

INFLUENCE OF OLIVE-GARLIC EXTRACT OIL DIETARY SUPPLEMENTATION ON GUT MORPHOMETRY AND MORPHOLOGY, CARCASS YIELD AND ORGAN WEIGHT OF BROILERS

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ABSTRACT

The continuous use of antibiotic growth promoters in broiler production has resulted in increased rate of antibiotic resistance in humans which necessitates the search for suitable alternatives without adverse effect on humans. The effect of dietary olive-garlic extract oil (OGEO) supplementation on gut morphometry and morphology, carcass yield and relative organ weights of broilers was investigated in this study. The experiment lasted for 42 days and broilers were fed diets supplemented with varying levels of OGEO at two phases (starter (0 – 21 days) and finisher (22 – 42 days)) using two hundred and forty unsexed Ross broilers. Four experimental diets were formulated for the starter and finisher phases and the diets were designated as diet 1 which is the control (without OGEO supplementation) while diets 2, 3 and 4 were supplemented with OGEO at 1 g/kg diet, 3 g/kg diet and 5 g/kg diet respectively. Individual treatment has four replicates containing fifteen birds per replicate (60 birds per treatment). Gut morphometry and morphology, carcass yield and relative organ weights, were determined on day 42. The data generated from the study were analysed in a one-way analysis of variance using SAS 2000 and significant differences among means were determined using Tukey's test in the same software. Increased duodenal villi height (Q, $p = 0.049$) (1786.78 μm) and villi height/crypt depth ratio (L, $p = 0.012$; Q, $p = 0.006$) (3.95) was significantly increased with dietary supplementation of 1 % OGEO for broilers. Higher dressed percentage (DP) (Q, $p = 0.013$) and breast meat weight (L, $p = 0.032$; Q, $p = 0.012$) was higher for broilers fed 1 g/kg OGEO-supplemented diet than other treatments. The weight of the bursa, thymus and spleen were not significantly influenced by dietary supplementation of OGEO. In conclusion, dietary supplementation of OGEO at 1 g/kg increased duodenal villi height and DP of broilers.

Key words: broiler; olive-garlic extract oil; gut morphometry; gut morphology; carcass; organ

INTRODUCTION

Modern breeding through advanced selection for improved growth, enhanced feed efficiency and tissue accretion has resulted in the production of broilers having 1.5 – 2.0 kg live weight within 5 – 6 weeks due to high feed conversion to meat. Hassanien (2011) reported a 28 % increase in breast meat yield of broilers due to

advanced selection for tissue accumulation. Despite the qualities, broiler production in the tropics and developing countries is challenged with diseases caused by different pathogenic microorganism. This has caused a continuous use of antibiotic growth promoters in broiler production to mitigate the effect of these microorganisms. However, the growing concerns of the residual effect of antibiotics in poultry

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birds resulting in antibiotic resistance in human has necessitated the search for alternatives without negative effect on the broilers and the final consumer. The common alternatives used are the phytogenic feed additives which include spices and herbs (Botsoglou *et al.*, 2002). These botanical products are potent due to their constituent bioactive compounds (Hernandez *et al.*, 2004). These bioactive compounds exhibit antimicrobial and anti-inflammatory properties and they also have the capacity to enhance digestion through the stimulation of digestive enzymes (Moorthy *et al.*, 2009). The recent strategy in the use of these botanical products is the combination of two or more phytogenic additives to enhance its activity through synergistic effect (Oso *et al.*, 2019). Recent reports indicate that phytogenic formulations are capable of improving performance and reducing mortality (Puvaca *et al.*, 2014; Abd El-Hack *et al.*, 2020). Improved performance and immune status of broilers was achieved with dietary supplementation of Ethiopian pepper and cloves composite (Williams *et al.*, 2023).

Garlic has been one of the commonly used phytogenic additives used in place of antibiotics due to inherent bioactive constituents against invading harmful micro-organisms (Demir *et al.*, 2003; Unigwe and Igwe, 2022). The constituent allicin and diallyl-sulphides which are sulphur-containing compounds exhibits antibacterial, antifungal and antioxidant functions (Bampidis *et al.*, 2005). Improved gut health through eradication of harmful microbes resulting in better performance has been achieved with dietary supplementation of garlic for broilers (Abujradah *et al.*, 2018). Olive oil is an essential oil containing poly-phenolic compounds which are capable of limiting the proliferation of harmful microorganisms within the digestive tract as well as stimulating the release of digestive enzymes (Tufarelli *et al.*, 2015). Olive oil also contains appreciable quantity of monounsaturated fatty acids (MUFA) with higher percentage of oleic acid which performs immune-modulatory functions and resists diseases that attack the immune system (Miles and Calder, 2015). In addition, dietary fats influence lipid composition and lipoprotein composition of tissues based on fatty acid framework and olive oil due to high content of MUFA improves the diet nutritionally and consequently positively influences fatty acid profile of muscles (Stark *et al.*, 2002; Bermudez *et al.*, 2011). Based on the qualities of these phytogenic products, we hypothesised that the combination of garlic extracts

and olive oil has the higher potential to exhibit these functions through synergistic effect than individual administration resulting in better meat yield without negative residual effect. Therefore, this study aimed to investigate the influence of dietary olive-garlic extract oil supplementation on gut morphometry and morphology, carcass yield and relative organ weight of broilers.

