Effect of pumpkin (*Cucurbita maxima*) seed meal on total cholesterol and fatty acids of laying hen eggs

Y. Martínez[1], M. Valdivié[2], G. Solano[3], Mirna Estarrón[4], O. Martínez[1] and J. Córdova[5]

[1]*Universidad de Granma, Apartado Postal 21, C.P 85300, Bayamo, Granma, Cuba*

[2]*Instituto de Ciencia Animal, Apartado Postal 24, San José de Las Lajas, Mayabeque, Cuba*

[3]*Instituto de Investigaciones Agropecuarias "Jorge Dimitrov", C.P 85300, Bayamo, Granma, Cuba*

[4]*Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Av. Normalistas 800, Guadalajara, Jalisco, México*

[5]*Universidad de Guadalajara, Departamento de Ing. Química. Blvd. Marcelino García Barragán 1421, Guadalajara, Jalisco, México*

Email: ymartinez@udg.co.cu

In order to evaluate the effect of four levels of inclusion (0, 3.3, 6.6 and 10 %) of pumpkin (*Cucurbita maxima*) seed meal on total cholesterol and fatty acids in laying hen eggs, one hundred and sixty White Leghorn (L-33 Hybrid) hens were allocated at the laying peak for 91 d, according to completely randomized design. One-way analysis of variance was applied, except for the ether extract concentration, using the factorial analysis with four treatments and 20 repetitions. The ether extract concentration in the egg was increased by effect of the laying weeks and the levels of inclusion of pumpkin seed meal (31.90 to 36.15 %). This oilseed enriched the egg with the fatty acids octadecanoic (152 to 450 mg/100 g), oleic (1282 to 1918 mg/100 g), linoleic (22 to 667 mg/100 g), and α-linolenic (457 to 649 mg/100 g); whereas it reduced the amount of arachidonic acid (62 to 50 mg/100 g). There was lower ratio of saturated/polyunsaturated fatty acids (0.18 to 0.13) and omega 6/omega 3 (7.65 a 6.47). Also, the inclusion of this feed diminished the total cholesterol in 28, at 30 mg/egg, in respect to the control. Including up to 10 % of pumpkin seed meal in the laying hen diets is recommended to increase the content of ether extract and the beneficial fatty acids and reduce the total cholesterol and the harmful fatty acids in the eggs.

Key words: *fatty acids, cholesterol, eggs, seeds, pumpkin.*

The egg has great nutritional value, because it is rich in proteins, fats, fat-soluble vitamins, and minerals. Its inclusion in the daily diet is very important for the human health. Despite the nutritive quality of the egg, its intake has been reduced in the last decades. This is related to the harmful effect of cholesterol and other lipids (Simopoulos 2002).

The cholesterol reduction in the egg is a complex task, because this substance is necessary for the embryo growth. In order to reduce the harmful lipids in the egg, several drugs have been used provoking adverse reactions in the birds (Yin *et al*. 2008). Nevertheless, the most objective and healthy solution is the incorporation of functional feeds in the diet, rich in phytosteroles, dietary fiber, and essential fatty acids that also provide omega 3 and omega 6 fatty acids. These conditions favor the nutritive quality of the egg and provide greater benefits to the consumer (Simopoulos 2002 and Martínez 2009).

The eggs enriched with omega 3 and omega 6 may contribute to the prevention and cure of cardiovascular diseases, malignant tumors and inflammatory processes; besides stimulating the brain development and the mental functions (Kremer 2000). Schumann *et al*. (2003) suggested that polyunsaturatedd fatty acids (PUFAs) in the diets diminished the fat liver syndrome in laying hens and stimulate the immunity response.

Caston and Leeson (1990) and Ayerza and Coates (2000) have managed to reduce the total cholesterol and enrich the eggs with the essential fatty acids omega 3 and omega 6, when including oilseeds such as colza (*Brassica napus*) and chia (*Salvia hispanica*), respectively.

Pumpkin belongs to the Cucurbitaceae family and it has seeds rich in proteins, polyunsaturated fats, phytosterols and dietary fiber (Martínez *et al*. 2008 and Martínez 2009). In laying hens, Martínez *et al*. (2010) found reduction in the harmful lipids and increment in serum essential fatty acids when including four levels of pumpkin seed meal in the diets, not affecting the principal productive indicators. The objective of this study was to assess the effect of four levels of inclusion (0, 3.3, 6.6 and 10 %) of pumpkin (*Cucurbita maxima*) seed meal on the total cholesterol and the profile of fatty acids in laying hen eggs.

