Response of Broiler Chickens to Oral Administration of *Ocimum Gratissimum* Leaf Extracts

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Abstract

The study was carried out on one hundred and ninety-five day-old broiler chicks of cobb500 to study the effect of *Ocimum gratissimum* leaf extracts on growth performance, blood profile, microbial population in the faecal and gut samples. The chicks were randomly selected into five groups with thirty-nine chicks per group and each group replicated thrice. The groups were: control (synthetic antibiotics), extracts from 200 g fresh leaf, 400 g fresh leaf, 40 g air-dried leaf and 80 g aid-dried leaf per litre of water respectively. Data collected were subjected to one-way Analysis of Variance. The growth performance except mortality was similar (P>0.05) across the groups. Albumin, urea, cholesterol, alkaline phosphate and sodium were influenced (P<0.05) at the starter phase while only alkaline phosphate was significantly (P<0.05) highest in birds on 400 g of fresh leaf extract at the finisher phase. Neutrophil was highest (P<0.05) in birds on 400 g of fresh leaf extract while those on 200 g had higher values of lymphocytes and eosinophil at starter phase. At finisher phase, birds on antibiotics and 80 g of air-dried leaf extract had higher (P<0.05) white blood cell. Faecal total microbial population was least (<0.05) in birds on antibiotics and 200 g fresh *Ocimum gratissimum* leaf extract at starter phase. The study concluded that the adoption of *Ocimum gratissimum* leaf extract as prophylactic treatment against bacteria should be encouraged among poultry farmers.

Introduction

Over the past few decades, antibacterial resistance has been a significantly concerning global health threat because bactericidal action of almost all known antibiotics is on the wane against pathogenic microorganisms in animal production (Reig and Toldra, 2008). Application of antimicrobial agents in poultry has been associated with the growth of resistant bacteria species. Resistant strains among poultry pathogens can lead to suppressed potency of antibiotics, translating to economic losses as resistant bacteria species pose significant risk to human health. Poultry is often raised under intensive conditions, primarily reared on large amounts of antimicrobials that are supplied to prevent, curtail or manage outbreak. (Marshall and Levy, 2011; Nhung, 2017).

Observed trend of research on application of antibiotic alternatives and feed additives to promote livestock growth have been explored with enhancement of gut health using low dosage of antibiotics. Classes of antibiotic alternatives available to boost productivity aid maximization of genetic potential under existing commercial conditions in poultry, such as enzymes, antimicrobial peptides, organic acids, phytogenics, prebiotics, probiotics, symbiotics, hyperimmune egg antibodies, bacteriophages, clay and metals (Gadde et al., 2017). Natural herbal product has been explored in pursuit of improved health status among birds coupled with consumer satisfaction that is increasingly associated with healthier product (Gardzielewska et al., 2003). An evaluation of performance of broiler chickens reveal novel herbal supplementations are incorporated to boost indices among experimental chickens since blood contribute a significant role in health profile assessment.

Scent leaf (*Ocimum gratissimum*) possesses bioactive principles potent against diverse bacterial species (Iwalokun, 2003; Akinyemi et al., 2004). The leaves, flowers, roots, and oils demonstrated antiviral, larvicidal, bactericidal, and insect repellant properties (Oparaocha et al., 2010; Sumitha and Thoppil, 2016; Pandey et al., 2017). The study of Junaid et al. (2006) showed that antimicrobial efficacy of cold-water extract of the fresh leaf of *O. gratissimum* applied on all test isolates had highest inhibitory activity. Mahomoodally (2013) likewise declared that the trend of application of medicinal substances points towards validating the efficacy and safety of African traditional medicines in relation to its form of application, signifying that the quality of principles utilized is dependent on the form of *Ocimum gratissimum*. On that premise, this study is designed to investigate the administration of raw and air-dried *Ocimum gratissimum* leaf extract on performance and blood profile of broiler chickens.

Material And Methods

Experimental Site: The experiment was carried out at the Poultry unit of the Teaching and Research Unit of Directorate of University farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of south – western Nigeria on Latitude 7^0 10' N and Longitude 3^0 2'E and altitude of 76m above the sea level (Google Earth, 2020). The climate is humid, with a mean annual rainfall of about 1037mm and mean temperature and humidity of 34.7^0C and 83%, respectively.

Preparation of the extracts: the extraction methods were two: the fresh and air-dried leave extracts. The fresh leaf extraction was prepared as follow; 200 and 400 g of fresh leaves was blended with six litre of water respectively. The procedure for the extraction of air-dried leaf extract is as follows; fresh leaves of the plant were harvested and spread in a room for air- drying. Air-drying method was adopted to prevent loss of volatile oils when spread under direct sunlight. The air-dried leaves were milled and dissolved in water at 40 and 80g (an equivalent of 200 and 400g fresh leaves, respectively) per six litres of water respectively. The solutions were stirred every 30 minutes for 3 hours and allowed to stand 24 hours. After the 24 hours, the solution was sieved to get extract according to the procedure of Jesuwenu and Okozi (2017).

