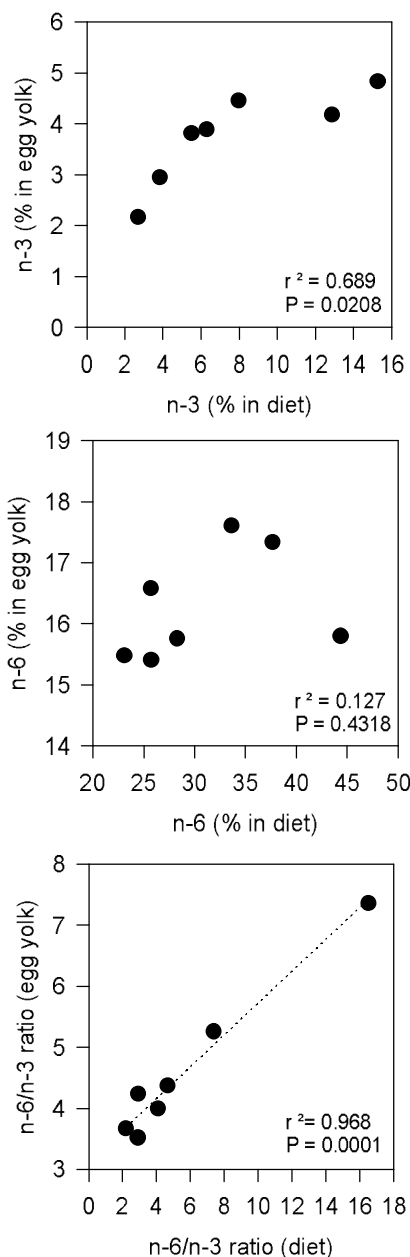


Figure 1. Regression curves for n-3 content, n-6 content, and n-6/n-3 ratio in the feed vs. the same variables in egg yolk.

Discussion

The effect of FO supplementation on performance parameters is in agreement with that observed in previous studies in which similar levels of fish oil (up to 4%) were included in the diet of layers (Hargis *et al.*, 1991; Van Elswyk *et al.*, 1992; Baucells *et al.*, 2000; Gonzalez-Esquerra and Leeson, 2000). Our results corroborate previous observations in which

no effect on layer performance are observed when diets enriched with n-3 fatty acids are given to the birds. Unfortunately, no previous reports on the use of TBPM as an ingredient for laying hen diets or as a source of n-3 fatty acids were found in the literature and therefore it is not possible to contrast our results with previous ones.

The levels of n-3 enrichment observed with the dietary supplementation of FO (and TBPM) are similar to those reported in previous studies. For example, layers fed 4% deodorized menhaden oil had omega-3 fatty acid content in egg yolk of 4.13% compared with 1.04% in the control group (Gonzalez-Esquerra and Leeson, 2000). In another study (Baucells *et al.*, 2000) layers fed a diet containing 4% FO laid eggs containing 5.06% omega-3 fatty acids compared with an omega-3 fatty acid content of 0.87% found in the eggs of hens fed a diet containing tallow instead of FO.

The decrease in the n-6/n-3 ratio observed in the present study in the eggs of birds supplemented with n-3 sources is consistent with the decrease reported in previous studies. For instance, Simopoulos (2000) reported an n-6/n-3 ratio of 19.9 in supermarket eggs versus a ratio of 6.6 in “fishmeal eggs”. Similarly, Baucells *et al.* (2000) reported that the n-6/n-3 ratio in egg yolk decreased from 15.58 in hens fed a diet containing tallow to 2.42 in hens fed a diet containing 4% FO. Interestingly, significantly higher levels of n-6 fatty acids were obtained in eggs from hens receiving 10% TBPM and 2% and 3% TFO; however, these levels were only between 6.2 and 8.4% greater than those of the control group, and therefore did not affect the n-6/n-3 ratio significantly.