Materials and Methods

The research was conducted at the Poultry Experimental Unit of the Institute of Animal Science, located in the Mayabeque province, Cuba. The average relative humidity was 78 %, the average minimum temperature, 16.6 °C, and the average maximum temperature, 27.6 °C.

Five samples of complete pumpkin seeds were collected and mixed from different lots that belonged to the variety INIVIT C-88, species maxima. Martínez *et al*. (2010) reported the chemical composition of these seeds.

A total of 160 laying hens of the White Leghorn breed (Hybrid L-33) of 33 weeks of age (at the laying peak) were allocated for 91 d according to completely randomized design into four treatments and 20 repetitions.

Composition and contribution of the diets. The diets were formulated according to the requirements recommended by the Union of Enterprises of the National Poultry Association (UECAN) (2007). The treatments consisted of four diets, with 0, 3.3, 6.6, and 10 % of pumpkin seed meal (PSM). The ingredients used for the elaboration of each diet are shown in table 1.

Experimental procedure. The unit was a metallic cage of 40 cm x 40 cm including two hens. The birds were given 108 g of feed/hen/d. Water was supplied *ad libitum* in two nipple drinkers/cage. The light was at 16 h/d. The adaptation period was of two weeks, according to Caballero (1982). The hens were not given medicines or veterinary therapeutical care throughout the research.

Analytical determinations. Since week 39 and up to the 45, 20 eggs/treatment were collected at 8:00 a.m. The yolks were separated from the albumen and they were weighed in group. Later, they were frozen at -20 °C until their analysis in the laboratory.

The ether extract from the yolks was determined according to AOAC (1995). The anhydrous ethylic ether was considered as solvent due to its lower boiling point to prevent the formation of trans fatty acids (Martínez 2009).

A Rotavapor Heidolph was used to remove the residual anhydrous ethylic ether in the samples, with temperature regulated at 38 °C, pressure at 5 mm Hg, and speed from 90 to 100 rpm. The samples were put in a bell of extraction of solvents in an oven at 38 ºC (AOAC 1995).

The total cholesterol was determined in the yolk after mixing the samples in anhydrous ethylic ether, at a rate of 1:50 v/v, in the week 45 through enzymatic kits. A Humalyzer 2000 ultraviolet spectrophotometer was used.

For quantifying the total fatty acids, a derivation (methylation) of the fatty acids was performed, according to AOAC (2002). Later, an Agilent Technologies 6890 gas chromatographer (Palo Alto, California) was used, fitted with flame ionization detector (FID) and fitted with HP 6890 auto-sampler. The equipment was controlled by a GC Chemstation data operator, version A.09.03. The

Table 1. Composition of the diets (% in humid basis)

Raw material (%)	Levels of pumpkin seed meal (%)			
	0	3.3	6.6	10
Corn meal	57.85	58.48	57.99	56.27
Soybean cake meal	28.00	25.20	22.89	20.72
Pumpkin seed meal	0.00	3.30	6.60	10.00
Plant oil	1.19	0.45	0.00	0.00
Dicalcium phosphate	1.90	1.90	1.90	1.90
Calcium carbonate	8.65	8.62	8.60	8.58
BHT (Antioxidant)	0.01	0.01	0.01	0.01
DL-Methionine	0.19	0.19	0.20	0.21
L-Lysine	0.04	0.05	0.06	0.06
Common salt	0.25	0.25	0.25	0.25
Premixture[1]	1.00	1.00	1.00	1.00
Zeolite	0.92	0.55	0.50	1.00
Calculated contribution (%)				
ME (MJ/kg DM)	11.63	11.63	11.63	11.66
Crude protein	17.00	17.00	17.00	17.00
Lysine	0.80	0.80	0.80	0.80
Methionine	0.47	0.47	0.50	0.51
Methionine + cystine	0.73	0.73	0.73	0.73
Calcium	3.80	3.80	3.80	3.80
Available phosphorus	0.40	0.40	0.40	0.40
Ether extract	2.20	3.30	4.40	5.40
Crude fiber	3.20	3.60	4.00	4.30