Management of Experimental Birds

A total of one hundred and ninety-five (195) day old chicks of Cobb 500 strain of broiler chickens was purchase from a reputable hatchery. The birds were randomly divided into five experimental groups. Each group was further sub-divided to three replicates of thirteen chicks per each. The groups were: control (use of synthetic antibiotics), extracts from 200 g fresh leaf, 400 g of fresh leaf, 40 g of air-dried leaf and 80 g of aid-dried leaf respectively. The extract was served to the birds at the rate of one-third of daily water intake throughout the experimental period expect a day prior and the day of vaccination. Water was served immediately the birds in each replicate finished the served extract. Brooding of chicks lasted for two weeks in individual pen with the use of charcoal pots and electrical bulbs as heat source and lightings. Synthetic antibiotic (enrofloxacin) was used for the birds in the control group. Commercial

broiler starter diet was supplied for the first four weeks of the study while commercial broiler finisher diet was used for the last three week. Feed and water were supplied *ad-libitum.* The experiment lasted for seven weeks.

Data collection: Growth performance indices (feed intake, weight gain) were taken weekly while feed conversion ratio was calculated by dividing feed intake by weight gain. Blood samples were collected on the 28^{th} (end of starter phase) and 49^{th} (finisher phase) days of the experiment from each replicate. Blood sample of 2.0ml was drawn via wing vein puncture with hypodermic syringes into bottles containing an anticoagulant; Ethylene diamine tetra acetate (EDTA) for the determination of haematological parameters. Packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cells (RBC) and white blood cells (WBC) and differential counts were determined according to the procedure of Schalm *et al*. (1975); mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived by calculation from the RBC, Hb, PCV values as described by Jain (1986). Another 2.0ml of blood was drawn into labelled sample bottles without EDTA for serum biochemical indices determination. Serum was separated from the clot blood by centrifuge. Serum indices; Total protein (TP) and albumin values were determined by Biuret method. Globulin was calculated as the difference between total protein and albumin. Cholesterol was determined according to the procedures of Thu *et al.* (2011), urea and creatinine were determined by the method Harr (2006), serum enzymes (ALT, ALP and AST) actions were determined spectrophotometrically (McComb *et al*., 1983).

Collection of faecal and gut samples: On the 28^{th} day of the experiment, black nylon was spread on the litter in each pen. Each pen was observed and the birds were allowed to roam freely over the nylon. The nylon was then withdrawn from the pen as soon as the feacal sample were collected on the nylon. Each sample collected was kept in a universal bottle. On the 49^{th} day of the experiment a chicken of average weight of each replicate was picked, fasted overnight and slaughtered. The gut sample was aseptically collected from the slaughtered bird for all replicates. All samples were taken to the laboratory for analysis to determine bacterial identification and count following procedure outlined by Adhikari and Kwon, (2017).

Determination of phytochemicals, microbial count and identification

Gram-positive and negative bacteria species were examined as caecal contents were serially diluted and plated, then incubated at 37°C under a microaerophilic condition for 15-18 hrs. Diluted samples were cultured on nutrient agar. Subsequently, adapted Mueller Hinton Agar (MHA) was modified according to Al-blooshi *et al*. (2021) to develop MHA-C15 (MHA containing 15% volume/volume water extract of clove). Gram-negative bacterial pathogens such as *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* grew on MHA-C15. However, none of the major Gram-positive bacterial pathogens such as *Streptococcus faecalis* and *Clostridium perfrigens* grew on it. To isolate bacteria in caecum-ilium mucosa, as well as the faeces, samples were rinsed in sterile Phosphate Buffered Saline (PBS) of pH 7.4 after luminal contents were separated three times, and homogenized in 20 ml of PBS. Samples extracted

were kept in cold storage and transported to the laboratory prior to subsequent analysis. Thereafter, Isolation and identification was carried out by observing colony morphology, grams staining and standard cultural and biochemical test.

Statistical Analysis: Data collected were subjected to One-way Analysis of variance using SAS (2010). The significant means were separated at $p<0.05$ using Duncan's Multiple Range Test in the software package.

Result

Activity of *Ocimum gratissimum* leaf extract on growth performance of broiler chickens is documented in Table 1. All growth performance parameters examined (final weight, weight gain, feed intake feed conversion ratio and water intake) were not affected ($p>0.05$) by extract offered. However, mortality was significantly ($P<0.05$) highest among birds on 400 g of fresh leaf extract while the least was obtained from those on 80 g of air-dried leaf extract.

Table 1
Effect of *Ocimum gratissimum* leaf extract on growth performance of broiler chickens

	Antibiotics	**Fresh leaf**		**Air-dried**		
Parameters	enrofloxacin	200	400	40	80	SEM
Initial weight (g)	38.56	38.54	38.67	38.59	38.08	0.11
Final weight (g)	2177.78	2322.05	2271.84	2274.15	2303.85	33.69
Weight gain (g)	2139.21	2283.51	2233.18	2235.56	2265.77	33.71
Daily weight gain (g)	43.6574	46.6022	45.5750	45.6236	46.2402	0.68
Daily feed intake (g)	106.24	108.52	111.31	114.46	116.49	1.31
Feed Conversion Ratio	2.43	2.33	2.44	2.50	2.52	0.03
Daily water intake (ml)	219.25	244.80	239.93	232.95	234.74	4.48
Mortality (%)	5.12^{ab}	7.69^{ab}	10.25^{a}	2.56^{ab}	0.00^{b}	1.43

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

Serum biochemical indices of broiler chickens on administration of *Ocimum gratissimum* leaf extract at starter phase is shown in Table 2. The results showed that albumin, urea, cholesterol ALP and sodium were significantly ($P<0.05$) different across the groups. Albumin content was higher ($P<0.05$) in birds supplied 200 g of fresh *Ocimum gratissimum* leaf extract and 80 g air-dried *Ocimum gratissimum* leaf extract. Birds on antibiotics had least value of urea and ALP. The result on Cholesterol showed that

highest value was obtained from the birds on 400 g of fresh *Ocimum gratissimum* leaf extract while least values were recorded among birds on antibiotic, 40 and 80 g of air-dried *Ocimum gratissimum* leaf extract. Birds on 80 g air-dried *Ocimum gratissimum* leaf extract had higher values of ALP and sodium content while the least value of sodium content was obtained from birds on 200 g fresh *Ocimum gratissimum* leaf extract.

