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Effects of Different Dietary Levels of Two Types of Olive Pulp and Exogenous Enzyme Supplementation on the Gastrointestinal tract size, Immunology and Hematology of Broilers

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■**Keywords**

Alternative feedstuffs, Immune responses, Natural antioxidants, Processing, Visceral physiology.

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ABSTRACT

The effects of the dietary inclusion of olive pulp (OP) and the supplementation of a commercial enzyme blend (ENZ) on the gastrointestinal tract (GIT) size, and immune and hematological parameters were evaluated. In total, 600 one-day-old Ross 308 male broiler chicks were divided into 6 treatments according to a completely randomized design, in a 2×2×2 factorial scheme, consisting of the dietary inclusion of two olive pulp levels (50 and 100 g/kg in diet), two pulp categories (processed and unprocessed), and the inclusion or not of an enzyme blend. On d 42, birds were euthanized and blood samples were collected, and lymphoid, hematologic, and GIT organs were measured. The inclusion of 100g/kg OP in the diets increased jejunum relative weight (J%) and jejunum length ($p \leq 0.05$). Processed OP reduced jejunum weight and length, J%, and left cecum length, serum triglycerides and VLDL cholesterol levels ($p \leq 0.05$). Enzyme supplementation did affect any of the studied parameters ($p>0.05$). The OP inclusion improved the GIT size, while processed OP reduced GIT measurements and serum lipid levels.

INTRODUCTION

Olive pulp (OP) is the dried residue of olive cake (the raw material resulting from the extraction of olive oil). OP is also a good source of several biologically active compounds, and has antioxidant, antifungal, and antibacterial properties (Al-Harthi, 2014). OP is considered a good source of protein, fat, calcium, copper and cobalt; however, its has nutritional value is poor due its low energy, digestible protein, and mineral content, and high lignin content. It is also poor in phosphorus, magnesium, and sodium, but has fair levels of manganese and zinc (Afsari *et al.*, 2013).

In the past, the utilization of crop residues and by-products as alternatives to cereal-soybean meal based diets was not successful, mainly due to their high fiber content and poor digestibility. Exogenous enzymes may be added to broiler diets containing these by-products to aid fiber digestion (carbohydrases) or to solubilize phytic phosphorus (phytase), thereby reducing their negative effects on broiler performance (Choct, 2006).

The ripening stage of the olives at harvest affects the levels of pectic polysaccharides found in the olive cell walls due to the presence of calcium chelating dimers, thereby changing the nutritional value of this by-product (Cardoso *et al.*, 2007). Most of those compounds are effective antioxidants. According to Kidd (2004), antioxidant substances are capable of reducing cellular free radical damage and enhance the immune response of broilers.

The occurrence of hydrophilic phenols in virgin olive oil is strictly related to the activities of various endogenous enzymes of olive fruits, as their concentrations in the oil are strongly affected by extraction conditions (Yorulmaz *et al.*, 2011). Crushing and malaxation are the most important critical points of the oil mechanical extraction process because the desirable compounds in OP residue can be inactivated by the crushing process (Clodoveo, 2012). The destoning processes are important to maintain the properties of the OP due to the removal of the enzymes that are contained in the seeds (stones). Lavelli and Bondesan (2005) observed an increase in the total secoiridoid polyphenol (anti-oxidant, anti-microbial, and anti-inflammatory compound) content and in the antioxidant activity of extra virgin olive oils when the fruits were pre-destoned.

OP is rich in fibers, and these contents change as a function of processing. It is known that digestion is affected by the physical and chemical characteristics of the feed (Le Goff and Noblet, 2001). In non-ruminants, the presence of fiber and its different characteristics, dependent of the source, is highly important for gastrointestinal (GIT) development (Macari, 2008).

Studies evaluating diets with olive pulp (destoned, or not) for commercial broilers are scarce. The objective of this experiment was to determine the effect of different dietary levels of processed and unprocessed olive pulp, with enzyme supplementation, on the gastrointestinal tract size, immune and hematological parameters of broiler chickens.

MATERIALS AND METHODS

Six hundred one-day-old Ross 308 male broilers were housed in cages (1.25 × 1.25m). The floor area provided per bird was 0.15 m^2. Cages were located in a poultry house with thermostatically-controlled side curtains. The cage floor was covered with paper litter, and the birds remained in the cages for the duration of the experiment, which ended at 42 days of age. Each cage of 10 chickens was assigned to a specific dietary treatment group. The diets (Tables 2 and 3) met or exceeded the recommendations of Ross 308 manual (Aviagen, 2009).

The OP was obtained by washing fresh olive fruit with water. Olives were then milled, placed in hot water (80°C) and centrifuged. At this stage, after "water + oil" emulsion was extracted removed from the olives, and the remaining residue was designated as "olive cake" (OC). In the next step, alpha-tocopherol (anti-oxidant) and an anti-fungal toxin-binder (adsorbent) were added to the OC. The OC was then dried at 70° Cusing hot air, resulting in OP. Olive processing consisted of passing the fruits through a sieve (1.5 mm mesh diameter). During this process, part of the stones (seeds) was removed to produce "partly destoned OP". Olive pulp (processed and unprocessed) chemical composition was determined according to the AOAC (1990).

Dried OP (processed = partly destoned, dried OP; unprocessed = dried OP) was added to the basal starter and grower diets at levels of 50 and 100 g/kg, respectively, which contained or not an enzyme blend (50 or 0 mg/kg diet). The enzyme (Natuzyme P50®, Australia) contained, per g of product, 1000,000 IU phytase, 700 IU β-glucanase,700 IU α-amylase, 6,000 UI cellulase, 700 IU pectinase, 10,000 IU xylanase, 30 IU lipase, and 3,000 IU protease.

A total of 600 one-day-old Ross 308 (Aviagen, New Bridge, Scotland, UK 35805) male chicks were allotted to 60 groups of 10 birds each. Treatments were distributed according to a completely randomized design, in a 2×2×2 factorial arrangement, with six treatments of six replicates of 10 birds each. Treatments consistent of two olive pulp levels (50 and 100 g/kg diet), two pulp types (processed and unprocessed), and two enzyme inclusion levels (0 and 50 mg/kg diet).

Table 1 – Chemical composition of two types of olive pulp used in the experiment.

Olive pulp types / (g/kg) Dry Matter	Partly destoned and dried (processed)	Dried only (unprocessed)
Dry matter	934.5	935.7
Energy (ME) (kcal/kg)	2980	1250
Crude protein	107.3	71.1
Crude fiber	256.0	350.0
Neutral detergent fiber (α-amylase)	716.0	744.0
Acid detergent fiber	550.0	584.0
Ash	85.0	62.0
Crude fat	130.0	85.0
Calcium	8.2	06.1
Phosphorus	0.7	0.6
Soluble sugars	1.7	1.4
Starch	9.7	10.5
Total polyphenols	3.7	1.9
Total tannins	22.9	17.9

Table 2 – Feed ingredients and chemical composition of the starter diets (1 to 21 days of age).