MATERIALS AND METHODS

Experimental location

The experiment was conducted at the Teaching and Research Farm, School of Agriculture, Lagos State University, Epe Campus, Lagos State, Nigeria. It adhered to the approved guidelines for Animal Research established by the Nigeria Institute of Animal Science (NIAS-NREP-2017). The farm is situated at coordinates 6°35'09.4"N latitude and 3°59'54.7"E longitude (Google Earth, 2020). This location falls within the lowland rainforest zone of the savannah agro-ecological region, characterized by an average annual rainfall of 1,694 mm and a mean temperature of 27.1 °C.

Test ingredients and preparation

Extra-virgin olive oil was sourced from Shoprite in Sangotedo, Lagos State, while garlic bulbs were obtained from the Mile 12 International Market, Lagos State. The garlic bulbs were peeled and chopped into smaller pieces using kitchen knives. The chopped garlic was then crushed and macerated with 70 % methanol. Following maceration, the mixture was filtered and combined with the olive oil at a ratio of 1 kg of garlic to 2 litres of olive oil. The resulting mixture was heated at 35 °C for 5 minutes with gentle stirring to ensure thorough blending. After heating, the garlic residues were separated from the oil through sieving. The garlic-infused olive oil (OGEO) was allowed to cool before being transferred into a plastic container for storage until use.

Chemical composition and antioxidative capacity of test ingredients

The chemical composition of the test ingredients was analysed, and the results are presented in Table 1. Phytochemical constituents were determined using established protocols: total polyphenols following the

Table 1. Chemical composition and antioxidative capacity of garlic, olive oil and olive garlic extract oil

Composition	Garlic	Oil of olive	Olive-garlic extract oil
Gross energy	113.21 kcal/100 g	875.42 kcal/100 g	8.94.35 kcal/100 g
Total phenol	601.24 mg/100 g	45.20 mg/100 ml	81.20 mg/100 ml
Flavonoids	6.46 mg/100 g	5.26 mg/100 ml	24.01 mg/100 ml
Oleic acid	36.43 %	56.58 %	52.31 %
Linolenic acid	35.48 %	15.48 %	0.51 %
Total antioxidant (% DPPH scavenged)	28.23 mg/ml	57.30 mg/100 ml	65.72 mg/ml
Alkaloids	5.32 mg/100 g	11.04 mg/ml	12.53 mg/ml
Saponin	1.78 mg/100 g	9.50 mg/ml	6.47 mg/ml
Tannin	4800 mg/100 g	5.23 mg/ml	7.35 mg/ml

DPPH= 2,2-diphenyl-1-picrylhydrazyl

method of Wright *et al.* (2000), flavonoids as described by Arvouet-Grand *et al.* (1994), extractable tannins using Hoff and Singleton's method (1977), alkaloids following Saddiqui and Ali (1997), and saponins as outlined by Edeoga *et al.* (2005). Fatty acids, including oleic and linolenic acids, were analyzed using the American Oil Chemists' Society (AOCS) standard procedure (1998). The energy content was measured using an adiabatic bomb calorimeter (Model 1261; Parr Instrument Co., Moline, IL, USA). Total antioxidant activity was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay based on the method described by Mimica-Dukic *et al.* (2004). Absorbance readings of the sample and blank solutions were recorded after one hour at 37 °C, and the reduction of DPPH was measured spectrophotometrically at 515 nm. The radical scavenging capacity (RSC) was calculated using the formula:

$$\text{RSC (\%)} = 100 \times (\text{A}_{\text{blank}} - \text{A}_{\text{sample}}) / \text{A}_{\text{blank}}.$$

Experimental birds and management

A total of 240-day-old Ross 308 broiler chicks were procured from Agric International Technology and Trade Limited (AGRITED) hatchery in Ibadan, Nigeria. Prior to their arrival, the poultry pens were thoroughly cleaned and disinfected. The pens were preheated using an external heat source, including electric bulbs and charcoal, to ensure a suitable environment for the chicks. The brooding house temperature was maintained at 3 °C for the first three days, after which it was gradually reduced by 2 °C per week until reaching 27 °C by the end of the second week. The birds were raised on a deep litter system with wood shavings provided as bedding material. Feeding trays

and water troughs were made available to the chicks upon arrival and were replaced with appropriately sized equipment as the birds matured. During the feeding trial, the average temperature and relative humidity recorded within the experimental pen were 31.2 ± 1.28 °C and 67.84 ± 2.50 %, respectively.

