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Original Research Article

Effect of full-fat sunflower or flaxseed seeds dietary inclusion on performance, egg yolk fatty acid profile and egg quality in laying hens

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ABSTRACT

A study was conducted to evaluate the effect of the dietary inclusion of full-fat flaxseed or full-fat sunflower seeds on performance parameters, egg quality parameters and egg yolk fatty acid profile in laying hens. A total of 150 Babcock Brown hens at 27 weeks of age were distributed in 3 experimental treatments, as follows: T1, control; T2, diet containing 13.5% full-fat whole flaxseed seeds; and T3, diet containing 13.5% full-fat ground sunflower seeds. Feed and water were provided ad libitum and the experiment lasted for 8 weeks. No significant differences were found on egg quality parameters, but total egg production and rate of lay were significantly ($P < 0.05$) lower in the group receiving sunflower seeds compared with the control, and significant differences ($P < 0.05$) in egg yolk saturated, monounsaturated, polyunsaturated, omega-6 (n-6) and omega-3 (n-3) fatty acids were observed, as well as in the n-6:n-3 ratio. The results show that the dietary inclusion of 13.5% flaxseed full-fat seeds significantly increases the n-3 polyunsaturated fatty acid (PUFA) content in the eggs and lowers the n-6:n-3 ratio without affecting performance parameters. Inclusion of full-fat sunflower seeds increases the n-6 PUFA content but affects total egg production and rate of lay. Further studies are needed to determine the level of inclusion of full-fat sunflower seeds that does not affect performance.

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1. Introduction

Polyunsaturated fatty acids (PUFA) are lipids with important physiological roles, with 2 of them considered essential to adult humans: linoleic acid (LA, C18:2 n-6) and α -linolenic acid (ALA, C18:3 n-3). Clinical studies have shown that replacing saturated fatty acids (SFA) by PUFA produces beneficial effects on the cardiovascular system (Jakobsen et al., 2009), and getting at least 5% to 10% of the total caloric intake from omega-6 (n-6) PUFA reduces the risk of coronary heart disease, compared with lower intakes (Harris et al., 2009; Kris-Etherton et al., 2010). On the other hand, in

prospective studies and properly randomized clinical trials, it has been found that omega-3 (n-3) fatty acids (FA) reduce the mortality associated with coronary heart disease and death by cardiac arrest (Mozaffarian and Wu, 2011). According to current international recommendations, the population in general should have a minimum of 250 mg/d of n-3 FA or 2 helpings of oily fish (Mozaffarian and Wu, 2011). Some researchers consider that current Western diets are generally deficient in n-3 FA compared with the diets of the human ancestors (Simopoulos, 2002). One way of increasing n-3 FA intake is through the consumption of natural sources rich in these FA such as oily fish and flaxseed (González-Esquerra and Leeson, 2000). Another possibility is through the intake of functional foods enriched with n-3 FA such as eggs (Lewis et al., 2000). By increasing the concentration of PUFA in the diet of the laying hens, it is possible to increase the amount of these FA in the eggs. In nutrition, it has become customary to use eggs as a means to increase the dietary intake of PUFA in humans (Stehle and Grimble, 1998). Higher amounts of PUFA in egg yolk fat can be obtained by supplementing the laying hen diets with flaxseed, fish or canola oils, or with marine algae (Gonzalez-Esquerra and Leeson, 2000;

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González-Esquerra and Leeson, 2001). Flaxseed, sunflower and canola meals and oils have also been used to modify the FA profile of egg yolk lipids in laying hens (Milinsk et al., 2003). However, very few studies have evaluated the inclusion of whole full-fat seeds such as linseed (flaxseed, *Linum usitatissimum* L.) or sunflower (*Helianthus annuus* L.). Linseed is rich in ALA (Lenzi Almeida et al., 2011), while sunflower seeds are a good source of LA (Karunajeewa et al., 1989).

The present study was conducted to evaluate the effect of the inclusion of 13.5% full-fat, whole flaxseed seeds and full-fat ground sunflower seeds on production parameters, egg quality and egg yolk FA profile in commercial laying hens. Whole seeds were chosen (instead of oil) because it is easier and more economical to include them in the diets. The working hypothesis is that the supplementation of either of these seeds will improve the FA profile of the eggs.

