



## RESEARCH ARTICLE

### Effect of Canola Oil and Vitamin A on Egg Characteristics and Egg Cholesterol in Laying Hens During Hot Summer Months

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#### ABSTRACT

Canola oil and vitamin A were evaluated for their effects on egg characteristics, egg cholesterol and egg triglycerides (TG) in laying hens prone to heat-stress during summer months. Four levels of canola oil (0, 2, 3 and 4% of diet) in combination with two levels of vitamin A (3,000 or 10,000 IU/kg of diet) were fed to laying hens for a period of 12 weeks. Various egg-quality parameters were measured on weekly basis while, serum TG, egg cholesterol and TG contents were analyzed during the last week of the trial. The results of the study showed that the egg weight, egg mass, yolk weight, Haugh unit score, shell thickness, shell weight and egg breaking-strength were similar ( $P>0.05$ ) for all canola oil levels supplemented to the laying hens. Higher egg weight and egg mass ( $P<0.05$ ) were noted for the hens at the diet with 10,000 IU vitamin A /kg of diet whereas, all other egg characteristics were not influenced by increasing the supplemental level of vitamin A. Serum TG, egg cholesterol and TG contents were not changed ( $P>0.05$ ) by increasing canola oil or vitamin A levels in the diet of laying hens. It might be concluded from the results of the present study that canola oil as a source of omega-3 fatty acids can be included in the diet of laying hens without compromising the quality characteristics of the eggs.

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#### INTRODUCTION

In the recent years, canola oil has been used by a number of researchers in the layer diets for egg-yolk fortification with omega-3 ( $\omega$ -3) polyunsaturated fatty acids (PUFA) (Gibbs *et al.*, 2010; Ahmad *et al.*, 2010; Ahmad *et al.*, 2012; Cheema *et al.*, 2012). Supplementation of dietary oils not only alters the egg-yolk fatty acids but also can change the lipid metabolism in the laying hens (Kakani *et al.*, 2012). Dietary  $\omega$ -3 PUFA can decrease the triglycerides (TG) synthesis and secretion from intestinal cells (Oliveira *et al.*, 2010; Heinze *et al.*, 2012). It is well-known that PUFA are structural components of all cell membranes in the body. With the increase in dietary  $\omega$ -3 PUFA contents, these are also increased in the membranes of the cells, including hepatocytes and reproductive organs (Yalçın and Ünal, 2010). As lipid peroxidation of PUFA in cell membranes and tissue linings is a problem associated with heat-stress

conditions, the increased amount of PUFA in the cell membranes may enhance this process in the laying hens during summer months. Heat stress, therefore, inversely affects the metabolic activity of hepatocytes which, consequently, is associated with a reduced synthesis and release of egg yolk precursors from the liver. As a result, there may be a serious decrease in the overall performance of the body organs involved in the synthesis of egg ultimately decreasing the weight and quality of egg and egg components in the hens fed on high PUFA diets in hot climate.

Vitamin A is an effective antioxidant as it can slow down the process of lipid peroxidation by breaking the chain reactions involved in this process and hence can check the process of membrane deterioration induced by the heat-stress (Sahin *et al.*, 2002; Ajakaiye *et al.*, 2011). Supplementation of vitamin A higher than NRC (1994) recommendations is reported to play an important role in restoration of membrane integrity and, hence, normal

functioning of reproductive organs in heat-stressed laying hens (Lin *et al.*, 2002; Kaya and Yildirim, 2011). Higher dietary level of vitamin A is reported to enhance the production performance and egg quality in the heat-stressed laying hens. Most of the previous studies on  $\omega$ -3 PUFA feeding to laying hens were conducted in temperate climates and usually overlooked the egg quality characteristics other than  $\omega$ -3 PUFA in the yolks. In the present study, canola oil was supplemented (as a source of  $\omega$ -3 PUFA) in combination with vitamin A to evaluate its effect on egg characteristics, and egg cholesterol and egg TG in laying hens during summer months.

