Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 767–773
Food Chemistry
www.elsevier.com/locate/foodchem

Enriching the egg yolk in $n-3$ fatty acids by feeding hens with diets containing horse fat produced in Uruguay

M.C. Cabrera [a,b,*], A. Saadoun [b,c], A. Grompone [d], T. Pagano [d], M. Salhi [b], R. Olivero [a], M. del Puerto [a]

[a] Laboratorio de Nutrición and Ciencia de los Alimentos, Facultad de Agronomía, Avda. Garzón 780, CP 12900 Montevideo, Uruguay
[b] Sección Fisiología and Nutrición, Facultad de Ciencias, calle Igua 4225 Montevideo, Uruguay
[c] Department Basico de Medicina, Unidad Asociada de la Facultad de Ciencias, Hospital de Clinicas Avda. Italia s/n Montevideo, Uruguay
[d] Laboratorio de Grasas y Aceites, Facultad de Química, Avda. General Flores Montevideo, Uruguay

Received 15 November 2004; received in revised form 5 July 2005; accepted 5 July 2005

Abstract

The present study was conducted to evaluate the ability of horse fat produced in Uruguay, compared with other lipid sources supplemented in the diet of laying hens, to modify the lipid composition and the $n-6/n-3$ fatty acid ratio of the produced eggs. For this purpose, 60 laying hens (*Gallus domesticus*) were fed for 30 days with five experimental diets (12 hens/diet) containing 3% sunflower oil (SO), rice oil (RO), beef tallow (BT), pressed-fat (PF), obtained by pressing fat from bovine viscera, and horse fat (HF) obtained from horse bone medulla. Throughout the trial, feeding rate, shell index, weight and total lipid contents of eggs, were not affected by the different diets. Feeding on the SO and RO diets significantly increased the amount of linoleic acid of the egg, although it was lower in the RO than in the SO eggs. Diet BT did not affect the saturated fatty acid content of the yolks. The diets with animal fats containing $18{:}3n-3$ (diets PF and HF), resulted in a significant increase in the $n-3$ fatty acid contents of the eggs, through an increase of linolenic ($18{:}3n-3$) and docosahexaenoic acid (DHA, $22{:}6n-3$) contents. Eggs from hens fed the HF diet showed increased linolenic acid (46 mg/yolk) and DHA (71 mg/yolk, 1.7% of total fatty acids) contents. These levels were obtained after two weeks of feeding. Moreover, the fatty acid profiles of eggs from treatment HF were not significantly affected by thermal treatment of the yolks. In conclusion, the fat from horse bone medulla, as produced in Uruguay, can be considered as a suitable lipid source for diets of laying hens, to modify the nutritional composition of the eggs in $n-3$ PUFA content, especially DHA, and consequently, the $n-6/n-3$ fatty acid ratio.

Keywords: Eggs; Horse fat; Fatty acids; $n-6/n-3$ ratio; DHA

1. Introduction

Dietary intake of $n-3$ polyunsaturated fatty acids (PUFA) is associated with reduced risk of coronary heart disease, one of the most important cause of death in Uruguay (Curto, Prats, & Ayestaran, 2004). Also, an adequate intake of $n-3$ PUFA seems to be associated with reduced risk of cancer. In this way, eicosapentaenoic acid (EPA, $20{:}5n-3$) and docosahexaenoic acid (DHA, $22{:}6n-3$) seem to be the most promising $n-3$ PUFA in regard to human health benefits. However, the observations are still controversial (Angerer, Kothny, Stork, & von Schacky, 2002; Horrocks & Yeo, 1999; Saadoun, Braida, Martinez, & Cabrera, 2003; Singer & Wirth, 2004; Stripp et al., 2003; Tapiero, Nguyen Ba, Couvreur, & Tew, 2002; Zock, 2001). As alfa-linolenic acid (ALNA, $18{:}3n-3$), a precursor of

* Corresponding author. Fax: +598 2 309 3004.
E-mail address: mcab@fagro.edu.uy (M.C. Cabrera).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.07.013

EPA and DHA, and linoleic acid (LA, 18:2$n-6$) compete for the same enzymatic system in the conversion to their biologically active molecules, a diet high in LA, as observed in most Western societies, reduces the possibility of converting the ALNA to EPA and DHA (Gurr & Harwood, 1991; Lands et al., 1992). Therefore, a dietary excess of linoleic acid could result in reduced tissue availabilities of EPA and DHA.

In the past, humans beings have consumed high levels of $n-3$ PUFA and a low level of $n-6$ PUFA and the ratio between the two groups of PUFA was near to 1:1. This ratio seems to be important to consolidate the beneficial effect of the $n-3$ PUFA (Simopoulos, 2002).

Unfortunately, at present, the ratio between $n-6$ and $n-3$ of consumed PUFA greater than 10:1, particularly in most western societies. Scientific reports support the conclusion that an elevated ratio of $n-6$ to $n-3$ PUFA is a major risk factor for thrombotic diseases, cancer and some chronic inflammatory diseases (Simopoulos, 2002). Also, an inadequate intake of $n-3$ PUFA, especially DHA, negatively influences brain growth and functional parameters in human infants (Anderson, 1994; Clandinin, 1999; Hoffman et al., 2004; Horrocks & Yeo, 1999; Innis, 1993).

