



# International Journal of **Biological Chemistry**

ISSN 1819-155X



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## **Effect of Dietary Supplementation of Canola Oil on Egg Production, Quality and Biochemistry of Egg Yolk and Plasma of Laying Hen**

<sup>1</sup>I.B. Ismail, <sup>1</sup>K.A. Al-Busadah and <sup>2,3</sup>S.M. El-Bahr

<sup>1</sup>Department of Physiology, Biochemistry and Pharmacology (Physiology), <sup>2</sup>Department of Physiology, Biochemistry and Pharmacology (Biochemistry), College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia

<sup>3</sup>Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt

*Corresponding Author: Sabry M. El-Bahr, Department of Physiology, Biochemistry and Pharmacology (Biochemistry), College of Veterinary Medicine and Animal Resources, King Faisal University, P.O. Box: 400, Al-Ahsa, 31982, Saudi Arabia Tel: (00966) (055) 8907894 Fax: (00966) (03) 5816635*

### **ABSTRACT**

Production of eggs of low cholesterol level is a prerequisite of modern studies to provide healthy food for human consumption. The present study was performed to assess the effect of canola oil (1 and 2%) on egg production, egg quality and biochemistry of plasma and egg yolk of laying hen. To achieve this goal, 75 hens (hisex, 26 weeks old) were divided into three treatments (25 hens for each) of 5 replicates (5 hens for each). In the first treatment, hens were fed basal diet free of canola whereas 1 and 2% canola oil were incorporated in the second and third treatment, respectively for eight weeks and all data were recorded on 4 and week 8 of the experiment. The current study revealed insignificant difference in feed intake, feed conversion ratio, egg production, egg weight, shell thickness, haugh units and egg yolk color among treatment groups. Plasma triacylglycerol and cholesterol values reduced significantly ( $p < 0.05$ ) in layers fed diet supplemented with canola oil after 8 weeks compare to control. Egg cholesterol values were reduced significantly ( $p < 0.05$ ) in eggs produced by hens supplemented with both concentrations of canola oil for 4 and 8 week compare to control. Liver and kidney function were not affected in all treatments throughout the study. Based on the current results, inclusions of 1% canola oil in layers diet were quiet enough to reduce plasma and egg cholesterol. These results open new perspectives on economy of poultry industry for production of healthy food (egg) for human consumption.

**Key words:** Cholesterol, triacyleglycerol, egg, layers, rapeseed

### **INTRODUCTION**

Egg is rich source of proteins, lipids, vitamins and minerals (Stadelman, 1999; Zeidler, 2002). However, its high cholesterol content and subsequent cardiovascular hazards restricted their human consumption. Many studies were performed to reduce egg cholesterol through genetic selection or inclusion of drugs in the diet of laying hen. The close relationship between lipid composition of egg and diet was well documented (Bavelaar and Beynen, 2004; Beynen, 2005). Therefore, many studies were conducted with marginal success to manipulate egg lipid particularly cholesterol through diet manipulation in laying hen (Beyer and Jensen, 1989; Van Elswyk *et al.*, 1992; Farran *et al.*, 1995; Hargis, 1988). The inclusion of canola oil in layers ration promoted the deposition of omega-3 (n-3) polyunsaturated fatty acid (PUFA) in egg yolk (Van Elswyk, 1997;

Yang *et al.*, 2000). The human health benefits of these fatty acids were reported as antihypertensive, anti-hyperlipidemic, anti-cardiac arrhythmia (Simopoulos, 2000; Tample, 1996), anti cancer (Rose, 1997), immunomodulator (Fernandes, 1995) and essential for normal brain and visual development in fetus (Neuringer *et al.*, 1998). Canola oil increases the n-3 PUFA contents in the form of linolenic acid. The linolenic acid is the parent of all n-3 family of fatty acids (Lopez-Ferrer *et al.*, 2001). Birds are known to be able to convert linolenic acid to longer chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA, C20:5), docosapentaenoic acid (DPA, C22:5) and docosahexaenoic acid (DHA, C22:6) through elongation and desaturation mechanisms. (Shakoor *et al.*, 2002; Rowghani *et al.*, 2007; Agah *et al.*, 2010; Ahmad *et al.*, 2010; Nobakht, 2011). However, these previous studies were of low economical values because it used high concentrations of canola that ranged from 3-5% and subsequent high ration cost. Moreover, canola oil is used extensively in human diet as mentioned above and therefore uses of higher concentrations of this oil in animal or bird rations may create a big challenge regarding human food that could be prevented. Therefore, the purpose of the current study was to investigate the effect of lower concentrations of canola oil on performance, egg quality and biochemistry of plasma and egg yolk of laying hen.

## MATERIALS AND METHODS

**Experimental design:** The current study was carried out using 75 mature commercial layers (Hisex; 26 weeks old) in a closed experimental poultry house at Researches station of King Faisal University, Al-Ahsa, Saudi Arabia in year 2012. Hens were kept in wire cages and divided into three treatments (25 birds each) with 5 replicate (5 hens/replicate). A basal diet (Table 1) was formulated to contain 17% crude protein and 2730 kcal kg<sup>-1</sup> Metabolizable Energy (ME). Dietary treatments consisted of 0% supplemented canola oil (basal diet), the basal diet with added 1% canola oil and the basal diet with added 2% canola oil. Basal diet was formulated according to NRC (1994). The three different experimental diets were given for eight weeks. Feed and water were provided *ad libitum*.

