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# Beneficial effect of feeding olive pulp and *Aspergillus awamori* on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails

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**ABSTRACT.** In the twelve-week study, the effect of feeding olive pulp (OP) without or with *Aspergillus awamori* on performance, egg quality, serum lipids and antioxidant status of 360 8-week-old laying Japanese quail hens was evaluated. Birds were randomly distributed to 6 groups: control (C) receiving maize-soybean basal diet with no additives, the other supplemented with: 0.1% *A. awamori* (A) containing $3\times10^6$ *A. awamori spores/g,* 5% OP ($OP_5$), 5% OP + 0.1% *A. awamori* ($OP_5A$), 10% OP ($OP_{10}$) or 10% OP + 0.1% *A. awamori* ($OP_{10}A$). It was shown that egg weight and number were increased ($P < 0.05$) in groups fed OP with or without *A. awamori* in comparison to C group, while final body weight, feed consumption, feed conversion ratio and egg mass did not differ between treatments. Yolk (%) and yolk:albumin ratio were enhanced ($P < 0.05$) in groups $OP_5A$, $OP_{10}$ and $OP_{10}A$, while egg shape index was improved in all treatment groups except $OP_{10}$ group during the last experimental period (16–20 week of age). The hypocholesterolemic effect of OP and *A. awamori* was greatly noticed, where yolk contents of cholesterol and total lipids and serum levels of triglycerides, cholesterol and LDL cholesterol were reduced in nearly all treated groups. Antioxidant status of birds from supplemented groups was improved ($P < 0.01$) as reduced glutathione content and glutathione reductase activity were increased, while lipid peroxidation was decreased. So, dietary supplementation of OP with or without *A. awamori* can improve performance, egg cholesterol content, serum lipid profile and antioxidant status of laying Japanese quail hens during early laying periods.

## Introduction

Poultry industry faces realistic challenge and ever-increasing problem of incessant rising in prices and limited amounts of high-quality feed materials. Thus, the feed costs can be reduced by using low-cost and locally available feedstuffs. So, it is required to constantly evaluate new feed sources, including agriculture by-products and food-industry wastes. Olive pulp (*Olea europaea* L., OP) represents one of the residues used as partially alternative to feed ingredients. OP is the remaining raw material from the olive oil extraction (mechanical or solvent method) after the removal of the seed fractions. Depending on oil extraction method, crude fat content can vary in the pulps. Indeed, the OP may be partly considered as

an energy source in poultry feed. It is a good source of α-linolenic acid with high free radicals scavenging activity because of its phenolic components (e.g., oleuropein and hydroxytyrosol) (Hashish and Abd El-Samee, 2005; Güçlü et al., 2008; Bulotta et al., 2014). Nevertheless, the relative high content of crude fibre in OP limits its use in poultry diets; so, further treatments (mechanical, chemical or biological) are required.

*Aspergillus awamori*, a variant of *Aspergillus niger,* has been used for the processing of Japanese distilled liquor (Shochu) called 'koji'. The products processed by or containing *A. awamori* have been recognized as safe by the USA Food and Drug Administration (Saleh et al., 2017). It is very provable that *A. awamori* had a superior ability to secrete enzymes (e.g., α-amylases, glucoamylases and proteases) enhancing digestions of carbohydrates and proteins, and produce a growth promoter during the fermentation as well as its role as probiotic (Panda et al., 2006; Saleh et al., 2017).

The aim of the present study was to evaluate the effect of feeding quails diets supplemented with OP with or without *A. awamori* addition. OP effect can be connected with the beneficial antioxidant components found in it. The possible mechanisms by which *A. awamori* exerts effect are: its superior ability to secrete enzymes able to degrade the non-starch polysaccharides (NSP) and fibre contents of OP, and its role as a probiotic. In addition, the ability to lower egg cholesterol levels had been previously reported for both OP (Hashish and Abd El-Samee, 2005; Saleh et al., 2017) and *A. awamori* (Imaizumi et al., 1992; Hara et al., 1999; Tang et al., 2015), and this coincides with the increasing demand for low-cholesterol or unsaturated fatty acids in eggs. Therefore, in this study, laying quails were selected as experimental subject to examine the effect of OP with or without *A. awamori* dietary inclusion on their productive performance, egg quality, and biochemical and antioxidant status.

## Material and methods

The experiment was carried out at the experimental poultry farm of the Poultry Research Unit, Biological Application Department, Radioisotopes Applications Division, Nuclear Research Center, Egyptian Atomic Energy Authority at Inshas (Egypt). All procedures were approved by Local Experimental Animals Care Committee and Institutional Ethics Committee. The birds were cared using husbandry guidelines derived from Egyptian Atomic Energy Authority standard operating procedures.

