Evaluation of olive pomace in the production of novel broilers with enhanced \textit{in vitro} antithrombotic properties\textsuperscript{†}

\textbf{Running Title:} Broilers fed experimental diets enriched with olive pomace.

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\textbf{Keywords:} Functional broiler meat/ Olive pomace/ Antithrombotic activity/ Sensory evaluation

\textbf{Abbreviations:} CSFF, commercial standard finisher feed, CSSF, commercial standard starter feed
DHA, docosahexanoic acid, EPA, eicosapentanoic acid, \textit{EC}\textsubscript{50}, equivalent concentration for 50\% aggregation,
EFF, experimental finisher feed, ESF, experimental starter feed, FCR, feed conversion ratio, OP, olive pomace,
PAF, platelet activating factor, PPP, platelet poor plasma, PRP, platelet rich plasma, TL, total lipids,
TNL, total neutral lipids, TPL, total polar lipids

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Abstract

Several attempts have been made not only to improve nutritional value of broilers but also to attenuate dependence on raw materials such as corn in compounded broilers feed. Therefore the aim of this study was to evaluate the impact of diets enriched with olive pomace (OP) on Ross 308 broilers growth performance, sensory characteristics and nutritional value in terms of cardioprotection. Broilers were fed four experimental diets containing 0 % OP (control group), 2.5 % OP (group A), 5.0 % OP (group B) and 7.5 % OP (group C). The obtained broiler meat samples were evaluated for their lipid and phenol content and their in vitro antithrombotic properties according to biological assay in human platelets. Groups B and C exhibited significantly increased ($P < 0.05$) growth rate compared to the ones of control group. Additionally group B exhibited significantly more potent ($P < 0.05$) in vitro antithrombotic properties ($EC_{50} = 10.5\pm0.92$) compared to the ones of control group ($EC_{50} = 420\pm21.3$). Grilled broiler meat of group B was found to have acceptable sensory properties. The overall conclusion of this paper is the potential use of OP in compounded broilers feed in the production of functional broilers meat.

Practical applications

The objective of this research is to assess the use of olive industry by-products as functional feed ingredients. For this purpose, broilers were fed experimental diets containing olive pomace (OP). Our results suggest that OP can be used in broiler feed to produce functional broilers meat with increased in vitro antithrombotic properties. These scientific data could have considerable practical value towards the valorisation of OP and increasing the sustainable production of functional broiler meat and therefore the overall food security.
1 Introduction

According to Food and Agriculture Organization (FAO) Food Outlook Biannual Report on Global Food Markets of 2014, poultry remains the main product traded, representing 43% of the total traded meat, followed by bovine, pig and ovine meat, respectively [1]. Poultry meat is distinguished for its low energy concentration and high nutrient density. Poultry meat is a valuable dietary source of proteins of high biological value due to the fact that it contains essential amino acids. Additionally poultry meat has a significant content of water-soluble vitamins B such as thiamin, riboflavin, niacin, vitamin B₆ and B₁₂, along with minerals like iron, zinc and copper of high bioavailability [2]. Poultry meat has been found to contain lower levels of saturated fat and more importantly higher polyunsaturated fatty acid contents than bovine, pork and lamb meat [2, 3].

