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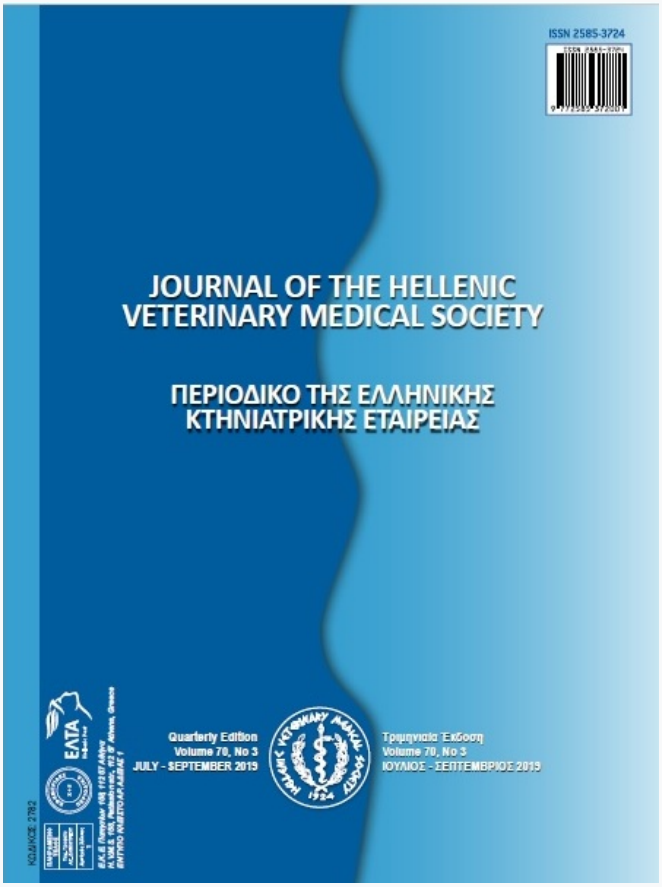

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Effects of olive pulp addition to broiler diets on performance, selected biochemical parameters and antioxidant enzymes

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Επίδραση της προσθήκης πάστας ελαιόκαρπου στο σιτηρέσιο ορνιθίων κρεοπαραγωγής στην ανάπτυξη, επιλεγμένες βιοχημικές παραμέτρους και αντιοξειδωτικά ένζυμα

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ABSTRACT. Olive oil production generates various by-products that can be used in animal nutrition. These by-products contain several polyphenolic compounds that may exhibit antioxidant properties. The present study was designed to evaluate the effects of adding olive pulp to the feed on broiler performance, carcass yield and antioxidant enzymes. Two hundred (200), as hatched, day-old, Cobb 500 broilers were reared in total for 42 days. There were 4 dietary treatments. In T1 treatment, no olive pulp was added to starter, grower and finisher diet. In T2 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 2.5 and 5% respectively. In T3 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 5% respectively. In T4 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 8% respectively. Performance, carcass yield and a number of biochemical parameters were examined. Oleuropein and hydroxytyrosol were present in the olive pulp at 952 and 216 mg/kg respectively. Broilers performed well and no differences were observed between treatments on final body weight, carcass yield, total antioxidant activity and expression of selected antioxidant enzymes. Discriminant analysis was further applied and revealed that samples clustered according to added level of olive pulp. Samples from broilers fed T2 and T3 diet were located in the middle of the plot away from other treatments exhibiting high values of carcass, breast yield and most of the antioxidant enzyme activities. In conclusion, olive pulp can be used up to 5% in diets of broilers and future studies conducted on-farm conditions may pronounce its impact on growth performance and antioxidant potential.

