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Original Research Article

Dietary olive-garlic extract oil supplementation: influence on performance and haematological indices of broilers

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Abstract

The use of antibiotic growth promoters in broiler production has been the norm in developing countries, however, the increased rate of antibiotic resistance in humans has resulted in the search for alternatives without negative residual effects. This study investigated the effect of dietary olive garlic extract oil (OGEO) supplementation on the performance and haematological indices of broilers. The experiment lasted for 42 days and broilers were fed varying levels of OGEO-supplemented diets at the starter (0–21 days) and finisher (22–42 days) phases using two hundred and forty unsexed Ross broilers. Four experimental diets were formulated for the two phases and the diets were designated as diet 1(control (without OGEO)) while diets 2, 3, and 4 were supplemented with OGEO at 1% (10 g/kg diet) 3% (30 g/kg diet) and 5% (50 g/kg diet). Each had four replicates containing fifteen birds per replicate. Performance was measured weekly and haematological indices were determined on days 21 and 42. Data collected were analysed in a one-way analysis of variance (SAS, 2000) and significant differences were determined using Tukey's test. Body weight (BW) increased linearly (L) ($p = 0.003$) and quadratically (Q) ($p = 0.006$) with dietary supplementation of OGEO at 1% for broilers on day 21. Similarly, at day 42, BW increased (L, $p = 0.006$; Q, $p = 0.029$) for the broiler on the same treatment. Mortality reduced (Q, $p = 0.035$) for broilers fed 1% OGEO-supplemented diet at day 42. At day 21, Packed cell volume (PCV, 47.33%) (Q, $p = 0.033$) and mean corpuscular volume (MCV, 130.83pg) (Q, $p = 0.008$) increased for broilers fed 1% and 3% OGEO supplemented diet respectively. Supplementation of OGEO at 3% increased (Q, $p = 0.036$) PCV (37.33%) and haemoglobin (Q, $p = 0.021$) (137.30 g/L) at day 42. In conclusion, the supplementation of OGEO in the diet of broilers at 1% resulted in improved WG and reduced mortality. The PCV and haemoglobin of broilers can be increased with the supplementation of OGEO up to 3% in the diet of broilers; it is thus a suitable alternative to antibiotics for improved performance and immunity.

Keywords: broiler; garlic; olive oil; weight gain; WBC; RBC; PCV; haemoglobin

INTRODUCTION

Broiler meat production plays a crucial role in the strategies for controlling shortage in protein intake and it stands as the fastest means of mitigating this problem because it is a fast-growing industry in the world (Bansal et al., 2011). The use of modern technologies and intensive systems of production has also helped the sector's advancement. However, broilers reared intensively cannot be separated from the challenges of diseases, and coupled with the fast metabolic rate of broilers for increased protein synthesis, their immune system heavily contends with diseases caused by

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

Composition	Garlic	Olive oil	OGEO
Total phenol	601.24 mg/100g	45.20 mg/100ml	81.20 mg/100ml
Flavonoids	6.46 mg/100g	5.26 mg/100ml	24.01 mg/100ml
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/100g	11.04 mg/ml	12.53 mg/ml
Saponin	1.78 mg/100g	9.50 mg/ml	6.47 mg/ml
Tannin	4800 mg/100g	5.23 mg/ml	7.35 mg/ml

OGEO = Olive garlic extract oil; DPPH = 2,2-diphenyl-1-picrylhydrazyl

bacteria, viruses, and fungi (Diaz Carrasco et al., 2019). Despite the growing concerns that the use of antibiotics causes harmful residues in meat leading to antibiotic resistance in consumers (Durairajan et al., 2021), it is still used as growth promoters to submerge infections for improved feed conversion and good economic return (Suresh et al., 2018). Therefore, there is a need to investigate suitable alternatives that can compete favourably with antibiotics without residual harmful effects on human consumption.

In recent times, phytogenic additives and essential oils have been explored as alternatives to antibiotics (Oso et al., 2019; Du et al., 2016). The novel strategy employed in the use of phytogenic additives is the combination of two or more phytogenic additives; the report by Cetin et al. (2016) indicates that the combination of two or more phyto-additives promotes the synergistic effect of active bio-constituents. Notwithstanding, contrasting results have been reported which creates a gap that needs to be bridged and this calls for strategies to improve the potency of these additives to compete effectively with antibiotics. Garlic has been used extensively in broiler diets because of the proven effects of the bioactive components (Hanieh et al., 2010). This spice contains sulphur-containing compounds such as allicin, diallyl-sulphides and allicin which perform antibacterial, antifungal, and antioxidant functions (Adibmoradi et al., 2006). The use of olive oil has been proven with many health benefits and as a carrier of essential oils, it exhibits various biological characteristics including immune-modulating properties, antibacterial, and antioxidant effects (Lee et al., 2020). Olive oil contains a high amount of monounsaturated fatty acids which is about 80% of the total oil especially oleic acid (Quintero-Flórez et al., 2015). Based on these properties, it was therefore hypothesised in the present study that a combination of the extract of garlic and olive oil may reduce the effect of pathogenic organisms for better immune status and performance. The study was therefore conducted to evaluate the effects of dietary olive-garlic extract oil supplementation on the performance and haematological indices of broilers.

MATERIALS AND METHODS

Experimental location

The experiment was executed at the Teaching and Research Farm, School of Agriculture, Lagos State University, Epe campus, Lagos State, Nigeria by approved guidelines for Animal Research by the Nigeria Institute of Animal Science in Nigeria (NIAS-NREP-2017). The farm is located on Latitude 6°35′32.4″N and Longitude 3°59′54.7″E. (Google Earth, 2020) It lies in the low land rain forest within the savannah agro-ecological zones with a mean annual rainfall of 1694 mm and temperature of 27.1 °C.

