



Received: 02-05-2024
Accepted: 12-06-2024

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Supplemental Value of Scent (*Ocimum gratissimum* Linn) Leaf Meal on Growth Performance and Haemo-biochemical Response of Broiler-chickens

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Abstract

The growth performance and haemo-biochemical response of broiler-chickens (n=200) to dietary supplementation of scent leaf meal (SLM) was investigated in a 42-day feeding trial. Birds on Diet 1 had *Ocimum gratissimum* leaf meal (OGLM) at 0 g/100kg feed and served as the positive control, diet 2 had OGLM at 0 g/100kg feed with 250 mg/kg antibiotic, diet 3 had OGLM at 250 g/100kg feed, diet 4 had OGLM at 500 g/100kg feed, diet 5 had 750 g/100kg feed and diet 6 had OGLM at 1000g/100kg feed. Feed intake,

growth rate and feed utilization were not significantly ($P>0.05$) influenced among birds fed the various test diets. Haemato-biochemical parameters such as packed cell volume, red blood counts, haemoglobin concentration, white blood counts, AST and ALT were influenced in broiler chickens fed dietary supplementation of OGLM. Supplementation of OGLM at 500-750 g/100kg diet was the optimal level as evidenced in growth while up to 1000 g/100kg feed in the haemato-biochemical parameters.

Keywords: Broiler Chickens, Haematology, *Ocimum Gratissimum*, Serum Enzymes

Introduction

Antibiotics have long been used as growth promoters and health maintenance additives in livestock industry. Pathetically, their indiscriminate use in livestock production has been reported to cause livestock to develop resistant to the drug leading to the creation of super bug bacterial with serious health complications (Lee *et al* 2013)^[17]. The overuse of antibiotics is also having indirect consequence on the health of humans upon the consumption of animals and animal products raised through this synthetic drug. This has hitherto led to increased antimicrobial resistance with severe infections, complications, increased morbidity and short life span. This prompted the European Union to ban the use of synthetic antibiotic as growth promoters in livestock industry. Other countries had since tolled the same line.

Herbs, shrubs and other botanicals have been identified to possess phytonutrients and pharmacological properties that could improve livestock growth, confer immunity as well as improve meat quality of the animal without the fear of residual effect on the consumption of the animal and, or their products (Diarra and Malouin 2014; Oloruntola *et al* 2018; Ogunsipe *et al* 2020)^[8, 27, 25]. Thus, these botanicals as reliable antibiotic alternatives (phytobiotic) are beginning to gain attention and acceptance in the livestock industry. Among the botanicals of nutritional and medicinal importance is scent (*Ocimum gratissimum*) leaves.

Scent (*Ocimum gratissimum*) leaf is noted for its nutritional value and phytomedicinal properties. It is high in magnesium, calcium, phosphorus, potassium, iron and vitamin A. The role of the vegetable leaf in digestive process could possibly be due to the presence of eugenol, methyl cinnamate, camphor and thymol (Nakamura *et al.*, 1999; Nweze and Ekwe, 2012)^[21, 24] that increase the feed aroma, stimulating the desire to eat more with enhanced growth performance.

The pharmacological properties of *Ocimum gratissimum* and its bioactive constituents are evident in their antioxidant, anti-inflammatory, anticancer, hepatoprotective, antidiabetic, antiseptic, antihypertensive, antidiarrhea and antimicrobial activities (Oloruntola *et al.*, 2021; Ezenwosu *et al.*, 2022; Onainor *et al.*, 2023)^[28, 9, 29] and this the leaf could be referred to as a therapy to multidimensional ailments. As phytobiotic agent, scent leaves have antibacterial activity against *Saphylococcus aureus*, *Salmonella enteritidis*, *Escherichia coli*. It is also having antifungal activity against *Penicillium notatum*, *Candida albicans*, *Microsporeum gyseum*. The leaf helps to lower blood sugar levels and thus act as antidiabetic supplement. The activity of *Ocimum gratissimum* leaves to lower blood sugar, reduce cholesterol (LDL cholesterol) and increase blood circulation with attendant proper nerve functioning could be due to its high calcium and magnesium levels. Calcium and magnesium as divalent

cations can form insoluble substance with fatty acids in the intestine to prevent the absorption of saturated fat leading to a reduction in serum cholesterol levels. Magnesium acts on the enzyme lecithin cholesterol acyl transferase (LCAT); a plasma enzyme that esterifies cholesterol by lowering low density lipoprotein (LDL) and triglyceride levels and raises high-density lipoprotein (HDL) levels.