Given that current nutritional recommendations include the reduction of saturated fat consumption in order to prevent the cardiovascular risk, poultry meat is a good dietary source for a healthier diet. Over the last two decades several attempts have been made in order not only to improve nutritional value of broilers but also to attenuate the limited supply of good quality raw materials such as corn [4, 5] and soybean [6] for the poultry feed industry, which resulted in a continuous increase in the production cost. These attempts were focused on poultry feed enrichment with components of either plant origin such as chia seeds [7], palm kernel [8] and herbs [9], or animal origin such as fermentatively recovered fish oil from fish visceral [10]. The type of fat used in formulating broiler diets has an impact on growth performance, the fatty acid composition and nutritional value of poultry meat [11]. Chia seeds were used as an ω-3 fatty acid dietary source for broilers which resulted in significantly lower levels of saturated fatty acids as well as lower ratios of saturated : polyunsaturated fatty acids and ω-6 : ω-3 of the broilers meat compared to the control diet [7]. Furthermore substituting 25% of soybean meal with palm kernel meal in growing cockerel diets has been found to induce the highest growth performance and the minimum production cost, suggesting the potentiality of palm kernel usage in compounded poultry feeds [8]. Thyme oil inclusion in female broilers diet exhibited the most positive effects on chick performance (body mass and feed consumption) [9]. The dietary inclusion of fish oil derived from fish by-products reduced the levels of total cholesterol in broilers meat and increased the levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in the carcass of broilers; these results demonstrated the nutritional potential of fish oil derived from fish by-products but also addressed the environmental issues related to disposal of such biological waste [10]. Olive pomace (OP) is one of the by-products of the olive oil extraction when using the three-phase centrifugal technology [12]. OP contains micro constituents with atheroprotective activity, such as Platelet Activating Factor (PAF)-inhibitors and phenolic/polyphenolic molecules with antioxidant and other pleiotropic actions [13]. Additionally OP polar lipids possess in vivo antiatherogenic properties [14]. There are limited scientific data on broilers compounded feed enrichment with olive oil production by-products such as olive pulp [15], whereas OP inclusion in animal feeds (such as lamb, goat and rabbit feeds) [16], cattle feeds [17], swine feeds [18] and fish feeds [19] is more common. Taken into consideration the functional
properties of OP, the aim of the present study was to expand our previous work on olive pomace and fish feeds [19] and to evaluate the potential dietary inclusion of OP in broiler diet by assessing broilers growth performance and the nutritional value of the obtained meat by assessing its in vitro antithrombotic properties and organoleptic characteristics.

2 Materials and methods

2.1 Broiler housing and diet formulation

The feeding trial was undertaken with Ross 308 broilers. The Ross 308 is a robust, fast growing, feed efficient broiler with good meat yield. Living conditions and broiler housing were in accordance to European Regulation 609/86 while the feeding trial was approved by local veterinary authorities and animal ethics committee. 400 broilers were distributed randomly into four groups of 100 broilers each, in an independent commercial facility located in Sparta, Greece. The broilers were housed in controlled temperature set at 28-30°C, while the relative humidity was 60-70% and the airflow was controlled by ventilation systems. Broilers were provided with 1-4 hours of darkness following a period of 20-23 hours of light per day, throughout the feeding trial, which was held for 42 days. On 43rd day, broilers were slaughtered, using the electrical water bath stunning method, and stored at 4°C for a few hours. At the end of the experiment, all broilers were individually weighed for growth performance determination. 50 broilers were randomly collected from each dietary treatment group, and 10 of them provided the broiler meat samples for the sensory, lipid and phenolic analysis and also the evaluation of their in vitro antithrombotic properties.

Feed supplementation plan was designed to meet the recommendations to achieve optimum growth performance. All four dietary treatment groups (control group and three experimental groups) were provided from day 1 to day 10 with commercial standard starter feed, containing 0% OP. Then the control group was further provided with commercial standard feed until day 24 following by the commercial standard finisher feed until the broilers reached 42 day of age, while the rest three experimental groups - were provided from day 11 to day 24 - with experimental starter feed which contained 2.5% OP. From day 25 to day 42, three experimental finisher feeds were provided to the respective three experimental dietary treatment groups containing 2.5% OP (group A; diet A), 5.0% OP (group B; diet B) and 7.5% OP (group C; diet C), respectively. The chemical composition of OP used for the three experimental broiler feeds formulation is shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Dietary Treatment</th>
<th>OP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Group A</td>
<td>2.5</td>
</tr>
<tr>
<td>Group B</td>
<td>5.0</td>
</tr>
<tr>
<td>Group C</td>
<td>7.5</td>
</tr>
</tbody>
</table>

The chemical composition of each experimental diet is shown in Table 2.

