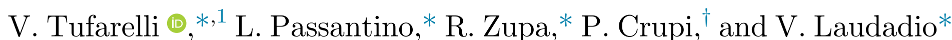
Suitability of dried olive pulp in slow-growing broilers: performance, meat quality, oxidation products, and intestinal mucosa features

V. Tufarelli ,*,[1] L. Passantino,* R. Zupa,* P. Crupi,[†] and V. Laudadio*

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ABSTRACT To assess the effect of dietary dried olive pulp (**DOP**) on growth performance, meat traits and oxidation, and intestinal mucosa features, a total of 180 male slow-growing broiler chickens (Hubbard) were divided into 3 groups and fed 3 isonitrogenous and isoenergetic diets from 14 d of age until slaughter (49 d). The treatments varied according to 3 DOP levels: a control diet without DOP (DOP0, 0%) and 2 test diets containing 5 and 10% of DOP (DOP5 and DOP10, respectively). Duodenal morphometric indices were measured at the end of the feeding period and included: villus height, crypt depth, villus-to-crypt ratio, and villus surface area. Dietary DOP had no adverse effect on growth performance, dressing percentage, or breast yield of broilers. The breast muscle pH at 24 h was significantly higher in birds fed DOP10 diet compared to those on DOP0 and DOP5 diets. Meat color was also affected by dietary treatments. Feeding DOP did not influence breast meat fatty acid composition, whereas meat from DOP-fed broilers resulted less susceptible to lipid and protein oxidation compared to control diet. Including DOP up to 10% in diet resulted in higher duodenal villus height, crypt depth, and villus height to crypt depth ratio as well as villus surface area. Based on our findings, dietary DOP supported productive traits of slow-growing broilers preserving meat from oxidation and improving intestinal morphometric features. As a result, the current study assessed that olive by-product can be used in broiler ration, resulting in a valuable ingredient as replacement for conventional feeds, which could reduce feeding costs due to the low cost of the olive by-product. Thus, using olive by-products as poultry feed may become economically feasible for producers where the olive oil industries play an important economic role.

Key words: broiler, olive pulp, growth, meat quality, gut morphology

2022 Poultry Science 101:102230
https://doi.org/10.1016/j.psj.2022.102230

INTRODUCTION

To date, the use of agroindustrial by-products in animal nutrition has been successfully assumed as a key-strategy to reduce feeding costs and to cope with the need to recycle waste material, which is costly to dispose of (Vasta et al., 2008; Brunetti et al., 2022; Vastolo et al., 2022). The olive (*Olea europaea*) and olive oil industries play significant economic and social role in many countries (International Olive Council, 2017) that generates wastes such as olive pomace, pulp, and leaves.

The extraction process has significant environmental impact due to the production of polluted wastewater and solid residue, depending on olive oil extraction or table olive processing methods (Tufarelli et al., 2013; Gerasopoulos et al., 2015; Branciari et al., 2017). One of the ways to take advantage from the olive oil industry wastes is its use as animal feed. Different ways to include olive by-products in animal diet have been described, varying from feeding it liquid, semi-solid, and solid (Molina-Alcaide and Yañez-Ruiz, 2008; Papadomichelakis et al., 2019). It has been also reported that the inclusion of solid by-products like dried olive pulp (**DOP**) could be of particular interest in broiler feeding with no adverse effects on productive performance (Papadomichelakis et al., 2019; Al-Harthi et al., 2020; Abd El-Moneim et al., 2022).

Moreover, recent interest is being generated in bioactive compounds (polyphenols, flavonoid, oleuropeoside, and simple phenolics) from olive by-products (including DOP) to enhance animal health and performance (Laudadio et al., 2015; Tufarelli et al., 2016; Herrero-Encinas et al., 2020). In this regard, it has been recently suggested that olive by-products might improve the intestinal health of many livestock species (Berbel and Posadillo, 2018; Mancini et al., 2018; Liotta et al., 2019;

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Received August 26, 2022.
Accepted September 29, 2022.

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Tzamaloukas et al., 2021). However, little information and inconsistent results are available on the effects of dried olive pulp on broiler growth responses and meat quality as well as on gut health.