Keywords: antioxidant, broilers, hydroxytyrosol, oleuropein, olive pulp

ΠΕΡΙΛΗΨΗ. Η παραγωγή ελαιόλαδου δημιουργεί ορισμένα υποπροϊόντα τα οποία μπορούν να χρησιμοποιηθούν στη διατροφή των ζώων. Αυτά τα υποπροϊόντα περιέχουν αρκετές πολυφαινολικές ενώσεις που ενδέχεται να παρουσιάζουν αντιοξειδωτικές ιδιότητες. Η παρούσα μελέτη σχεδιάστηκε για να αξιολογήσει την επίδραση της προσθήκης πάστας ελαιόκαρπου στο σιτηρέσιο ορνιθίων κρεοπαραγωγής στην ανάπτυξη, στην απόδοση σε σφάγιο και στην ενεργότητα ορισμένων αντιοξειδωτικών ενζύμων. Διακόσιοι (200), νεοσσοί κρεοπαραγωγής Cobb 500, ηλικίας μιας ημέρας, εκτράφηκαν για συνολικά 42 ημέρες. Υπήρξαν 4 διατροφικές επεμβάσεις. Στην επέμβαση Τ1 δεν προστέθηκε πάστα ελαιόκαρπου στο εναρκτήριο, ανάπτυξης και τελικό σιτηρέσιο. Στην επέμβαση Τ2, η πάστα ελαιόκαρπου προστέθηκε στο εναρκτήριο, ανάπτυξης και τελικό σε επίπεδο 0, 2,5 και 5% αντιστοίχως. Στην επέμβαση Τ3, η πάστα ελαιόκαρπου προστέθηκε στα τρία σιτηρέσια σε επίπεδο 0, 5 και 5% αντιστοίχως, ενώ στην επέμβαση Τ4 σε επίπεδο 0, 5 και 8% αντιστοίχως. Μελετήθηκε η ανάπτυξη, η απόδοση σε σφάγιο και ένας αριθμός βιοχημικών παραμέτρων. Η ολευρωπεΐνη και η υδροξυτυροσόλη ανιχνεύτηκαν στην πάστα ελαιόκαρπου σε επίπεδα 952 και 216 mg/kg αντιστοίχως. Τα ορνίθια αναπτύχθηκαν καλά και δεν παρατηρήθηκαν διαφορές μεταξύ των επεμβάσεων στο τελικό σωματικό βάρος, την απόδοση σε σφάγιο, την ολική αντιοξειδωτική ικανότητα και την ενεργότητα των αντιοξειδωτικών ενζύμων. Εφαρμογή της διακριτικής ανάλυσης έδειξε ότι τα δείγματα διακρίνονται βάση του επιπέδου προσθήκης πάστας ελαιόκαρπου. Τα δείγματα των επεμβάσεων Τ2 και Τ3 εντοπίστηκαν στο κέντρο του διαγράμματος απομακρυσμένα από τις άλλες επεμβάσεις παρουσιάζοντας υψηλές τιμές σε απόδοση σε σφάγιο, αναλογία βάρους στήθους προς σωματικό βάρος και ενεργότητα των περισσοτέρων αντιοξειδωτικών ενζύμων. Συμπερασματικά, η πάστα ελαιόκαρπου μπορεί να χρησιμοποιηθεί στα σιτηρέσια ορνιθίων κρεοπαραγωγής έως 5% και μελλοντικές μελέτες σε πραγματικές συνθήκες εκτροφής ίσως αναδείξουν περαιτέρω τις θετικές επιδράσεις αυτού στην απόδοση και αντιοξειδωτική προστασία των ορνιθίων.

Λέξεις Κλειδιά: αντιοξειδωτικά, ελιά, ολευρωπεΐνη, ορνίθια, πάστα ελαιόκαρπου, υδροξυτυροσόλη

INTRODUCTION

In the Mediterranean area, olive tree (*Olea europaea L.*) is cultivated for the production of table olives and edible olive oil. Long known to many generations that olive oil is an essential component of the healthy Mediterranean diet, it is now widely appreciated by consumers in Europe and many parts of the world for its unique aroma and nutritional properties (Frankel et al., 2013). The European Union is the leading producer of olive oil producing more than two thirds of world production. Four member states, namely Spain, Italy, Greece and Portugal produce 99% of the total EU olive oil production (European Commission, 2017).

Olive oil production generates various by-products that can be used in animal nutrition. By-products of olive oil extraction process include but not limited to crude olive cake, exhausted olive cake, partly destoned olive cake either crude or exhausted, olive pulp, vegetation waters and leaves (Sansoucy, 1985; Heuzé et al., 2015). The fruit of the olive tree (Kalogeropoulos and Tsimidou, 2014) as well as the by-products (Botsoglou et al., 2013; King et al., 2014; Gerasopoulos et al., 2015) contain several antioxidants that can potentially scavenge free radicals and provide antioxidant protection.

Table 1. Composition (g/kg) and calculated analysis of the experimental broiler diets