The World Health Organization (WHO) is now recommending a ratio between 3:1 and 4:1 for $n-6$ to $n-3$ fatty acids in the diet and the best way to achieve this is by the identification of foods with an adequate $n-3$ and $n-6$ PUFA composition.

In Uruguay, soy and rice oils provide $n-3$ PUFA to consumers. However, these sources are high in $n-6$ PUFA and they may have some detrimental side effects. Fish or fish-oil consumption is probably the best way to improve the intake of $n-3$ PUFA in Uruguay. However, the consumption of fish is low among some population groups in this country who dislike this product. On the other hand, the consumption of eggs is high and grows rapidly because of their affordable cost, easy cooking for most people and availability all over the country.

Although the cholesterol content of eggs is high, research results indicate that most people can consume eggs without experiencing a significant increase in serum total or LDL cholesterol levels (Lewis, Schalch, & Scheideler, 2000). Also, breast milk $n-3$ PUFA content can be increased without altering plasma cholesterol and triacylglycerol when $n-3$ PUFA eggs are consumed by nursing women (Cherian & Sim, 1996). For this, enriching eggs with specific fatty acids can be considered as a good and alternative way of increasing the intake of $n-3$ PUFA in Uruguay.

Egg lipid contents can be modified by feeding hens specific diets (Leclercq, 1972; Stadelman & Pratt, 1989). The diet can include some ingredients rich in $n-3$ lipids, such as linseed, fish-oil and others (Farrell, 1998; Hargis, Van Elswyk, & Hargis, 1991; Jiang, Ahn, & Sim, 1991; Marshall, Kubena, Hinton, Hargis, & Van Elswyk, 1994; Maurice, 1994; Milinsk, Murakami, Gomes, Matsushita, & de Souza, 2003; Nash, Hamilton, & Hulan, 1995). Also, the diet can include purified and commercially available $n-3$ fatty acid mixtures (Cloughley, Noble, Speake, & Sparks, 1997; Oh, Lin, Ryue, & Bell, 1994; Oh, Ryue, Hsieh, & Bell, 1991). All these products and ingredients need to be imported, at high cost, in Uruguay, which consequently causes a rise in hen diet costs. For this reason we propose to use locally produced by-products and ingredients with $n-3$ fatty acid contents. The objective of this work was to evaluate horse fat, a cheap feedstuff produced in Uruguay, in comparison with other lipid sources, such as vegetable oil, beef tallow and pressed-fat (most often used as supplements in diets for laying hens in Uruguay) for its ability to modify the fatty acid composition and the $n-6/n-3$ fatty acid ratio of eggs. All these by-products are available in the animal food market in Uruguay.

2. Materials and methods

2.1. Lipids sources

The lipids sources, from a local market, were used without modification or further purification. The fat horse (diet HF) used in this work was produced in Uruguay and comes from the defatting of bone (bone medulla) before mineralization in order to obtain ash bone for inclusion in animal diets. Other lipids from horses were used in a different industrial process, but the costs did not permit their inclusion in animal diet. Beef tallow (diet BT) was obtained from the spare fat. The pressed-fat (diet PF) was obtained by pressing residual fat from viscera. Sunflower oil (SO) and rice oil (diet RO) were obtained from a local broker. Table 1 shows the fatty acid composition of the lipid sources used in this work.

2.2. Experimental procedure

For 30 days, 60 38 week-old Hy-Line laying hens (*Gallus domesticus*) from a commercial breeder were housed individually and fed with five experimental diets (12 hens/diet), containing 3% of each of the above-mentioned lipid sources. Ingredients and fatty acid composition of the experimental diets are shown in Tables 2 and 3. The protocol and use of the animals were approved by the Ethical Committee of the Faculty of Agronomical Sciences (University of R.O. Uruguay).

Before starting the trial, hens were fed with a corn-based diet (containing 3.48% dw lipids; initial diet). Hen live weight was recorded at the beginning and at the end of the feeding trial. A 16:8 h photoperiod and

Table 1
Fatty acid[a] contents of the oils and fats sources as a percentage of total lipids

Fatty acid	SO	RO	BT	PF	HF
14:0	1.2	nd	3.20	2.01	2.14
16:0	6.8	16.7	26.5	17.3	19.5
16:1	1.5	nd	2.46	3.06	3.93
18:0	4.1	1.33	22.6	14.2	9.17
18:1	23.9	40.5	33.7	27.5	28.5
18:2$n-6$	61.9	35.0	2.49	3.32	5.55
18:3$n-3$	0.6	1.43	nd	2.87	9.49
$\sum$SAFA	12.1	18.0	52.3	33.5	30.8
$\sum$MUFA	25.4	40.5	36.2	30.6	32.5
$\sum n-6$ PUFA	61.9	35.0	2.49	3.32	5.55
$\sum n-3$ PUFA	0.6	1.43	nd	2.87	9.47
Total Lipids (% DW)[b]	–	–	96.7	90.7	95.6

[a] C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2$n-6$, linoleic acid; C18:3$n-3$, alfa-linolenic acid; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; nd, not detectable; PUFA, polyunsaturated fatty acids; SO, sunflower oil; RO, rice oil; BT, beef tallow; PF, pressed-fat; HF, horse fat.
[b] DW, dry weight of animal product.