Experimental design and dietary treatment

The broilers were distributed into four dietary treatments based on weight equalization. A completely randomized design was implemented, dividing the birds into 16 pens (3 × 3 m each) separated by corrugated wire mesh. Each treatment was replicated four times, with 15 birds per replicate. Experimental diets for the starter phase (0–21 days) and finisher phase (22–42 days) were formulated according to the nutrient requirements specified in the Ross Nutrition Specification (2019). Diet 1 served as the control diet without OGEO supplementation, while diets 2, 3, and 4 included OGEO supplementation at levels of 1 g/kg, 3 g/kg, and 5 g/kg, respectively. For the starter phase, the energy content of diet 1 (control) was 2930.00 kcal/kg, diet 2 was 2938.80 kcal/kg, diet 3 was 2956.40 kcal/kg, and diet 4 was 2974.00 kcal/kg. During the finisher phase, diet 1 (control) provided 3097.00 kcal/kg, diet 2 contained 3105.80 kcal/kg, diet 3 had 3123.40 kcal/kg, and diet 4 offered 3141.00 kcal/kg (Table 2). The levels of OGEO supplementation were informed by findings from prior studies on olive oil (Zhang *et al.*, 2013) and garlic (Unigwe and Igwe, 2022) in broiler diets. The trial lasted six weeks, with birds raised intensively on a deep litter system at a stocking density of 12 ft² per bird. Feed and water were provided *ad libitum* throughout the study period. No antibiotics or antimicrobials were

Table 2. Gross composition of experimental diet

Ingredients	Starter ^a	Finisher ^b
Maize	55.00	63.00
Soya bean meal	18.00	13.50
GNC	15.50	13.50
Fish meal	2.50	2.00
Wheat offal	5.00	4.00
Oyster	1.00	1.00
Lysine	0.25	0.25
Methionine	0.25	0.25
Vitamin/Mineral premix*	0.25	0.25
Salt	0.25	0.25
Total	100	100
Determined nutrients		
Metabolisable energy (kcal/kg) **	2930.00	3097.00
Crude protein (%)	22.70	19.84
Fat (%)	3.98	4.36
Crude fibre (%)	4.30	4.58
Nitrogen free extract (%)	57.29	59.07
Calcium	1.20	1.20
Phosphorous (%)	0.50	0.40
Lysine (%)	1.30	1.10
Methionine (%)	0.60	0.60
Ash (%)	2.20	1.90
*Starter premix: vit. A 10,000,000 IU; vit. D 32,500,000 IU; vit. E 23,000 mg; vit. K3 2,000 mg; vit. B1 1,800 mg; vit. B2 5,500 mg; niacin 27,500 mg; pantothenic acid 7,500 mg; vit. D6 3,000 mg; vit. B12 15 mg; folic acid 750 mg; biotin H2 60 mg; chlorine chloride 300,000 mg; cobalt 200 mg; copper 3,000 mg; iodine 1,000 mg; iron 20,000 mg; manganese 40,000 mg; selenium 200 mg; zinc 30,000 mg. *Finisher phase: vit. 8,500,000 IU; vit. D3 1,500,000 IU; vit. E 10,000 mg; vit K3 1,500 mg; vit. B1 1,600 mg; vit. B2 4,000 mg; niacin 20,000 mg; pantothenic acid 5,000 mg; vit. D6 1,500 mg; vit. B12 10 mg; folic acid 500 mg; biotin H2 750 mg; chlorine chloride 175,000 mg; cobalt 200 mg; copper 3,000 mg; iodine 1,000 mg; iron 20,000 mg; manganese 40,000 mg; selenium 200 mg; zinc 30,000 mg. **Estimated using the Nutrient Requirements of Poultry, NRC (1994) formulae, ME = 26.7 (% dry matter) + 77 (% ether extract) – 51.22 (% crude fibre). ^aAt Starter phase, Diet 1 (control) had energy of 2930.00 kcal/kg, diet 2 had 2938.80 kcal/kg, diet 3 had 2956.40 kcal/kg and diet 4 had 2974 kcal/kg. ^bAt Finisher phase, diet 1 (control) had energy of 3097 kcal/kg, diet 2 had 3105.80 kcal/kg, diet3 had 3123.40 kcal/kg and diet 4 had 3141.00 kcal/kg.		

used or incorporated into the broiler diets during the experiment.

