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Production Performance, Egg Quality Characteristics, Fatty Acid Profile and Health Lipid Indices of Produced Eggs, Blood Biochemical Parameters and Welfare Indicators of Laying Hens Fed Dried Olive Pulp

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Abstract: This study aimed to evaluate the long-term dietary effects of dried olive pulp (OP) on production performance, fatty acid profile and health lipid indices and quality characteristics of produced eggs, health and welfare indicators of laying hens. It was carried out in a commercial poultry farm using 300 Isa Brown layers at 23 weeks of age. The hens were randomly and equally divided in six dietary groups CON, OP2, OP3, OP4, OP5 and OP6, according to the inclusion rate of OP in the ration (0%, 2%, 3%, 4%, 5% and 6%, respectively). OP feeding increased the percentage of polyunsaturated fatty acids (PUFA) in eggs, decreased that of saturated fatty acids (SFA) and improved the PUFA to SFA ratio and health lipid indices, as indicated by the decrease of AI and TI and the increase in the h/H ratio of produced eggs, in a dose-dependent way. OP-fed layers presented a lower percentage of broken eggshells compared to controls. No adverse effects on birds' performance, egg quality traits, health and welfare parameters were observed but a positive impact on Keel Bone Damage (KBD) incidence and belly plumage damage was recorded. OP feeding at the rates of 5% and 6% seems to be beneficial in improving egg nutrition quality.

Keywords: olive pulp; layers; performance; egg quality; egg lipid profile; health; welfare

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1. Introduction

It is well known that the major cost in poultry farming is feed, representing 70% of the total production costs [1]. In order to reduce this cost, the research has recently focused on exploring and evaluating new sources of raw materials from agricultural and industrial by-products for use as animal feed. Key benefits of this practice include lower dependence of animal production on human consumed seeds and reduced waste management costs [2]. Around 2.1 million tons of olive oil is produced annually in Europe, contributing to around 68% of the world's total production, with Spain, Italy and Greece having the leading role in olive oil production [3]. However, the olive oil industry generates considerable amounts of by-products with a harmful environmental impact [4]. The utilization of olive by-products as feedstuff is a promising strategy of recycling this waste, assisting the transition to an efficient circular waste-based economy [2]. It also perfectly fits with the EU Green Deal Strategy for boosting the efficient use of resources by moving to a clean, circular economy and stopping climate change, reversing biodiversity loss and cutting pollution [5].

Olive pulp (OP) is the residue remaining after olive cake (the raw material resulting from the extraction of olive oil) is dried. It is characterized by a high level of essential fatty acids (73% oleic acid, 13% palmitic acid and 7% linoleic acid) as well as high residual oil [6]. Furthermore, it contains oleuropeoside beneficial compounds such as oleuropein and phenolic compounds such as tyrosol [7]. Extensive investigations confirm that dietary polyphenols are strong antioxidants and can be used in poultry for enhancing health and ameliorating the growth performance and quality of animal products [8]. Olive pulp can therefore provide animals with energy and, in particular, polyunsaturated fatty acids, being also a source of many biologically active ingredients with antioxidants, antifungals, antibacterial and anti-tumoral properties [9–14]. Moreover, it is considered a good source of protein, calcium, copper and cobalt [1]. Its high nutritive value and chemical composition makes OP an interesting and low-cost nutrient for productive animals [15,16].

Olive pulp has been previously used at various inclusion rates in laying hens' diet in a limited number of studies with inconsistent results regarding birds' performances, egg quality and health indices [1,2,17–23]. Most of these feeding trials have been carried out in experimental units, evaluating the dietary effect of OP on hens' productivity for a short period of time (6–12 weeks). However, there is lack of research evidence in the available literature regarding the optimal inclusion rate in hens' diet as well as the dietary impact of OP on the lipid profile of egg as well as on hens' welfare. Therefore, the present study was designed in order to investigate the dietary effect of dried OP on laying hens' performance, egg quality characteristics, fatty acid composition and health lipid indices of produced eggs, blood biochemical parameters and welfare indicators of hens, under field conditions, during an entire production cycle. A complimentary goal of this study was to assess the optimal inclusion rate of OP in birds' feed.

2. Materials and Methods

The experimental protocol of the study and implemented animal care procedures were approved by the Committee for Research Ethics of Hellenic Agricultural Organization-DIMITRA (52216/23 October 2020). The study was conducted in accordance with the Declaration of Helsinki.

2.1. Experimental Design

This study was carried out in a commercial poultry farm in Greece and lasted 45 weeks. In total, 300 Isa Brown layers, 23 weeks of age, with initial body weight (BW) 1.58 ± 0.01 kg, were randomly accommodated in 30 enriched cages (10 hens/cage) that were fully equipped and met the requirements of EU Directive 1999/74/EC [24]. Hens were vaccinated and managed according to the breed standards. The light program provided 14 h light (15 lux) per day (14 h light-10 h dark cycle) and kept constant to the end of the experiment. The average temperature in the laying house during the trial was 26 ± 3 °C. Birds were fed with a basal layer diet, formulated according to breed recommendations in mash form. Feed and water were offered ad libitum. After 2 weeks of adaptation, hens were randomly divided in 6 dietary groups CON, OP2, OP3, OP4, OP5 and OP6, with 50 hens/group, 5 replicates-cages/group, 10 hens/replicate-cage. The CON group was fed the basal layer diet provided during the adaptation period and served as control, while the OP groups were fed the basal diet supplemented with dried olive pulp, at the rates of 2%, 3%, 4%, 5% and 6%, respectively. In OP diets, dried olive pulp replaced mainly maize and a small amount of soya meal of the control diet so as to make all rations isonitrogenous and isocaloric (Table 1). The dried OP used in the experiment was a commercial animal feed supplement in the form of flour (*Sparta* INNOLIVE®, Sparta Life S.A., Sparta, Greece). The nutrient and fatty acid composition of OP used in the feeding trial is presented in Table 2.

Table 1. Formulation and nutrient composition of diets containing olive pulp (OP) compared with the control diet (CON).

	CON	OP2	OP3	OP4	OP5	OP6
Ingredients %						
Maize	54.2	53.2	52.2	51.2	50.2	49.2
Soyameal-48	11	10	10	10	10	10
Limestone	9.6	9.6	9.6	9.6	9.6	9.6
Layer concentrate 25% [1]	25	25	25	25	25	25
Olive pulp	0	2	3	4	5	6
MCP	0.2	0.2	0.2	0.2	0.2	0.2
Calculated analysis						
Crude protein (%)	17.68	17.55	17.57	17.59	17.60	17.62
Crude fiber (%)	5.62	5.72	5.68	5.78	5.68	6.48
Fat (%)	6.33	6.48	6.54	6.53	6.50	6.58
Ash (%)	12.70	12.66	12.60	12.59	12.61	12.73
Metabolizable energy (Kcal/kg)	3100	3100	3100	3100	3100	3100
Lysine (%)	0.80	0.80	0.80	0.80	0.80	0.80
Methionine + Cystine (%)	0.66	0.66	0.66	0.66	0.66	0.66
Ca (%)	4.20	4.20	4.20	4.20	4.20	4.20
Av. P (%)	0.33	0.33	0.33	0.33	0.33	0.33

[1] Layer concentrate 25% is a protein/fat mixture for layers including vitamins, minerals, enzymes, yolk coloring, organic acids and mycotoxin binder (Supplementary Table S1). MCP: Monocalcium Phosphate, Av. P: Available phosphorus.

Table 2. Nutrients and fatty acid composition of olive pulp (OP).

Items	Olive Pulp
Moisture (g/100 g)	8.3
Proteins (g/100 g)	9.5
Fat (g/100 g)	14.5
Crude fiber % (*w*/*w*)	25.3
Carbohydrates (g/100 g)	61.0
Ash (g/100 g)	6.7
Lysine %	0.04
Methionine %	0.03
Threonine %	0.37
Ca % (*w*/*w*)	1.52
Mg % (*w*/*w*)	0.15
P % (*w*/*w*)	0.15
ME (Kj/100 g)	1735
Phenolic compounds (mg/kg dry matter)	2410
Fatty acids (% of total fat)	
Lauric (dodecanoic) acid (C12:0)	0.05
Myristic acid (C14:0)	0.05
Myristoleic acid (C14:1)	11.09
Palmitic acid (C16:0)	0.69
Palmitoleic acid (C16:1)	0.19
Margaric acid (C17:0)	0.09
Stearic acid (C18: 0)	2.71
Oleic acid (C18:1)	70.77
α-Linoleic acid (C18:2)	10.77
Linolenic acid(C18:3)	0.48
Arachidic acid (C20:0)	0.85
Eicosenic acid (Gadoleic, C20: 1)	0.11
cis-11,14,17-Eicosatrienoic acid (C 20:3 ω3)	<0.02 *
cis-8,11,14-Eicosatrienoic acid (C 20:3 ω6)	<0.02 *
Arachidonic acid (C 20:4 ω6)	0.13
EPA (C 20:5 n3)	<0.02 *
Behenic acid (C22:0)	0.12

Table 2. *Cont.*

Items	Olive Pulp
Erucic acid (C22:1)	<0.02 *
Docosapentaenoic acid (DPA n3 (C22:5 n3)	<0.02 *
DHA (C 22:6 n3)	<0.02 *
Lignoceric acid (C24:0)	0.18

* This value is the detection limit of the assay. P: Phosphorus, ME: Metabolizable energy, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

2.2. Hen Productivity and Egg Quality

Laying hens were individually weighted at the onset (23 weeks of age) and at the end of the experiment (68 weeks of age). From 27 to 68 weeks of age, feed intake was measured weekly per replicate, weighing the amount of feed distributed and that of residual and scattered feed and was calculated as daily feed intake per hen. The number of eggs produced as well as those with dirty eggshells or deficits (broken, cracked or without eggshell) were recorded daily per replicate pen. Individual egg weights were recorded per replicate every week. Mortality rate was recorded daily. The following were calculated per each replicate of each treatment group: Hen Day-Egg Production % (HDEP = total number of eggs produced on a day/number of hens present on that day) × 100; Feed Intake (FI = total FI/number of days of the trial period); egg mass (Egg mass = (HDEP × egg weight)/100) and Feed Conversion Ratio (FCR = g feed/g egg mass).