Ingredient (g/kg) \ Treatment	50[3] p[4]	50 p + ENZ[5]	100 p	100 p + ENZ	50 u[6]	50 u + ENZ	100 u	100 u + ENZ
Processed olive meal	50	50	100	100	0	0	0	0
Unprocessed olive meal	0	0	0	0	50	50	100	100
Enzyme	0	0.05	0	0.05	0	0.05	0	0.05
Corn	507.3	507.3	456.6	456.6	482.5	482.5	407	407
Soybean meal	370.6	370.6	370.6	370.6	377.2	377.2	383.7	383.7
Soybean oil	30	30	32.1	32.1	47.6	47.6	67.4	67.4
Wheat bran	0.1	0.05	0.1	0.05	0.1	0.05	0.1	0.05
Dicalciumphosphate	19.3	19.3	19.6	19.6	19.4	19.4	19.7	19.7
Limestone	10.9	10.9	9.1	9.1	11.5	11.5	10.3	10.3
Vitamin Mixture[1]	3	3	3	3	3	3	3	3
Mineral Mixture[2]	3	3	3	3	3	3	3	3
Salt	2.3	2.3	2.1	2.1	2.4	2.4	2.4	2.4
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine	1.4	1.4	1.5	1.5	1.4	1.4	1.5	1.5
Lysinehydrochloride	0.6	0.6	0.8	0.8	0.4	0.4	0.4	0.4
Total	1000	1000	1000	1000	1000	1000	1000	1000
Dry Matter (%)	90.32	90.32	90.49	90.49	90.48	90.48	90.82	90.82
Energy (ME) (kcal/kg)	3025	3025	3025	3025	3025	3025	3025	3025
Crude Protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Ether Extract (%)	5.90	5.90	6.52	6.52	7.47	7.47	9.68	9.68
Linoleic Acid (%)	2.79	2.79	2.79	2.79	3.64	3.64	4.48	4.48
Crude Fiber (%)	4.58	4.58	6.49	6.49	5.07	5.07	7.47	7.47
Calcium (%)	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Phosphorus (%)	0.74	0.74	0.73	0.73	0.73	0.73	0.73	0.73
Available Phosphorus (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Potassium (%)	0.95	0.95	0.99	0.99	0.94	0.94	0.95	0.95
Chlorine (%)	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.19
Manganese (mg/kg)	474.27	474.27	474.11	474.11	475.36	475.36	476.25	476.25
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Zinc (mg/kg)	383.69	383.69	383.21	383.21	385.60	385.60	387.01	387.01
Choline (mg/g)	1.59	1.59	1.56	1.56	1.59	1.59	1.56	1.56
Folic acid (mg/kg)	2.19	2.19	2.18	2.18	2.21	2.21	2.20	2.20
Arginine (%)	1.48	1.48	1.46	1.46	1.49	1.49	1.49	1.49
Glycine (%)	0.92	0.92	0.91	0.91	0.93	0.93	0.92	0.92
Serine (%)	1.10	1.10	1.09	1.09	1.11	1.11	1.10	1.10
Gly+Ser (%)	2.02	2.02	2.00	2.00	2.04	2.04	2.02	2.02
Histidine (%)	0.59	0.59	0.58	0.58	0.59	0.59	0.58	0.58
Isoleucine (%)	0.93	0.93	0.92	0.92	0.94	0.94	0.93	0.93
Leucine (%)	1.89	1.89	1.84	1.84	1.89	1.89	1.84	1.84
Lysine (%)	1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Methionine (%)	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Cysteine (%)	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Met+Cys (%)	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Phenylalanine (%)	1.06	1.06	1.04	1.04	1.06	1.06	1.05	1.05
Tyrosine (%)	0.87	0.87	0.86	0.86	0.88	0.88	0.87	0.87
Phe+Tyr (%)	1.93	1.93	1.90	1.90	1.94	1.94	1.92	1.92
Threonine (%)	0.84	0.84	0.83	0.83	0.84	0.84	0.83	0.83
Tryptophan (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Valine (%)	1.02	1.02	1.01	1.01	1.03	1.03	1.01	1.01

[1] Vitamin A: 3,600,000 IU/kg; Vitamin D_3: 800,000 IU/kg; Vitamin E: 7,200IU/kg; Vitamin K_3: 800 mg/kg; Vitamin B_1: 720 mg/kg; Vitamin B_2: 2,640 mg/kg; Vitamin B_3 (Calcium Pantothenate): 4,000 mg/kg; Vitamin B_5 (Niacin): 12,000 mg/kg; Vitamin B_6: 1,200 mg/kg; Vitamin B_9 (Folic acid): 400 mg/kg; Vitamin B_{12}: 6 mg/kg; Vitamin H_2 (Biotin): 40 mg/kg; Choline: 100,000 mg/kg; Antioxidant: 40,000 mg/kg and 1mg/kg Excipient.

[2] Mn: 39,680 mg/kg; Fe: 20,000 mg/kg; Zn: 33,880 mg/kg; Cu: 4,000 mg/kg; I: 400 mg/kg; Se: 80 mg/kg; Choline: 100,000 mg/kg and 1 mg/kg Excipient.

[3] 50 g/kg olive pulp inclusion.

[4] Processed olive pulp inclusion, [5]Enzyme inclusion, [6]Unprocessed olive pulp inclusion.

Table 3 – Feed ingredients and chemical composition of the finisher diets (22-42 days of age).

Ingredient (g/kg) \ Treatment	50[3] p[4]	50 p + ENZ[5]	100 p	100 p + ENZ	50 u[6]	50 u + ENZ	100 u	100 u + ENZ
Processed olive meal	50	50	100	100	0	0	0	0
Unprocessed olive meal	0	0	0	0	50	50	100	100
Enzyme	0	0.05	0	0.05	0	0.05	0	0.05
Corn	547.6	547.6	496.8	496.8	522.6	522.6	447.1	447.1
Soybean meal	323.2	323.2	323.2	323.2	329.8	329.8	336.3	336.3
Soybean oil	42.3	42.3	44.5	44.5	60	60	79.8	79.8
Wheat bran	0.1	0.05	0.1	0.05	0.1	0.05	0.1	0.05
Dicalciumphosphate	17	17	17.3	17.3	17.1	17.1	17.4	17.4
Limestone	8.7	8.7	6.9	6.9	9.3	9.3	8.2	8.2
Vitamin Mixture[1]	3	3	3	3	3	3	3	3
Mineral Mixture[2]	3	3	3	3	3	3	3	3
Salt	2.3	2.3	2.1	2.1	2.5	2.5	2.4	2.4
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine	1.1	1.1	1.2	1.2	1.1	1.1	1.2	1.2
Lysinehydrochloride	0.2	0.2	0.4	0.4	0	0	0	0
Total	1000	1000	1000	1000	1000	1000	1000	1000
Dry Matter (%)	90.36	90.36	90.53	90.53	90.52	90.52	90.85	90.85
Energy (ME) (kcal/kg)	3150	3150	3150	3150	3150	3150	3150	3150
Crude Protein (%)	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Ether Extract (%)	7.24	7.24	7.87	7.87	8.82	8.82	11.02	11.02
Linoleic Acid (%)	3.49	3.49	3.49	3.49	4.34	4.34	5.18	5.18
Crude Fiber (%)	4.49	4.49	6.39	6.39	4.97	4.97	7.38	7.38
Calcium (%)	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Phosphorus (%)	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67
Available Phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Potassium (%)	0.87	0.87	0.90	0.90	0.85	0.85	0.87	0.87
Chlorine (%)	0.18	0.18	0.17	0.17	0.18	0.18	0.18	0.18
Manganese (mg/kg)	471.74	471.74	471.58	471.58	472.83	472.83	473.72	473.72
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Zinc (mg/kg)	381.73	381.73	381.25	381.25	383.63	383.63	385.05	385.05
Choline (mg/g)	1.48	1.48	1.45	1.45	1.48	1.48	1.46	1.46
Folic acid (mg/kg)	2.04	2.04	2.02	2.02	2.05	2.05	2.05	2.05
Arginine (%)	1.33	1.33	1.31	1.31	1.34	1.34	1.34	1.34
Glycine (%)	0.84	0.84	0.83	0.83	0.84	0.84	0.84	0.84
Serine (%)	1.00	1.00	0.99	0.99	1.01	1.01	1.00	1.00
Gly+Ser (%)	1.84	1.84	1.82	1.82	1.85	1.85	1.84	1.84
Histidine (%)	0.54	0.54	0.53	0.53	0.54	0.54	0.53	0.53
Iso-Leucine (%)	0.84	0.84	0.83	0.83	0.85	0.85	0.84	0.84
Leucine (%)	1.75	1.75	1.71	1.71	1.75	1.75	1.70	1.70
Lysine (%)	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11
Methionine (%)	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Cysteine (%)	0.33	0.33	0.32	0.32	0.33	0.33	0.32	0.32
Met+Cys (%)	0.75	0.75	0.74	0.74	0.75	0.75	0.74	0.74
Phenylalanine (%)	0.96	0.96	0.95	0.95	0.97	0.97	0.96	0.96
Tyrosine (%)	0.79	0.79	0.78	0.78	0.80	0.80	0.79	0.79
Phe+Tyr (%)	1.75	1.75	1.73	1.73	1.77	1.77	1.75	1.75
Threonine (%)	0.76	0.76	0.75	0.75	0.76	0.76	0.76	0.76
Tryptophan (%)	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Valine (%)	0.94	0.94	0.92	0.92	0.94	0.94	0.93	0.93