Therefore, the objective of the present study was to evaluate an alternative feeding strategy for broiler producers by comparing the feeding value of diets containing different levels DOP on growth performance, meat quality, and intestinal mucosa features.

MATERIALS AND METHODS

The current trial was conducted at the Experimental Poultry Research Center of the University of Bari "Aldo Moro", Valenzano, Bari, Italy. Authors adhere that procedures imposed on the animals were carried out according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Authors also adhere to the EU regulations on feed legislation, such as regulation EC No 767/ 2009 of the European Parliament Council of July 13, 2009.

Birds, Management and Dietary Treatments

A total of 180 male slow-growing broiler chickens (Hubbard) on day of hatch were purchased. On d 14, broiler chickens were individually weighed and divided among pens and randomly assigned to one of the three dietary treatment groups and grown up to 49 d of age. There were a total of 18 floor pens, 6 replicate pens (2.5 × 1.5 m) per treatment with ten broiler chickens in each pen. The pens were in a closed house with controlled environment. Each pen was equipped with a pan feeder and a manual drinker. Broiler chickens were raised on a concrete floor with straw as litter and stocking density was according to EU legislation (EC, 2007). For the first 2 wk of age, chickens were fed the same mashed diet without dried olive pulp (DOP). Then, broilers were fed a grower-finisher pelleted diet containing 0, 5, or 10% of DOP, respectively. Diets were formulated to meet NRC (1994) nutrient recommendations for broiler chickens. The ingredient and chemical composition of the diets are reported in Table 1. All broiler chickens were reared under similar conditions and feed and water were provided ad libitum throughout the whole feeding trial. The body weight (**BW**) and average daily feed intake (**ADFI**), from which average daily gain (**ADG**) and feed conversion ratio (**FCR**) were calculated, were measured. Mortalities were taken into account when calculating the FCR.

Preparation of Dried Olive Pulp

The olive pulp was a pomace obtained from *Coratina* cultivar supplied by the farm "*Le Tre Colonne*" (Giovinazzo, Bari, Italy). The dehydration of the olive pulps was carried out in a pilot plant by a fluid bed dryer with an operative capacity of 30 kg/h. The inlet temperature of the air was 110 to 120°C, whereas the mean temperature for drying was 50 to 60°C. The DOP was grounded by using a hammer mill, then stored in polyethylene bags until used for feed formulations. The DPO was chemically analyzed at the Animal Nutrition Laboratory, University of Bari "Aldo Moro" (Valenzano, BA, Italy) to assess dry matter (**DM**), crude protein, crude lipids, ash, lignin, calcium, total phosphorous, and potassium according to (AOAC, 2005). Metabolizable energy was estimated according to Van Der Klis and Fledderus (2007). Total phenolic compounds were extracted from the

Table 1. Ingredients and chemical composition of experimental diets fed to slow-growing broiler chickens.

Item[1]	DOP0	DOP5	DOP10
Ingredients (g/kg)			
Corn	565.0	565.0	565.0
Soybean meal (48% CP)	175.0	175.0	195.0
Sunflower meal (38% CP)	75.0	65.0	45.0
Wheat	75.0	75.0	45.0
Wheat middlings	60.0	30.0	10.0
Dicalcium phosphate	20.0	20.0	20.0
Soybean oil	10.0	-	-
Olive pulp	-	50.0	100.0
Calcium carbonate	10.0	10.0	10.0
L-Lysine HCl	2.0	2.0	2.0
Sodium chloride	2.0	2.0	2.0
Sodium bicarbonate	2.0	2.0	2.0
Vitamin-mineral premix[2]	2.0	2.0	2.0
L-Threonine	1.0	1.0	1.0
DL-Methionine	1.0	1.0	1.0
Chemical composition, % DM			
CP	19.90	19.70	19.70
Crude fat	4.90	4.00	4.10
Ash	6.50	6.60	6.70
Calculated analysis			
ME (MJ/kg)	13.80	13.60	13.50
Calcium	1.13	1.15	1.16
Available phosphorus	0.49	0.49	0.50
Lysine	1.09	1.15	1.23
Methionine	0.45	0.49	0.52
Methionine + Cysteine	0.83	0.87	0.91
Threonine	0.83	0.88	0.93

[1]DOP0 = control diet without dried olive pulp; DOP5 = diet containing 5% of dried olive pulp; DOP10 = diet containing 10% of dried olive pulp.

