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**Original Article**

# *Effects of Oil Source on Egg Quality and Yolk Fatty Acid Profile of Layer Hens*

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## ABSTRACT

The effects of different supplemental oils on the performance, egg quality, and fatty acid (FA) profiles of eggs produced by 52 week-old Hy-Line laying hens were investigated. A total of two hundred ninety-two laying hens were assigned to three treatments with four replicates each, according to the source of oil supplemented to their diets: corn oil (CO), restaurant spent oil (RO), and a control diet (with soybean oil, SO) using a traditionally used oil in hens' diets in local conditions. Egg production was not affected by the type of oil fed and did not change during the course of the study. Eggs mean weight were not affected by type of supplemented oil. A similar trend was observed for shell thickness, yolk heights, Haugh units, and albumen heights. The results demonstrated that both fatty acid C8:0 and C12:0, present in RO and SO respectively, do not appear in the egg yolk in any treatment. Incorporated RO significantly reduced ($p<0.05$) the percentage of unsaturated fatty acid in egg yolk as compared to the SO or CO. Conversely, replacing SO with high quality oil like CO in the feed of laying hens significantly enhanced ($p<0.05$) egg quality.

## INTRODUCTION

Oils and fats are supplemented to livestock (poultry) diets to increase its energy density, maintain the calorie/ protein ratio, and improve diets' palatability. However, it can modify the fatty acid (FA) profile of eggs yolks (Brugalli *et al.*, 1998; Mohamed *et al.*, 2019; Reda *et al.*, 2019; Santtos *et al.*, 2019; Kralic *et al.*, 2020; Reda *et al.*, 2020; Batkowski *et al.*, 2021; Panite *et al.*, 2021).

Meat, milk, and eggs are very important in achieving food security. However, the quality of these products and their low fat content have come under consumers' focus, with demands for low fat animal products. Quality of products of animal origin have recently been the target of several studies in an attempt to improve its marketing and nutritional quality (Newman *et al.*, 2002; Basmacıoğlu *et al.*, 2003; Güçlü *et al.*, 2008; Huang, 2016; Batkowski *et al.*, 2021; Panite *et al.*, 2021). There are several factors, internal and external, that affect fatty acids profile of the animal products. Fatty acid concentration in egg yolk is influenced by the diet, as well as by genetics, age, and production level (Güçlü *et al.*, 2008). Moreover, Popova *et al*, (2020) evaluated the quality of eggs from layers reared under alternative and conventional systems and found that rearing in alternative system considerably reduced the content of C14:0, C16:0, C18:0, C16:1n-7, and C18:1n-9, as well as saturated and monounsaturated fatty acids. The fatty acid profile of egg yolk can be changed by feeding different oil supplements which are routinely used as part of layers' diets to correct energy requirements (Shafey *et al.,* 2003; Cabrera *et al.,* 2005; Güçlü *et al.*, 2008; Huang, 2016; Batkowski *et al.,* 2021; Panite *et al.*, 2021). Polyunsaturated fatty acids change yolk lipids profile, as indicated by previous research (Eseceli & Kahraman, 2004; Güçlü *et al.*, 2008). It is well known that plant oils used in poultry feed have similar lipid profiles. However, they differ in their physical characteristics and effects on fat properties of animal products (Güçlü *et al.*, 2008). Şenköylü, (2001) supposed that the reason behind these differences could be type of soil and the genetic makeup of plant sources.