### Animals and diets

In total, 360 8-week-old laying Japanese quail hens with nearly the same body weight were randomly divided into six groups. Each group had three replicates (20 quails each) which were caged in wire battery cages (100 × 60 × 50 cm; length × width × height) under the same managerial, hygienic and environmental conditions. The experiment was conducted in autumn season and ambient temperature ranged from 20–25 °C. Cages were equipped with stainless steel nipple drinker. Feed in mash form and water were available *ad libitum* for each cage throughout the whole study. The light cycle was 16 h during the experiment period (three months) from October to December. The trial started at 8 week of age, and lasted for 12 weeks (20 week of age). The control group (C) received a maize-soybean basal diet with no additives. The basal diet was formulated to meet the bird dietary nutrient requirements (NRC, 1994). The other treatment groups were fed the basal diet supplemented with: 1 g Tomoko/kg (A), 5% olive pulp ($OP_5$), 5% olive pulp +1 g Tomoko/kg ($OP_5A$), 10% olive pulp ($OP_{10}$) or 10% olive pulp +1 g Tomoko/kg ($OP_{10}A$). Tomoko is the product of Biogenkoji Research Institute (Kagoshima, Japan), which contains $3\times10^6$ *A. awamori* spores per g. The composition and the calculated analysis of the experimental diets are shown in Table 1.

### Nutrients and fatty acid composition of olive pulp

De-stoned, dried and grounded olive pulp was analysed for chemical composition (Table 1). Dry matter (DM) (2000 #930.15), crude protein (CP) (2000 #984.13), crude fibre (CF) (2000 #973.18), ether extract (EE) (2003 #2003.05) and ash (2000 #923.03) contents in OP were analysed according to the AOAC International procedures (AOAC International, 2000, 2003). The following formula was used to calculate nitrogen free extract (NFE) according to Scott et al. (1976):

$$NFE = DM\% - (CP\% + EE\% + CF\% + Ash\%)$$

Fatty acids were determined by gas chromatography coupled with mass spectrometry (GC-MS) (Thermo Fisher, Waltham, MA, USA) after its conversion to fatty acid methyl esters according to Saleh et al. (2012). Relative composition of fatty acids was calculated as the % of the total fatty acids (Table 2).

### Data collection and egg parameters

Body weight was recorded at the beginning and at the end of the experiment, while feed consump-

**Table 1.** Composition and calculated analysis of experimental diets, and the proximate composition of the olive pulp

| Indices | Olive pulp concentrations | | |
|---|---|---|---|
| | control (0%) | 5% | 10% |
| Ingredients, % | | | |
| yellow maize | 53.9 | 47.6 | 40.6 |
| soybean meal (44%) | 34.5 | 34.5 | 34.9 |
| dicalcium phosphate | 1.20 | 1.10 | 1.10 |
| limestone | 5.70 | 5.70 | 5.70 |
| sodium chloride | 0.30 | 0.30 | 0.30 |
| vitamin-mineralpremix[1] | 0.30 | 0.30 | 0.30 |
| dl-methionine | 0.15 | 0.15 | 0.15 |
| soybean oil | 4.00 | 5.35 | 7.00 |
| olive pulp | 0.00 | 5.00 | 10.0 |
| Calculated values[2], % | | | |
| crude protein | 20.07 | 20.02 | 20.06 |
| metabolizable energy (ME) MJ/kg | 12.20 | 12.15 | 12.15 |
| crude fibre | 3.60 | 4.46 | 5.73 |
| lysine | 1.14 | 1.13 | 1.12 |
| methionine | 0.48 | 0.47 | 0.46 |
| methionine + cysteine | 0.80 | 0.78 | 0.76 |
| calcium | 2.51 | 2.51 | 2.53 |
| available phosphorus | 0.36 | 0.35 | 0.35 |
| Proximate composition of the olive pulp, analyzed | | | |
| dry matter (DM), % | | 87.2 | |
| crude protein, g/kg DM | | 97 | |
| crude fibre, g/kg DM | | 200 | |
| nitrogen free extract, g/kg DM | | 378 | |
| crude fat, g/kg DM | | 107 | |
| ash, g/kg DM | | 80 | |

[1] vitamin-mineral premix provided per kg diet: IU: vit. A 4 000 000, vit. $D_3$ 500 000; g: vit. E 16.7, vit. K 0.67, vit. $B_1$ 0.67, vit. $B_2$ 2, vit. $B_6$ 67, vit. $B_{12}$ 0.004, nicotinic acid 16.7, pantothenic acid 6.67, biotin 0.07, folic acid 1.67, choline chloride 400, Zn 23.3, Mn 10, Fe 25, Cu 1.67, I 0.25, Se 0.033, Mg 133.4; [2] calculated according to National Research Council (NRC, 1994)

**Table 2.** Fatty acid composition of olive pulp[1]

| Indices | Olive pulp |
|---|---|
| Saturated fatty acids (SFAs), % | |
| C16:0 (palmitic) | 14.24 ± 1.12 |
| C18:0 (stearic) | 2.51 ± 0.19 |
| C20:0 (arachidonic) | 0.46 ± 0.07 |
| ΣSFAs | 17.21 |
| Monounsaturated fatty acids (MUFAs), % | |
| C16:1 (palmitoleic) | 1.51 ± 0.08 |
| C18:1 (oleic) | 68.02 ± 5.76 |
| C20:1 (eicosenoic) | 0.25 ± 0.02 |
| ΣMUFAs | 69.78 |
| Polyunsaturated fatty acids (PUFAs), % | |
| C18:2 (linoleic) | 12.18 ± 0.92 |
| C18:3 (α-linolenic) | 0.83 ± 0.09 |
| ΣPUFAs | 13.01 |
| PUFAs/SFAs | 0.756 |
| MUFAs/PUFAs | 5.364 |

[1] values are presented as means ± SEM (n = 5)

tion (FC) was recorded weekly. Feed conversion ratio (FCR) was calculated as: g feed/g egg. Egg number (EN) and egg weight (EW) were monitored daily to calculate the egg mass (EM = EN ×EW).