Ingredients (g/kg)	Starter 0%	Grower 0%	Grower 2.5%	Grower 5%	Finisher 0%	Finisher 5%	Finisher 8%
Maize	622.3	660.2	627.4	600.7	696.1	639.2	607.9
Soybean meal	268.9	234.6	235.1	241.3	184.8	193.1	197.5
Olive pulp	0	0	25.0	50.0	0	50.0	80.0
Gluten	50.0	36.5	43.9	39.9	55.0	55.1	55.4
Soybean oil	12.8	26.0	26.0	26.0	25.0	25.0	25.0
Monocalcium phosphate	13.5	12.4	12.5	12.7	10.6	10.9	11.0
Limestone	15.2	14.3	14.2	14.2	13.0	12.9	12.9
Lysine	5.8	5.5	5.4	5.3	5.1	4.9	2.0
Methionine	2.6	2.5	2.4	2.5	1.8	1.9	1.9
Threonine	0.7	0.6	0.6	0.6	0.3	0.3	0
NaCl	5.7	5.0	5.0	4.4	5.7	4.2	3.9
Premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Calculated Analysis							
ME (MJ/kg)	12.7	13.2	13.1	13.0	13.5	13.3	13.2
CP (g/kg)	217.6	196.1	200.0	200.0	187.2	190.0	190.0
Sodium (g/kg)	2.3	2.0	2.0	1.8	2.3	1.7	1.6
Ca (g/kg)	9.0	8.4	8.4	8.4	7.6	7.6	7.6
Available P (g/kg)	4.5	4.2	4.2	4.2	3.8	3.8	3.8
Lysine (g/kg)	13.2	11.9	11.9	11.9	10.5	10.5	9.0
Methionine+ cysteine (g/kg)	9.8	8.9	8.9	8.9	8.2	8.2	8.2
Threonine (g/kg)	8.6	7.8	7.8	7.8	7.1	7.1	6.8
Arginine (g/kg)	13.8	12.5	12.5	12.5	11.3	11.3	11.3

[1]Premix supplied per kg of diet: 13,000 IU vitamin A (retinyl acetate), 5,000 IU vitamin D_3 (cholecalciferol), 100 mg vitamin E (DL-α-tocopheryl acetate), 4 mg vitamin K_3, 2.6 mg thiamin, 8 mg riboflavin, 3 mg pyridoxine, 0.015 mg vitamin B_{12}, 85 mg nicotinic acid, 22 mg pantothenic acid, 2 mg folic acid, 0.2 mg biotin, 10 mg ascorbic acid, 400 mg choline, 1 mg iodine, 40 mg iron, 120 mg manganese, 20 mg copper, 0.2 mg cobalt, 0.3 mg selenium, 100 mg zinc

In the latter years the contribution of the feed component of total costs for broiler production increased to approximately 70% (Donohue and Cunningham, 2009). Competition for plant sources between food, feed and biofuel producers may intensify the problem (Popp et al., 2014). Therefore, there is a need to successfully adopt a strategy to reduce feeding costs and find alternative, low-cost feedstuffs.

Three issues were taken into account during the design of the present study. Firstly, many of the olive oil's beneficial effects on human health are attributed to the polyphenolic compounds that may exhibit potent antioxidant properties (Kalogeropoulos and Tsimidou, 2014). Secondly, the availability of local olive oil by-products since Greece is a major olive oil producer (European Commission, 2017) and thirdly the need to use alternative low cost feedstuffs in order to reduce feeding costs (Donohue and Cunningham, 2009). The present study was designed to evaluate the effects of adding olive pulp to the feed on broiler performance and carcass yield.

MATERIALS AND METHODS

Animals, diets and experimental design

Two hundred (200), as hatched, day-old, Cobb 500 broilers were used in total. The broilers were obtained from a commercial hatchery. The duration of the experiment was 42 days with housing and care of broilers, conforming to the guidelines of the Ethical Committee of the Faculty of Animal Science and Aquaculture of the Agricultural University of Athens and complying with directive 2010/63/EC on the protection of animals used for scientific purposes. Pen was the experimental unit. There were five replicate pens of four (4) dietary treatments namely T1, T2, T3 and T4, randomly allocated in the house. There were 10 broilers per pen, 50 per treatment. Birds were assigned to a pen (2 m^2) with wheat straw shavings litter. The maximum stocking density in the pens did not

at any time exceed 33 kg/m^2 following EU directive 2007/43/EC. In house environmental conditions (light and ventilation) were controlled. Heat was provided with a heating lamp per pen.

Dried olive pulp was supplied by Sparta Life S.A, (Sparta, Greece). Chemical analysis revealed that the content of dry matter was 945 g/kg and the content of major nutrients (expressed in dry matter basis) was for crude protein (CP), crude fat, crude fibre (CF) and ash 85.7, 174.6, 276.0, and 61.4 g/kg respectively. Metabolisable energy was estimated at 11.2 MJ/kg (Van Der Klis and Fledderus, 2007).

Broilers were fed three different diets, namely starter (0 - 10 days), grower (11 - 22 d) and finisher (23 - 42 d). In T1 treatment, no olive pulp was added to starter, grower and finisher diet. In T2 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 2.5 and 5% respectively. In T3 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 5% respectively. In T4 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 8% respectively. Feed and water were provided *ad libitum*. Diets were isonitrogenous, isocaloric, with similar content of other oil sources (soybean oil). Composition (g/kg) and calculated analysis of the experimental broiler diets are shown in Table 1.