Although much research was conducted to investigate the effect of different supplemental oils on animal products' fatty acid profiles (Balevi and Coşkun, 2000; Grobas *et al.,* 2001; Shafey *et al.,* 2003; Basmacıoğlu *et al.*, 2003; Guo *et al.*, 2004; Saghir, *et al.,* 2012; Abo Omar & Zaza, 2016; El Qub and Abo Omar, 2016), results of some works were inconsistent (Grobas *et al.,* 2001; Shafey *et al.*, 2003; Murata *et al.*, 2003; Cabrera *et al.,* 2005). Unsaturated fatty acid enriched vegetable oils are better digested by poultry, as compared to fatty acid enriched animal fats (Zduńczyk *et al.*, 2001; Dvorin *et al.*,1998). In pigs, feeding conjugated linoleic acid (CLA) increased the rate of gain (Thiel-Cooper *et al.*, 2001; Lauridsen *et al.,* 2005), and improved feed efficiency (Lauridsen *et al.*, 2005; Bee, 2001; Ramsay *et al.*, 2001; Wiegand *et al.*, 2001). However, in several other investigations, no growth-enhancing effect of CLA in pigs was observed (Ramsay *et al.*, 2001; Stangl *et al.*, 1999; Muller *et al.*, 2000; Gatlin *et al.*, 2002). Body weight gain and feed intake of broiler chickens were significantly reduced by dietary CLA (Szymczyk *et al.*, 2001). An increase in weight gain and feed intake was reported by Bolukbasi (2006), whereas Sirri *et al.* (2003) found no influence of dietary CLA on productive performance in poultry.

It is necessary to understand the effect of different oils on the performance and external and internal egg quality factors better, as well as investigate the effect of serum lipid profile under local conditions, especially when using RO and CO in poultry diets instead of the traditionally used SO.

The objective of this study was investigating the effect of feeding RO and CO as supplementary oils on layers performance, egg quality, and fatty acid composition of the egg yolk, as compared to traditionally used SO.

## MATERIALS AND METHODS

The present study was conducted at the An-Najah National University farm, Tulkarm, Palestine, in the period between March and June, 2019. One hundred ninety two 52 week old Hy-Line laying hens were used in the study. Hens were placed in individual cages with dimensions of 45 x 45 x 30 cm and randomly distributed among three groups with four replicates per group, containing 16 hens each. Diets were formulated according to the nutrient requirements of laying hens presented in NRC (1985). Experimental rations including three rations were as follows: Diet with soybean oil (SO) as a control diet, diet with spent restaurant oil (RO), diet with corn oil, (CO) at level of 2% of the experimental rations (Table 1).

**Table 1 –** The experimental ration fed to laying hens with soybean (SO), restaurant (RO), and corn (CO) oils, g/kg.

| Item | Content |
|---|---|
| Ingredient (kg) | |
| Corn | 447 |
| Wheat | 167 |
| Soybean meal | 243 |
| Experimental oil* | 20 |
| Limestone | 100 |
| Methionine | 1.5 |
| Lysine | 0.8 |
| Layers premix** | 5 |
| Sodium carbonate | 2.2 |
| DCP | 13 |
| Salt | 8 |
| Chemical composition (%) | |
| Dry matter | 88 |
| Crude protein | 17 |
| Crude fat | 4.5 |
| Crude fiber | 4.1 |
| Total Ca | 3.1 |
| Total P | 0.8 |
| ME, MJ/ kg | 11.0 |

*oil was supplemented to diets as SO, soybean oil (control); RO, restaurant oil; CO, corn oil.

**Premix provided per kg of premix: 6 000 000 IU vitamin A; 6 000 IU vitamin D3; 20 000 IU vitamin E; 2 g vitamin K2; 1.3 g vitamin B1; 2.2 g vitamin B2; 1.9 g vitamin B6; 14 mg vitamin B12; 8 g niacin; 320 mg folic acid; 3 g calcium pantothenic acid; 46 mg D-Biotin.: 75 g Mn; 28 g Fe; 65 g Zn; 5 g Cu; 0.2 g I.

All hens were under similar management and environmental conditions such as lighting program (16 hours light: 8 hours darkness).

The study comprised three periods with 2 weeks each, starting from 52 weeks of age. The first period lasted from 52 to 54 weeks (1-14 d); the second period from 54 to 56 weeks (15-28 d); and the third period from 56 to 58 wk (29-42 d) of birds' age.