[1]Each kg contains: vit. A, 10 x 10^6 I.U.; vit. D_3, 1.5x 10^6 I.U. ; vit. K_3, 2100 mg; vit. E, 10000 mg; thiamine, 800 mg; riboflavin, 2500 mg; panthotenic acid, 10000 mg; pyridoxine, 2500 mg; folic acid, 250 mg; biotin, 100mg; vit. B_{12}, 15 mg; manganese, 60000 mg; copper,8000 mg; iron, 60000 mg; zinc, 50000mg; selenium, 200 mg; iodine, 800 mg; cobalt, 500 mg; antioxidant, 125000 mg.

fatty acids in the form of methyl esters were separated in an HP-23 cis\trans capillary column (60 m x 250 µm ID x 0.25 µm of layer width).

A thin layer chromatography was run to corroborate the fat transformation into the respective methyl esters (AOAC 2002). The determinations were conducted in the laboratories of the Technological Center and State Assistance of the Jalisco Sate (CIATEJ), Mexico. The preparation of the samples was carried out in the University of Guadalajara, Mexico, and in the Institute of Animal Science in Cuba. The analyses of ether extract and of the profile of fatty acids in the yolks were performed ten times. The total cholesterol was determined five times.

A completely randomized design was applied with one-way analysis of variance, except for the variable ether extract, using analysis of variance with 7 x 4 factorial fit (seven weeks and four levels of pumpkin seed meal). The test of Duncan (1955) was used when necessary to determine the differences between means. All the data from the statistical analysis were processed with the SPSS software, version 17.0.

Results and Discussion

Table 2 provides the interaction (P < 0.05) of the laying weeks with the levels of inclusion of pumpkin seed meal for the concentration of ether extract in the yolk of laying hen eggs. The concentration was outstanding including pumpkin seed meal and with the increase in the experimental weeks.

The inclusion of 10 % of pumpkin seed meal in laying hen diets provided larger amount of ether extract to the diets (5.40 %) (table 1). This could provoke larger concentration of ether extract in the yolk (36.15 %). Besides, the high concentration of polyunsaturated fatty acids in the pumpkin seed (Martínez *et al*. 2010) could determine the greater addition of ether extract to the yolk. According to Dolz (1996), the greatest unsaturation of the fatty acids is proportional to the potential of formation of micelles, which brings about greater efficacy in fat absorption. However, the total serum lipids in the hens did not show statistical differences between treatments (P < 0.05), regardless the fact that the harmful lipids decreased (Martínez *et al*. 2010). Apparently, the high concentration of the unsaponifiable material in the INIVIT C-88 pumpkin seed (34.70 g/kg) (Martínez *et al*. 2011) could contribute to the concentration of ether extract in the yolk.

Ayerza and Coates (2000) noted that the ether extract of the yolk was increased when including the chia seed up to 7 %. However, when including 28 %, the effect was the opposite, being associated with the crude fiber contribution. According to Dolz (1996), crude fiber is antagonist to the fat absorbed. Thus, the laying hens, when fed pumpkin seed meal, have crude fiber content lower than 5 %.

The laying weeks and the age of the hens determine higher concentration of ether extract in the yolk, related to the increment in the egg size, and the weight and height of the yolk with age (Martínez 2009 y Grobas *et al*. 2001). Besides, for the maintenance of the bird with higher body weight, the lipid synthesis *in novo* (liver), and the fat transport in the very low density lipoproteins increase with higher incorporation of lipids to the yolk (Grobas *et al*. 2001).

The total cholesterol in the yolk of the eggs from laying hens was superior (P < 0.05) for the control compared with the 3.3, 6.6, and 10 % of pumpkin seed meal inclusion. The mg/egg and mg/g of cholesterol were reduced when including the pumpkin seed meal in the diets (table 3).

The reduction of 1.71 mg of cholesterol/g in yolk was positive, considering the several diseases caused by the excessive intake of this lipid (Elkin 2009). The inclusion of 3.3, 6.6, and 10 % of pumpkin seed meal decreased in 28, 30, and 27 mg of cholesterol in each egg, respectively. With the inclusion of pumpkin seed meal in the feedstuffs, the same cholesterol reduction is attained in the egg.