Table 2
Composition of the experimental diets on an as-fed basis

Ingredient	Diet (g/kg)
Oil/fat[a]	30.0
Corn grain	551.6
Soybean meal	279.0
Blood meal	20.0
L-Lysine HCl	1.5
DL-methionine	1.0
Calcium carbonate	95.0
Dicalcium phosphate	15.0
Salt	3.0
Vitamin–mineral premix[b]	2.5
Zinc bicitrate	0.8
Antioxidant (BHT)	0.6

[a] Containing 30 g from sunflower oil or rice oil or beef tallow or pressed-fat or horse fat/kg of diet.
[b] Supplied per kg of diet: vitamin A, 12000 IU; vitamin D3, 2400 IU; vitamin E, 30 IU; vitamin K, 3 mg; thiamin, 2 mg; riboflavin, 6 mg; niacin, 50 mg; calcium pantothenate, 12 mg; pyridoxin, 4 mg; biotin, 100 μg; folic acid, 1 mg; vitamin B12; 20 μg choline chloride, 430 mg; and the trace minerals (mg per kg of diet): Mn, 80; Zn, 60; Fe, 50; Cu, 5; I, 1.5; Se, 0.2; Co, 0.15.

18 °C temperature were maintained through the feeding trial.

Once a day, 150 g of the experimental diets were offered to each bird and un consumed food was weighed in order to determine the daily rate of diet intake.

Eggs were collected every morning and weighed after 24 h. Once a week, eggs collected from three consecutive days were sampled; 24 h after collection, yolk fresh weight and shell dry weight were recorded. Each week, three isolated yolks (from the same hen), coming from consecutive days, were pooled and stored at −20 °C for further biochemical analysis. Individual egg shells, were washed with tap water, dried at 110 °C for 24 h and weighed after being cooled to room temperature. An initial sample, consisting of 30 eggs, collected just before starting the feeding trial, was sampled in the same way.

Table 3
Lipid and fatty acid[a] contents (% dry weight) of the experimental diets[b]

Fatty acid	Initial	SO	RO	BT	PF	HF
14:0	0.02	0.01	0.03	0.11	0.09	0.07
16:0	0.45	0.45	0.75	0.84	0.84	0.66
16:1	0.03	0.03	0.02	0.07	0.08	0.09
18:0	0.26	0.18	0.14	0.82	0.63	0.33
18:1	0.61	1.16	1.49	1.38	1.35	0.99
18:2$n-6$	0.78	2.05	1.76	0.87	0.66	0.78
18:3$n-3$	0.03	0.04	0.07	0.06	0.12	0.21
$\sum$SAFA	0.73	0.64	0.92	1.77	1.56	1.06
$\sum$MUFA	0.64	1.19	1.51	1.45	1.43	1.08
$\sum n-6$ PUFA	0.78	2.05	1.76	0.87	0.66	0.78
$\sum n-3$ PUFA	0.03	0.04	0.07	0.06	0.12	0.21
TFA	2.18	3.92	4.26	4.15	3.77	3.13
Total lipids	3.48	6.14	6.13	5.84	5.84	5.70

All data are based on laboratory analysis result.
[a] C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2$n-6$, linoleic acid; C18:3$n-3$, alfa-linolenic acid; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, total fatty acids; PUFA, polyunsaturated fatty acids.
[b] Initial, diet the hens received before the experimental trial; SO, diet containing 30 g sunflower oil/kg; RO, diet containing 30 g rice oil/kg; BT, diet containing 30 g beef tallow/kg; PF, diet containing 30 g pressed-fat/kg; HF, diet containing 30 g horse fat/kg.

Moisture content of egg yolks was determined by drying the samples for 24 h at 110 °C. Fat sources and yolk total lipids were extracted in a chloroform: methanol (2:1) solution, as described by Folch, Lees, and Sloane-Stanley (1957) and quantified gravimetrically. After alkaline hydrolysis, fatty acids were methylated with BF_3 (AOCS, 1990). The obtained fatty acid methyl esters were analyzed by gas chromatography, using a Hewlett Packard GC equipped with a flame ionization detector (FID) and fitted with a fused capillary column (50 m × 0.25 mm i.d. and 0.20 μm of Carbowax 20 M). Injector and detector temperatures were 220 and 240 °C, respectively. C19:0 (Sigma Chemical Co.) was used as an internal standard for fatty acid quantification. Yolk samples from the HF diet were cooked for 3 min at 100 °C and the fatty acid content was measured as previously described. The data were expressed as mg of fatty acids per yolk.

Experimental data was analyzed by a completely randomized one-way ANOVA (Sokal & Rohlf, 1981) and means were compared using the Tukey's test (Zar, 1984).