**Performance and egg quality parameters:** Feed intake (g/hen/week), Egg production/hen/week and Feed conversion ratio were recorded on week 4 and week 8 as an average for the period (WK1-Wk4) and (WK5-WK8), respectively. Feed conversion ratio was calculated as g feed/g egg. Egg quality parameters (egg weight, egg shell thickness, yolk color and haugh unit) were assessed also two times with four weeks interval. For this purpose, the eggs were collected, stored for 24 h and then measurements were performed. Shell thickness was measured at three locations on the egg (air cell, equator and sharp end) using a micrometer (Mitutoyo, 0.01 mm, Japan; Wells, 1968) and albumin height was measured by tripod micrometer (Mitutoyo, 0.01 mm, Japan). Haugh unit was calculated according to Card and Nesheim (1972) using this formula:

$$\text{Haugh unit} = 100 \log \text{HA} + 7.57 - 1.7 \text{WE}^{0.37}$$

where, the (HA) is albumen height and (WE) is egg weight.

**Determination of egg cholesterol:** Every four weeks, three eggs, randomly taken from each replicate, the cholesterol content of the egg yolk was determined by using the methods of Hammad *et al.* (1996) and Berrio and Hebert (1990). The eggs were hard-boiled and yolks were

Table 1: Composition of experimental diets supplemented with various concentration of canola oil

| Ingredients                                   | Diets (canola oil) |         |         |
|---|--------------------|---------|---------|
|   | T1 (0%)            | T2 (1%) | T3 (2%) |
| Yellow corn                                   | 62.61              | 62.57   | 62.57   |
| Soya bean                                     | 24.73              | 24.74   | 24.74   |
| Lime stone                                    | 10.10              | 9.13    | 8.20    |
| Dicalcium phosphate                           | 1.25               | 1.75    | 1.75    |
| Canola oil                                    | 0.00               | 1.00    | 2.00    |
| Sodium bicarbonate                            | 0.23               | 0.23    | 0.37    |
| Salt (NaCl)                                   | 0.21               | 0.21    | 0.21    |
| Vitamin premix <sup>1</sup>                   | 0.10               | 0.10    | 0.10    |
| Mineral premix <sup>2</sup>                   | 0.10               | 0.10    | 0.10    |
| Methionine 99%                                | 0.10               | 0.10    | 0.10    |
| Choline chloride 60%                          | 0.07               | 0.07    | 0.07    |
| Total   | 100.00             | 100.00  | 100.00  |
| <b>Calculated nutrient (%)</b>                |                    |         |         |
| Crude protein                                 | 17.00              | 17.00   | 17.00   |
| Crude fat                                     | 2.25               | 3.16    | 4.00    |
| Crude fiber                                   | 2.36               | 2.36    | 2.36    |
| Methionine                                    | 0.38               | 0.38    | 0.38    |
| Lysine  | 0.92               | 0.92    | 0.92    |
| Calcium                                       | 4.30               | 4.00    | 3.60    |
| Phosphorus                                    | 0.37               | 0.37    | 0.37    |
| Sodium  | 0.16               | 0.16    | 0.16    |
| Linoleic acid                                 | 1.26               | 1.26    | 1.26    |
| Metabolizable energy (kcal kg <sup>-1</sup> ) | 2730.00            | 2730.00 | 2730.00 |

<sup>1</sup>Vitamin premix supplied the following kg<sup>-1</sup> of complete feed: Vitamin A, 12,000IU, Vitamin D<sub>3</sub> 2,500 IU, Vitamin E 30 IU, Vitamin K<sub>3</sub>, 2 mg, Thiamin 2.25 mg, Riboflavin 7.5 mg, Pyridoxine 3.5 mg, Vitamin B<sub>12</sub> 0.02 mg, Niacin 45 mg, Pantothenic acid 12.5 mg, Biotin 0.125 mg, Folic acid 1.5 mg. <sup>2</sup>Mineral premix supplied the following: Zinc 50 mg kg<sup>-1</sup>, Copper 12 mg kg<sup>-1</sup>, Iodine 0.3 mg kg<sup>-1</sup>, Cobalt 0.2 mg kg<sup>-1</sup>, Iron 100 mg kg<sup>-1</sup>, Selenium 0.1 mg kg<sup>-1</sup>, Manganese 110 mg kg<sup>-1</sup>, T<sub>1</sub>: Control group; basal diet with 0% canola oil, T<sub>2</sub>: Basal diet supplemented with 1% canola oil, T<sub>3</sub>: Basal diet supplemented with 2% canola oil

separated and 0.1 g samples of yolks were weighed accurately. Yolk lipids were extracted with isopropanol (4 mL) then vortexed and centrifuged at 3000 rpm for 10 min. The yolk cholesterol concentration was determined in the filtered samples by UV spectrophotometer using commercial kits (Human Gesellschaft fur Biochemica and Diagnostica mbH, Wiesbaden, Germany). The cholesterol concentration of the egg yolk (mg cholesterol g<sup>-1</sup> of egg yolk) was calculated by the method of Boehringer Mannheim GmbH Biochemica (1989). Briefly, Results were read at spectrophotometer in 520 nm wavelength and evaluated with the following formula:

$$\text{Cholesterol content in extract (ECV) (mg dL}^{-1}\text{)} = \frac{\text{Read value in sample}}{\text{Read value in standard}} \times \text{CS}$$

(CS: Concentration of standard)

$$\text{Egg yolk cholesterol (mg g}^{-1}\text{)} = \frac{(\text{ECV}/100) \times 4}{\text{Sample values (g)}}$$