Table 2
Effect of *Ocimum gratissimum* leaf extract on Serum biochemical parameters of broiler chickens at starter phase

		Fresh leaf		**Air-dried leaf**		
Parameters	Antibiotics	200g	400	40	80	SEM
Glucose (mg/dl)	150.00	150.26	143.10	140.50	160.67	8.84
Protein (g/L)	38.30	41.17	42.70	40.97	42.53	0.89
Albumin (g/L)	21.87^{b}	24.80^{a}	24.13^{ab}	24.07^{ab}	24.90^{a}	0.39
Globulin (g/L)	16.43	16.36	18.56	16.90	17.63	0.72
Urea (mg/dl)	4.00^{b}	5.13^{ab}	6.87^{a}	5.30^{ab}	5.00^{ab}	0.37
Creatine (mg/dl)	0.80	0.83	0.70	0.70	0.70	0.02
Cholesterol (mg/dl)	60.46^{b}	74.70^{ab}	89.97^{a}	69.13^{b}	62.23^{b}	3.46
Aspartate transaminase (IU/L)	167.87	175.03	157.83	163.73	151.20	6.23
Alanine aminotransaminase (IU/L)	11.73	11.93	10.00	11.60	8.53	1.22
Alkaline phosphate (IU/L)	76.30^{b}	83.20^{ab}	90.40^{a}	80.60^{ab}	91.43^{a}	2.06
Calcium (mg/dl)	8.60	9.25	9.04	8.79	9.58	0.18
Phosphate (mg/dl)	5.88	5.87	5.45	5.54	5.52	0.26
Sodium (mmol/L)	117.20^{ab}	106.46^{b}	117.10^{ab}	108.33^{ab}	120.46^{a}	2.06
Potassium (mmol/L)	2.77	3.23	3.56	3.20	3.40	0.15
Bilirubin (mg/dl)	0.66	0.60	0.73	0.63	0.70	0.03
Chloride (mg/dl)	81.06	90.66	86.86	84.83	79.56	2.56

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

Among haematological parameters obtained, blood neutrophil, lymphocytes and eosinophil were influenced (P<0.05) by the oral administration of *Ocimum gratissimum* leaf extract (Table 3). It was observed that neutrophil was higher in birds on 400 g fresh *Ocimum gratissimum* leaf extract but the least value was from the counterpart on 200 g fresh *Ocimum gratissimum* leaf extract. Lymphocytes and eosinophil were higher in bird on 200 g fresh *Ocimum gratissimum* leaf extract while the least value of the two measurements were obtained from birds on 400 g fresh *Ocimum gratissimum* leaf extract and 80 g air-dried *Ocimum gratissimum* leaf extract.

Table 3
Haematological indices of broiler chicken on administration of *Ocimum gratissimum* leaf extract at starter phase

		Fresh leaf (g)		Air-dried leaf (g)		
Parameters	Antibiotics	200	400	40	80	SEM
Pack Cell Volume (%)	23.66	24.33	23.66	24.33	26.66	0.79
Haemoglobin (g/dl)	8.00	8.10	8.03	8.00	8.96	0.27
White Blood Cell ($x10^9$/L)	14.66	13.60	11.10	11.20	13.36	0.95
Red Blood Cell ($x10^{12}$/L)	2.78	3.06	2.65	3.04	3.39	0.13
Neutrophil (%)	24.33^{ab}	14.66^{b}	36.00^{a}	25.66^{ab}	18.00^{ab}	2.88
Lymphocytes (%)	73.00^{ab}	79.33^{a}	60.00^{b}	71.67^{ab}	81.00^{a}	2.92
Basophil (%)	0.00	0.33	0.66	0.33	0.00	0.11
Eosinophil (%)	1.33^{ab}	2.66^{a}	1.33^{ab}	0.66^{b}	0.33^{b}	0.28
Monocytes (%)	1.33	3.00	1.67	1.67	0.67	0.42

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

The faecal microbial population of broiler chickens is presented in Table 4. The total bacterial count and clostridium spp and proteus were significantly (P<0.05) affected extract offered. Total microbial population was highest in birds on 40 g of air-dried *Ocimum gratissimum* leaf extract while the birds on antibiotics and 200 g fresh *Ocimum gratissimum* leaf extract had least value. There was no presence of Clostridium Spp in birds on antibiotics. *Proteus mirabilis* was also not present in the faecal samples of birds on 200 g fresh and 80 g air-dried *Ocimum gratissimum* leaf extract.