[1] Vitamin A: 3,600,000 IU/kg; Vitamin D3: 800,000 IU/kg; Vitamin E: 7,200IU/kg; Vitamin K3: 800 mg/kg; Vitamin B1: 720 mg/kg; Vitamin B2: 2,640 mg/kg; Vitamin B3 (Calcium Pantothenate): 4,000 mg/kg; Vitamin B5 (Niacin): 12,000 mg/kg; Vitamin B6: 1,200 mg/kg; Vitamin B9 (Folic acid): 400 mg/kg; Vitamin B12: 6 mg/kg; Vitamin H2 (Biotin): 40 mg/kg; Choline: 100,000 mg/kg; Antioxidant: 40,000 mg/kg and 1mg/kg Excipient.

[2] Mn: 39,680 mg/kg; Fe: 20,000 mg/kg; Zn: 33,880 mg/kg; Cu: 4,000 mg/kg; I: 400 mg/kg; Se: 80 mg/kg; Choline: 100,000 mg/kg and 1 mg/kg Excipient.

[3] 50 g/kg olive pulp inclusion.

[4] Processed olive pulp inclusion, 5Enzyme inclusion, 6Unprocessed olive pulp inclusion.

Gastrointestinal traits

On day 42, birds were fasted for 4 h for the complete evacuation of the gut, and three birds per replicate were selected and euthanized by cervical dislocation. The average obtained by the three birds for each carcass and gastrointestinal tract parameter was calculated and considered as one experimental unit. Birds were plucked using the dry plucking method. Carcasses were weighed before and after evisceration. The following gastrointestinal parts were dissected and collected: crop, proventriculus, pancreas, duodenum, ileum, jejunum, left and right ceca, colon, thymus, liver, spleen, bursa of fabricius, brain, kidneys, and testes. The jejunum and the ileum were identified as the intestinal segments cranial and caudal to Meckel's diverticulum, respectively. All dissected organs were also weighed. The length (cm), width (mm), and wall thickness (mm) of the duodenum, ileum, jejunum, left and right ceca, and colon were recorded. All dimensions were rounded to integers. Organ width was measured when organs were completely empty and flattened. The relative weight of all dissected segments of the digestive tract was calculated as a percentage of the completely eviscerated carcass, according to the following formula: [(weight of the component(s)/ eviscerated carcass weight)×100].

Data were submitted to analysis of variance (ANOVA) using a two-way ANOVA procedure (SAS Institute, Inc., 2000), according to the following model: $Y_{ijk}= \mu+A_i+B_j+C_k+AB_{ij}+AC_{ik}+BC_{jk}+ABC_{ijk}+e_{ijkl}$; where μ = general mean, A_i = effect of olive pulp levels, B_j = effect of oliver pulp processing, C_k = effect of the enzyme inclusion, AB_{ij}= effect of the interaction between olive pulp level and processing, AC_{ik}= effect of the interaction between olive pulp level and enzyme complex inclusion, BC_{jk}= effect of the interaction between olive pulp processing and enzyme inclusion, ABC_{ijk}= effect of the interaction among olive pulp level, processing, and enzyme inclusion, and e_{ijkl} = random residual effect of observation.

After statistical differences were confirmed, the General Linear Model (PROC GLM) was used, and the differences among means ($p\leq0.05$) were evaluated via Duncan's multiple range test (SAS, 2000).

Hematological parameters

Before blood collection, birds were feed-fasted for 4 h in an attempt to allow for the stabilization of the various plasma constituents. Blood was collected in the morning to further reduce the variability of the measured plasma constituents. At 42 days of age, 5 mL venous blood was collected from the ulnaris vein in the wing of one randomly selected bird per replicate. The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulant ethylene diamine tetra acetic acid (EDTA). Blood samples were centrifuged at 3000 rpm for 20 minutes to assure separation of the blood cells from the plasma. Plasma was collected and stored at -20°C until plasma constituent analyses were performed according to standard protocols (Weiss and Wardrop, 2010). The levels of the following hematological parameters were determined: uric acid, total cholesterol, triglycerides, very low density lipoprotein (VLDL), high density lipoprotein (HDL), low density lipoprotein (LDL), LDL/ HDL ratio, aspartate amino transferase (AST), alanine amino transferase (ALT), total protein, albumin, and globulin.

Immunological parameters

A vaccination program against avian influenza virus (1 day of age), infectious bronchitis virus (1 and 18 days of age), Gumboro virus (16 day of age), and Newcastle disease virus (1, 6, and 20 days of age) was applied.

Humoral immune response to the Newcastle vaccine was measured in blood samples collected on days 7 and 21 by antibody titering by hemagglutination-inhibition test (HI). Humoral immune response to the avian influenza vaccine was measured in blood samples collected on days 21 and 28 by antibody titering by HI. Humoral immune response to the infectious bronchitis vaccine was measured in blood samples collected on day 25 by antibody titering by ELISA.

Primary immune response was determined in two birds per replicate injected with sheep red blood cells (SRBC) on day 14. Ten mL SRBC were thoroughly mixed with 1 mL phosphate buffered saline (PBS) solution, and the 0.5 mL of the obtained solution was drawn into a syringe and injected under the breast skin. Blood samples were collected in days 24 and 33 to measure the immune response against SRBC.

RESULTS AND DISCUSSION

Gastrointestinal traits

No significant interactions were observed between olive pulp (OP) level and enzyme supplementation, OP level and pulp processing, or enzyme supplementation and pulp processing for the evaluated gut parameters ($p>0.05$, Table 4, 5 and 6). Crop weight, relative crop weight (CP%), proventriculus weight, and relative proventriculus weight (PV%) were not influenced

by OP dietary levels (p>0.05, Table 4); however, the inclusion of 100g OP/kg increased jejunum relative weight (J%) and length (p≤ 0.05, Table 4). This indicates that up 100 g OP/kg can be included in broiler diets, with no negative effects on gut parameters. Both processes and unprocessed OP have high fiber levels (unprocessed FB= 350g/kg, and processed FB= 256 g/kg, Table 1). High dietary inclusion levels of fiber-rich feedstuffs interfere with organ size and weight, as seen in the present study. The inclusion of fibrous by-products in non-ruminant feeds affects the size and weight of digestive organs (Le Goff and Noblet, 2001). Engberg *et al.* (2004) concluded that whole wheat feeding, which is richer in fiber than ground dehulled wheat, stimulates the function and size of broiler digestive organs. Other researchers observed significant increase in the relative weights of the digestive organs of broilers fed a whole-wheat diet, which is richer in fiber than ground dehulled wheat (Ravindran *et al.*, 2006).