The cholesterol reduction in the yolk could be determined by the presence of unsaturated fatty acids in the pumpkin seed meal. Martínez *et al*. (2010) proved

Table 2. Interaction of different levels of inclusion of pumpkin seed meal and the laying weeks in the concentration of ether extract of the eggs of laying hens for seven weeks

	Levels of pumpkin seed meal (%)				
Laying weeks	Control	3.3	6.6	10	SE ±
39	31.74^{a}	33.94^{e}	34.91^{g}	35.02^{g}	0.10***
40	31.82^{ab}	33.93^{e}	34.87^{g}	36.06^{hi}	
41	31.98^{ab}	33.93^{e}	34.86^{g}	35.76^{h}	
42	31.87^{ab}	33.06^{d}	34.72^{g}	36.14^{i}	
43	32.05^{bc}	34.06^{ef}	35.01^{g}	36.15^{i}	
44	32.30^{c}	34.25^{f}	34.96^{g}	35.83^{h}	
45	32.30c	34.25^{f}	34.96^{g}	35.83^{h}	

^{a,b,c,d,e,f,h,i} Means with different letters differ at P < 0.05 (Duncan 1955) *** P < 0.001

Table 3. Total cholesterol in the yolk of eggs from laying hens fed different levels of pumpkin seed meal

Total cholesterol	Levels of pumpkin seed meal (%)				
	0	3.3	6.6	10	E± Sig
mg/egg	249.00[a]	221.00[b]	219.00[b]	222.00[b]	0.43***
mg/g yolk	14.91[a]	13.24[b]	13.28[b]	13.57[b]	0.14***

[a,b] Medias with different letters in the same row differ at P < 0.05 (Duncan 1955)
*** P<0.001

that the fatty acids linoleic (13894.00 mg/100 g) and oleic (8616.98 mg/100 g) in the pumpkin seed meal reduce the serum circulation of the low density lipoproteins (LDL) and the total cholesterol. This could provoke lower amount of total cholesterol in the yolk.

Cherian and Sim (1991), by using linseed (*Linum usitatissimum*) rich in α-linolenic (C18:3n-3), in laying hen diets, proved that the cholesterol was not reduced in the yolk ($P < 0.05$). Rowghani *et al*. (2007) noted that the linoleic acid has more evident hypo-cholesterol effects than the α-linolenic acid. This could affect the result with linseed.

The pumpkin seed meal INIVIT C-88 is rich in phytosterols (233.08 mg/100 g) (Martínez *et al*. 2011). Campesterol and beta-sitosterol in pumpkin seed meal contribute to the reduction of the total serum cholesterol in the hens, with possible reducing effect on the egg (Martínez *et al*. 2010). Liu *et al*. (2010) observed reduction of the cholesterol in the egg since the sixth week of experimentation, by using high levels of phytosterols. This proved the hypocholesterol effectiveness of the sterols in the pumpkin seed. Elkin and Lorenz (2009) reported low incorporation of sterols in the egg yolk, associated with the cholesterol reduction in the yolk, and with the low esterification by the enzyme 3-hydroxy-3-methylglutaril-CoA reductase.

According to Martínez (2009), the dietary fiber in the pumpkin seed meal (41.4 %) could decrease the cholesterol in the egg yolk. Ayerza and Coates (2000), by including chia seed in the diets of laying hens up to 28 %, noted reduction in the yolk cholesterol, which is related to the dietary fiber and the high percentages of unsaturated fatty acids in the diets. Savón *et al*. (2007) reported that the fiber reduces the cholesterol absorption by binding with the biliary salts in the gastrointestinal tract, with reduction in the time of the intestinal passage and increase in the excretion de sterols in the feces.

Despite the hypocholesterol characteristics of the pumpkin seed meal, the total cholesterol in the yolk was not reduced in progressive form. Ayerza and Coates (2000), Elkin and Lorenz (2009) and Liu *et al*. (2010) found similar results. If it is considered that the cholesterol is essential for birds, the synthesis of endogenous cholesterol (liver) increases, apparently, to create homeostasis, and it is deposited in the egg for the embryo (Yin *et al*. 2008). At the same time, the rise in the ether extract in the egg yolk with levels of pumpkin seed meal increases the lipids in the yolk, among them the cholesterol.

Table 4 shows the profile of fatty acids in the yolk of eggs from laying hens fed pumpkin seed meal contents. Palmitic (C16:0) is the most representative saturated fatty acid in the yolk, which was increased when including pumpkin seed meal. Also, the octadecanoic (C18:0) showed high values, with higher amounts for the 3.3 % of pumpkin seed meal.