Sampling

On onset and at the end of each phase, broilers body weight (BW) was recorded and the mean body weight gain (MWG) was calculated. Furthermore, feed intake was measured (MFC) and feed to gain ratios (FCR) were calculated. Broilers were inspected daily and mortality was recorded on the appropriate data capture form. Total mortality was calculated as the number of broilers that died throughout the study compared to the initial number of broilers placed. At the end of the 6th week, 10 chickens per treatment were sacrificed to investigate treatment effects on carcass yield. The birds were weighed, anesthetized with a mild electric current, slaughtered, plucked, eviscerated and stored in the refrigerator for 24 h. The new weight was used for cold carcass yield calculation. Moreover, breast yield (boneless or with keel) was calculated as percentage of body weight.

At the end of the trial, the litter in each pen was scored to assess the degree of wetness based on method of Murakami et al. (2000) with minor modifications and representative samples were collected for dry matter determination. Furthermore, representative samples of freshly voided excreta were obtained for dry matter and wetness determination. Scoring was undertaken by a single operator using a scale based on shape and white cap definition of excreta, ranging from 0 to 3 with 0 referring to normal droppings with white caps in definition while 3 referred to completely liquid droppings. Dry matter determination was carried out according to standard procedures (AOAC International, 2005; method 930.15).

Haematology and Activity of Antioxidant Enzymes

At the end of the trial, blood samples were collected (n=5) for determination of haematocrit (%), aspartate aminotransferase (SGOT-AST) (IU/l), alanine aminotransferase (SGPT-ALT) (IU/l), blood urea nitrogen (BUN) (mg/dl), γ-glutamyltransferase (γ-GT) (IU/l), alkaline phosphatase (IU/l), cholesterol (mg/dl), total proteins (g/dl) and fractions of albumins (g/dl) and globulins (g/dl). Analysis was performed using an automated ABX Pentra 400 bench top analyser (Horiba-ABX, Montpellier, France).

Total antioxidant activity and selected antioxidant enzymes were determined in plasma. In detail, glutathione peroxidase (GPx), glutathione transferase (GST), superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) were determined according to Paglia and Valentine (1967), Habig et al. (1974), McCord and Fridovich (1969), Mavis and Stellwagen (1968) and commercial kit (CAT 100, Sigma-Aldrich, USA) respectively. Total antioxidant activity was determined with the ABTS (2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid)) and the FRAP (Ferric Reducing Ability of Plasma) assay according to Pellegrini et al. (1999) and Benzie and Strain (1996) respectively.

Oleuropein and hydroxytyrosol determination

All solvents were purchased from Baker as analytical (extraction) or HPLC (chromatographic analyses) grades. For the chromatographic analyses HPLC-grade water was prepared using a Milli-Q system, while all HPLC-solvents were filtered prior to use through cellulose acetate membranes of 0.45 μm pore size.

Calibration curves for oleuropein and 3-hydroxytyrosol standards (both obtained from Sigma–Aldrich) were constructed using the following concen-

tration levels (10, 50, 100, 150, 200, 250 and 300 μg/mL for oleuropein and 2, 20, 50, 100 and 150 μg/mL for 3-hydroxytyrosol).

The sample was prepared as follows: 10 g were poured into 60 mL Methanol (MeOH) and sonicated in an ultrasonic bath for 10 min. The solvent was separated by centrifugation (10 min, 7000 rpm) and the remaining solid was re-extracted for two additional times. The combined extracts were evaporated under vacuum and slurry obtained was purified with Solid Phase Extraction (SPE) using Methanol as eluent. The Methanolic solution was concentrated under vacuum to provide a semi-solid residue which was weighed, dissolved in Methanol/Water (1:1 v/v), membrane filtered (0.45 μm) and subjected to HPLC analysis. To avoid the degradation of bioactive molecules, all the aforementioned activities were performed in temperatures below 40 °C.

HPLC analysis was carried out using an Agilent 1100 system equipped with quaternary pump, degasser and diode array detector (DAD). The column used was a Kromasil C18 column (250 mm x 4.1 mm, particle size 5 μm) with a guard column of the same material (8 x 4 mm). Injection was by means of a Rheodyne injection valve (model 7725I) with a 20 μL fixed loop. Chromatographic data were acquired and processed using the Chemstation software.

The HPLC analyses was carried out at 40 °C (maintained by a column thermostat) and 120-140 bar pressure. The gradient eluted consisted of solvents A (aqueous solution with 0.2% v/v acetic acid) and B (Acetonitrile, ACN). Run time was 50 min, with a constant flow-rate of 1.0 mL/min in accordance with the following gradient time table: at zero-time, 98% A and 2% B; after 40 min, the pumps were adjusted to 70% A and 30% B. After the end of the run, a 30 min equilibration period was followed utilizing the zero-time mixture, prior to injection of the next sample. Peaks were identified by comparing their retention time and UV–Vis spectra with reference compounds and data were quantified in respect to the corresponding curves of the reference compounds which were used as standards. The peak area values (measured at 280 nm) constitute the average of three measurements. Results were expressed as mg/kg of olive pulp.