Test ingredients and preparation

The extra-virgin olive oil was purchased from Shoprite Sangotedo, Lagos State. The garlic was purchased from Mile 12 international market, Lagos State. The garlic bulb was peeled and slashed to pieces using kitchen knives. The slashed garlic was crushed and macerated with the use of methanol (70%). After maceration, it was filtered and infused into the olive oil and heated for 5 minutes at a controlled temperature of 35 °C while stirring gently to ensure a proper mix. The garlic and olive oil were mixed at a ratio of extract from 1 kg garlic bulb to 2 litres of olive oil. After heating, the remains of the garlic bulb were sieved from the oil while the olive-garlic extract oil (OGEO) was left to cool and then stored in a plastic container before use.

Chemical composition and antioxidative capacity of test ingredients

Samples of the test ingredients were taken and analysed for their chemical composition

Table 2. Gross composition of experimental diet

Ingredients	Starter	Finisher
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
Phosphorus (%)	0.50	0.40
Lysine (%)	1.30	1.10
Methionine (%)	0.60	0.60
Ash (%)	2.20	1.90

*Starter premix: vit. A10,000,000 IU, vit. D 32,500,000 IU, vit. E 23,000 mg, vit. K_3 2,000 (mg), vit. B1 1,800 (mg), vit. B_2 5,500 (mg), niacin 27,500 (mg), pantothenic acid 7,500 (mg), vit. D_6 3,000 (mg), vit. B_{12} (15 mg), folic acid (750 mg), biotin H_2 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. D_3 1,500,000 IU, vit. E 10,000 mg, vit K_3 1,500 mg, vit. B_1 1,600 mg, vit. B_2 4,000 mg, niacin 20,000 mg, pantothenic acid 5,000 mg, vit. D_6 1,500 mg, vit. B_{12} 10 mg, folic acid 500 mg, biotin H_2 750 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.
**Calculated using the formulae, ME = 26.7 (% dry matter) + 77 (% ether extract) – 51.22 (% crude fibre) Nutrient Requirements of Poultry, NRC (1994)

(Table 1). The phytochemical constituents were analysed following standard procedures; total polyphenol (Wright et al., 2000), flavonoid (Arvouet-Grand et al., 1994), extractable tannin (Hoff and Singleton, 1977), alkaloids (Saddiqui and Ali, 1997) and saponin content (Edeoga et al., 2005). The fatty acid (oleic and linolenic acid) was determined using the standard method of the American Oil Chemists' Society (AOCS) 1998. The total antioxidant analysis using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out using the methods previously described by Mimica-Dukic et al. (2004). The absorbance of the obtained solutions and the blank were recorded after one hour at 37 °C. The disappearance of DPPH was measured spectrophotometrically at 515 nm. The radical scavenging capacity (RSC) percentage was calculated using the equation below:

$$RSC\ (\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

Experimental birds and management

Two hundred and forty (240) day-old broilers (Ross 308) were purchased from Agric International Technology and Trade Limited (AGRITED) hatchery in Ibadan Nigeria. Cleaning and disinfection of the poultry pens were done before the arrival of birds. The pens were preheated before the arrival of the birds with the use of an external heat source (electric bulb and charcoal). The temperature of the brooding house was regulated at 31 °C for the first three days and was reduced by 2 °C each week to an ambient temperature of 27 °C at the end of two weeks. The birds were reared on a deep litter system, with wood shavings provided as bedding to the birds. Feeding trays and water troughs were provided for the chicks on arrival and they were changed to appropriate sizes as the birds advanced in age. The range of temperature and relative humidity within the experimental pen during the feeding trial was 31.2 ± 1.28 °C and 67.84 ± 2.50% respectively.

Experimental design and dietary treatment

The broilers were allotted on a weight equalisation basis to four dietary treatments. A completely randomised design was used to allot the birds to 16 pens (each measuring 3 × 3 m) partitioned using corrugated wire mesh. Each treatment was replicated 4 times with 15 birds per replicate. The experimental diets formulated for the starter (0–21 days) and finisher phase (22–42 days) were based on the nutrient requirement guide (Ross Nutrition Specification, 2019). 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% (10 g/kg diet) 3% (30 g/kg diet) and 5% (50 g/kg diet), respectively (Table 2). The dietary supplementation of OGEO was based on reports from previous literature on the use of olive oil (Zhang et al., 2013) and garlic (Unigwe and Igwe, 2022) in the diet of broilers. The experiment lasted for six weeks and all birds were raised intensively using a deep litter system with a spacing of 12 feet per bird while feed and water were supplied ad-libitum during the experimental period. Antimicrobials or antibiotics were not administered or added to the feed of the broilers throughout the feeding trial.

Performance measurements

The performance of broilers was monitored and determined by measuring initial weight (IW), weight gain (WG), feed intake (FI), calculation of feed conversion ratio (FCR), and mortality. The experiment commenced from a day old and the IW of broiler chicks were taken on arrival and subsequent WG were recorded at the end of every week for six weeks of the experiment. The WG was calculated as the difference between the body weights for the given week and the previous week and the final weight was taken and recorded at the end (42nd day) of the experiment. The FI was measured by giving a known quantity of feed to broiler chicken while the leftover feed was weighed to determine FI for each treatment. The FCR was determined by calculating the ratio of feed intake to weight gain. The occurrence of mortalities was recorded throughout the experimental period and expressed in percentage.