Egg production, egg weight, and hens' body weight were measured during 42 days, as well as yolk color, shell quality, and shell thickness. Egg shell thickness (mm) was measured using a micrometer and the mean of the total period was calculated. Moreover, Haugh

**Table 2 –** Effect of oil source on egg production and egg weight.

| | Days | SO | RO | CO |
|---|---|---|---|---|
| Egg production (%) | 1-14 | 67.5±6.4 | 71.3±7.0 | 70.0±8.2 |
| | 15-28 | 63.8±8.0 | 65.0±7.9 | 66.7±6.1 |
| | 29-42 | 58.8±8.3 | 58.8±7.5 | 60.0±4.9[a] |
| Egg weight | 1-14 | 66.98±2.66 | 65.8±4.36 | 67.9±2.9 |
| | 15-28 | 63.0±3.87 | 64.36±4.63 | 64.85±4.45 |
| | 29-42 | 61.34±4.06 | 62.74±3.35 | 61.7±4,24 |

[a] Indicated significant difference when compared to week 42 in the same column ($p<0.05$).

unit (HU) was calculated using the following equation, introduced by Raymond Haugh in 1937:

$$HU = 100^* \log (H + 7.57)-(1.7 * W^{0.37})$$

Where HU= Haugh unit, H= Albumen height in (mm), W= Egg weight in (gm).

Measurement of the fatty acid profile was conducted for both the oil used in the feed and egg yolk, being determined through gas chromatography analysis (PerkinElmer Claru 500 GCMS). After lipid extraction following to Bligh & Dyer (1959), fatty acid separations were achieved in a BPX70 capillary column (30 m × 250µm diameter). Oven temperature was programmed from 100 °C to 240 °C at a rate of 3 °C/min, kept at 240 °C for 15 min, and then elevated to 350 °C at a rate of 3 °C/min. The final temperature was maintained for 30 min. Helium was used as carrier gas at a constant pressure of 260 kPa.

Experimental diets were analyzed for proximate nutrients using AOAC (1990) procedures.

Dry matter (DM) was determined by drying at 105°C until obtaining constant weight. The mineral content was then determined by ashing at 600°C for 8 h. Nitrogen was determined by the Kjeldahl method (CP = x 6.25); Association of Analytical Communities (1990). NDF and ADF were determined according to Van Soest *et al.* (1991).

The samples were analyzed for the mineral elements using an atomic absorption spectrophotometer (Shimadzu 650 model), whereas K and Na were determined using flame photometry.

### Statistical analysis

All data were recorded and sorted using Excel 2013. Then, data were analyzed by one-way ANOVA with Statistical Analysis System (SAS, JMP 2008, version 8). When the ANOVA showed statistical significance, Tukey multiple range test was conducted. $p<0.05$ indicated significant difference.

## RESULTS AND DISCUSSION

Egg production was not affected by type of oil fed (Table 3) and did not change during the course of the study. Güçlü (2008) reported no significant difference in egg production in the treatment groups of olive, soybean, and grape seed oils and that of the control group at the end of a 7 weeks period (0-42).