**Determination of plasma parameters:** About 3 mL of blood were collected from brachial vein of layers of all treatments in EDTA vacutainers every four weeks. After centrifugation, plasma

samples were kept frozen at -20°C until the time of analysis. Plasma total cholesterol was determined by using the kit manufactured by Human Gesellschaft fur Biochemica and Diagnostica mbH, Wiesbaden, Germany (Cat. No. 10019). The same commercial kits were used for determination of triacylglycerol (Trinder, 1969) and High Density Lipoprotein cholesterol (HDL-c) (Cat. No. 10084). Very Low Density Lipoprotein cholesterol (VLDL-c) was calculated by division of Triacylglycerol (TAG) by 5 (Bauer, 1982) and Low Density Lipoprotein cholesterol (LDL-c) was determined by calculation method with the help of formula reported by Friedewald *et al.* (1972) as:

$$\text{LDL-c} = \text{Total cholesterol} - (\text{VLDL-c} + \text{HDL-c})$$

Plasma samples were used also for spectrophotometric determination of the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) as directed by Reitman and Frankel (1957). Blood urea, uric acid and creatinine were determined according to the method described by Tabacco *et al.* (1979) and Todd *et al.* (1984), respectively.

**Statistical analysis:** The obtained data was compared between groups within different periods by using student-t-test. All data was presented as Mean±standard Error of Mean (SEM). The analysis was done on samples from each replicate of birds per treatment. All tests will perform using computer package of the statistical analysis system (SAS Institute, 2004). Statements of statistical significance are based on  $p < 0.05$ .

## RESULTS

Mean values for performance parameters in layers fed with diets of 1 and 2% canola or control diet every four weeks are shown in Table 2. The results showed insignificant ( $p > 0.05$ ) difference in feed intake values (g/hen/week) in hen fed diet free of canola, T1 (696.2±9.4); diet mixed with 1% canola oil, T2 (698.6±3.1) and diet mixed with 2% canola oil, T3 (707.9±8.3) after 4 weeks whereas these values were (799.6±11), (819.6±10) and (829±8.3), respectively after 8 weeks from the start of the experiment. The present findings (Table 2) also showed insignificant ( $p > 0.05$ ) difference in egg production, feed conversion ratio (g of feed  $g^{-1}$  of egg weight) in all treatments. Insignificant ( $p > 0.05$ ) difference in egg weight (g) was recorded in T1 (367.4±4.9), T2 (368.1±3.4) and T3 (359.1±5.2) after 4 and 8 weeks (401.8± 5.5), (381.1± 8.0) and (395.6±3.0) of the study, respectively (Table 3). Data summarized in Table 3 showed also that, the effect of inclusion of canola oils (1 and 2%) on egg shell thickness and egg yolk color were non-significant ( $p > 0.05$ ) compare to control. The present findings as shown in Table 4 indicated that, the inclusion of canola oil 1% in layers ration reduced their plasma TAG (53.2±1.5 mmol L<sup>-1</sup>) and total cholesterol level

Table 2: Performance of commercial layers supplemented with canola oil for 8 weeks

| Parameters   | Time     | T <sub>1</sub> | T <sub>2</sub> | T <sub>3</sub> |
|--|----------|----------------|----------------|----------------|
| Feed intake g/hen/week                               | 4th week | 696.2±09.40    | 698.6±03.1     | 707.9±8.30     |
|  | 8th week | 799.6±11.00    | 819.6±10.0     | 829.0±8.30     |
| Egg production/hen/week                              | 4th week | 6.4±0.200      | 6.2±0.20       | 6.1±0.20       |
|  | 8th week | 6.7±0.100      | 6.1±0.20       | 6.4±0.10       |
| Feed conversion ratio<br>(g of feed/g of egg weight) | 4th week | 1.89±0.02      | 1.9±0.02       | 2.0±0.03       |
|  | 8th week | 2.00±0.10      | 2.2±0.10       | 2.1±0.10       |

T<sub>1</sub>: Control group; basal diet with 0% canola oil, T<sub>2</sub>: Basal diet supplemented with 1% canola oil, T<sub>3</sub>: Basal diet supplemented with 2% canola oil, Values are Mean±SE

Table 3: Egg quality of commercial layers supplemented with canola oil for 8 weeks

| Parameters               | Time     | T <sub>1</sub> | T <sub>2</sub> | T <sub>3</sub> |
|--------------------------|----------|----------------|----------------|----------------|
| Egg weight (g)           | 4th week | 367.40±4.9     | 368.1±3.4      | 359.1±5.2      |
|                          | 8th week | 401.80±5.5     | 381.1±8.0      | 395.6±3.0      |
| Egg shell thickness (mm) | 4th week | 35.20±0.2      | 35.8±0.2       | 35.6±0.5       |
|                          | 8th week | 36.60±0.1      | 36.4±0.3       | 35.9±0.3       |
| Yolk color               | 4th week | 5.04±0.1       | 4.6±0.2        | 4.8±0.1        |
|                          | 8th week | 4.70±0.2       | 5.2±0.2        | 6.1±0.2        |
| Haugh units              | 4th week | 68.20±0.8      | 64.7±0.9       | 68.3±0.9       |
|                          | 8th week | 65.80±0.6      | 59.8±0.4       | 54.5±0.6       |

T<sub>1</sub>: Control group; basal diet with 0% canola oil, T<sub>2</sub>: Basal diet supplemented with 1% canola oil, T<sub>3</sub>: Basal diet supplemented with 2% canola oil, Values are Mean±SE