3. Results and discussion

Inclusion of 3% of horse fat (HF) does not modify the diet consumption of animals. All diets were equally

Table 4
Moisture, egg and yolk weight and total lipids at the beginning of the trial (initial) and after 30 days of feeding the experimental diets[a]

Diet	Moisture (% of yolk)	Egg weight (g)	Yolk weight (g)[b]	Total lipids (g/egg)
Initial	46.54 ± 0.97a	63.6 ± 5.7a	15.7 ± 1.5a	5.06 ± 0.54a
SO	48.37 ± 0.51b	67.4 ± 5.1a	17.1 ± 1.2b	4.95 ± 0.44a
RO	48.43 ± 0.46b	64.9 ± 4.1a	17.1 ± 1.3b	5.11 ± 0.46a
BT	48.69 ± 0.57b	64.8 ± 5.7a	16.6 ± 1.6ab	4.95 ± 0.43a
PF	48.63 ± 0.63b	66.9 ± 4.1a	16.7 ± 1.0ab	5.07 ± 0.40a
HF	48.41 ± 0.60b	65.4 ± 4.1a	16.9 ± 1.8ab	5.14 ± 0.63a

All values shown are the means ± SD of 12 hens (a pool of 3 consecutive eggs for each hen). Values with different letters within each column were significantly different ($p < 0.05$) by one-way ANOVA, followed by Tukey's test.

[a] Initial, diet the hens received before the experimental trial; SO, diet containing 30 g sunflower oil/kg; RO, diet containing 30 g rice oil/kg; BT, diet containing 30 g beef tallow/kg; PF, diet containing 30 g pressed-fat/kg; HF, diet containing 30 g horse fat/kg.

[b] Fresh weight.

accepted by the birds, resulting in no significant differences ($p > 0.05$) among treatments in dietary intake nor in hen live weight at the end of the trial (data not presented).

After 30 days of treatment with the diet containing HF, no significant differences ($p > 0.05$) were observed for eggs total lipid weight expressed as g/egg (Table 4). However, the yolk weight was significantly affected by the inclusion of oil coming from sunflower and rice when compared with the initial diet. In this sense, increased yolk weight was observed when 3% of sunflower or rice oil was incorporated into the hen's diet (Table 4). The content of yolk moisture increased significantly ($p < 0.05$) compared with the initial diet, with no differences among treatments (Table 4). No negative effects in egg parameters were observed when 3% of HF was used in comparison with other sources.

After feeding the hens for 30 days with HF, the C14:0 and C16:0 did not vary significantly when compared with the other treatments (Table 5). The C18:0 content varied only with the SO treatment. Although the amounts of saturated fatty acid (% dry weight) in diets BT (1.77%), PF (1.56%), HF (1.06%), RO (0.92%) and SO (0.64%) were very different (Table 3), this did not affect the concentration of these fatty acids in the eggs (Table 4). This fact is probably related, to a lower absorption of dietary 18:0 compared to the higher absorption of C18:2$n-6$ and C18:3$n-3$ (Sklan, 1979). Also, the hen maintain an adequate saturated:unsaturated ratio of the egg yolk in order to ensure the growth and hatchability of chicks. For example, the ingestion of conjugated linoleic acid (CLA) by laying hens resulted in increased levels of saturated fatty acids and in reduced levels of monounsaturated fatty acids, without modification of the amount of polyunsaturated fatty acids in egg yolk (Alvarez, Garcia-Rebollar, Calchadora, Mendez, & de Blas, 2005;

Table 5
Fatty acid contents (mg/yolk) of the eggs at the beginning of the trial and after 30 days of feeding the experimental diets[a]

Fatty acids	Initial	SO	RO	BT	PF	HF
14:0	17.0 ± 2.5a	16.4 ± 5.3a	18.1 ± 5.8a	20.6 ± 4.8a	23.4 ± 13.6a	22.1 ± 6.6a
16:0	1087 ± 129a	1025 ± 99a	1056 ± 126a	1004 ± 97a	1001 ± 114a	1026 ± 173a
16:1	185 ± 34.3ab	116 ± 16.1c	137 ± 27.5c	175 ± 21.3b	183 ± 28.2b	194.2 ± 36.3b
18:0	319 ± 31.5ab	353 ± 34.3a	324 ± 45.6ab	306 ± 33.2b	308 ± 30.5b	303 ± 45.7b
18:1	1748 ± 217a	1594 ± 139a	1717 ± 191a	1779 ± 187a	1779 ± 190a	1752 ± 286a
18:2$n-6$	515 ± 70.5a	802 ± 66.5b	670 ± 79.9c	429 ± 54.9d	452 ± 44.1ad	508 ± 79.9ad
18:3$n-3$	1.8 ± 3.7a	1.7 ± 3.9a	8.6 ± 4.3ab	11.7 ± 2.7bc	18.5 ± 3.2bc	46.5 ± 8.1c
20:4$n-6$	94.4 ± 10.6a	97.0 ± 10.3a	93.0 ± 19.3ab	78.1 ± 5.0bc	71.0 ± 17.3bc	64.4 ± 17.9c
20:5$n-3$	nd	nd	nd	nd	nd	nd
22:4$n-6$	16.5 ± 5.1a	14.9 ± 7.0ab	8.9 ± 6.8bc	10.6 ± 4.1ac	3.8 ± 5.9c	2.4 ± 5.5c
22:6$n-3$	17.2 ± 12.6a	nda	10.8 ± 12.0a	23.6 ± 20.7ab	40.0 ± 23.1bc	71.2 ± 29.6c
∑SAFA	1427 ± 158a	1394 ± 121a	1398 ± 167a	1331 ± 124a	1332 ± 142a	1351 ± 214a
∑MUFA	1935 ± 247a	1710 ± 145a	1854 ± .209a	1954 ± 202a	1961 ± 206a	1946 ± 317a
∑$n-6$[b]	635 ± 84.7a	918 ± 76.6b	781.0 ± 97.6d	526 ± 60.2c	536 ± 53.0c	576 ± 95.2ac
∑$n-3$[b]	18.5 ± 15.0ab	1.7 ± 3.9a	19.4 ± 14.7abd	35.2 ± 21.6bc	58.5 ± 24.5cd	118.7 ± 36.5c
TFA	4016 ± 0.45a	4023 ± 0.31a	4052 ± 0.44a	3846 ± 0.37a	3890 ± 0.39a	3990 ± 0.64a
$n-6/n-3$	34.3	554.8	40.3	14.9	9.2	4.9