Statistical Analysis

Data were analysed using the Statgraphics Centurion statistical package (version 16.1) and are presented as least squares means ± pooled standard errors. Pen was the experimental unit. Dietary treatment effects on biochemical and haematological parameters, enzyme activities, carcass yield and all the other parameters were explored using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. Kolmogorov–Smirnov test revealed that all variables, except score and the percentages of mortality, followed a normal distribution. Differences between these variables among the four treatments were examined using the Kruskal–Wallis non-parametric test, followed by Dunn's multiple range test. Moreover, principal components analysis was used to reduce the dimensionality of the data and to detect the relationships between the variables. Discriminant analysis was also applied to pooled data to establish those variables capable of distinguishing and classifying samples among the four treatments. Wilk's lambda (λ) criterion was used for selecting discriminant variables. For all tests, the significance was set at ≤0.05.

RESULTS

Broiler performance and carcass yield

Performance of broilers is presented in Table 2. Overall, broilers performed well with final body broiler weight at day 42 being about 2.4 kg. Statistically, no differences were observed between treatments. The FCR of broilers fed olive pulp up to 5% (treatments T2 and T3) did not differ with that of broilers fed the control diet. Broilers fed the diet with the highest inclusion level of oil pulp (T4) numerically consumed more feed and had lower weight gain and this was reflected in the FCR which was statistically higher compared to broilers fed the control or the olive pulp diets up to 5%. No difference between treatments were noted on mortality. It is worth noting that mortality rate of treatment T4 was nil.

Carcass yield is presented in Table 2. No differences were observed between treatments and average carcass yield (grand mean) was 74.5% of body weight. No differences were observed on breast yield between treatments.

Litter and excreta of broilers fed diets with increased levels of olive pulp revealed a tendency to be drier compared to those of broilers fed the control diet (data not shown) but this was not confirmed by data on dry matter since statistically no differences were observed between treatments (Table 2).

Table 2. Performance of broilers during the total experimental period (0-42 d), carcass yield (%) and dry matter content of excreta and litter (%) at the end of the trial

Parameter	Treatments[1]				SEM	*P-value*
	T1	T2	T3	T4		
BW[2] (g)	2411.9	2397.8	2369.2	2320.2	77.10	0.896
Mortality (%)	3.33	3.33	4.44	0	2.827	0.745
MFC[3] (g)	4419.2	4513.6	4454.2	4699.7	153.3	0.737
MBWG[4] (g)	2375.8	2361.9	2332.8	2283.7	77.08	0.894
FCR[5]	1.86[a]	1.91[ab]	1.91[ab]	2.06[b]	0.036	0.039
Carcass Yield (% live weight)	74.05	74.19	75.55	74.25	0.676	0.376
Breast Yield (% live weight)	28.27	28.95	29.55	27.76	0.671	0.269
Boneless Breast Yield (% live weight)	19.91	20.39	20.93	19.51	0.580	0.349
Excreta Dry Matter (%)	17.60	19.69	19.57	16.39	0.644	0.273
Litter Dry Matter (%)	44.64	47.25	43.11	43.85	1.890	0.811

Values are means of five replicate pens (n = 5). Means with different superscripts (a, b) in each row indicate significant differences (P≤0.05) between treatments.

[1]Broilers were fed three different diets, namely starter (0 - 10 days), grower (11 - 22 d) and finisher (23 - 42 d). In T1 treatment, no olive pulp was added to starter, grower and finisher diet. In T2 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 2.5 and 5% respectively. In T3 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 5% respectively. In T4 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 8% respectively.

[2]BW: body weight of 42 days old broilers.

[3]MFC: Mean feed intake of the total experimental period (0-42 days).

[4]MBWG: Mean body weight gain of the total experimental period (0-42 days).

[5]FCR: Feed conversion ratio of the total experimental period (0-42 days).

Biochemical parameters and antioxidant enzymes

Several biochemical and haematological parameters were examined in order to investigate potential effects on broiler's health. In Table 3, SGOT-AST, SGPT-ALT, BUN, γ-GT, alkaline phosphatase, cholesterol, total protein, albumins, globulins and haematocrit measurements are presented. No major differences were noticed in broilers fed olive pulp compared to broilers fed the control diet.

Oleuropein and hydroxytyrosol were present in the olive pulp at 952 and 216 mg/kg respectively. In Table 3, total antioxidant activity and expression of selected antioxidant enzymes is presented. No major differences were noticed among treatments.