Blood collection and haematological indices

On the 21st and 42nd days of the study, blood samples (2.5 ml) were collected from the brachial wing of three birds per replicate into bottles with ethylene diamine tetra-acetate (EDTA) for the determination of haematological variables. The Packed Cell Volume (PCV) was determined using the microhaematocrit method in capillary tubes and centrifuged for 5 minutes at 12,000 × *g* according to Van Beekvelt et al. (2001). The haematology cell counter MS4® (Melet Schloesing Laboratories, France) was used for the counting of the Red Blood Cell (RBC). Hemoglobin concentration (Hb) was determined using the Randox® reagent and evaluated using a spectrophotometer (Spectronic 20 Genesys). Total White blood cell (WBC) count and WBC differentials were carried out on blood smears stained with May-Grunwald-Giemsa stain (Blue-Mclendon and Green, 2010). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Feldman et al. (2000).

Statistical analysis

All data collected were analysed using one-way analysis of variance (ANOVA) in a completely randomised design using Statistical Analysis System (SAS 2000). The significant means among treatments were separated using the Tukey test in the SAS statistical tool. The level of significance was considered at $p < 0.05$. Orthogonal polynomial contrast analysis was also done to determine the linear and quadratic response trend to varying levels of OGEO supplementation.

Table 3. Performance of broilers fed the diet supplemented with olive-garlic extract oil

Parameters	Olive-garlic extract oil supplementation levels				S.E.M.	P-value	
	0%	1%	3%	5%		Linear	Quadratic
Body weight, g/bird							
1 d	40.00	39.50	39.75	39.25	0.20	0.309	1.000
21 d	533.00^{b}	583.25^{a}	530.75^{b}	536.25^{b}	7.98	0.003	0.006
42 d	1404.83^{b}	1591.84^{a}	1458.71^{b}	1419.59^{b}	43.45	0.006	0.029
Weight gain, g/bird							
1–21 d	473.00^{b}	501.11^{a}	472.09^{b}	496.67^{b}	7.93	0.003	0.006
22–42 d	871.83	889.41	777.04	883.53	25.47	0.840	0.452
1–42 d	1344.83	1390.53	1249.13	1380.19	30.36	0.419	0.230
Feed intake, g/bird							
1–21 d	1043.87	1096.60	1035.80	1052.50	21.95	0.871	0.709
22–42 d	1996.07	2080.87	1970.93	2046.30	17.96	0.778	0.884
1–42 d	3039.93	3177.47	3006.73	3098.80	29.88	0.981	0.688
Feed: gain, g/g							
1–21 d	2.19	2.18	2.19	2.12	0.05	0.210	0.115
22–42 d	2.23	2.33	2.54	2.32	0.07	0.818	0.280
1–42 d	2.30	2.28	2.41	2.25	0.05	0.470	0.148
Mortality, %							
1–21 d	2.50	1.75	1.75	1.75	0.84	0.531	0.640
22–42 d	5.00^{a}	0.00^{d}	1.64^{c}	3.50^{b}	1.04	0.770	0.027
1–42 d	7.42^{a}	1.75^{d}	3.39^{c}	5.14^{b}	1.20	0.430	0.035

$^{a–b}$Means on the same row with differing superscripts are significantly different ($p < 0.05$)
S.E.M. = Standard error of the mean

RESULTS

Effect of olive-garlic extract oil supplementation on performance of broilers

Table 3 shows the performance of broilers fed the diet supplemented with varying levels of olive-garlic extract oil (OGEO). Their body weight (BW) was significantly ($p < 0.05$) affected on days 21 and 42 by supplementation of OGEO. Broilers fed the diet containing 1% OGEO had increased (linear, $p = 0.003$; quadratic, $p = 0.006$) BW at day 21. A similar pattern was observed for BW at day 42, broilers fed the diet with OGEO at 1% had higher (linear, $p = 0.006$; quadratic, $p = 0.029$) BW compared to other treatments. Weight gain (WG) of broilers varied significantly linearly and quadratically during the 21 days rearing period in which broilers fed the diet containing 1% OGEO had higher (linear, $p = 0.003$; quadratic, $p = 0.006$) WG compared to other treatments. Mortality during 22–42 days was highest for broilers fed the diet supplemented with 0% OGEO while those fed the diet containing 1% OGEO had the least mortality (quadratic, $p = 0.027$). Mortality during the entire rearing period (1–42 days) was significant quadratically ($p = 0.035$) among treatments. Broilers fed the diet with 0% OGEO had the highest (7.42%) mortality whereas those fed the diet with 1% OGEO had the least (1.75%) mortality. Feed intake (FI) and feed conversion ratio (FCR) were not affected significantly ($p > 0.05$) by dietary treatment throughout the entire rearing period.

Effect of olive-garlic extract oil supplementation on haematological indicators of broilers

The haematological indicators of broilers fed diets supplemented with OGEO are shown in Table 4. At day 21, packed cell volume (PCV), red blood cell (RBC), mean corpuscular haemoglobin (MCV), and mean corpuscular haemoglobin concentration (MCHC) were significantly ($p < 0.05$) affected by dietary supplementation of OGEO while other parameters were not significantly ($p > 0.05$) affected. Supplementation of 1% OGEO resulted in a quadratic increase ($p = 0.033$) in PCV and broilers fed diet without OGEO supplementation had reduced PCV. RBC was

Table 4. Haematological parameters of broilers fed diet supplemented with olive-garlic extract oil

