agriculture

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Olive Pulp and Exogenous Enzymes Feed Supplementation Effect on the Carcass and Offal in Broilers: A Preliminary Study

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Received: 16 July 2020; Accepted: 12 August 2020; Published: 14 August 2020

Abstract: Nowadays, there is an increasing interest in the exploitation and valorization of agricultural food waste and by-products. At the same time, the growing demand by markets worldwide, especially in Africa and Southeast Asia, can justify the growing interest in the use of by-products for the poultry industry. Olive pulp is one of the most interesting by-products of olive tree farming (typical of the Mediterranean area), being a good source of many biologically active compounds with antioxidant, antifungal, and antibacterial properties. The presence of processed olive pulp in the diet showed to be effective in increasing the weight of specific carcass and offal traits. This work aims at studying olive pulp as a feed supplement in poultry nutrition, by focusing on the effects on broiler carcass and offal. Olive pulp (OP) is one of the by-products of olive tree farming, being the residue of olive cake after it is dried. To evaluate the effects of OP in a diet supplemented with different levels of a commercial enzyme (ENZ) blend on broiler carcass and offal traits, three hundred male broiler chicks (Ross 308 lineage; one-day-old) were divided into ten treatment groups according to a completely randomized design. The treatments diets contained: unprocessed OP (50 g/kg, 100 g/kg, 50 g/kg with ENZ, 100 g/kg with ENZ), processed OP (50 g/kg, 100 g/kg, 50 g/kg with ENZ, 100 g/kg with ENZ), and control groups (without OP, and without OP

with ENZ). The OP processing increased breast percentages in broilers. Supplementation with ENZ did not change any of the studied carcass or offal trait values. The presence of OP (50 g/kg) in broiler diets increased the eviscerated carcass, leg, and neck percentage values. The presence of processed OP (50 g/kg) in the diet showed to be effective in increasing the weight of specific carcass and offal traits.

Keywords: by-products; feed processing; feedstuffs; olive pulp; poultry; supplements.

1. Introduction

Olive pulp (OP) is the remainder of olive cake (raw material resulting from olive oil extraction) after the cake is dried. It is also one of the most interesting by-products of olive tree farming, being a good source of several biologically active compounds with antioxidant, antifungal, and antibacterial properties [1–5] with a great nutraceutical potential [6–14]. The use of by-products from different vegetal origins to supplement feed is, on the other hand, attracting growing interest due to environmentally friendly use, efficacy, and sustainability, other than avoiding the necessity to dump potentially useful and valuable by-products of the agro food system [15,16]. Nonetheless, attention should be given to potential secondary metabolites present in the olive [17–20] adopting the necessary precaution in the field to avoid their presence [21].

The OP is considered a good source of protein, fat, calcium, copper, and cobalt, but it is low in its nutritive value (energy, digestible proteins, and minerals) and it has a high lignin content. It is also poor in some metals, e.g., phosphorus, magnesium, and sodium, but it contains reasonable levels of manganese and zinc [22–24]. The ripening stage at harvest interferes with pectic polysaccharides, which are found in the OP cell walls due to the presence of calcium chelating dimers, thereby changing the nutritional value of this by-product [25].Whereas in the past, the use of crop residues and by-products as alternatives to cereals–soybean meal-based rations for broilers diet was not successful, mainly due to the high fiber content and poor digestibility, now it is increasing in interest [26–28]. Some exogenous enzymes may be added to broiler diets containing these by-products as an aid for fiber digestion (carbohydrases) or phytic phosphorus solubilization (phytase), thereby reducing their negative effects on broiler production [29]. Lavelli and Bondesan [30] observed an increase in the total secoiridoid polyphenol (antioxidant, antimicrobial, and anti-inflammatory compounds) content and antioxidant activity in extra virgin olive oil when the fruits were pre-destoned. Most of these compounds are effective antioxidants; according to Kidd [31], antioxidant substances can reduce cellular free radical damage and improve the broilers' immunology, performance, and carcass. A recent study by Debbou-Iouknane et al. [32] investigated the in vitro anticoccidial effect of olive pulp (*Olea europaea* L var. Chemlal) extract on the destruction of *Eimeria* spp. *Oocysts* isolated from infected chickens. The findings of this study showed that phenolic compounds of OP extract tested separately possess an anti-*Eimeria* spp. effect. The recent study of Papadomichelakis et al. [33] on the effects of dietary dried olive pulp inclusion on growth performance and meat quality of broiler chickens, showed that broiler chickens utilize dried olive pulp (DOP) supplemented diets more efficiently when dietary DOP inclusion is increased gradually with age, i.e. by using a combination of grower and finisher diets with a maximum of 25 and 50 g DOP/kg, respectively. The dietary addition of olive cake to broilers up to the level of 150g/kg did not affect performance parameters [34]. Zarei et al. [35] also reported that the inclusion of up to 86g/kg of olive pulp in the diet of laying hens had no negative effects on production parameters. Other researchers found positive effects of the nutritional use of olive pulp. Abo Omar [36] reported an increase in broiler feed intake (and a decrease in feed efficiency) with the inclusion of about 60g of olive pulp/kg diet. This author related this high feed intake to the fiber content of the olive pulp and the consequent increase in passage rate in the gastrointestinal tract. However, the dietary addition of 75g/kg of OP has a negative effect on weight gain, according to Rabayaaet al. [37]. On the other hand, the feasibility of including olive pulp up to the level of 160g/kg in broiler diets was reported [38]. The

use of enzymes to improve the nutritional value of olive pulp was studied in laying hens, but no positive effects were observed on production or egg quality parameters [39].