Table 4
Microbial population in the faeces of broiler chickens on oral administration of *Ocimum gratissimum* leaf extract at starter phase

		Fresh leaf (g)		Air-dried leaf (g)		
Parameters	Antibiotics	200	400	40	80	SEM
Total Bacterial Count (cfu/ml)	1.00^{b}	1.06^{b}	1.56^{ab}	1.70^{a}	1.53^{ab}	0.99
Escherichia coli (cfu/ml)	0.46	0.36	0.23	0.63	0.50	0.06
Streptococcus faecalis (cfu/ml)	0.33	0.23	0.83	0.46	0.70	0.11
Pseudomonas Spp (cfu/ml)	0.10	0.13	0.27	0.30	0.23	0.04
Clostridium Spp (cfu/ml)	0.00^{c}	0.33^{a}	0.07^{bc}	0.13^{abc}	0.27^{ab}	0.04
Proteus (cfu/ml)	0.10^{ab}	0.00^{b}	0.16^{a}	0.16^{a}	0.00^{b}	0.02

^{abc} Means with different superscripts along the same row are significantly (P<0.05) different

The effect of oral administration of *Ocimum gratissimum* leaf extract on the serum biochemical parameters at finisher phase (Table 5) indicated that ALP was the only measurement that was influenced (P<0.05). The highest value was recorded from chickens on 400 g fresh *Ocimum gratissimum* leaf extract while the least value was obtained from their counterpart on antibiotics.

Table 5
Serum biochemical indices of broiler chicken on *Ocimum gratissimum* leaf extract at finisher phase

		Fresh leaf (g)		Air-dried leaf (g)		
Parameters	Antibiotics	200	400	40	80	SEM
Glucose (mg/dl)	179.93	185.76	193.53	186.36	181.93	5.15
Protein (g/L)	65.00	72.26	64.96	68.33	68.86	1.49
Albumin (g/L)	39.03	42.16	36.46	38.16	40.00	0.91
Globulin (g/L)	25.96	30.10	28.50	30.16	28.86	0.77
Urea (mg/dl)	7.30	7.23	7.16	7.26	6.50	0.16
Creatine (mg/dl)	0.90	0.80	0.83	0.86	0.80	0.02
Cholesterol (mg/dl)	70.66	67.16	67.63	79.40	60.90	2.90
Aspartate transaminase (IU/L)	95.90	119.33	112.90	124.20	120.70	3.99
Alanine aminotransaminase (IU/L)	26.66	33.43	23.83	30.93	31.10	2.54
Alkaline phosphate (IU/L)	67.80^{b}	91.40^{ab}	101.47^{a}	76.77^{ab}	86.47^{ab}	4.48
Calcium (mg/dl)	9.17	9.76	9.61	10.08	9.96	0.19
Phosphate (mg/dl)	5.68	6.74	5.07	5.53	5.71	0.29
Sodium (mmol/L)	126.73	129.97	123.03	110.83	122.97	3.49
Potassium (mmol/L)	4.70	4.83	3.87	4.63	3.97	0.26
Bilirubin (mg/dl)	0.57	0.50	0.60	0.60	0.57	0.02
Chloride (mg/dl)	88.83	104.07	93.00	102.47	94.07	2.64

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

The effect of oral administration of *Ocimum gratissimum* leaf extract on haematological parameters of broiler chickens at the finishing phase is presented in Table 6. White blood cell count was the only parameter significantly (P<0.05) different across the treatment groups. Chickens on antibiotics and those on 80 g air-dried *Ocimum gratissimum* leaf extract had increased count than groups given 40 g air-dried *Ocimum gratissimum* leaf extract.

Table 6
Haematological indices of broiler chicken on *Ocimum gratissimum* leaf extract at finisher phase

		Fresh leaf (g)		Air-dried leaf (g)		
Parameters	Antibiotics	200	400	40	80	SEM
Pack Cell Volume (%)	28.00	31.00	28.67	30.67	31.33	0.62
Haemoglobin (g/dl)	9.47	10.47	9.63	10.33	10.53	0.20
White Blood Cell ($x10^9$/L)	12.03^a	9.50^{ab}	7.80^{ab}	4.97^b	13.37^a	1.16
Red Blood Cell ($x10^{12}$/L)	3.11	3,57	3.32	3.51	3.59	0.08
Neutrophil (%)	14.00	16.00	21.33	17.33	16.67	1.67
Lymphocytes (%)	83.33	79.00	73.00	79.67	76.67	1.76
Basophil (%)	0.00	0.00	1.00	0.00	1.00	0.16
Eosinophil (%)	1.00	3.33	1.67	1.00	2.67	0.37
Monocytes (%)	1.67	1.67	3.00	2.00	1.67	0.40

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

Gut microbial count of broiler chickens offered *Ocimum gratissimum* leaf extract at finisher phase presented in Table 7 reveal extract did not affect bacterial species (*E. coli*, *Streptococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Clostridium perfrigens* and *Proteus mirabis*) and the subsequent total count.