Scent leaves also contain bioactive components and essential oils that are of both nutritional and health benefits. Such essential oils and bioactive compounds of importance include thymol, eugenol, geraniol and linalool, flavonoids, saponins, tannins, alkaloids, terpenoids, quercetin, phenols, glycosides, steroids, rosmarinic acid and oxalic acid. These compounds have varied phytonutrient and pharmacological benefits that could be exploited in livestock production. The thrust of this study is to assess the supplemental levels of *Ocimum gratissimum* leaf meal as phyto-genic feed supplement in broiler chicken production.

Materials and methods

Ethics approval: The right to conduct the experiment was approved by the Research Committee of the Department of Agricultural Science, Adeyemi Federal University of Education, Ondo, Nigeria.

Collection and processing of scent leaf: Leaves from healthy *Ocimum gratissimum* plants were collected from the University environment. The leaves were washed under running water to remove dirt, air-dried under shade, milled to produce leaf meal and stored under air tight condition before use. The air-dried leaves were analyzed for their proximate composition and mineral contents (AOAC 2002) [5].

Feed preparation: A basal diet was formulated for each phase of the birds using the conventional feed ingredients as shown in Table 1. The basal diet was divided into five equal portions, designated diets 1-5, and supplemented with scent leaf meal at graded levels. The diets for the starter and finisher birds were prepared to meet the nutrient requirements of broiler chickens (NRC 2012) [23].

Table 1: Feed composition of broiler chickens (g/100g)

	Broiler starter	Broiler finisher
Ingredients		
Maize	54.1	58.6
Rice bran	5.72	5.43
Wheat offal	6.16	6.41
Groundnut cake	10.1	9.41
Soybean meal	15.7	13.5
Fish meal	5.12	3.55
Lime stone	1.50	1.55
Dicalcium Phosphate	0.50	0.50
Premix	0.30	0.25
Lysine	0.15	0.15
Methionine	0.15	0.15
Salt	0.50	0.50
Nutrient composition		
Crude protein (%)	23.1	20.3
ME (kcal/kg)	3125	2918

Note: The diets were supplemented to have: Diet 1 (0 g SLM /100kg diet), diet 2 (250 g SLM /100kg diet), diet 3 (500 g SLM /100kg diet), diet 4 (750 g SLM /100kg diet), diet 5 (1000 g SLM /100kg diet).

Experimental design: The design of the experiment was complete randomization involving 200 Arbor-Acre one-day old unsexed broiler chicks distributed to 5 dietary treatments of 40 birds to a treatment. Each dietary treatment was replicated 5 times of 8 birds to a replicate.

Bird's management: The birds were housed in their group pens with wood shaving as the litter material. The temperature of the house was within the range of $32 \pm 2^\circ\text{C}$ on the first day to day 7 after which the temperature was reduced by 2°C on each successive week until a temperature of $28 \pm 2^\circ\text{C}$ was maintained till the end of the experiment. Fresh feeds and clean cool water were served the birds ad libitum throughout the 6 weeks experimental period.

Blood collection and haemato-biochemical determination: At 42 days of the experiment, blood were collected from 3 birds per replicate ($n=75$) via the wing vein into two separate bottles. Blood collected into EDTA bottle was used for haematological determination while blood collected without EDTA was used for the serum biochemical determination using commercial kits (Reflection® Plus 8C79 (Roche Diagnostic, GmbH Mannheim, Germany).

Data collection and analysis: Data collected on daily feed consumption, weight gain, haematological indices, serum lipids and data calculated on feed conversion ratio were subjected to one-way analysis of variance using General Linear Model (GLM) of SPSS (2006) [32]. Means were compared using Duncan option of the statistical software.

Results

Judging from the nutrient composition (Table 2), *Ocimum gratissimum* leaf contains appreciable amount of nutrients that can justify its supplementation in broiler chicken diet.