[2]Supplied per kilogram of diet: vitamin A 12,000 IU; vitamin E, 10 mg; vitamin D 2,200 IU; niacin 35.0 mg; d-pantothenic acid 12 mg; riboflavin 3.63 mg; pyridoxine 3.5 mg; thiamine 2.4 mg; folic acid 1.4 mg; biotin 0.15 mg; vitamin B 0.03 mg; Mn 60 mg; Zn 40 mg; Fe 1,280 mg; Cu 8 mg; I 0.3 mg; Se 0.2 mg.

Table 2. Proximate chemical composition of dried olive pulp (DOP) included into broiler diets.

Dry matter, %	88.5
Crude protein, %	11.5
Crude lipids, %	5.5
Ash, %	7.5
Lignin, %	13.5
Cellulose	32.0
Phosphorus, %	0.4
Calcium, %	1.5
Potassium, %	1.8
Metabolizable energy, MJ/kg DM[1]	11.1
Total phenolic compounds[2]	7,900.0

[1]Estimated according to Van Der Klis and Fledderus (2007).

[2]mg/kg DM.

DOP according to the procedure of Papadomichelakis et al. (2019). The proximate chemical composition of the DPO included into broiler diet is reported in Table 2.

Sample Collection

On d 49 of the feeding trial, 5 broilers of average BW were randomly selected from each pen following a 12-h fasting period, weighed individually, and slaughtered. The abdominal fat (consisting of fat surrounding the gizzard, proventriculus, and in the abdominal body cavity), breast (*Pectoralis major*) muscle were removed and weighed immediately. Samples of breast meat muscle were immediately stored at −80°C for assessing crude fat content, and others were individually stored in plastic bags at 4°C for meat quality analysis.

Meat Quality Parameters

At 24 h after slaughter, the breast muscle pH was measured at a depth of 2.0 cm below the surface. This was performed using a combined glass-penetrating electrode (Ingold, Mettler Toledo, Greifensee, Switzerland).

Meat samples were anal for moisture, ash, and protein by oven, muffle furnace, and Kjeldahl methods, respectively, as described in AOAC (2005). Total lipids were extracted according to the method of Folch et al. (1957).

Color measurements were assessed on the carcass surface over the breast muscles and on a freshly exposed cut surface of muscle. A Minolta CR-300 chromameter (Minolta, Osaka, Japan) was set to the L^* (lightness), a^* (redness), and b^* (yellowness) Commission Internationale d'Eclairage scale, as described by Combes et al. (2008).

Meat Fatty Acids

In preparation for the analysis of fatty acid (**FA**) composition, samples of breast meat (5 g each) were freeze-dried and then ground. Methyl heptadecanoate was dissolved into n-hexane (1 mg/mL) as an internal standard. Methyl esters of the FA were prepared by incubating samples (300 mg each) and 5 ml internal standard 2 h at 80°C with methanolic acetyl chloride in a total volume of 9 mL (Sukhija and Palmquist, 1988). After cooling to room temperature, 7 mL of 7% (w/v) K2CO3 was added with mixing, and then the organic phase was collected after centrifuging at 1,500 *g* for 2 min at 4°C. Fatty acid methyl esters were separated over a CP-SIL883 column (100 m × 0.25 mm i.d., film thickness 0.20 μm fused silica; Varian, Palo Alto, CA) in a Shimadzu (Model 2GC17A, Kyoto, Japan) gas chromatograph with a HP GC Chem Station Rev. A.05.04 data handling system and using flame ionization detection. Helium was used as the carrier gas at a constant flow rate of 1.7 mL/min. The oven temperature was programmed as follows: 175°C, held for 4 min; 175 to 250°C at 3°C/min; and then maintained for 20 min at 250°C. The injector port and detector temperature were 250°C. Samples (1 μL) were injected with an auto-sampler. Output signals were identified and quantified from the retention times and peak areas of known calibration standards. Composition was expressed as weight percentages of the total fatty acids. To assess the nutritional implications, the ratio of n-6 PUFA to n-3 PUFA (n-6/n-3) and the ratio of polyunsaturated fatty acids (**PUFA**) to saturated fatty acids (**SFA**) were calculated.