## MATERIALS AND METHODS

White Leghorn laying hens (n=240), at 48<sup>th</sup> week of age and with initial body weight of 1648±100 grams were randomly divided into 24 replicates. These replicates were randomly allotted to eight treatment groups (8\*3replicates\*10birds = 240 birds) which were fed diets with 0, 2, 3 and 4% canola oil with 3,000 or 10,000 IU vitamin A/kg of diet (4 × 2 factorial, under Completely Randomized Design). The hens were kept in cages and at high ambient environmental temperature (diurnal temperature: Average min. 27.5°C, Average max. 39.0°C) throughout the experimental period of 12 weeks. The layer diets were formulated according to NRC (1994) recommendations for dietary needs of laying hens. The hens under all treatment groups had *ad libitum* access to feed and water throughout the experiment.

The egg weight, egg mass, shell weight and shell thickness were measured on weekly basis for each replicate, separately. The egg compression tests were conducted on an egg shell intensity meter's machine (Ogawa Seiki Co Ltd, Japan) for measuring egg shell breaking strength. Haugh unit score and yolk indices were calculated according to the formula described by Singh and Kumar (1994). Prior to the start of the trial, feed ingredients used in the formulation of the diets were analyzed for proximate components (AOAC, 1990). Ingredients and chemical composition of layer rations are shown in Table 1. Egg cholesterol and TG (mg/dl) were extracted according to the methods of the AOAC (1990). The data were analyzed by the two-way ANOVA using GLM and means were compared by Tukey's honestly significant difference test (Minitab 13.1, Minitab Inc., State College, PA).

## RESULTS

The increase in canola oil level from 0 to 4% of the diet had no effect (P>0.05) on the egg weight and egg mass. However, the higher (P<0.05) weight and mass were noted for the eggs laid by the hens at the diets with higher vitamin A supplementation (Table 2). Yolk weight, yolk weight as % of egg weight, yolk index, albumen weight as % of egg weight, Haugh Unit Score, shell weight, shell thickness and shell breaking strength were also similar for the all canola oil and vitamin A levels (Table 3). The serum TG, egg cholesterol and TG contents did not change (P>0.05) with the increase in canola oil or vitamin A levels in the diets of the laying hens (Table 4). No significant interaction was found between canola oil and vitamin A for these parameters.

**Table 1: Ingredients and Nutrient Composition of Layer Diets**

Diets <sup>a</sup>	R <sub>1</sub> <sup>a</sup> & R <sub>2</sub> <sup>b</sup>	R <sub>3</sub> <sup>a</sup> & R <sub>4</sub> <sup>b</sup>	R <sub>5</sub> <sup>a</sup> & R <sub>6</sub> <sup>b</sup>	R <sub>7</sub> <sup>a</sup> & R <sub>8</sub> <sup>b</sup>
Ingredients				
Corn	65.0	53.0	50.0	48.0
Rice broken	4.40	10.9	11.6	11.5
Soybean meal 44 %	13.0	19.8	23.4	27.1
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-methionine	0.03	0.07	0.85	0.10
Vit./min premix <sup>1</sup>	0.35	0.35	0.35	0.35
Total	100	100	100	100
Nutrient Composition				
CP (%)	17.0	17.0	17.0	17.0
ME (Kcal/kg)	290	290	290	290
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 <sup>2</sup> (%)	1.50	1.66	1.81	1.98
LNA <sup>3</sup> (%)	0.07	0.26	0.36	0.46

<sup>1</sup>Provided per kilogram of diet: Cholecalciferol, 1,250 IU; Vitamin E (dl-alpha-tocopherylacetate), 12 IU; menadione, 2.5mg; riboflavin, 6 mg; calcium pantothenate, 8 mg; niacin, 15 mg; pyridoxine 2 mg; folic acid, 1 mg; vitamin B<sub>12</sub>, 7µg; Mn, 50 mg; Zn, 55 mg; Fe 40 mg; Cu, 4 mg; I, 2 mg; Co, 0.3 mg; ethoxyquin, 150 mg; <sup>2</sup>R<sub>1</sub> to R<sub>8</sub> is the rations offered to eight treatment groups; <sup>a</sup> rations containing 3000 IU/kg of diet vitamin A; <sup>b</sup> rations containing 10000 IU kg of diet vitamin A; <sup>3</sup> LA = Linoleic Acid; <sup>3</sup> LNA = Linolenic Acid.

