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Performance and Fatty Acid Compositions of Yolk Lipid from Laying Hens Fed with Locally Produced Canola Seed (*Brassica napus* L.)

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Abstract: An experiment was carried out to determine the influence of feeding different levels of Locally Produced Canola Seed (LPCS) on performance and fatty acid compositions of egg yolk lipid. In a completely randomized design, four treatments including control and three levels of whole canola seed (5, 10 and 15%) were fed to 108 laying hens (Hy-line w-36 at the age of 30 weeks) in four groups with three replications for 12 weeks. Fatty acid compositions of egg yolk were measured at the end of 41 weeks. Usage of LPCS in all levels decreased total amount of saturated fatty acid and the ratio of n-6 to n-3 of the yolk in comparison with control diet. The diets had significant effects on egg shell weight, haugh unit and yolk color index. With 15% LPCS in diet, significantly decreased daily haugh unit in comparison with control diet. Supplementation of LPCS >10% in diet resulted to decreased egg shell weight and yolk color comparing to control diet. There were significant differences in daily feed intake, egg production and egg mass. There was no difference in daily produced egg mass between levels of 5 and 10% LPCS in comparison with control diet (51.94 and 50.57 vs. 51.67). The using of 10% LPCS in diet had the best result between treatments due to better performance and ratio of n-6 to n-3 polyunsaturated fatty acid in egg yolk in comparison with control diet (8.28 vs. 16.94).

Key words: Fatty acid, canola seed, performance, laying hen, egg yolk, Iran

INTRODUCTION

Canola is a winter crop that is widely grown around the world. Canola was originally derived from rapeseed varieties; its component have been altered through genetic selection which markedly reduced it's detrimental components, erucic acid and the glucosinolates to a negligible level and to <20 μM g^{-1} (Leeson and Summers, 2001). These levels are low enough to be of little or no harm to poultry. Other toxins such as tannin, sinapine may also cause some problems if present in high level such in case of sinapine may cause a fishy odor in some brown egg birds (Leeson and Summers, 2001). Full-fat canola seed is not ordinarily used as a feedstuff for poultry. However, with 42% fat and 21% protein, it could be an alternative energy and protein source when it is economically priced (Nwokolo and Sim, 1989).

Flax and canola have been recognized as rich plant sources of Linolenic Acid (LNA), the parent fatty acid of n-3 Polyunsaturated Fatty Acids (PUFA) (Cherian and Sim, 1991). The beneficial effects of n-3 PUFA such as their antithrombotic and anti-inflammatory properties have been established by clinical and epidemiological studies (Herod and Kinsella, 1986; Simopoulos, 2000). According to Simopoulos (2004), the optimal n-6: n-3 ratio in human nutrition is 3:1 but as mentioned by Simopoulos (2000), this ratio is actually between 10:1-15:1 in industrialized countries. This imbalance is being linked to such problems as heart attacks, diabetes, cancer, etc. (Yannakopoulos *et al*., 2005).

Because there is a limited consumption of these n-3 PUFA in the human diet, researchers have encouraged the alternative production of foods with high n-3 PUFA content (Mazalli *et al*., 2004). Increased consumption of n-3 PUFA requires identification of a food source that the public would eat in sufficient amounts to meet recommended intake. Eggs are a potential source of n-3 PUFA because they can be easily enriched with n-3 PUFA by dietary modifications of the laying hens (Lewis *et al*., 2000). According to Van Elswyk (1997), PUFA-enriched eggs can be obtained by enriching layer feeds with marine or oilseed oils such as linseed, sunflower and canola as these easily promote the incorporation of n-3 acids in the egg yolk. However, Cherian and Sim (1991) suggested that the natural antioxidants in the full fat seeds protect the PUFA better than the extracted oil. Thus, if fully utilized,

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canola and linseeds should serve as an excellent dietary source of energy, protein and n-3 fatty acids. The aim of the present study was to evaluate the effect of incorporating locally produced canola seed (var. Talaye) in layer diets of on performance and fatty acid compositions of yolk lipid.

MATERIALS AND METHODS

Birds and diets: This research was carried out at the Animal Research Station of Agricultural Research and Education Center in Ali Abad-e Kamin, Shiraz, Iran. Used canola seeds, var. Talaye (00) had been provided by Department of Seed Production and Improvement, Agricultural Research and Education Center. About 108 Hy-Line w-36 commercial laying hens were divided into 4 treatments with 3 replicates of 9 birds. Hens were housed in cages (3 hens per cage of 30×40 cm) in an open-sided house given artificial light (16L:8D). The age of the hens at the start of the experiment was 30 weeks. The treatments were assigned randomly and consisted of the incorporation of 5, 10 and 15% whole Locally Produced Canola Seed (LPCS) in commercial corn-soybean meal diets in which the corn and soybean meal were partially replaced. A control diet was used which contained 0% LPCS. All diets were isoenergetic and isonitrogenous (Table 1). Crude protein and ether extract were determined by analytical procedure of AOAC (1990) and metabolizable energy of LPCS analyzed according to the method described by Sibbald (1976). The diets were calculated to meat recommendations of National Research Council (NRC, 1994). The experiment was conducted for 12 weeks. Feed and water were provided *ad libitum*.

