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Effect of Feeding Canola Oil and Vitamin A on the Fatty Acid Profile of Egg Yolks in Laying Hens

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Abstract: Two hundred and forty White Leghorn laying hens at 48th week of age were randomly divided into 24 experimental units. These experimental units were allotted to eight treatment groups which were fed diets with 0, 2, 3 and 4% canola oil with 3000 or 10000 IU vitamin A/kg of diet (4 x 2 factorial design), for a period of 12 weeks in order to observe the effects of feeding canola oil and vitamin A on the fatty acid profile of egg yolks. Two eggs per replicate were collected at the end of trial and analyzed for fatty acid contents of egg yolks. The increase in dietary canola oil levels increased ($p<0.001$) both n-6 and n-3 Polyunsaturated Fatty Acids (PUFA) but the increase in n-3 PUFA was more pronounced. The n-6/n-3 ratio decreased with the increase in dietary canola oil levels. However, the increase in dietary vitamin A level did not influence ($p>0.05$) the egg yolk fatty acid composition.

Key words: Canola oil, n-3 PUFA, laying hens

INTRODUCTION

Omega-3 polyunsaturated fatty acids (n-3 PUFA) play an important role in reducing blood viscosity and pressure, platelet aggregation, cardiac arrhythmia and plasma triglycerides (Simopoulos, 2000) in humans. Dietary intake of n-3 PUFA decreases risk of heart disease (Tample, 1996), provide an inhibitory effect on the growth of prostate and breast cancer (Rose, 1997), delays the loss of immunological functions (Fernandes, 1995) and is required for normal fetal brain and visual development (Neuringer *et al*., 1998). Considering the potential health benefits of n-3 PUFA, these should be increased in the human diet. In this regard, eggs are potential source of n-3 fatty acids because they can be easily enriched with n-3 PUFA by dietary modifications of the laying hens. The enrichment of laying-hen rations with vegetable oils, such as canola oil, readily promotes the deposition of n-3 PUFA into egg yolk (Van Elswyk, 1997). Canola oil may clearly increase the n-3 PUFA contents in the form of Linolenic Acid (LNA), the precursor of whole n-3 family of fatty acids (Lopez-Ferrer *et al*., 2001). Canola oil has what is now considered to be an almost perfect balance of n-6 to n-3 PUFA; the n-6 to n-3 ratio in canola oil is 2:1 which perfectly matches with human requirements. In PUFA-enriched eggs, the control of lipid per-oxidation is required to prevent the loss of nutritional values (Chow, 1992). Supplementation of hens' diet with vitamin A may increase the content of this vitamin in eggs (Mendonca *et al*., 2002) and can prevent any possible lipid per-oxidation in eggs and egg products. Moreover, considering the potential health benefits described for vitamin A (Food and Nutrition Board, 2000), enriched poultry eggs can be a useful source of this vitamin in human diet.

Most of the trials conducted in the past used linseed or marine oils for enrichment of eggs with n-3 PUFA. However, A little information is available on use of canola oil to enhance the n-3 PUFA contents of egg. So, the present trial was conducted to evaluate the supplemental effects of canola oil, in combination of vitamin A, on egg yolk fatty acid composition in laying hens.

MATERIALS AND METHODS

Two hundred and forty White Leghorn laying hens at 48th week of age were randomly divided into 24 basic experimental units; each comprising of 10 laying hens and was designated as a replicate. These replicates were allotted to eight treatment groups (three replicates/treatment) which were fed diets with 0, 2, 3 and 4% canola oil with 3000 or 10000 IU vitamin A /kg of diet (4 x 2 factorial design). The hens were kept in cages (2 birds/cage) providing 0.093 m^2 floor space area to each, throughout the experimental period of 12 weeks. All the diets were isocaloric and isoproteinous formulated according to the recommendations of National Research Council (1994). Hens had ad libitum access to feed and water throughout the experimental period. The light regime was 16L:8D for all treatment groups. Two eggs per replicate were collected at the end of trial and analyzed for fatty acid contents of egg yolks.