The fatty acid oleic (C18:1n-9), the most abundant mono-unsaturated in the yolk, had values superior to the control, when including up to 10 % of pumpkin seed meal in the diets. Among the mono-unsaturated fatty acids, the linoleic acid (C18:2n-6) was the predominant in the yolk. The same occurred with the poly-unsaturated fatty acid α-linolenic (C18:3n-3). Both showed significant differences ($P < 0.05$) compared with the control. Besides, the arachidonic acid decreased in respect to the level of inclusion of pumpkin seed meal.

The important ratios of n6/n3 and of saturated fatty acids/poly-unsaturated fatty acids (table 4) were lower ($P < 0.05$) with 3.3, 6.6, and 10 % of pumpkin seed meal, compared with the control.

The pumpkin seed is within the reduced and privileged group of oilseeds having essential fatty acids, although the variability in the omega 3 and omega 6 should be considered, according to the species and varieties (Martínez 2009).

The eggs of the hens fed 10 % of pumpkin seed meal were enriched in 617 mg of linoleic acid/100 g of yolk compared with the control (table 4). Caston and Leeson (1990) and Cherian and Sim (1991) obtained similar results in the content of omega-6 in the egg yolks, when supplementing the diets of the hens with linseed and colza, respectively. This proves that the level of inclusion of omega 6 in the diets affects the percentage incorporated to the egg.

The content of arachidonic fatty acid (C20:4n-6) in the egg yolk decreased with the inclusion of 6.6 and 10 % of pumpkin seed meal (0.39 and 0.40 g/ 100 g of yolk) compared with the control (0.45 g/ 100 g of yolk) (table 4). Martínez *et al*. (2010) confirmed decline in arachidonic acid in the blood serum of the hens, when including up to 10 % of pumpkin seed meal. This could reduce the incorporation of arachidonic acid to the egg. Researches with levels of inclusion of chia seed and linseed in laying hens brought about similar results by

Table 4. Profile of fatty acids in the yolk of eggs from laying hens fed different levels of pumpkin seed meal (mg/100g).

Fatty acids	Levels of pumpkin seed meal (%)				SE± Sig
	0	3.3	6.6	10	
C12:0	3.87 ±0.30[a]	3.66 ±0.30[ab]	3.93 ±0.20[a]	2.97 ±0.20[b]	*
C13:0	120.27[c]	127.72[b]	133.78[b]	140.61[a]	0.26***
C15:0	24.24	25.68	23.90	24.03	1.04
C16:0	8348.00[b]	8612.00[ab]	8756.00[a]	8833.00[a]	42.83*
C17:0	65.39[b]	75.87[a]	73.83[a]	79.74[a]	2.87*
C18:0	2910.00[c]	3360.00[a]	3062.00[b]	3198.00[ab]	91.03*
C24:0	28.90[ab] ±2.90	22.90[b] ±3.67	28.10[ab] ±2.80	34.80a ±3.10	**
C15:1	22.25[b]	21.15[b]	24.63[a]	23.28[a]	0.91*
C16:1n-7	884.91	896.60	964.00	904.00	31.26
C18:1n-9	12007.00[c]	13289.00[b]	13379.00[b]	13925.00[a]	192.70***
C18:1n-5	542.05	577.16	557.77	548.39	12.47
C20:1	78.60[c]	85.90[ab]	88.30[a]	84.27[b]	1.32***
C18:2n-6	5825.00[b]	5847.00[b]	6236.00[a]	6492.00[a]	101.10***
C18:3n-3	327.72[c]	784.69[b]	950.00[a]	976.00[a]	87.77***
C20:4n-6	449.90[a]	461.16[a]	387.50[b]	399.72[b]	15.89**
C22:6n-3	115.00	117.00	126.00	128.00	15.53
∑ AGS	11499.00[b]	12484.00[a]	12092.00[ab]	12171.00[ab]	240.20**
∑ AGMI	13535.00[c]	14870.00[b]	14852.00[b]	15485.00[a]	211.00***
∑ AGPI	6729.00[c]	7215.00[b]	7659.00[ab]	7987.00[a]	163.10***
AGS/AGPI	1.71[ab]	1.78[a]	1.58[ab]	1.53[b]	0.06*
∑n-6	6275.00[b]	6308.00[b]	6623.00[ab]	6888.00[a]	117.90**
∑n-3	454.04[b]	907.57[a]	1035.00[a]	1095.00[a]	89.69***
n6/n3	15.74[a]	9.27[b]	8.09[b]	8.71[b]	1.35***

[a,b,c,d] Means with different letters in the same row differ at $P < 0.05$ (Duncan 1955)

*$P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

FA: fatty acid, SFA= saturated fatty acids, MUFA= mono-unsaturated fatty acids, PUFA= poly-unsaturated fatty acids, n-6=Omega 6, n-3=Omega 3.

decreasing the arachidonic acid in the egg (Cherian and Sim 1991 and Ayerza and Coates 2000).