Meat Oxidation Products

Thiobarbituric acid-reactive substances (**TBARS**) were determined after 7 d of storage at 4°C meat samples as described by McDonald and Hultin (1987). Tissue samples (2 g) were weighed into test tubes each with 18 mL of 3.86% perchloric acid; samples were homogenized with a Polytron (IKA Labortechnik T25-B, Selangor, Malaysia) 3 × 15 s at high speed. Fifty microliters of butylated hydroxyl anisole (**BHA**) (4.5% BHA in ethanol) was added to the sample prior to homogenization. The homogenate was filtered through a filter paper. The filtrate (2 mL) was mixed with 2 mL of 20 mM TBA in distilled water and incubated in a boiling water bath for 30 min. After cooling, the absorbance of filtrate was determined at 531 nm against a blank containing 2 mL of 3.86% perchloric acid and 2 mL of 20 mM thiobarbituric acid-reactive solution. The thiobarbituric acid-reactive substances values were expressed as milligrams of malonaldehyde per kg of meat.

For meat lipid hydroperoxides determination, about 1 g of meat muscle was homogenized in 5 ml of chloroform/methanol (1:1) for 30 s. Subsequently, the Polytron was washed for 30 s with 5 ml solvent. The homogenates and wash solutions were then combined. Three mL of 0.5% NaCl was added, and the mixture was vortexed for 30 s before centrifugation for 10 min to separate the mixture into 2 phases. Ice cold chloroform/methanol (1:1) (1.3 mL) was added to 2 mL of the lower phase and briefly vortexed. Twenty-five microliters of ammonium thiocyanate (4.38 M) and 25 μL ferrous chloride (18 mM) were added to assay for lipid hydroperoxides according to Shantha and Decker (1994). Samples were incubated for 20 min at room temperature before the absorbances at 500 nm were determined.

Meat protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with dinitrophenylhydrazine as described by Oliver et al. (1987) with slight modifications. Burger patties (1 g) were minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 6 M NaCl (pH 6.5) using an Ultraturrax homogenizer (IKA-Werke, Staufen, Germany) 2 × 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL Eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 mL) and subsequent centrifugation for 5 min at 4,200 *g*. One pellet was treated with 1 mL 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) dinitrophenylhydrazine in 2 M HCl (carbonyl concentration measurement). Both samples were incubated

for 1 h at room temperature. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed 3 times with 1 ml ethanol:ethyl acetate (1:1, v/v) to remove excess dinitrophenylhydrazine. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5), stirred and centrifuged for 2 min at 4,200 g to remove insoluble fragments. Protein concentration was calculated from the absorption at 280 nm using bovine serum albumin as the standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones.

Histological Examination

At d 49, on the same slaughtered broilers, intestinal segment samples (approximately 2 cm in length) of duodenum were excised and flushed with 0.9% saline to remove the contents. Segments were fixed in 10% neutral-buffered formalin for histological examinations. The segments collected were the loop of the duodenum (at 5 cm from the pylorus). Moreover, also liver samples were collected from the same subjects. Samples were dehydrated, cleared, and paraffin embedded. Intestinal segments from 12 birds per dietary treatment were sectioned at a 5 to 7 μm thickness, placed on glass slides, and processed in Masson's trichrome stain for examination by light microscopy according to Culling et al. (1985). Each sample section on slides were stained with haematoxylin and eosin (**H&E**; Merck, Darmstadt, Germany) and Azan Mallory for morphological observations and morphometric measurements. The morphometric indices evaluated were: villus height from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio (Laudadio et al., 2012). The apparent villus surface area was calculated by the following formula: [(villus width at one-third + villus width at two-thirds of the height of the villus) $\times 2^{-1} \times$ villus height], according to Iji et al. (2001). Morphometric investigations were performed on 20 intact villi and 30 crypts chosen from each duodenal segment of broiler chickens and evaluated at 10× and 25× magnification by using an image analysis system (X-Series, Alexasoft).