## Egg quality criteria

Egg and eggshell quality examinations (eggshell thickness, shell, yolk and albumen weights (%), yolk index, yolk:albumen ratio, egg shape index and Haugh Unite (HU) score were conducted using an average of 10 eggs laid between 12:00 and 15:00 from each treatment replicate which were randomly collected at the ends of 12, 16, and 20 week of age. After eggs weighing and measuring their length and width, the eggs were carefully broken on a glass plate (35 × 35 cm) to measure external and internal egg quality criteria. Yolks were isolated from albumen and weighed. To measure shell weight, eggshells were cleaned of any clinging albumen; eggshells were then dried at room temperature and weighed. Albumen weight was ascertained by subtracting the weights of yolk and shell from the whole egg weight. Yolk, albumen and shell weights were expressed as a percentage of the whole egg. Shell thickness (with shell membrane) was determined from mean estimations of shell thickness at three areas on the egg (air cell, equator and sharp end) using a micrometer. Yolk diameter was measured to the nearest 0.05 mm by a vernier caliper, however yolk height was measured to the nearest 0.01 mm by means of tripod micrometer reading. The yolk index was calculated as the yolk height divided by yolk diameter while egg shape index was calculated as the ratio of egg width to length. HU score was computed according the following equation:

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

where: H and W refer to albumen height and egg weight, respectively. Yolk total lipids and cholesterol were determined by 10 eggs from each treatment and measured by the methods of Folch et al. (1957) and Washburn and Nix (1974).

## Blood sampling and biochemical analysis

At the end of the experimental period, six quails from each group were randomly chosen and blood samples were collected from the brachial vein in tube without anticoagulant for separate serum; then the samples were immediately centrifuged at 4500 rpm for 15 min and the obtained serum was stored at −20 °C

until further analysis. The total protein, albumin, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol serum concentrations and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) serum activities were analysed using spectrophotometer (Spectronic 1201, Milton Roy, Ivyland, PA, USA) using commercial kits (Spinreact Co., Girona, Spain) according to the manufacturer's instructions. The reduced glutathione (GSH) and malondialdehyde (MDA) contents, and glutathione reductase (GSR) activity in the serum were analysed using commercial kits (Cell Biolabs Inc., San Diego, CA, USA).

### Statistical analysis

Data were analysed by two-way analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of the statistical software SPSS (ver. 18.0; IBM Corp., Armonk, NY, USA). Tukey's multiple comparison tests were used to identify which treatment conditions were significantly different from each other at a significance level of $P < 0.05$.

## Results and discussion

### Productive performance

There were no significant differences in initial and final body weights, daily FC, FCR and EM of quails among experimental treatments (Table 3). However, EW was increased in $OP_5A$ and $OP_{10}$ groups during the periods from 16–20 week of age and only in $OP_5A$ in the overall one. The EN was increased in all groups receiving OP regardless *A. awamori* addition in periods 12–16, 16–20 week of age and the overall period, meanwhile there was no difference between groups A and C. The highest values of EW and EN were recorded in $OP_5A$ and $OP_{10}$ groups followed by $OP_5$, mainly in overall period. There were no mortality in all treatment groups. These increment in EW and EN may be due to the residues of olive oil and its high contents of polyunsaturated fatty acids, mainly α-linoleic acid (March and MacMillan, 1990; Grobas et al., 1999, 2001; Güçlü et al., 2008). In addition, the eminent ability of *A. awamori* to produce cellulase and xylanase, and digest raw starches as well as soluble NSP (Saleh et al., 2017) found in the diet and OP, besides its role as probiotic, may enhance the utilization of OP and diet ingredients which in result improves quail productive performance. The previous aspects may be considered as explanations of the enhancement effect observed in the aforementioned groups. Our results are in agreement with recent reports investigating the productive performance. Many studies observed the insignificant effect of OP inclusion up to 10% into laying hen diets on productive performance except EW (Zangeneh and Torki, 2011; Zarei et al., 2011; Al-Harthi and Attia, 2015). Moreover, the dietary addition of *A. awamori* increased EW and egg mass with no noticeable effect on the average body weight gain of hens (Saleh et al., 2017). Egg production in hens fed diets with probiotic content of bacteria and fungi was not significantly affected in hens aged from 40 to 52 week (Ramasamy et al., 2009), but it was significantly higher during the late laying period, from 54 to 65 week of age (Yörük et al., 2004).