Parameters	Olive-garlic extract oil supplementation levels				Reference values	S.E.M.	P-value	
	0%	1%	3%	5%			Linear	Quadratic
Day 21								
PCV (%)	32.67^{c}	47.33^{a}	34.33^{bc}	41.00^{ab}	#22.00–35.00	1.87	0.123	0.033
Haemoglobin (g/L)	111.70	147.00	124.30	135.30	#70.00–130.00	0.49	0.146	0.108
Red blood cell (× 10^{12}/L)	1.97^{b}	3.67^{ab}	2.10^{ab}	4.00^{a}	#2.50–3.50	0.33	0.047	0.819
White blood cell (× 10^{9}/L)	13.33	14.87	13.93	14.80	#21.18–22.17	0.34	0.279	0.631
Heterophil (%)	25.33	26.67	31.00	29.00	NR	1.09	0.134	0.441
Lymphocyte (%)	73.00	71.67	67.00	69.67	#45.00–70.00	1.10	0.142	0.350
Eosinophil (%)	0.33	0.33	0.33	0.33	ǂ1.50–6.00	0.14	1.000	1.000
Basophil (%)	0.33	0.33	0.67	0.67	NR	0.15	0.397	1.000
MCV (fl)	107.03^{b}	122.85^{ab}	130.83^{a}	116.27^{ab}	ǂ104.00–135.00	3.21	0.102	0.008
MCH (pg)	39.05	40.34	42.11	36.59	ǂ32.00–43.90	0.90	0.444	0.061
MCHC (%)	31.16^{b}	32.65^{ab}	36.27^{a}	31.73^{b}	ǂ30.20–36.20	0.70	0.195	0.007
Day 42								
PCV (%)	28.33^{b}	30.33^{ab}	37.33^{a}	30.00^{ab}	#22.00–35.00	1.31	0.185	0.036
Haemoglobin (g/L)	99.30^{b}	103.00^{b}	137.30^{a}	106.00^{b}	#70.00–130.00	0.53	0.083	0.021
Red blood cell (× 10^{12}/L)	2.53	2.70	2.90	2.90	#2.50– 3.50	0.09	0.836	0.142
White blood cell (×10^{9}/L)	14.33^{a}	13.23^{ab}	11.53^{b}	12.80^{ab}	#21.18–22.17	0.39	0.037	0.069
Heterophil (%)	37.00^{a}	26.67^{b}	25.00^{b}	29.00^{ab}	NR	1.60	0.016	0.005
Lymphocyte (%)	78.67	69.67	67.00	68.33	#45.00–70.00	1.83	0.440	0.009
Eosinophil (%)	0.00	0.67	0.33	0.33	ǂ1.50–6.00	0.14	0.620	0.282
Basophil (%)	0.67	0.33	0.00	0.33	NR	0.14	0.332	0.282
MCV (fl)	111.35	112.9	111.72	103.17	ǂ104.00–135.00	2.33	0.258	0.316
MCH (pg)	34.14^{c}	35.96^{bc}	38.24^{ab}	40.22^{a}	ǂ32.00–43.90	0.81	<0.001	0.483
MCHC (%)	33.32^{b}	34.03^{ab}	34.35^{ab}	36.12^{a}	ǂ30.20–36.20	0.78	0.009	0.128

$^{a–b}$Means on the same row with differing superscripts are significantly different ($p < 0.05$), S.E.M. = Standard error of the mean, PCV = Packed Cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, #Abdulazeez et al. (2016), ǂHarrison and Lightfoot (2005), NR = Not reported significantly higher linearly ($p = 0.047$) for broilers fed the diet supplemented with 5% OGEO than broilers fed the diet without OGEO supplementation. Quadratic increase ($p = 0.008$) in MCV resulted in broilers with OGEO supplementation. Broilers fed diet with 3% OGEO supplementation had higher MCV compared to those fed diet with 0% OGEO supplementation however, similar to those fed diet containing 1% OGEO and those fed diet containing 5% OGEO. Dietary supplementation of OGEO at 3% also resulted in increased (quadratic, $p = 0.007$) MCHC compared to that fed diet with 5% OGEO and that fed diet with 0% OGEO.

At day 42, PCV, haemoglobin, white blood cell (WBC), heterophil, MCH and MCHC were significantly ($p < 0.05$) influenced by OGEO supplementation. Broilers fed the diet supplemented with 3% OGEO had increased (quadratic, $p = 0.036$) PCV compared to that fed diet without OGEO supplementation. Haemoglobin was significantly higher (quadratic, $p = 0.021$) in broilers fed the diet supplemented with OGEO at 3% than in other treatments. WBC count Increased linearly ($p = 0.037$) in broilers fed the diet without OGEO supplementation while those fed the diet with OGEO at 3% supplementation had reduced WBC count. Heterophil count was also increased (linear, $p = 0.016$; quadratic, $p = 0.005$) in broilers fed the diet without OGEO supplementation but reduced for broilers fed the diet supplemented with 1% OGEO and those fed diet supplemented with OGEO at 3%. MCH varied significantly (linear, $p < 0.001$) across treatments in which broilers fed diets supplemented with 3 and 5% OGEO had increased MCH whereas broilers fed the diet with 0% OGEO had reduced MCH. Dietary supplementation of OGEO resulted in a linear increase ($p = 0.009$) in MCHC. Broilers fed the diet supplemented with 5% OGEO had the highest MCHC but MCHC reduced significantly for broilers fed the diet without OGEO supplementation.