Statistical Analysis

Data were analyzed as one-way ANOVA design, using the GLM procedure of SAS (version 9.2; SAS, 2008). All the studied traits were analyzed by using univariate linear model as the response variables and diet (0, 5, and 10% DOP, respectively) as the factor explanatory variables, assuming that the random residual variance follows a normal distribution. Duncan's multiple range test was applied to compare the significance of differences between the means (Steel and Torrie, 1980). Statistical significance was considered at $P \leq 0.05$.

Table 3. Effect of dietary treatments on performance of slow-growing broiler chickens.

	Diet[1]				
Item	DOP0	DOP5	DOP10	SEM	*P*-value
Final BW,[2] g	2,098	2,144	2,161	33.8	0.101
ADG, g/d	50.1	50.4	50.6	0.89	0.168
ADFI, g/d	115.2	114.4	114.3	2.01	0.085
FCR, g/g	2.30	2.27	2.26	0.03	0.115
Mortality, %	1.57	1.49	1.50	0.32	0.622

[1]DOP0 = control diet without dried olive pulp; DOP5 = diet containing 5% of dried olive pulp; DOP10 = diet containing 10% of dried olive pulp.

[2]Abbreviations: ADG, average daily gain (14−49 d); ADFI = average daily feed intake (14−49 d); BW, final body weight at 49 days of age; FC, feed conversion ratio (14−49 d).

RESULTS AND DISCUSSION

The mean performance traits of slow-growing broilers fed diets containing different levels of DOP are presented in Table 3. Growth traits of broilers fed the control or DOP diets were not different ($P > 0.05$). Likewise, mean mortality was very low and was not different among dietary treatments. Thus, the findings of the present study indicated that DOP can be included to broiler diets up to 10% without impairment of feed efficiency. In a recent study, Pappas et al. (2019) reported that olive pulp added to broiler diets up to 5% supported health and carcass traits without negative effect on feed to gain ratio; moreover, in the same study no difference among treatments were noted on mortality rate. Furthermore, it was observed in ducks that olive pulp at 12% of diet with or without enzyme complex resulted in improved growth and feed efficiency (Said et al., 2015). The same trend was also observed by Sateri et al. (2017), where a broiler diet containing 4% of olive by-product (as olive meal) with the addition of an enzyme supplement resulted in appropriate combination to support growth performance and carcass characteristics without influence on blood biochemistry, humoral immunity response, as well as caecal microbiota. In laying hens, including olive cake in diets up to 20% did not affect performance and egg quality, but increased feed intake and improved feed efficiency (Al-Harthi et al., 2020).

Eviscerated carcass yield, determined after the removal of the head, neck, and feet, was approximately 74.0% and it was not different ($P = 0.066$) among groups (Table 4). Further, the breast and abdominal fat pad yields were similar among treatments ($P = 0.095$ and $P = 0.069$, respectively). Similarly, increasing level of olive pulp in diet of broilers at 5% (Papadomichelakis et al., 2019) or 10% (Pappas et al., 2019) had no negative effects on dressing percentage and carcass composition of 35-day-old broilers. The present results also indicated that the mean values of pH in chicken breast meat were 5.63, 5.87, and 6.01, respectively, and there were significant increases ($P = 0.021$) in pH values with the increase of DPO in diet. While meat moisture, protein, or ash percentage of slow-

Table 4. Effect of dietary treatments on carcass traits and breast muscle quality of slow-growing broiler chickens.

	Diet[1]				
Item	DOP0	DOP5	DOP10	SEM	*P*-value
Carcass traits[2]					
Eviscerated carcass	73.0	74.1	74.3	0.36	0.066
Breast	20.0	20.5	20.7	0.23	0.095
Abdominal fat	1.6	1.8	2.0	0.05	0.069
Proximate composition					
Moisture, %	73.2	72.9	73.0	0.29	0.235
Protein, %	23.9	24.1	24.0	0.44	0.113
Fat, %	2.06	2.13	2.12	0.06	0.087
Ash, %	0.84	0.87	0.88	0.08	0.058
pH_{24}	5.63[c]	5.87[b]	6.01[a]	0.16	0.021
Color at 24 h					
L^*	48.88[b]	51.94[a]	52.25[a]	0.41	0.028
a^*	3.12[c]	3.69[a]	3.87[a]	0.10	0.031
b^*	12.31[a]	11.17[b]	11.30[b]	0.29	0.037