Considering this context, further studies evaluating the carcass and offal of commercial broilers fed diets with OP seems to be needed, and for this reason, the objective of this preliminary study was to assess the effect of different dietary levels of processed and unprocessed OP with enzyme supplementation on carcass and offal of broiler chickens during a 6-week trial.

2. Material and Methods

Experimental protocols were approved by the Animal Care Committee of the Islamic Azad University, Iran (process #17–33–5–9013; 93–12–7). Three hundred day-old Ross 308 male broilers (Aviagen; New Bridge, Scotland, UK; 35805) were divided into 30 groups of 10 birds. Each cage (10 chickens) was assigned to a specific dietary treatment group. The experimental design had a total of 10 treatments (10 birds/treatment), and three replicates per treatment, as follows:

- 50 p: processed OP (50 g/kg) without enzyme blend;
- 50 p + ENZ: processed OP (50 g/kg) with enzyme blend (50 mg/kg);
- 100 p: processed OP (100 g/kg) without enzyme blend;
- 100 p + ENZ: processed OP (100 g/kg) with enzyme blend (50 mg/kg);
- 50 u: unprocessed OP (50 g/kg) without enzyme blend;
- 50 u + ENZ: unprocessed OP (50 g/kg) with enzyme blend (50 mg/kg);
- 100 u: unprocessed OP (100 g/kg) without enzyme blend;
- 100 u + ENZ: unprocessed OP (100 g/kg) with enzyme blend (50 mg/kg);
- Ctrl: control diet without OP and without enzyme blend;
- Ctrl + ENZ: control diet without OP with enzyme (50 mg/kg) blend.

The birds were housed in cages (dimensions: 1.25 × 1.25 m; floor area: 0.15 m^2 per bird), which were located in a thermostatically-controlled curtain side-wall poultry barn. The cage floors were covered with paper roll litter, and the birds remained in the cages for the duration of the experiment, which ended at their 42nd day of age. A two-phase feeding schedule, which consisted of starter (1–21 days) and grower (22–42 days) feed periods, was adopted in this experimental study. Table 1 and Table 2 report the ingredients and chemical composition of diets used during the starter period and the finishing period, respectively. The diets met or exceeded the Ross 308 catalog recommendations for the starter and grower phases [40].

Table 1. Feed ingredients and chemical composition of diets used during the starter period (1st –21st days of age, as-fed basis).

	Processed Olive Pulp (OP_p)				**Unprocessed Olive Pulp (OP_u)**					
	50[3] p [4]	**50 p + ENZ [5]**	**100 p**	**100 p + ENZ**	**50 u [6]**	**50 u + ENZ**	**100 u**	**100 u + ENZ**	**Ctrl [7]**	**Ctrl +ENZ**
Ingredients (g/kg)										
Processed OP	50.00	50.00	100.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Unprocessed OP	0.00	0.00	0.00	0.00	50.00	50.00	100.00	100.00	0.00	0.00
Enzyme	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Corn	507.30	507.30	456.60	456.60	482.50	482.50	407.00	407.00	558.00	558.00
Soybean meal	370.60	370.60	370.60	370.60	377.20	377.20	383.70	383.70	370.70	370.70
Soybean oil	30.00	30.00	32.10	32.10	47.60	47.60	67.40	67.40	27.80	27.80
Wheat bran	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
Dicalcium phosphate	19.30	19.30	19.60	19.60	19.40	19.40	19.70	19.70	19.00	19.00
Limestone	10.90	10.90	9.10	9.10	11.50	11.50	10.30	10.30	12.70	12.70
Vitamin mixture [1]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral mixture [2]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	2.30	2.30	2.10	2.10	2.40	2.40	2.40	2.40	2.50	2.50
Sodium bicarbonate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
DL-Methionine	1.40	1.40	1.50	1.50	1.40	1.40	1.50	1.50	1.30	1.30
Lysine hydrochloride	0.60	0.60	0.80	0.80	0.40	0.40	0.40	0.40	0.40	0.40
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Nutrient analysis (g/kg)										
Dry matter	903.20	903.20	904.90	904.90	904.80	904.80	908.20	908.20	901.50	901.50
Metabolizable energy (kcal/kg)	3.025	3.025	3.025	3.025	3.025	3.025	3.025	3.025	3.025	3.025
Crude protein	230.00	230.00	230.00	230.00	230.00	230.00	230.00	230.00	230.00	230.00
Ether extract	59.00	59.00	65.20	65.20	74.70	74.70	96.80	96.80	52.70	52.70
Linoleic acid	27.90	27.90	27.90	27.90	36.40	36.40	44.80	44.80	27.90	27.90
Crude fiber	45.80	45.80	64.90	64.90	50.70	50.70	74.70	74.70	26.70	26.70
Calcium	10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50	10.50
Phosphorus	7.40	7.40	7.30	7.30	7.30	7.30	7.30	7.30	7.40	7.40
Available phosphorus	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium	9.50	9.50	9.90	9.90	9.40	9.40	9.50	9.50	9.20	9.20
Chlorine	1.80	1.80	1.80	1.80	1.90	1.90	1.90	1.90	1.90	1.90
Manganese (mg/kg)	474.27	474.27	474.11	474.11	475.36	475.36	476.25	476.25	474.44	474.44
Sodium	0.16	0.16	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	384.17	384.17