Table 4: Plasma biochemical parameters of commercial layers supplemented with canola oil for 8 weeks

| Parameters                           | Time     | T <sub>1</sub> | T <sub>2</sub> | T <sub>3</sub> |
|--------------------------------------|----------|----------------|----------------|----------------|
| Triglyceride (mmol L <sup>-1</sup> ) | 4th week | 57.2±1.1       | 57.1±1.0       | 59.3±2.4       |
|                                      | 8th week | 58.3±0.8       | 53.2±1.5*      | 49.7±1.7*      |
| Cholesterol (mg dL <sup>-1</sup> )   | 4th week | 92.8±1.5       | 90.1±2.0       | 94.2±3.5       |
|                                      | 8th week | 115.3±1.2      | 99.1±2.1*      | 102.4±3.2*     |
| HDL-c (mg dL <sup>-1</sup> )         | 4th week | 48.8±2.1       | 48.8±1.2       | 50.6±2.3       |
|                                      | 8th week | 48.8±3.1       | 45.1±5.1       | 44.7±4.1       |
| LDL-c (mg dL <sup>-1</sup> )         | 4th week | 35.7±3.1       | 34.2±2.1       | 38.1±3.2       |
|                                      | 8th week | 50.1±2.1       | 45.2±5.1       | 52.7±4.1       |
| VLDL-c (mg dL <sup>-1</sup> )        | 4th week | 8.3±1.2        | 8.4±1.4        | 8.8±1.4        |
|                                      | 8th week | 9.5±1.1        | 10.0±1.0       | 10.1±1.1       |
| ALT (U L <sup>-1</sup> )             | 4th week | 12.1±1.1       | 12.3±1.2       | 11.1±1.2       |
|                                      | 8th week | 12.8±1.1       | 13.0±1.0       | 11.0±2.1       |
| AST (U L <sup>-1</sup> )             | 4th week | 88.2±3.4       | 91.0±3.2       | 85.0±5.1       |
|                                      | 8th week | 87.1±3.7       | 93.0±5.1       | 93.0±5.6       |
| Creatinine (mg dL <sup>-1</sup> )    | 4th week | 4.8±1.1        | 3.5±1.3        | 3.8±1.2        |
|                                      | 8th week | 3.7±1.5        | 4.1±1.1        | 3.0±1.1        |
| Uric acid (mg dL <sup>-1</sup> )     | 4th week | 5.1±1.3        | 4.1±1.0        | 4.0±1.0        |
|                                      | 8th week | 4.4±1.0        | 3.6±0.9        | 4.7±1.0        |
| Urea (mg dL <sup>-1</sup> )          | 4th week | 11.3±2.2       | 10.9±2.1       | 13.6±3.4       |
|                                      | 8th week | 10.5±2.1       | 10.7±2.1       | 12.9±2.6       |

T<sub>1</sub>: Control group; basal diet with 0% canola oil, T<sub>2</sub>: Basal diet supplemented with 1% canola oil, T<sub>3</sub>: Basal diet supplemented with 2% canola oil, Values are Mean±SE, \*Statistically significant when compared to control (T<sub>1</sub>) at p<0.05, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol, VLDL-c: Very low density lipoprotein cholesterol, ALT: Alanine transaminase and AST: Aspartate transaminase

(99.1±2.1 mg dL<sup>-1</sup>) significantly (p<0.05) compare to control value (58.3±0.8 mmol L<sup>-1</sup>) and (115.3±1.2 mg dL<sup>-1</sup>), respectively after 8 weeks (Table 4). In addition, the inclusion of canola oil 2% in layers ration reduced their plasma TAG (49.7±1.7 mmol L<sup>-1</sup>) and total cholesterol level (102.4±3.2 mg dL<sup>-1</sup>) significantly (p<0.05) compare to control values after 8 weeks. It is clear that, the reduction of both TAG and total cholesterol was time dependent as it was observed only at the end of the experiment, after 8 weeks whereas, this reduction in TAG and Total cholesterol was not influenced by the concentrations of canola oil. The present results (Table 4) indicated also that HDL-c, LDL-c and VLD-c concentrations were not affected (p>0.05) by dietary inclusion of canola oil compare to control (Table 5). The present findings indicated that dietary inclusion of canola oil

Table 5: Egg cholesterol of commercial layers supplemented with canola oil for 8 weeks

| Parameters                           | Time     | T <sub>1</sub> | T <sub>2</sub> | T <sub>3</sub> |
|--------------------------------------|----------|----------------|----------------|----------------|
| Cholesterol (mg g <sup>-1</sup> egg) | 4th week | 15.7±0.4       | 13.8±0.5*      | 12.5±0.2*      |
|                                      | 8th week | 13.0±0.2       | 11.0±0.5*      | 12.0±0.5*      |

T<sub>1</sub>: Control group; basal diet with 0% canola oil, T<sub>2</sub>: Basal diet supplemented with 1% canola oil, T<sub>3</sub>: Basal diet supplemented with 2% canola oil, Values are Mean±SE, \*Statistically significant when compared to control (T<sub>1</sub>) at p<0.05

in ration of laying hens was safe as reflected on comparable values of liver enzymes activities (ALT, AST) and renal injury biomarkers level (BUN, uric acid and creatinine) in all treatments (Table 4). The data summarized in Table 5 showed that, inclusion of 1% canola oil in ration of laying hen reduced significantly egg cholesterol (13.8±0.5 mg g<sup>-1</sup> egg) after 4 and 8 weeks (11.0±0.5 mg g<sup>-1</sup> egg) compare to control treatment (15.7±0.4; 13.0±0.2 mg g<sup>-1</sup> egg, respectively). The inclusion of 2% canola oil in ration of laying hen reduced significantly (p<0.05) egg cholesterol (12.5±0.2 mg g<sup>-1</sup> egg) after 4 weeks and 8 weeks (12.0±0.5 mg g<sup>-1</sup> egg) compare to control treatment (Table 5). The reduction of egg cholesterol was induced by both concentrations of canola oil after 4 and 8 weeks of the experiment (Table 5).