2. Materials and methods

2.1. Birds, housing and experimental diets

Animal use and care were conducted according to the “Guide for the care and use of laboratory animals” of the National Research Council (NRC, 2011). The study was conducted at “La bonita” poultry farm, county “La Bruja”, city of Pacho, Cundinamarca, Colombia (5°07'50" North; 74°09'30" West). The mean altitude is 1,400 m and the temperature ranges between 14 and 25 °C. One hundred and fifty Babcock Brown hens at 27 wk of age were randomly distributed in 30 floor pens, each one with 5 birds. The experimental unit was the replicate of 5 birds/pen and the experimental treatments were replicated 10 times. The treatments consisted of 3 different diets, as follows: T1, control; T2, diet containing 13.5% whole full-fat flaxseed seeds; and T3, diet containing 13.5% ground full-fat sunflower seeds. A 13.5% inclusion level was chosen because in a previously published study, 15% ground flaxseed caused a significant decrease in egg weight (Scheideler and Froning, 1996). The same inclusion level was chosen for full-fat sunflower seeds since only one study was found in the literature reporting the use of full-fat sunflower seeds in layers and the dietary level used was only 2.35% (Karunajeewa et al., 1989). The ingredients and calculated analysis of the experimental diets is shown in Table 1. The diets reached or exceeded the nutritional requirements of laying hens (NRC, 1994). The diets were fed *ad libitum* and the experiment lasted for 8 weeks.

2.2. Performance parameters

The body weight of each hen was recorded at the beginning and at the end of the experiment. Feed intake, rate of lay, egg weight, and feed conversion rate (kg of feed/kg of egg) were determined on a weekly basis. Total egg production was calculated for the 8 weeks of the experiment. During 31 to 34 weeks of age (5 to 8 weeks of the experiment) 10% of the eggs were collected at random to determine egg quality parameters (egg weight, yolk weight, shell weight and thickness, albumen weight and height, Haugh units and yolk color).

2.3. Determination of the egg yolk fatty acid profile

The FA profile in egg yolk was determined in a pool of 5 eggs randomly selected from 3 replicate pens per treatment, as previously described (Riaño et al., 2011). In brief, 1 g of pooled egg yolk was extracted with 40 mL of a mixture of chloroform and methanol (2:1, vol/vol), homogenized and filtered. To 20 mL of the filtered extract, 4.5 mL of water was added, and the mix was homogenized again and centrifuged at 1,500 × g for 10 min. The supernatant was removed and discarded, and 1 mL of the lower layer was transferred to a

Table 1

Ingredients and calculated analysis of the experimental diets (% fresh weight basis).

Item	Experimental diets		
	Control	Flaxseed	Sunflower
Ingredients			
Corn	48.9	51.7	51.7
Wheat bran	11.1	2.0	2.0
Soybean meal (48%)	26.4	19.0	19.0
Quinoa seeds	2.0	2.2	2.2
Full-fat flaxseed	–	13.5	–
Full-fat sunflower	–	–	13.5
Calcium carbonate	10.4	10.2	10.2
Bicalcium phosphate	0.7	0.93	0.93
Salt	0.3	0.3	0.3
Methionine	0.09	0.09	0.09
Choline chloride	0.01	0.01	0.01
VMP ¹	0.1	0.1	0.1
Nutrient analysis (calculated)			
Protein	18.2	17.8	17.4
Metabolizable energy, kcal/kg	2,810	2,860	2,880
C18:2 (linoleic acid)	1.33	2.96	5.86
C18:3 (linolenic acid)	0.05	7.81	0.05
Ether extract	3.02	7.30	7.93
Calcium	4.49	4.44	4.45
Available phosphorus	0.39	0.41	0.42
Methionine	0.45	0.44	0.45
Sulphur amino acids	0.77	0.76	0.75

VMP = vitamin-mineral premix.