Analysis: Chemical analysis of the layer ration was run using international procedures of AOAC (1990). Ingredients and chemical composition of layer ration are shown in Table 1. The fatty acid contents of egg yolks

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Table 1: Ingredient and nutrient composition of layer diets

Diets	T_1^a &T_2^b	T_3^a &T_4^b	T_5^a&T_6^b	T_7^a &T_8^b
Ingredients	---------- % ----------			
Corn	65.00	53.00	50.00	48.00
Rice broken	4.40	10.85	11.60	11.45
Soybean meal	13.00	19.80	23.40	27.10
Fish meal 52%	5.50	0.00	0.00	0.00
Corn gluten	4.00	4.90	2.50	0.00
Canola oil	0.00	2.00	3.00	4.00
Limestone	6.92	7.25	7.35	7.40
DCP	0.72	1.65	1.65	1.60
L-lysine	0.08	0.14	0.06	0.00
Dl-methyonine	0.03	0.07	0.85	0.10
Vit./min. premix[1]	0.35	0.35	0.35	0.35
Total	100	100	100	100
Nutrients				
CP (%)	17.00	17.00	17.00	17.00
ME (Kcal/Kg)	2900	2900	2900	2900
EE (%)	3.22	4.30	5.14	6.00
CF (%)	3.88	3.70	3.91	4.07
Ca (%)	3.24	3.27	3.30	3.26
Av.P (%)	0.41	0.43	0.42	0.44
Lysine (%)	0.90	0.92	0.91	0.90
Methionine (%)	0.38	0.37	0.36	0.4
Threonine (%)	0.64	0.66	0.65	0.63
LA[2] (%)	1.50	1.66	1.81	1.98
LNA[3] (%)	0.07	0.26	0.36	0.46

[1]Provided per kilogram of diet: Cholecalciferol, 1,250 IU; Vitamin E (dl-alpha-tocopheryl acetate), 12 IU; menadione, 2.5 mg; riboflavin, 6 mg; calcium pantothenate, 8 mg; niacin, 15 mg; pyridoxine 2 mg; folic acid, 1 mg; vitamin B_{12}, 7 µg; Mn, 50 mg; Zn, 55 mg; Fe 40 mg; Cu, 4 mg; I, 2 mg; Co, 0.3 mg; ethoxiquin, 150 mg. [a]rations containing 3000 IU/kg of diet vitamin A; [b]rations containing 10000 IU kg of diet vitamin A; [2]LA = Linoleic Acid;[3]LNA = Linolenic Acid

were determined by gas chromatography. The egg yolks were separated by breaking the eggs. Yolk lipids were extracted according to AOAC (1995) by using chloroform and absolute alcohol (1:1). Fatty acids were converted into Fatty Acid Methyl Esters (FAME) according to the method described by Chin *et al*. (1992) by using 4 ml HCl in 100 ml methanol. FAME were Separated and quantified by gas Chromatograph (Varian 3900) using a fused silica capillary column (30 M, 0.25 mm diameter).

Statistical analysis: The statistical analysis was performed using the two-way ANOVA by GLM and Tukey's honestly significant difference test was used to compare means (Minitab 13.1, Minitab Inc., State College, PA).

RESULTS

The amount of oleic acid (OA; an n-9 fatty acid) was increased ($p<0.001$) by feeding canola oil (Table 2). The total n-6 and n-3 PUFA in egg yolks of laying hens increased ($p<0.001$) with the increase in dietary canola oil level (Table 2 and 3). The supplementation of 4% canola oil produced best results with highest n-6 and n-3 PUFA level in the yolks. The ratio of n-6 to n-3 PUFA decreased ($p<0.001$) with the increase in canola oil level

Table 2: Effect of canola oil and vitamin A on egg yolk Oleic Acid (OA), Linoleic Acid (LA), Linolenic Acid (LNA) and Arachidonic Acid (AA) contents (% of total fatty acids) in laying hens