## DISCUSSION

The insignificant difference in feed intake, egg production and feed conversion ratio (p>0.05) in all treatments come in accordance with Eder *et al.* (1998), Shakoor *et al.* (2002, 2003) and Kucukersan *et al.* (2010) in layers. In addition, Roth-Maier *et al.* (1998) found that egg production, egg weight and feed conversion were not influenced by the inclusion of fat sources. Significant reduction was reported in egg production and feed intake of laying hen fed ration supplemented with Iranian canola seed over 10% concentration compare to control (Agah *et al.*, 2010). Furthermore, reduction in feed intake, failing in fat retention and decrease in Metabolizable energy were observed in birds fed ration supplemented with fat sources (Summers *et al.*, 1982). Low performance was obtained in laying hen fed high level of full fat canola seed diet (over 10%) which passed through intestine indigested and subsequent low nutrients were utilized by the birds (Najib and Al-Khateeb, 2004). In addition, 50% of dietary fat was retained in birds fed 20% full fat canola (Leeson *et al.*, 1987). The reduction in fat retention might be attributed to formation of insoluble soap of fatty acid and minerals (Atteh and Leeson, 1984). Feed consumption of hens obtained in this study was higher than reported previously (Balevi and Coskun, 2000). Difference between the studies might be due to the level of ME in the ration.

The insignificant difference in egg weight, egg shell thickness and egg yolk observed in all treatment agree with previous findings. Eder *et al.* (1998), Roth-Maier *et al.* (1998), Shakoor *et al.* (2002, 2003) and Kucukersan *et al.* (2010) showed non-significant changes of egg quality characters in laying hens fed diet supplemented with oils. However, supplementation of canola oil over 10% in diet of layers induced significant decrease in yolk color and egg shell weight compare to control (Agah *et al.*, 2010). As low concentrations of canola oil were used in the present study, the percentage of corn oil in ration formulation was not affected. Therefore, the xanthophyll and carotene content of corn which gives the yellow color of the egg yolk (Yannakopoulos *et al.*, 2005) was the same in all treatments and this can interpret the insignificant changes of yolk color observed in all treatments of the current study. Haugh unit is widely accepted indicator of internal egg quality. There are an inverse relationship between haugh unit value and time of storage (Williams, 1992). The insignificant difference in haugh units as observed in the present study may

owed to the same elapsed time of storage and examination or due to absence of relation between layers feed and haugh units (Naber, 1979). Previous studies (Farrel, 2002; Rowghani *et al.*, 2007) reported similar findings regarding the insignificant changes of egg weight in all treatments of the present study. Similar findings (Shakoor *et al.*, 2002) were demonstrated that inclusion of canola oil 5% in the diet of laying hens reduced serum TAG significantly. Interestingly, concentrations of canola oil used in the current study represented 20-40% of canola concentrations that used previously (Shakoor *et al.*, 2002). In the contrary, MUFA and PUFA were not able to reduce TAG in hypertriglyceridemic patient (Mattson and Grundy, 1985) whereas sunflower and fish oils increased TAG level in laying hens (An and Kang, 1999). The hypocholesterolemic effect of canola oil may be attributed to its higher content of MUFA and PUFA that has inhibitory effect either on the cholesterol biosynthesis in liver (Mattson and Grundy, 1985) or lipogenesis (Leveille *et al.*, 1975). Similar findings (Rays *et al.*, 1998; An and Kang 1999; Grundy, 1987; Lewis *et al.*, 2000) reported a significant decrease in serum cholesterol level due to inclusion of MUFA and PUFA in layers diet. In addition, inclusion of canola oil 5% and soya bean in layer ration was found to decrease serum total cholesterol level (Shakoor *et al.*, 2002). Previous study (Shafey, 1998; Botsoglou *et al.*, 1998; Meluzzi *et al.*, 2000) have been demonstrated that cholesterol level was not affected by inclusion of dietary oil in ration of laying hens. In the same concern, plasma cholesterol values have not been affected by dietary treatment of fish oil, canola oil and poultry byproduct in laying hen (Murata *et al.*, 2003). Plasma cholesterol of laying hen is of singular characteristics (Chapman, 1980) and is not related to egg yolk lipid level, although synthesized in the liver and transported by the blood (Sutton *et al.*, 1984). The present findings indicated that, both concentration of canola oil (1 and 2%) induced the same hypolipidemic and hypocholesterolemic effects, however, lower concentration is favorable economically. Interestingly, the desired hypolipidemic and hypocholesterolemic effects was attained in the present study by using 20 or 40% of the canola concentrations used earlier (Shakoor *et al.*, 2002), respectively.