Choline (mg/g)	1.59	1.59	1.56	1.56	1.59	1.59	1.56	1.56	1.62	1.62
Folic acid (mg/kg)	2.19	2.19	2.18	2.18	2.21	2.21	2.20	2.20	2.21	2.21
Arginine	14.80	14.80	14.60	14.60	14.90	14.90	14.90	14.90	15.00	15.00
Glycine	9.20	9.20	9.10	9.10	9.30	9.30	9.20	9.20	9.40	9.40
Serine	11.00	11.00	10.90	10.90	11.10	11.10	11.00	11.00	11.20	11.20
Gly + Ser	20.20	20.20	20.00	20.00	20.40	20.40	20.20	20.20	20.60	20.60
Histidine	5.90	5.90	5.80	5.80	5.90	5.90	5.80	5.80	6.00	6.00
Isoleucine	9.30	9.30	9.20	9.20	9.40	9.40	9.30	9.30	9.40	9.50
Leucine	18.90	18.90	18.40	18.40	18.90	18.90	18.40	18.40	19.40	19.40
Lysine	12.70	12.70	12.70	12.70	12.70	12.70	12.70	12.70	12.70	12.70
Methionine	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70
Cysteine	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.70	3.70
Met + Cys	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.30	8.40	8.40
Phenylalanine	10.60	10.60	10.40	10.40	10.60	10.60	10.50	10.50	10.80	10.80
Tyrosine	8.70	8.70	8.60	8.60	8.80	8.80	8.70	8.70	8.90	8.90
Phe + Tyr	19.30	19.30	19.00	19.00	19.40	19.40	19.20	19.20	19.70	19.70
Threonine	8.40	8.40	8.30	8.30	8.40	8.40	8.30	8.30	8.50	8.50
Tryptophan	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Valine	10.20	10.20	10.10	10.10	10.30	10.30	10.10	10.10	10.40	10.40

[1]Vitamin A: 3,600,000 IU/kg; Vitamin D_3: 800,000 IU/kg; Vitamin E: 7200 IU/kg; Vitamin K_3: 800 mg/kg; Vitamin B_1: 720 mg/kg; Vitamin B_2: 2640 mg/kg; Vitamin B_3 (Calcium pantothenate): 4000 mg/kg; Vitamin B_5 (Niacin): 12,000 mg/kg; Vitamin B_6: 1200 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; Excipient: 1 mg/kg. [2] Mn: 39,680 mg/kg; Fe: 20,000 mg/kg; Zn: 33,880 mg/kg; Cu: 4000 mg/kg; I: 400 mg/kg; Se: 80 mg/kg; Choline: 100,000 mg/kg; Excipient: 1 mg/kg.; [3] OP=50 g/kg.; [4] Diet with OPp; [5] Diet with ENZ; [6] Diet with OPu; [7] Control diet without OP.

Table 2. Feed ingredients and chemical composition of diets used during the finishing period (22nd–42nd days of age, as-fed basis).

	Processed Olive Pulp (OP_p)				**Unprocessed Olive Pulp (OP_u)**					
	50 [3] p [4]	**50 p + ENZ [5]**	**100 p**	**100 p + ENZ**	**50 u [6]**	**50 u + ENZ**	**100 u**	**100 u + ENZ**	**Ctrl [7]**	**Ctrl +ENZ**
					Ingredients (g/kg)					
Processed OP	50.00	50.00	100.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Unprocessed OP	0.00	0.00	0.00	0.00	50.00	50.00	100.00	100.00	0.00	0.00
Enzyme	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05
Corn	547.60	547.60	496.80	496.80	522.60	522.60	447.10	447.10	598.20	598.20
Soybean meal	323.20	323.20	323.20	323.20	329.80	329.80	336.30	336.30	323.30	323.30
Soybean oil	42.30	42.30	44.50	44.50	60.00	60.00	79.80	79.80	40.20	40.20
Wheat bran	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
Dicalcium phosphate	17.00	17.00	17.30	17.30	17.10	17.10	17.40	17.40	16.70	16.70
Limestone	8.70	8.70	6.90	6.90	9.30	9.30	8.20	8.20	10.50	10.50
Vitamin mixture[1]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral mixture[2]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	2.30	2.30	2.10	2.10	2.50	2.50	2.40	2.40	2.50	2.50