[1]DOP0 = control diet without dried olive pulp; DOP5 = diet containing 5% of dried olive pulp; DOP10 = diet containing 10% of dried olive pulp.
[2]Percentages of body weight at slaughter.
[a-c]Means within a row with no common superscript differ significantly ($P < 0.05$).

growing broilers fed either of the 3 dietary treatments were not significantly different ($P > 0.05$). Including DOP in the broilers diet significantly increased the lightness and redness of breast meat ($P = 0.028$ and $P = 0.031$, respectively), as depicted by higher L^* and a^* values, and reduced the yellowness of meat ($P = 0.037$) as indicated by b^* values (Table 4). Meat color is an important indicator of meat quality and is one of the first characteristics noted by customers, especially in boneless products. Inclusion of DOP in slow-growing broiler diet led to darker breast meat and also increased redness of meat. Nevertheless, these values were in the normal range, and therefore, these meats would not be considered to be excessively dark (Woelfel et al., 2002). A possible explanation for meat color observed both in the present study and in literature could be the dark color of the DOP that could influence coloration of meat (Tsala et al., 2020). Furthermore, also in rabbits, dietary supplementation with olive by-products (Dal Bosco et al., 2012) did not affect values of meat pH, cooking loss, tenderness, and color parameters.

The breast meat FA compositions of slow-growing broilers fed different levels of DOP in diet are presented in Table 5. Meat from broilers under all the 3 dietary treatments had similar amounts of total saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, as well as the level of n-6 and n-3 polyunsaturated fatty acids. The n-6/n-3 ratio was also similar among dietary groups as well as for the PUFA to SFA ratio. Therefore, alternative and friendly dietary treatments including by-product of olive oil industry up to 10% of diet did not negatively affect meat fatty acids profile of slow-growing broilers. At this regard, it was assessed by Papadomichelakis et al. (2019) that in broilers (Cobb 500) dietary DOP increased the intramuscular 18:1 n-9 and MUFA proportionally to the inclusion rate in finisher diet. In addition,

Table 5. Effect of dietary treatment on breast meat fatty acid composition and oxidation products of slow-growing broiler chickens[1].

	Diet[2]				
Item	DOP0	DOP5	DOP10	SEM	*P*-value
Fatty acids, % on total FA					
Σ SFA	35.04	34.06	33.96	0.49	0.095
Σ MUFA	27.20	27.12	28.84	0.57	0.123
Σ PUFA	37.76	38.82	36.92	0.38	0.086
Σ n-6	34.48	35.29	33.57	0.40	0.075
Σ n-3	3.28	3.53	3.35	0.09	0.119
n-6/n-3	10.51	10.00	10.02	0.13	0.097
PUFA/SFA	1.08	1.14	1.09	0.03	0.085
Meat oxidation					
TBARS, mg MDA/kg of meat	0.51[b]	0.41[a]	0.40[a]	0.02	0.005
Lipid hydroperoxides, μmol/g of meat	0.40[b]	0.35[a]	0.33[a]	0.02	0.006
Protein carbonyls, nmol DNPH/mg protein	1.27[b]	1.12[ab]	1.03[a]	0.08	0.027

Abbreviations: DNPH, 2,4-dinitrophenyl hydrazine; MDA, malonaldehyde; MUFA monounsaturated fatty acid; PUFA polyunsaturated fatty acid; SFA saturated fatty acids; TBARS, thiobarbituric acid reactive substances.
[1]Each value represents the mean of 12 birds per treatment.
[2]DOP0 = control diet without dried olive pulp; DOP5 = diet containing 5% of dried olive pulp; DOP10 = diet containing 10% of dried olive pulp; Σ SFA = sum of all even chain fatty acid up to 22:0; Σ MUFA = sum of 14:1, 16:1, 18:1, 20:1 and 22:1; Σ PUFA = sum of 18:2, 18:3, 20:2, 20:3, 20:4, 20:5, 22:4, 22:5 and 22:6; Σ n-6 = sum of 18:2, 18:3 n-6, 20:2, 20:3 n-6, 20:4 and 22:2; Σ n-3 = sum of 18:3 n-3, 20:3 n-3, 20:5, 22:5 and 22:6.
[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

Chamruspollert and Sell (1999) reported that the presence of olive oil residue in by-products can cause modifications in unsaturated FAs by inhibiting the delta-9 desaturase enzyme system, which is responsible for desaturating saturated FAs and converting them to unsaturated FAs in the muscles.