Gut morphometry and morphology

At day 42, the gastrointestinal tract of the broilers used for carcass measurements was excised and intestinal samples (2 cm segments) were taken from the mid-region of the intestinal segments (duodenum, jejunum and ileum). The duodenum was considered from the gizzard outlet to the end of the pancreatic loop, the jejunum from the pancreatic loop to Meckel's diverticulum and the ileum from Meckel's diverticulum

to the cecal junction. The samples of the intestinal segments were cleaned with 0.1 M phosphate-buffered saline having a pH of 7.4 and the tissues were fixed in 10 % neutral-buffered formalin. Sections from each segment were embedded in paraffin wax using a standard technique to obtain 4µm thick paraffin sections and were stained with hematoxylin and eosin. Villus height and crypt depth was measured using a Nikon Phase Contrast Microscope Integrated Digital Imaging Analyses (Nikon Tec, Shinagawa, Tokyo, Japan). The villi length was measured from the tip to the villi base, and the crypt depth was measured from

the base of the villi to the base of the crypt. Likewise, the villus widths were measured at the basal and apical parts to obtain the basal, and apical widths, respectively (Jamroz *et al.*, 2006).

Carcass measurements and relative organ weights

On day 42, broilers whose weight is representative of the average weight of broilers in each pen were selected and placed in the slaughterhouse. The broilers were fasted for approximately 7 hrs to prevent the contamination of the carcasses with digesta and faeces but were given water during this period. The birds were re-weighed before being slaughtered to obtain the live body weight as described by Moses *et al.* (2022). The body weight and dressed weight were measured, while the dressing percentage was calculated. Cut parts, which include breast, back, thighs, wings, drumsticks, and abdominal fat were weighed using a digital sensitive scale and expressed as percentages of respective body weights. The organs, which include the kidney, lungs, gizzard, liver, heart, caecum, bursa, and spleen, were also weighed and calculated as percentages of respective body weights.

Statistical analysis

The collected data were subjected to a one-way analysis of variance (ANOVA) using a completely randomized design, performed with the Statistical Analysis System (SAS, 2000). Treatment means showing significant differences were separated using Tukey's test within the SAS software. A significance threshold of $P < 0.05$ was applied. Additionally, orthogonal polynomial contrast analysis was conducted to evaluate the linear and quadratic response trends associated with varying levels of OGEO supplementation.

RESULTS

Gut morphometry of broilers

Table 3 shows the gut morphometry of broilers fed the diet supplemented with OGEO at day 42. The duodenal villi height was higher (quadratic, $p = 0.049$) for broilers fed the diet supplemented with 1 g/kg OGEO as shown in Figure 1. Supplementation of OGEO at 3 g/kg resulted in higher (linear, $p = 0.041$) duodenal cryptal depth than that fed diet without OGEO

Table 3. Gut morphometry of broilers fed diet supplemented with olive-garlic extract oil

	Olive-garlic extract oil supplementation level				Pooled	P-value	
Parameters	0 g/kg	1 g/kg	2 g/kg	5 g/kg	SEM	Linear	Quadratic
Duodenum (μm)							
Villi height	1346.51 ^b	1786.78 ^a	1382.66 ^b	1596.46 ^{ab}	71.68	0.555	0.049
Villi width	166.76	173.51	213.73	176.19	9.79	0.442	0.276
Cryptal depth	416.33 ^b	452.33 ^{ab}	527.67 ^a	503.00 ^{ab}	29.52	0.041	0.079
Cryptal width	187.33	171.67	198.33	182.67	10.16	0.904	1.000
Villi height/crypt depth	3.23 ^{ab}	3.95 ^a	2.62 ^b	3.17 ^{ab}	2.05	0.012	0.006
Jejunum (μm)							
Villi height	1369.73 ^b	1342.6 ^{ab}	1463.23 ^{ab}	1727.11 ^a	55.16	0.006	0.078
Villi width	151.01	207.14	191.48	156.28	14.23	0.999	0.153
Cryptal depth	570.44	498.22	509.43	424.80	44.92	0.364	0.951
Cryptal width	158.83	205.75	182.45	168.60	11.69	0.958	0.249
Villi height/crypt depth	2.40 ^c	2.69 ^b	2.87 ^b	4.07 ^a	1.30	0.030	0.231
Ileum (μm)							
Villi height	1055.21 ^b	1228.69 ^{ab}	1501.30 ^a	1356.05 ^{ab}	57.96	0.006	0.054
Villi width	140.85 ^b	141.93 ^{ab}	158.69 ^{ab}	206.15 ^a	10.03	0.010	0.142
Cryptal depth	494.00	547.88	737.34	603.73	51.88	0.295	0.392
Cryptal width	138.22 ^b	140.75 ^{ab}	148.13 ^{ab}	201.65 ^a	9.75	0.012	0.099
Villi height/crypt depth	2.14	2.24	2.04	2.25	1.20	0.230	0.107