Diet	OA (%)	LA (%)	LNA (%)	AA (%)
Canola oil (%)				
0	37.90[d]	13.70[c]	0.89[b]	1.86
2	41.98[c]	14.76[b]	1.99[a]	1.82
3	43.39[b]	14.79[b]	1.92[a]	1.89
4	44.28[a]	15.64[a]	1.98[a]	1.97
SEM	0.193	0.079	0.035	0.044
Vit. A (IU/kg diet)				
3000	41.79	14.75	1.69	1.88
10,000	41.99	14.70	1.69	1.89
SEM	0.137	0.056	0.025	0.031
ANOVA	---------- Probabilities ----------			
Oil	0.000	0.000	0.000	0.196
Vit. A	0.329	0.587	0.917	0.913
Oil x Vit. A	0.366	0.665	0.637	0.981

[a-d]Means within a column with different superscripts differ significantly ($p<0.001$)

of the diet in laying hens (Table 2). The lowest ratio was observed at the level of 4% canola oil in the diet. However, vitamin A level higher than NRC recommendations did not produce any significant ($p>0.05$) affect on egg yolk n-6 PUFA, n-3 PUFA or n-6/n-3 PUFA ratio in laying hens. No dietary interaction ($p>0.05$) was observed between canola oil and vitamin A for egg yolk n-6 PUFA, n-3 PUFA or n-6/n-3 PUFA ratio in laying hens.

DISCUSSION

The n-6 PUFA: Linoleic Acid (LA) and total n-6 PUFA of egg yolks followed an increasing trend with the increase in canola oil in the diet of hens. Canola oil contains almost 20% LA (Rowghani *et al*., 2007) (Which is double than LNA in canola oil), so the dietetic increase in canola oil resulted into an increase in LA content of egg yolks. The Arachidonic Acid (AA) content in yolks was not increased with the increase in canola oil of the diet. It could be suggested that LNA have more preference than LA for desaturation and elongation in the liver metabolism of hens (Bean and Leeson, 2003). That's why AA level could not be increased instead of increased level of LA in the diet. On overall basis, total n-6 PUFA increased in egg yolks with the increase in canola oil in the diet, mainly because of increased LA contents of yolks. The n-6 PUFA contents remained similar for both dietary vitamin A levels. Similar to the present results, the addition of canola oil to the hens' diet increased the concentration of LA in the egg yolks (Da Silva Filardi *et al*., 2005). Canola oil supplementation resulted into an increase in LA content of thigh and breast muscles of broilers (Nobar *et al*., 2007). Shafey *et al*. (2003) had reported an increase in LA contents in yolks of hens fed on sunflower oil in the diet. The results of current study are not in agreement with Aydin (2005) who fed various

Table 3: Effect of canola oil and vitamin A on Eicosapantanoic Acid (EPA), Docosapantanoic Acid (DPA), Docosahexanoic Acid (DHA), total n-3 and n-6 PUFA (% of total fatty acids) and n-6:n-3 in laying hens

Diet	EPA	DPA	DHA	n-3 PUFA	n-6 PUFA	n-6:n-3
	(%)					
Canola oil (%)						
0	0.07[b]	0.23[c]	1.24[d]	2.44[d]	15.57[c]	6.39[a]
2	0.13[a]	0.26[b]	1.70[c]	4.08[c]	16.59[b]	4.07[b]
3	0.15[a]	0.25[bc]	2.30[b]	4.63[b]	16.69[b]	3.60[c]
4	0.13[a]	0.30[a]	2.97[a]	5.39[a]	17.61[a]	3.27[d]
SEM	0.005	0.006	0.025	0.027	0.094	0.036
Vit. A (IU/kg diet)						
3000	0.12	0.26	2.04	4.12	16.63	4.31
10,000	0.11	0.26	2.06	4.14	16.59	4.35
SEM	0.003	0.004	0.017	0.019	0.066	0.026
ANOVA	Probabilities					
Oil	0.000	0.000	0.000	0.000	0.000	0.000
Vit. A	0.195	1.000	0.342	0.628	0.681	0.414
Oil x Vit. A	0.352	0.539	0.670	0.119	0.787	0.224

[a-d]Means within a column with different superscripts differ significantly ($p<0.001$)

canola oil levels (0.5-10%) in the diet of laying hens and concluded that oil supplementation did not produce any change in n-6 PUFA of egg yolk. On other hand, Nobar *et al*. (2007) reported a decrease in total n-6 PUFA in the muscles of broilers fed diets with canola oil. The increase in OA contents of egg yolks is due to the fact that canola oil has more than 50% OA in it which favoured the deposition of this fatty acid in the egg yolks.