Principal Components Analysis and Discriminant Analysis

Principal components analysis (PCA) was applied to pooled data in order to reduce the dimensionality of the data and detect the most important causes of variability, since a great correlation between the variables was noticed. PCA of the 26 variables (variables of broiler performance, carcass yield, dry matter, biochemical and haematological parameters and antioxidant enzymes) resulted in nine principal components with eigen-values greater than 1.0, a common statistical cut-off point. The nine selected components accounted for 83.23% of the total variability. In Figure 1 a plot of both first and second principal components is presented. The first principal component (PC) explained 19.05% of the total variability and was mainly defined by Total proteins, Globulins and Albumins. These haematological parameters were placed close together on the negative side of the horizontal axis, indicating that they were positively correlated with each other. They were away from the axis origin, suggesting that they were well represented from the first PC, which could be considered as representative of the haematological parameters. The second PC explained another 16.47% of the total variability and was mainly defined by the body weight (BW), the mean body weight gain (MBWG) and some enzyme activities (GR, GPx). BW and MBWG were located close together on the positive side of PC2, indicating a high positive correlation. GR and GPx were also close together and therefore they were positively correlated with each other. The second PC can be considered as a representative of the body weight and enzyme activities. The third PC explained another 10.67% of the total variability and it was mainly defined by FCR, MFC, Carcass yield and Breast yield. Carcass and Breast yield were placed close together indicating that they were positively correlated with each other. The third PC can be considered as a representative of feed intake and carcass and breast yield.

Table 3. Treatment effects on selected biochemical and haematological parameters and on total antioxidant activity and activity of enzymes

Parameter	Treatments[1]				SEM	*P-value*
	T1	T2	T3	T4		
Haematocrit (%)	33.33	33.17	32.83	33.50	1.127	0.978
SGOT-AST[2] (IU/l)	279.2	437.7	390.4	360.2	51.78	0.209
SGPT-ALT[3](IU/l)	13.67	13.50	14.67	13.67	0.834	0.748
BUN[4] (mg/dl)	0.77	0.64	0.94	0.60	0.167	0.484
γ-GT[5] (IU/l)	16.33	16.67	20.33	21.00	1.902	0.215
Alkaline phosphatase (IU/l)	1106.5	2094.3	1319.5	1653.5	294.4	0.127
Cholesterol (mg/dl)	109.2	107.7	112.5	95.17	7.242	0.374
Total proteins (g/dl)	3.12	3.30	3.08	3.05	0.174	0.748
Albumins (g/dl)	1.78	1.82	1.75	1.65	0.072	0.416
Globulins (g/dl)	1.33	1.48	1.33	1.40	0.125	0.808
FRAP[6] (μmol ascorbic acid)	6.47	6.25	6.33	6.30	0.480	0.989
ABTS[7] (% inhibition)	30.21	32.08	32.41	32.15	1.935	0.835
GPx[8] (U/ml)	2.43	2.922	2.965	2.812	0.270	0.537
SOD[9] (U/ml)	11.90	12.00	11.74	12.20	0.269	0.697
GR[10] (U/ml)	0.020	0.023	0.023	0.023	0.002	0.341
CAT[11] (U/ml)	102.7	107.7	98.77	90.87	16.478	0.905
GST[12] (U/ml)	0.35	0.41	0.42	0.41	0.024	0.146

Values are means of five replicate pens (n = 5).
[1]Broilers were fed three different diets, namely starter (0 - 10 days), grower (11 - 22 d) and finisher (23 - 42 d). In T1 treatment, no olive pulp was added to starter, grower and finisher diet. In T2 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 2.5 and 5% respectively. In T3 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 5% respectively. In T4 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 8% respectively.
[2]SGOT-AST: aspartate aminotransferase.
[3]SGPT-ALT: alanine aminotransferase.
[4]BUN: Blood urea nitrogen.
[5]γ-GT: γ-glutamyltransferase.
[6]FRAP: Ferric Reducing Ability of Plasma
[7]ABTS: 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid)
[8]GPx: Glutathione Peroxidase
[9]SOD: Superoxide Dismutase
[10]GR: Glutathione Reductase
[11]CAT: Catalase
[12]GST: Glutathione Transferase

Since analysis of variance did not reveal remarkable differences on biochemical and haematological parameters, enzyme activities, carcass yield and the other parameters among the dietary treatments, discriminant analysis was further applied to the pooled data in order to investigate if the samples can be distinguished according to the type of diet that they were fed. Twenty six variables (performance, carcass yield, dry matter, biochemical and haematological parameters and antioxidant enzymes) were entered to develop a model to discriminate the samples. Even though one discriminant function was statistically significant (p=0.024), it can be seen in Figure 2, a discriminant plot of the first two discriminant functions, more readable than a one-dimensional plot. All the samples were correctly classified according to the treatment (100% of the total cases). On the left side of the plot, it can be seen a cluster of the samples that originated from treatment T1. These samples may be related to high body weight, body weight gain, percentages of mortality and cholesterol and on the contrary to low values of feed intake, FCR, SGOT-AST, γ-GT and phosphatase. Moreover, it must be mentioned that most of the enzyme activities had lower values in the samples treated with the control diet T1. On the other hand, samples from treatment T4 were clustered on the right side of the plot and they had the opposite characteristics. Samples originating from dietary treatments T2 and T3 were located in the middle of the plot separately from each other. These samples had high values of total proteins, albumins, globulins, carcass and breast yield and most of the antioxidant enzyme activities.