DISCUSSION

Performance

The BW was influenced by OGEO supplementation in the diet of broilers and dietary supplementation of 1% OGEO at day 21 resulted in higher BW than other treatments. The increase in BW is a result of the growth-promoting properties of garlic and olive oil. The use of garlic in the diet of broilers has been recognised for its role in the stimulation of digestive enzymes by acting as a prebiotic for increased nutrient utilisation in poultry birds (Al-Shuwaili et al., 2015). A similar increase in BW was also observed on day 42 in which broilers fed the diet supplemented with 1% OGEO had increased BW. This is still connected to the beneficial effect of the use of garlic in diets; the result obtained in this study is in agreement with the report of Puvaca et al. (2014) who observed an increase in BW of broilers fed diets containing garlic supplemented at 0.5 and 1.0%. Makwana et al. (2015) also reported improved BW of broilers with dietary supplementation of garlic at 0.1% compared to those fed the control diet. The increased BW obtained for broilers fed a 1% OGEO-supplemented diet could also be attributed to the influence of olive oil which provides nutrients through the constituent polyunsaturated fatty acids (PUFA) also containing fat-soluble vitamins (Stark et al., 2002). The study of Hadi and Al-Khalisy (2018) revealed that the inclusion of 2% olive oil in the diet of broiler chickens significantly increased BW with improved FCR. The WG significantly increased with supplementation of 1% OGEO in the diet of broilers on day 21 which resulted from the synergistic effect of garlic and olive oil. This is in agreement with the report of Elbaz et al. (2022) who observed increased WG for broilers fed diets supplemented with 200 mg/kg of garlic essential oil compared to the control. The use of extract from plants and spices has been reported to be effective when used in isolation or mixtures of two or three in promoting the performance and health status of animals (Cabuk et al., 2006). It is important to point out that in this study, the inclusion of OGEO beyond 1% in the diet of broilers resulted in reduced BW and WG. The weight reduction observed with higher inclusion of OGEO is an expression of the synergistic effect of the functional constituent of garlic and olive oil in promoting lean tissue despite the roles they play in promoting growth. The investigations by Zangeneh and Torki (2011) revealed that the inclusion of olive meal (9%) in the diet of broilers did not adversely affect performance and increased intake of fibre which promotes lean tissue formation. In addition, the phenols contained in olive oil manipulate cholesterol metabolism to exhibit hypocholesterolemic function (Sarica and Toptas, 2014). Garlic having a similar attribute also contains 3-hydroxy-3-methylglutaryl reductase which has hypocholesterolemic effects and inhibits peroxidation of lipids (Stanacev et al., 2011). The feed intake and FCR were not significantly influenced by dietary supplementation of OGEO throughout the experimental period. This is similar to the report of Unigwe and Igwe (2022) who reported no significant difference in the feed intake of broilers fed a diet containing 1% of garlic powder compared to the control. However, a contrary report by Rastad (2020) indicates increased feed intake with the inclusion of garlic powder in the diet of broilers. The discrepancies in the reports could be due to the differences in diet composition and modes of administration of the additives. Furthermore, Zhang et al. (2013) reported a decrease in feed intake of broilers fed diets containing 2 and 5% olive oil. Tufarelli et al. (2015) also reported lower feed intake with improved FCR for broilers fed a diet containing 2.5% extra virgin olive oil compared to those fed sunflower oil. In discussing the influence of olive oil in the present study, better performance was observed for broilers fed diets supplemented with 1% OGEO without an increase in feed intake and most reports have indicated no significant effect of olive oil supplementation on feed intake. This corroborates the findings of Poorghasemi et al. (2013) who reported that the positive influence on performance arising from the use of olive oil is not linked to feed intake but due to reduced passage rate of digesta in the gastrointestinal tract permitting better nutrient utilisation and also due to the important nutrients contained in olive oil including essential fatty acids, essential amino acids and polyphenols (Bilal et al., 2021). Mortality was significantly reduced with dietary supplementation of OGEO and the group of broilers fed 1% OGEO diet had the least mortality. The reduced mortality observed in this study with the use of OGEO in the diet of broilers reveals the prominent synergistic effect of garlic and olive oil in maintaining the health status of birds owing to their active constituents. The functional constituents of garlic such as allicin, diallyl disulfide and S-methylcysteine sulfoxide are known to be effective against harmful pathogenic intestinal bacteria (Gram-positive and Gram-negative) (Tatara et al., 2005). The observation on mortality in the current study is in agreement with the report of Mulugeta et al. (2019) who reported the lowest mortality for broilers fed a diet containing 3% garlic. The phenolic constituents of olive oil which are bioactive molecules are known to exhibit antimicrobial and antioxidant properties (Zbakh and Abbassi, 2012). The observed reduced mortality in the current study with the supplementation of OGEO corroborates the report of El-Bahra and Ahmed (2012) who observed increased antibody titers against the Newcastle disease virus with the inclusion of olive oil in the diet of broiler chickens. The findings from the current study and previous reports suggest that the combined use of olive oil and garlic will be effective in promoting the positive health status of broilers resulting in better performance. It is worthy of note that the groups of broilers fed 3 and 5% OGEO-supplemented diets had higher mortality than those fed 1% OGEO-supplemented diet although lower than the control group. This observation indicates that dietary OGEO supplementation beyond 1% did not result in a positive effect on mortality. This outcome could be due to the antagonistic reaction of an inherent bioactive constituent of garlic and olive oil resulting in nonactivity. It has been reported that phytogenic plants contain heterogeneous mixtures of bioactive compounds and their activity is influenced by the varying proportion of individual components which may exhibit positive or negative synergistic effects due to their combination (Bourgaud et al., 2001).

Haematology

At day 21, the supplementation of OGEO in the diet of broilers irrespective of supplemental level resulted in increased PCV. However, the broilers fed diets supplemented with 1 and 5% OGEO had significantly higher PCV counts than the control. The PCV values obtained for these treatments were higher than the normal values reported by Abdulazeez et al. (2016) and this suggests increased protein intake by the birds resulting in improved quality of the blood. The increased PCV could also be associated with increased nutrient availability resulting in increased blood protein. Garlic contains a compound called allicin which stimulates digestive enzyme secretion for improved nutrient digestion and utilisation (Esmail, 2012). The RBC was also significantly higher for broilers fed the diet supplemented with 5% OGEO than the control group. The observed increase in RBC suggests that garlic and olive oil possess some erythropoietic and haematinic properties which promote the production of red blood cells. The nutrients contained in olive oil such as vitamins, oleic acid, and linolenic acid could also encourage erythropoiesis (Ranalli et al., 2002). In agreement with the current study, Unigwe and Igwe (2022) reported a higher RBC count for the broiler group fed the diet supplemented with 1% garlic than the control group.