By increasing the levels of inclusion of pumpkin seed meal, there was progressive rise in the omega-3 in the egg, from 454 mg/100 g of yolk for the control up to 1095 mg/100 g of yolk for the inclusion of 10 % of pumpkin seed meal (table 4). In the reports on the introduction of feeds rich in alpha-linolenic fatty acids in diets of laying hens it is proved the trend toward the enrichment of the egg with the omega 3 (Caston and Leeson 1990, Cherian and Sim 1991, Ayerza and Coates 2000 and Rowghani *et al*. 2007).

The sum of the mono-unsaturated fatty acids and the poly-unsaturated fatty acids in the egg yolk (table 4) was increased compared with the level of inclusion of pumpkin seed meal, due to the concentration of the fatty acids oleic, linoleic and alpha-linolenic in the pumpkin seed meal, and to the serum circulation and its incorporation to the egg (Martínez *et al*. 2010). Suksombat *et al*. (2006) obtained similar results; when supplementing the feedstuff with sunflower oil they reported rise in the mono-unsaturated fatty acids in the egg yolk.

The docosahexonoic fatty acid was not increased in the yolk by increasing the levels of pumpkin seed meal in the feedstuff (table 4), regardless its higher concentration in the blood serum of the hens when including the pumpkin seed meal (Martínez *et al*. 2010). According to Grobas *et al*. (2001), due to the physiological peculiarities of the birds, the incorporation of the fatty acids resulting from elongation and desaturation is poor. Ayerza and Coates (2000) found increment of docosahexonoic fatty acid by using up to 28 % of chia seed. The high concentration of omega 3 in the chia seed (65 %) and the level of inclusion could affect this outcome.

The European Commission Community Research (2000) noted requirements of fatty acids omega 3 of the order of 1 g d^{-1}. However, the National Food and Nutrition Board (2005) reported intake of 110-160 mg d^{-1}. Simopoulos (2002) recommended 2.2 g d^{-1} of the α-linolenic fatty acid. An egg of laying hens fed up to 10 % of pumpkin seed meal has approximately 186 mg of omega 3. This turns it into a functional feed that may fulfill the omega 3 requirements in humans.

The omega 6/omega 3 ratio (n6/n3) decreased as the content of pumpkin seed meal was increased in the feedstuffs. This ratio diminished from 15.74 for the control up to 8.09 for the diet with 6.6 % of pumpkin seed meal (table 4). The World Health Organization (1995) in its report on fats and oils in human nutrition recommended n-6/n-3 ratios of 5-10/1 in the diets to prevent atherosclerosis and cardiovascular risks. The values in table 4 are within the range of reference proposed. Nevertheless, the British Nutrition Foundation (1992) reported a ratio of omega 6/omega 3 of 6/1. Simopoulos (2002) recommended ratios of 2.1/1.

When including up to 10 % of pumpkin seed meal (INIVIT C-88) in the diets of laying hens, the ether extract was increased with interaction with the laying weeks and the beneficial fatty acids. On the contrary, the total cholesterol and the harmful fatty acids wee reduced in the eggs.