Table 2

The reagents, solvents and silica gel G-60 were supplied from Merck (Darmstadt, Germany). Bovine serum albumin, PAF and the fatty acid methyl ester standards were supplied by Sigma-Aldrich (St. Louis, MO, USA). Platelet aggregation was measured in a Chrono-Log (Havertown, PA, USA) aggregometer (model 400-VS) coupled to a Chrono-Log recorder (Havertown, PA, USA).

2.3 Isolation of total lipids, total polar and total neutral lipids of broilers meat

Total lipids (TL) were extracted from 100 g of broiler fillets from each dietary treatment according to the Bligh-Dyer method [20]. TL were further separated into total polar lipids (TPL) and total neutral lipids (TNL) using the counter-current distribution method [21]. Countercurrent distribution chromatography allows excellent recovery of TPL from TNL, while the obtained TPL contain glycolipids and phospholipids. TPL were weighted and kept at 4°C for further analysis. The procedure was carried out in triplicates.

2.4 In vitro biological assay on human platelet rich plasma (PRP)

The in vitro biological activity of TPL of broiler meat samples was evaluated on human platelet rich plasma (PRP) against 2.5 x 10⁻⁶ M PAF (final concentration in the cuvette). Fasting blood
samples were obtained from healthy volunteers (n=6) via venepuncture from the median cubital vein using a 20G needle (Sarstedt, Nümbrecht, Germany) attached to S-monovettes containing 0.106 mol/L trisodium citrate solution in a 1:10 ratio of citrate to blood (Sarstedt, Nümbrecht, Germany). Blood was centrifuged at 180 x g for 15 minutes at 24°C to obtain the supernatant platelet rich plasma (PRP). The lower phase was centrifuged at 1,465 x g for 20 minutes at 24°C to obtain the platelet poor plasma (PPP). PRP was standardised to 5.0 x 10^6 platelets mL^-1, by diluting with PPP prior to analysis on a Chronolog-490 two channel platelet aggregometer (Havertown, PA, USA) using the PPP as a blank. The EC50 value, namely equivalent concentration for 50% aggregation, was estimated for each biologically active broiler meat sample, as described elsewhere [19].

2.5 Determination of total phenolics

An amount of 1 g dry meat sample was extracted twice with 20 mL of 80% aqueous methanol as described by Kähkönen et al. [22] combined supernatants were evaporated to a volume of about 1.0 ml. The total phenol content was measured using the Folin-Ciocalteu assay and gallic acid was used as a reference standard for plotting calibration curve [22]. A volume of 0.1 ml of the meat extracts were mixed with 1.0 ml Folin-Ciocalteu reagent (diluted 1:10 with distilled water). The solution was kept at dark for 5 min and then 1.0 ml sodium carbonate (7.5% w/v) was added, prior to neutralization. The reaction mixture was incubated at room temperature at dark for 1 hour for color development. The absorption of the developed blue color was measured at 765 nm using spectrometer UV-vis (Shimadzu V-1601). The results were expressed in mg gallic acid per g of dry sample. Each assay was carried out in triplicate.

2.6 Grilling procedure and sensory evaluation

The assessors’ training regarding the identification and evaluation of: a) the attributes taste, flavour, aftertaste and odour and b) the basic senses of taste and flavour (namely sweet, salty, bitter and sour) of the grilled broiler fillets was carried out as described elsewhere [23]. A panel of ten assessors (five females and five males), aged 21 - 29 years, from the University of Athens participated in the sensory evaluation. Two samples of conventional diet grilled broiler breast fillets and conventional diet grilled broiler thigh fillets, different from the ones used for the analysis of the present study, were used as a frame of reference in order to develop terminology based on the attribute differences [23]. Broiler fillets of breast and thigh were grilled for ten minutes on each side at 217°C in a Black and Decker contact griller (Black and Decker, Beachwood, 100 OH, USA). Portions of approximately 100 g of grilled broiler samples were provided to each assessor and assessed as described elsewhere [23]. The sensory evaluation of all attributes was carried out using twenty two attributes: seven for taste (namely, grilled chicken, grilled fat of chicken, fatty, sweety, salty, rich, delicious), eight for aftertaste (namely, grilled chicken, fatty, sweety, salty, astringent, rich, oily, lasting), and seven for odour (namely, grilled chicken, grilled fat of chicken, chicken fat, odorous, oily, animal fat, fatty odour). The assessors scored the samples of grilled chicken using values between zero (0, least liked) and ten (10, most liked) for attributes describing taste, aftertaste and odour properties.