A total of 90 eggs (15 eggs/group, 3 eggs/replicate) were randomly selected at the end of the experiment, in order to determine some internal (albumen and yolk weights and percentages, Haugh unit, yolk color, albumen and yolk height and pH, yolk diameter and index) and external (shape index, eggshell weight, percentage and thickness) egg quality traits. Egg weight was measured on a digital scale with accuracy to the nearest 0.01 g. The egg yolk, egg white (albumen), and eggshell of the cracked egg were weighed on the same digital scale. Then, the proportions of yolk ((yolk weight/egg weight) × 100), albumen ((albumen weight/egg weight) × 100), and shell ((shell weight/egg weight) × 100) in each egg were calculated. The egg shape index value ((width/height) × 100) was calculated using the height and width values of the egg measured with an electronic caliper. To calculate the egg yolk index ((height/diameter) × 100), the height, width and length of the yolk were measured with a tripod micrometer and an electronic caliper, respectively. Haugh unit values were calculated using the egg weight (g) and albumen height (mm) (albumen height + 7.57 − 1.7 × egg weight $^{0.37}$). Albumen height was measured using a tripod micrometer. Yolk color was determined according to the Roche yolk color fan ranging from pale yellow (1) to deep orange (15). Shell thickness (mm) was measured using a dial gauge micrometer. Finally, albumen and yolk pH were measured with a waterproof pH meter.

2.3. Determination of Egg Fatty Acid Composition, Phenols Content and Health Lipid Indices

A total of 180 eggs (30 eggs/group, 6 eggs/replicate) were randomly collected from laying hens of intermediate (50 weeks) age, in order to determine fatty acid (FA) profile, total fat and phenols content. In each dietary treatment, 6 final egg samples were formulated after mixing and homogenizing 5 eggs out of the 30 initially collected eggs. The 6 representative egg samples from each group, were analyzed twice for their total phenolic acids content, fatty acids (FA) profile and, the percentage of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), in the whole edible parts of eggs (albumen + yolk).

All reagents used were of GC-grade and were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The reagents for acid hydrolysis consisted of petroleum ether, boiling area: 40–60 °C, anhydrate sodium sulphate, auxiliary filtration (Celite 545), hydrochloric acid solution (HCl analytical grade). The reagents for Soxhlet Extraction were iso-octane of chromatographic purity, potassium hydroxide, methanolic solution of potassium hydroxide 2 M, methanol with a water content of not more than 0.5% (m/m),

plastic syringes with disk filters (cellulose acetate) of 0.45 μm and glass vials with caps. Samples were homogenized by a knife mill. A corresponding quantity depending on the sample was used (±0.1 mg) and was placed in a 250 mL spherical flask. Generally, 5 to 10 g of sample was used. Then, 100 mL of aqueous solution of HCl 4N was added with 2–3 boiling stones and was boiled gently in a 6-position heating mantle at a vertical reflux condenser for 60 min. Then it was left at room temperature to cool down. Afterwards, the solution was filtered and was rinsed with deionized water until neutral pH. The filter paper was dried on a watch glass, at 55 ± 2 °C for 16–18 h (overnight) and the procedure of soxhlet extraction was followed. In a subsequent step, extracted fatty acids were trans-esterified in a methanol potassium hydroxide solution and the fames samples were analyzed by Gas Chromatography - Flame Ionization Detector (GC-FID) [25].

Fatty acid contents were determined by gas chromatography (GC-FID) using a Shimadzu GC-2010 Plus High-End Gas Chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) (GC), equipped with Flame Ionization Detector (Shimadzu Europa GmbH, Duisburg, Germany) (FID), after lipid extraction by the soxhlet procedure, as mentioned. The column used was a Supelco SP2560 (Merck KGaA, Darmstadt, Germany), 100 m × 0.25 mm × 0.20 μm. Helium was used as a carrier gas at a flow rate of 2 mL/min [26]. Injection volume was 1 μL with split ratio 1:50 and injector temperature at 250 °C. The detector temperature was set at 250 °C. The temperature program applied was: initial oven temperature at 110 °C (7 min), increasing at 3 °C/min to 190 °C (2 min), then in a first step at 0.5 °C/min to 205 °C, in a second at 5 °C/min to 230 °C (5 min) and in a third at 5 °C/min to a final temperature of 240 °C for 5 min. The total run time was: 82.67 min. The results were identified using GC solution software comparing mass spectra with retention time peaks. The FA, SFA, MUFA and PUFA values were expressed as weight percentages (% of total FAs).

The phenolic acid content of eggs was determined by Ultra Violet -Visible (UV-VIS) spectrophotometry method using Folin Ciocalteu reagent 2N (Merck KGaA, Darmstadt, Germany). Initially, the sample was extracted with 70% methanol and then was stirred and filtered with a Whatman No 2 filter. Afterwards, 5 mL Folin Ciocalteu 10% was added to the filtrate solution and was stirred. After 3–8 min had passed since the addition of the 10% Folin Ciocalteu reagent, 4 mL of the 7.5% sodium carbonate was added, and the tubes were stirred again. They were left to rest for 60 min and then their optical density was measured with glass cells 10 mm at 765 nm by UV-VIS method. A standard solution of 1000 ppm Gallic acid was used for the calibration curve in the following concentrations: 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm. Total phenolic acid was expressed as mg Gallic Acid Equivalent (GAE)/g.

Based on the proportions of particular FAs and their groups, the health quality of egg lipids was assessed by calculating: Atherogenic Index (AI), Thrombogenic Index (TI) and the ratio between hypocholesterolemic (h)/hypercholesterolemic (H) fatty acids (h/H). The following equations were used to calculate these indexes:

Atherogenic Index [27,28]:

$$\begin{aligned} AI &= (4 \times C14{:}0 + C16{:}0 + C18{:}0)/(\Sigma MUFA + \Sigma PUFA\text{-}n\text{-}6 + \Sigma PUFA\text{-}n\text{-}3) \\ AI^{**} &= (4 \times C14{:}0) + C16{:}0/(\Sigma MUFA + \Sigma PUFA\text{-}n\text{-}6 + \Sigma PUFA\text{-}n\text{-}3) \end{aligned} \quad (1)$$

Thrombogenic Index [28]:

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/(0.5 \times \Sigma MUFA + 0.5 \times \Sigma PUFA\text{-}n\text{-}6 + 3 \times \Sigma PUFA\text{-}n\text{-}3 + \Sigma PUFA\text{-}n\text{-}3/\Sigma PUFA\text{-}n\text{-}6) \quad (2)$$

Ratio between hypocholesterolemic and hypercholesterolemic fatty acids [29]:

$$h/H = C18{:}1n9c + C18{:}2n6c + C18{:}3n3c + C18{:}3n6c + C20{:}2n6 + C20{:}3n6 + C20{:}4n6 + C22{:}6n3/C14{:}0 + C16{:}0 \quad (3)$$

where:

Σ = Summatory,
MUFA = monounsaturated FAs and
PUFA = polyunsaturated FAs

The atherogenic index was calculated by using two equation formulas either by including C18:0 fatty acid to the numerator of equation formula (IA), or not (IA**) since both methods are reported to the literature.

2.4. Blood Biochemical Parameters

At 50 weeks of age, blood samples were collected from 2 randomly selected birds per replicate (a total of 60 blood samples, 10 per group) for the determination of selected biochemical parameters. Approximately 2 mL of blood were taken from the brachial vein of each hen and were collected in plastic vacuum tubes (BD Vacutainer® SST™ II Advance, Becton Dickinson, NJ, USA). After clotting, the serum was separated by centrifugation (3000× *g* for 15 min), transferred into plastic vials and forwarded on ice to an ISO-certified commercial veterinary laboratory for analysis. The serum samples were analyzed for Cholesterol, Triglycerides, Aspartate Aminotransferase (AST), Gamma-Glutamyl Transferase G-GT, Uric acid, Blood Urea Nitrogen (BUN) and Glutamate Dehydrogenase (GLDH) using an automatic biochemical analyzer (Advia® 1800 chemistry analyzer—Siemens Healthineers Headquarters, Erlangen, Germany) and commercially available diagnostic kits.

2.5. Welfare Indicators

In the middle of the production cycle (50 week of age) the hens of each group were individually evaluated for the presence of keel bone damage (KBD), plumage damage, comb abnormalities, skin lesions, claws, foot pad dermatitis and toe damage. Assessment of KBD for all laying hens was performed by the same person according to the palpation technique [30]. Each hen was gently held by one person, and another trained person examined and palpated the keel bone. It was only determined whether KBD was present (fracture, deformation, or both score 1) or not (completely straight and flat keel bone, score 0). Moreover, the % of hens in each group presenting deformation, fractures or both was calculated.