¹ Proving per kilogram of diet the following amounts of vitamins and minerals: 8000 I.U. of vitamin A, 3500 I.U. of vitamin D3, 50 I.U. of vitamin E, 3 I.U. of vitamin K, 2 mg of thiamin, 5 mg of riboflavin, 3 mg of pyridoxamine, 10 mg of pantothenic acid, 1 mg of folic acid, 100 µg of biotin, 40 mg of niacin, 400 mg of choline, 10 µg of vitamin B12, 60 mg of manganese, 30 mg of iron, 5 mg of copper, 50 mg of zinc, 1 mg of iodine and 0.3 mg of selenium.

previously weighed test tube. The solvent was evaporated under a gentle stream of nitrogen and the remaining dry film was dissolved with toluene to a concentration of 25 mg/mL. From this solution, 20 µL was taken and added to 160 µL of toluene and 20 µL of Meth-Prep II transesterification reagent (Alltech Associates, Inc., Deerfield, IL, USA). The mix was left at room temperature for 30 min and then 1 µL of the solution was injected into the gas chromatograph for the determination of the fatty acid methyl esters (FAME). The FAME were separated on an SGE BPX70 capillary gas chromatograph (GC) column (SGE Analytical Science, Australia) with a 30 m × 0.32 mm inside diameter × 0.25 µm film thickness using a Shimadzu GC-14A Gas Chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector. Separation was obtained with a temperature ramp (initial temperature 80 °C for 2 min, 30 °C/min until 140 °C, then 10 min at 140 °C, 2.9 °C/min to 200 °C, and finally 2.9 min at 200 °C) using helium as the carrier gas and nitrogen as the make-up gas. The injection was made in split mode with a split ratio of 1:30. Retention times were compared with those of known standards (Supelco, Inc., Bellefonte, PA, USA).

2.4. Statistical analysis

Data were analysed by an ANOVA under a completely randomized design using the General Linear Models (GLM) procedure of SAS (SAS user's guide: statistics 2013). The means of all variables were compared using a Tukey test and probability values less than 0.05 were considered significant.

3. Results

3.1. Performance parameters

With the exception of total egg production during the experiment (8 weeks) and rate of lay, no significant differences in

performance parameters were observed (Table 2). Total egg production and rate of lay were significantly ($P < 0.05$) lower in the group fed the diet containing full-fat sunflower seeds compared with the control. However, total egg production and percent rate of lay did not differ significantly between the flaxseed group and the other 2 groups. Initial and final body weights did not differ significantly among treatments. None of the egg quality parameters measured showed significant differences among the experimental groups (Table 3).

3.2. Fatty acid composition of seeds and egg yolk fat

The percent FA composition of the flaxseed and sunflower seeds used in the experimental diets is shown in Table 4. Five FA were the major components in each of the seeds (myristic, palmitic, stearic, oleic and linoleic acids in sunflower seeds; palmitic, stearic, oleic, linoleic and α -linolenic acids in flaxseed seeds). Sunflower seeds had 1.51% myristic acid, which was absent in flaxseed seeds, whereas flaxseed seeds contained a high percentage of ALA (57.5%), a FA not found in sunflower seeds. Palmitic and stearic acid content was similar in both types of seeds, but the LA content was 2.6 times higher in sunflower seeds compared with flaxseed seeds (34.6% vs. 13.1%).

The egg yolk FA composition for the 3 experimental treatments is summarized in Table 5. All FA presented significant differences ($P < 0.05$) among treatments except for myristic, stearic and oleic acids. The egg yolk of the eggs from the control group had a significantly higher percentage of palmitic acid and a greater content of total saturated FA than that from the other 2 groups. The percentage of LA in the eggs from the hens fed sunflower seeds was 19.8%; this value was 4.9% and 6.1% higher compared with the control group and the group receiving flaxseed seeds, respectively. On the other hand, the percentage of the n-3 FA ALA was much greater in the eggs from the birds fed flaxseed seeds (4.19%) compared with the control eggs (0.59%) or the eggs from the hens fed sunflower seeds (0.27%). When the FA profiles of all treatments were analyzed, it was observed that the percentage of ALA was correlated in a quadratic fashion with the percentage of docosahexaenoic acid (DHA), whereas DHA was linearly correlated with docosapentaenoic acid (DPA) (Fig. 1).

The percentage of PUFA was significantly higher in the eggs from the hens fed full-fat sunflower seeds (22.9%) compared with the control hens (18.3%) but did not differ from those fed the flaxseed seeds (21.8%). On the other hand, the n-6:n-3 ratio was significantly

Table 2
Effect of full-fat sunflower or flaxseed seeds dietary inclusion at 13.5% on performance parameters in Babcock Brown laying hens.¹