**Table 2: Effect of canola and vitamin A on egg weight, egg mass, yolk weight, yolk % and yolk index in laying hens**

Diet	Egg weight (g)	Egg mass (g)	Yolk weight (g)	Yolk % (of egg)	Yolk index
Canola oil (%)					
0	61.32	42.81	16.87	27.87	0.41
2	59.54	41.25	16.88	27.70	0.36
3	58.75	40.13	16.14	27.20	0.40
4	60.71	42.46	16.32	26.53	0.41
SEM	0.950	0.815	0.338	0.889	0.029
Vit. A (IU/kg diet)					
3000	58.98 <sup>b</sup>	40.42 <sup>b</sup>	16.50	27.43	0.38
10,000	61.18 <sup>a</sup>	40.91 <sup>a</sup>	16.60	27.22	0.41
SEM	0.672	0.576	0.239	0.628	0.021
ANOVA	Probabilities				
Oil	0.260	0.123	0.325	0.715	0.538
Vit. A	0.034	0.007	0.769	0.821	0.335
Oil × Vit. A	0.115	0.109	0.396	0.325	0.428

<sup>a,b</sup> means within a column with different superscripts differ significantly (P<0.05).

**Table 3: Effect of canola oil and vitamin A on albumen weight (Alb. Wt), Haugh Unit (HU) score, shell weight, shell strength (St.) and shell thickness (Th.) in laying hens**

Diet	Alb. Wt (as % of egg)	HU score(g)	Shell weight (kg/cm <sup>2</sup> )	Shell St. (mm)	Shell Th.
Canola oil (%)					
0	58.63	92.67	7.30	1.83	0.37
2	58.99	94.65	6.88	2.37	0.36
3	59.41	94.72	7.05	2.60	0.38
4	59.64	93.94	7.50	2.50	0.38
SEM	1.07	1.899	0.330	0.508	0.007
Vit. A (IU/kg diet)					
3000	58.92	94.51	7.20	2.33	0.38
10,000	59.42	93.48	7.17	2.32	0.37
SEM	1.52	1.343	0.233	0.359	0.005
ANOVA	Probabilities				
Oil	0.914	0.859	0.578	0.720	0.096
Vit. A	0.648	0.594	0.921	0.974	0.384
Oil × Vit. A	0.621	0.995	0.487	0.951	0.296

Means within a column with no superscripts differ non-significantly (P>0.05).

**Table 4:** Effect of canola oil and vitamin A on serum triglycerides, egg cholesterol and egg triglycerides in laying hens

Diet	Serum triglycerides (mg/dl)	Egg cholesterol (mg/dl)	Egg triglycerides (mg/dl)
Canola oil (%)			
0	160.80	362.20	10.37
2	159.30	355.80	10.39
3	159.53	349.80	10.42
4	158.92	357.50	10.51
SEM	3.142	11.164	0.054
Vit. A (IU/kg diet)			
3000	163.70	355.30	10.43
10,000	163.00	357.40	10.42
SEM	2.202	7.894	0.038
ANOVA	Probabilities		
Oil	0.974	0.889	0.280
Vit. A	0.675	0.849	0.915
Oil × Vit. A	0.569	0.628	0.778

Means within a column with no superscripts differ non-significantly ( $P > 0.05$ ).

## DISCUSSION

The results of the present study depicted no differences in the egg weight and egg mass produced by the hens on the diets with various canola oil levels. It was reported earlier that dietary PUFA can alter the composition of yolk fatty acids but the total yolk lipids remain unaffected with no change in egg weight or yolk weight (Pekel *et al.*, 2009). In the present study, the serum and egg TG levels in laying hens were similar for the all dietary canola oil levels that's why, the egg weight was not different for various treatment groups. The present results confirmed the findings of many researchers who reported no change in egg weight and egg mass by feeding various dietary sources of  $\omega$ -3 PUFA in the diets of laying hens (Rowghani *et al.*, 2007; Petrović *et al.*, 2012). In the present study, the increase in dietary vitamin A level increased the egg weight and egg mass in laying hens. It was reported earlier that vitamin A maintain the integrity of epithelial tissue and possibly the lining of reproductive tract including magnum and ovaries (Squires and Naber, 1993) so, higher dietary vitamin A can improve the overall integrity and functioning of reproductive tract resulting into increased egg weight especially in heat-stressed laying hens. Similar results were reported by Lin *et al.* (2002).