Table 2: Chemical composition of the *Ocimum gratissimum* leaf

Proximate composition, g/100g		Mineral contents, mg/100g			
		Macro minerals		Micro minerals	
Dry matter	94.61	Ca	2451	Fe	602
Crude protein	13.22	P	45.63	Mn	10.32
Crude fibre	8.23	Na	128	Zn	31.74
Crude fat	2.21	K	248	Cu	1.09
Ash	4.33	Mg	2714		

The result of the performance of broiler chickens on Table 3 reveals that feed intake and feed conversion were not significantly ($P<0.05$) affected by dietary supplementation of *Ocimum gratissimum* leaf meal but at 500 and 750 g/100kg *Ocimum gratissimum* leaf meal supplementation, final live and weight gain of broiler chickens recorded higher significant ($P < 0.05$) difference compared with birds on 0 and 1000 g/100kg *Ocimum gratissimum* leaf meal supplementation ($P>0.05$). It is worthy of note to report that the final live and weight gain of broiler chickens on antibiotic treated diet did not differ significantly ($P>0.05$) compared with birds on the control (0 g/100kg and 250-1000 g/100kg) *Ocimum gratissimum* dietary supplementation. This thus implied that *Ocimum gratissimum* leaf meal could be a viable alternative (phyto-genic feed additive) to the synthetic antibiotics.

Table 3: Performance of broiler chickens fed OGLM (g/100kg) in the diet

	0 OGLM	0 OGLM + A	250 OGLM	500 OGLM	750 OGLM	1000 OGLM	SEM	P
Live weight/bird								
Initial, g	37.99	37.87	37.96	37.91	37.80	37.88	0.14	0.86
Final, g	1654.31 ^b	1663.88 ^{ab}	1656.16 ^b	1684.21 ^a	1683.21 ^a	1658.92 ^b	7.66	0.03
Daily gain, g	38.49 ^b	38.71 ^{ab}	38.53 ^b	39.20 ^a	39.18 ^a	38.59 ^b	0.18	0.03
Feed intake, g/d	84.12	84.29	83.87	84.77	85.09	84.71	0.32	0.11
Feed conversion	2.17	2.17	2.18	2.16	2.17	2.19	0.01	0.38

Note: The diets were supplemented to have: Diet 1 (0 g SLM /100kg diet), diet 2 (0 g SLM /100kg diet + A: 250mg antibiotic), diet 3 (250 g SLM /100kg diet), diet 4 (500 g SLM /100kg diet), diet 5 (750 g SLM /100kg diet), diet 6 (1000 g SLM /100kg diet)

Haematological and serum total serum protein parameters increased while blood cholesterol and serum enzymes decreased in broiler chickens fed Ocimum gratissimum leaf meal supplemented diets compared with those on control and antibiotic treated diets. This shows the haematopoietic potential of the Ocimum gratissimum leaf meals to ensure

formation of blood cells without the risk of hypercholesterolemia and transaminitis or hypertransaminasemia as evidenced in the low blood cholesterol and serum enzymes of the broiler chickens on Ocimum gratissimum leaf meal supplemented diets.

Table 4: Haemato-biochemical of broiler chickens fed OGLM (g/100kg) in the diet

	0 OGLM	0 OGLM + A	250 OGLM	500 OGLM	750 OGLM	1000 OGLM	SEM	P
Haematological parameters								
PCV, %	31.30 ^c	32.31 ^a	31.72 ^{bc}	32.16 ^{ab}	32.37 ^a	32.38 ^a	0.17	0.001
RBC counts, 10 ⁹ /ml	3.19 ^b	3.27 ^b	3.37 ^a	3.42 ^a	3.41 ^a	3.43 ^a	0.03	0.001
Hb, g/dL	10.19 ^c	10.25 ^c	10.46 ^b	10.79 ^a	10.80 ^a	10.81 ^a	0.05	0.001
WBC counts, 10 ⁹ /ml	8.90	9.04	8.65	9.04	8.97	9.02	0.13	0.34
Serum metabolites								
TSP, g/dL	3.80 ^c	3.82 ^c	3.85 ^{bc}	3.92 ^{ab}	3.95 ^a	3.93 ^{ab}	0.03	0.01
Chol, mg/dL	137.82 ^a	137.49 ^a	131.54 ^b	128.36 ^{bc}	127.39 ^{bc}	126.38 ^c	1.52	0.001
AST, u/l	256.12 ^a	249.54	243.27 ^b	243.11 ^b	241.67 ^b	238.48 ^b	2.39	0.004
ALT, u/l	2.17 ^a	2.14 ^a	2.10 ^a	1.95 ^b	1.84 ^{bc}	1.79 ^c	0.03	0.001

^{ab} Means without common superscripts along the same row are different at P < 0.05