### Egg quality criteria

All examined egg quality parameters were insignificantly affected by supplemented OP and *A. awamori* either separated or combined during the experimental periods from 8–12 and 12–16 week of age (Table 4). However, during the last experimental period (16–20 week of age) yolk (%) and yolk:albumen ratio increased significantly in groups $OP_{10}$, $OP_5A$ and $OP_{10}A$ in comparison to C group. Furthermore, egg shape index was elevated significantly in all treatment groups except $OP_{10}$, while HU score was increased only in $OP_{10}$ and $OP_5A$ groups as compared with control one. Feeding OP and *A. awamori* showed a remarkable decrease ($P < 0.05$) effect in yolk total lipids and yolk cholesterol during the third experimental period and the overall one (Table 5). These results indicate that prolongation of the feeding with OP and/or *A. awamori* could enhance the egg quality. Present results are in agreement with the reports of Zarei et al. (2011) and Al-Harthi and Attia (2015) who stated that dietary inclusion of OP with levels 10 and 20% into laying diets had an increment effect on yolk index, yolk:albumin ratio and HU score. The effect of feeding *A. awamori* was demonstrated by Saleh et al. (2017) who showed that egg quality parameters were not significantly altered in groups receiving different levels of *Aspergillus* or other probiotic microorganisms.

To the best of our knowledge, studies investigating the effect of feeding OP and *A. awamori* on egg yolk total lipids and cholesterol are very limited. OP and *A. awamori* showed noteworthy decrement effect on yolk total lipids and yolk cholesterol during the third experimental period (16–20 week of age) and the overall one (8–20 week of age) (Table 5). Little

**Table 3.** Effect of different diets supplementation (olive pulp with or without *Aspergillus awamori*) on performance of laying quails from 8 to 20 week of age

| Indices | Treatment group[1] | | | | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | C | A | $OP_5$ | $OP_5A$ | $OP_{10}$ | $OP_{10}A$ | | |
| Body weight, g | | | | | | | | |
| initial | 198 | 197 | 199 | 198 | 196 | 205 | 1.676 | 0.700 |
| final | 280 | 271 | 286 | 278 | 274 | 285 | 3.510 | 0.491 |
| Feed consumption, g/bird/day | | | | | | | | |
| weeks 8–12 | 20.50 | 20.20 | 22.77 | 22.80 | 21.37 | 20.30 | 0.364 | 0.068 |
| weeks 12–16 | 24.30 | 24.03 | 27.37 | 25.00 | 25.57 | 24.67 | 0.419 | 0.218 |
| weeks 16–20 | 26.17 | 26.43 | 30.03 | 28.27 | 28.40 | 27.27 | 0.457 | 0.111 |
| overall | 23.67 | 23.56 | 26.72 | 25.36 | 25.11 | 24.08 | 0.395 | 0.132 |
| Feed conversion ratio, g feed/g egg | | | | | | | | |
| weeks 8–12 | 2.48 | 2.47 | 2.18 | 2.55 | 2.31 | 2.49 | 0.056 | 0.399 |
| weeks 12–16 | 2.63 | 2.62 | 2.34 | 2.48 | 2.44 | 2.65 | 0.049 | 0.377 |
| weeks 16–20 | 2.73 | 2.81 | 2.48 | 2.70 | 2.62 | 2.85 | 0.056 | 0.487 |
| overall | 2.62 | 2.64 | 2.34 | 2.58 | 2.46 | 2.67 | 0.052 | 0.456 |
| Egg weight, g | | | | | | | | |
| weeks 8–12 | 10.69 | 10.53 | 13.34 | 11.56 | 11.89 | 10.77 | 0.367 | 0.213 |
| weeks 12–16 | 12.22 | 12.75 | 12.72 | 14.30 | 12.94 | 13.46 | 0.260 | 0.267 |
| weeks 16–20 | $11.98^{b}$ | $12.17^{ab}$ | $12.29^{ab}$ | $14.04^{a}$ | $14.10^{a}$ | $13.78^{ab}$ | 0.300 | 0.050 |
| overall | $11.63^{b}$ | $11.82^{b}$ | $12.78^{ab}$ | $13.30^{a}$ | $12.98^{ab}$ | $12.67^{ab}$ | 0.183 | 0.018 |
| Egg number | | | | | | | | |
| weeks 8–12 | 21.03 | 21.36 | 21.56 | 21.57 | 21.50 | 21.21 | 0.075 | 0.226 |
| weeks 12–16 | $24.74^{c}$ | $25.15^{bc}$ | $25.37^{ab}$ | $25.55^{ab}$ | $25.70^{a}$ | $25.44^{ab}$ | 0.085 | 0.001 |
| weeks 16–20 | $25.72^{d}$ | $25.90^{cd}$ | $26.31^{ab}$ | $26.54^{a}$ | $26.57^{a}$ | $26.14^{bc}$ | 0.080 | <0.001 |
| overall | $23.83^{c}$ | $24.14^{bc}$ | $24.41^{ab}$ | $24.55^{a}$ | $24.59^{a}$ | $24.26^{ab}$ | 0.070 | <0.001 |
| Egg mass, g/hen/day | | | | | | | | |
| weeks 8–12 | 8.27 | 8.19 | 10.47 | 9.09 | 9.31 | 8.27 | 0.296 | 0.165 |
| weeks 12–16 | 9.24 | 9.18 | 11.73 | 10.23 | 10.58 | 9.42 | 0.332 | 0.174 |
| weeks 16–20 | 9.59 | 9.45 | 12.14 | 10.62 | 10.93 | 9.67 | 0.350 | 0.174 |
| overall | 9.03 | 8.94 | 11.45 | 9.98 | 10.27 | 9.12 | 0.326 | 0.171 |