Table 7

Gut microbial counts of broiler chicken on *Ocimum gratissimum* leaf extract at finisher phase

		Fresh leaf (g)		Air-dried leaf (g)		
Parameters	Antibiotics	200	400	40	80	SEM
Total Bacteria Count (cfu/ml)	1.36	1.27	1.17	1.10	1.37	0.07
Escherichia coli (cfu/ml)	0.43	0.50	013	047	037	0.05
Streptococcus faecalis (cfu/ml)	0.40	0.37	0.50	0.33	0.37	0.06
Pseudomonas spp (cfu/ml)	0.27	0.20	0.27	0.10	0.10	0.03
Staphylococcus spp (cfu/ml)	0.167	0.10	0.17	0.07	0.20	0.04
Entrobacta spp (cfu/ml)	0.10	0.10	0.10	0.133	0.20	0.02

Discussion

The similarities in the growth performance indices across the groups is an indication that the *O. gratissimum* leaf extract has antibacterial properties and this resulted into similar final live weight. The oral administration air-dried leaf extract further established that the processing method (air-drying) might have reduced the toxicity of phytochemicals and therefore reduced the mortality percentage. Various antinutritional factors are present in plants reduce the digestibility of proteins and mineral absorption. Anti-nutrients are one of the key factors that reduce the bioavailability of various components in diets (Samtiya et al., 2020). Njoku *et al.* (2017) reported that air-drying method of *O. gratissimum* leaf is the best processing method to preserve yield and chemical composition of the essential oil for the plant to retain the components responsible for its biological activity.

The higher albumin content obtained from birds on extract from 200 g of fresh *Ocimum gratissimum* leaf and 80 g of air-dried *Ocimum gratissimum* leaf is an indication that the extract stimulated increased albumin. This supports the report of Hashemipour et al. (2014) who established an increase in plasma albumin concentrations among chickens offered carboxy methyl cellulose and thymol + carvacrol, and consequently adduced effect to essential oil stimulating better digestion and absorption of nutrients. Similarly, Nweze and Ekwe, (2012) declared that eugenol methyl cinnamate and thymol in *Ocimum gratissimum* positively influenced feed digestive properties, resulting in increased appetite of birds, thereby effecting increased plasma albumin. Urea concentration in the blood was high among groups offered 400 g of *Ocimum gratissimum* leaf extract at the starter phase. Urea – according to Liu et al. (2016) is a major nitrogenous waste product of protein metabolism gotten from high-protein diet that is circulated in the blood prior to excretion. Posit from the author above agrees with this study as seen in the numerical rise in blood protein among chickens offered 400 g of fresh Ocimum gratissimum leaf. Also, at starter phase, lowered serum cholesterol was reported among birds given antibiotics and both levels of air-dried *Ocimum gratissimum* leaf. The cholesterol lowering effect of air-dried *Ocimum gratissimum* had could be attributed to processing method employed. Modifications exists from processing of *Ocimum gratissimum* leaf. Hence, hydration properties, viscosity and gel formation of *O. gratissimum* leaf affect the potency of plants – as properties related to the microstructure of dried leaf changes with processing (Dawkins et al., 2001), thereby suggesting that the availability of eugenol, known to be slightly water soluble as well as niacin in extract from air-dried of *Ocimum gratissimum* leaf with cholesterol lowering properties (Harb et al., 2019) was affected. On the contrary, cholesterol lowering capability among birds offered antibiotics was likely made possible by the improved gut health. Gut microbes metabolize and modify dietary and host-derived molecules in the small intestine known to impact serum cholesterol levels (Kriaa et al., 2019; Kenny et al., 2020). Notably, cholesterol value for the group was lower than the values reported by Adegoke et al. (2018) and Galli et al. (2020). Significant rise in alkaline phosphatase (ALP) as *Ocimum gratissimum* dosage increased for both forms reveal increased antinutritional content in relation to dosage. Tannins, phenols and phlobatannins were reported from the phytochemical screening of *Ocimum gratissimum* by Shuaib et al. (2015). USNND (2010), established tannins as bitter principles or plant polyphenols that bind, precipitate and diminish proteins and various organic compounds in living systems – an impact not observed in the liver of groups offered antibiotics at the

starter phase. Additionally, serum ALP values in this study were higher than the values documented by Chand et al. (2018) with 15.5 – 21.36 U/I ALP values declared for broiler chickens (Ross, Hubbard, Cobb and Arbor acer) reared under thermoneutral and high ambient temperature zones. Further, alkaloid was more prominent in *Ocimum tenuiflora* than phenols, tannins and flavonoid according to the findings of Mousavi et al. (2019), which could have been contributed to increased ALP levels. Significant increase in sodium content of air-dried 80 g of *Ocimum gratissimum* extract can be attributed to increased mineral content upon drying – a fact confirmed by Zaharaddeen *et al*. (2019). Neutrophil count was progressively higher in chickens offered 400 g of fresh *Ocimum gratissimum* leaf extract than 200 g, implying presence of foreign bodies in the blood, leading to phagocytotic action (Nworgu et al., 2013). Air – drying slightly modified the chemical composition of *Ocimum gratissimum*. An inverse relationship exists between neutrophil and lymphocyte count when fresh *Ocimum gratissimum* extract was offered. Increased lymphocyte among groups offered 200 g of fresh *Ocimum gratissimum* extract had identical count as group given 80 g of air-dried *Ocimum gratissimum* extract. Findings on supplementation of Basil leaf and seed to pullets by Nworgu et al. (2013) reveal suppressed lymphocyte count as inclusion increased, which is similar as the outcome from this study when 400 g of fresh *Ocimum gratissimum* was given. That higher immunity was conferred when higher dosage of fresh *Ocimum gratissimum* extract was offered indicate that the nutrient and vitamin availability in fresh leaf boosted the immunity of birds in a complementary way, whereas, increase in lymphocyte as extract from air-dried *Ocimum gratissimum* was offered indicate progressive depletion of bio-active principles that could have complemented immune development in broiler chicks. At the starter phase, eosinophil count was lowered in chicks given air – dried *Ocimum gratissimum* extract. Notably, leaf form had an effect. Concentration of alkaloids and tannins increase with decreased moisture. Olanrewaju et al. (2017) evaluated phytochemicals in dry, wet and oil extracts of the Leaf of Morinda lucida and reported that dried extract of leaves had higher total phenolic, flavonoids, saponin and tannins than wet extracts. Also, Hossain et al. (2016) concluded that air drying had the highest effect on steroidal alkaloid contents, followed by freeze drying and vacuum oven drying. Consequently, it is safe to allude that form of drying positively complemented eosinophil production and action at the starter phase. Significantly, eosinophil lowering activity of both air-dried extracts effected count lower than the 0.9 – 1.04 x 10^3 U/L declared by Talebi et al. (2005) from 5 strains of broiler chickens at the starter phase.