The rest of the welfare parameters were determined according to the Welfare Quality Network (2019) protocol [31]. Briefly, in each hen, the plumage of 3 different body parts (head-neck, back-rump and belly) was given a score on a 3-point scale: 0—All body parts had no or slight wear, (nearly) complete feathering (only single feathers lacking); 1—One or more body parts had moderate wear, i.e., damaged feathers (worn, deformed) or one or more featherless areas <5 cm in diameter at the largest extent; 2—One or more body parts had at least one featherless area ≥5 cm in diameter at the largest extent. The percentage of hens presenting each score was then calculated. In order to achieve a single general plumage score per bird the aforementioned scores of the 3 body parts were combined according to the following classification: 0—All body parts had score '0'; 1—One or more body parts had score '1', but no body part had score '2'; 2—One or more body parts had score '2'. Percentage of birds with scoring categories 0, 1, 2 was recorded.

The hens of each group were individually inspected at the rear end, legs and underneath feathers for the presence of skin lesions (3 or more pecks and/or scratches, large unhealed wounds). An individual score was assessed ranging from 0 to 2 as follow: 0—No lesions, only single (<3) pecks (punctiform damage < 0.5 cm diameter) or scratches; 1—At least one lesion < 2 cm diameter at largest extent or ≥3 pecks or scratches; 2—At least one lesion ≥ 2 cm diameter at largest extent. Both feet of each hen were examined for the presence of foot pad dermatitis (swelling-bubble foot) or toe damage (wounds on one or more toes and/or missing (parts of) one or more toes) and scores were assessed according to the following: (a) Foot pad dermatitis: 0—Feet intact, no or minimal proliferation of epithelium; 1—Necrosis or proliferation of epithelium or chronic bumble foot with no or moderate swelling; 2—Swollen (dorsally visible); (b) toe damage: 0—no toe damage; 1—presence of toe damage. The percentage of birds within each scoring category was recorded. Additionally, the percentage of hens in each group presenting normal or long claws was estimated. Comb individual examination was also performed for the presence of

abnormalities (blue or black spots or areas, very pale combs, wounds–not being punctiform pecking wounds–or missing parts)—score 1 or not—score 0.

2.6. Statistical Analysis

The data were analyzed using the statistical software Jeffreys's Amazing Statistics Program JASP (JASP v 0.14. https://jasp-stats.org/download/ (accessed on 20 January 2020)); [32]. The significance of the differences of KBD incidence rate, mortality rate, plumage damage, claw length, comb abnormalities, skin lesions, foot pad dermatitis and toe damage among groups was assessed by Chi-square test. For the analysis of laying hens' performance, egg quality traits, egg yolk fatty acid composition, phenols content, health yolk lipid indices and blood biochemical parameters the normality of the data was tested using the Shapiro–Wilk test and the homogeneity of variance was evaluated with Levene's test. One-way Analysis of Variance (ANOVA)was used to compare the average values of the parameters evaluated among dietary treatments. Post hoc analysis was performed using the Tukey test. In cases where the distribution was not normal the comparisons were made with the non-parametric tests Kruskal–Wallis and Mann–Whitney. All comparisons were made at a significance level of $p \leq 0.05$.

3. Results

3.1. Laying Performance

The incorporation of OP in laying hens' diet had a significant impact ($p < 0.05$) on HDEP %, egg weight, percentage of eggs with broken shell and egg mass (Table 3). However, final BW, percentage of eggs with dirty shells, feed consumption and FCR were not significantly different among dietary treatments ($p > 0.05$) as indicated in Table 3. Hens of group OP4 presented significantly lower HDEP % compared to that of hens belonging to the CON, OP2, OP5 and OP6 groups ($p < 0.05$), whereas OP6 hens had significantly higher HDEP % compared to OP2, OP3 and OP4 hens ($p < 0.05$). The highest egg weight was recorded in the CON group followed by the OP6, OP3, OP2, OP5 and OP4 groups, respectively. The differences in egg weight among dietary treatments were significant ($p < 0.05$) except those observed between the OP5 and OP4 groups. Eggs produced by hens that were fed diets with OP at the rate of 2%, 3%, 5% and 6% had a significantly lower percentage of broken shells compared to those produced by the CON and OP4 hens ($p < 0.05$). The percentage of broken eggshells between the CON and OP4 groups did not differ significantly ($p > 0.05$). Eggs produced by hens of the OP4 and OP5 groups had significantly lower egg mass compared to that recorded in eggs of the CON group ($p < 0.05$). The eggs produced by the CON, OP2, OP3 and OP6 hens presented similar egg mass ($p > 0.05$). Overall mortality rate ranged from 2% (OP6 group) to 12% (OP4 group) and the differences among treatments were not significant ($p > 0.05$).

Table 3. The overall performance of laying hens (27–68 week of age). Data are presented as mean ± SE.

Items	Dietary Treatments					
	CON	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
Final BW (Kg)	2.02 ± 0.04	1.94 ± 0.04	1.95 ± 0.02	1.99 ± 0.04	1.93 ± 0.04	1.91 ± 0.02
HDEP (%)	86.75 ± 0.43 ^{ac}	85.43 ± 0.54 ^{a}	83.35 ± 0.75 ^{ab}	83.88 ± 0.53 ^{b}	85.66 ± 0.60 ^{ac}	86.41 ± 0.59 ^{c}
Egg weight (g)	66.23 ± 0.17 ^{a}	64.19 ± 0.16 ^{b}	64.73 ± 0.15 ^{c}	63.78 ± 0.18 ^{d}	63.90 ± 0.16 ^{d}	65.26 ± 0.16 ^{e}
Eggs with broken shell %	1.57 ± 0.16 ^{a}	0.65 ± 0.09 ^{b}	0.74 ± 0.10 ^{b}	1.32 ± 0.15 ^{a}	0.68 ± 0.11 ^{b}	0.75 ± 0.11 ^{b}
Eggs with dirty eggshells %	0.13 ± 0.05	0.07 ± 0.05	0.19 ± 0.06	0.26 ± 0.10	0.09 ± 0.04	0.13 ± 0.07
Feed consumption (g/h/d)	112.42 ± 4.37	116.7 ± 5.23	103.18 ± 3.10	114.28 ± 4.67	113.12 ± 4.90	102.42 ± 3.09
FCR	2.00 ± 0.08	2.18 ± 0.10	2.00 ± 0.07	2.18 ± 0.09	2.12 ± 0.09	1.88 ± 0.06
Egg mass	56.45 ± 0.75 ^{a}	54.05 ± 1.11 ^{ab}	52.59 ± 1.33 ^{ab}	52.87 ± 0.87 ^{b}	53.69 ± 1.00 ^{b}	54.95 ± 1.02 ^{ab}

$^{a–e}$ Means within a row with different superscripts differ significantly ($p < 0.05$).

3.2. Egg Quality Traits

Egg quality traits of 68-week-old laying hens that received the control and diets containing different levels of OP are presented in Table 4. No differences were found in shape index, shell weight, albumen weight, albumen and yolk ratio (%), Haugh units, albumen height and yolk pH ($p > 0.05$). Hens of the OP2 group laid eggs with thinner shells than those produced in the rest of the groups and the differences noticed between the OP2 group and the OP3, OP4 and OP5 groups were significant ($p < 0.05$). The lowest shell ratio (%) was observed in the OP6 group and differed significantly ($p < 0.05$) with the highest that was found in the OP2 group. Eggs from the OP5 group presented significantly lower yolk weight compared to eggs from the OP6, OP3 and OP4 groups ($p < 0.05$). The addition of OP in laying hens' diet increased egg yolk diameter, with significant differences being observed between the CON group with the OP4 and OP5 groups, as well as between the OP4 and OP6 groups ($p < 0.05$). Moreover, eggs from the OP6 group had significantly higher yolk height compared to the OP4 eggs ($p < 0.05$). The aforementioned differences in yolk diameter and height reflected in the egg yolk index. In particular, a significantly increased yolk index was found in the OP6 eggs compared to the OP3 and OP4 eggs ($p < 0.05$). A greater yolk color score was recorded in the OP4 and OP6 groups compared to the rest of the dietary treatments, however significant differences were only found between the OP3 and OP4 groups ($p < 0.05$). The albumen pH of the OP5 and OP6 eggs was significantly higher than that recorded in the eggs of the rest of the groups.

Table 4. Egg quality traits of laying hens at 68 weeks of age fed the control and diets containing olive pulp. Data are presented as mean ± SE.