Values are the mean of 12 hens (a pool of 3 consecutive eggs for each hen) followed by the SE. Values not having a common letter within each file were significantly different ($p < 0.05$) by the one-way ANOVA followed by the Tukey's test.

[a] Initial, diet the hens received before the experimental trial; SO, diet containing 30 g sunflower oil/kg; RO, diet containing 30 g rice oil/kg; BT, diet containing 30 g beef tallow/kg; PF, diet containing 30 g pressed-fat/kg; HF, diet containing 30 g horse fat/kg. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, total fatty acids; nd, not detectable.

[b] Polyunsaturated fatty acids.

Raes et al., 2002). This imbalance between the fatty acids observed in yolk, induces severe embryonic mortality (Aydin, Pariza, & Cook, 2001; Raes et al., 2002).

In our work, the monounsaturated fatty acid C18:1 and total monounsaturated fatty acids did not vary among treatments (Table 5) . Table 5 also presents the polyunsaturated fatty acid composition of the yolk, and shows a reduced level of C18:2n6 with the diet containing HF and other animal fats such as PF and BT, in comparison with the vegetal diet containing SO and RO.

Supplementation of diets, with animal fats containing 18:3$n-3$ (diets HF and PF), resulted in a significant increase of the $n-3$ fatty acid content in the eggs, compared with the initial sample and vegetable oils, due to an increase of 18:3$n-3$ and 22:6$n-3$ fatty acid contents (Table 5). On the other hand, 20:4$n-6$ yolk content decreased significantly when compared to the initial sample, although the 18:2$n-6$ content was not affected by these treatments (Table 5). Several studies (e.g., Cherian & Sim, 1992a, 1992b, 1997; Hargis et al., 1991) have shown a reduction of the 20:4$n-6$ content in egg yolk through the inclusion of lipids rich in $n-3$ fatty acids in the diet of laying hens. This phenomenon can be explained by the enzyme desaturase competing for substrates such as linoleic and linolenic acids. The order of preference for the substrate is, as established for rats, 18:3$n-3$ > 18:2$n-6$ > 18:1$n-9$ (Sprecher, 1989).

Considering that 20:5$n-3$ was not detected in the egg yolks and that 22:6$n-3$ is important for embryonic development, especially in the formation of neural tissues and vision organs (Anderson, 1994; Hoffman et al., 2004; Horrocks & Yeo, 1999; Innis, 1993), it was hypothesized that 22:6$n-3$ was preferentially incorporated into the eggs to the detriment of 20:5$n-3$ or 20:4$n-6$. In newly hatched chickens, a tendency to accumulate DHA in the brain, related to the $n-3$ fatty acids in maternal diet, has been demonstrated (Cherian & Sim, 1992a, 1993, 1997; Speake, Murray, & Noble, 1998). The use of a more concentrated $n-3$ fatty acids sources such as flaxseed meal and oil, can induce a detectable amount of C20:5$n-3$, as observed in the work of Milinsk et al. (2003). In mammals, DHA is highly concentrated in the synaptic membranes and the photoreception cell membranes of the retina (Bazan, 1990), suggesting that, during development and differentiation of the nervous central system, DHA is required for synaptogenesis, biogenesis of photoreception membranes and vision (Hoffman et al., 2004). Also, DHA is considered to be promising ingredient against the cancer, as observed in colon, prostate, breast, (Chen & Istfan, 2000; Kato et al., 2002; Rose, Connolly, Rayburn, & Coleman, 1995; Yang, Lee, Hong, & Chung, 1999) and cardiovascular diseases (Angerer et al., 2002; Horrocks & Yeo, 1999; Singer & Wirth, 2004; Tapiero et al., 2002).