The hens in the current study produced eggs with similar yolk weight and yolk indices regardless of dietetic canola oil or vitamin A levels. It was reported earlier that the rate of hepatic synthesis of fats is sufficient to supply the amount of lipids needed to achieve optimum performance, egg and yolk weight, and exogenous fat might not influence to meet these requirements (Baucells *et al.*, 2000; Shafey *et al.*, 2003). No differences were observed in lipids (TG) circulating in the blood of the hens at various dietary canola oil levels which seemed to direct similar yolk-fat deposition hence similar yolk weights, in the present study.

Egg white quality, in terms of Haugh Unit Score and albumen weight as a % of egg weight in laying hens, proved to be indifferent for the all dietary canola oil and vitamin A levels. These results suggested that PUFA might not have any relation with egg white quality. Rowghani *et al.* (2007) stated that the egg white quality was not altered by the inclusion of 5% canola oil in the

diets of the laying hens. Horniakova (1997) observed no change in the egg white weight with 2 and 6% canola oil supplementation in the layers diet. In the present study, the higher vitamin A supplementation did not change the egg white quality which confirmed the findings of previous studies (Mori *et al.*, 2003).

Egg shell thickness, egg shell weight and egg shell breaking strength was same for various canola oil and vitamin A levels. These results depicted that dietary PUFA had no relation with egg shell quality. The present study confirmed the results of trial by Filardi *et al.* (2005) in which canola oil at supplemental level of 3.12% in the ration of laying hens did not produce any difference in shell thickness and shell weight when compared to other groups. Mori *et al.* (2003) reported no change in eggshell quality in the hens fed vitamin A above NRC (1994) recommendations of 3000 IU/kg diet; same were the results of present study.

**Egg cholesterol and egg triglycerides:** Cholesterol biosynthesis happens in the liver and ovary in the laying hens. Cholesterol is readily transferred from the blood to the developing ova and, therefore, almost all of egg-yolk cholesterol originates from blood cholesterol (McLachlan *et al.*, 1996). In the current trial, as the serum TC was same at various dietary canola oil levels, the egg cholesterol was also unchanged. So, it might be suggested that the cholesterol deposition in the egg-yolks was dependent on the serum cholesterol levels. Existing studies reported that the genetic programs as well as a vast array of dietary treatments had resulted in only slight reductions (generally 5% or less) in the egg cholesterol contents. It has been hypothesized earlier that when whole-egg cholesterol content is reduced below that is sufficient to support an embryo, the hen will cease egg production. In the present study, the egg TG contents also showed dependency to the serum TG level. As serum TG contents did not change with the increase in dietary canola oil level, the egg TG contents were also similar for the all dietary canola oil levels. This proved that it is very difficult to change the total-fat contents of egg yolk by dietary manipulation. It was suggested earlier by Baucells *et al.* (2000) that PUFA in the diet can change only yolk fatty acids composition but cannot alter the total amount of lipids in yolks. Shafey *et al.* (2003) and Hosseini-Vashan *et al.* (2011) investigated the relationship between the type of oil-supplement and blood and yolk cholesterol and found that the type of dietary oil-supplement altered the fatty acid profile of yolks and composition of plasma lipoproteins without having a significant effect on the overall yolk contents of TG and cholesterol. The results of present study showed that dietary vitamin A levels have no effect on cholesterol and TG contents of eggs.

**Conclusion:** It might be concluded from the results of the present study that canola oil can be used for the fortification of eggs with  $\omega$ -3 PUFA without any detrimental effects on egg characteristics. The supplementation of vitamin A higher than NRC recommended level of 3000 IU/kg of diet can enhance the egg weight and egg mass in heat-stressed laying hens.

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