Hen Age: 68 wk	**Dietary Treatments**					
Items	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
Shape Index (%)	76.50 ± 0.86	76.79 ± 1.48	77.63 ± 0.85	78.00 ± 0.79	77.97 ± 0.88	76.07 ± 1.50
Shell thickness (mm)	0.38 ± 0.01 ^{ab}	0.36 ± 0.01 ^{a}	0.41 ± 0.01 ^{b}	0.41 ± 0.01 ^{b}	0.41 ± 0.02 ^{b}	0.40 ± 0.02 ^{ab}
Shell weight (g)	9.42 ± 0.27	9.62 ± 0.44	9.59 ± 0.14	9.29 ± 0.32	9.35 ± 0.29	9.14 ± 0.24
Shell ratio (%)	13.78 ± 0.23 ^{ab}	15.10 ± 0.63 ^{a}	14.07 ± 0.22 ^{ab}	14.17 ± 0.36 ^{ab}	14.61 ± 0.31 ^{ab}	13.17 ± 0.33 ^{b}
Albumen weight (g)	42.10 ± 1.25	38.59 ± 1.10	41.47 ± 1.01	38.51 ± 1.43	39.39 ± 1.21	43.05 ± 1.08
Albumen ratio (%)	61.51 ± 0.93	60.41 ± 0.60	60.76 ± 1.27	58.72 ± 1.28	61.45 ± 0.68	61.90 ± 0.50
Yolk weight (g)	15.75 ± 0.53 ^{ab}	15.27 ± 0.30 ^{ab}	16.67 ± 0.34 ^{b}	16.45 ± 0.40 ^{b}	14.83 ± 0.33 ^{a}	16.74 ± 0.29 ^{b}
Yolk ratio (%)	23.05 ± 0.62	23.97 ± 0.34	24.45 ± 0.57	25.23 ± 0.79	23.22 ± 0.55	24.14 ± 0.48
Haugh units	94.84 ± 0.95	95.48 ± 0.93	93.93 ± 0.86	94.81 ± 0.97	96.00 ± 0.59	96.22 ± 1.01
Albumen height (mm)	7.11 ± 0.16	7.09 ± 0.19	6.92 ± 0.18	7.01 ± 0.18	7.19 ± 0.13	7.43 ± 0.18
Yolk diameter (mm)	41.88 ± 0.16 ^{a}	42.94 ± 0.33 ^{abc}	43.11 ± 0.29 ^{abc}	43.31 ± 0.28 ^{b}	43.24 ± 0.40 ^{bc}	42.01 ± 0.26 ^{ac}
Yolk height (mm)	18.43 ± 0.18 ^{ab}	18.47 ± 0.24 ^{ab}	18.26 ± 0.17 ^{ab}	18.06 ± 0.21 ^{a}	18.61 ± 0.24 ^{ab}	19.03 ± 0.14 ^{b}
Yolk index	44.01 ± 0.49 ^{ab}	43.05 ± 0.68 ^{ab}	42.36 ± 0.32 ^{b}	41.72 ± 0.47 ^{b}	43.11 ± 0.85 ^{ab}	45.32 ± 0.44 ^{a}
Yolk color	10.50 ± 0.22 ^{ab}	10.60 ± 0.22 ^{ab}	10.20 ± 0.20 ^{a}	11.10 ± 0.23 ^{b}	10.70 ± 0.15 ^{ab}	11.00 ± 0.21 ^{ab}
Albumen pH	8.55 ± 0.04 ^{b}	8.55 ± 0.04 ^{b}	8.57 ± 0.02 ^{b}	8.62 ± 0.02 ^{b}	8.76 ± 0.03 ^{a}	8.82 ± 0.02 ^{a}
Yolk pH	6.30 ± 0.05	6.18 ± 0.03	6.14 ± 0.06	6.22 ± 0.03	6.23 ± 0.05	6.17 ± 0.02

$^{a–c}$ Means within a row with different superscripts differ significantly ($p < 0.05$).

3.3. Egg Fatty Acid Composition, Phenols Content and Health Lipid Indices

Significant differences ($p < 0.05$) were detected among dietary treatments on egg total fat, FA composition, total phenols and health lipid indices that were determined at the whole edible parts (albumen and yolk) of eggs obtained from laying hens of intermediate age (Table 5). Eggs from the OP6 group had significantly ($p < 0.05$) lower total fat content than that recorded in the eggs from the rest of the groups, whereas the highest fat content was found in eggs from group OP3 ($p < 0.05$). Hens from the CON and OP2 groups produced eggs with a similar percentage of total fats ($p > 0.05$). The highest phenol content among treatments was found in the OP4 and OP6 eggs ($p < 0.05$) and the lowest in the OP3 eggs ($p < 0.05$). The total phenol content of eggs produced by the CON, OP2 and OP5 hens was not significantly different ($p > 0.05$).

Table 5. The effect of dietary olive pulp (OP) supplementation on egg total fat, fatty acid (FA) composition (g/100 g FA) total phenols and health lipid indices as evaluated in the whole edible parts (albumen and yolk) of eggs obtained from 50-week-old laying hens. Data are presented as mean ± SE.

Hen Age: 50 wk	**Dietary Treatments**					
Item	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
% Fat	9.09 ± 0.10 ^{a}	9.06 ± 0.09 ^{a}	10.93 ± 0.08 ^{b}	10.26 ± 0.09 ^{c}	9.49 ± 0.09 ^{d}	7.66 ± 0.07 ^{e}
MUFA % (g/100 g Fat)	47.01 ± 0.40 ^{ab}	46.61 ± 0.28 ^{ab}	45.56 ± 0.55 ^{a}	47.42 ± 0.47 ^{b}	45.43 ± 0.32 ^{a}	47.74 ± 0.52 ^{b}
PUFA % (g/100 g Fat)	7.33 ± 0.08 ^{a}	9.06 ± 0.08 ^{b}	11.13 ± 0.13 ^{c}	10.70 ± 0.13 ^{c}	22.21 ± 0.49 ^{d}	20.67 ± 0.16 ^{e}
SFA % (g/100 g Fat)	45.67 ± 0.37 ^{a}	44.33 ± 0.36 ^{ab}	43.31 ± 0.58 ^{bc}	41.86 ± 0.41 ^{c}	32.35 ± 0.72 ^{d}	31.59 ± 0.45 ^{d}
Total Phenols (mg GAE/g)	36.05 ± 0.30 ^{a}	36.10 ± 0.35 ^{a}	31.15 ± 0.23 ^{b}	39.49 ± 0.41 ^{c}	35.47 ± 0.24 ^{a}	39.08 ± 0.32 ^{c}
PUFA/SFA	0.16 ± 0.00 ^{a}	0.21 ± 0.00 ^{ab}	0.26 ± 0.01 ^{b}	0.26 ± 0.00 ^{b}	0.69 ± 0.03 ^{c}	0.66 ± 0.01 ^{c}
PUFA n6	7.42 ± 0.14 ^{a}	9.46 ± 0.17 ^{b}	11.59 ± 0.16 ^{c}	10.83 ± 0.09 ^{d}	22.36 ± 0.13 ^{e}	19.30 ± 0.10 ^{f}
PUFA n3	ND	ND	ND	ND	0.94 ± 0.01 ^{a}	0.83 ± 0.01 ^{b}
PUFA n6/PUFA n3					23.71 ± 0.24 ^{a}	23.31 ± 0.13 ^{a}
AI	0.84 ± 0.01 ^{a}	0.82 ± 0.01 ^{a}	0.74 ± 0.01 ^{b}	0.69 ± 0.02 ^{c}	0.47 ± 0.01 ^{d}	0.49 ± 0.01 ^{d}
AI**	0.69 ± 0.01 ^{a}	0.67 ± 0.01 ^{a}	0.59 ± 0.01 ^{b}	0.58 ± 0.02 ^{b}	0.39 ± 0.00 ^{c}	0.41 ± 0.00 ^{c}
TI	1.63 ± 0.01 ^{a}	1.60 ± 0.02 ^{a}	1.44 ± 0.02 ^{b}	1.33 ± 0.04 ^{b}	0.85 ± 0.01 ^{c}	0.89 ± 0.01 ^{c}
h/H	1.40 ± 0.01 ^{a}	1.43 ± 0.02 ^{a}	1.66 ± 0.02 ^{b}	1.65 ± 0.05 ^{b}	2.52 ± 0.03 ^{c}	2.39 ± 0.03 ^{d}
Fatty acids						
Caproic acid (C6:0)	0.028 ± 0.003	0.027 ± 0.002	0.018 ± 0.003	0.023 ± 0.002	ND	ND
Caprylic acid (C8:0)	0.180 ± 0.003 ^{a}	0.173 ± 0.002 ^{a}	0.112 ± 0.003 ^{b}	0.130 ± 0.003 ^{c}	ND	ND
Myristic acid (C14:0)	0.460 ± 0.003 ^{a}	0.400 ± 0.003 ^{b}	0.362 ± 0.003 ^{c}	0.480 ± 0.003 ^{d}	0.347 ± 0.003 ^{e}	0.350 ± 0.003 ^{ec}
Myristoleic acid (C14:1)	0.058 ± 0.003 ^{a}	0.058 ± 0.003 ^{a}	0.038 ± 0.003 ^{b}	0.088 ± 0.003 ^{c}	0.067 ± 0.002 ^{ad}	0.077 ± 0.003 ^{cd}
Pentadecylic acid (C15:0)	0.070 ± 0.003 ^{ac}	0.223 ± 0.135 ^{b}	0.080 ± 0.004 ^{ab}	0.073 ± 0.003 ^{a}	0.062 ± 0.003 ^{c}	0.060 ± 0.004 ^{c}
Palmitic acid (C16:0)	35.710 ± 0.257 ^{a}	35.408 ± 0.180 ^{a}	32.683 ± 0.218 ^{b}	32.882 ± 0.619 ^{b}	25.372 ± 0.232 ^{c}	26.142 ± 0.193 ^{c}
Palmitoleic acid (C16:1)	3.582 ± 0.124 ^{a}	3.558 ± 0.101 ^{a}	2.713 ± 0.152 ^{b}	4.367 ± 0.118 ^{c}	3.457 ± 0.095 ^{a}	4.393 ± 0.159 ^{c}
Margaric acid (C17:0)	0.250 ± 0.003 ^{a}	0.238 ± 0.003 ^{a}	0.280 ± 0.003 ^{b}	0.190 ± 0.003 ^{c}	0.173 ± 0.004 ^{d}	0.142 ± 0.003 ^{e}
Ginkgolic acid (C17:1)	0.040 ± 0.003 ^{a}	0.030 ± 0.003 ^{a}	0.040 ± 0.003 ^{a}	0.040 ± 0.003 ^{a}	0.090 ± 0.003 ^{b}	0.088 ± 0.003 ^{b}
Stearic acid (C18:0)	8.492 ± 0.153 ^{a}	8.368 ± 0.149 ^{a}	8.505 ± 0.111 ^{a}	6.393 ± 0.134 ^{b}	5.557 ± 0.176 ^{c}	5.440 ± 0.164 ^{c}
Elaidic acid (C18:1n9t)	0.110 ± 0.003 ^{a}	0.048 ± 0.003 ^{b}	0.048 ± 0.003 ^{b}	0.070 ± 0.003 ^{c}	ND	ND
Oleic (C18:1n9c)	43.258 ± 0.262 ^{ab}	41.613 ± 0.462 ^{ac}	43.087 ± 0.334 ^{abc}	43.965 ± 0.568 ^{b}	41.555 ± 0.275 ^{c}	43.155 ± 0.382 ^{abc}
Linoleic acid (C18:2n6c)	7.380 ± 0.139 ^{a}	9.403 ± 0.169 ^{b}	11.430 ± 0.155 ^{c}	10.573 ± 0.093 ^{d}	21.437 ± 0.125 ^{e}	18.598 ± 0.102 ^{f}