As a result of the changes in the yolk fatty acid profile after 30 days of experimental diet feeding, the $n-6/n-3$ fatty acid ratio in the egg yolks was strongly modified (Table 5). The best ratio (4.9) was obtained when diets were supplemented with horse fat. The next best ratio observed (9.2) was obtained with the PF diet . As expected, the use of sunflower oil (diet SO) rich in 18:2$n-6$, resulted in an elevated $n-6/n-3$ ratio (554.8). The addition of rice oil (diet RO) to the diet

Table 6
Fatty acid contents (mg/yolk) of the eggs of treatment HF (horse fat) during the course of the trial

	Initial	Week 1	Week 2	Week 3	Week 4
14:0	17.0 ± 2.5a	19.2 ± 22.4a	29.7 ± 25.5a	12.4 ± 19.3a	22.1 ± 6.6a
16:0	1087 ± 129a	1038 ± 136ab	1087 ± 166ab	885 ± 172.9b	1026 ± 173ab
16:1	184.8 ± 34.3a	151 ± 37.8a	170 ± 32.1a	158 ± 43.2a	194 ± 36.3a
18:0	319 ± 31.5a	340 ± 46.0ab	375 ± 38.4a	284 ± 51.4a	303 ± 45.7a
18:1	1748 ± 217a	1815 ± 169a	2017 ± 232.0a	1599 ± 307a	1752 ± 286a
18:2$n-6$	515 ± 70.5a	500 ± 50.8a	527 ± 64.2a	470 ± 87.0a	508 ± 79.9a
18:3$n-3$	1.8 ± 3.7a	43.9 ± 5.3b	53.8 ± 8.0b	54.7 ± 14.6b	46.5 ± 8.1b
20:4$n-6$	94.4 ± 10.6a	67.0 ± 12.1b	71.2 ± 10.1b	54.3 ± 14.4b	64.4 ± 17.9b
22:3$n-6$	8.9 ± 14.6a	nd	nd	nd	1.8 ± 2.4a
22:4$n-6$	16.5 ± 5.1a	nd	nd	nd	2.4 ± 5.5a
22:6$n-3$	17.2 ± 12.6a	52.1 ± 10.9ab	69.0 ± 11.2b	59.0 ± 13.8b	71.2 ± 29.6b
∑SAFA	1427 ± 158a	1397 ± 169ab	1492 ± 214a	1182 ± 224b	1351 ± 214ab
∑MUFA	1935 ± 247a	1966 ± 206a	2187 ± 254a	1757 ± 343a	1946 ± 317a
∑$n-6$[a]	635.2 ± 84.7a	567 ± 60.3ab	598 ± 72.6ab	524 ± 100.1b	576.2 ± 95.2ab
∑$n-3$[a]	18.5 ± 15.0a	96.0 ± 15.3ab	123 ± 16.4b	114 ± 24.6b	118 ± 36.5b
Ratio $n-6/n-3$	34.3	5.9	4.9	4.6	4.9
Yolk weight (g)	15.7 ± 1.5a	15.9 ± 1.4a	15.9 ± 1.5a	16.2 ± 1.8a	16.9 ± 1.8a

Values are the means of 12 eggs. Values having different letters within each file were significantly different ($p < 0.05$) by the Tukey's test. nd, not detectable; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids.

[a] Polyunsaturated fatty acids.

Table 7
Effects of heating egg yolks (100 °C for 3 min) on the fatty acid contents (mg/egg) of final HF eggs

	HF	HF cooked
14:0	22.1 ± 6.6a	16.2 ± 16.7a
16:0	1026 ± 173a	983 ± 190a
16:1	194 ± 36.3a	175 ± 44.8a
18:0	303 ± 45.7a	305 ± 48.0a
18:1	1752 ± 286a	1747 ± 337a
18:2$n-6$	508 ± 79.9a	486 ± 91.8a
18:3$n-3$	46.5 ± 8.1a	53.0 ± 11.5a
20:4$n-6$	64.4 ± 17.9a	57.5 ± 13.3a
20:5$n-3$	nd	nd
22:3$n-6$	1.8 ± 4.0	nd
22:4$n-6$	2.4 ± 5.5	nd
22:6$n-3$	71.2 ± 29.6a	61.6 ± 13.6a
∑SAFA	1351 ± 214a	1305 ± 240a
∑MUFA	1946 ± 317a	1922 ± 379a
∑$n-6$ PUFA	576 ± 95.2a	544 ± 104.3a
∑$n-3$ PUFA	118 ± 36.5a	115 ± 24.2a
$n-6/n-3$	4.9	4.7

Values are the means of 12 eggs followed the SD.Values having similar letters within each file were not significantly different ($p > 0.05$) by one-way ANOVA. nd ,not detectable. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid.

gave $n-6/n-3$ ratio of 40.3, since the increase of the $n-6$ fatty acid in the RO eggs was accompanied by a slight increase of the $n-3$ fatty acid content.

One week after starting to feed the hens with the horse fat-supplemented diet, a significant increase of 18:3$n-3$ and a reduction of 20:4$n-6$ content were observed. There was a slower increase in the content of 22:6$n-3$ that became significant after the second week of feeding diet HF (Table 6). The fatty acid profile of eggs from treatment HF was not significantly modified by the thermal treatment of yolks (Table 7).