The n-3 PUFA: Canola oil has a handsome quantity of LNA (more than 10%) in it (Da Silva Filardi *et al*., 2005). The increase in dietary canola oil caused a significant increase in LNA and its derivatives Eicosapantanoic Acid (EPA), Docosapantanoic Acid (DPA), Docosahexanoic Acid (DHA) and total n-3 PUFA in egg yolks of hens, as depicted in the present results. Although canola oil have no EPA, DPA and DHA in it, the increase in these fatty acids with the increase in canola oil in the diet confirmed the ability of laying hens to convert LNA into its long chain metabolites as reported earlier (Schumann *et al*., 2000). With the increase in dietary canola oil levels, the total n-3 PUFA increased at a higher magnitude than total n-6 PUFA in the egg yolks. The present results are in line with Rowghani *et al*. (2007) who observed an increase in LNA, EPA, DPA and total n-3 PUFA contents of egg yolk by the supplementation of 3% and 5% canola oil to the laying hens. The addition of canola oil to the hens' diet promoted the enrichment of egg with LNA, DHA and n-3 PUFA as a whole, to some extant in the egg yolk (Da Silva Filardi *et al*., 2005). Hens fed diets supplemented with combination of fish oil (4%) and rape seed oil (2%) laid eggs with higher content of n-3 PUFA (LNA, EPA, DPA and DHA) in yolk lipids (Skrtic *et al*., 2007). Aydin (2005) fed various canola oil levels (0.5-10%) in the diet of laying hens and concluded that as the proportion of canola oil increased in the diet of layers, the concentrations of LNA and total n-3 PUFA in the yolk increased. Cherian and Sim (1991) reported increased LNA and DHA content of egg yolks with feeding 16% canola seed in the diet. Canola oil supplementation dramatically increased the LNA, EPA and DHA content of thigh and breast muscles of broilers (Nobar *et al*., 2007). As for as vitamin A; its higher level in the diets did not affect the n-3 fatty acid composition of egg yolks.

n-6/n-3 PUFA ratio: Canola oil has an ideal LA/LNA ratio of 2:1 in it (Simopoulos, 2000). Laying hens in this study showed the ability to deposit fatty acids in accordance to what is fed in the ration. The LA/LNA ratio in yolks decreased with the increase in dietary canola oil level. The increase in n-3 PUFA contents at a high magnitude than n-6 PUFA resulted into decreased n-6/n-3 PUFA ratio in egg yolks of hens fed on canola oil in the diet. According to the previous studies also, the addition of canola oil to the hens' diet can lower the n-6:n-3 ratio in the egg yolk (Da Silva Filardi *et al*., 2005). Similarly, Aydin (2005) offered various canola oil levels (0.5-10%) to the laying hens and concluded that n-6:n-3 ratio in the egg yolk decreased in a linear fashion with the increase in canola oil level. Pita *et al*. (2006) found that 6% canola oil in the diet of hens resulted n-6/n-3 ratio of 6.48 in the egg yolks. However, in the present study, vitamin did not produce any favorable effect on n-6/n-3 fatty Acid ratio of egg yolks.

Conclusion: The inclusion of canola oil in the diet of laying hens promoted the deposition of n-6 and n-3 FUPA in egg yolks. The increase in n-3 PUFA was more pronounced. So, the inclusion of canola oil in the diet of laying hens resulted into the eggs with better proportion of n-3 PUFA. As n-3 PUFA are more beneficial for human health than other type of fatty acids, n-3 enriched eggs produced by canola oil feeding to laying hens are more valuable for human beings than ordinary commercial eggs.

REFERENCES

AOAC, 1990. Official Methods of Analysis. Association of Agricultural Chemists. Virginia, DC., USA.

AOAC, 1995. Official Methods of Analysis. 16 Edn., Association of official analytical chemists, Arlington, VA.

Aydin, R., 2005. The effects of Conjugated Linoleic Acid (CLA) and canola oil on the fatty acid composition and egg quality of eggs from laying hens. South African J. Anim. Sci., 35: 172-180.