The insignificant difference in HDL-c, LDL-c and VLDL-c concentrations of all treatment as reported in the present study disagree with previous results (Shakoor *et al.*, 2002) reported significant increase in serum HDL-C and decreased LDL-C in layers fed diet mixed with 5% canola oil compare to the control. The confliction might be attributed to the difference in concentrations of canola used in previous and current experiments. It has been reported that, HDL-C concentrations were not affected by any of oil sources such as fish, canola and soybean oils in rat (Radcliffe *et al.*, 2001) and laying hen (Murata *et al.*, 2003; Guclu *et al.*, 2008). Previous studies were performed to study the effect of dietary inclusion of canola oil in ration of laying hen from lipid metabolism point of view particularly cholesterol and fatty acid analysis. Therefore, literatures concerning the effect of canola oil on liver and kidney function were not available, therefore the present study might be the first study reported the effect of canola oils (1 and 2%) on liver and kidney functions. Avian cholesterol biosynthesis has been occurred in the liver and transferred into developing yolk via blood several days before ovulation and deposited in the growing follicle. In domestic fowl, average yolk cholesterol concentration was found to be 16.13 mg g<sup>-1</sup> wet yolk during the first year of egg production (Hall and McKay, 1993). Data concerning the effect of inclusion of fatty acids in the diet of layers on plasma and egg cholesterol levels were contradictory. In agreement with current study, some publications (Hollands *et al.*, 1980; Mori *et al.*, 1999; Aydin, 2005) showed that dietary PUFA decreased the concentration of plasma and yolk cholesterol in laying hens. The significant decrease in egg yolk cholesterol as observed in the present study might be secondary to the decreased plasma cholesterol (El Bagir *et al.*, 2006). Other publications



(Caston and Leeson, 1990; Beyer and Jensen, 1992; Simopoulos, 2000; Milinsk *et al.*, 2003; Agah *et al.*, 2010; Kucukersan *et al.*, 2010) demonstrated that no difference in concentration of egg yolk cholesterol in the layers diets mixed with different dietary oils. These studies demonstrated that, the amount of egg cholesterol is affected by yolk lipoprotein cholesterol not by cholesterol density in its plasma (Agah *et al.*, 2010). Moreover, no relation between plasma and egg cholesterol was reported (Beyer and Jensen, 1992). Major part of lipoproteins cholesterol is on their superficial layer and therefore the egg cholesterol reduction is only feasible by raising the size of lipoproteins (Agah *et al.*, 2010). Egg cholesterol concentration was higher in hen fed diet mixed with canola oil compare to control (Rowghani *et al.*, 2007). In addition, total cholesterol concentration of egg yolk was differed according to the type of dietary oil sources (fish, canola or poultry byproduct) in laying hen (Murata *et al.*, 2003). Although, Leeson and Summers (2005) and Agah *et al.* (2010) reported that decrease in egg cholesterol content was only possible via genetic modification, the present study demonstrated the possibility of reduction of egg cholesterol via dietary manipulation using lower concentration of canola oil (1%).

## CONCLUSION

The present study concluded that, inclusions of 1% canola oil in layer diet were quiet enough to reduce plasma and egg cholesterol. These results open new perspectives on economy of poultry industry for production of healthy food (egg) for human consumption.

## ACKNOWLEDGMENT

This research was supported by the Deanship of Scientific Research (DSR 120055), King Faisal University, Saudi Arabia.

## REFERENCES

- Agah, M.J., H. Nassiri-Moghaddam, A.M. Tahmasbi and H. Lotfollahian, 2010. Performance and fatty acid compositions of yolk lipid from laying hens fed with locally produced canola seed (*Brassica napus* L.). *Res. J. Biol. Sci.*, 5: 228-232.
- Ahmad, S., Ahsan-ul-Haq, M. Yousaf and H. Nawaz, 2010. Effect of feeding canola oil and vitamin a on the fatty acid profile of egg yolks in laying hens. *Pak. J. Nutr.*, 9: 191-194.
- An, B.K. and C.W. Kang, 1999. Effects of dietary fat sources containing  $\omega$ -3 or  $\omega$ -6 polyunsaturated fatty acids on fatty acid composition of egg yolk in laying hens. *Korean J. Anim. Sci.*, 41: 293-310.
- Atteh, J.O. and S. Leeson, 1984. Effects of dietary saturated or unsaturated fatty acids and Ca levels on performance and mineral metabolism of broiler chicks. *Poult. Sci.*, 63: 2252-2260.
- Aydin, R., 2005. The effects of Conjugated Linolenic Acid (CLA) and canola oil on the fatty acid composition and quality of eggs from laying hens. *South Afr. J. Anim. Sci.*, 35: 172-179.
- Balevi, T. and B. Coskun, 2000. Effects of some dietary oils on performance and fatty acid composition of eggs in layers. *Revue Med. Vet.*, 151: 847-854.
- Bauer, J.D., 1982. *Clinical Laboratory Methods*. 9th Edn., The C.V. Mosby Co., St. Louis, MO.
- Bavelaar, F.J. and A.C. Beynen, 2004. Relationships between the intake of n-3 polyunsaturated fatty acids by hens and the fatty acid composition of their eggs. *Int. J. Poult. Sci.*, 3: 690-696.
- Berrio, L.F. and J.A. Hebert, 1990. The effect of adding cholesterol to laying hen diets as powder or predissolved in fat. *Poult. Sci.*, 69: 972-976.
- Beyer, R.S. and L.S. Jensen, 1989. Overestimation of the cholesterol content of eggs. *J. Agric. Food Chem.*, 37: 917-920.