The present study shows that feeding hens with horse fat is a cheap, fast and appropriate method for enriching eggs with $n-3$ fatty acid in Uruguay.

Acknowledgement

This work was supported in part by grants from Comision Sectorial de Investigacion Cientifica CSIC – Universidad de la Republica, Uruguay.

References

Alvarez, C., Garcia-Rebollar, P., Calchadora, P., Mendez, J., & de Blas, J. C. (2005). Effects of dietary conjugated linoleic acid and high-oleic sunflower oil on performance and egg quality in laying hens. *British Poultry science, 46*, 80–86.

Anderson, G. J. (1994). Developmental sensitivity of the brain to dietary $n-3$ fatty acids. *Journal of Lipids Research, 35*, 105–111.

Angerer, P., Kothny, W., Stork, S., & von Schacky, C. (2002). Effect of dietary supplementation with ω-3 fatty acids on progression of atherosclerosis in carotid arteries. *Cardiovascular Research, 54*, 183–190.

AOCS. (1990). *Fatty acid composition by gas chromatography. Official methods and recommended practices.* American Oil Chemists Society.

Aydin, R., Pariza, M. W., & Cook, M. E. (2001). Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. *Journal of Nutrition, 131*, 800–806.

Bazan, N. G. (1990). Supply of $n-3$ polyunsaturated fatty acids and their significance in the central nervous system. In R. J. Wurtman & J. J. Wurtman (Eds.), *Nutrition and the brain*. New York: Raven Press Ltd..

Chen, Z. Y., & Istfan, N. W. (2000). Docosahexaenoic acid is a potent inducer of apoptosis in HT-29 colon cancer cells. *Prostaglandins Leukotrienes and Essential Fatty acids, 63*, 301–308.

Cherian, G., & Sim, J. S. (1992a). Preferencial accumulation of $n-3$ fatty acids in the brain of chicks from eggs enriched with $n-3$ fatty acids. *Poultry Science, 71*, 1658–1668.

Cherian, G., & Sim, J. S. (1992b). Omega-3 fatty acid composition of the newly hatched chicks from the α-linolenic acid enriched eggs. *Lipids, 27*, 706–710.

Cherian, G., & Sim, J. S. (1993). The net transfer and incorporation of yolk $n-3$ fatty acids into developing chick embryo. *Poultry Science, 72*, 98–105.

Cherian, G., & Sim, J. S. (1996). Changes in the breast milk fatty acids and plasma lipids of nursing mothers following consumption of $n-3$ polyunsaturated fatty acid enriched eggs. *Nutrition, 12*, 8–12.

Cherian, G., & Sim, J. S. (1997). Egg yolk polyunsaturated fatty acids and vitamin E content alters the tocopherol status of hatched chicks. *Poultry Science, 76*, 1753–1759.

Clandinin, M. T. (1999). Brain development and assessing the supply of polyunsaturated fatty acid. *Lipids, 34*, 131–137.

Cloughley, J., Noble, R., Speake, B., & Sparks, N. (1997). Manipulation of docosahexaenoic (22:6 $n-3$) acid in the chicken's egg. *Prostaglandins Leukotrienes and Essential Fatty acids, 57*, 222.

Curto, S., Prats, O., & Ayestaran, R. (2004). Investigacion sobre factores de riesgo cardiovascular en Uruguay. *Revista Medica Uruguay, 20*, 61–71.

Farrell, D. J. (1998). Enrichment of hen eggs with $n-3$ long-chain fatty acids and evaluation of enriched eggs in humans. *American Journal Clinical Nutrition, 68*, 538–544.

Folch, J., Lees, M., & Sloane-Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Gurr, M. I., & Harwood, J. L. (1991). *Lipid biochemistry. An introduction* p. 406. London: Chapman and Hall.

Hargis, P. S., Van Elswyk, M. E., & Hargis, B. M. (1991). Dietary modification of yolk lipid with menhaden oil. *Poultry Science, 70*, 874–883.

Hoffman, D. R., Theuer, R. C., Castaneda, Y. S., Wheaton, D. H., Bosworth, R. H., O'Connor, A. R., et al. (2004). Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. *Journal of Nutrition, 134*, 2307–2313.

Horrocks, L. A., & Yeo, Y. K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research, 40*, 211–225.

Innis, S. M. (1993). Borden award lecture. Fatty acid requirements of the newborn. *Canadian Journal of Physiology and Pharmacology, 72*, 1483–1492.

Jiang, Z., Ahn, D. U., & Sim, J. S. (1991). Effects of feeding flax and two types of sunflower seeds on fatty acid compositions of yolk lipid classes. *Poultry Science, 70*, 2467–2475.

Kato, T., Hancock, R. L., Mohammadpour, H., McGregor, B., Manalo, P., Khaiboulina, S., et al. (2002). Influence of omega-3 fatty acids on the growth of human colon carcinoma in nude mice. *Cancer Letter, 187*, 169–177.