Bean, I.D. and S. Leeson, 2003. Long-term effect of feeding linseed on performance and egg fatty acid composition of brown and white hens. Poult. Sci., 82: 388-394.

Cherian, G. and J.S. Sim, 1991. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of eggs, embryo and newly hatched chickens. Poult. Sci., 70: 917-992.

Chin, S.F., W. Liu, J.M. Storkson, Y.L. Ha and M.W. Pariza, 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Compos. Anal., 5: 185-197.

Chow, C.K., 1992. Biological effects of oxidized fatty acids. Pages 689-705 in: Fatty Acids in Food and Their Health Implications. C.K. Chow, Ed. Marcel Dekker, Inc., New York, NY.

Da Silva Filardi, R., O.M. Junqueira, A.C. de Laurentiz, E.M. Casartelli, E. Aparecida Rodrigues and L. Francelino Araujo, 2005. Influence of different fat sources on the performance, egg quality and lipid profile of egg yolks of commercial layers in the second laying cycle. J. Applied Poult. Res., 14: 258-264.

Fernandes, G., 1995. Effects of calorie restriction and omega-3 fatty acids on autoimmunity and aging. Nutr. Rev., 53: S72-S79.

Food and Nutrition Board, 2000. Dietary reference intakes for vitamin A, vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zink. National Academy Press, Washington, DC.

Lopez-Ferrer, S., M.D. Baucells, A.C. Barroeta, J. Galobart and M.A. Grashorn, 2001. n-3 enrichment of chicken meat using fish oil: 2. Use of precursors of long chain polyunsaturated fatty acids: linseed oil. Poult. Sci., 80: 753-761.

Mendonca, Jr. C.X., C.R.M. Almeida, A.V. Mori and C. Watanbe, 2002. Effects of dietary vitamin A on egg yolk retinol and tocopherol levels. J. Applied Poult. Res., 11: 373-378.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., National Academy Press, Washington DC.

Neuringer, M., G.J. Anderson and W.E. Conner, 1998. The essentiality of ω-3 fatty acids for the development and function of the retina and brain. Annu. Rev. Nutr., 8: 517-541.

Nobar, R.S.D., K. Nazeradl and A. Gorbani, 2007. Effect of canola oil on saturated fatty acids contents in broiler meat. J. Anim. Vet. Advances, 6: 1204-1208.

Pita, M.C.G., E. Piber Neto, P.R. Carvalho and C.X. Mendonça Junior, 2006. Effect of dietary supplementation of flaxseed, canola oil and vitamin E upon polyunsaturated fatty acids in chicken eggs. Arq. Bras. Med. Vet. Zootec., 58: 925-931.

Rowghani, E., M. Arab, S. Nazifi and Z. Bakhtiari, 2007. Effect of canola oil on cholesterol and fatty acid composition of egg-yolk of laying hens. Int. J. Poult. Sci., 6: 111-114.

Rose, D.P., 1997. Dietary fatty acids and prevention of hormoneresponsive cancer. PSEBM, 216: 224-233.

Schumann, B.E., E.J. Squires and S. Leeson, 2000. Effect of dietary flaxseed, flax oil and n-3 fatty acid supplementation on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. Br. Poult. Sci., 41: 465-472.

Shafey, T.M., J.G. Dingle, M.W. McDonald and K. Kostner, 2003. Effect of type of grain and oil supplement on the performance, blood lipoproteins, egg cholesterol and fatty acids of laying hens. Int. J. Poult. Sci., 2: 200-206.

Simopoulos, A.P., 2000. Human requirement for n-3 polyunsaturated fatty acids. Poult. Sci., 79: 961-970.

Skrtic, Z., G. kralik, Z. gajcevic, I. Bogut and D. Hanzek, 2007. The increase of the n-3 PUFA contents in eggs. Izvorni Znanstveni Clank, 58: 644-647.

Tample, N.J., 1996. Dietary fats and coronary heart disease. Biomed. Pharmacother., 50: 261-268.

Van Elswyk, M.E., 1997. Comparison of n-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review. Br. J. Nutr., 78: 61-S69 (Suppl.1).