**Table 3 –** Effect of oil source on eggs quality of layers[1].

| | Week | SO | RO | CO |
|---|---|---|---|---|
| Shell thickness (mm) | 42 | 0.43 ± 0.004 | 0.41 ± 0.05 | 0.42 ± 0.05 |
| | 46 | 0.39 ± 0.04 | 0.40 ± 0.04 | 0.40 ± 003 |
| | 48 | 0.44 ± 0.04 | 0.42 ± 0.03 | 0.44 ± 0.03 |
| Yolk height (mm) | 42 | 19.16 ± 0.84 | 19.85 ± 1.31 | 19.11 ± 1.05 |
| | 46 | 19.92 ± 1.2 | 19.67 ± 1.42 | 19.75 ± 0.84 |
| | 48 | 19.29 ± 1.41 | 19.01 ± 1.55 | 18.27 ± 1.58 |
| Albumen height (mm) | 42 | 8.40 ± 0.86 | 8.07 ± 1.17 | 8.32 ± 1.31 |
| | 46 | 9.58 ± 0.98 | 9.73 ± 1.65 | 9.16 ± 1.27 |
| | 48 | 9.14 ± 1.12 | 9.55 ± 1.46 | 8.73 ± 1.45 |
| Haugh unit | 42 | 89.77 ± 4.77 | 88.01 ± 6.59 | 88.73 ± 7.38 |
| | 46 | 96.67 ± 4.36 | 97.50 ± 8.91 | 94.06 ± 5.94 |
| | 48 | 94.87 ± 5.22 | 96.26 ± 6.76 | 92.47 ± 7.30 |
| Color | 42 | 10.58 ± 0.95 | 10.80 ± 0.83 | 10.67 ± 0.62 |
| | 46 | 7.17 ± 0.90 [a] | 7.17 ± 0.90 [a] | 7.17 ± 0.99 [a] |
| | 48 | 7.00 ± 0.71 [a] | 7.00 ± 0.91 [a] | 7.17 ± 0.80 [a] |

[a] indicating the difference within the same column is significant ($p<0.05$).

[1] The egg quality of 16 laying hens per treatment.

Similar reports showed that there was no significant difference in egg production between laying hens with diets containing olive and soybean oil and the control group (Shafey *et al.*, 2003). Other research showed that soybean and grape seed oil had no significant effect on egg production (Filardi *et al.*, 2007). Similar findings were reported when feeding canola oil (Costa *et al.* 2008). Moreover, Hosseini-Vashan & Afzali (2008) reported that palm oil had no effect on egg yield when fed at rate of 4.5% of ration. Results of this study are consistent with those reported by Brugalli *et al.* (1998) and Muramatsu *et al.* (2005), who did not observe any effect of dietary oil inclusion levels on egg production. Grobas *et al.* (2004), studying different PUFA sources in layer feeds, observed that although fat source did not influence feed intake, egg production, or feed efficiency, diets containing soybean oil produced heavier eggs as compared with tallow, olive oil, or linseed oil being used as PUFA sources. Similarly, heavier eggs and albumens were observed in layers fed with palm oil (Santtos *et al.*, 2019). In contrast, Costa *et al.* (2008) reported a linear effect of different soybean levels on egg production. Egg production was increased with increasing level of soybean oil in the diet (Rodrigues *et al.* 2005). The inconsistency in the effects of different oil sources in egg yield could be due to several factors such as the crops' production level, climate, and the vegetation stage of the crop.

Eggs mean weight were not affected by different types of supplemented oil. A similar trend was observed for shell thickness, yolk heights, Haugh unit, and albumen heights (Table 3). Güçlü (2008) showed that oil source had significant positive effects on egg weights with addition of olive, soybean, and grape seed oils to layer hens rations as compared to control rations at the end of a 6 weeks feeding trial. A similar effect was observed when feeding linoleic acid to laying hens, whereas this acid improved the metabolism of estradiol of plasma that leads to more synthesis of fat and proteins, improving egg production and weights.

Supplemental oils significantly reduced the index of yolk color as compared to the first period of the study (Table 3). This result is in disagreement with previous research were oil source had no effect on the yolk index (Güçlü, 2008).

Shell thickness was not affected by the oil source fed, with similar results reported by Güçlü (2008) when olive, soybean, and grape seed oils had no effect on shell thickness when compared with control. In the present study, slight improvements in eggshell thickness were observed in all diets that did not contain maize, sesame, and fish oil ($p>0.05$). Grobas *et al.* (2000) observed better egg quality in layers that were fed diets containing soybean oil. On the other hand, Mazalli *et al.* (2004) found an effect of fat sources on eggshell percentage, but only when vitamin E levels were increased from 12 IU to 100 IU, with the lowest eggshell percentage being determined by the addition of a mixture of fish oil and linseed oil (1:1) and the addition of sunflower oil.