Lands, W. E. M., Libelt, B., Morris, A., Kramer, N. C., Prewitt, T. E., Bowen, P., et al. (1992). Maintenance of lower proportions of ($n-6$) eicosanoid precursors in phospholipids in human plasma in response to added dietary ($n-3$) fatty acids. *Biochimica Biophysica Acta, 1180*, 147–162.

Leclercq, B. (1972). Etude de la biosynthèse et de l'utilisation des acides gras chez la pondeuse. *Annales de Biologie Animale Biochimie Biophysique, 12*, 441–452.

Lewis, N. M., Schalch, K., & Scheideler, S. E. (2000). Serum lipid response to $n-3$ fatty acid enriched eggs in persons with hypercholesterolemia. *Journal of the American Dietetic Association, 100*, 365–367.

Marshall, A. C., Kubena, K. S., Hinton, K. R., Hargis, P. S., & Van Elswyk, M. E. (1994). $n-3$ Fatty acid-enriched table eggs – a survey of consumer acceptability. *Poultry Science, 73*, 1334–1340.

Maurice, D. V. (1994). Dietary fish oils. Feeding to produce designer eggs. *Feed Management, 45*, 29–32.

Milinsk, M. C., Murakami, A. F., Gomes, S. T. M., Matsushita, M., & de Souza, N. E. (2003). Fatty acid profile of eggs yolk lipids from hens fed dits rich in $n-3$ fatty acids. *Food Chemistry, 83*, 287–292.

Nash, D. M., Hamilton, R. M. G., & Hulan, H. W. (1995). The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens. *Canadian Journal of Animal Science, 75*, 247–253.

Oh, S. Y., Lin, C. H. H., Ryue, J., & Bell, D. E. (1994). Eggs enriched with omega-3 fatty acids as a wholesome food. *Journal of Applied Nutrition, 46*, 15–23.

Oh, S. Y., Ryue, J., Hsieh, C. H., & Bell, D. E. (1991). Eggs enriched with ω-3 fatty acids and alterations in lipid concentrations in plasma and lipoproteins and in blood pressure. *American Journal of Clinical Nutrition, 54*, 689–695.

Raes, K., Huyghebaert, G., De Smeet, S., Nollet, L., Arnouts, S., & Demeyer, D. (2002). The deposition of conjugated linoleic acid in eggs of laying hens fed diets varying in fat level and fatty acid profile. *Journal of Nutrition, 132*, 182–189.

Rose, D. P., Connolly, J. M., Rayburn, J., & Coleman, M. (1995). Influence of diet containing eicosapentaenoic or docosahexaenoic acid on growth and metastasis of breast cancer cells in Nude mice. *Journal of National Cancer Institute, 87*, 587–592.

Saadoun, A., Braida, C., Martinez, M., & Cabrera, C. (2003). Metastasis experimentales de melanoma B16-F1 en ratones Balb/c: Implicancia del nivel energetico y de los acidos grasos $n-6$ y $n-3$ de la dieta. *Revista Uruguaya de Patologia Clinica, 37*, 15–22.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy, 56*, 365–379.

Singer, P., & Wirth, M. (2004). Can $n-3$ PUFA reduce cardiac arrhythmias? Results of a clinical trial. *Prostaglandins Leukotrienes and Essential Fatty acids, 71*, 153–159.

Sklan, D. (1979). Digestion and absorption of lipids in chicks fed triglycerides or free fatty acids: synthesis of monoglycerides in the intestine. *Poultry Science, 58*, 885–892.

Sokal, R. R., & Rohlf, F. J. (1981). *Biometry. The principles and practice of statistics in biological research* (2nd ed.). New York: W.H. Freeman & Co..

Speake, B. K., Murray, A. M. B., & Noble, R. C. (1998). Transport and transformations of yolk lipids during development of the avian embryo. *Progress in Lipid Research, 37*, 1–32.

Sprecher, H. (1989). ($n-3$) and ($n-6$) fatty acid metabolism. In C. Galli & A. P. Simopoulos (Eds.), *Dietary omega 3 and omega 6 fatty acids. Biological effects and nutritional essentiality*. *Nato ASI Series. Serie A: Life Sciences* (Vol 171). New York and London: Plenum Press.

Stadelman, W. J., & Pratt, D. E. (1989). Factors influencing composition of the hens's eggs. *World Poultry Science, 45*, 247–266.

Stripp, C., Overvad, K., Christensen, J., Thomsen, B. L., Olsen, A., Moller, S., et al. (2003). Fish intake is positively associated with breast cancer incidence rate. *Journal of Nutrition, 133*, 3664–3669.

Tapiero, H., Nguyen Ba, G., Couvreur, P., & Tew, K. D. (2002). Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomedicine and Pharmacotherapy, 56*, 215–222.

Yang, Y. J., Lee, S. H., Hong, S. J, & Chung, B. C. (1999). Comparison of fatty acid profiles in the serum of patients with prostate cancer and bening prostatic hyperplasia. *Clinical Biochemistry, 32*, 405–409.

Zar, J. H. (1984). *Biostatistical analysis* (2nd ed.). New Jersey: Prentice-Hall.

Zock, P. L. (2001). Dietary fats and cancer. *Current Opinion in Lipidology, 12*, 5–10.