Sample collection: Egg were collected daily and records of feed intake, egg production, egg weight, egg mass and feed conversion were obtained weekly. Egg were collected over 2 days at the end of experiment period for measurements of weight, egg shell thickness, shell weight, haugh unit, shell strength and yolk color (Roche fan). The analysis of the yolk fatty acid profile performed with the collection of yolks of 5 eggs to compose a pool for each replicate. Approximately 2 g of the egg yolk sample was weighed out into a 50 mL centrifuge tube and C13 internal standard (synthetic triglyceride with 13 carbons) was added. Yolk lipid was extracted according the method proposed by Folch *et al*. (1957). Approximately 50-200 μL of the lower chloroform phase was added to a test tube along with 2.0 mL of boron trichloride in 14% methanol. The tube was then capped and methylated in a boiling water bath for 15 min. Tubes were allowed to cool and then vortexed. Approximately, 1 μL of the upper hexane layer was then withdrawn into gas chromatography vials for injection. The fatty acid methyl esters were analyzed using a gas chromatograph with a 60 m×0.32-mm inside diameter, DB-23 capillary column, 0.10 μM film thickness. The quantification of fatty acid methyl esters was based on comparison to a known internal standard. Yolk cholesterol was measured following the methodologies of Froning *et al*. (1990).

Table 1: Composition of experimental diets supplemented with 0, 5, 10 and 15% Locally Produced Canola Seed (LPCS)

	Diet (LPCS)			
Ingredients	0%	5%	10%	15%
Yellow corn	66.89	64.13	61.43	58.69
Soybean meal	17.66	15.80	13.80	11.80
Canola seeds	0.00	5.00	10.00	15.00
Alfalfa meal	1.00	1.00	1.00	1.00
Fish meal	2.00	2.00	2.00	2.00
Oyster shell	9.45	9.38	9.36	9.28
Vegetable oil	0.68	0.41	0.16	0.00
Salt (NaCl)	0.38	0.38	0.38	0.38
Dicalcium phosphate	1.21	1.17	1.15	1.13
Vitamin premix1	0.25	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25	0.25
L-Lysine-HCl	0.06	0.06	0.06	0.06
DL-methionine	0.17	0.17	0.16	0.16
Total	100.00	100.00	100.00	100.00
Calculated nutrient (%)				
Crude protein	14.9	14.90	14.90	14.90
Calcium	3.86	3.87	3.87	3.87
Available P	0.39	0.39	0.39	0.39
Methionine + cysteine	0.69	0.69	0.69	0.69
Lysine	0.79	0.79	0.79	0.79
ME (kcal kg^{-1})	2770.00	2770.00	2770.00	2770.00

[1]Vitamin premix supplied the following kg^{-1} of complete feed: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 30 IU; vitamin K3, 2 mg; thiamine, 2.25 mg; riboflavin, 7.5 mg; pyridoxine, 3.5 mg; cobalamine, 0.02 mg; niacin, 45 mg; D-pantothenic acid, 12.5 mg; biotin, 0.125 mg; folic acid, 1.5 mg, [2]Mineral premix supplied the following as mg kg^{-1} of complete feed: zinc, 50; copper, 12; iodine, 0.3; cobalt, 0.2; iron, 100; selenium 0.1; manganese, 110

Statistical analysis: Data were analyzed as a complete randomized design with four treatments and three replicates. Statistical analysis of data was carried out using SAS statistical package program (SAS institute, 2004). Means were compared using least square means adjusted for Duncan ($p \leq 0.05$).

RESULTS AND DISCUSSION

The amounts for crude protein and ether extract of LPCS were estimated 22 and 39%, respectively (AOAC, 1990) and Metabolizabel Energy (AMEn) of LPCS was 3141 kcal kg^{-1} by method of Sibbald (1976). Mean values for performance parameters in layers fed with diets of LPCS or control diet at the end of trial period (age of 42 weeks) are shown in Table 2. The results showed significant difference between experimental diets in egg

Table 2: Performance parameters and egg characteristics of hens fed Locally Produced Canola Seed (LPCS) at different levels