The MCV and MCHC increased in broilers fed the diet supplemented with 3% OGEO and this could be connected to the increased PCV and RBC. The values obtained for these haematological indicators were also within the normal range reported by Harrison and Lightfoot (2005) and this suggests better nutritive and health status of the group of broilers. The increased MCH and MCV values observed imply no trace of anaemia could be found in the broilers as the main indicators of examining the amount of circulating red blood cells are the PCV, haemoglobin and MCH which are essential in anaemia diagnosis, and RBC-producing capacity of the bone marrow (Thachil and Bates, 2017). A significant difference did not exist for WBC and its differentials at day 21, which reveals that the birds' health was not challenged negatively at this stage and it also confirms the reason for the non-significant difference in mortality across treatment at day 21. It is also important to note that the values across treatment for WBC were below the normal range reported by Abdulazeez et al. (2016) and this indicates that the immune system was not challenged with foreign agents that might necessitate the proliferation of WBCs. Although, Iwuji and Herbert (2012) stated that animals having low WBC count are at risk of infection; however, low WBC could not necessarily mean impaired health status as the increase in WBC could be an initiation of phagocytosis to generate antibodies in response to foreign agents.

At day 42, the PCV and haemoglobin increased for broilers fed the diet supplemented with 3% OGEO which suggests improved nutrient utilisation, particularly protein. The PCV (also called haematocrit) is essentially involved in oxygen and nutrient transport thereby increasing nutrient availability and utilisation (Isaac et al., 2013). The PCV value obtained for broilers fed the diet containing 3% OGEO was slightly higher than the normal range and this indicates there was no case of polycythemia. The increased haemoglobin count also implies increased oxygen transport to tissues which will result in increased oxidation of ingested feed for energy release (Ugwuene, 2011). The WBC and heterophil count increased in broilers fed the control diet but decreased in broilers fed the 3% OGEO-supplemented diet. The increased WBC and heterophil count observed in broilers in the control group on day 42 suggests the invasion by foreign agents. The reduction in WBC count has been reported to be due to reduced production of leucocytes which signifies better health status without infection from foreign agents (Odesanmi et al., 2010). The increased heterophil count also indicates the occurrence of stress which could be nutritional and pathological (Etim et al., 2014). It is noteworthy that the increased mortality observed between days 22 to 42 for broilers in the control group could be associated with the increased WBC and heterophil count obtained on day 42 for broilers of the same group. Although research reports on the effect of olive oil on haematological variables are rare, those obtained in broilers fed the diet supplemented OGEO reveal the beneficial effect of garlic and olive oil in promoting good health status. The active ingredient in garlic known as allicin may have suppressed the effect of harmful microorganisms as it is known to exhibit antimicrobial properties (Adibmoradi et al., 2006). The reduced WBC count observed for the broiler group fed the 3% OGEO-supplemented diet could also be attributed to the health-promoting effect of olive oil owing to the constituent polyphenol such as oleocanthal and oleuropein which possess potent anti-inflammatory and antioxidant properties (Shi et al., 2017).

The MCH and MCHC increased for broilers fed the 5% OGEO-supplemented diet and this can also be linked to the positive influence of OGEO in the diet. The values obtained for MCH and MCHC were within the normal range which implies that the dietary supplementation of OGEO up to 5% did not influence these parameters negatively. When MCHC values are extremely below the normal range, hypochromasia is said to have occurred (Olafadehan, 2011) which is not the case in this study. The MCH and MCHC are indicators used to determine the conditions of blood level and a situation of low level indicates anaemia (Aster, 2004). The increase in MCH and MCHC obtained for broilers with the use of OGEO confirms the roles of these additives in improving health status. It has been reported that extracts of phytogenic plants and spices when used solely or in combination with the diets of animals are capable of improving the health status resulting in improved performance (Cabuk et al., 2006).

CONCLUSION AND RECOMMENDATION

The supplementation of OGEO at 1% in the diet of broilers increased BW and WG but the supplementation at 3 and 5% level resulted in reduced BW and WG. The dietary supplementation of OGEO irrespective of supplemental level reduced mortality however, those fed the 1% OGEO-supplemented diet had the least mortality. Improved PCV was achieved with OGEO supplementation at 1 and 3% levels for day 21 and day 42, respectively. Therefore, this study reveals that OGEO can be supplemented in the diet of broilers at 1% as an alternative to antibiotics for increased performance, and improved immunity.

CONFLICT OF INTEREST

The authors declared no conflicts of interest concerning research, authorship, and publication of this article.

REFERENCES

Abdulazeez H., Adamu S.B., Igwebuike J.U., Gwayo G.J., Muhammad A.I. (2016): Haematology and serum biochemistry of broiler chickens fed graded levels of baobab (*Adansonia digitata L.)* Seed

Meal. IOSR Journal of Agriculture and Veterinary Science 9: 48–53.

Adibmoradi M., Navidshad B., Seifdavati J., Royan M. (2006): Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. The Journal of Poultry Science 43: 378–383.

Al-Shuwaili M.A., Ibrahim E.I., AlBayati M.T. (2015): Effect of dietary herbal plants supplement in turkey diet on performance and some blood biochemical parameters. Global Journal of Bio-science and Biotechnology 4: 85–89.

AOCS American Oil Chemists' Society (1998): Official methods and recommended practices of the American Oil Chemists' Society Method Ce 2–66. GLC ranges of Fatty acid composition. 5th Edition, Champaign, III. AOCS Press.

Arvouet-Grand A., Vennat B., Pourrat A., Legret, P. (1994): Standardization of propolis extract and identification of principal constituents. Journal of Pharmacy Belgium 49: 462–468.