It was also interesting to note that DOP had significant effect on meat oxidation products (Table 5). The meat from DOP-fed broilers had a lower thiobarbituric acid-reactive substances level compared to control broilers ($P = 0.005$). The concentrations of lipid hydroperoxides muscle were significantly reduced when broilers fed DOP compared to control-diet (0.35 and 0.33 vs. 0.40 μmol/g; $P = 0.006$). A significant effect on protein oxidation ($P = 0.027$) related to the dietary DOP in breast meat was observed. The carbonyl levels resulted to be higher in broilers under control-diet compared to DOP-fed animals. The protein carbonyl content was used as a measure of the extent of oxidative reactions affecting muscle proteins during storage of meat patties. Carbonyl compounds are formed as a result of the oxidative degradation of side chains of lysine, proline, arginine, and histidine residues (Mercier et al., 1998; Laudadio and Tufarelli, 2011). The level of protein carbonyls of broiler meat fed DOP indicated optimal oxidative reactions. Moreover, protein oxidation seems to be influenced by the level of lipid oxidation in meat (Tufarelli et al., 2022). The levels of thiobarbituric acid-reactive substances observed in the present study were correlated with carbonyl proteins

Table 6. Effect of dietary treatments on duodenum mucosa morphometry of slow-growing broiler chickens[1].

	Diet[2]				
Item	DOP0	DOP5	DOP10	Pooled SEM	P-value
Villus height, μm	1,020[c]	1,127[b]	1,341[a]	43.7	<0.001
Crypt depth, μm	183[c]	201[b]	218[a]	10.1	0.003
Villus height/ crypt depth	5.57[b]	5.61[b]	6.15[a]	0.38	0.002
Villus surface area, mm^2	0.132[c]	0.158[b]	0.234[a]	0.09	0.001

[1]Each value represents the mean of 12 birds per treatment.
[2]DOP0 = control diet without dried olive pulp; DOP5 = diet containing 5% of dried olive pulp; DOP10 = diet containing 10% of dried olive pulp.
[a-c]Means within a row with no common superscript differ significantly ($P < 0.05$).

levels. The results of the present study agree those of Tsala et al. (2020) in pig meat fed dried olive pulp, where the MDA values tended to be improved in the DOP group after one and seven days of refrigerated storage. Reduced oxidative stability was observed after the inclusion of olive by-products into the diet of broilers (Papadomichelakis et al., 2019) and rabbits (Dal Bosco et al., 2012). These inconsistencies imply that many factors may affect the antioxidant potential of olive by-products, including peroxide value and polyphenol levels. Moreover, high levels of DOP in diet may have negative effect on the equilibrium between pro- and antioxidative meat content due to its high auto-oxidation rates.

Table 6 showed the effects of different levels of DOP on the duodenum morphology of broilers. The duodenal villus height was significantly lower in the control group than that in the DOP groups ($P < 0.001$). At this intestinal tract, crypts were deeper in broilers fed a DOP10 diet compared with those on DOP5 and DOP0 diets (218 vs. 201 and 183 μm, respectively; $P = 0.003$). As a consequence, the villus height to crypt depth ratio and villus surface area differed significantly ($P = 0.002$ and $P = 0.001$, respectively) among groups. Deeper crypts indicate higher intestinal cell turnover (Ząbek et al., 2020) and faster tissue replacement (Laudadio et al., 2012; Lee et al., 2022). Therefore, a deeper nest depth will increase nutrient require gut maintenance and reduce bird performance. In contrast, increased villus height and villus height/crypt depth ratio reflect an increase replacement and well-differentiated intestinal mucosal epithelial cells hence increase digestibility and absorptivity (Wu et al., 2020). Thus, it was confirmed that DOP may improve the duodenal tissue type, promote digestion and absorption, also supporting broiler chickens growth. The features of the duodenal mucosa of broilers did not show any alterations; moreover, as reported in Figure 1, in broiler fed 10% of DOP the duodenal mucosa was characterized by several longitudinal microscopic villi without central lymphatic vessel at the base of which there were deep crypts. The epithelium resulted to be of simple cylindrical type, with the presence of goblet cells (Figures 1A and 1B). The hepatic parenchyma of broilers did not show any morphological changes in all groups (data not showed), supporting further that DOP can be successfully included into broiler diet up to 10% the without leading possible metabolic alterations.