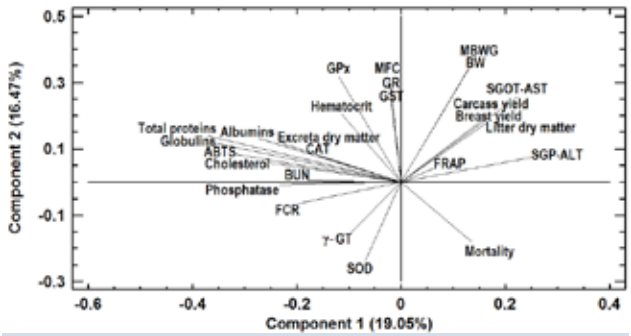

Figure 1. Plot of the first and second principal component

Principal components analysis of the 26 variables. 9 components have been extracted that account for 83.23% of the variability in the original data. First two are presented in the plot.
1. BW: body weight of 42 days old broilers. 2. Mortality: Average mortality of the total experimental period (0-42 days). 3. MFC: Mean feed intake of the total experimental period (0-42 days). 4. MBWG: Mean body weight gain of the total experimental period (0-42 days). 5. FCR: Feed conversion ratio of the total experimental period (0-42 days). 6. Carcass: carcass weight expressed as a percentage of live bird weight 7. Breast yield: Breast weight with keel expressed as a percentage of live bird weight. 8. Excreta dry matter: dry matter of excreta (%). 9. Litter dry matter: dry matter of litter (%). 10. SGOT-AST: aspartate aminotransferase. 11. SGPT-ALT: alanine aminotransferase. 12. BUN: Blood urea nitrogen. 13. γ GT: γ-glutamyltransferase. 14. Phosphatase: Alkaline phosphatase 15. Cholesterol. 16. Total proteins. 17. Albumins. 18. Globulins. 19. Hematocrit. 20. GPx: Glutathione peroxidase 21. SOD: Superoxide Dismutase. 22. GR: Glutathione Reductase. 23. CAT: Catalase. 24. GST: Glutathione Transferase. 25. FRAP: Ferric Reducing Ability of Plasma. 26. ABTS: 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid).

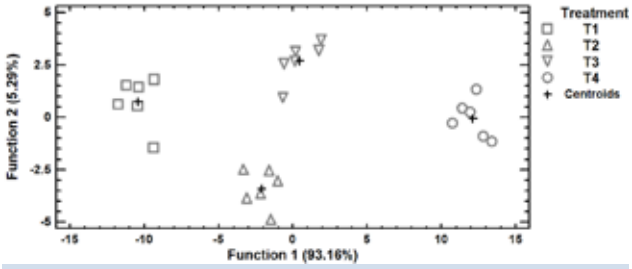

Figure 2. Discriminant plot separating samples according to the dietary treatment

Broilers were fed three different diets, namely starter (0 - 10 days), grower (11 - 22 d) and finisher (23 - 42 d). In T1 treatment, no olive pulp was added to starter, grower and finisher diet. In T2 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 2.5 and 5% respectively. In T3 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 5% respectively. In T4 treatment, olive pulp was added to starter, grower and finisher diet at a level of 0, 5 and 8% respectively.

DISCUSSION

The results of the present study indicate that olive pulp (8.6% CP; 27.6% CF) can be added to diets up to 5% in order to be utilised by broilers without impairment of feed efficiency. Previously, Taklimi et al. (1999) reported improved feed efficiency at 5% inclusion level of olive pulp (6.6% CP; 41.3% CF) in layer diets but declined at higher rates due to increased feed intake attributed to the increased crude fibre content. Similarly, in the present study, when broilers fed 8% olive pulp showed poorer FCR and numerically higher feed intake level. In ducks, Fathalla et al. (2015) examined the effects of an olive pulp (10.2% CP; 24% CF) on the growth performance and concluded that olive pulp added at 12% with or without enzyme complex resulted in improvement of weight gain and feed conversion ratio compared to that of ducks fed the control diet. In hens, inclusion of olive cake (5.2% CP) in diets at a ratio of up to 20% did not affect negatively performance and egg quality, but increased feed intake and impaired FCR compared with control group (Al-Harthi, 2015). Reported differences between studies may be related to differences in chemical composition and most notably the fibre content of olive by-products used. Recently it was reported that moderate amounts of fibre may improve the development of organs, enzyme production, and nutrient digestibility in poultry due to alterations in solubility, viscosity, and fermentation capability that in turn affects microbiota diversity and counts (Mateos et al., 2012).