Sodium bicarbonate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
DL-Methionine	1.10	1.10	1.20	1.20	1.10	1.10	1.20	1.20	1.00	1.00
Lysine hydrochloride	0.20	0.20	0.40	0.40	0.00	0.00	0.00	0.00	0.00	0.00
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
				Nutrient analysis (g/kg)						
Dry matter	90.36	90.36	90.53	90.53	90.52	90.52	90.85	90.85	90.19	90.19
Metabolizable energy (kcal/kg)	3150.0	3150.0	3150.0	3150.0	3150.0	3150.0	3150.0	3150.0	3150.0	3150.0
Crude protein	21.00	21.00	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	6.62	6.62
Linoleic acid	3.49	3.49	3.49	3.49	4.34	4.34	5.18	5.18	3.49	3.49
Crude fiber	4.49	4.49	6.39	6.39	4.97	4.97	7.38	7.38	2.58	2.58
Calcium	0.90	0.90	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	0.68	0.68
Available phosphorus	0.45	0.45	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	0.84	0.84
Chlorine	0.18	0.18	0.17	0.17	0.18	0.18	0.18	0.18	0.19	0.19
Manganese (mg/kg)	471.74	471.74	471.58	471.58	472.83	472.83	473.72	473.72	471.91	471.91
Sodium	0.16	0.16	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	382.21	382.21
Choline (mg/g)	1.48	1.48	1.45	1.45	1.48	1.48	1.46	1.46	1.51	1.51
Folic acid (mg/kg)	2.04	2.04	2.02	2.02	2.05	2.05	2.05	2.05	2.06	2.06
Arginine	1.33	1.33	1.31	1.31	1.34	1.34	1.34	1.34	1.35	1.35
Glycine	0.84	0.84	0.83	0.83	0.84	0.84	0.84	0.84	0.86	0.86
Serine	1.00	1.00	0.99	0.99	1.01	1.01	1.00	1.00	1.02	1.02
Gly + Ser	1.84	1.84	1.82	1.82	1.85	1.85	1.84	1.84	1.88	1.88
Histidine	0.54	0.54	0.53	0.53	0.54	0.54	0.53	0.53	0.55	0.55
Isoleucine	0.84	0.84	0.83	0.83	0.85	0.85	0.84	0.84	0.85	0.85
Leucine	1.75	1.75	1.71	1.71	1.75	1.75	1.70	1.70	1.80	1.80
Lysine	1.11	1.11	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	0.42	0.42
Cysteine	0.33	0.33	0.32	0.32	0.33	0.33	0.32	0.32	0.34	0.34
Met + Cys	0.75	0.75	0.74	0.74	0.75	0.75	0.74	0.74	0.76	0.76
Phenylalanine	0.96	0.96	0.95	0.95	0.97	0.97	0.96	0.96	0.98	0.98
Tyrosine	0.79	0.79	0.78	0.78	0.80	0.80	0.79	0.79	0.81	0.81
Phe + Tyr	1.75	1.75	1.73	1.73	1.77	1.77	1.75	1.75	1.79	1.79
Threonine	0.76	0.76	0.75	0.75	0.76	0.76	0.76	0.76	0.77	0.77
Tryptophan	0.27	0.27	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	0.95	0.95

[1]Vitamin A: 3,600,000 IU/kg; Vitamin D_3: 800,000 IU/kg; Vitamin E: 7200 IU/kg; Vitamin K_3: 800 mg/kg; Vitamin B_1: 720 mg/kg; Vitamin B_2: 2640 mg/kg; Vitamin B_3 (Calcium pantothenate): 4000 mg/kg; Vitamin B_5 (Niacin):12,000 mg/kg; Vitamin B_6: 1200 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 1 mg/kg Excipient. [2] Mn: 39,680 mg/kg; Fe: 20,000 mg/kg; Zn: 33,880 mg/kg; Cu: 4000 mg/kg; I: 400 mg/kg; Se: 80 mg/kg; Choline: 100,000 mg/kg; Excipient: 1 mg/kg.; [3] OP=50 g/kg;.[4]Diet with OPp; [5]Diet with ENZ; [6]Diet with OPu; [7] Control diet without OP.

The OP was obtained by washing fresh olive fruit with water and milling them. Table 3 reports the chemical composition of two types of olive meal used in the experimental procedures. After these steps, the olives were added to hot water (80 °C) and centrifuged. At this stage, the water–oil emulsion was removed from the milled olive, which we named "olive cake" (OC). In the next step, α-tocopherol (antioxidant) and an antifungal toxin-binder (zeolite adsorbent) were added to the OC, which was then dried with hot air (70 °C), thereby resulting in the OP. Olive processing consisted of passing the milled fruits through a sieve (pore diameter: 1.5 mm). During this process, parts of the stones (seeds) were removed to produce "partly destoned" OP. In the diets with enzyme (ENZ), this product (50 mg/kg) was added to the OP (Natuzyme P50®; Sydney, Australia). The nutritional chemical analysis of processed (OPp) and unprocessed (OPu) dried OP was performed [41].

Table 3. Chemical composition of two types of olive meal used in the experiment (as-fed basis).

Types of Olive Meal	Dried Processed Olive Pulp (Partly Destoned)*	Original Dried Unprocessed Olive Pulp
Dry matter (g/kg)	934.50	935.70
Metabolizable energy (kcal/kg)	2980.00	1250.00
Crude protein (g/kg)	107.30	71.10
Crude fiber (g/kg)	256.00	350.00
Neutral detergent fiber (α-amylase) (g/kg)	716.00	744.00
Acid detergent fiber (g/kg)	550.00	584.00
Ash (g/kg)	85.00	62.00
Crude fat (g/kg)	130.00	85.00
Calcium (g/kg)	8.20	6.10
Phosphorus (g/kg)	0.70	0.60
Soluble sugars (g/kg)	1.70	1.40
Starch (g/kg)	9.70	10.50
Total polyphenols (g/kg)	3.70	1.90
Total tannins (g/kg)	22.90	17.90

* Seeds partially removed.