Table 5. *Cont.*

Hen Age: 50 wk	Dietary Treatments					
Item	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
γ -Linolenic (C18:3n6)	ND	ND	ND	ND	0.120 ± 0.003 ^{b}	0.088 ± 0.003 ^{c}
α-Linolenic (C18:3n3)	ND	ND	ND	ND	0.872 ± 0.003 ^{b}	0.778 ± 0.003 ^{c}
Arachidic acid (C20:0)	0.040 ± 0.003 ^{a}	0.040 ± 0.003 ^{a}	0.038 ± 0.003 ^{a}	0.030 ± 0.003 ^{ab}	0.020 ± 0.003 ^{b}	0.022 ± 0.003 ^{b}
Gondoic acid (C20:1n9)	0.302 ± 0.003 ^{a}	0.348 ± 0.003 ^{b}	0.398 ± 0.003 ^{c}	0.440 ± 0.003 ^{d}	0.000 ± 0.000	0.000 ± 0.000
Eicosadienoic acid (C20:2n6)	0.040 ± 0.003 ^{a}	0.058 ± 0.003 ^{b}	0.092 ± 0.003 ^{c}	0.098 ± 0.003 ^{c}	0.198 ± 0.003 ^{d}	0.172 ± 0.003 ^{e}
Dihomo-γ-linolenic acid (C20:3n6)	ND	ND	ND	ND	0.097 ± 0.003 ^{a}	0.078 ± 0.003 ^{b}
Arachidonic acid (C20:4n6)	ND	ND	0.065 ± 0.013 ^{a}	0.078 ± 0.003 ^{a}	0.507 ± 0.003 ^{b}	0.367 ± 0.003 ^{c}
Docosadienoic acid (C22:2n6)	ND	ND	ND	ND	ND	ND
Docosahexaenoic acid (DHA) (C22:6n3)	ND	ND	ND	ND	0.072 ± 0.003 ^{a}	0.050 ± 0.003 ^{b}

$^{a–f}$ Means within a row with different superscripts differ significantly ($p < 0.05$), ND: Not Detected, AI: Atherogenic Index [27], AI**: Atherogenic Index [28], TI: Thrombogenic Index, h/H: hypocholesterolemic (h)/hypercholesterolemic (H) fatty acids.

The percentage of MUFA, PUFA, PUFA n6, PUFA n3 and SFA of eggs as well as the PUFA/SFA and PUFA n6/PUFA n3 ratios were significantly affected ($p < 0.05$) by dietary treatments (Table 5). In the OP4 and OP6 eggs, MUFA constituted about 47.74% of the total amount of FAs. This percentage was similar with that recorded for the CON and OP2 groups ($p > 0.05$) but it was significantly higher than that observed in eggs of the OP3 and OP5 groups ($p < 0.05$). The corresponding value was similar between eggs from the CON, OP2, OP3 and OP5 groups ($p > 0.05$). Hens that were fed diets with OP produced eggs with a significantly higher amount of PUFA and PUFA n6 compared to CON hens ($p < 0.05$). Among the groups, the highest percentage of PUFA and PUFA n6 was recorded in the OP5 eggs ($p < 0.05$). The OP3 and OP4 eggs presented similar levels of PUFA ($p > 0.05$) however, the PUFA n6 percentage was significantly different among all groups ($p < 0.05$). In the eggs derived from CON, OP2, OP3 and OP4 hens, PUFA n3 was not detected. On the other hand, hens fed diets with 5% OP produced eggs with significantly higher amount of PUFA n3 than that detected in the OP6 eggs ($p < 0.05$). Consequently, the PUFA n6/PUFA n3 ratio was calculated only for the OP5 and OP6 eggs and did not differ between these groups. As the incorporation rate of OP increased in the laying hens' diet, the percentage of SFA in produced eggs decreased (Table 5). The eggs derived from OP5 and OP6 hens displayed the lowest SFA level compared with that found in the eggs produced by hens of the other treatments ($p < 0.05$). A similar percentage of SFA was found between CON and OP2 eggs, between OP2 and OP3 eggs, as well as between OP3 and OP4 eggs ($p > 0.05$). The significant differences of MUFA, PUFA and SFA levels observed among eggs of all investigated groups resulted in relevant differences of the PUFA/SFA ratio. In particular, the OP5 and OP6 eggs presented the highest PUFA/SFA ratio whereas the CON eggs presented the lowest one ($p < 0.05$). The corresponding ratio was 0.26 for the OP3 and OP4 eggs which was significantly higher than that of the CON eggs ($p < 0.05$) but similar to that found in the OP2 eggs ($p > 0.05$).

The Atherogenic Index, calculated either with the inclusion of C18:0 fatty acid to the numerator of equation formula (IA), or without it (IA**), was found significantly reduced in the OP5 and OP6 eggs than that recorded in eggs of all the other treatments ($p < 0.05$). The corresponding value in the OP3 and OP4 eggs was higher than that in the CON and OP2 eggs ($p < 0.05$). The lowest values of TI were recorded in the OP5 and OP6 eggs, followed by that of the OP3 and OP4 eggs whereas the highest corresponding value was found in the CON and OP2 eggs ($p < 0.05$). Among experimental groups, the OP6 eggs presented the highest h/H ratio followed by that of the OP5 eggs ($p < 0.05$). At the OP3 and OP4 eggs the corresponding ratio was similar ($p > 0.05$) but differed significantly with the lowest one observed in CON and OP2 eggs ($p < 0.05$).

All of the individual egg's FAs, except caproic acid (C6:0), were affected by the addition of OP in laying hens' diet. The differences observed among dietary treatments were significant ($p < 0.05$) and are presented in detail in Table 5. Generally, the most abundant fatty acid among SFAs recorded in eggs of all investigated groups was the palmitic acid (C16:0). The lowest concentration of palmitic acid, approximately 25.37%, was found in the OP5 and OP6 eggs and the highest, about 35.7%, in the CON and OP2 eggs ($p < 0.05$). The intermediate level of the corresponding value observed in the OP3 and OP4 eggs (about 32.7%) differed significantly from that found in the eggs of the rest of the treatments ($p < 0.05$). According to the results, oleic acid was the most abundant of the MUFAs recorded in eggs of all the studied groups. The highest levels of oleic acid were recorded in the OP4 eggs and differed significantly from the lowest levels observed in the OP5 and OP2 groups ($p < 0.05$). Data analysis showed that, among PUFAs, the most abundant fatty acid in eggs of all treatments was linoleic acid. The hens that were fed diets with OP produced eggs with significantly higher levels of linoleic acid compared to the controls ($p < 0.05$). Moreover, as the incorporation rate of OP in the birds' feed was increasing, a significant elevation of linoleic acid concentration in produced eggs was recorded ($p < 0.05$).

3.4. Blood Biochemical Constituents

Table 6 shows the effect of experimental diets on blood profile of 50-week-old laying hens. The egg-layers that consumed diets containing OP at the rate of 3%, 5% and 6% had a significantly higher serum uric acid concentration compared to the CON hens ($p < 0.05$). Serum cholesterol, triglycerides and BUN levels, were not affected by the addition of olive pulp in hens' feed ($p > 0.05$). Data analysis revealed that no significant effect ($p > 0.05$) on liver enzymes' concentration levels was recorded among dietary groups.

Table 6. The effect of experimental diets on biochemical parameters of laying hens in the middle of production cycle. Data are presented as mean ± SE.

Items	Dietary Treatments					
Hen Age: 50 wk	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
Cholesterol (mg/dL)	64.30 ± 6.15	73.90 ± 5.92	80.20 ± 5.60	98.20 ± 19.80	89.70 ± 6.82	92.80 ± 8.67
Triglycerides (mg/dL)	459.46 ± 120.02	663.92± 104.76	783.41 ± 113.05	881.30 ± 141.00	894.67 ± 137.12	951.78 ± 158.59
Uric acid (mg/dL)	2.19 ± 0.39 ^{a}	3.30 ± 0.27 ^{ab}	4.16 ± 0.41 ^{b}	2.88 ± 0.38 ^{ab}	4.25 ± 0.36 ^{b}	4.29 ± 0.28 ^{b}
BUN (mg/dL)	13.75 ± 4.52	17.44 ± 2.76	21.29 ± 4.00	26.58 ± 4.46	21.71 ± 4.39	25.19 ± 5.34
AST (IU/L)	203.40 ± 6.25	198.90 ± 4.68	187.20 ± 8.38	192.30 ± 6.11	190.80 ± 6.12	195.00 ± 2.82
G-GT (IU/L)	26.28 ± 2.04	27.28 ± 2.39	28.34 ± 1.69	30.39 ± 2.87	26.71 ± 2.65	27.88 ± 3.32
GLDH (U/L)	9.71 ± 2.04	11.15 ± 2.39	10.72 ± 1.69	7.83 ± 2.87	7.67 ± 2.65	8.25 ± 3.32

^{a,b} Means within a row with different superscripts differ significantly ($p < 0.05$). G-GT: Gamma-Glutamyl Transferase

3.5. Welfare Parameters

Data in Table 7 display the dietary influence of OP on KBD incidence as evaluated in middle-aged laying hens and the percentage of birds in each group with deformation, fractures or both. The lowest KBD incidence rate was recorded in the OP3 hens and was significantly different among all groups ($p < 0.05$). Birds of the OP2 group presented the highest KBD incidence rate and was significantly different ($p < 0.05$) than that found in the CON, OP3 and OP6 hens. The incidence of KBD in the OP4 and OP5 hens was significantly different only in comparison with that recorded in the OP3 hens ($p < 0.05$). From the birds with damaged keels, research findings revealed that 2.04% of hens in the CON and OP6 groups had both deformation and fractures, while in the OP3 group, 2.08% of the hens had only fractures and 10.42% had only deformation. Only deformed keels were recorded in the hens of the groups OP2, OP4 and OP5. The differences in keel bone deformation rate found among the dietary treatments followed the same pattern with those concerning the KBD incidence among experimental groups (Table 7).