2.7. Statistical analysis

The experimental analysis was carried out in triplicate and the results were expressed as mean value ± SD. The statistical analysis was carried out using one-way analysis of variance (ANOVA) and the differences were considered to be statistically significant when P was less than 0.05. The data were analysed using a statistical software package (PASW 18 for Windows, SPSS Inc., Chicago, 215 IL, USA).

3 Results and discussion

3.1 Growth performance of broiler samples

Feed supplementation was designed to meet recommendations of the National Research Council to achieve optimum growth and performance. Growth performance factors of broilers fed control
diet (0% OP), diet A (2.5 % OP), diet B (5.0 % OP) and diet C (7.5 % OP) are shown in Table 3.

Groups B and C (broilers fed experimental diets B and C containing 5.0 % and 7.5 % OP, respectively) have been found to exhibit significant lower (P < 0.05) mortality levels than control group (Table 3). Moreover, feed intake and feed conversion ratio (FCR) of groups B and C have been found to be significantly lower (P < 0.05) when compared to control group, while growth rate has been found to be significantly increased (P < 0.05) in groups B and C compared to control (Table 3). These findings are rather promising highlighting the potential of OP as functional feed ingredient in the future development of functional broiler feeds.

Table 3

### 3.2 Phenolic and lipid contents of broiler samples

The levels of total phenolics in broiler samples are given in Table 4. The inclusion of OP in the experimental diets has resulted in statistically significant increased levels of gallic acid (expressed as mg/g of dry sample) in broilers of group B compared to control group (Table 4). This result could be attributed to the fact that OP - a natural by-product of olive oil production - has increased total phenolic content [13]. Moreover total phenolics possess antibacterial properties [13], protecting broilers from bacteria and prolonging shelf-life.

TL, TPL and TNL contents of all broiler groups expressed as g / (100 g chicken tissue) are given in Table 4. Broilers fed the experimental diets B and C were found to contain significantly higher amounts (P < 0.05) of TL when compared to the ones fed control diet (Table 4). A similar trend to the one of TL of broilers of groups B and C was found for TPL and TNL; the levels of both lipid classes have been found to be significantly increased (P < 0.05) compared to control group (Table 4).

Table 4

### 3.3 Biological activity of TPL of broiler samples

TPL of broilers fed control diet (0% OP), diet A (2.5 % OP), diet B (5.0 % OP) and diet C (7.5 % OP) were examined for their ability to induce washed rabbit platelet aggregation or inhibit the PAF-induced platelet aggregation. The tested samples were found to exhibit only aggregatory effect, therefore the EC50 values were calculated, expressed as μg of EC50 (Table 5).

Table 5

The aggregatory activities of TPL suggest the presence of PAF agonists, i.e. lipid microconstituents that antagonise PAF in binding to the PAF receptors and therefore preventing PAF’s action to cause platelet aggregation.

Given that the lower the EC50 value of a lipid sample is the stronger PAF agonistic activities this sample contains; the aggregatory activities of TPL of groups A and B were found to be significantly more potent (P < 0.05) compared to the aggregatory activities of TPL of control group (Table 5). Therefore OP inclusion in the experimental diets of broilers has resulted in a statistically significant decrease of EC50 values which corresponds to more potent in vitro antithrombotic properties of broilers of groups A and B (Table 5). Group B was found to exert the strongest (P < 0.05) aggregatory activities (Table 5), which could be attributed a) to the increased levels of TPL of group B since it is the polar lipid fraction in foods that exhibit strong in vitro antithrombotic activity of fish tissue [24] and b) to OP inclusion in the experimental diet. It could be thus suggested that the experimental diet B has been shown as the most beneficial diet to increasing the nutritional value of broilers in terms of antithrombotic properties.