Proper litter conditions are crucial for the survival of broilers and when not met bacterial growth and ammonia production may negatively affect health (Atapattu et al., 2008). Moisture of litter is of paramount importance for broiler growth since the decomposition of uric acid releases ammonia to the environment (Shah et al., 2007). It has been reported that dietary fibre may reduce the growth of pathogenic microorganisms and the occurrence of digestive disturbances, such as wet litter (Mateos et al., 2012), but in the present study, excreta and litter dry matter did not differ between treatments.

In the present study, carcass yield was similar between treatments and close to Cobb's yield for as hatched broilers (Cobb-Vantress, 2015). Similarly, increasing level of olive pulp in the diet of broilers up to 10% had no significant effects on dressing percentage and carcass composition of 35 day old broilers (Abo Omar, 2005). Previous study with olive cake added at 5 or 10% in broiler diets, with or without the presence of enzymes did not affect carcass yield and internal organs (Al-Harthi et al., 2017). Similarly, olive cake in broiler diets up to 10% did not adversely affect car-

cass traits and inner organs (Al-Harthi et al., 2016). In ducks, Fathalla et al. (2015) examined the effects of an olive pulp on the growth and carcass traits concluded that olive pulp added at 12% without enzyme complex did not affect dressing carcass weight but in the presence of enzyme increased dressing weight.

Olive tree fruits and olive by-products have gained considerable attention due to the interest on phenolic compounds as potential antioxidants (Silva et al., 2006). In detail, it has been shown that oleuropein, the main glycoside present in olive fruit, and hydroxytyrosol a major degradation product of oleuropein exhibit antioxidant and anti-inflammatory properties (Cardoso et al., 2006; Omar, 2010). In the present study, total antioxidant activity determined in blood was not altered by olive pulp addition to broiler diets. Contrary to our findings, Oke et al. (2017) reported that inclusion of olive leaf extract in the water of broilers, reared in a hot and humid tropical climate, improved performance and increased plasma SOD activity. Similarly to our results, Tarek et al. (2013) reported no significant difference in the performance of broiler chickens fed different doses of olive leaf extract in feed. Furthermore, Branciari et al. (2017) observed that dietary administration of a semi-solid olive cake improved the oxidative stability of broiler meat when added at a high dose but did not have any effect at a lower dose. Differences between studies regarding performance and response to olive tree extracts or by products may be attributed to experimental conditions, the presence of stress factors, inclusion level and duration of supplementation. Previous studies examined the polyphenol content and the antioxidant capacity of several by-products and reported that addition of by-products from olive mill wastewater processed using ceramic membrane microfiltration to chicken diet improved their redox status (Gerasopoulos et al., 2015). Furthermore, it was reported that olive leaves included on pig diets at 2.5% may improve the tocopherol content of meat without excessively compromising growth performance (Paiva-Martins et al., 2014). The noted differences may be attributed to different content of polyphenols between examined olive by-products. In detail, it was reported that total phenolic content of fresh olive leaves is 17 g/kg polyphenols while pulps may contain up to 30 g/kg (Silva et al., 2006). However, Paiva-Martins et al. (2014) reported that leaves and branches of olive tree may contain polyphenols up to 67 g/kg Under this context, it was reported that changes in phenolic composition may appear during olive processing, oil extraction and storage (Frankel et al., 2013).

In the present study, the determined values of several biochemical parameters examined were in line with normal values (Campbell, 2004) indicating that good health is maintained when olive pulp is added into broiler diets. Similarly, Sayehban et al. (2015) reported that processed olive pulp fed to broiler diets had no effect on hematological parameters. In contrast, Al-Harthi (2015) reported that plasma albumin was increased when olive cake was added to layer diets at 10 and 20% compared to control. However, in the present study total plasma protein concentration ranged within the normal range from 2.5 to 4.5 g/dl reported for birds by Carpenter (2004).

CONCLUSION

In conclusion, the present study showed that olive pulp added to broiler diets up to 5% can maintain good health and carcass yield without negatively affecting feed to gain ratio. This trial was a small scale one with low levels of stress however, under commercial conditions, any potential differences as those noted in the discriminant analysis of the present study could be more pronounced. Future studies may optimise the use of olive pulp in broiler nutrition in terms of both growth performance and antioxidant potential.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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