Table 7. Percentage of hens in the middle of production cycle observed in the 6 dietary treatments (CON, OP2, OP3, OP4, OP5, OP6) for the absence or presence (SCORE 0, 1) of KBD and % of hens presenting deformation, fractures or both.

KBD	Dietary Treatments					
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	53.06 ^{a}	29.17 ^{b}	87.50 ^{c}	44.90 ^{ab}	48.00 ^{ab}	59.18 ^{a}
1	46.94 ^{a}	70.83 ^{b}	12.50 ^{c}	55.10 ^{ab}	52.00 ^{ab}	40.82 ^{a}
Keel Bone Damage % of hens	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
Deformation	44.90 ^{a}	70.83 ^{b}	10.42 ^{c}	55.10 ^{ab}	52.00 ^{ab}	38.78 ^{a}
Fractures	0.00	0.00	2.08	0.00	0.00	0.00
Deformation & Fractures	2.04	0.00	0.00	0.00	0.00	2.04

$^{a–c}$ Means within a raw for a particular score with different superscripts differ significantly ($p < 0.05$).

Feather damage evaluation revealed significant differences ($p < 0.05$) among experimental groups either on individual body areas or on total plumage score (Table 8). Overall, the higher percentage of hens with the best plumage condition (score 0) was recorded in the OP5 group while the lowest one was found in the OP2 group. The percentage of birds

evaluated with score 0 differed significantly between the OP2 and OP5 groups ($p < 0.05$). The percentage of CON hens with a score 1 was significantly lower ($p < 0.05$) than that recorded for hens of the OP6 group. Finally, a significantly higher percentage of birds with score 2 for total feather damage was observed in the hens of the CON and OP2 groups in comparison to the OP4, OP5 and OP6 hens ($p < 0.05$). During the evaluation for total feather damage, none of the hens of the OP5 and OP6 groups was given a score 2.

Table 8. Percentage of hens in the middle of production cycle observed in the 6 dietary treatments (CON, OP2, OP3, OP4, OP5, OP6) scoring for 0, 1, 2 for feather damage in 3 body regions.

Head-Neck	**Dietary Treatments**					
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	34.69 ^{a}	14.58 ^{b}	27.08 ^{ab}	30.61 ^{ab}	38.00 ^{a}	22.45 ^{ab}
1	51.02 ^{a}	58.34 ^{ab}	62.50 ^{ab}	69.39 ^{ab}	62.00 ^{ab}	77.55 ^{b}
2	14.29 ^{a}	27.08 ^{a}	10.42 ^{ab}	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}
Back-Rump						
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	93.88	89.58	100.00	100.00	100.00	100.00
1	6.12	10.42	0.00	0.00	0.00	0.00
Belly						
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	77.55 ^{a}	87.50 ^{ab}	87.50 ^{ab}	97.96 ^{bc}	100.00 ^{c}	100.00 ^{c}
1	18.37 ^{a}	12.50 ^{a}	10.42 ^{ab}	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}
2	4.08	0.00	2.08	2.04	0.00	0.00
Total plumage						
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	30.61 ^{ab}	14.58 ^{b}	27.08 ^{ab}	30.61 ^{ab}	38.00 ^{a}	22.45 ^{ab}
1	51.02 ^{a}	58.34 ^{ab}	62.50 ^{ab}	67.35 ^{ab}	62.00 ^{ab}	77.55 ^{b}
2	18.37 ^{a}	27.08 ^{a}	10.42 ^{ab}	2.04 ^{b}	0.00 ^{b}	0.00 ^{b}

$^{a–c}$ Means within a raw for a particular score with different superscripts differ significantly ($p < 0.05$); 0—All body parts have no or slight wear, (nearly) complete feathering (only single feathers lacking); 1—One or more body parts have moderate wear, i.e., damaged feathers (worn, deformed) or one or more featherless areas < 5 cm in diameter at the largest extent; 2—One or more body parts have at least one featherless area ≥ 5 cm in diameter at the largest extent.

No significant differences in plumage conditions in the back/rump area were observed among groups ($p > 0.05$). A significantly higher percentage of hens with score 0 in the head/neck area was recorded in the CON and OP5 groups than the OP2 group ($p < 0.05$). Moreover, for the same body area, the highest percentage of hens with score 1 was found in the OP6 group. The differences observed among groups in hens scoring 1 for feather damage in the head/neck area were significant ($p < 0.05$) between the OP6 and CON groups. None of the hens consuming diets with OP at the dose of 4%, 5% and 6% were given a score 2 for feather damage in the head/neck area. On the contrary, similar percentages of the CON and OP2 hens ($p > 0.05$) were evaluated with a score 2 in this body area. The percentages of CON and OP2 hens evaluated with a score 2 for feather damage at the head/neck area were significantly different ($p < 0.05$) from those observed in the OP4, OP5 and OP6 hens (Table 8).

Laying hens that received OP at the rates of 4%, 5% and 6% presented the best plumage condition in the belly area (Table 8). For score 0 in feather damage evaluation, the differences observed among experimental groups were significant ($p < 0.05$) between the CON group with the OP4, OP5 and OP6 groups as well as between the OP2 and OP3 groups with the OP5 and OP6 groups. Significantly higher was the percentage of hens evaluated for belly feather damage with score 1 in the CON and OP2 groups than the 0% observed in the OP4, OP5 and OP6 groups ($p < 0.05$). The percentage of hens evaluated for belly feather damage with score 2 was similar in all groups ($p > 0.05$).

In the present study, no problems with foot pad dermatitis or toe damage were recorded in hens of all dietary groups. The birds of all treatments were evaluated with very good scores for comb abnormalities and skin lesions, indicating no evidence of such welfare issues (Table 9). The percentages of hens in each score category for cob abnormalities and skin lesions did not differ among the groups ($p > 0.05$). The addition of OP in laying hens' diet had a significant impact on birds' claws (Table 9). The highest percentages of hens with long nails were found in the OP4, OP5 and OP6 groups, followed by the OP3 and CON groups. All hens of the OP2 group had normal nails in respect of their length. The differences regarding nail length of hens were similar between the CON and OP2 groups ($p > 0.05$). However, significant differences ($p < 0.05$) in the length of hens' nails were observed between the CON and OP2 groups with the OP3 group, between the CON and OP2 groups with the OP4, OP5 and OP6 groups, as well as between the OP3 group with the OP4, OP5 and OP6 groups.

Table 9. Percentage of hens in the middle of production cycle observed in the 6 dietary treatments (CON, OP2, OP3, OP4, OP5, OP6) scoring for comb abnormalities and skin lesions (0, 1, 2) as well as claw length (Normal/Long).

Comb Abnormalities	**Dietary Treatments**					
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	97.96	100.00	100.00	100.00	98.00	100.00
1	2.04	0.00	0.00	0.00	2.00	0.00
2	-	-	-	-	-	-
Skin lesions						
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
0	100.00	100.00	100.00	97.96	100.00	100.00
1	0.00	0.00	0.00	2.04	0.00	0.00
2	-	-	-	-	-	-
Claw length						
score	**CON**	**OP2**	**OP3**	**OP4**	**OP5**	**OP6**
Normal	93.88 ^{a}	100.00 ^{a}	77.08 ^{b}	22.45 ^{c}	42.00 ^{c}	30.61 ^{c}
Long	6.12 ^{a}	0.00 ^{a}	22.92 ^{b}	77.55 ^{c}	58.00 ^{c}	69.39 ^{c}

$^{a–c}$ Means within a raw with different superscripts differ significantly ($p < 0.05$).

4. Discussion

A large proportion of consumers in today's health conscious society is seeking for properly balanced diets in order to prevent and minimize adverse health problems [33,34]. At the same time, in the last decade, there has been an increased consumer demand for meat and egg products that focuses on animal welfare during production as well as on product safety and quality [35,36]. As a result of this direction, adequate supplementation of poultry diets with novel and beneficial feed additives or supplements is gaining importance as it significantly improves overall poultry production and performance as well as safeguarding the health of birds [37,38]. Previous studies indicate that manipulating the laying hens' diet, by adding different by-products rich in fatty acids and antioxidants, can alter the FA profile of eggs [39–43]. A review of literature highlights that OP is not just an oil by-product, but a source of functional ingredients exploitable to obtain high value-added foods, thus increasing their shelf-life and/or formulating more nutritious and healthy products [44]. The reuse of waste to recover functional compounds is in line with consumer and society's requirements for high quality, safe, processed foods and the reduction of waste to exert a lower environmental impact [45].