Item	Experimental diets			SE	P-value
	Control	Sunflower	Flaxseed		
Feed intake, g/d	101.5 ± 5.6	101.3 ± 4.3	99.9 ± 5.3	0.93	0.745
Total egg production	36.2 ± 6.5 ^a	29.9 ± 5.2 ^b	30.9 ± 3.0 ^{ab}	1.03	0.022
Rate of lay, %	65.9 ± 11.8 ^a	54.4 ± 9.4 ^b	56.3 ± 5.5 ^{ab}	1.88	0.022
Total egg weight, kg	2.10 ± 0.36	1.80 ± 0.40	1.83 ± 0.20	0.32	0.107
FCR, kg feed/kg egg	2.66 ± 0.40	3.13 ± 0.40	3.00 ± 0.25	0.05	0.251
Egg weight, g/egg	57.9 ± 1.8	59.5 ± 3.8	59.4 ± 4.0	0.60	0.501
Egg mass, g/hen per d	38.1 ± 6.7	32.6 ± 7.2	33.4 ± 3.8	1.16	0.108
Initial body weight, g	1,828 ± 104	1,823 ± 106	1,797 ± 105	18.7	0.785
Final body weight, g	2,205 ± 183	2,171 ± 195	2,119 ± 177	33.4	0.587

FCR = feed conversion rate.

^{a, b} Within a row, means with different superscripts are significantly different ($P < 0.05$).

¹ Values are the mean ± standard error (SE) of 10 replicate pens per treatment for the 8-week experimental period.

Table 3
Effect of full-fat sunflower or flaxseed seeds dietary inclusion at 13.5% on egg quality parameters in Babcock Brown laying hens.¹

Item	Experimental diets			SE	P-value
	Control	Sunflower	Flaxseed		
Shell thickness, mm	0.198 ± 0.02	0.196 ± 0.02	0.198 ± 0.01	0.0042	0.9769
Eggshell strength, N	11.72 ± 1.35	11.09 ± 1.03	11.63 ± 0.62	0.1900	0.3582
Yolk color (Roche scale)	6.9 ± 0.31	7.0 ± 0.47	6.8 ± 0.42	0.0735	0.5560
Egg weight, g	58.8 ± 2.19	58.6 ± 1.58	58.6 ± 1.72	0.3268	0.9529
Egg yolk weight, g	15.6 ± 0.95	15.8 ± 0.71	15.5 ± 0.84	0.1505	0.6845
Albumen weight, g	35.0 ± 2.80	35.8 ± 1.29	35.6 ± 2.62	0.4172	0.7395
Shell weight, g	7.46 ± 0.14	7.51 ± 0.36	7.50 ± 0.23	0.0482	0.9229
Egg length, mm	55.6 ± 0.54	55.6 ± 0.85	55.4 ± 0.95	0.1429	0.8041
Egg width, mm	42.5 ± 0.82	42.7 ± 0.77	42.3 ± 0.92	0.1523	0.5272
Albumen height, mm	0.86 ± 0.10	0.86 ± 0.10	0.85 ± 0.14	0.0210	0.9702
Albumen diameter, mm	90.6 ± 5.53	87.1 ± 4.71	89.3 ± 0.90	1.0541	0.4074
Albumen index, %	0.95 ± 0.15	1.01 ± 0.15	0.97 ± 0.21	0.0313	0.7373
Haugh units	92.4 ± 0.55	92.4 ± 0.54	92.4 ± 0.73	0.1085	0.9705

¹ Values are the mean ± standard error (SE) of 10 replicate pens per treatment for the 8-week experimental period.

($P < 0.05$) lower in the eggs from the hens fed the flaxseed diet (2.2), compared with the control (10.5) and sunflower diet (22.4).

4. Discussion

Polyunsaturated fatty acids are FA with important physiological functions, being 2 of them essential to humans: LA and ALA. In laying hens, the egg yolk FA composition depends on the liver lipid synthesis, the lipid components of the diet and the liver uptake of dietary lipids (Sim and Qi, 1995). Through manipulation of the diet it is possible to modify the FA profile of the egg and increase its PUFA content. In the present trial, the inclusion of full-fat seeds rich in LA or ALA (sunflower and flaxseed, respectively) resulted in a significant increase of these FA in the egg yolk.