^{a,b}Means on the same row with differing superscripts are significantly different ($P < 0.05$)

SEM = Pooled standard error of mean

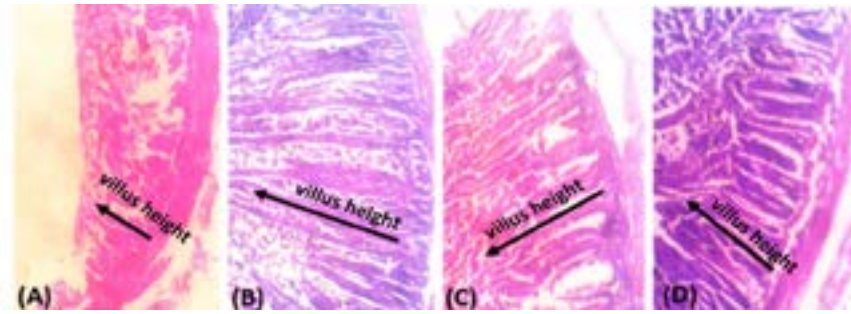


Figure 1. Morphological slides showing the villus in the duodenum of broilers of (A) control group; (B) 1 g/kg OGEO group; (C) 3 g/kg OGEO group; (D) 5 g/kg OGEO group ($\times 10$)

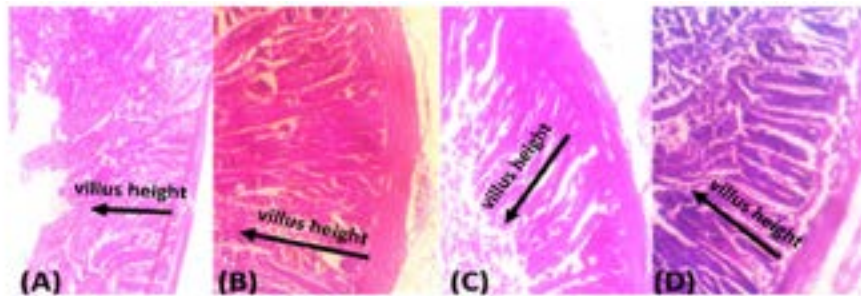


Figure 2. Morphological slides showing the villus in the jejunum of broilers of (A) control group; (B) 1 g/kg OGEO group; (C) 3 g/kg OGEO group; (D) 5 g/kg OGEO group ($\times 10$)

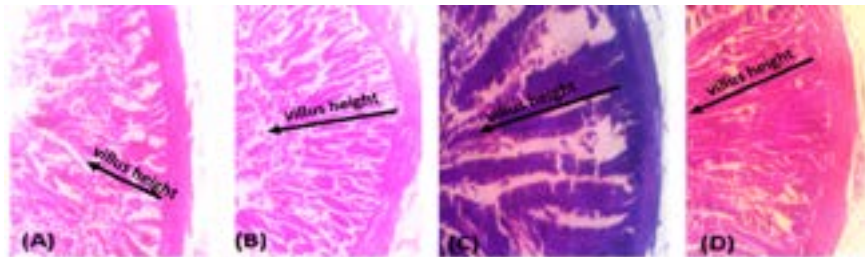


Figure 3. Morphological slides showing the villus in the ileum of broilers of (A) control group; (B) 1 g/kg OGEO group; (C) 3 g/kg OGEO group; (D) 5 g/kg OGEO group ($\times 10$)

supplementation. Broilers fed the diet supplemented with OGEO at 1 g/kg had increased (linear, $p = 0.012$; quadratic, $p = 0.006$) Villi height/crypt depth ratio (VH: CD) while those fed 3 g/kg OGEO supplemented diet had reduced VH: CD. Duodenal villi width and cryptal width were not significantly ($p > 0.05$) influenced by OGEO supplementation. In the jejunum, villi height and VH: CD was significantly ($p < 0.05$) affected by dietary supplementation of OGEO. Higher (linear, $p = 0.006$)

jejunal villi height was observed for broilers fed the diet supplemented with OGEO at 5 g/kg than those fed diet without OGEO supplementation as shown in Figure 2. Jejunal VH: CD increased (quadratic, $p = 0.031$) with supplementation of OGEO at 5 g/kg in the diet of broilers but those fed the control diet had the lowest VH: CD. In the ileum, cryptal depth and VH: CD were not significantly ($p > 0.05$) influenced by the supplementation of OGEO. Supplementation of OGEO

at 3 g/kg in the diet of broilers resulted in higher (linear, $p = 0.006$; quadratic, $p = 0.054$) ileal villi height than those fed the diet supplemented without OGEO as shown in Figure 3. The broilers fed the diet supplemented with 3 g/kg OGEO had higher (linear, $p = 0.010$) ileal villi width and higher (linear, $p = 0.012$) ileal cryptal width compared to those fed the control diet.