**Table 4 –** Fatty acid profile of the oil used to feeding the hens.

| | Fatty acid (%) of supplemental oils | | |
|---|---|---|---|
| Fatty acid | SO | RO | CO |
| C8:0 Caprylic acid | 0.00 | 0.50 | 0.00 |
| C12:0 Lauric acid | 0.33 | 0.00 | 0.00 |
| C14:0 Myristic acid | 0.36 | 0.05 | 0.00 |
| C16:0 Palmitic acid | 18.77 | 8.34 | 13.36 |
| C18:0 Stearic acid | 5.13 | 3.94 | 2.19 |
| C16:1 7-hexadeconoic acid | 0.00 | 0.00 | 0.00 |
| C16:1 Palmitoleic acid | 0.00 | 0.00 | 0.00 |
| C18:1 Cis oleic acid | 34.37 | 38.34 | 36.51 |
| C18:1 Trans oleic acid | 2.14 | 0.98 | 0.84 |
| C18:2 Linoleic acid | 38.90 | 47.85 | 46.84 |

The mean value of the percentage of yolk fatty acids in different dietary groups is shown in Table 5. The results demonstrated that both fatty acid C8:0 and C12:0, present in restaurant and stock oil respectively, do not present in the egg yolk in all the treatments. However, the obtained results showed that the saturated fatty acid (C14:0) significantly increased its presence in the yolk of laying hens that consumed feed prepared with RO after 46 weeks, and with CO after 48 weeks, as compared to SO (control). However, the level of unsaturated fatty acid (C16:1) 7-hexadeconoic acid was significantly reduced in the egg yolk of the laying hens that consumed the feed containing RO, while no significant difference appeared when corn oil was used to feed the hens as compared to control. Moreover, fatty acid (C18:2) level was significantly reduced after the laying hens consumed the feed that contained RO instead of SO. In contrast, (C18:2) level was significantly increased after feeding the hens with the feed contain CO at 46 weeks (Batkowski *et al.*, 2021; Panite *et al.*, 2021)

The effect of feeding weeks at the same treatment on the fatty acid profile of egg yolk was also evaluated and the results showed that the level of fatty acid C14:0 significantly increased at 48 weeks when hens were fed with feed containing RO, as compared to 42 weeks for the same treatment. Whereas, the level of unsaturated fatty acid C18:1 Cis, significantly reduced and that of C18:1 Trans significantly increased, due to