Among bacteria species examined, clostridium spp growth was inhibited by antibiotics when compared with count from birds offered extracts of *Ocimum gratissimum*. For proteus mirabilis, 200 g and 80 g of fresh and air-dried *Ocimum gratissimum* extracts respectively hampered the growth of *Proteus mirabilis*. Eugenol, according to Nakamura et al. (1999) suppress *Proteus mirabilis* growth. Hence, both level of extract sufficiently hinder occurrence of *Proteus sp*. At the finisher phase, serum ALP followed the trend of the starter phase as birds on antibiotics, having suppressed ALP value at finisher phase. Reason proposed at the starter phase consequently extends to the finisher phase. Total bacteria count was lowered when birds were offered antibiotic and 200 g of air-dried *Ocimum gratissimum* extract – a consequence of the broad acting capacity of both substances. At the finisher phase, higher WBC among groups given antibiotics, 400 g of fresh *Ocimum gratissimum* extract and 80 g of air-dried *Ocimum*

gratissimum extract than the 9.33 x 10^9/L declared by Ajayi and Imouokhome (2015) was documented. WBC among chickens given 40 g air-dried *Ocimum gratissimum* extract was likewise lower than the 6.87 – 9.57 x 10^3/L declared by Galli et al. (2020), implying that nutrients provided was inadequate to aid production in the bone marrow, however, higher WBC suggests defence by the immune system against pathogenic or exogenous invasions.

Conclusion

It was concluded that the use of *O. gratissimum* leaf extract favourable compete with the conventional antibiotics on the growth performance of broiler chicken. However, extract from air-dried leaf improved survivability of the chickens.

Declarations

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Competing interests

The authors declare that they have no known competing conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

The study was approved by the research ethics committee of College of Animal Science and Livestock Production of the University in line with the principle of Livestock research

Consent to participate

All the authors willingly participated in the research work when they were approached and their commitment to the success of the work showed that no one was coarse.

Consent for publication

All the authors gave their consent and agreed that the manuscript should be submitted to journal of Tropical Animal Health and Production for publication.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Authors' Contributions

All authors contributed to the study. Conception and design was performed by [Egbeyale, L. T.], [Adegoke, V. A.], [Ayo-Ajasa, O. Y.] and [Adewole, F. A],. Material preparation and data collection were performed by [Yusuf, A. O.], [Amusan, O. A.], [Gbegbin, A. P.] and [Taylor, S. O]. Data analysis was performed by [Egbeyale, L. T.]. The first draft of the manuscript was written by [Egbeyale, L. T.] and [Adegoke, V. A.] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

1. Adegoke, A.V., Abimbola, M.A., Sanwo, K.A., Egbeyale, L.T., Abiona, J.A., Oso, A.O. and Iposu, S.O., 2018. Performance and blood biochemistry profile of broiler chickens fed dietary turmeric (*Curcuma longa*) powder and cayenne pepper (*Capsicum frutescens*) powders as antioxidants. Veterinary and Animal Science, 6, 95–102. doi.org/10.1016/j.vas.2018.07.005
2. Adhikari, B., and Kwon, Y.M., 2017. Characterization of the Culturable Subpopulations of 323 Lactobacillus in the Chicken Intestinal Tract as a Resource for Probiotic Development. Frontiers in Microbiology, 8, 324
3. Ajayi, H., and Imouokhome, J., 2015. Blood parameters and performance of broiler chickens fed diets containing feather meal at three crude protein levels, with or without protease supplementation. Nigerian Journal of Agriculture, Food and Environment, 11, 146–149.
4. Akinyemi, K.O., Mendie, U.E., Smith, S.T., Oyefolu, A.O. and Coker, A. O., 2004. Screening of some medicinal plants for anti-salmonella activity. Journal of Herb Pharmocothereraphy, 5(1), 45–60.
5. Al-blooshi, S.Y., Latif, M.A.A., Sabaneh, N.K., Mgaogao, M., and Hossain, A., 2021. Development of a novel selective medium for culture of Gram-negative bacteria. Biomed Central Research Notes 14, 211. https://doi.org/10.1186/s13104-021-05628-2