Table 4 – Cranial gut segments characteristics of experimental broilers (mean±standard deviation)*

Treatment \ Trait		Crop (g)	CP (%)	Pro-V (g)	PV (%)	Liver (g)	Liver (%)
ENZ	No	11.72±1.34	0.47±0.05	9.05±0.41	55.62±2.28	2.22±0.07	2.22±0.07
	Yes	10.60±1.34	0.42±0.05	9.84±0.41	60.02±2.28	2.36±0.07	2.36±0.07
OP	50	10.25±1.28	0.40±0.05	9.54±0.41	57.42±2.28	2.28±0.07	2.28±0.07
	100	12.07±1.40	0.49±0.05	9.35±0.41	58.22±2.28	2.30±0.07	2.30±0.07
OP	p	10.79±1.34	0.43±0.05	8.89±0.41	57.15±2.28	2.31±0.07	2.31±0.07
	u	11.53±1.34	0.45±0.05	10.00±0.41	58.49±2.28	2.27±0.07	2.27±0.07
50 p		8.28±2.29	0.33±0.09	8.48±0.85	0.34±0.03	56.53±4.34	2.29±0.14
50 p+ENZ		10.97±2.81	0.42±0.11	9.10±0.85	0.36±0.03	59.32±4.34	2.40±0.14
100 p		14.35±2.81	0.60±0.11	9.41±0.85	0.37±0.03	57.41±4.34	2.31±0.14
100 p+ENZ		9.57±2.81	0.40±0.11	8.58±0.85	0.35±0.03	55.34±4.34	2.26±0.14
50 u		12.55±2.81	0.50±0.11	9.79±0.85	0.38±0.03	56.87±4.34	2.22±0.14
50 u+ENZ		9.21±2.29	0.35±0.09	10.81±0.85	0.42±0.03	56.98±4.34	2.21±0.14
100 u		11.72±2.81	0.47±0.11	8.53±0.85	0.34±0.03	51.67±4.34	2.08±0.14
100 u+ENZ		12.64±2.81	0.50±0.11	10.88±0.85	0.41±0.03	68.45±4.34	2.58±0.14

Treatment \ Trait		Pancreas (g)	P (%)	Duodenum (g)	D (%)	D length (mm)	D width (mm)	D diameter (mm)
ENZ	No	6.39±0.28	0.25±0.01	20.16±0.84	0.80±0.02	383±9.8	6.90±0.29	0.54±0.04
	Yes	6.39±0.28	0.25±0.01	20.55±0.84	0.80±0.02	373±10.4	7.26±0.29	0.61±0.04
OP	50	6.48±0.28	0.24±0.01	20.40±0.84	0.80±0.02	392±9.8	7.26±0.29	0.54±0.04
	100	6.27±0.28	0.26±0.01	20.32±0.84	0.80±0.02	364±10.4	6.90±0.29	0.61±0.04
OP	p	6.59±0.28	0.26±0.01	19.32±0.84	0.78±0.02	374±10.4	7.39±0.29	0.60±0.04
	u	6.45±0.28	0.25±0.01	21.40±0.84	0.83±0.02	382±9.8	6.77±0.29	0.55±0.04
50 p		6.08±0.55	0.24±0.01	19.80±1.65	0.80±0.04	393±18.6	7.11±0.58	0.55±0.08
50 p+ENZ		5.96±0.55	0.23±0.01	19.38±1.65	0.77±0.04	373±18.6	7.91±0.58	0.56±0.08
100 p		7.08±0.55	0.28±0.01	19.09±1.65	0.77±0.04	351±18.6	6.89±0.58	0.57±0.08
100 p+ENZ		6.68±0.55	0.27±0.01	19.02±1.65	0.77±0.04	380±22.8	7.67±0.58	0.71±0.08
50 u		6.54±0.55	0.25±0.01	19.99±1.65	0.78±0.04	408±18.6	7.16±0.58	0.52±0.08
50 u+ENZ		6.52±0.55	0.25±0.01	22.43±1.65	0.87±0.04	396±18.6	6.87±0.58	0.53±0.08
100 u		5.86±0.55	0.23±0.01	21.79±1.65	0.87±0.04	381±18.6	6.45±0.58	0.52±0.08
100 u+ENZ		6.75±0.55	0.25±0.01	21.37±1.65	0.80±0.04	343±18.6	6.60±0.58	0.65±0.08

*Means (± standard error) within each column with no common superscript differ significantly at p≤0.05. Means (± standard error) within each column without superscript did not differ significantly at p>0.05. ENZ= enzyme blend inclusion; OP= olive pulp; p=processed; u=unprocessed; CP(%)= relative crop weight; Pro-V=proventriculus weight; PV (%)=relative proventriculus weight; P (%)=relative pancreas weight; D (%)=relative duodenum weight and D= duodenum

Table 5 – Small intestine segments characteristics of experimental broilers (mean±standard deviation)*

Treatment \ Trait		Jejunum (g)	J (%)	Jejunum length (mm)	Jejunum width (mm)	Jejunum diameter (mm)
ENZ	No	85.0^{a}±5.4	3.39^{a}±0.18	1238^{a}±34	8.03±0.54	0.60±0.05
	Yes	85.2^{a}±5.4	3.33^{a}±0.18	1288^{a}±34	8.57±0.54	0.59±0.05
OP	50	77.8^{a}±5.4	3.06^{b}±0.18	1205^{b}±34	8.92±0.54	0.59±0.05
	100	92.5^{a}±5.4	3.66^{a}±0.18	1321^{a}±34	7.68±0.54	0.61±0.05
OP	p	71.3^{b}±5.4	2.87^{b}±0.18	1200^{b}±34	8.17±0.54	0.60±0.05
	u	99.0^{a}±5.4	3.85^{a}±0.18	1326^{a}±34	8.43±0.54	0.60±0.05
50 p		62.2^{a}±10.4	2.49^{b}±0.34	1126^{a}±78	8.26±0.99	0.61±0.11
50 p + ENZ		71.9^{a}±10.4	2.88^{ab}±0.34	1176^{a}±78	8.97±0.99	0.59±0.11
100 p		83.8^{a}±10.4	3.38^{ab}±0.34	1190^{a}±78	7.16±0.99	0.59±0.11
100 p+ ENZ		67.3^{a}±10.4	2.74^{ab}±0.34	1306^{a}±78	8.28±0.99	0.60±0.11
50 u6		84.3^{a}±10.4	3.29^{ab}±0.34	1233±78	8.33±0.99	0.62±0.11
50 u + ENZ		92.6^{a}±10.4	3.58^{ab}±0.34	1283±78	10.11±0.99	0.53±0.11
100 u		109.8^{a}±10.4	4.41^{a}±0.34	1403^{a}±78	8.35±0.99	0.57±0.11
100 u+ ENZ		109.1^{a}±10.4	4.11^{ab}±0.34	1386^{a}±78	6.93±0.99	0.66±0.11
Treatment \ Trait		Ileum (g)	I (%)	Ileum length (g)	Ileum width (mm)	Ileum diameter (mm)
ENZ	No	8.27±0.59	0.33±0.02	145.0^{a}±4.6	7.42±0.26	0.60^{a}±0.03
	Yes	9.22±0.59	0.36±0.02	152.7^{a}±4.9	6.83±0.26	0.62^{a}±0.03
OP	50	8.33±0.59	0.32±0.02	159.5^{a}±4.6	6.98±0.26	0.69^{a}±0.03
	100	9.16±0.59	0.36±0.02	138.1^{b}±4.9	7.27±0.26	0.54^{b}±0.03
OP	p	8.28±0.59	0.33±0.02	147.7^{a}±4.9	7.21±0.26	0.58^{a}±0.03
	u	9.21±0.59	0.35±0.02	150.0^{a}±4.6	7.04±0.26	0.65^{a}±0.03
50 p		8.09±1.09	0.32±0.04	156.6^{a}±9.4	8.11±0.58	0.67^{a}±0.06
50 p + ENZ		8.95±1.09	0.36±0.04	170.0^{a}±9.48	6.92±0.58	0.59^{a}±0.06
100 p		8.32±1.09	0.33±0.04	136.6^{a}±9.4	7.15±0.58	0.40^{a}±0.06
100 p+ ENZ		7.76±1.09	0.32±0.04	127.5^{a}±11.6	6.66±0.58	0.68^{a}±0.06
50 u6		7.37±1.09	0.28±0.04	145.0^{a}±9.4	6.60±0.58	0.83^{a}±0.06
50 u + ENZ		8.90±1.09	0.34±0.04	166.6^{a}±9.4	6.29±0.58	0.68^{a}±0.06
100 u		9.28±1.09	0.37±0.04	141.6^{a}±9.4	7.83±0.58	0.52^{a}±0.06
100 u+ ENZ		11.27±1.09	0.42±0.04	146.6^{a}±9.4	7.44±0.58	0.56^{a}±0.06