- Beyer, R.S. and L.S. Jensen, 1992. Cholesterol concentration of egg yolk and blood plasma and performance of laying hens as influenced by dietary  $\alpha$ -ketoisocaproic acid. *Poult. Sci.*, 71: 120-127.
- Beynen, A.C., 2005. Fatty acid composition of eggs produced by hens fed diets containing groundnut, soya bean or linseed. *NJAS-Wageningen J. Life Sci.*, 52: 3-10.
- Boehringer Mannheim GmbH Biochemica, 1989. *Methods of Biochemical Analysis and Food Analysis Using Test-Combinations*. Boehringer Mannheim, Germany, pp: 26-28.
- Botsoglou, N.A., L.A. Yannakopoulos, D.J. Fletouris, A.S. Tserveni-Goussi and I.E. Psomas, 1998. Yolk fatty acid composition and cholesterol content in response to level and form of dietary flaxseed. *J. Agric. Food Chem.*, 46: 4652-4656.
- Card, L.E. and M.C. Nesheim, 1972. *Poultry Production*. 11th Edn., Lea and Febiger, Philadelphia.
- Caston, L. and S. Leeson, 1990. Research note: Dietary flax and egg composition. *Poult. Sci.*, 69: 1617-1620.
- Chapman, M.J., 1980. Animal lipoproteins: Chemistry, structure and comparative aspects. *J. Lipid Res.*, 21: 789-853.
- Eder, K., D.A. Roth-Maier and M. Kirchgessner, 1998. Laying performance and fatty acid composition of egg yolk lipids of hens fed diets with various amounts of ground or whole flaxseed. *Archiv fuer Gefluegelkunde*, 62: 223-228.
- El Bagir, N.M., A.Y. Hama, R.M. Hamed, A.G. Abd-El-Rahim and A.C. Beynen, 2006. Lipid composition of egg yolk and serum in laying hens fed diets containing black cumin (*Nigella sativa*). *Int. J. Poult. Sci.*, 5: 574-578.
- Farran, M.T., M.G. Uwayjian, A.M.A. Miski, F.T. Slieman, F.A. Adada and V.M. Ashkarian, 1995. Effect of feeding raw and treated common vetch seed (*Vicia sativa*) on the performance and egg quality parameters of laying hens. *Poult. Sci.*, 74: 1630-1635.
- Farrel, D.J., 2002. Adding value to the hens egg. *Nut. Rep. Int.*, 45: 1052-1057.
- Fernandes, G., 1995. Effects of calorie restriction and omega-3 fatty acids on autoimmunity and aging. *Nutr. Rev.*, 53: S72-S77.
- Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. *Clin. Chem.*, 18: 499-502.
- Grundy, S.M., 1987. Monounsaturated fatty acids, plasma cholesterol and coronary heart disease. *Am. J. Clin. Nutr.*, 45: 1168-1175.
- Guclu, B.K., F. Uuank and K.M. Iscan, 2008. Effects of dietary oil sources on egg quality, fatty acid composition of eggs and blood lipids in laying quail. *South Afr. J. Anim. Sci.*, 38: 91-100.
- Hall, L.M. and J.C. Mckay, 1993. The relationship between yolk cholesterol and total lipid concentration throughout the first year of egg production in the domestic fowl. *Br. Poult. Sci.*, 34: 487-495.
- Hammad, S.M., H.S. Siegel and H.L. Marks, 1996. Dietary cholesterol effects on plasma and yolk cholesterol fractions in selected lines of Japanese quail. *Poult. Sci.*, 75: 933-942.
- Hargis, P.S., 1988. Modifying egg yolk cholesterol in domestic fowl-a review. *World's Poult. Sci. J.*, 44: 17-29.
- Hollands, K.G., A.A. Grunder and C.J. Williams, 1980. Response to five generations of selection for blood cholesterol levels in White Leghorn. *Poult. Sci.*, 59: 1316-1323.
- Kucukersan, K., D. Yesilbag and S. Kucukersan, 2010. Influence of different dietary oil sources on performance and cholesterol content of egg yolk in laying hens. *J. Biol. Environ. Sci.*, 4: 117-122.