6. Chand, N., Naz, S., Rehman, Z., Khan, R.U., 2018. Blood biochemical profile of four fast-growing broiler strains under high ambient temperature. Applied Biological Chemistry, 61 (3), 273–279. doi:10.1007/s13765-018-0358-4
7. Dawkins, N.L., Gager, J., Cornillon, J.P., Kim, Y., Howard, H., and Phelps, O., 2001. Comparative Studies on the Physicochemical Properties and Hydration Behaviour of Oat Gum and Oat Rim in Meat-based Patties. Journal of Food Science, 66, 1276–1282.
8. Gadde, U., Kim, W., Oh, S., and Lillehoj, H., 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Animal Health Research Reviews, 18, 1–20. 10.1017/S1466252316000207.
9. Galli, G.M., Gerbet, R.R., Griss, L.G., Fortuoso, B.F., Petrolli, T.G., Boiago, M.M., Souza, C.F., Baldissera, M.D., Mesadri, J., Wagner, R., Rosa, G., Mendes, R.E., Gris, A. and Da Silva, A.S., 2020. Combination of herbal components (curcumin, carvacrol, thymol, cinnamaldehyde) in broiler chicken feed: Impacts on response parameters, performance, fatty acid profiles, meat quality and control of coccidia and bacteria. Microbial Pathogenesis, 103916. doi:10.1016/j.micpath.2019.103916
10. Gardzielewska, J., Pudyszak, K., Majewska, T., Jakubowska, M. and Pomianowski, J., 2003. Effect of plant-supplemented feeding on fresh and frozen storage quality of broiler chicken meat. Electron Journal of Polish Agricultural University, 6, 12–12
11. Google Earth Pro 7.3.4.8248. 2020. Alabata Region Nigeria. 7° 12' 36.42"N, 3° 26' 30.51"E, Ele 147m alt 1.15km. Borders and labels; places layers. NOAA, Digital Globe 2020 (Accessed July 25, 2020), http://www.google.com/earth/ index.html.
12. Harb, A.A., Bustanji, Y.K., Almasri, I.M. and Abdalla, S.S., 2019. Eugenol Reduces LDL Cholesterol and Hepatic Steatosis in Hypercholesterolemic Rats by Modulating TRPV1 Receptor. Scientific Reports 9, 14003. https://doi.org/10.1038/s41598-019-50352-4
13. Harr, K. E., 2006. Diagnostic value of biochemistry. In: Harrison GJ, Lightfoot TL, editors, Clinical Avian Medicine. www.clinicalavianmedicine.com
14. Hashemipour, H., Kermanshadi, H., Golian, A., and Khaksar, V., 2014. Effects of carboxy methyl cellulose and thymol + carvacrol on performance, digesta viscosity and some blood metabolites of broilers. Journal of Animal Physiology Animal Nutrition, 98, 672–679.
15. Iwalokun, B.A., Gbenle, G.O., Adewole, T.A., Smith, S.I., Akinsinde, K.A., Omonigbehin, E.O., 2003. Effects of *Ocimum gratissimum* L. essential oil at subinhibitory concentrations on virulent and multidrug-resistant Shigella strains from Lagos, Nigeria. Acta Pathologica, Microbiologica, et Immunologica Scandinavica, 111, 477–82
16. Jain, N.C., 1986. Schalm's veterinary haematology 4th edition (N.C. Jain) Lea and Febiger, Philadelphia. Pp 1221.
17. Jesuwenu, A.D. and Okozi, O.M., 2017. Antibacterial activity of *Ocimum gratissimum* l. against some selected human bacterial pathogens. Journal of Pharmaceutical Research International, 20(1), 1–8. DOI: 10.9734/JPRI/2017/37897