At the age of 42 days (after 4 h-fasting for complete evacuation of the gut), one bird from each replicate were randomly selected, weighed, and euthanized (stunned and slaughtered). In the slaughter method, stunning by electronarcosis and subsequent bleeding (by the jugular court) were used. The averages of these birds were calculated and used as one experimental unit. The experimental units were used to measure carcass yield and offal characteristics. Birds were fully defeathered via the dry method. Feet were separated from the carcass at the tibiotarsal joint. Neck, wingtips, gut, and liver were removed, and the carcass was weighed (cold carcass weight, after chilling). Economically relevant parts of the carcass and offal were separated. First, breast muscle (including the skin and sternum) was dissected free from the carcass. Legs (thighs and drumsticks were dissected by disarticulation at the hip joint and dissection of tissue from the iliac bone. All abdominal fat (including that around the rectum, gizzard, and proventriculus) was collected. Various parts of the carcasses were dissected and weighed separately. All parts, including the head, breast, wings, neck, thighs and drumsticks (legs), gizzard, heart, liver, lung, and abdominal fat, were weighed and weight recorded. The total weight of all dissected parts was related to the whole eviscerated carcass. Relative percentage ratios were calculated according to the following equation: 100 x (weight of component(s)/eviscerated carcass weight).

Data analysis of variance used the two-way ANOVA procedure. Data were submitted to two-way analysis of the variance [42]. The following equation was applied:

$$Yijk = \mu + Ai + Bj + Ck + ABij + ACik + BCjk + ABCijk + eijkl \quad (1)$$

where μ = general average, Ai = olive pulp levels, Bj = effect of processing, Ck = effect of enzyme supplementation, ABij = effect of the interaction between olive pulp levels and processing, ACik = effect of the interaction between olive pulp levels and enzyme supplementation, BCjk = effect of the interaction between olive pulp processing and enzyme complex interaction effect, ABCijk = effect of

the interaction among olive pulp levels, olive pulp processing, and enzyme supplementation, and eijkl = incidental residual effect of observation.

After the statistical differences were confirmed, the general linear model (PROC GLM) was used, and the differences between means ($p \leq 0.05$) were assessed via Duncan's multiple range test.

3. Results

Higher values for OP did not affect the following characteristics: live body weight (LW), defeathered body weight (DW), full abdomen carcass weight (FC), empty abdomen carcass weight (EC), eviscerated carcass weight (ECr), breast weight, thigh and drumstick weight (legs), wing weight, and relative breast and wing weights ($p > 0.05$; Table 4). The relative breast weight was increased by OP processing (partial destoning) ($p > 0.05$; Table 4). However, a significant difference, which was not found in the values for LW, DW, FC, EC, relative ECr, breast, wing, relative wing, thigh and drumstick, and relative thigh and drumstick weights was due to the OP processing ($p > 0.05$) as shown in Table 4.

Table 4. Carcass traits of broilers fed diets containing different amounts of processed (OP_P) and unprocessed (OP_u) olive pulp, with and without enzyme (ENZ), in the period 1–6 weeks of age.

	LW (g)	DW (g)	FC (g)	EC (g)	EC_r (%)	BrW (g)	BrW_r (%)	TDW (g)	TDW_r (%)	WW (g)	WW_r (%)
Diets											
OP_P (50 g/kg)	2770 ^{a}	2464 ^{a}	2228 ^{a}	1743 ^{a}	78.3 ^{a}	842 ^{a}	34.2 ^{a}	718 ^{a}	29.2 ^{a,b}	95 ^{a}	3.87 ^{a}
OP_P (50 g/kg) + ENZ	2752 ^{a}	2476 ^{a}	2295 ^{a}	1752 ^{a}	76.3 ^{a}	857 ^{a}	34.5 ^{a}	680 ^{a}	27.5 ^{b}	108 ^{a}	4.35 ^{a}
OP_P (100 g/kg)	2807 ^{a}	2482 ^{a}	2315 ^{a}	1763 ^{a}	76.2 ^{a}	857 ^{a}	34.5 ^{a}	707 ^{a}	28.5 ^{b}	110 ^{a}	4.40 ^{a}
OP_P (100 g/kg) + ENZ	2782 ^{a}	2456 ^{a}	2288 ^{a}	1738 ^{a}	75.9 ^{a}	847 ^{a}	34.4 ^{a}	690 ^{a}	28.1 ^{b}	106 ^{a}	4.33 ^{a}
OP_u (50 g/kg)	2910 ^{a}	2564 ^{a}	2365 ^{a}	1847 ^{a}	78.0 ^{a}	862 ^{a}	33.5 ^{a}	845 ^{a}	32.8 ^{a}	112 ^{a}	4.34 ^{a}
OP_u (50 g/kg) + ENZ	2952 ^{a}	2572 ^{a}	2368 ^{a}	1800 ^{a}	76.1 ^{a}	849 ^{a}	33.0 ^{a}	749 ^{a}	29.2 ^{a,b}	111 ^{a}	4.32 ^{a}
OP_u (100 g/kg)	2827 ^{a}	2490 ^{a}	2278 ^{a}	1687 ^{a}	74.0 ^{a}	801 ^{a}	32.1 ^{a}	673 ^{a}	27.0 ^{b}	105 ^{a}	4.22 ^{a}
OP_u (100 g/kg) + ENZ	3033 ^{a}	2635 ^{a}	2433 ^{a}	1780 ^{a}	73.2 ^{a}	842 ^{a}	32.0 ^{a}	735 ^{a}	27.9 ^{b}	107 ^{a}	4.04 ^{a}
Without OP	2797 ^{a}	2447 ^{a}	2215 ^{a}	1674 ^{a}	75.6 ^{a}	775 ^{a}	31.7 ^{a}	672 ^{a}	27.5 ^{b}	100 ^{a}	4.06 ^{a}
Without OP + ENZ	2872 ^{a}	2642 ^{a}	2348 ^{a}	1820 ^{a}	77.5 ^{a}	902 ^{a}	34.2 ^{a}	681 ^{a}	25.7 ^{b}	109 ^{a}	4.16 ^{a}
Standard errors	±114	±99	±90	±70	±1.4	±39	±0.9	±35	±0.9	±6	±0.20

LW: Live body weight; DW: Defeathered body weight**;** FC**:** Full abdomen carcass weight; EC: Empty abdomen carcass weight; EC_r: Eviscerated carcass; BrW: Breast weight; BrW_r: Relative breast weight; TDW: Thigh and drumstick weight; TDW_r: Relative thigh and drumstick weight; WW: Wing weight; WW_r: Relative wing weight. ^{a,b} Means (± standard errors) within each column of dietary treatments with no common superscript differ significantly at $p \leq 0.05$.