[1] treatment groups: C – corn-based diet, A – 1 g/kg *A. awamori*, $OP_5$ – 5% olive pulp, $OP_5A$ – 5% olive pulp + 1 g/kg *A. awamori*, $OP_{10}$ – 10% olive pulp, $OP_{10}A$ – 10% olive pulp + 1 g/kg *A. awamori*; [2] SEM – standard error of means; $^{a–d}$– means with different superscripts are significantly different

is known about the mechanism by which probiotics reduce the cholesterol content in eggs (Tang et al., 2015). However, the proposed mechanism by which *A. awamori* decrease yolk cholesterol content might be attributed to the fermentation occurred by *A. awamori*, which produces short-chain fatty acids that suppress hepatic cholesterol synthesis (Hara et al., 1999) and stimulate bile acid synthesis (Imaizumi et al., 1992). Moreover, *A. awamori*, as a probiotic, has the ability to alter the pathways of cholesteryl-esters and lipoprotein transporters (Liong et al., 2007), which might reduce the availability of cholesterol for deposition into egg yolks (Tang et al., 2015). Whereas, yolk cholesterol-lowering effect of OP may be due to its high contents of unsaturated fatty acids, especially n-3, n-6 and n-9, which might affect the quality of yolk-lipids contents (Hashish and Abd El-Samee, 2005). The authors reported decrease in egg yolk concentrations of total lipids, TC, TG, LDL-cholesterol and phospholipids in group fed olive cake compared to the control group. Moreover, Saleh et al. (2017) found a significant decrease in the yolk cholesterol in groups fed *A. awamori*. Other researchers observed also the beneficial effects of probiotic on egg quality such as lower cholesterol content (Mikulski et al., 2012; Tang et al., 2015).

Nowadays, the demand for low-cholesterol or low-saturated fat eggs has been increased because of the growing passion of health-conscious customers for healthier foods. Therefore, it would be convenient financially for egg producers to be able to produce and market low-cholesterol eggs. Egg producers have accomplished the decrease in egg cholesterol and fat by choosing eggs from hens of specific ages that are nourished on special diets, and these eggs are tagged as ‘lowered fat and lowered

**Table 4.** Effect of different diets supplementation (olive pulp with or without *Aspergillus awamori*) on egg quality criteria of laying quails from 8–12, 12–16 and 16–20 week of age