**Table 5 –** Effect of different oil sources on fatty acid composition of egg yolk.

| Fatty acid (%) | Stock oil | | | Oxidized oil | | | Corn oil | | |
|---|---|---|---|---|---|---|---|---|---|
| | Feeding weeks | | | Feeding weeks | | | Feeding weeks | | |
| | 42 | 46 | 48 | 42 | 46 | 48 | 42 | 46 | 48 |
| C8:0 Caprylic acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C12:0 Lauric acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C14:0 Myristic acid | 0.35 ± 0.00 | 0.18 ± 0.07 | 0.24 ± 0.03 | 0.15 ± 0.00 | 0.24 ± 0.03[a] | 0.29 ± 0.04[b] | 0.24 ± 0.11 | 0.17 ± 0.00 | 0.36 ± 0.11[a] |
| C16:0 Palmitic acid | 28.01 ± 1.51 | 26.99 ± 0.88 | 27.05 ± 0.61 | 26.68 ± 0.68[a] | 26.75 ± 0.31 | 29.4 ± 2.26 | 37.21 ± 1.69[a] | 27.66 ± 2.66[b] | 26.71 ± 0.97[b] |
| C18:0 Stearic acid | 7.52 ± 0.26 | 6.32 ± 0.63 | 8.88 ± 0.93 | 7.47 ± 0.30 | 8.48 ± 1.01 | 8.19 ± 1.96 | 7.67 ± 1.77 | 8.07 ± 1.97 | 8.44 ± 0.50 |
| *Sum of SFA* | *35.88 ± 1.77* | *33.49 ± 1.58* | *36.17 ± 1.57* | *34.3 ± 0.98* | *35.47 ± 1.35* | *37.88 ± 4.26* | *45.12 ± 3.57* | *35.9 ± 4.63[b]* | *35.51 ± 1.58[b]* |
| C16:1 7-hexadeconoic acid | 0.36 ± 0.07 | 0.36 ± 0.03 | 0.48 ± 0.10 | 0.23 ± 0.02 | 0.35 ± 0.07 | 0.28 ± 0.18[a] | 0.29 ± 0.08 | 0.36 ± 0.06 | 0.52 ± 0.09[b] |
| C16:1 Palmitoleic acid | 1.96 ± 0.50 | 1.90 ± 0.23 | 1.89 ± 0.45 | 1.82 ± 0.06 | 1.82 ± 0.25 | 2.25 ± 1.06 | 1.72 ± 0.48 | 1.83 ± 0.55 | 1.68 ± 0.45 |
| C18:1 Cis oleic acid | 44.16 ± 1.60 | 48.02 ± 1.50 | 43.0 ± 1.65 | 47.65 ± 0.91 | 48.87 ± 0.63 | 43.06 ± 1.95[b] | 36.31 ± 3.36[a] | 46.09 ± 2.89 | 45.89 ± 0.72[b] |
| C18:1 Trans oleic acid | 3.12 ± 1.24 | 2.27 ± 0.32 | 3.72 ± 0.96 | 2.28 ± 0.67 | 3.99 ± 1.27 | 4.03 ± 1.35[b] | 4.87 ± 0.73 | 2.88 ± 0.89 | 1.99 ± 0.24[b] |
| C18:2 Linoleic acid | 14.28 ± 0.45 | 12.95 ± 0.76 | 13.29 ± 1.90 | 11.97 ± 2.46[a] | 8.89 ± 2.69[a], | 11.16 ± 2.75[a] | 11.86 ± 0.85[a] | 14.71 ± 1.61[a] | 14.58 ± 1.03 |
| *Sum of USFA* | *63.88 ± 3.86* | *65.5 ± 2.84* | *62.38 ± 5.06* | *63.95 ± 4.12* | *63.92 ± 4.91* | *60.78 ± 7.29* | *55.05 ± 5.50* | *65.87 ± 6.00* | *64.66 ± 2.53* |

a, Mean value in the same row are significant different as compared to the control (stock oil) ($p<0.05$), the mean value with letter "b" are significantly different as compared to the week 42 for the same treatment ($p<0.05$).

the influence of the heat treatment that was applied to the oil before its use as feed.

Replacing SO with corn oil resulted in significant reduction in levels of saturated fat acid C14:0 in yolk, as well as in total saturated fatty acids. Saturated fatty acids levels were reduced in yolks of layer hens consuming flaxseed oil (Huang, 2016). The reduction in SFA was mainly due to the decline in C16:0 fatty acid concentration while the increase in n-3 PUFA were likely due to the elevated concentration of C18:3n-3 and C22:6n-3. Similar to our findings, Jia *et al.* (2008) reported that feeding 15% of Linpro to layers increased n-3 PUFA concentrations in eggs by 106%. However, the unsaturated fatty acids C16:1 7-hexadeconoic acid and C18:1 Cis significantly increased. C18:1 Trans significantly reduced in comparison to week 42 in the same treatment. In conclusion, feeding laying hens with high quality oil such as corn oil increases polyunsaturated fatty acids in egg yolk.

## CONFLICT OF INTERESTS

The authors declare no potential conflict of interest.

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