The addition of enzyme (ENZ) to the diet did not increase the values for any of the studied carcass traits (p>0.05; Table 4). Higher values for OP did not significantly change the following offal values: abdominal fat weight (FW), relative abdominal fat (FWr), gizzard weight, relative gizzard weight, heart weight, relative heart weight, neck weight, relative neck weight, head weight, relative head weight, liver weight, relative liver weight, lung weight, and relative lung weight (p >0.05), as shown in Table 5. The relative neck weight was higher in birds fed 50 g/kg of the OP diet (p >0.05), as per Table 5. The OP partial destoning did not change any offal traits p >0.05), as reported in Table 5. Furthermore, the addition of ENZ did not change any of the offal values (p >0.05), as can be seen in Table 5.

Table 5. Offal traits of broilers fed diets containing different amounts of processed (OP_p) and unprocessed (OP_u) olive pulp, with and without enzymes (ENZ), in the period 1–6 weeks of age.

	FW (g)	FW_r(%)	G (g)	G_r (%)	Ht(g)	Ht_r(%)	N(g)	N_r(%)	Hd(g)	Hd_r(%)	Lv(g)	Lv_r(%)	Lg (g)	Lg_r(%)
						Diets								
OP_p (50 g/kg)	49.79[a]	2.02[a]	64.25[a]	2.60[a]	16.74[a]	0.68[a]	58.74[a]	2.38[a]	76.19[a]	3.09[a]	56.53[a]	2.29[a]	11.27[a]	0.45[a]
OP_p (50 g/kg) + ENZ	39.95[a]	1.60[a]	69.49[a]	2.81[a]	13.08[a]	0.53[a]	56.64[a]	2.28[a]	72.46[a]	2.91[a]	59.32[a]	2.40[a]	12.68[a]	0.51[a]
OP_p (100 g/kg)	42.50[a]	1.70[a]	81.33[a]	3.29[a]	15.51[a]	0.62[a]	56.92[a]	2.29[a]	70.45[a]	2.83[a]	57.41[a]	2.31[a]	11.51[a]	0.46[a]
OP_p (100 g/kg) + ENZ	42.31[a]	1.71[a]	66.48[a]	2.72[a]	15.82[a]	0.65[a]	51.81[a]	2.11[a]	74.03[a]	3.02[a]	55.34[a]	2.26[a]	13.14[a]	0.55[a]
OP_u (50 g/kg)	59.44[a]	2.34[a]	67.80[a]	2.64[a]	15.49[a]	0.60[a]	60.68[a]	2.36[a]	71.96[a]	2.81[a]	56.87[a]	2.22[a]	10.37[a]	0.40[a]
OP_u (50 g/kg) + ENZ	45.99[a]	1.81[a]	70.31[a]	2.71[a]	13.07[a]	0.51[a]	60.80[a]	2.36[a]	76.65[a]	2.97[a]	56.98[a]	2.21[a]	10.68[a]	0.43[a]
OP_u (100 g/kg)	40.82[a]	1.63[a]	70.91[a]	2.85[a]	13.30[a]	0.53[a]	56.34[a]	2.26[a]	71.52[a]	2.87[a]	51.67[a]	2.08[a]	11.91[a]	0.47[a]
OP_u (100 g/kg) + ENZ	56.34[a]	2.14[a]	66.03[a]	2.49[a]	14.86[a]	0.57[a]	55.29[a]	2.11[a]	74.88[a]	2.85[a]	68.45[a]	2.58[a]	10.90[a]	0.41[a]
Without OP level	59.87[a]	2.44[a]	68.52[a]	2.79[a]	13.14[a]	0.53[a]	55.14[a]	2.25[a]	62.11[a]	2.54[a]	56.55[a]	2.30[a]	10.57[a]	0.43[a]
Without OP level + ENZ	57.28[a]	2.16[a]	69.82[a]	2.65[a]	14.38[a]	0.54[a]	59.70[a]	2.25[a]	71.72[a]	2.71[a]	64.43[a]	2.44[a]	11.03[a]	0.41[a]
Standard errors	±7.47	±0.30	±5.91	±70.23	±1.16	±0.05	±3.07	±0.09	±5.12	±0.17	±4.34	±0.14	±1.24	±0.05

FW: Abdominal fat weight; FW_r: Relative abdominal fat; G: Gizzard weight; G_r: Relative gizzard weight; Ht: Heart weight; Ht_r: Relative heart weight; N: Neck weight; N_r: Relative neck weight; Hd: Head weight; Hd_r: Relative head weight; Lv: Liver weight; Lv_r: Relative liver weight; Lg**:** lung weight; Lg_r: Relative lung weight. [a] Means (± standard errors) within each column of dietary treatments with no common superscript differ significantly at $p \leq 0.05$.