| Indices | Treatment group[1] | | | | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | C | A | $OP_5$ | $OP_5A$ | $OP_{10}$ | $OP_{10}A$ | | |
| Albumen, % | | | | | | | | |
| weeks 8–12 | 58.60 | 58.74 | 58.84 | 58.40 | 57.01 | 58.62 | 0.375 | 0.797 |
| weeks 12–16 | 56.80 | 58.86 | 58.79 | 57.59 | 55.85 | 57.64 | 0.376 | 0.139 |
| weeks 16–20 | 58.36 | 60.08 | 59.32 | 57.57 | 57.23 | 57.84 | 0.373 | 0.192 |
| Yolk, % | | | | | | | | |
| weeks 8–12 | 29.35 | 29.95 | 28.69 | 29.04 | 29.41 | 29.96 | 0.323 | 0.891 |
| weeks 12–16 | 30.91 | 29.65 | 29.68 | 30.98 | 30.86 | 30.27 | 0.245 | 0.121 |
| weeks 16–20 | 29.59$^{b}$ | 29.78$^{b}$ | 29.70$^{b}$ | 31.19$^{a}$ | 31.45$^{a}$ | 31.01$^{a}$ | 0.263 | 0.039 |
| Shell, % | | | | | | | | |
| weeks 8–12 | 12.05 | 11.31 | 12.47 | 12.57 | 13.58 | 11.42 | 0.353 | 0.501 |
| weeks 12–16 | 12.29 | 11.49 | 11.53 | 11.43 | 13.29 | 12.09 | 0.274 | 0.365 |
| weeks 16–20 | 11.15 | 10.14 | 10.99 | 11.24 | 11.31 | 12.05 | 0.293 | 0.662 |
| Egg shape index | | | | | | | | |
| weeks 8–12 | 80.70 | 79.26 | 80.34 | 81.49 | 76.82 | 83.21 | 0.809 | 0.333 |
| weeks 12–16 | 76.70 | 78.31 | 79.84 | 78.72 | 80.77 | 82.90 | 0.795 | 0.310 |
| weeks 16–20 | 76.57$^{b}$ | 79.16$^{a}$ | 80.72$^{a}$ | 79.98$^{a}$ | 76.91$^{b}$ | 80.49$^{a}$ | 0.570 | 0.048 |
| Yolk index | | | | | | | | |
| weeks 8–12 | 45.86 | 41.98 | 40.56 | 40.50 | 46.64 | 45.02 | 1.031 | 0.334 |
| weeks 12–16 | 47.99 | 45.11 | 44.05 | 39.56 | 41.27 | 44.61 | 1.589 | 0.771 |
| weeks 16–20 | 37.55 | 38.05 | 40.05 | 41.32 | 40.36 | 39.06 | 0.723 | 0.714 |
| Shell thickness, mm | | | | | | | | |
| weeks 8–12 | 0. 376 | 0.305 | 0.330 | 0.309 | 0.383 | 0.324 | 0.015 | 0.586 |
| weeks 12–16 | 0.410 | 0.339 | 0.350 | 0.399 | 0.398 | 0.335 | 0.013 | 0.404 |
| weeks 16–20 | 0.323 | 0.307 | 0.371 | 0.333 | 0.342 | 0.321 | 0.010 | 0.558 |
| Yolk: albumen ratio | | | | | | | | |
| weeks 8–12 | 0.503 | 0.510 | 0.488 | 0.498 | 0.516 | 0.511 | 0.008 | 0.944 |
| weeks 12–16 | 0.544 | 0.505 | 0.505 | 0.538 | 0.553 | 0.526 | 0.007 | 0.112 |
| weeks 16–20 | 0.507$^{b}$ | 0.497$^{b}$ | 0.501$^{b}$ | 0.542$^{a}$ | 0.550$^{a}$ | 0.536$^{a}$ | 0.007 | 0.032 |
| Haugh unit score | | | | | | | | |
| weeks 8–12 | 91.98 | 92.88 | 88.21 | 87.94 | 88.88 | 91.83 | 1.142 | 0.754 |
| weeks 12–16 | 88.61 | 88.82 | 90.26 | 89.42 | 92.29 | 90.54 | 0.773 | 0.818 |
| weeks 16–20 | 87.38$^{b}$ | 84.98$^{b}$ | 86.54$^{b}$ | 93.00$^{a}$ | 93.63$^{a}$ | 89.47$^{ab}$ | 1.064 | 0.046 |

[1] treatment groups: C – corn-based diet, A – 1 g/kg *A. awamori*, $OP_5$ – 5% olive pulp, $OP_5A$ – 5% olive pulp + 1 g/kg *A. awamori*, $OP_{10}$ – 10% olive pulp, $OP_{10}A$ – 10% olive pulp + 1 g/kg *A. awamori*; [2] SEM – standard error of means; [ab]– means with different superscripts are significantly different

**Table 5.** Effect of different diets supplementation (olive pulp with or without *Aspergillus awamori*) on yolk total lipids and total cholesterol content of laying quails from 8 to 20 week of age

| Indices | Treatment group[1] | | | | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | C | A | $OP_5$ | $OP_5A$ | $OP_{10}$ | $OP_{10}A$ | | |
| Yolk total lipids, mg/g yolk | | | | | | | | |
| weeks 8–12 | 352.8 | 347.1 | 346.4 | 348.7 | 352.3 | 347.0 | 1.450 | 0.736 |
| weeks 12–16 | 357.9$^{a}$ | 316.0$^{cd}$ | 329.6$^{b}$ | 309.5$^{cd}$ | 321.4$^{bc}$ | 306.8$^{d}$ | 4.268 | <0.001 |
| weeks 16–20 | 352.3$^{a}$ | 274.9$^{c}$ | 302.7$^{b}$ | 260.3$^{d}$ | 280.5$^{c}$ | 256.6$^{d}$ | 7.973 | <0.001 |
| overall | 354.3$^{a}$ | 312.7$^{cd}$ | 326.2$^{b}$ | 306.2$^{cd}$ | 318.1$^{bc}$ | 303.5$^{d}$ | 4.247 | <0.001 |
| Yolk total cholesterol, mg/gyolk | | | | | | | | |
| weeks 8–12 | 18.54 | 18.37 | 18.59 | 18.00 | 18.52 | 17.67 | 0.136 | 0.312 |
| weeks 12–16 | 19.89 | 18.60 | 18.90 | 18.19 | 18.54 | 17.71 | 0.240 | 0.140 |
| weeks 16–20 | 19.62$^{a}$ | 17.21$^{b}$ | 17.57$^{b}$ | 16.76$^{b}$ | 16.93$^{b}$ | 16.12$^{b}$ | 0.297 | 0.001 |
| overall | 19.35$^{a}$ | 18.06$^{b}$ | 18.35$^{ab}$ | 17.65$^{b}$ | 18.00$^{b}$ | 17.17$^{b}$ | 0.189 | 0.003 |