The present study revealed that the addition of dried OP in laying hens' diet increased the percentage of PUFA in produced eggs and decreased that of SFA in a manner proportional to the inclusion rate in hens' diet. On the other hand, the concentration of MUFA remained relatively stable in the eggs produced by OP-fed layers compared to the control.

The reduced SFAs concentration recorded in the eggs produced by OP-fed layers could be mainly attributed to a proportional reduction of palmitic acid, which was found to be the most abundant among the SFAs recorded in the eggs of all dietary groups and, to a lesser extent, to a similar reduction of the stearic and myristic acid concentrations. The increased concentration in PUFAs observed in the eggs laid by hens receiving OP is attributed to a similar proportional elevation of linoleic acid concentration, the most abundant fatty acid among PUFAs found in the eggs of all experimental groups. The FA composition analysis of OP used in this study revealed the presence of linoleic acid in a percentage of 10.77%. It could be supported that the increase in the OP incorporation rate in the hens' diet resulted in a concomitant increase of linoleic acid concentration in produced eggs. Since there is lack of research evidence regarding the effect of OP in the lipid profile of eggs when supplementing laying hens' feed, the comparison of our results with those in literature is not possible. However, similar studies carried out with other farm animals have shown that the incorporation of OP in their diet ameliorates the FA profile by decreasing SFAs and increasing unsaturated fatty acids in the final products such as broiler meat [46,47], pork meat [48], rabbit meat [49], lamb meat [50] and small ruminants' milk [51,52] and cheese [52]. The decreased concentration of SFAs in OP eggs observed in this study could be attributed to the high concentration of phenolic compounds of the OP used in the experiment that act as antioxidants and suppress egg lipid oxidation. The antioxidant effect of polyphenols on the final product when supplemented in animals' diet has been previously documented [53]. In this investigation, the increase of OP incorporation rate in the hens' diet did not result in a similar elevation of egg total phenol content. This is not surprising however, since it has been recently shown that the content of polyphenols in body tissues is not directly related to their dietary levels [53].

In line with our findings, Laudadio et al. [54] noticed that feeding high-polyphenols extra-virgin olive oil to laying hens raised the PUFAs and linoleic acid composition, reduced SFAs in egg yolks and improved the PUFA/SFA ratio. Ratios of PUFA/SFA are commonly used to assess the nutritional value of fat. Dietary ratios of PUFA to SFA above 0.45 are considered safe for human consumption [55] and appropriate in order to protect against the development of ischemic heart disease [56]. The current study revealed that feeding laying hens with dried OP increased the PUFA/SFA ratio in a manner proportional to the inclusion rate in the hens' diet. Furthermore, the optimal ratios from a nutritive point of view were achieved with the higher doses of olive pulp (5% and 6%). The differences of MUFA, PUFA and SFA levels observed among the eggs of all the investigated groups resulted in relevant differences of the PUFA/SFA ratio. Additionally, our results indicated that feeding laying hens with the higher dose of OP (6%) significantly reduced the total fat content of eggs. Similar reduction of egg fat composition has been documented by Abd El-Moneim and E.M. Sabic [57], after the inclusion of 5% and 10% of OP in the diet of Japanese laying quails in a twelve-week feeding trial.

Even though PUFA/SFA is the most commonly used index for evaluating the nutritional value of dietary foods, it is considered too general and unsuitable for assessing the atherogenicity of foods [58] since specific SFA and PUFA have different metabolic effects [56]. Fatty acids can either promote or prevent atherosclerosis and coronary thrombosis, based on their effects on serum cholesterol and low density lipoprotein cholesterol concentrations [54]. For this reason, the index of atherogenicity (AI) was developed [28] and characterizes the atherogenic potential of FA. From the main classes of the SFAs, the C14:0 and C16:0 fatty acids are known to be among the most atherogenic, while C18:0 is thought to be neutral with respect to atherogenicity, but is instead considered to be thrombogenic [54,58]. Unsaturated Fatty Acids (UFAs) are considered to be anti-atherogenic as they inhibit the accumulation of plaque and reduce the levels of phospholipids, cholesterol, and esterified fatty acids [27,59]. Therefore, the consumption of foods or products with a lower AI can reduce the levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) in human blood plasma [60]. Another important index commonly used in many FA composition studies in order to assess the degree of thrombogenicity is TI [58].

This index characterizes the thrombogenic potential of FAs, indicating the tendency to form clots in blood vessels and provides the contribution of different FAs, which denotes the relationship between the pro-thrombogenic FAs (C12:0, C14:0, and C16:0) and the anti-thrombogenic FAs (MUFAs and the n-3 and n-6 families) [28]. It has been reported that animal products with a low index of thrombogenicity decrease the threat of atrial fibrillation [61]. In brief, both the AI and the TI can be used to assess the potential effects of FA composition on Cardiovascular Health (CVH). A FA composition with a lower AI and TI has a better nutritional quality, and its consumption may reduce the risk of coronary heart disease (CHD), but no organization has yet provided the recommended values for the AI and TI [58].

The results of this study indicated that the addition of OP in laying hens' diet decreased both AI and TI in a proportional manner. At the same time incorporation rates of OP above 3% reduced significantly the h/H ratio of produced eggs, especially at the higher doses (5% and 6%). According to Santos-Silva et al. [62], the higher the ratio between the hypocholesterolemic and the hypercholesterolemic fatty acids, the more the oil or fat is appropriate to human nutrition. These observations demonstrate the health beneficial potential associated with the fat intake from eggs produced from OP-fed layers since they presented higher nutrition quality than the control conventional eggs. It is difficult to compare the values of the AI, TI, and h/H indexes obtained in this research for the eggs laid by hens that were fed OP-supplemented diets to those of other studies because, in the literature, there is a lack of work related to the assessment of health lipid indexes of hens' eggs following OP dietary incorporation. However, similar to our results, Laudadio et al. [54] denoted a significant decrease of AI in the yolk of eggs produced by laying hens fed with extra-virgin olive oil, rich in polyphenols. Previous feeding trials performed in other farm animals have shown that the addition of OP in their diet improves the health lipid indices in final products such ewe milk [63] and rabbit meat [49] by reducing both AI and TI indexes.

Regarding laying hens' performance, our findings indicated that including OP up to 6% in laying hens' diet during a production cycle does not compromise their final body weight and does not affect feed consumption, FCR or the percentage of eggs with dirty eggshells. Results concerning body weight are in line with those obtained by other investigators who evaluated the in feed inclusion of OP in hens' diet, at rates ranging from 1% [23] up to 20% [1,21,22] for a period of 6, 12 and 16 weeks, respectively. In respect of feed consumption, our results confirm those observed in previous feeding trials [2,18–20,23]. However, increased feed intake has been previously demonstrated in laying hens that were fed diets containing 4–20% OP compared to birds fed OP-free diets [1,17,21,22]. In terms of FCR, our results are in agreement with those recorded by other authors [2,18,21]. According to Ghasemi et al. [20], the addition of 20% OP in a corn-based diet of laying hens had no impact on FCR which is consistent with our findings. However, when the same amount of OP was incorporated in a wheat-based diet the FCR was increased. Deterioration of FCR was also observed in layers that were fed diets with 10–20% OP compared to controls [1,17,19,22]. The non-significant differences to the produced number of eggs with dirty shells observed among the dietary groups could be an indirect indicator that OP does not cause diarrhea when added to laying hen's diet at the inclusion levels studied.

The present study demonstrated that hens that were fed diets with OP produced lighter eggs compared to controls. Despite this reduction however, OP eggs did not weight less than 63 g. According to Commission Regulation (EC) No 589/2008 [64], eggs should be graded by weight as follows: XL-very large: 73 g and more; L-large: from 63 g up to 73 g; M-medium: from 53 g up to 63 g; S-small: under 53 g. Taking into consideration the European guidelines, hens of all the experimental groups produced L-large eggs as graded by their weight. A numerical decrease in egg weight was also reported by Rezar et al. [23] in hens that were fed a diet with 1% OP compared to those that received a control diet (62.6 g and 64.3 g, respectively). Contrary to our results, other authors found that the addition of OP at the rate of 4.5% [2], 9% [2,18], 12% and 16% [1] in the layers' diet significantly

increased the egg weight of produced eggs. On the other hand, no differences in egg weight were recorded by other authors when OP was included in laying hens' diet at levels from 10% up to 20% [17,20–22].

In the current study, a drop of HEDP% of about 3% was recorded in hens of the OP3 and OP4 groups compared to controls. This is considered as a random finding, since the laying hens that were fed the higher dosage rate of OP presented similar HDEP% with the CON hens. A decrease of egg production (%) has also been observed in previous feeding trials in which OP was incorporated in laying hens ratio at the levels of 9% [18], 12% and 16%, respectively [1]. However, other investigators observed no differences on egg production performance of laying hens that were fed diets with OP compared to the control hens [2,17,19–23]. This trial also revealed that hens of the OP4 and OP5 groups laid eggs with a lower egg mass than that recorded in eggs of the CON group. The differences in egg mass observed between the CON and the OP4 and OP5 groups is attributed to the highest HDEP% of CON hens as well as to the heaviest eggs produced by them, compared to the corresponding values recorded for the other two groups. According to some researchers, supplementing laying hens' diet with OP did not affect the egg mass of produced eggs [2,17–22]. However, Abd-El Galil et al. [1] observed a significant decrease of egg mass by 5.8% in hens that were fed diets with 16% olive cake compared to those that were fed a control diet.