*Means (± standard error) within each column with no common superscripts differ significantly at p≤0.05.ENZ= enzyme blend inclusion; OP= olive pulp; p=processed; u=unprocessed; J (%)=relative jejunum weight and I (%)=relative ileum weight.

Dietary enzyme inclusion and OP processing did not improve any of the studied carcass, cranial gut segment, or liver parameters (p>0.05, Table 4). Malayoğlu *et al.* (2010) did not verify any influence of enzyme supplementation on broiler internal organ weights either. Engberg *et al.* (2004) found that whole wheat feeding did not interfere with digestive size in broilers, but reduced the activities of amylase in the pancreatic tissue; on the other hand, the addition of xylanase increased chymotrypsin and lipase activities. Other researchers reported contradictory results. For instance, Lázaro *et al.* (2004) found that dietary enzyme inclusion reduced crop size in broilers, whereas enzyme supplementation reduced the relative weight of certain segments of the gastrointestinal tract in the study of Józefiak *et al.* (2007). According to Wang *et al.* (2005), the inclusion of enzymes in broiler diets decreased the size of the digestive organs and of the gastrointestinal tract. Another explanation for these contradictory results is the type of experimental design that was used. The experimental design used to evaluate enzymes generally take into account their nutritional value, which is discounted from the diets (Choct, 2006). In the present experiment, however, feeds were formulated to supply the broilers' requirements according to the genetic company manual, regardless of the inclusion or not of the enzyme blend, which was added "on top" of the broilers' requirements. Therefore, the nutrients supplied by enzymes exceeded the requirements of the animals, which may explain the lack of digestive organ differences.

The inclusion levels of OP did not affect pancreas absolute or relative weights (P%); duodenum absolute and relative weight (D%), length, or width; jejunum weight, width, or diameter; ileum ansorlute

Table 6 – Large intestine segments characteristics of experimental broilers (mean±standard deviation)*

Treatment \ Trait		Colon (g)	CL (%)	CL length (mm)	CL width (mm)	CL diam. (mm)	Cecum (g)	
							Right	Left
ENZ	No	5.90±0.46	0.23±0.01	88.66±3.40	8.20±0.83	0.67±0.03	8.00±0.59	8.15±0.77
	Yes	6.03±0.46	0.24±0.01	94.79±3.61	8.86±0.88	0.64±0.04	8.03±0.59	7.82±0.77
OP	50	5.52±0.46	0.21±0.01	93.75±3.40	8.85±0.83	0.68±0.04	8.65±0.59	8.14±0.77
	100	6.41±0.46	0.25±0.01	89.70±3.61	8.21±0.88	0.63±0.04	7.38±0.59	7.83±0.77
OP	p	6.52±0.46	0.26±0.01	93.45±3.61	8.61±0.88	0.67±0.04	7.87±0.59	7.41±0.77
	u	5.42±0.46	0.21±0.01	90.00±3.40	8.45±0.83	0.64±0.04	8.17±0.59	8.56±0.77
50 p		4.27±0.93	0.17±0.03	96.66±6.84	7.88±1.49	0.72±0.08	6.93±1.25	6.86±1.57
50 p+ENZ		8.10±0.93	0.32±0.03	98.33±6.84	9.21±1.49	0.68±0.08	9.71±1.25	8.06±1.57
100 p		7.50±0.93	0.29±0.03	86.33±6.84	8.66±1.49	0.67±0.08	9.52±1.25	8.99±1.57
100p+ENZ		6.20±0.93	0.25±0.03	92.50±8.38	8.68±1.83	0.61±0.09	5.30±1.25	5.74±1.57
50 u		5.35±0.93	0.20±0.03	91.66±6.84	8.55±1.49	0.67±0.08	8.58±1.25	8.63±1.57
50 u+ENZ		4.38±0.93	0.17±0.03	88.33±6.84	9.77±1.49	0.67±0.09	9.40±1.25	9.01±1.57
100 u		6.50±0.93	0.26±0.03	80.00±6.84	7.70±1.49	0.63±0.08	6.98±1.25	8.14±1.57
100u+ENZ		5.45±0.93	0.20±0.03	100.00±6.84	7.79±1.49	0.61±0.08	7.71±1.25	8.47±1.57

Treatment \ Trait		Cecum (%)		CC length (mm)		Cecum width (mm)		Cecum diameter (mm)	
		Right	Left	Right	Left	Right	Left	Right	Left
ENZ	No	0.32±0.02	0.32±0.03	186±4.6	186^{a}±4.1	9.73±0.49	9.43±0.88	0.55±0.03	0.40±0.03
	Yes	0.31±0.02	0.31±0.03	184±4.6	186^{a}±4.1	9.87±0.49	9.77±0.94	0.49±0.03	0.40±0.03
OP	50	0.34±0.02	0.32±0.03	185±4.6	189^{a}±4.1	9.98±0.49	9.81±0.94	0.55±0.03	0.44±0.03
	100	0.29±0.02	0.31±0.03	185±4.6	183^{a}±4.1	9.62±0.49	9.40±0.88	0.49±0.03	0.37±0.03
OP	p	0.32±0.02	0.30±0.03	181±4.6	179^{b}±4.1	10.08±0.49	9.42±0.88	0.53±0.03	0.41±0.03
	u	0.31±0.02	0.33±0.03	189±4.6	194^{a}±4.1	9.52±0.49	9.78±0.94	0.50±0.03	0.40±0.03
50 p		0.28±0.05	0.28±0.06	181±8.8	175^{a}±8.1	9.50±1.10	8.98±1.74	0.55±0.08	0.35±0.07
50 p+ENZ		0.39±0.05	0.32±0.06	180±8.8	188^{a}±8.1	10.99±1.10	10.58±1.74	0.44±0.08	0.42±0.07
100 p		0.38±0.05	0.36±0.06	183±8.8	180±8.1	9.95±1.10	9.56±1.74	0.51±0.08	0.41±0.07
100p+ENZ		0.22±0.05	0.24±0.06	181±8.8	173^{a}±8.1	9.90±1.10	8.58±1.74	0.65±0.08	0.46±0.07
50 u		0.33±0.05	0.34±0.06	191±8.8	198±8.1	9.25±1.10	9.45±1.74	0.76±0.08	0.54±0.07
50 u+ENZ		0.36±0.05	0.35±0.06	190±8.8	196^{a}±8.1	10.19±1.10	10.21±2.13	0.46±0.08	0.43±0.07
100 u		0.28±0.05	0.32±0.06	190±8.8	193^{a}±8.1	10.25±1.10	9.76±1.74	0.37±0.08	0.31±0.07
100u+ENZ		0.29±0.05	0.33±0.06	186±8.8	188^{a}±8.1	8.39±1.10	9.72±1.74	0.42±0.08	0.31±0.07

*Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at p≤0.05. ENZ= enzyme blend inclusion; OP= olive pulp; p=processed; u=unprocessed; CL (%)=relative colon weight; diam.=diameter and CC=cecum.

and relative weight (I%), width, or diameter; colon absolute or relative weights (CL%), length, width, and diameter; or the right and left cecum absolute and relative weights (right= RC% and left =LC%), length, width and diameter (p>0.05, Tables 4, 5 and 6). Other studies achieved similar results. González-Alvarado *et al.* (2007) did not verify any changes in visceral organ size in broilers were fed a high fiber diet, except for the gizzard, who presented hypertrophy. When comparing raw rice with raw corn, González-Alvarado *et al.* (2008) observed an increase in PV% and G% in broiler fed raw corn, but not in all of the other visceral organs that were studied, as also found in the present experiment. In their study, González-Alvarado *et al.* (2008) reported a higher fiber content in raw corn than raw rice, thereby explaining their results.

Broilers that were fed with 100 g/kg of OP presented with a higher relative jejunum weight (J%) and longer jejunum length, but a shorter ileum length and small erileum diameter (EC%) (p≤0.05, Table 5). Contrary to what is commonly thought about the digestive effect of fiber in non-ruminants (Macari, 2008), González-Alvarado *et al.* (2007) reported that high fibrous feedstuff improved the apparent retention of most nutrients, and increased total digestive tract weight; however, they detected a reduction in small intestine (duodenum, jejunum, and ileum) length. On the other hand, González-Alvarado *et al.* (2008) observed that high fiber diets did not affect the relative length of the intestines, but low fiber diets reduced the presence of digesta in the intestines and increased the presence of feed in the gizzard. Digesta retention affects intestinal tract size and weight due mechanical stimulation and contact of nutrients (Wilfarta *et al.*, 2007). The jejunum is the main site of nutrient absorption in the gastrointestinal tract, and increasing its size is highly

desirable and correlated with greater absorption of nutrients (Macari, 2008). Another substance contained in OP that can interfere with gastrointestinal size is tannin. Dietary tannins are thought to reduce diet digestibility and metabolizable energy levels through a direct interaction with proteins and carbohydrates from both exogenous and endogenous sources (Mansoori and Acamovic, 2007). Therefore, OP inclusion in the present experiment may have influenced the size and weight of different parts of the small intestine due to its tannin content.

Processed OP (partial destoning) resulted in lower jejunum absolute and relative weights (J%)and length, and left cecum length compared with unprocessed OP ($p \leq 0.05$, Tables 5 and 6). These results may be attributed to the lower fiber content of the processed material. Olive seeds are highly lignified and have a low fat content (García-Ayuso and Luque de Castro, 1999). Dietary fiber affects the development of the different segments of GIT, and the effects differ according to the physical-chemical characteristics of the fiber source. Plant by-products, like OP, are good sources of fiber in broiler diets. The cecum of birds is a known site of allozyme digestion, which is performed by bacteria (Macari, 2008). These bacteria use as substrate fibrous material that is not digested in the most cranial part of the intestine, and are thus stimulated by the fiber content of the diet. Fiber fermentation produces volatile fatty acids, including butyrate, which is consumed by the intestinal wall as an energy source, thereby stimulating the growth of cecum enterocytes (Macari, 2008). González-Alvarado *et al.* (2007) verified an increase in the cecum weight of broilers fed high fiber by-product diets (oat hulls and soy hulls). Based on these facts, we may infer that the presence of fiber in the unprocessed material may have stimulated the development of the jejunum and cecum of birds. Other factors may also have influenced these variables. Crushing and malaxation are the most important critical points in the olive oil mechanical extraction process (Yorulmaz *et al.*, 2011). Lavelli and Bondesan (2005) observed an increase in the total secoiridoid polyphenol (anti-oxidant, anti-microbial, and anti-inflammatory) content and in the antioxidant activity in extra virgin olive oils when the fruits were pre-destoned. The GIT is the largest organ of the body's immune defense in animals, which is no different for birds (Macari, 2008). The presence of anti-oxidants and anti-microbial substances may influence immune organsize and weight, thus resulting in smaller, less inflammatory, and chemotactic organs. One portion of the cecum is more sensitive to these substances: the cecal tonsils. These segments of the cecum have lymphoepithelial cells, which characterizes them as lymphoid organs (Macari, 2008). Thus, the processing of the OP may contribute to less inflammation of the organ as a whole, consequently explaining their smaller size in birds.

No significant interaction was observed among the evaluated treatments on the studied hematological variables ($p>0.05$, Table 7). OP levels and ENZ inclusion did not interfere with any of the studied blood parameters, including ($p>0.05$, Table 7). Uric acid, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), LDL/HDL ratio, aspartate amino transferase (AST), alanine amino transferase (ALT), total protein, albumin, and globulin levels were not affected by OP processing ($p>0.05$, Table 7). Processed OP increased the triglyceride and VLDL concentrations in broiler blood ($p \leq 0.05$, Table 7). No studies on the interference of dietary OP on broiler hematological parameters were found in literature. However, a series of papers studying antioxidant and functional herb compounds, with OP-related properties, have been published. Ginger extract has antioxidative properties, since it can scavenge superoxide anions and hydroxyl radicals, as shown by Saeid *et al.* (2010). Those authors did not find any influence of the addition of ginger extract to broiler diets on total protein, albumin, globulin, serum HDL, LDL, and VLDL blood levels, except lower cholesterol levels. According to Mehala and Moorthy (2008), blood total cholesterol, HDL, LDL, and triglyceride levels were not affected by the inclusion of *Aloe vera* (rich in saponins and antimicrobial properties) or *Curcuma longa* powder in broiler feeds.