These findings are in accordance with previous results of our team where OP inclusion in experimental fish diets of gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) enriched in vitro antithrombotic activity of fish tissue [19].

The beneficial activity of OP could be due to the fact that this by-product of olive oil production contains biological active lipid micro-constituents with both in vitro antithrombotic and in vivo anti-atherosclerotic properties [14]. The structure and the mechanism of action of these microconstituents has not yet been clarified however previous study of our team regarding the structural elucidation of polar lipids obtained from olive pomace fed sea bass showed that the improved in
*in vitro* antithrombotic properties could be attributed to the presence of diacyl-glycerophospholipids species [25]. Olive pomace’s potential as a functional feed ingredient brings a double positive effect: on one hand, it is a by-product of olive industry of low cost but high environmental impact and on the other hand it is highly bioactive against inflammation [16]. The valorization of OP should enable chicken industry to have access to a sustainable olive industry by-product. By using OP, the production of broilers could be regarded as environmentally friendly and sustainable but also the final product would have improved *in vitro* antithrombotic properties. Our past research in aquaculture species has demonstrated the functional potential of OP in fish feeds [19] and this potential of OP is demonstrated, with this paper, for broilers, as well.

### 3.4 Sensory analysis

The aim of this analysis was to detect possible sensory differences between grilled breast and thigh meat of broilers of control group (marked as “conventional broiler”) and of group B (marked as “enriched broiler”) which was found to be the most biologically active group. Grilling cooking method was chosen since it is one of the most popular cooking methods with minimal impact on overall flavour, texture, acceptability of the cooked meat and its *in vitro* anti-thrombotic properties of lipids as shown previously [26].

The panelists scored the grilled broiler samples using values between zero (0) and ten (10) (the lowest “0”, the highest “10”) for attributes describing taste, aftertaste and odour characteristics. The sensory evaluation of grilled broiler samples in terms of taste, aftertaste and odour are given in Fig. 1a and 1b, where the scores for the different attributes of each sample are given in the form of spider-web plots.

Fig. 1a

Fig. 1b

The assessors indicated significant organoleptic differences between control and group B. Grilled breast meat of group B broilers exhibited higher scores for all taste and odour attributes when compared to grilled breast meat of control group broilers (Fig. 1a). Grilled breast meat of enriched broilers fed diet B were found to be fattier, sweeter, saltier, richer, more delicious and of more intensive grilled taste compared to grilled breast meat of control group (conventional broiler) (Fig. 1a). Furthermore, grilled breast meat of enriched broilers fed diet B were found to have fattier, sweeter, saltier, richer, oilier and more grilled aftertaste than grilled breast meat of control group (Fig. 1a); while grilled breast meat of control broilers was found to exhibit more intensive metallic and astringent aftertaste attributes (Fig. 1a). The odour attributes oily, odorous, fatty odour, spicy and grilled chicken have been found to score higher for the grilled breast meat of enriched broilers fed diet B when compared to the ones of control group (Fig. 1a). Regarding the grilled thigh meat, broilers of group B have been found to exhibit higher scores for almost all taste, aftertaste and odour attributes when compared to the ones of control group broilers (Fig. 1b). Grilled thigh meat of enriched broilers fed diet B were found to exert fattier, sweeter, saltier, richer, more delicious and more grilled taste than the respective taste attributes of grilled thigh meat of control broilers (conventional broilers) (Fig. 1b). Grilled thigh meat of enriched broilers fed diet B were found to have fattier, sweeter, saltier, richer, oilier, more lasting and more grilled aftertaste than grilled thigh meat of control group (Fig. 1b), while grilled thigh meat of control group broilers were found to be more astringent than grilled thigh meat of group B broilers (Fig. 1b). Grilled thigh meat of group B broilers scored higher in all odour attributes compared to the ones of control group (Fig. 1b).