[1] treatment groups: C – corn-based diet, A – 1 g/kg *A. awamori*, $OP_5$ – 5% olive pulp, $OP_5A$ – 5% olive pulp + 1 g/kg *A. awamori*, $OP_{10}$ – 10% olive pulp, $OP_{10}A$ – 10% olive pulp + 1 g/kg *A. awamori*; [2] SEM – standard error of means; [a–d] – means with different superscripts are significantly different

cholesterol' and marketed (Bradley and King, 2016). It has been declared that hen age can influence the cholesterol content of egg yolk (Zemková et al., 2007) and younger hens tend to lay eggs with elevated cholesterol level (Oloyo, 2003). The ability of OP and *A. awamori* to reduce the yolk total lipids and cholesterol levels at periods of 16–20 and 8–20 weeks of age in this study could be exploited by egg producers for lowering the yolk cholesterol in layers during the early laying period. Egg consumption has been reported to contribute to 32% of TC intake (Hu et al., 1999), subsequently; a diminishment in yolk cholesterol might be beneficial to consumers, particularly to hyper-responders to dietary fats.

## Serum biochemical parameters

AST, ALT and ALP activities, total protein and albumin values were insignificantly affected by all treatment groups (Table 6). Nevertheless, TG, TC and LDL-cholesterol values were decreased ($P < 0.01$) in all treatment groups except group $OP_5$ for TG and A group for TC, while the significant increase ($P < 0.01$) in HDL value was observed only in $OP_{10}A$ group in comparison with the control one. Moreover, antioxidant status of laying Japanese quails fed OP and/or *A. awamori* was improved ($P < 0.01$); significant increase in serum GSH value and GSR activity, and decrease in TBARS content were noticed in groups fed OP with or without *A. awamori*. Earlier, previous researches had proved that some oil sources (e.g., fish, soybean and olive oil), especially that have high content of oleic acid, exert potential detrimental effects on animals by increasing susceptibility to lipid or protein peroxidation in liver mitochondria (Huertas et al., 1992; Ochoa-Herrera et al., 2001; Quiles et al., 2006; Dong et al., 2018). So, we measured the serum values of AST and ALT as biomarkers for liver health in order to ensure the absence of any side effects on the liver exerted from the tested supplemental doses. In line with our results, Zangeneh and Torki (2011) stated that dietary OP inclusion did not exert any significant effect on the blood parameters. Hashish and Abd El-Samee (2005) demonstrated that using 10 and 20% of olive cake had no significant effect on TG content but level of 5% reduced plasma cholesterol concentration. While, Zarei et al. (2011) reported that the inclusion of OP in hens diet decreased blood level of TG but not affected levels of TC, HDL and LDL. The reason for the hypocholesterolemic effect of OP may be due to its high content of fibre, which increases dietary fibre which in turn may reduce blood fat levels but the mechanisms are not fully understood (Razdan and Pettersson, 1994). It has been proposed that the increased viscosity associated with soluble fibres may postpone the emptying of the gastrointestinal tract, decrease intestinal motility and fat absorption thereby reducing lipid absorption (Razdan and Pettersson, 1994).

**Table 6.** Effect of different diets supplementation (olive pulp with or without *Aspergillus awamori*) on blood components and oxidative status of laying quails at 20 week of age

| Blood components | Treatment group[1] | | | | | | SEM[2] | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | C | A | $OP_5$ | $OP_5A$ | $OP_{10}$ | $OP_{10}A$ | | |
| Protein fractions, g/dl | | | | | | | | |
| total protein | 3.028$^{ab}$ | 2.563$^{b}$ | 3.191$^{ab}$ | 4.085$^{a}$ | 4.004$^{a}$ | 3.379$^{ab}$ | 0.155 | 0.007 |
| albumin | 1.810 | 1.760 | 1.789 | 2.430 | 2.098 | 1.675 | 0.092 | 0.131 |
| Enzymes activity, U/l | | | | | | | | |
| AST | 119.2 | 117.2 | 115.8 | 116.6 | 109.9 | 108.8 | 2.023 | 0.664 |
| ALT | 90.11 | 89.86 | 105.05 | 106.93 | 105.75 | 96.88 | 2.586 | 0.147 |
| ALP | 229.7 | 222.9 | 195.2 | 212.9 | 221.9 | 229 | 4.741 | 0.296 |
| Lipid profile, mg/dl | | | | | | | | |
| triglycerides | 2667$^{a}$ | 1934$^{bc}$ | 2343$^{ab}$ | 1905$^{bc}$ | 1719$^{bc}$ | 1653$^{c}$ | 106.2 | 0.002 |
| total cholesterol | 275.9$^{a}$ | 228.5$^{ab}$ | 239.2$^{bc}$ | 197.1$^{bc}$ | 201.9$^{bc}$ | 187.7$^{c}$ | 8.00 | <0.001 |
| HDL-cholesterol | 27.8$^{b}$ | 38.65$^{b}$ | 36.27$^{b}$ | 39.38$^{b}$ | 41.8$^{b}$ | 56.6$^{a}$ | 2.536 | 0.001 |
| LDL-cholesterol | 134$^{a}$ | 87.42$^{b}$ | 83.9$^{b}$ | 73.0$^{b}$ | 92.10$^{b}$ | 67.02$^{b}$ | 7.111 | 0.018 |
| Oxidative status | | | | | | | | |
| GSH, ng/ml | 0.181$^{b}$ | 0.220$^{a}$ | 0.222$^{a}$ | 0.236$^{a}$ | 0.233$^{a}$ | 0.253$^{a}$ | 0.007 | 0.001 |
| GSR, mU/ml | 0.182$^{b}$ | 0.203$^{ab}$ | 0.216$^{a}$ | 0.218$^{a}$ | 0.230$^{a}$ | 0.239$^{a}$ | 0.006 | 0.001 |
| MDA, µmol | 0.307$^{a}$ | 0.161$^{c}$ | 0.196$^{bc}$ | 0.221$^{bc}$ | 0.228$^{b}$ | 0.211$^{bc}$ | 0.012 | <0.001 |