	Diet (LPCS)				
Parameters	0%	5%	10%	15%	SEM
Egg production (%)	89.02^{ab}	90.72^{a}	88.55^{b}	85.45^{c}	0.36
Egg weight (g)	58.08^{a}	57.27^{b}	57.12^{b}	57.46^{b}	0.09
Egg mass (g/hen/day)	51.67^{ab}	51.94^{a}	50.57^{b}	49.10^{c}	0.21
Feed intake (g/hen/day)	95.26^{a}	92.92^{b}	90.33^{c}	86.54^{d}	0.33
Feed conversion (g of feed/g of egg mass)	1.85^{a}	1.80^{ab}	1.80^{ab}	1.77^{b}	0.01
Egg shell weight (g)	5.44^{a}	5.19^{ab}	5.04^{b}	4.96^{b}	0.05
Egg shell thickness (mm)	0.298	0.292	0.287	0.285	0.23
Shell strength (kg cm^{-2})	2.98	3.10	2.94	3.01	0.09
Haugh units	73.88^{a}	70.13^{a}	74.18^{a}	64.28^{b}	1.01
Yolk color	6.20^{a}	6.00^{ab}	5.70^{b}	5.30^{c}	0.06

$^{a-d}$Means within a row with no common superscript differ significantly ($p<0.05$)

production, daily egg weight, egg mass, dietary intake ($p<0.01$) and feed conversion ratio ($p\leq0.0529$). The use of 15% dietary LPCS caused a significant decrease ($p<0.01$) in egg production (85.45%) comparing with control diet (89.02%). Usage of LPCS in all levels declined layer daily feed intake ($p<0.01$). Replacement of 15% LPCS in diet resulted in a decline of egg mass (49.1 g) than the control (51.67 g) ($p<0.01$). Overall, these performance characteristics showed a decrease in diets >10% dietary LPCS. According to Dora (1999)'s report feeding with canola seed with levels over 10% of diets based on wheat and barley decreased egg weight and egg production percent significantly.

The reason for destructive effects on performance of broilers while using diets containing >10% canola seed can be expressed by dietary intake decline as well as failing in fat retention and decline in level of metabolizable energy (Summers *et al*., 1982). In such condition, chickens may not make maximum utilization of this fat. For instance, Leeson *et al*. (1987) observed that birds fed with 20% full-fat canola retained only about 50% of dietary fat. The reduction in fat retention might owe to the formation of insoluble soaps involving fatty acids and minerals (Atteh and Leeson, 1984). On the other hand, Najib and Al-Khateeb (2004) noticed from the feces of birds fed with high levels (>10%) of full-fat canola that higher levels of these seeds passed through the intestine indigested which means lower nutrients were utilized by the birds and consequently lower performance and birds weight were obtained. They suggested that the depression in performance of hens fed with high levels of canola could be due to the presence of low levels of glucosinolates in canola seeds.

As shown in Table 2, there were significant differences in qualitative traits comprising egg shell weight, haugh unit ($p<0.05$) and yolk color index ($p<0.01$) among diets containing LPCS and the control diet. Duncan's mean comparison test indicated that using high levels of LPCS in diets (15%) has fairly decreased shell weight and yolk color. There are no difference between experimental diets and control in characteristics of egg shell strength and thickness. The lighter color in yolks from layers fed with high levels of LPCS may be due to the presence of anti-nutritional factors in canola seed. Investigations have also proved that some anti-nutritional and managerial factors are able to reduce the efficiency of xanthophyll supply in yolk and to lessen the yolk color. Also, some feed containing oxidizing agents such as specific minerals and fatty acids led to lightening in yolk color (Leeson and Summers, 2001). On the other hand, increments of LPCS replacement in diets lessen the amount of corn used and consequently its contribution in providing pigments (xanthophyll and carotene) through diet. This may also be another reason for lighter yolk color in hens fed with experimental diets rather than with control (Yannakopoulos *et al*., 2005).

Table 3 shows mean values for percentage of yolk fatty acids at the end of experiment period (end of 41 week) in different experimental and control diets. It was found that using LPCS in amounts >5% of diets caused a significant rise in percent of yolk LNA comparing with control diet ($p<0.01$) as the ratio of linoleic to linolenic acid (LA:LNA) in yolk from eggs produced by layers feeding with control diet (29.96) was approximately halved in those produced by layers feeding with diets of 10% LPCS (16.74). By increments in level of LPCS in layer rations the total amount for n-3 fatty acids increased ($p<0.01$).