These sensory data suggest that the inclusion of OP in the broilers diet has improved the sensory properties of the final product, especially in the case of grilled thighs where the enriched broiler has been found to have stronger “sweet” taste, “rich” aftertaste and “grilled chicken” odour. It could be thus deduced that the inclusion of OP has improved broilers sensory characteristics. Past research has shown that OP can improve the sensory properties of Awassi sheep [27]. To the best of our knowledge, this is the first report on the effect of OP to the sensory properties of broilers.
4 Conclusions

In conclusion this paper indicates that the experimental diets B and C which were enriched with 5.0% and 7.5% OP respectively, exhibited satisfactory growth performance factors for broiler Ross 308 at the end of 42nd growth day, along with low mortality. TPL of group B broilers demonstrated the strongest in vitro antithrombotic ability, indicating that OP has the potential to enrich the in vitro cardioprotective properties of broiler. Furthermore, regarding the organoleptic characteristics of the two examined samples (control and group B), group B exhibited higher scores for almost all the attributes of taste, aftertaste and odour when compared to control group. These results indicate that 5.0% OP inclusion in broilers feed contributes to a broiler product formulation (breast and thigh meat) with enriched in vitro antithrombotic properties and also more appealing sensory profile. Our current research efforts are towards the structural elucidation of the cardioprotective lipids in broiler TPL fraction.

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The authors have declared no conflict of interest.

References


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**Tables**

**Table 1. Chemical composition of olive pomace (OP).**

<p>| | |</p>
<table>
<thead>
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<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Energy (Kcal/100g)</strong></td>
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</tr>
<tr>
<td><strong>Fat (g/100g)</strong></td>
<td>18.3</td>
</tr>
<tr>
<td><strong>Carbohydrates (total) (g/100g)</strong></td>
<td>58.9</td>
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<tr>
<td><strong>Proteins (g/100g)</strong></td>
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<td><strong>Moisture (g/100g)</strong></td>
<td>5.9</td>
</tr>
<tr>
<td><strong>Ash (total) (g/100g)</strong></td>
<td>7.1</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Control Diet</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Crude fiber (g/100g)</td>
<td>21.5</td>
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<tr>
<td>Total phenolics (mg/kg)</td>
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</tr>
<tr>
<td>Calcium (Ca) (mg/kg)</td>
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<td>Phosphorus (P) (mg/kg)</td>
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<td>Magnesium (Mg) (mg/kg)</td>
<td>785</td>
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<tr>
<td>Iron (Fe) (mg/kg)</td>
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<tr>
<td>Lysine (%)</td>
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<td>Threonine (%)</td>
<td>0.49</td>
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<td>NaCl (g/100g)</td>
<td>0.46</td>
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Table 2. Chemical composition of control diet and three experimental diets.

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Diet A</th>
<th>Diet B</th>
<th>Diet C</th>
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<tbody>
<tr>
<td>CSSF Energy (kcal/g)</td>
<td>347.1</td>
<td>349.4</td>
<td></td>
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<tr>
<td>CSFF Fat (g/100g)</td>
<td>4.6</td>
<td>2.4</td>
<td>4.6</td>
<td>3.9</td>
</tr>
<tr>
<td>CSSF Total Carbohydrates (g/100g)</td>
<td>55.5</td>
<td>60.1</td>
<td>55.5</td>
<td>52.5</td>
</tr>
<tr>
<td>CSSF Proteins (g/100g)</td>
<td>22.5</td>
<td>19.8</td>
<td>22.5</td>
<td>17.5</td>
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<td>CSSF Moisture (g/100g)</td>
<td>10.1</td>
<td>9.91</td>
<td>10.1</td>
<td>10.79</td>
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<tr>
<td>EFF Ash (g/100g)</td>
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<td>4.1</td>
<td>4.0</td>
<td>3.8</td>
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<tr>
<td>CSSF Crude Fiber (g/100g)</td>
<td>3.5</td>
<td>8.0</td>
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<td>5.7</td>
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<tr>
<td>CSSF Total phenolics (mg/kg)</td>
<td>691</td>
<td>724</td>
<td>691</td>
<td>855</td>
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</tbody>
</table>