[1] treatment groups: C – corn-based diet, A – 1 g/kg *A. awamori*, $OP_5$ – 5% olive pulp, $OP_5A$ – 5% olive pulp + 1 g/kg *A. awamori*, $OP_{10}$ – 10% olive pulp, $OP_{10}A$ – 10% olive pulp + 1 g/kg *A. awamori*; AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, HDL – high-density lipoprotein, LDL – low-density lipoprotein, GSH – glutathione reduced, GSR – glutathione reductase, MDA – malondialdehyde, [2] SEM – standard error of means; [a–d] – means with different superscripts are significantly different

*A. awamori* inclusion into laying diets showed hypocholesterolemic effect while serum AST and ALP were not influenced (Saleh et al., 2017). Comparable lipid-lowering effects were considered in chickens fed diet containing *A. oryzae* (Kim et al., 2003; Panda et al., 2006; Sallh and Al Hussary, 2009) and *A. niger* (Yoon et al., 2004; Al-Kassie et al., 2008). The postulated mechanisms by which *A. awamori*, as a probiotic, decreases serum cholesterol include the retardation of cholesterol synthesis *via* the inhibition of 3-hydroxyl-3-methylglutaryl-coenzyme (HMG-CoA) reductase (Hajjaj et al., 2005), its ability to produce bile salt hydrolase enzyme (BSH) (EC 3.5.1.24) for bile salt deconjugation in the enterohepatic circulation (Klaver and Van der Meer, 1993), the assimilation of cholesterol by probiotics to incorporate dietary cholesterol into their cellular membrane (St-Onge et al., 2000), and the conversion of cholesterol by probiotics in the intestine into coprostanol, which is directly excreted with the feaces (Ooi and Liong, 2010).

To our knowledge, studies conducted to investigate the effect of dietary OP supplementation in antioxidant system of poultry are very limited. The amelioration effect of OP on quail antioxidant status may be due to its contents of polyphenols and unsaturated fatty acids what could be attributed to increasing lipoprotein synthesis in the liver which in turn boosted the antioxidant defence system of birds (Al-Harthi and Attia, 2015). As well as, Visioli et al. (1995) and Aldini et al. (2006) identified olive mill waste as a potential source for the recovery of antioxidant and anti-atherogenic (Léger et al., 2000) because its contents of triterpenes, pectins, oligosaccharides, oleuropeinand hydroxytyrosol (HT) (Bulotta et al., 2014) which are the main phenolic compounds. HT is one of major phenolic compounds present in olive fruit and it has been revealed to be the most interesting, because of its remarkable pharmacological and antioxidant activity (Fabiani et al., 2002; Visioli et al., 2004). The antioxidant properties of HT can be attributed to the presence of orthodihydroxy moiety, called orthodiphenol. Besides its capacity or ability for donating electrons, its high antioxidant efficiency is due to the scavenger capacity of free radicals during the process of oxidation. However, concerning the antioxidant effect of *A. awamori,* Saleh et al. (2011; 2012) stated that the addition of *A. awamori* (0.05% or 0.1%) or *A. niger* (0.05%) to the diet decreased the TBARS value in the broiler breast muscle, indicating that the fungus has antioxidative properties. These results indicate that *A. awamori* produces antioxidative substances. In addition, feeding diets containing *A. awamori* increased the mRNA expressions of antioxidant enzymes (i.e., glutathione peroxidase, catalase and superoxide dismutase) (El-Deep et al., 2014).

## Conclusions

The present study demonstrated for the first time the effect of the combination of olive pulp (OP) and *Aspergillus awamori* in laying Japanese quail diets. The supplementation of OP and/or *A. awamori* caused a significant increases in egg weight, egg number, yolk content (%), and egg shape index as well as improved antioxidant status, serum lipid profile and yolk cholesterol content without altering the rest of productive performance and egg quality parameters. Present study indicates that supplementation of OP and *A. awamori* alone or in a combination into laying Japanese quail diet could lead to the production of low-total lipids and low-cholesterol eggs, during the early laying periods, which will be beneficial for the consumer health.

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