Carcass characteristics of broilers

The carcass characteristics of broilers fed the diets supplemented with OGEO are presented in Table 4. The supplementation of OGEO irrespective of inclusion level did not significantly affect feed intake (linear, $p = 0.981$; quadratic, $p = 0.688$). Supplementation of OGEO at 1 g/kg resulted in higher live weight (LW) (linear, $p = 0.006$; quadratic, $p = 0.029$), dressed weight (DW) (quadratic, $p = 0.003$) and dressing percentage (DP) (quadratic, $p = 0.013$) compared to other treatments. The breast weight was highest (linear, $p = 0.032$; quadratic, $p = 0.012$) for broilers fed the diet supplemented with 1 g/kg OGEO than other treatment groups. Supplementation of OGEO at 1 g/kg resulted in the highest (linear, $p = 0.005$; quadratic, $p = 0.043$) thigh weight, while those fed the control diet, had the lowest thigh weight. Supplementation of 5 g/kg OGEO resulted in increased (linear, $p = 0.033$; quadratic $p = 0.035$) drumstick weight and those fed 1 g/kg OGEO-supplemented diet had reduced drumstick weight. Broilers fed the diet supplemented with 5 g/kg OGEO had the lowest (linear, $p = 0.007$) abdominal fat while those fed diet

without OGEO supplementation had the highest abdominal fat while those fed diets supplemented 1 g/kg and 3 g/kg OGEO had intermediate abdominal fat.

Organ weight of broilers

Table 5 shows the relative organ weight of broilers fed the diets supplemented with OGEO. Empty gizzard weight (EGW) was higher (quadratic, $p = 0.015$) for broilers fed diets supplemented with 1 g/kg and 3 g/kg OGEO. Broilers fed the diet supplemented with 3 g/kg OGEO had increased (linear, $p = 0.008$) proventriculus weight. Whole GIT liver, heart, bursa, thymus and spleen weight, were not significantly ($p > 0.05$) affected by OGEO supplementation.

DISCUSSION

The observed changes in intestinal morphology can reveal the effects and benefits of the combined influence of olive oil and garlic extract on the digestive tract. In the current study, broilers fed the diet supplemented 1 g/kg OGEO had increased duodenal villi height and duodenal VH: CD. The increased villi height and VH: CD implies more surface area that will permit increased absorption of nutrients (Iji *et al.*, 2001). Precisely, the increased VH: CD observed for the group of broilers fed diets supplemented with 1 g/kg OGEO suggests improved nutrient utilization capacity. It was stated by Montagne *et al.* (2003) that

Table 4. Carcass measurements of broilers fed diet supplemented with olive-garlic extract oil

Parameters	Olive-garlic extract oil supplementation level				Pooled SEM	P-value	
	0 g/kg	1 g/kg	2 g/kg	5 g/kg		Linear	Quadratic
Feed intake (g)	3039.93	3177.47	3006.73	3098.80	29.88	0.981	0.688
Live weight (g)	1404.83 ^b	1591.84 ^a	1458.71 ^b	1419.59 ^b	43.45	0.006	0.029
Dressed weight (g)	1095.33 ^b	1349.33 ^a	1152 ^b	1064.67 ^b	37.45	0.142	0.003
Dressing percentage (%)	70.49 ^b	76.53 ^a	70.65 ^b	71.69 ^b	0.82	0.539	0.013
Cut parts (% LW)							
Breast	18.78 ^b	19.59 ^a	18.92 ^b	17.61 ^b	19.69	0.032	0.012
Wings	9.80	8.32	8.23	8.13	7.36	0.217	0.328
Thigh	9.90 ^c	12.07 ^a	11.43 ^b	11.18 ^b	12.08	0.005	0.043
Back	16.36	16.44	15.89	16.01	6.83	0.613	0.463
Drumstick	10.51 ^{ab}	9.17 ^b	9.90 ^{ab}	10.74 ^a	4.04	0.033	0.035
Abdominal fat	1.91 ^a	1.32 ^{ab}	0.80 ^{ab}	0.53 ^b	1.24	0.007	0.502

^{a,b}Means on the same row with differing superscript are significant ($P < 0.05$)