The FA composition of the flaxseed seeds used in the present trial was similar to that of a previous report (Yalçın et al., 2007), in which the same 5 FA were found at similar percentages: palmitic (6.39%), stearic (4.94%), oleic (16.52%), linoleic (14.62%), and linolenic (56.90%) acids; in the present trial, the corresponding values for these FA were 4.64%, 3.48%, 20.5%, 13.1% and 57.5%, respectively. On the other hand, the FA composition of the sunflower seeds was similar to that reported for the so-called “high-oleic” sunflower oil, in which the oleic acid content is higher than the LA content (Ramos et al., 2009). Values reported for high-oleic sunflower oil were 4.6%, 3.4%, 62.8% and 27.5% for palmitic, stearic, oleic and linoleic acids, respectively, whereas for regular sunflower oil these values were 6.2%, 3.7%, 25.2%, and 63.1%, respectively (Ramos et al., 2009). In the present trial the values for palmitic, stearic, oleic and LA were 4.38%, 4.24%, 54.5%, and 34.6%, respectively. Interestingly, one study reported 33.43% trans-oleic and 54.74% trans-linoleic acids in sunflower oil (Oliveira et al., 2010); this is unusual since

Table 4
Fatty acid composition of the full-fat sunflower and flaxseed seeds used in the experimental diets (%).

Fatty acid	Sunflower ¹	Flaxseed
Myristic acid (C14:0)	1.51	—
Palmitic acid (C16:0)	4.38	4.64
Stearic acid (C18:0)	4.24	3.48
Oleic acid (C18:1 n-9c)	54.5	20.5
Linoleic acid (C18:2 n-6c)	34.6	13.1
α -linolenic acid (ALA, C18:3 n-3)	—	57.5

¹ High oleic sunflower seeds.

Table 5
Effect of full-fat sunflower or flaxseed seeds dietary inclusion at 13.5% on egg yolk fatty acid composition (%).

Item	Experimental diets			P-value
	Control	Sunflower	Flaxseed	
Myristic acid (C14:0)	0.25 ± 0.03	0.22 ± 0.01	0.22 ± 0.02	0.351
Palmitic acid (C16:0)	24.5 ± 0.48 ^a	22.4 ± 0.28 ^b	22.6 ± 1.09 ^b	0.020
Palmitoleic acid (C16:1)	2.98 ± 0.21 ^a	1.35 ± 0.49 ^b	2.71 ± 0.43 ^a	0.005
Stearic acid (C18:0)	7.95 ± 0.33	8.24 ± 0.08	8.46 ± 0.35	0.159
Oleic acid (C18:1 n-9c)	43.2 ± 1.10	42.3 ± 0.71	42.0 ± 0.67	0.228
Vaccenic acid (C18:1 n-7)	1.95 ± 0.32 ^a	1.06 ± 0.06 ^b	1.55 ± 0.13 ^{ab}	0.005
Linoleic acid (C18:2 n-6)	14.9 ± 1.92 ^b	19.8 ± 0.31 ^a	13.7 ± 1.45 ^b	0.004
Linolenic acid (ALA, C18:3 n-3)	0.59 ± 0.03 ^b	0.27 ± 0.02 ^b	4.19 ± 0.82 ^a	0.001
Arachidonic acid (AA, C20:4 n-6)	1.82 ± 0.06 ^b	2.14 ± 0.00 ^a	1.18 ± 0.10 ^c	0.001
Eicosapentaenoic acid (EPA, C20:5 n-3)	nd	nd	0.09 ± 0.02	–
Docosapentaenoic acid (DPA, C22:5 n-3)	0.13 ± 0.01 ^b	0.08 ± 0.01 ^b	0.38 ± 0.06 ^a	0.001
Docosahexaenoic acid (DHA, C22:6 n-3)	0.89 ± 0.04 ^b	0.63 ± 0.05 ^b	2.26 ± 0.25 ^a	0.001
SFA	32.7 ± 0.5 ^a	30.9 ± 0.2 ^b	31.3 ± 0.8 ^b	0.019
MUFA	48.1 ± 1.6 ^a	44.7 ± 0.3 ^b	46.3 ± 1.0 ^{ab}	0.046
PUFA	18.3 ± 2.0 ^b	22.9 ± 0.4 ^a	21.8 ± 1.6 ^{ab}	0.022
n-6:n-3 ratio	10.5 ± 0.5 ^b	22.4 ± 0.3 ^a	2.2 ± 1.1 ^c	<0.0001
n-3	1.6 ± 0.02 ^b	0.99 ± 0.1 ^b	6.92 ± 0.9 ^a	<0.0001
n-6	17.0 ± 2.0 ^b	22.2 ± 0.3 ^a	15.1 ± 1.6 ^b	0.003

nd = not detected; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

^{a-b} Within a row, means with different superscripts are significantly different ($P < 0.05$).

it has been reported that all FA present in sunflower oil have a cis configuration.