Aster J.C. (2004): Anaemia of diminished erythropoiesis. In V. Kumar, A. K. Abbas, N. Fausto, S. L. Robbins, and R. S. Cotran (Eds.), Robbins and Cotran Pathologic Basis of Disease. Philadelphia, pp. 638–649.

Bansal G.R., Singh V.P., Sachan N. (2011): Carcass quality characteristics of broilers as affected by dried poultry excreta supplementation. Asian Journal of Poultry Science 5: 116–123.

Bilal R.M., Liu C., Zhao H., Wang Y., Farag M.R., Alagawany M., Hassan F., Elnesr S.S., Elwan H. A.M., Qiu H., Lin Q. (2021): Olive Oil: Nutritional Applications, Beneficial health aspects and its prospective application in poultry production: A review. Frontiers in Pharmacology 12: 723040.

Blue-Mclendon A., Green R.A. (2010): Hematology of ratites. In: D.J. Weiss and K.J. Wardrop, eds. Schalm's veterinary hematology. 6th ed. Ames: Lippincott Williams & Wilkins, pp. 987–993.

Bourgaud, F., Gravot, A., Milesi, S., Gontier, E. (2001): Production of plant secondary metabolites: A historical perspective. Plant Science 161: 83–851. https://doi.org/10.1016/S0168-9452(01)00490-3

Cabuk M., Bozkurt M., Alcicek A., Akbap Y., Kucukyllmaz K. (2006): Effect of a herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. South African Journal of Animal Science 36: 135–141.

Cetin E., Yibar A., Yesilbag D., Cetin I. Cengiz S.S. (2016): The effect of volatile oil mixtures on the performance and ilio-caecal microflora of broiler chickens, British Poultry Science 57: 780–787.

Diaz Carrasco J.M., Casanova M., Fernández N.A., Miyakawa M.E. (2019): Microbiota, Gut Health and Chicken Productivity: What Is the Connection? Microorganisms 7: 10–374.

Du E., Wang W., Gan L., Li Z., Guo S., Guo Y. (2016): Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with Clostridium perfringens. Journal of Animal Science and Biotechnology 22: 7–19.

Durairajan R., Murugan M., Karthik K., Porteen K. (2021): Farmer's Stance on Antibiotic Resistance to E. coli and extended spectrum – β -lactamase producing (ESBL) E. coli isolated from poultry droppings. Asian Journal of Dairy and Food Research 40: 88–93.

Edeoga H.O., Okwu D.E., Mbaebie B.O. (2005): Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology 4: 685–688.

El-Bahra S.M., Ahmed A.S. (2012): Effect of vegetable oil on growth, lipid profile and immunological response in broiler chicken fed iso-energetic diet. Revista Cientifica UDO Agricola 12: 202–207.

Elbaz A.M., Ashmawy E.S., Salama A.A., Abdel-Moneim A.E., Badri F.B., Thabet H.A. (2022): Effects of garlic and lemon essential oils on performance, digestibility, plasma metabolite, and intestinal health in broilers under environmental heat stress. BMC Veterinary Research 18: 430.

Esmail S.H. (2012): Analytics, Black cumin and garlic powder in poultry diets. Poultry World 28: 7–9.

Etim N.N., Williams M.E., Akpabio U., Offiong E.E. (2014): Haematological parameters and factors affecting their values. Agricultural Science 2: 37–47.

Feldman B.F., Zinkl J.G., Jain N.C. (2000): Schalm's Veterinary Hematology. 5th Edition, Philadelphia, Lippincott Williams & Wilkins, pp. 1120–1124.

Google Earth (2020): (Location of Experimental Farm, School of Agriculture, LASU, Epe Campus) Retrieved November 10th, 2020 from https://earth.google.com

Hadi D.S., Al-khalisy A.F. (2018): The effect of different levels of olive oil in ration supplementation on some biochemical and productive traits in broilers. International Journal of Natural Science 9: 137–142.

Hanieh H., Narabara K., Piao M., Gerile C., Abe A., Kondo Y. (2010): Modulatory effects of two levels of dietary Alliums on immune response and certain immunological variables, following immunization, in White Leghorn chickens. Animal Science Journal 81: 673–680.

Harrison G.J., Lightfoot T. (2005): Clinical Avian Medicine. ISBN 00-9754994-0-8

Hoff J.E., Singleton K.E. (1977): A method for the determination of tannin in food. Journal of Food Science 42: 1566–1569.

Isaac L.J., Abah G., Akpan B., Ekaette I.U. (2013): Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria, pp. 24–27.

Iwuji T.C., Herbert U. (2012): Haematological and serum biochemical characteristics of rabbit bucks fed diets containing garcimiola kola seed meal Proceedings of 37th Annual Conference of Nigerian Society for Animal Production, pp. 87–89.

Lee J.W., Kim D.H., Kim Y.B., Jeong S.B., Oh S.T., Cho S.Y., Lee K.W. (2020): Dietary encapsulated essential oils improve production performance of coccidiosis-vaccine-challenged broiler chickens. Animals (Basel) 10: 3–481.

Makwana R.B., Raval A.P., Chauhan H.D., Kulkarni R.C., Srivastava A.K., Bhagwat S.R., Rajgor B. B. (2015): Effects of garlic (*Allium sativum*) supplementation on growth performance, carcass characteristics and economics of broilers. Journal of Animal Research 5: 843–848.

Mimica-Dukic N., Bozin B., Sokovic M., Simin N. (2004): Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. Journal of Agricultural and Food Chemistry 52: 2485–2489.

Mulugeta M., Worku Z., Seid A., Debela L. (2019): Effect of garlic powder (*Allium sativum*) on performance of broiler chicken. Livestock Research for Rural Development 31, article 58.

NRC. (1994): Nutrient Requirements of Poultry 9th ed. National Academic Press, Washington, D.C., USA.