4. Discussion

The abovementioned results allowed the observation that the OP diet levels as high as 100 g/kg can be included in the fodder without changing carcass traits. Abo Omar et al. [43] found the same results for weight percentages of carcass cuts from broilers fed OP up to 100 g/kg. However, they reported the lowest LW values at this OP maximum level (100 g/kg). Similarly, Rabayaa et al. [37] also observed the lowest LW values at this same OP level. One possible explanation for these lower LW values could be that the birds in these last two experiments were slaughtered at 35 days of age (driller type chicken). At this age, the birds have little capacity to digest fibrous by-products, such as OP, thereby resulting in a lower LW value. When working with olive cake (not dried OP), El Hachemi et al. [34] reached inclusion levels of 150 g/kg in the diet without changing broiler carcass weights. Our results are in agreement with those obtained in that study, where the authors reported that the OP diet affects one qualitative characteristic of poultry products.

Broilers fed with 50 g/kg of OP presented higher relative thigh and drumstick, leg, and EC weight percentages (EC%) (p>0.05) (see Table 4). Results of leg weight percentages probably contributed to a higher proportion of ECr, as the EC comprised the legs. Other researchers reported conflicting results. Abo Omar et al. [43] found no difference in leg weight or EC percentages when feeding birds with 100 g/kg of OP in the fodder. Suksombat et al. [44] reported a higher fatty acid presence in the thigh muscles of broilers when their diets were supplemented with conjugated linoleic acid (an unsaturated fatty acid).

The oil residue present in OP diets is rich in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), as it contains about 82.2% of the total fatty acid content [45,46]. Thus, the presence of MUFA and PUFA may have influenced the fatty acid deposition in broiler thighs and drumsticks to result in this higher leg percentage and, consequently, in the higher EC values. Panda et al. [47] reported that linseed oil (LO) based diets had higher levels of PUFA than sunflower oil (SFO) based diets, and this could be the reason that a lower abdominal fat content was observed. The total saturated fatty acid (TSFA) and monounsaturated fatty acid (MUFA) content of breast and thigh meat decreased progressively with higher amounts of LO, as expected according to the fatty acid profiles of the diet. The total polyunsaturated fatty acid (TPUFA) content increased linearly with the levels of LO in the diet. The total n-3 fatty acids (FA) contents in breast and thigh meats were significantly higher in the LO based diet compared to SFO.

In addition to that, the total n-3 FA content increased linearly with the increase in the level of dietary incorporation of LO in diets. However, no difference in n-6 FA content of meat was observed due to variation in the levels of LO in the diet. The ratio of n-6:n-3 FA decreased linearly by increasing the replacement of SFO by LO in the diet. Amongst the treatment, significantly higher n-3 FA content in breast and thigh meat and the lowest ratio of n-6: n-3 FA was observed due to complete replacement of SFO with LO in the diet. In addition, Panda et al. [48] showed that abdominal fat content was significantly reduced by incorporating 2% or 3% of fish oil (FO) in the diet. The saturated fatty acid content decreased, and the polyunsaturated fatty acid (PUFA) content increased linearly in breast and thigh meat by replacement of sunflower oil (SFO) with FO at graded levels. The dietary replacement of SFO with FO completely resulted in an increase in the accumulation of n-3 long chain PUFA, particularly linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in the muscle tissues. The highest concentration of n-3 FA and lowest concentration of n-6 FA in breast and thigh meat was observed in the diet where LO was completely replaced with FO in the diet. The ratio of n-6 to n-3 FA also decreased linearly with increasing levels of FO in the diet. The abdominal fat content was significantly lower in the birds fed the low energy diet compared to either control or enzyme supplemented diets [49].

The increase in breast percentage by OP processing (partial destoning) can be explained by crushing and malaxation, as the most important critical points of the oil's mechanical extraction process and its influence in the final product [50]. Lavelli and Bondesan [30] observed an increase in the total secoiridoid polyphenol (an antioxidant, antimicrobial, and anti-inflammatory compound) content and the antioxidant activity in extra virgin olive oils when the fruits were pre-destoned. These

authors concluded that a better knowledge of the reactions occurring during olive processing, especially regarding the involvement of endogenous pulp and stone enzymes, is essential to predict the effect of destoning on extra virgin olive oil quality.

The olive oil residue contributes to most of the energy provided by the OP in the bird diets, as both oil and the beneficial dietary factors are present. Thus, the presence of these antioxidants, antimicrobial, and anti-inflammatory compounds (not denatured by seed enzymes) may have contributed to this superior poultry breast percentage associated with feed containing processed OP. On the other hand, a significant difference due to the OP processing was not found in several offal traits. Furthermore, the non-inactivated secoiridoid polyphenols did not seem to influence these traits. However, a higher breast percentage was observed, although the OP processing did not influence the EC or breast weights. The properties of crushing and olive processing, as well as the effects on the quality of OP oil residue in animal feed, are still largely unknown, and further research is necessary to clarify these issues fully.