SEM = Pooled standard error of mean

Table 5. Relative organ weight of broilers fed diet supplemented with olive-garlic extract oil

Parameters (% LW)	Olive-garlic extract oil supplementation level				Pooled SEM	P-value	
	0 g/kg	1 g/kg	2 g/kg	5 g/kg		Linear	Quadratic
Whole GIT weight	14.11	14.83	13.81	12.95	0.36	0.092	0.316
Empty gizzard weight	1.72 ^b	2.50 ^a	2.54 ^a	1.98 ^b	0.01	0.087	0.015
Proventriculus	0.33 ^b	0.33 ^b	2.00 ^a	1.00 ^{ab}	0.23	0.008	0.067
Pancreas	0.25	0.27	0.35	0.31	0.02	0.377	0.345
Liver	2.32	2.45	2.63	2.48	0.09	0.160	0.282
Heart	0.55	0.44	0.57	0.58	0.02	0.760	0.067
Bursa	0.15	0.14	0.21	0.15	0.01	0.056	0.347
Thymus	0.93	0.93	0.40	0.40	0.01	0.620	1.000
Spleen	0.13	0.13	0.17	0.12	0.01	0.058	0.500

^{a,b}Means on the same row with differing superscript are significant ($P < 0.05$)

SEM = Pooled standard error of mean

the VH: CD is an important index in evaluating nutrient utilization efficacy of the intestine. It is important to note that the group of broilers with increased duodenal villi height also recorded improved BW and WG which indicates that there is a direct linkage between villi height and body weight gain. Solis de los Santos *et al.* (2007) observed from the investigations on the effect of mannan-oligosaccharide yeast extract supplementation in turkey poults that increased body weight gain is associated with increased villi height. Reduced duodenal crypt depth was also observed for the broilers fed the 1 g/kg OGEO-supplemented diet and this indicates reduced cell turnover which is beneficial for intestinal health. The energy consumed during cellular turnover reduces the nutrient resources that should be channeled towards increased growth and therefore lower crypt depth enhance performance (Marković *et al.*, 2009). The jejunal villi height of broilers fed the diet supplemented with 5 g/kg OGEO was significantly higher than those fed the control diet. In the jejunum also, the VH: CD was higher for broilers fed the 5 g/kg OGEO-supplemented diet than other treatments although, the group fed the control diet had the least VH: CD. The increase in jejunal villi height and VH: CD following the use of OGEO implies that the functional constituents of these additives positively influence the microstructures of the intestine to enhance nutrient utilisation. The linear increase in jejunal villi height is of great advantage to the broilers since a substantial portion of digested food products are absorbed and completed in the jejunum (Oso *et al.*, 2019). The observation in the present study

is similar to the report of Mohammadi *et al.* (2014) who observed increased villi height in the jejunum of broilers fed a diet supplemented with clove essential oil at 100–500 mg/kg diet at day 42. Su *et al.* (2021) also observed linear and quadratic increases in villus-crypt depth ratio when a combination of essential oils ((3.05 % thymol, 2.3 % carvacrol and 0.26 % cinnamaldehyde, the carrier is dextrin) was supplemented in the diet of broilers at 50 to 400 mg/kg on day 21. Increased ileal VH was obtained for broilers fed the 3 g/kg OGEO-supplemented diet while the villi width and cryptal width increased for broilers fed the 5 g/kg OGEO-supplemented diet. The increase observed signifies improved intestinal morphology which will result in increased secretion of brush board enzymes for improved nutrient intake (Viveros *et al.*, 2011).

The carcass measurements show that supplementation of 1 g/kg OGEO in the diet of broilers resulted in a significantly higher increase in LW, DW and DP than in other treatments. However numerically, the group of broilers fed the control diet had the least LW, DW and DP. The increase in these carcass parameters as obtained with the group of broilers fed the 1 g/kg OGEO supplemented diet is a reflection of increased performance in which broilers fed 1 g/kg OGEO supplemented diet had significantly higher BW and WG than other treatments. The increased DW and DP further reveal the beneficial effect of garlic and olive oil in positively influencing nutrient assimilation for improved performance. Increased breast meat weight and thigh weight were also observed for broilers fed the 1 g/kg OGEO supplemented diet. The nutrient content