References

AOAC. 1995. Official Methods of Analysis. Ass. Off. Agric. Chem. 16th Ed. Washington. D.C. USA

AOAC. 2002. Methyl esters of fatty acids in oils and fats 969.33. En: Official Methods of Analysis. 17th Ed. Gaithersburg, Maryland. p. 19

Ayerza, R. & Coates, W. 2000. Dietary levels of chia: Influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. Poult. Sci. 78:724

Caballero, A. 1982. Folleto de diseño experimental. Instituto Nacional de Ciencias Agrícolas. La Habana, Cuba. p. 30

Caston, L. & Leeson, S. 1990. Dietary flax and egg composition. Poult. Sci. 2:1617

Cherian, G. & Sim. J. S. 1991. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of eggs, embryos and newly hatched chicks. Poult. Sci. 70: 917

Dolz, S. 1996. Utilización de las grasas y subproductos lipídicos en monogástricos. XII Curso de especialización FEDNA. p. 11

Duncan, B. 1955. Multiple ranges and multiple F test. Biometrics 11:1

Elkin, R.G. 2009. Additional perspectives on analytical techniques and standardization: Cholesterol and fatty acid contents of eggs, tissues, and organs. Poult. Sci. 88:249

Elkin, R. & Lorenz, E. 2009. Feeding laying hens a bioavailable soy sterol mixture fails to enrich their eggs with phytosterols or elicit egg yolk compositional changes. Poult. Sci. 88:152

European Commission Community Research. 2000. Project report: functional food science in Europe. Office for official publications of the European Communities L-2985, Luxembourg

Grobas, S., Méndez, J., Lázaro, R., Blas, C. & Mateos, G. 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poult. Sci. 80:1171

Kremer, J. M. 2000. N-3 fatty acid supplements in rheumatoid arthritis. Am. J. Clin. Nutr. Suppl. 1:349

Liu, X., Zhao, H. L. Thiessen, S., House, J. D. & Jones, H. 2010. Effect of plant sterol-enriched diets on plasma and egg yolk cholesterol concentrations and cholesterol metabolism in laying hens. Poult. Sci. 89: 270

Martínez, Y. 2009. Caracterización química de la harina de semilla de calabaza y su empleo en la alimentación de gallinas ponedoras y pollos de ceba. PhD Thesis. Instituto de Ciencia Animal. La Habana, Cuba

Martínez, Y., Martínez, O., Córdova, J., Valdivié, M. & Estarrón, M. 2011. Fitoesteroles y escualeno como hipocolesterolémicos en cinco variedades de semillas de *Cucurbita maxima* y *Cucurbita moschata* (calabaza). Rev. Cubana Plant. Med. 16:72

Martínez, Y., Valdivié, M., Estarrón, M., Solano, G & J. Córdova. 2010. Serum lipid profile of laying hens fed pumpkin (*Cucurbita maxima*) seed level. Cuban J. Agric. Sci. 44:393

Martínez, Y., Valdivié, M., La O., A. L. & Leyva, E. 2008. Potencialidades de la semilla de calabaza como alimento para monogástricos. Rev. ACPA. 4: 20

National Food and Nutrition Board. 2005. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Available: <http://www.iom.edu/CMS/3788/4576/4340.aspx> [Consulted: 03/06/2010]

Rowghani, E., Arab, M., Nazifi, S. & Bakhtiari, Z. 2007. Effect of Canola Oil on Cholesterol and Fatty Acid Composition of Egg-yolk of Laying Hens. Inter. J. Poult. Sci. 2: 111

Savón, L., Scull, I. & Martínez, M. 2007. Integral foliage meals of three tropical legumes for poultry feeding. Chemical composition, physical properties and phytochemical screening. Cuban J. Agric. Sci. 41:339

Schumann, E., Squires, J. & Leeson, S. 2003. Effect of dietary flaxseed, flax oil and n-3 fatty acid supplement on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. Brit. Poult. Sci. 44: 234.

Simopoulos, A. P. 2002. The importance of the ratio of omega- 6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy. 56: 365

Suksombat, W., Samitayotin, S. & Lounglawan, P. 2006. Effect of conjugated linoleic acid supplementation in layer diet on fatty acid compositions of egg yolk and layer performances. Poult. Sci. 85:1603

Unión Estatal del Centro Avícola Nacional. 2007. Requerimientos nutritivos de las ponedoras White Leghorn L-33 cubanas. Ed. Ministerio de la Agricultura. p. 1

World Health Organization. 2008. Fats and oils in human nutrition: report of a joint expert consultation. Food and agriculture Organization of the United Nations and the World Health Organization. FAO Food Nutr. 57. p. 147

Yin, J. D., Shang, X., Li, D. F., Wang, F. L., Guan, F. & Wang, Z. Y. 2008. Effects of dietary conjugated linoleic acid on the fatty acid profile and cholesterol content of egg yolks from different breeds of layers. Poult. Sci. 87:284

Received: February 23, 2011