No effect on voluntary intake was associated with the experimental diets; however, the dietary inclusion of sunflower seeds caused a significant decrease in percent and total egg production compared with the control group. A possible explanation for this effect could be the presence of antinutritional factors, since it has been reported that sunflower meal may contain up to 3.0% to 3.5% phenolic acids, which can generate o-quinones capable of binding lysine and methionine residues in proteins (Diaz, 2001). No effect on rate of lay or egg weight were observed in a previous study conducted with layers that were supplemented with full-fat sunflower seeds (Karunajeewa et al., 1989); however, the inclusion level in this study was only 2.35%, not 13.5%. Total and percent egg production were also affected by the inclusion of full-fat flaxseed seeds. Although the difference failed to reach statistical significance, numerical differences were high (36.2 eggs/hen and 65.9% egg production in control hens vs. 30.9 eggs/hen and 56.3% egg production in hens fed flaxseed). Flaxseed contains antinutritional and toxic compounds such as cyanogenic glycosides and the dipeptide linatine (Diaz, 2001), which might have played a role in the numerically lower egg production compared to the control

group. In regards to egg production, it is important to note that the total and percent egg production in all experimental groups was lower than expected for the breed and age of the hens. This decreased performance was attributed to the presentation of cutaneous fowl pox, which affected the hens for most of the experiment.

The inclusion of either sunflower or flaxseed seeds had no significant effect on any of the egg quality parameters evaluated. This finding agrees with a previous trial in which inclusion levels of up to 24.84% sunflower seed meal (not whole seed, however) did not affect any egg quality parameter (Shi et al., 2012). Similarly, in another trial, the inclusion of 10% flaxseed in laying hen diets did not affect any of the egg quality parameters evaluated (egg weight, yolk weight, shell weight, albumen weight and height, Haugh units, yolk color, and shell thickness) (Hayat et al., 2009).

In regard to the FA composition of the egg yolk lipids, a decrease in SFA has been observed with flaxseed oil. In one study, the inclusion of 0.5%, 1.0%, 1.5% or 2.0% flaxseed oil caused a lineal decrease in myristic (0.74%, 0.66%, 0.62% and 0.52%, respectively) and palmitic (29.4%, 29.2%, 28.6% and 25.7%, respectively) acids (Souza et al., 2008). Significantly lower myristic and palmitic acids percentages were also observed in the present trial in the egg yolk

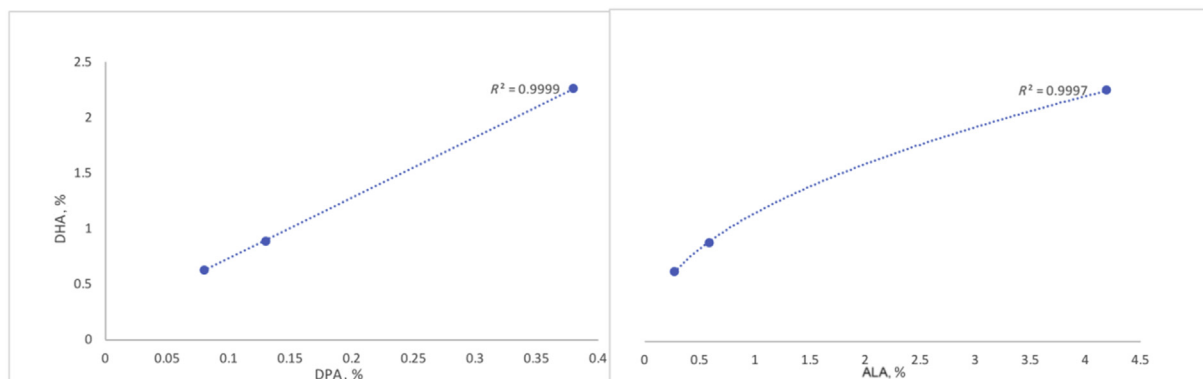


Fig. 1. Relationship between docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) and relationship between DHA and α -linolenic acid (ALA) in egg yolk fat. Each point represents the mean of 3 observations per experimental treatment (control eggs and eggs from hens receiving 13.5% flaxseed or sunflowers seeds). A linear relationship was observed between DHA and DPA, whereas a quadratic relationship was observed between DHA and ALA.

fat of the hens fed either flaxseed or sunflower seeds, compared with the control group.