These results are comparable with those of other researchers indicating that the values of yolk LNA and total n-3 fatty acids increased with higher levels of canola seed in diet ($p<0.05$) (Cherian and Sim, 1991; Baucells *et al*., 2000). Studies carried out with poultry have confirmed that LNA from canola seeds as the unique source of n-3 fatty acids can serve as the precursor of longer-chain n-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) through

Table 3: Lipid content of egg yolk from hens fed Locally Produced Canola Seed (LPCS) at different levels[1]

	Diet (LPCS)				
Fatty acid (%)	0%	5%	10%	15%	SEM
Linoleic acid (C18:2 n-6)	16.870	19.020	17.530	19.26	0.380
α-Linolenic acid (C18:3 n-3)	0.580^{c}	0.850^{bc}	1.060^{b}	1.52^{a}	0.070
Arachidonic acid (C20:4 n-6)	0.310^{a}	0.190^{b}	0.210^{b}	0.18^{b}	0.009
Docosapentaenoic acid (C22:5 n-3)	0.087	0.070	0.087	0.10	0.005
Eicosapentaenoic acid (C20:5 n-3)	0.000	0.007	0.007	0.01	0.001
Docosahexaenoic acid (C22:6 n-3)	0.370^{b}	0.490^{b}	1.010^{a}	1.03^{a}	0.050
Total saturated fatty acid	39.040^{a}	34.050^{b}	34.490^{b}	28.90^{c}	0.600
Total n-3 fatty acid	19.440	17.740	19.210	17.18	0.390
Total n-6 fatty acid	1.030^{c}	1.410^{c}	2.160^{b}	2.66^{b}	0.070
Ratio (C18:2 n-6):(C18:3 n-3)	29.960^{a}	22.580^{b}	16.740^{c}	13.16^{c}	0.820
Ratio n-6:n-3	16.940^{a}	13.620^{b}	8.280^{c}	7.33^{c}	0.360
Cholesterol (mg g^{-1})	10.000	9.670	10.300	9.60	0.400

$^{a-c}$Means within a row with no common superscript differ significantly ($p<0.05$), [1]All values are as percent of total fatty acids except cholesterol

an elongation and desaturation pathway, thus enriching the egg yolk with n-3 fatty acids (Cherian and Sim, 1991). In this study, the amounts of total saturated fatty acids, Arachidonic Acid (AA) and ratio of n-6 to n-3 fatty acids were reduced by adding LPCS ($p<0.05$). The fall in total saturated fatty acids in egg yolks from hens fed with diets containing LPCS is consistent with observation of other researchers, as they also reported a decline in amount of saturated fatty acids in egg yolks enriched by n-3 fatty acid (Scheideler and Lewis, 2001; Filardi *et al*., 2005).

In addition, in a study, Aydin (2005) pointed that an increase of 5-10% in canola oil in layer rations prevented the build-up of fatty acids including palmitic and stearic acid and decreased the concentration of oleic acid while increasing LNA concentration in yolk. So, one can deduce that the rise of unsaturated n-3 fatty acids in yolk accompanies with notable depletion of saturated fatty acids leading to development of a healthier profile of lipids and therefore, to increase of egg nutritional value (Yannakopoulos *et al*., 2005). The lower concentration of AA can due to the competition between LNA and LA for the Δ-6 desaturase enzyme; LNA is the preferred substrate over LA, thus limiting the synthesis of AA from LA (Cherian and Sim, 1991; Simopoulos, 2000). The dietary n-6 to n-3 fatty acid ratio may be more important than the absolute amount of dietary n-3 fatty acids in the inhibition of AA metabolism and in maintaining the essential balance for optimum health (Simopoulos, 2000).

Researcher evinced that there was no difference between experimental and control diets in the context of yolk cholesterol ($p>0.05$). Results from assays about the effect of using dietary fatty acids on levels of plasma and egg cholesterol are contrary where some researchers demonstrated that dietary PUFA decreased the concentration of plasma and yolk cholesterol (Hollands *et al*., 1980; Mori *et al*., 1999) while others findings (Caston and Leeson, 1990; Milinsk *et al*., 2003) in agreement, shown no difference in concentration of egg yolks in experimental diets. No absolute achievement in reduction of egg cholesterol might be for physical structure of egg lipids. The amount of egg cholesterol is affected by yolk lipoprotein cholesterol not by cholesterol density in its plasma. Major part of lipoprotein cholesterol is on their superficial layer. So, the egg cholesterol reduction is only feasible by raising the size of lipoproteins.

This condition results in a decrease in the ratio of superficial molecules of cholesterol to the total lipids. Unfortunately, larger lipoproteins cause lower efficiency of transferring molecules across follicle wall. So, notable cut in egg cholesterol content seems to be only possible via genetic modification in processes related to lipoprotein production and its transfer to follicle (Leeson and Summers, 2005).

CONCLUSION

In this study, it was observed that incorporation of LPCS in layer rations could induce a reduction in the ratio of n-6 to n-3 fatty acids in lipids isolated from yolk. Given its recently developing cultivated area throughout Iran, this oil seed can be utilized as a proper ingredient for layer rations towards supplying part of dietary energy and protein besides scaling up the ratio of n-3 to n-6 fatty acids in yolks. However, according to our findings, the application of LPCS over 10% in layer diets is not recommended, as it causes considerable reductions in performance characteristics (egg production and egg mass) and qualitative (Haugh unit and yolk color index) in comparison with control diet.

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