Odesanmi S.O., Lawal R.A., Ojokuku S.A. (2010): Haematological effects of Ethanolic fruit extract of *Tetrapleura tetraptera* in Male Dutch White rabbits. Research Journal of Medical Plants 4: 213–217.

Olafadehan O.A. (2011): Haematological parameters, serum constituents and organ development of growing rabbits as affected by feeding processed cassava peels. Animal Nutrition and Feed Technology 11: 41–51.

Oso A.O., Suganthi R.U., Reddy G.B.M., Malik P. K., Thirumalaisamy G., Awachat V.B., Selvaraju S., Arangasamy A., Bhatta R. (2019): Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. Poultry Science 98: 4755–4766.

Poorghasemi M., Seidavi A., Qotbi A.A.A., Laudadio V., Tufarelli V. (2013): Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. Asian-Australasian Journal of Animal Science 26: 705–710.

Puvaca N., Kostadinovic L.J., Ljubojevic D., Lukac D., Popovic S., Dokmanovc B., Stanacev V. S. (2014): Effects of dietary garlic addition on productive performance and blood lipid profile of broiler chickens. Biotechnology in Animal Husbandry 30: 669–676.

Quintero-Flórez A., Sinausia Nieva L., Sánchez-Ortíz A., Beltrán G., Perona, J.S. (2015): The fatty acid composition of virgin olive oil from different cultivars is determinant for foam cell formation by macrophages. Journal of Agricultural and Food Chemistry 63: 6731–6738.

Ranalli A., Pollastri L., Contento S., Di Loreto G., Iannucci E., Lucera L., Russi F. (2002): Acylglycerol and fatty acid components of pulp, seed, and whole olive fruit oils. Their use to characterize fruit variety by chemometrics. Journal of Agricultural and Food Chemistry 50: 3775–3779.

Rastad A. (2020): Effects of antibiotic replacement with garlic powder and probiotic on performance, carcass characteristics, oxidative enzymes and intestinal morphology of broiler chickens. Journal of Sciences: Animal Science. 42. DOI:10.4025/actascianimsci.v42i1.48734

Saddiqui A.A., Ali M. (1997): Pharmaceutical Chemistry. 1st ed., CBS Publishers and Distributors, New Delhi, India, pp. 126–131.

Sarica S., Toptas S. (2014): Effects of dietary oleuropein supplementation on growth performance, serum lipid concentrations and lipid oxidation of Japanese quails. Journal of Animal Physiology and Animal Nutrition 98: 1176–1186.

SAS Institute, (2000). SAS/STAT(R) Inc. User's Guide, Version 9, SAS Institute, Inc. Cary, NC.

Shi C., Chen X., Liu Z., Meng R., Zhao X., Liu Z., Guo N. (2017): Oleuropein protects L-02 cells against H2O2-induced oxidative stress by increasing SOD1, GPx1 and CAT expression. Biomedicine & Pharmacotherapy 85: 740–748.

Stanacev V., Glamocic D., Milosevic N., Puvaca N., Stanacev V., Plavsa N. (2011): Effect of garlic (*Allium sativum L.*) in fattening chicks' nutrition. African Journal of Agricultural Research 6: 943–948.

Stark A. H., Madar Z. (2002): Olive oil as a functional food: epidemiology and nutritional approaches. Nutrition Reviews 60: 170–176.

Suresh G., Das R. K., Kaur Brar S., Rouissi T., Avalos Ramirez A., Chorfi Y., Godbout S. (2018): Alternatives to antibiotics in poultry feed: molecular perspectives. Critical Reviews in Microbiology 44: 318–335.

Tatara M.R., Liwa Ś.E., Dudek K.K., Mosiew C.Z.J., Studziński T. (2005): Effect of aged extract and allicin administration to sows during pregnancy and lactation on body weight gain and gastrointestinal tract development of piglets. Bulletin – Veterinary Institute in Pulawy 49: 349–355.

Thachil J., Bates I. (2017): Approach to the Diagnosis and Classification of Blood Cell Disorders. Dacie and Lewis Practical Haematology, pp. 497–510.

Tufarelli V., Laudadio V., Casalino E. (2015): An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in meat-type broiler chickens. Environmental Science and Pollution Research 23: 6197–6204.

Ugwuene M.C. (2011): Effect of dietary palm kernel meal for maize on the haematological and serum chemistry of broiler turkey. Nigerian Journal of Animal Science 13: 93–103.

Unigwe C.R., Igwe, I.R. (2022): Effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) powders on the growth performance and haematology of broiler chickens. Nigerian Journal of Animal Science 24: 141–153.

Van Beekvelt M. C., Colier W. N., Wevers R. A., Van Engelen B. G. (2001): Performance of near-infrared spectroscopy in measuring local $O(_2)$ consumption and blood flow in skeletal muscle. Journal of Applied Physiology 2: 511–519.

Wright L.P., Mphangwe N.I.K., Nyirenda H.E., Apostolides Z. (2000): Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinensis* for predicting the quality of black tea produced in Central and Southern Africa. Journal of the Science of Food and Agriculture 80: 1823–1830.

Zangeneh S., Torki M. (2011): Effects of B-Mannanase supplementing of olive pulp-included diet on performance of laying hens, egg quality characteristics, humoral and cellular immune response and blood parameters. Global Veterinaria 7: 391–398.

Zbakh H., El Abbassi A. (2012): Potential use of olive mill wastewater in the preparation of functional beverages: A Review. Journal of Functional Foods 4: 53–65.

Zhang Z. F., Zhou T. X., Kim I. H. (2013): Effects of dietary olive oil on growth performance, carcass parameters, serum characteristics, and fatty acid composition of breast and drumstick meat in broilers. Asian-Australasian Journal of Animal Science 26: 416–422.

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