The total egg yolk saturated fatty acid (SFA) content in the eggs produced by the hens receiving PUFA sources was slightly but significantly lower compared with the control group. In a previous study, the inclusion of 2% flaxseed or sunflower oils to laying hen diets resulted in an egg yolk fat SFA content of 27.7% and 30.7%, respectively (Celebi and Macit, 2008); these values are close to those found in the present trial for the eggs produced by the hens receiving sunflower and flaxseed supplementation (30.9% and 31.3% SFA, respectively). In the same trial (Celebi and Macit, 2008), total monounsaturated FA (MUFA) in the egg yolk fat were 45.4% and 46.6% for sunflower and flaxseed oil supplementation, respectively; these values are very close to the 44.7% and 46.3% found in the present study for the diets supplemented with sunflower or flaxseed seeds, respectively.

In regard to the PUFA profile in egg yolk fat, it was observed that the inclusion of flaxseed significantly increased the percentage of n-3 PUFA, an effect that has been previously reported for whole flaxseed (Aymond and Van Elswyk, 1995). Interestingly, flaxseed supplementation (oil or seeds) increases not only egg yolk ALA content, but also DPA and DHA (Scheideler and Froning, 1996). Further, it has been reported that supplementation of flaxseed results in the production of eggs enriched in n-3 PUFA as follows: ALA > DHA > DPA > eicosapentaenoic acid (EPA) (Sim and Qi, 1995). These results agree with those from the present trial, since flaxseed supplementation led to ALA, DHA, DPA and EPA concentrations of 4.19%, 2.26%, 0.38% and 0.09%, respectively. Our results confirm that laying hens can convert dietary ALA into EPA, DPA and DHA through elongation and desaturation reactions (Carrillo-Domínguez et al., 2005). Further, the percentages of ALA and DHA in egg yolk fat were highly correlated ($R^2 = 0.9997$), and also the percentages of DPA and DHA ($R^2 = 0.9999$). It is important to note that PUFA elongation and desaturation reactions are considered to be very limited in humans (Baucells et al., 2000), and therefore the supplementation of full-fat flaxseed seeds or flaxseed oil to human diets is of limited value; however, by supplementing flaxseed to hens, eggs enriched in long-chain n-3 FA can be obtained. In regards to the content of n-6 PUFA in egg yolk fat, it was observed that supplementation of full-fat sunflower seeds was associated with a significant increase in the n-6 FA LA and its product of elongation and desaturation arachidonic acid (C20:4 n-6, AA) (Grynberg, 2005). Similar results have been reported in egg fat yolk from hens fed sunflower oil (Baucells et al., 2000).

Reduction in the n-6:n-3 ratio in human diets has been promoted since the 1990s (Simopoulos, 2002) and eggs enriched with n-3 PUFA from flaxseed have become a common product in several countries (Surai and Sparks, 2001). Further, it has been recommended that a healthy ratio of n-6:n-3 FA should be lower than 4 (Valencak et al., 2015). In the present trial, the n-6:n-3 ratio obtained in the egg yolk fat from the hens fed whole full-fat flaxseed seeds was 2.2, which is well below the recommended healthy ratio of 4. It is important to note, however, that although n-3 PUFA intake is considered to be important to keep a healthy status in humans, n-6 PUFA intake is no less important. Recently, the American Heart Association (AHA) dismissed prior concerns about n-6 PUFAs and their potential role in inflammation, thrombosis and low-density lipoprotein oxidation; further, the AHA scientific advisory committee has recommended that at least 5% to 10% of the daily energy intake comes from n-6 PUFA (Khandelwal et al., 2013).

5. Conclusions

The results of the present study confirm that the supplementation of sunflower or flaxseed oil in the diet of laying hens is able to

modify the lipid profile of the egg yolk, making it healthier according to current recommendations. The results also show the feasibility of using full-fat ground sunflower or full-fat whole flaxseed seeds, which is more convenient and economical than using oils. No studies have been reported previously reporting the use of a 13.5% inclusion level of full-fat sunflower seeds (only 2.35%). More studies are needed in order to determine the dietary inclusion levels that do not affect egg laying rate and egg production.

Conflict of Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare there is no conflict of interest.

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