of olive oil including the fat content which is composed majorly of monounsaturated fatty acid is essential for energy production thereby promoting weight gain (Chiofalo *et al.*, 2004; Serafini and Tonetto, 2019). Olive is also known to contain phenolic compounds such as gallic acid, oleuropein, tyrosol and hydroxytyrosol (Cioffi *et al.*, 2010) which inhibits the growth of intestinal harmful microorganisms to enhance nutrient availability thereby promoting tissue accretion. Garlic with its complementary effect contains bioactive compounds such as diallyl polysulphides which makes it a potent antimicrobial additive and it is known to exhibit a strong effect on the digestive and the immune system of animals (Amagese *et al.*, 2001; Al-Shuwaili *et al.*, 2015). However, Zhang *et al.* (2013) observed no significant effect of olive oil inclusion at 2 and 5 % levels in the diet of broilers on eviscerated carcass weight and breast meat weight. The contrary report could be due to differences in the diet composition, management procedures, olive oil source and processing methods which may influence the composition of active constituents. It is important to state that in the current study that the dietary supplementation of OGEO beyond 1 g/kg resulted in reduced DW, DP, breast meat weight and thigh weight. The reduction observed at 3 g/kg and 5 g/kg supplemental levels of OGEO suggests that the inhibitory activity of the constituents of olive oil and garlic on fat accumulation were synergistically potent at this level which can reduce weight gain. The active components of phytogenic additives called essential oils are known to exhibit antioxidative, anti-inflammatory and hypolipidemic properties (Viuda-Martos *et al.*, 2010). The mono-unsaturated fatty acids of olive oil are also capable of altering the fatty acid profile of the animal tissues (Nakbi *et al.*, 2010). This is in agreement with the findings of Onu *et al.* (2010) and Stanačev *et al.* (2011), the authors reported that the use of garlic in higher doses of 3 % and above have a depressive effect on weight gain due to the effect of antinutritional substances. The abdominal fat was reduced with the supplementation of OGEO in the diet of broilers but the group of broilers fed the 5 g/kg OGEO-supplemented diet had the lowest abdominal fat. The reduction in abdominal fat is associated with the impeding effect of garlic and olive oil on fat accumulation. Oleuropein and hydroxytyrosol which are active constituents of olive oil are active against low-density lipoprotein oxidation as well as able to reduce 3-hydroxy-3-methylglutaryl coenzyme A secretion which is responsible

for cholesterol synthesis (Patrick and Uzick, 2001). In addition, the phenolic constituents of olive oil interfere with cholesterol synthesis by reducing serum and liver triglyceride (Sarica and Toptas, 2014). The reduced abdominal fat observed for broilers with the supplementation of OGEO is in accordance with the report of Milošević *et al.* (2013) who reported lower abdominal fat for broilers fed a diet supplemented with 1.5 and 3 % garlic compared to those fed a control diet. The outcome of the present study further confirms the hypolipidemic property of garlic and also reveals the synergistic effect of olive oil and garlic on inhibiting fat accumulation.

The result of the organ weights shows that the EGW was higher with the supplementation of 1 g/kg and 3 g/kg OGEO in the diet of broilers. The reason behind the rise in EGW is not clear but it could be associated with the rate of feed retention or adherence to the gizzard wall due to oil content which may increase pressure in the gizzard causing thickening of the muscles. Increased proventriculus weight was also observed for broilers fed the 3 g/kg OGEO-supplemented diet. The increase in proventriculus weight may not pose or indicate any form of danger to the birds since mortality was not high in this group. The lymphoid organs (bursa, spleen and thymus) including the pancreas weight were not significantly influenced by OGEO supplementation in the diet of broilers. This observation indicates that the use of OGEO had no adverse effect on the organs because negative changes in these organs make the animal to be susceptible to infections. The organs especially the lymphoid organs and precisely the bursa is essential for the production of the lymphocyte cells (B and T) that are responsible for the defense of the body against diseases (Latif *et al.*, 2014). The result of the current study is similar to the report of Elbaz *et al.* (2022) who observed no significant effect of dietary supplementation of garlic essential oil (200 mg/kg diet) on the spleen and thymus weight of broilers. The outcome of the current investigation on organ weights corroborates the report of Sayehban *et al.* (2015) who did not observe a significant influence of olive pomace inclusion at 5 and 10 % on lymphoid organ weight of broilers. Odunowo and Olumide (2019) also reported no effect of dietary garlic powder supplementation on bursa and thymus weight. The non-significant difference in liver and heart weight across treatments further affirms that the use of OGEO did not disrupt the normal health status of the birds.

CONCLUSION

The supplementation of OGEO at 1 g/kg and 3 g/kg in the diet of broilers increased duodenal and ileal villi height respectively. Furthermore, the DW, DP and breast meat weight increased with dietary 1 g/kg OGEO supplementation while abdominal fat was reduced. In addition, the organs were not negatively affected by dietary OGEO supplementation. Therefore, this study reveals that OGEO can be supplemented in the diet of broilers at 1 g/kg as an alternative to antibiotics for improved gut health and carcass yield.

AUTHOR'S CONTRIBUTIONS

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All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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