In conclusion, based on findings, DOP in diets up to 10% supported the productive traits of slow-growing broilers preserving meat from oxidation and improving intestinal mucosa features. As a result, the current study

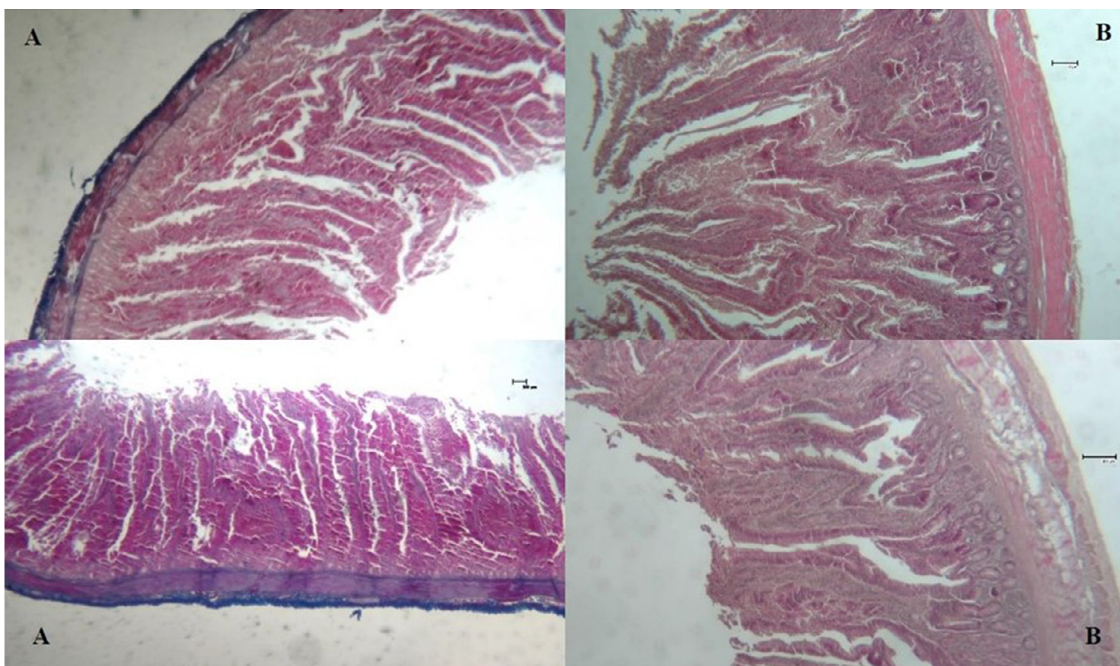

Figure 1. Cross section of small intestine of broiler chickens fed dried olive pulp (at 10% of diet). (A) Section of duodenum showing mucosa with several long villi and deep crypts (Azan Mallory, 10×); (B) magnification of some villi without central lymphatic vessels, their epithelium was simple cylindrical (H&E, 25×).

assessed that olive by-product can be successfully used in broiler rations, resulting in a valuable ingredient as partial replacement for conventional feeds, which could further reduce feeding costs because of the lower cost of the olive by-product. Thus, use of olive by-products as poultry feed may become economically feasible for producers where the olive oil industries play an important economic role.

ACKNOWLEDGMENTS

The authors would like to thank all the people facilitating the implementation of this research including students and technical staff.

DISCLOSURES

No conflict of interest exits, and the manuscript was approved by all authors.

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