- Leeson, S., J.O. Atteh and J.D. Summers, 1987. Effects of increasing dietary levels of full-fat canola on performance, nutrient retention and bone mineralization. *Poult. Sci.*, 66: 875-880.
- Leeson, S. and J.D. Summers, 2005. *Commercial Poultry Nutrition*. 3rd Edn., University Books, Guelph, Ontario, Canada Pages: 398.
- Leveille, G.A., D.R. Romsos, Y. Yeh and E.K. O'Hea, 1975. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. *Poult. Sci.*, 54: 1075-1093.
- Lewis, N.M., S. Seburg and N.L. Flanagan, 2000. Enriched eggs as a source of N-3 polyunsaturated fatty acids for humans. *Poult. Sci.*, 79: 971-974.
- 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.
- Mattson, F.H. and S.M. Grundy, 1985. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.*, 26: 194-202.
- Meluzzi, A., F. Sirri, G. Manfreda, N. Tallarico and A. Franchini, 2000. Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. *Poult. Sci.*, 79: 539-545.
- Milinsk, M.C., A.E. Murakami, S.T.M. Gomes, M. Matsushita and N.E. de Souza, 2003. Fatty acid profile of egg yolk lipids from hens fed chicks rich in n-3 fatty acids. *Food Chem.*, 83: 287-292.
- Mori, A.V., C.X. Mendonca, jr. and C.O.F. Santos, 1999. Effect of dietary lipid lowering drugs upon plasma lipids and egg yolk cholesterol levels of laying hens. *J. Agric. Food Chem.*, 47: 4731-4735.
- Murata, L.S., J. Ariki, C.R. Machado, L.P.G. da Silva and M.J.M. Rezende, 2003. Effect of oils sources on blood lipid parameters of commercial laying hens. *Braz. J. Poult. Sci.*, 5: 203-206.
- NRC, 1994. *Nutrient Requirements of Poultry*. 9th Edn., National Academy Press, Washington, DC., USA.
- Naber, E.C., 1979. The effect of nutrition on the composition of eggs. *Poult. Sci.*, 58: 518-522.
- Najib, H. and S.A. Al-Khateeb, 2004. The effect of incorporating different levels of locally produced canola seeds (*Brassica napus*, L.) in the diet of laying hen. *Int. J. Poult. Sci.*, 3: 490-496.
- Neuringer, M., G.J. Anderson and W.E. Conner, 1998. The essentiality of  $\omega$ -3 fatty acids for the development and function of the retina and brain. *Ann. Rev. Nutr.*, 8: 517-541.
- Nobakht, A., 2011. Effects of using different levels of vegetable oils and their blends on egg yolk fatty acids profile of laying hens. *Adv. Environ. Biol.*, 5: 2728-2731.
- Radchiffe, J.D., C.C. King, D.M. Czajka-Narins and V. Imrhan, 2001. Serum and liver lipids in rats fed diets containing corn oil, cottonseed oil, or a mixture of corn and cottonseed oils. *Plant Foods Human Nutr.*, 56: 51-60.
- Rays, R., J. Koreleski, M. Kuchata, A. Sieradzka and Z. Zegarek, 1998. The influence of marine originated fat in diet for hens on the polysaturated fatty acids in egg yolk and cholesterol content in blood serum. *Roezniki-Naukowe-Zootechniki*, 25: 75-90.
- Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
- Rose, D.P., 1997. Dietary fatty acids and prevention of hormone-responsive cancer. *PSEBM*, 216: 224-233.
- Roth-Maier, A., A. Dora and M. Kirchgessener, 1998. Feeding of DD-rapeseed to fattening chicken and laying hens. *Landwirtsch Forschung*, 41: 140-150.

- 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.
- SAS Institute, 2004. *SAS Users Guide: Statistics*. SAS Institute, Cary, NC.
- Shafey, T.M., 1998. Effects of dietary retinol and sunflower oil on the performance and on the lipoproteins, lipids, cholesterol and retinol concentrations of plasma and eggs of laying hens. *Arab Gulf J. Sci. Res.*, 16: 331-345.
- Shakoor, H.I., M.L. Khan, Z. Nasir, N. Mukhtar and M.S. Rehman, 2002. Effect of feeding canola and soybean oils on serum lipid profile in commercial layers. *Pak. Vet. J.*, 22: 48-51.
- Shakoor, H.I., M.A. Javed, Z. Iqbal, Z. Nasir and N. Mukhtar, 2003. Comparative study on the effects of feeding canola and soybean oils on egg production and cholesterol in commercial layers. *Pak. Vet. J.*, 23: 22-26.
- Simopoulos, A.P., 2000. Human requirement for N-3 polyunsaturated fatty acids. *Poult. Sci.*, 79: 961-970.
- Stadelman, W.J., 1999. The incredibly functional egg. *Poult. Sci.*, 78: 807-811.
- Summers, J.D., H. Shen and S. Leeson, 1982. The value of canola seed in poultry diets. *Can. J. Anim. Sci.*, 62: 861-868.
- Sutton, C.D., W.M. Nuir and G.E. Mitchell Jr., 1984. Cholesterol metabolism in laying hen as influenced by dietary cholesterol, caloric intake and genotype. *Poult. Sci.*, 63: 972-980.
- Tabacco, A., F. Meattini, E. Moda and P. Tarli, 1979. Simplified enzymatic/colorimetric serum urea nitrogen determination (letter). *Clin. Chem.*, 25: 336-337.
- Tample, N.J., 1996. Dietary fats and coronary heart disease. *Biomed. Pharmacother.*, 50: 261-268.
- Todd, J.C., J.B. Henry, A.H. Sanford and I. Davidsohn, 1984. *Clinical Diagnosis and Management by Laboratory Methods*. 17th Edn., Saunder Publisher, America, ISBN: 9780721646572, Pages: 1502.
- Trinder, P., 1969. Enzymatic calorimetric determination of triglycerides by GPO-PAP method. *Ann. Clin. Biochem.*, 6: 24-27.
- Van Elswyk, M.E., A.R. Sams and P.S. Hargis, 1992. Composition, functionality and sensory evaluation of eggs from hens fed dietary menhaden oil. *J. Food Sci.*, 57: 342-344.
- 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: S61-S69.
- Wells, R.G., 1968. A Study of the Hen's Egg. In: *British Egg Marketing Board Symposium*, Carter, T.C. (Ed.). WHO, Edinburg, UK., pp: 207-249.
- Williams, K.C., 1992. Some factors affecting albumen quality with particular reference to Haugh unit score. *World's Poult. Sci. J.*, 48: 5-16.
- Yang, C.X., C. Ji, L.M. Ding. and Y. Rong, 2000. N-3 fatty acid metabolism and effects of alpha-linolenic acid on enriching n-3 FA eggs. *J. Chi. Agric. Univ.*, 95: 117-122.
- Yannakopoulos, A., A. Tserveni-Gousi and E. Christaki, 2005. Enhanced egg production in practice: The case of bio-omega-3 egg. *Int. J. Poult. Sci.*, 4: 531-535.
- Zeidler, G., 2002. Shell Eggs and their Nutritional Value. In: *Commercial Chicken Meat and Egg Production*, Bell, D.D. and W.D. Jr. Weaver (Ed.). Kluwer Academic Publisher, New York, Pages: 1109-1128.