The results of the present study are consistent with the findings of Toghyani *et al.* (2010a), who reported that the inclusion of *Thymus valgaris* (rich in phenols, essential oils, and saponins) powder at a 10 g/kg diet did not influence blood protein, albumin, total and LDL cholesterol concentrations, and albumin to globulin ratio in broilers. According to Toghyani *et al.* (2011), serum protein, albumin, and triglycerides were not affected by the dietary addition of garlic (antibacterial-rich compound) and cinnamon (rich in antibacterial compounds and anti-inflammatory compounds) powder in broilers. Toghyani *et al.* (2010b), when evaluating the supplementation of black seed and peppermint (both rich in essential oils) in broiler diets, did not find any effects on serum protein, albumin, triglyceride, LDL, HDL and total cholesterol levels. OP compounds have similar functions and characteristics as those natural organic compounds, including high

Table 7 – Hematological parameters (mean±standard deviation)*

Treatment \ Trait		Uric acid (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
ENZ	No	4.10±0.61	132.75±4.50	69.25^{a}±5.24	13.83^{a}±1.03	90.66±5.58	28.25±4.05
	Yes	5.07±0.61	140.33±4.50	75.50^{a}±5.24	14.91^{a}±1.03	102.58±5.58	22.83±4.05
OP	50	4.25±0.61	137.75±4.50	74.33^{a}±5.24	14.75^{a}±1.03	98.25±5.58	24.75±4.05
	100	4.91±0.61	135.33±4.50	70.41^{a}±5.24	14.00^{a}±1.03	95.00±5.58	26.33±4.05
OP	p	4.54±0.61	137.00±4.50	81.50^{b}±5.24	16.16^{b}±1.03	98.91±5.58	21.91±4.05
	u	4.63±0.61	136.08±4.50	63.25^{a}±5.24	12.58^{a}±1.03	94.33±5.58	29.16±4.05
50 p		3.76±1.15	137.66±9.09	58.33^{ab}±10.04	11.66^{ab}±1.98	109.66±11.70	16.33±8.26
50 p+ENZ		4.66±1.15	139.33±9.09	88.33^{ab}±10.04	17.33^{ab}±1.98	102.33±11.70	19.66±8.26
100 p		4.50±1.15	131.00±9.09	94.00^{b}±10.04	18.66^{b}±1.98	91.33±11.70	21.00±8.26
100 p+ENZ		5.23±1.15	140.00±9.09	85.33^{ab}±10.04	17.00^{ab}±1.98	92.33±11.70	30.66±8.26
50 u		4.33±1.15	134.00±9.09	83.66^{ab}±10.04	16.66^{ab}±1.98	80.66±11.70	36.66±8.26
50 u+ENZ		4.26±1.15	140.00±9.09	67.00^{ab}±10.04	13.33^{ab}±1.98	100.33±11.70	26.33±8.26
100 u		3.80±1.15	128.33±9.09	41.00^{a}±10.04	8.33^{a}±1.98	81.00±11.70	39.00±8.26
100 u+ENZ		6.13±1.15	142.00±9.09	61.33^{ab}±10.04	12.00^{ab}±1.98	115.33±11.70	14.66±8.26

Treatment \ Trait		LDL/HDL	AST (U/L)	ALT (U/L)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
ENZ	No	0.29±0.05	267.16±13.09	1.50±0.16	3.63±0.13	1.35±0.04	2.28±0.08
	Yes	0.23±0.05	246.66±13.88	1.25±0.17	3.73±0.13	1.35±0.04	2.37±0.08
OP	50	0.22±0.05	269.66±13.09	1.50±0.16	3.71±0.13	1.35±0.04	2.36±0.08
	100	0.30±0.05	244.16±13.88	1.25±0.17	3.65±0.13	1.35±0.04	2.29±0.08
OP	p	0.23±0.05	253.91±13.88	1.58±0.17	3.62±0.13	1.34±0.04	2.28±0.08
	u	0.30±0.05	259.91±13.09	1.16±0.16	3.74±0.13	1.36±0.04	2.37±0.08
50 p		0.15±0.11	250.00±28.72	1.66±0.32	3.76±0.24	1.36±0.09	2.40±0.16
50 p+ENZ		0.19±0.11	285.66±28.72	1.66±0.32	3.73±0.24	1.36±0.09	2.36±0.16
100 p		0.23±0.11	271.00±28.72	2.00±0.32	3.33±0.24	1.33±0.09	2.00±0.16
100 p+ENZ		0.34±0.11	209.00±35.18	1.00±0.39	3.66±0.24	1.30±0.09	2.36±0.16
50 u		0.29±0.13	308.66±28.72	1.33±0.32	3.60±0.24	1.33±0.09	2.26±0.16
50 u+ENZ		0.27±0.11	234.33±28.72	1.33±0.32	3.76±0.24	1.33±0.09	2.43±0.16
100 u		0.52±0.11	239.00±28.72	1.00±0.32	3.83±0.24	1.36±0.09	2.46±0.16
100 u+ENZ		0.12±0.11	257.66±28.72	1.00±0.32	3.76±0.24	1.43±0.09	2.33±0.16

*Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at p≤0.05. Means (± standard error) within each column of dietary treatments without superscript did not differ significantly at p>0.05. ENZ= enzyme blend inclusion; OP= olive pulp; p=processed; u=unprocessed.

instability. Harvesting, processing, and crushing affect the stability of OP compounds (Clodoveo, 2012). Some OP compunds present potent antioxidant activity by direct scavenging of reactive oxygen and nitrogen species and by acting as chain-breaking peroxyl radical scavengers, which have a low stability in the presence of air (Russo *et al.*, 2010). Thus, those compounds may not have had the chance to express their properties due to the OP and the broiler diets processing.

The high VLDL and triglyceride serum concentrations observed in the broilers fed the processed OP maybe explained by improvements in energy digestibility. The processing of OP consists in partial destoning, thereby resulting in a lower fiber material. Dietary fiber is responsible for accelerating digesta passage rate (Macari *et al.*, 2008). Due the low fiber content of the diet (Table 1), broilers that were fed the processed OP presented may have presented lower digesta passage rate than those fed with unprocessed OP, remaining longer in the intestinal tract, possibly resulting in better feed digestion. As a consequence, the triglycerides in soybean oil and in the OP processed oil residue were better absorbed. The VLDL are the main lipids that are transported via avian blood, and triglyceride is the main component the principal content of the VLDL (Macari *et al.*, 2008), which may explain its highlevels in the blood of broilers fed the OP diet.

No significant interactions were observed between olive pulp (OP) levels and enzyme supplementation, OP levels and pulp processing, or enzyme supplementation and pulp processing on the studied immunity traits (p>0.05, Table 8). Antibody titers against avian influenza on d21 (lg2), and d 28 (lg2), Newcastle disease on d27 (lg2) or infectious bronchitis (lgT-IBV) were not affected by OP levels, processing, or ENZ inclusion (p>0.05, Table 8). Likewise, total antibodies

(IgT) from the humoral immune response to injections with sheep red blood cells (SRBC) on d 7 and 14 after injection were not influenced by OP levels or processing (p>0.05, Table 8). Some authors reported the inefficacy of some herb components to modulate the immune system of broiler modulations. Toghyani *et al.* (2011) reported that garlic and cinnamon powder had no influence on any immune-related parameters. In another study, Toghyani *et al.* (2010a) observed that thyme powder at a 10 g/kg diet did not affect antibody titers against Newcastle, influenza, or SRBC. However, other authors observed that medicinal herbs may affect the immune response of broilers. The addition of a medicinal plant blend (alfalfa, liquorice root, great burdock, and cinnamon) to a broiler diet resulted in improvements in antibody titers against Newcastle disease virus (Khaligh *et al.*, 2011).