18. Junaid, S., Olabode, A.O., Onwuliri, F.C., Okwori, A.E.J., and Agina, S.E., 2006. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. African Journal of Biotechnology, 5, 2315–2321.
19. Kenny, D.J., Plichta, D.R., Shungin, D., Koppel, N., Hall, A.B., Fu, B., Xavier, R.J., 2020. Cholesterol Metabolism by Uncultured Human Gut Bacteria Influences Host Cholesterol Level. Cell Host and Microbe, 28 (2), 245–257 *doi*: 10.1016/j.chom.2020.05.013.
20. Kriaa, A., Bourgin, M., Potiron, A., Mkaouar, H., Jablaoui, A., Gérard, P., Maguin, E. and Rhimi, M., 2019. Microbial impact on cholesterol and bile acid metabolism: current status and future prospects. Journal of Lipid Research, 60 (2), 323–332. https://doi.org/10.1194/jlr.R088989.
21. Liu, M., Li, M., Liu, J., Wang, H., Zhong, D., Zhou, H., and Yang, B., 2016. Elevated urinary urea by high-protein diet could be one of the inducements of bladder disorders. Journal of Translational Medicine, 14, 53. https://doi.org/10.1186/s12967-016-0809-9
22. Mahomoodally, M.F., 2013. "Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants", Evidence-Based Complementary and Alternative Medicine. Article ID 617459, 14 pages. https://doi.org/10.1155/2013/617459
23. Marshall, B.M., and Levy S.B., 2011. Food animals and antimicrobials: Impacts on human health. Clinical Microbiology Reviews, 24, 718–733
24. McComb, R.B., Bowers, G.N. and Rosen, S. 1983. Alkaline phosphate. Plenum Press, New York.
25. Hossain, M.B., Nigel, B., and Dilip, R., 2016. Effect of Drying Methods on the Steroidal Alkaloid Content of Potato Peels, Shoots and Berries. Molecules, 21(4),403. 10.3390/molecules21040403.
26. Mousavi, L., Mohd S.R., and Murugaiyah, V., 2019. Nutritional and antinutritional values of leaves and stems of *Ocimum tenuiflorum* L. Food Research, 3(6), 798–807. 10.26656/fr.2017.3(6).160.
27. Nakamura, C., Ueda-Nakamura, T., Bando, É., Melo, A. Cortez, D. and Filho, B., 1999. Antibacterial Activity of *Ocimum gratissimum* L. Essential Oil. Memórias do Instituto Oswaldo Cruz, 94, 675–8. 10.1590/S0074-02761999000500022.
28. Nhung, N.T., Chansiripornchai, N., and Carrique-Mas, J.J., 2017. *Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review.* Frontiers in Veterinary Science, 4(126), 1--16, doi:10.3389/fvets.2017.00126
29. Njoku, N.E., Ubbaonu, C.N., Alagbaoso, S.O., Eluchie, C.N., Umelo, M.C., 2015. Amino acid profile and oxidizable vitamin content of *Synsepalum dulcificum* berry (miracle fruit) pulp. Food Sciences and Nutrition, 3(3), 252–256. doi: 10.1002/fsn3.213.
30. Nweze, B.O. and Ekwe, O.O., 2012 Growth performance, gut and haemo-microbial study of finishing broilers fed African sweet basil (*Ocimum gratissimum*) leaf extract. Ozean Journal of Applied Sciences, 5 (2), 185–191.
31. Nworgu, F.C., Yekini, B.O. and Oduola, O.A., 2013. Effects of basil leaf (*Ocimum gratissimum*) supplement on some blood parameters of growing pullets. International Journal of Agriculture: Research and Review, 3 (3), 480–488.

32. Olanrewaju, R.A., Ale, E.M., and Akinwale, S.O. 2017. Qualitative and Quantitave Evaluation of the Phytochemicals in Dry, Wet and Oil Extracts of the Leaf of Morinda lucida. Journal of Biology, Agriculture and Healthcare, 6, 22–25.
33. Oparaocha, E.T., Iwu, I., and Ahanakuc, J.E., 2010. Preliminary study on mosquito repellent and mosquitocidal activities of *Ocimum gratissimum* (L.) grown in eastern Nigeria. Journal of Vector Borne Disease, 47(1), 45–50. PMID: 20231773.
34. Pandey, S., Singh, S., Kumar, N., and Botany, R., 2017. Antiviral, antiprotozoal, antimalarial and insecticidal activities of *Ocimum gratissimum*. Asian Journal of Pharmaceutical Research and Development, 5, 1–9.
35. Reig, M., and Toldra, F., 2008. Veterinary drug residues in meat: concerns and rapid methods for detection. Meat Science, 78:60–7. doi:10.1016/j.meatsci.2007.07.029
36. Samtiya, M., Aluko, R. E. and Dhewa, T. 2020. Plant food anti-nutritional factors and their reduction strategies: an overview. Food Production, Processing and Nutrition 2, 6. https://doi.org/10.1186/s43014-020-0020-5
37. SAS Institute Inc 2013. SAS/ACCESS® 9.4 Interface to ADABAS: Reference. Cary, NC: SAS Institute Inc.
38. Schalm, O.W., Jain, N.C. and Caroll, E.J., 1975. Veterinary haematology. 3rd Edn., Lea and Fabiger, Philadelphia. Pp. 15–218.
39. Shuaib, O.R., Adeniran, O.I., Musah, M., Yerima, H., Sani, H. and Amusat, K., 2015. Comparative nutritional and anti-nutritional analysis of *Ocimum grattissimum and Ocimum basilicum*. Academic Arena, 7 (7), 77–81. (ISSN 1553-992X). http://www.sciencepub.net/academia. 10
40. Sumitha, K.V., and Thoppil, J.E., 2016. Larvicidal efficacy and chemical constituents of *O. gratissimum* L. (Lamiaceae) essential oil against Aedes albopictus Skuse (Diptera: Culicidae). Parasitology research, 115(2), 673–80. doi: 10.1007/s00436-015-4786-3. PMID: 26462801.
41. Talebi, A., Rezaei, S., Rozeh-Chai, R. and Sahraei, R., 2005. Comparative Studies on Haematological Values of Broiler Strains (Ross, Cobb, Arbor-acres and Arian). *International Journal of Poultry Science*, 4, 573–579.
42. Thu, T.N.D., Thompson, L.D., Galyean, M.L., Brooks, C.J., Patterson, K.Y. and Boylan, M.L., 2011. Cholesterol Content and Methods for Cholesterol Determination in Meat and Poultry Comprehensive Reviews. Food Science and Food Safety, 10(5), 269–289. https://doi.org/10.1111/j.1541-4337.2011.00158.x
43. United State National Nutritional Data. 2010. United State Document on Nutrition.
44. Zaharaddeen, N.G., and Samuel, O., 2019. The effect of different drying methods on the elemental and nutritional composition of *Vernonia amygdalina* (bitter leaf), Journal of Taibah University for Science, 13 (1), 396–401, DOI: 10.1080/16583655.2019.1582148