CSSF: commercial standard starter feed
CSFF: commercial standard finisher feed
ESF: experimental starter feed
EFF: experimental finisher feed
Control diet: 0% olive pomace
Diet A: 2.5 % olive pomace
Diet B: 5.0 % olive pomace
Diet C: 7.5 % olive pomace

Table 3. Growth performance factors (mean ± SD, n=3) of broilers fed control diet (control group), diet A (group A), diet B (group B) and diet C (group C).

<table>
<thead>
<tr>
<th></th>
<th>Mortality</th>
<th>Feed Intake (Kg)</th>
<th>Growth Rate (Kg)</th>
<th>FCR*</th>
</tr>
</thead>
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<tr>
<td>Control group</td>
<td>4.20 ± 0.21&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.49 ± 0.22&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.04 ± 0.10&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.20 ± 0.12&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>
Table 4. Contents of total phenolics (mean ±SD, n=3), expressed in mg gallic acid per g of dry sample (ds) and total lipids (TL), total polar lipids (TPL) and total neutral lipids (TNL) of broilers fed control diet (control group), diet A (group A), diet B (group B) and diet C (group C) expressed in g per 100g of sample.

<table>
<thead>
<tr>
<th></th>
<th>mg gallic acid/g ds</th>
<th>g TL/100g sample</th>
<th>g TPL/100g sample</th>
<th>g TNL/100g sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.26±0.01</td>
<td>3.08 ± 0.15</td>
<td>0.85 ± 0.05</td>
<td>2.24 ± 0.11</td>
</tr>
<tr>
<td>Group A</td>
<td>0.29±0.02</td>
<td>3.43 ± 0.20</td>
<td>0.78 ± 0.02</td>
<td>2.15 ± 0.11</td>
</tr>
<tr>
<td>Group B</td>
<td>0.29±0.01</td>
<td>5.89 ± 0.29</td>
<td>1.14 ± 0.06</td>
<td>4.47 ± 0.22</td>
</tr>
<tr>
<td>Group C</td>
<td>0.28±0.01</td>
<td>5.27 ± 0.26</td>
<td>1.33 ± 0.02</td>
<td>4.74 ± 0.24</td>
</tr>
</tbody>
</table>

Control diet: 0 % olive pomace
Diet A: 2.5 % olive pomace
Diet B: 5.0 % olive pomace
Diet C: 7.5 % olive pomace

* Indicate significant differences among different diets (control diet vs. diet B and control diet vs. diet C, respectively; P < 0.05) according to Anova analysis.

Table 5. EC50 values expressed in μg of total polar lipids (TPL) of broilers fed control diet (control group), diet A (group A), diet B (group B) and diet C (group C).

<table>
<thead>
<tr>
<th></th>
<th>EC50 (μg)</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>420±21.3</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Group A</td>
<td>122±6.08</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Group B</td>
<td>10.5±0.92</td>
<td>Aggregation</td>
</tr>
<tr>
<td>Group C</td>
<td>450±33.2</td>
<td>Aggregation</td>
</tr>
</tbody>
</table>

Control diet: 0 % olive pomace
Diet A: 2.5 % olive pomace

* Indicate significant differences among different diets (control diet vs. diet B and control diet vs. diet C, respectively; P < 0.05) according to Anova analysis.
Diet B: 5.0 % olive pomace
Diet C: 7.5 % olive pomace

\textsuperscript{a,b} Indicate significant differences among different diets (control diet vs. diet A, control diet vs. diet B and diet A vs. diet B respectively; $P < 0.05$) according to Anova analysis.
Figure 1a. Attributes of taste, aftertaste and odour of grilled breast meat of conventional broiler (control group) and enriched broiler fed diet B (group B).
Figure 1b. Attributes of taste, aftertaste and odour of grilled thigh meat of conventional broiler (control group) and enriched broiler fed diet B (group B).