All of these previous results support the efficacy of medicinal herbs in the immunological modulation in broilers. So why were these results not observed in this experiment? Duarte *et al.* (1993), in a classical experiment to elucidate the phytotherapy properties of oleuropeosides, proved that these substances are capable of inhibiting peak contractile forces in electrically driven left atria of guinea pig hearts. However, the oleopeouside doses used in that experiment were much higher compared to their content in the OP evaluated in the present study. Therefore, the low dose-response hypothesis cannot be ruled out, as it may explain the low effects of OP compounds on most of the evaluated hematological parameters. The OP compounds were possibly too diluted in the diets to cause any effect on the broilers' immune system. In an extensive review about the beneficial effects

Table 8 – Immune response to vaccination and SRBC injection, and organ weights (mean±standard deviation)*

Treatment \ Trait		Antibody titer					Total antibody	
		Influenza 21 days (lg2)	Influenza 28 days (lg2)	Newcastle 7 days (lg2)	Newcastle 27 days (lg2)	Infectious bronchitis virus (IgT-IBV)	SRBC at 7th days (IgT)	SRBC at 14th days (IgT)
ENZ	No	2.08±0.26	2.41±0.44	3.00±0.13	3.58±0.17	367.00±117.51	2.25±0.44	0.91±0.22
	Yes	2.50±0.26	2.75±0.44	2.75±0.13	3.50±0.17	356.66±117.51	3.91±0.44	1.41±0.22
OP	50	2.08±0.26	2.66±0.44	2.75±0.13	3.50±0.17	211.58±117.51	3.16±0.44	1.41±0.22
	100	2.50±0.26	2.50±0.44	3.00±0.13	3.58±0.17	512.08±117.51	2.00±0.44	0.91±0.22
OP	p	2.50±0.26	2.08±0.44	2.91±0.13	3.41±0.17	278.08±117.51	2.75±0.44	1.00±0.22
	u	2.08±0.26	3.08±0.44	2.83±0.13	3.66±0.17	445.58±117.51	2.41±0.44	1.33±0.22
50 p		2.00±0.57	2.66±0.90	3.00±0.27	3.66±0.38	250.00±215.05	1.00±0.92	0.33±0.49
50 p+ENZ		3.00±0.57	1.66±0.90	2.66±0.27	3.00±0.38	240.00±215.05	4.66±0.92	1.33±0.49
100 p		2.33±0.57	2.00±0.90	3.33±0.27	3.66±0.38	339.00±215.05	1.66±0.92	0.66±0.49
100p+ENZ		2.66±0.57	2.00±0.90	2.66±0.27	3.33±0.38	283.33±215.05	4.66±0.92	1.66±0.49
50 u		2.33±0.57	2.33±0.90	2.66±0.27	3.66±0.38	281.00±215.05	2.66±0.92	2.00±0.49
50 u+ENZ		1.00±0.57	4.00±0.90	2.66±0.27	3.66±0.38	75.33±215.05	4.33±0.92	2.00±0.49
100 u		1.66±0.57	2.66±0.90	3.00±0.27	3.33±0.38	598.00±215.05	1.66±0.92	0.66±0.49
100u+ENZ		3.33±0.57	3.33±0.90	3.00±0.27	4.00±0.38	828.00±215.05	2.00±0.92	0.66±0.49

Treatment \ Trait		Thymus (g)	Spleen (g)	Bursa (g)	Brain (g)	Kidneys (g)	Testes (g)
ENZ	No	4.13±0.65	2.80±0.17	4.19±0.37	2.62±0.06	12.58±0.75	0.57±0.07
	Yes	5.64±0.65	2.99±0.17	4.00±0.37	2.45±0.06	12.73±0.75	0.64±0.07
OP	50	5.14±0.65	2.77±0.17	4.03±0.37	2.53±0.06	12.73±0.75	0.62±0.07
	100	4.63±0.65	3.02±0.17	4.16±0.37	2.54±0.06	12.58±0.75	0.58±0.07
OP	p	5.25±0.65	2.96±0.17	4.02±0.37	2.57±0.06	11.87±0.75	0.59±0.07
	u	4.53±0.65	2.83±0.17	4.17±0.37	2.50±0.06	13.44±0.75	0.62±0.07
50 p		4.02±1.35	2.97±0.40	4.55±0.70	2.47±0.13	12.73±1.43	0.58±0.15
50 p+ENZ		6.92±1.35	2.88±0.40	3.37±0.70	2.55±0.13	12.44±1.43	0.67±0.15
100 p		3.40±1.35	2.90±0.40	4.20±0.70	2.79±0.13	11.51±1.43	0.44±0.15
100p+ENZ		6.65±1.35	3.11±0.40	3.96±0.70	2.48±0.13	10.81±1.43	0.68±0.15
50 u		5.14±1.35	2.88±0.40	4.06±0.70	2.68±0.13	12.61±1.43	0.72±0.15
50 u+ENZ		4.51±1.35	2.37±0.40	4.13±0.70	2.42±0.13	13.15±1.43	0.53±0.15
100 u		3.98±1.35	2.44±0.40	3.94±0.70	2.55±0.13	13.47±1.43	0.53±0.15
100u+ENZ		4.48±1.35	3.63±0.40	4.54±0.70	2.35±0.13	14.53±1.43	0.68±0.15

*Means (± standard error) within each column of dietary treatments with no common superscript differ significantly at p<0.05.Means (± standard error) within each column of dietary treatments without superscript did not differ significantly at p>0.05.ENZ= enzyme blend inclusion; OP= olive pulp; p=processed; u=unprocessed; SRBC= Sheep red blood cell at 7th or 14th days after injection; Influenza = titer against influenza 21 and 28 days after vaccination and Newcastle= titer against Newcastle disease 7 and 27 days after vaccination.

of extracts from olive leaves on human health, Sabry (2014) showed that oleuropeoside presents antioxidant activity against reactive oxygen and nitrogen species produced by physiological mechanisms of energy supply, detoxification, chemical signaling, and immune function. Other OP compounds emphasized in that study were the flavonoids and their anti-microbial activity. Most data collected by Sabry (2014) referred to extracts from olive tree leaves, which contain higher concentrations of these components compared with OP, which may explain why the OP had no effect on the immune system of broiler chickens.

The inclusion of the enzyme blend did not affect the evaluated immune parameters ($p>0.05$, Table 8). Mushtaq *et al.* (2007) did not observe any pronounced effects of a glucanase and xylanase blend on antibody titers against Newcastle and infectious bursal diseases either, and no effect on immune response of broilers was found with the use of various enzyme blends (Malayoğlu *et al.*, 2010). The main enzyme mechanisms of immune modulation by enzymes are reducing digesta viscosity and increasing its dry content, resulting in less substratum available for bacterial development. Thus, the main organ affected by these additives is the gut, thereby hindering the observation of their effects in blood samples. Liu *et al.* (2008) observed that the percentage of CD4+ and CD8+ T lymphocyte subsets in the intestine of broilers were increased by phytase, but the CD4+ to CD8+ ratio was not affected. Those authors also found higher levels of intestinal secretory IgA with the addition of phytase in broilers on d 14, 21, and 28.

No significant interactions or effects of the evaluated treatments on the weights of the thymus, spleen, bursa, brain, kidneys, or testes were detected ($p>0.05$, Table 8). These results are in agreement with those of Kırkpınar *et al.* (2011), who evaluated the inclusion of oregano and garlic essential oils in broiler chickens' diets and did not observe effects on liver, spleen, or bursa weight. Soltan *et al.* (2008), when testing the addition of anise seeds, an aromatic antioxidant-rich herb, to broiler feeds did not verify any effects on bursa, thymus, and liver weights, or the spleen index. Toghyani *et al.* (2010a) reported that thyme powder at a 10 g/kg level did not affect liver, bursa, or spleen weights in broiler chickens. Toghyani *et al.* (2010b) did not verify any influence of black seeds and peppermint on bursa, spleen, or thymus weights of broilers. Most of the results indicate the lack of effects of these plant components on lymphoid and hematological organs.

CONCLUSION

The inclusion of 100g OP /kg in broiler diets increases jejunum absolute and relative weights and jejunum length. Processed OP increased serum triglyceride and VLDL levels, and reduces jejunum absolute and relative weights and length, and left cecum length. The dietary inclusion OP increases GIT size in broilers, but not when it is added in its processed form. The inclusion of processed OP in broiler diets increases lipid absorption and serum levels. The dietary addition of OP and of an enzyme blend did not present any immuno modulatory effects in broilers.

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