A 15–58% lower percentage of broken eggshells was recorded to the OP-fed groups compared to control hens. This finding is very important from a financial point of view because cracked and broken eggshells are regarded as major source of economic loss for egg producers [65]. These results indicate that OP might increase the eggshell strength. Shell strength is influenced by shell thickness and shell matrix organization [66]. Even though eggshell quality data were similar between the OP and CON eggs, a numerical increase of shell thickness and percentage (%) was observed in the OP groups compared to controls. To our knowledge, there are no research findings regarding the dietary effect of OP on eggshell-breaking strength. However, Zhang and Kim [67] showed that supplementing laying hens' diet with 2% and 5% olive oil increased eggshell breaking strength and the shell thickness of produced eggs in comparison to controls. These researchers supported that olive oil, which is considered to be a good solvent of vitamin D, could improve calcium concentrations in eggshells. Vitamin D is known to be a fat-soluble vitamin and has a direct effect on calcium absorption. On the other hand, it has been previously documented that the addition of organic or inorganic sources of combined Zn, Mn and Cu to hens' feed does not significantly influence the amount of eggshell material deposited during eggshell formation but can enhance some mechanical properties like, improved breaking strength and fracture toughness (resistance to fracture) regardless of the source of the trace elements [68]. Olive pulp is regarded as a good source of minerals like Ca, P, Zn Mn and Cu [69]. Thus, it could be supported that the mineral content of OP fed to laying hens could have a positive impact on the eggshell strength of laid eggs, but further research is needed in order to verify this mechanism of action.

Both internal and external egg quality traits were not adversely affected by the incorporation of OP in laying hens' diet. A minor but significant increase of albumen pH was recorded in eggs produced by hens that were fed with 5% and 6% OP compared to CON eggs. Currently, there are no data regarding the dietary effect of OP in albumen pH so the elevation of the corresponding value observed here cannot be explained. The increase in albumen pH has been associated to a loss of CO_2 via eggshell pores [70]. However, all eggs used in our trial for the evaluation of their quality were one day old and they were collected and analyzed at the same day. In general, eggs recently laid have an initial albumen pH of 7.4 to 8.6 [71]. In this study, albumen pH documented in eggs from all dietary treatments was found slightly higher than the reported ranges. From the rest of the quality parameters evaluated in present trial, an increase in yolk diameter was observed in OP4 and OP5 eggs compared to CON eggs which could be due to the numerically higher yolk ratio (%) documented in OP4 and OP5 eggs. Yolk diameter is an important

measurement taken for the calculation of yolk index which provides indication on the freshness of the egg. Despite the recorded increase in yolk diameter observed in OP4 and OP5 eggs, the yolk index of eggs produced by OP-fed layers was similar to that recorded in CON eggs. These findings are in agreement with those obtained by other investigators in relevant feeding trials [17,19,20,22]. However, higher inclusion rates of OP in hens' diet than those used in this study, such as 9% [18] and 10% [21] resulted in an increased yolk index of produced eggs. This improvement of yolk index was attributed to the beneficial effect of the unsaturated fatty acids and polyphenols in the OP used [21].

The results concerning the assessment of dietary effect of OP in egg quality traits of laying hens in available literature are inconsistent. According to some researchers, egg shape index [18–21], Haugh unit (HU) [18,20–22], yolk color [2,20–22], shell thickness [2,18,21], shell weight and ratio % [18,21,22], yolk weight and ratio % [21,22] and albumen weight and ratio % [21,22] were not affected by the incorporation of OP in birds' diet, which is consistent with our findings. On the other hand, Zangeneh and Torki [2] observed that the supplementation of 4.5% and 9% of OP in hens' diet increased the shell weight of produced eggs, whereas hens receiving 9% OP laid eggs with a decreased HU compared to the controls and to those fed diets containing 4.5% OP. Increased shell weight and shell ratio (%) of produced eggs was also reported in the study of Al-Harthi and Attia [22] after the supplementation of hens' diet with 10% OP. Contrary to our results, decreased shell thickness by 7.97%, 5.83% and 10.50% has been documented in eggs produced by laying hens that were fed with higher levels of olive cake, 8%, 12% and 16%, respectively [1]. Moreover, previous feeding trials have shown that when OP is incorporated into layers' diet at even higher inclusion rates (16–20%) the produced eggs presented a lower shape index [17,22], decreased HU, paler yolk color as well as reduced shell weight and shell thickness compared to controls [17,19].

The current study revealed that the addition of OP in hens' diet has no adverse effect on the birds' liver and kidney function, as indicated from the evaluation of the biochemical parameters selected. The higher serum uric acid concentration recorded to the layers that consumed diets containing OP at the rate of 3%, 5% and 6% compared to the CON hens is of no clinical significance since it remained within the reported normal ranges of 2–7 mg/dL for this parameter [72]. In accordance with our results, other authors also did not notice any dietary impact of OP on hens' serum concentration of cholesterol [18], triglycerides [19] or both [2,20]. However, other researchers observed a reduction in the serum concentration of cholesterol [19], triglycerides [18] or both [1]. Consistent with our findings, Al-Harthi et al. [21] observed no negative effect in hens' liver function after the supplementation of their diet with 10% and 20% of olive cake. However, AST serum concentration has been shown to increase when OP was incorporated in hens that were fed diets at higher rates (16–17%) compared to controls and to those receiving lower levels of OP [1,17].

The variability of our results regarding the dietary effect of OP in laying hens' performance, egg quality characteristics and health parameters evaluated with those previously recorded in similar feeding trials, could be possibly attributed to differences in the composition of OP and diets used, the inclusion rate and the hens' age and hybrid.

The present investigation revealed a positive dietary effect of OP on KBD at an inclusion level of 3%, as hens of the OP3 group presented the lowest KBD incidence rate among all experimental groups. This finding is highly valued since KBD is a well-recognized health and welfare issue of the modern poultry sector, with a high prevalence in commercial laying hens globally [73,74], that has been shown to cause stress in birds, reduce their productivity and compromise egg quality, posing financial concerns for producers [75–77]. The bone demineralization process seems to play a key role to the pathogenesis of keel fractures [78]. Recent research evidence indicated that keel bone fractures are associated with differences in the concentrations and activities of bone metabolism-related indexes, as well as bone mineral density in laying hens [79] and thus abnormal bone metabolism could be a causative factor of KBD [80]. The exact mechanism implicated in the recorded decrement

of KBD incidence in OP3 hens is currently unknown and needs further investigation since there are not similar studies in the available literature. Possibly, the PUFA and phenolic compounds of OP used in the present trial could play a role. Evidence presented over the past 20 years has shown that long chain polyunsaturated fatty acids (LCPUFAs) are beneficial to bone health [81]. Similar action has been attributed to dietary polyphenols [82]. In particular, the phenolic compounds in olive oil have been shown to possess antioxidant properties in vivo and in vitro and influence bone mineral density by acting as free radicals, preventing oxidation-induced damage to bone cells [83,84]. Moreover, a recent work carried out in ovariectomized rats fed with 100 μL and 200 μL/day of olive oil for a period of 3 months revealed an improvement of their bones' biomechanical parameters [85].

Regarding the rest of the welfare parameters evaluated, it was shown that OP improved belly plumage condition of hens fed with 4–6% incorporation rates compared to controls. The feather loss in this body area has been linked to abrasion in housing equipment like perches [86]. Moreover, those hens presented the lowest incidence of score 2 compared to controls, as indicated from the total plumage evaluation. No welfare issues in respect of comb abnormalities, skin lesions, foot pad dermatitis and toe damage were recorded in the present trial. Finally, it was shown that feeding laying hens with OP at a percentage of 3% or more increases the length of their claws. The results regarding belly feather condition and claw length observed in this study could be attributed to the skin health beneficial effect of the bioactive compounds of OP, such as PUFA and polyphenols. Oils rich in essential fatty acids has been shown to improve skin hydration, have a regenerative effect on the damaged epidermal lipid barrier and regulate skin metabolism [87]. Both omega -3 and omega -6 fatty acids are important cell membrane components, essential for the function of epidermal barrier; they exhibit anti-inflammatory and anti-allergic effects, enhance repair processes and soothe irritation [87]. Plant polyphenols are considered as important substances for skin function, with hydrating, smoothing and softening effects [88–90]. Additionally, they soothe irritation and reduce the redness of skin, accelerating the natural regeneration of the epidermis, stabilizing the capillaries, improving microcirculation and elasticity in the skin and protecting against harmful external factors [87]. However, since no data are currently available regarding the dietary effect of OP in feather condition and claw length of laying hens, the results observed here need further investigation.

5. Conclusions

The current study revealed that feeding laying hens a diet with dried OP increased the percentage of PUFA in eggs, decreased that of SFA and improved the PUFA to SFA ratio in a manner proportional to the inclusion rate in the hens' diet. Moreover, an amelioration of health lipid indices was recorded in a dose-depended way, as indicated by the decrease of AI and TI and the increase in the h/H ratio of produced eggs. These observations demonstrate the potential health benefits associated with the fat intake from eggs produced from OP-fed layers, especially those fed with higher inclusion rates, since they presented higher nutrition quality than the control conventional eggs. Olive pulp-fed layers presented 15–58% lower percentage of broken eggshells compared to controls indicating a potential cost benefit from the use of OP. The addition of OP in laying hens' diet for a long period of time did not adversely affect the birds' performance, internal and external egg quality traits or health and welfare parameters evaluated. A positive impact on KBD incidence and belly plumage damage was shown to be possibly due to the bone and skin health beneficial effects of the OP bioactive compounds like PUFA and polyphenols, however further investigation is necessary to verify the exact mechanism implicated in those results. Finally, from the evaluated dietary levels of OP, 5% and 6% seems to be more advantageous for the consumer in terms of egg nutrition quality so they are highly recommended.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14063157/s1, Table S1: Nutritional analysis of Layer concentrate 25%.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

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