Discussion

The phytonutrient properties of Ocimum gratissimum leaf meal typified by the levels of moisture, protein, lipid, fibre and minerals reveal the potential of this leaf meal as possible feed supplement in broiler chicken nutrition. Moisture content is an important index to measure the stability, shelf life and deteriorative tendency of a substance or food. The moisture content of Ocimum gratissimum in the present study is slightly below the 10.30-10.72% reported in previous studies (Adewole, 2014; Ajayi 2017) [2, 3]. The low moisture content suggests the tendency of the leaf to be less prone to bacterial spoilage thus ensuring the longer shelf life. Proteins as molecules function to repair damaged and build body tissues, drives metabolic reactions, maintains pH and fluid balance, transports and stores nutrients, keeps the immune system strong and can act as an energy source (through the process of gluconeogenesis) when carbohydrate intake is low. The protein content obtained for Ocimum gratissimum leaf in this study (13.22%) is within the values 12.98-16.51% reported for leafy vegetables (Abu *et al.*, 2014; Fagbohun *et al.*, 2012; Mlitan *et al.*, 2014; Ajayi, 2017) [1, 10, 19, 3]. Dietary fibre has been reported to regulate blood cholesterol, attenuate blood sugar level, maintain bowel health and integrity, lower the risk of hemorrhoids, diverticular disease (small pouches in the colon) and colorectal cancer (Ishida *et al.*, 2000; Bach Knudsen, 2001) [14, 6]. It also ensures healthy digestive process through smooth digestion, prevention of constipation, emptiness of bowel and soft passage of stool (Bach Knudsen, 2001) [6]. The crude fibre reported in this study (9.32%) is within the values 9.07-11.38% reported by previous authors (Fagbohun *et al.*, 2012; Adewole, 2014; Ajayi, 2017) [10, 2, 3]. Fat/lipid has great influence in

contributing to gross energy as it yields over 9 Kcal/g compared to carbohydrate and over 5 Kcal compared to protein (Nagao and Yanagita, 2010) [20]. The crude fat/lipid obtained in this study is within the range 2.78-4.81% earlier reported (Adewole, 2014; Ajayi, 2017) [2, 3] but lower than the 10.25% reported for Vernonia amygdalina leaf (Tsado *et al.*, 2015) [34]. The sensory activity of fat/lipid to enhance texture, flavour and aroma of food makes Ocimum gratissimum leaf a condiment in feed/food preparation. Ash content of feed reflects the presence of inorganic constituents that could dictate the composition and levels of minerals present. Ash as a good source of potassium, magnesium and phosphorus exhibits various biochemical and physiological activities in living cells. Ash contains mannitol that provides a diuretic effect helping eliminate wastes and toxins and increase urine production with overall effect on kidney health. Thus, the dietary ash in Ocimum gratissimum leaf meal could function to promote the health status of broiler birds.

The ash content obtained in this study is lower than the values (10.95-13.67%) earlier reported (Fagbohun *et al.*, 2012; Ajayi, 2017) [10, 3] but higher than the 2.45% reported by Mlitan *et al.* (2014) [19]. The differences observed could be factors arising from dry ashing temperature and duration, sample size, moisture content of the sample, surrounding influences or other extraneous conditions. Minerals are important components in the skeletal frame work of animal body, they are also essential components of many enzymes, hormones, vitamins and pigments and as cofactors for many enzymes involved in metabolism (Fox and Zimba, 2018) [11]. Not only does calcium function in bone and teeth formation and development, it also essential for metabolic process, nerve function, blood clotting and even release hormones

that affect many functions in the animal body (Klimecha and Muszyńska, 2007; Perez-Gallardo *et al.*, 2008; Krebs *et al.*, 2015)^[15, 31, 16].

Mg is an important element responsible for physiological, biological and the chemical functions in muscle and nerves, activation of many enzymes in metabolism and secretion of parathyroid hormones essential for calcium metabolism and energy production, particularly in bone, kidney and adipose tissues. The levels of Mg in this study is higher than the value reported by Okunlola *et al.* (2019)^[26]. Differences observed could be attributed to stage of harvest and method of processing the leaf meal. The high levels of Mg in *Ocimum gratissimum* leaf could make the leaf meal a desirable feed supplement to help keep blood pressure, maintain bone rigidity ensure good heart rhythm (Inoue, 2005)^[13]. Adequate intake of magnesium and potassium are essential for energy production, prevents the development of chronic metabolic complications and powerful support for cardiovascular health (Inoue, 2005)^[13]. Sodium, potassium and chloride ions function to regulate osmotic pressure, maintain membrane potential, promote acid-base equilibrium and transmission of impulses in the body system. Iron in connection with copper and zinc are critical for blood formation and renewal. Thus, the presence of these minerals in *Ocimum gratissimum* leaf meal could have aided stability, healthiness and influenced the growth and blood profile of the birds.