In general, it is accepted that the amount of long chain polyunsaturates within the yolk is a reflection of the amount within the diet (Leskanich and Noble, 1997). In the present study, this fact was true for n-3 fatty acids, but not for n-6 fatty acids (Figure 1). We cannot provide an explanation for this observation but it might be possible that the reason lies on the essentiality of linoleic acid (18:2n6), the main n-6 fatty acid. The only essential fatty acid for chickens is linoleic acid (Watkins, 1995) and it is possible that this acid is being used as a precursor of other

compounds (for instance prostaglandins 1 and 2) instead of being transported entirely into the egg yolk. This fact would explain why the n-6 fatty acid content in the diet is not linearly related to the n-3 fatty acid content in egg yolk.

The fact that a linear relationship exist for the n-6/n-3 ratio in feed and yolk indicates that the n-6/n-3 ratio of the diet could be used as a good predictor of the n-6/n-3 ratio in yolk. However, dietary n-3 content is not as accurate as a predictor of the n-3 yolk content, and the dietary n-6 content is not linearly related to the egg yolk n-6 content as it was previously discussed.

The results of the present study indicate that TBPM, a product potentially available in the Colombian market, with an n-3 fatty acid content of 15.13% and an n-6/n-3 ratio of 0.923, is an adequate source for the enrichment of eggs with n-3 fatty acids. Supplementing diets with levels of 5-20% TBPM, results in the production of egg yolks containing between 1.97 and 3.54 times more n-3 fatty acids than those from non-supplemented diets.

References

- Baucells MD, Crespo N, Barroeta AC, Lopez-Ferrer S, Grashorn MA. Incorporation of different polyunsaturated fatty acids into eggs. *Poult Sci* 2000; 79:51-59.
- Betancourt L, Díaz GJ, Aguilar X, Ríos J. Efecto del ensilaje de vísceras de trucha (*Oncorhynchus mykiss*) sobre el comportamiento productivo y el contenido de ácidos omega-3 en hígado, muslos y pechuga, de pollos de engorde. *Livest Res Rural Develop* 2005; [17: Article #106, fecha de acceso: Abril 26 de 2011] URL: <http://www.lrrd.org/lrrd17/9/beta17106.htm>
- Folch J, Lees M, Stanley GSH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; 226:497-509.
- Gonzalez-Esquerra R, Leeson S. Effect of feeding hens regular and deodorized Menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. *Poult Sci* 2000; 79:1597-1602.
- Hargis PS, Van Elswyk ME, Hargis BM. Dietary modification of yolk lipids with menhaden oil. *Poult Sci* 1991; 70:874-883.
- Howe PR, Downing JA, Grenyer BF, Grigonis-Deane EM, Bryden WL. Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and eggs. *Lipids* 2002; 37:1067-1076.
- Kris-Etherton PM, Harris S, Appel LJ. Omega-3 fatty acids and cardiovascular disease: New recommendations from the American Heart Association. *Arterioscler Thromb Vasc Biol* 2003; 23:151-152.
- Leskanich CO, Noble RC. Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. *Worlds Poult Sci J* 1997; 53:155-183.
- Leeson S, Summers JD. *Scott's Nutrition of the Chicken*. 4th ed. Guelph (ON): University Books; 2001.
- Lewis NM, Seburg S, Flanagan NL. Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans. *Poult Sci* 2000; 79:971-974.
- SAS Institute Inc. *SAS/STAT® 9.2 User's Guide*. Cary (NC): SAS Institute Inc.; 2008.
- Simopoulos AP. Human requirement for n-3 polyunsaturated fatty acids. *Poult Sci* 2000; 79:961-970.
- Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 2002; 56:365-379.
- Van Elswyk ME, Sams AR, Hargis PS. Composition, Functionality, and sensory evaluation of eggs from hens fed dietary menhaden oil. *J Food Sci* 1992; 57:342-344, 349.
- Watkins BA. Biochemical and physiological aspects of polyunsaturates. *Poult Av Biol Rev* 1995; 6:1-18.