The ENZ ineffectiveness in increasing carcass weight values could be due to the amount of xyloglucans in the diets containing 100 g/kg of OP, which may not be enough to decrease the carcass values. Thus, this seems to be a safe limit for use in diets without ENZ requirements. Other anti-nutritional factors, such as pectic polysaccharides [25], did not appear to negatively affect the traits at this level of ENZ inclusion. Brenes et al. [51], at the beginning of their enzyme study, gathered information that enzyme supplementation reduced the relative weights of the proventriculus (39%), pancreas (24%), liver (8%), duodenum (16%), jejunum (20%), ileum (18%), and colon (29%), but not the gizzard percentage. Other researchers reported conflicting results, as they found that the enzymes reduced the gizzard and crop percentages [52]. However, the enzyme amounts in the diets were considered in these studies. Again, this result may be explained by the type of experimental design. All the nutrient requirements were fulfilled, including the ENZ "on top" of these, without subtracting their nutritional contribution to diet calculation. One could also expect a smaller gizzard weight associated with diets after the addition of ENZ since they contribute to fiber digestion. However, the results were different from the expectations. Again, we conclude that the fiber level in the diet was not very high, and OP levels higher than 100 g/kg could be included.

In addition, El Hachemi et al. [34] did not find reduced values for FW and FW% when they used OC levels up to 150 g/kg. However, they reported a higher linoleic fatty acid proportion in fat deposition. Abd el-Samee and Hashish [45] also observed changes in the fatty acid profile, but not in the deposition amount of layer hens' yolk when they were fed with 57 g/kg of OC. Other researchers concluded that including OP in hen diets increased the yolk index and fatty acid deposition [35], but our results do not support this conclusion. This is a polemic discussion, which requires further studies, especially regarding the effects of including OP on the fatty acid deposition profile in birds. If we can show a greater deposition of unsaturated fatty acids in the fat of birds fed OP, this feed would be of strategic importance as an ally to human health. Thus, OP-based broiler diets can be sustainably enriched with PUFA, preventing OP (a potentially environment polluting waste) from being improperly discarded.

The lack of effect of the OP diet on gizzard weights and percentages is unexpected, because this diet is rich in fiber, which can increase gizzard contraction, thus bringing greater muscular tonus, hypertrophy, and weight gain. González-Alvarado et al. [53] observed gizzard hypertrophy in birds fed a high fiber diet that then resulted in a gizzard percentage increase. In another study, a gizzard percentage increase was observed in birds fed diets including oat hulls [54]. These facts support the hypothesis that, at the level of 100 g/kg of OP, the amount of fiber contained in OP is so small that it does not influence the carcass traits or results in hypertrophy of the gizzard in poultry birds. Thus, further research should be conducted by increasing the OP level in broiler diets to estimate the inclusion threshold. Abo Omar et al. [43] also observed an increase in the head proportion (relative to body weight), but not in the neck percentage. This result of neck traits is a novelty in broiler research, being difficult to explain. One hypothesis is that the neck percentage was proportional to the values for EC and leg percentages, which were also higher at this level of OP. Studies reporting offal values are rare in the literature due to their low commercial interest. Further research about the

influence of feeding on these parts, which are less desirable to the consumer, is necessary, and should not be forgotten. In the broiler production chain, the profit margin is becoming increasingly narrow to the point that no gain opportunity should be overlooked. Demand in rising markets, such as Africa and Southeast Asia, can add value to these by-products from the poultry industry. The OP partial destoning (processing) did not change any offal traits (p>0.05), as can be observed in Table 5.

Unlike the breast percentages, the offal values did not seem to be affected by the processing, despite the decrease in the fiber fraction in the diet. One would expect greater gizzard weights and percentages in birds fed processed OP due to this increase in the fiber fraction [53,54], but this was not observed. It could be speculated that the level of OP fiber (either processed or not processed one), and the inclusion level (up to 100 g/kg) was not enough to influence the proportion and size of these organs. Another issue is that the current broiler chicken strains have a high disposition towards carcass deposition, as they are being selected for this. Most of the ingested nutrients are expected to meet the demands of large muscle groups, and the internal organs (except the gastrointestinal tract) should be proportionally smaller as they require less maintenance.

5. Conclusions

This study focused on the evaluation of the effect of feed supplementation with olive pulp and exogenous enzymes on the carcass and offal in broilers. Further research is ongoing to explore other aspects. Our findings indicated how the dietary addition of OP at an amount of 50 g/kg increased the eviscerated carcass, leg, and neck percentage values. However, the dietary addition of processed OP increased the weight of specific carcass and offal traits. The data observed also indicated that the inclusion of ENZ did not affect the values of any of the studied carcass traits.

Author Contributions: Conceptualization, P.S., A.S. (Alireza Seidavi), and A.S. (Antonello Santini); methodology, P.S., W.A.G.A., L.F.T.A.; validation, M.D., A.G., P.S., A.S. (Alireza Seidavi); formal analysis, P.S., W.A.G.A., L.F.T.A.; data curation, P.S., B.O., and P.G.; writing—original draft preparation, P.S., A.S. (Alireza Seidavi), A.D., M.L.; A.S. (Antonello Santini); writing—review and editing, P.S., A.S. (Alireza Seidavi), M.D., A.G., E.B.S., A.D., M.L., A.S. (Antonello Santini); supervision, A.S. (Alireza Seidavi) and A.S. (Antonello Santini). All authors have read and agreed to the published version of the manuscript.

Funding: Financial support by Rasht Branch, Islamic Azad University; Grant number 17.16.4.18418 is gratefully acknowledged.

Acknowledgments: This manuscript was prepared from the MSc thesis presented by the first author to the Islamic Azad University, Rasht Branch, Rasht, Iran. We are grateful to the Islamic Azad University, Rasht Branch, Rasht, Iran, and to the Minas Gerais Research Foundation (FAPEMIG), Brazil, for their support.

Conflicts of Interest: The authors declare that they have no conflict of interest with regard to the study presented in this report.

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