Iron especially, but also copper and zinc are critical for blood function and renewal.

The higher final live weight and body weight gain of broiler chickens on *Ocimum gratissimum* leaf meal, particularly at 500-1000 g/100kg diet could be attributed to the readily available phytonutrients in the diets. Meleese *et al.* (2011)^[18] reported that the better weight gain of birds fed *Moringa oleifera* leaf meal was attributed to the biologically available proteins and high levels of methionine and amino acids in the leafy vegetable. The similarities in the feed intake and feed conversion of birds on the dietary treatments shows the potency of the substances (eugenol, camphor, thymol and methyl cinnamate) in *Ocimum gratissimum* leaf as phytobiotic feed supplement to support adequate feed intake and feed utilization in broiler chicken production. Supplementation of broiler chicken diets with leafy vegetables have been reported to exert various biological activities such as good growth, better feed utilization (Negi, 2012; Vallenzuela-Grijalva *et al.*, 2017; Oloruntola *et al.*, 2021)^[22, 35, 28].

The role of phytochemicals to scavenge toxic radicals, prevent oxidative cell damage, repair DNA, strengthen immune reaction system, modulate metabolism to activate muscle development and promote gut microbiota resulting in increased membrane permeability to enhance nutrient digestion and absorption (Al-Harrasi *et al.*, 2014; Dhama *et al.* 2015; Gonzalez-Rios *et al.*, 2016)^[4, 7, 12] could have enhanced better weight gain in broiler chickens fed *Ocimum gratissimum* leaf meal supplementation. The improved blood profile and decreased blood cholesterol (hypolipidaemia) and serum enzymes of broiler chickens on *Ocimum gratissimum* leaf meal supplemented diets show the property of the leaf meal to promote better erythropoiesis and haematopoiesis of the birds while the values obtained in WBC counts of birds on leaf meal supplements compared to birds the control shows the immunomodulatory effect of *Ocimum gratissimum* leaf

meal to confer immunity on birds (Dhama *et al.* 2015)^[7]. The reduced concentration of cholesterol is an indication of the potency of *Ocimum gratissimum* leaf meal to metabolize fat and reduce the effect of hyperlipidemia or hypercholesterolemia. The potency of *Ocimum gratissimum* leaves to reduce cholesterol (hypcholesterolemia) and increase blood formation (erythropoiesis) with proper nerve functioning could be attributed to its high calcium and magnesium levels. Calcium and magnesium ions can form insoluble substance with fatty acids in the gut to inhibit the absorption of saturated fat leading to a reduction in serum cholesterol levels. Calcium specifically attenuate adipocyte lipid accretion and thus play a pivotal role in the regulation of energy metabolism while magnesium limit adipose tissue accumulation, improve glucose and insulin metabolism, enhance endothelium-dependant vasodilation, normalize lipid profile and attenuate inflammatory response (Pelczyńska *et al.*, 2022)^[30]. Magnesium acts on the enzyme lecithin cholesterol acyl transferase (LCAT); a plasma enzyme that esterifies cholesterol by lowering low density lipoprotein (LDL) and triglyceride levels and raises high-density lipoprotein (HDL) levels (Inoue *et al.*, 2005; Pelczyńska *et al.*, 2022)^[13, 30]. Reduction in ALT and AST may provide evidence for the hepatoprotective effect of the bioactive components in *Ocimum gratissimum* leaf meal (Toghyani *et al.* 2015)^[33]. Thus, the biological activities (as antioxidant, anti-inflammatory, anti-diabetic, antimicrobial) in *Ocimum gratissimum* leaf meal could have enhanced good growth and blood synthesis of broiler chickens placed on *Ocimum gratissimum* leaf meal supplementation.

Conclusion

Supplementation of *Ocimum gratissimum* leaf meal at the optimal level of 500 and 750 g/100kg diet enhanced broiler growth and up to 1000 g/100kg diet enhanced blood cell formation.

Broiler farmers in this part of the world are encouraged to utilize this leaf meal as phytobiotic feed supplement in broiler chicken diet.

Acknowledgment

The authors appreciate the financial support of Tetfund towards the research work.

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