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Can Dietary Supplementation of Clinopodium brownei oil Influence the Growth Performance, Haematology and Serum Biochemical Indices of Hubbard Broiler Chickens?

Alagbe J.O
Adjunct Lecturer, Department of Biochemistry, Gandhi College of Agriculture, Rajasthan, India
Assistant Professor, Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India
Department of Animal Science, University of Abuja, Nigeria

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Annotation: This experiment was undertaken to examine the influence of dietary supplementation of Clinopodium brownei oil on growth performance and some blood parameters of Hubbard broiler chickens. 400 – 1 day old Hubbard broilers of mixed sex were randomly distributed into four groups (T1, T2, T3 and T4) of 100 birds. Each group was further divided into five replicate consisting of twenty birds. Treatment T1 (Negative group/control): experimental diet without any additive, T2 (Positive control): experimental diet with 2.5 g Adriamycin® /kg diet, T3: experimental diet with 2.0 mL Clinopodium brownei oil per kg diet, T4: experimental diet with 4.0 mL Clinopodium brownei oil per kg diet. Experimental diet was formulated to meet the nutrient requirements for broilers requirements according to NRC (2012) recommendations. The experiment lasted for 42 days and a completely randomized design was adopted. Feed and fresh water was always made available. Overall result obtained reveals that in T3 and T4, the body weight gain and feed consumption was higher; in T2, it was intermediate; and in T1, it was lower

($p<0.05$). Dietary supplementation of significantly enhanced ($p<0.05$) red blood cell, pack cell volume, haemoglobin and white blood cell count. Clinopodium brownei oil supplementation decreased serum cholesterol concentration, mortality, feed conversion ratio and increase ($p<0.05$) concentrations of total protein, albumin, globulin, alanine amino transferase and aspartate transaminase. In conclusion, Clinopodium brownei oil supplementation at 2.0 mL or 4.0 mL/kg diet improved body weight gain, feed consumption, feed conversion ratio and some blood constituents without compromising the health status of birds.

Keywords: Clinopodium brownei oil, growth, performance, blood, nutrients.

Introduction

As the demand for antibiotic free feed is increasing globally due to the rising cases of antimicrobial resistance which threatens both human and animals (Alagbe, 2024). Incorporation of antibiotics in feed even at a lower concentration can foster resistant genes in bacteria over time and thus need to be prevented (Alagbe et al., 2022). After the ban by the European Union in 2006, the use of medicinal plants have been suggested as one of the potential alternatives to antibiotics (Musa et al., 2020). Herbs offer a promising tool to reduce antibiotic use because they contain bioactive compounds, eco-friendly, non-toxic and are generally regarded as safe (Daniel et al., 2023).

Clinopodium brownei is a plant that carries a unique spectrum of bioactive compounds found in other trees. It is an aromatic, herbaceous, perennial plant which belongs to the family Lamiaceae and native to Tropical and sub-tropical America (Noriega et al., 2023; Aguilar et al., 2015). Every part of the plant holds a therapeutic value. The leaves heals fever, cough, arthritis, diarrhea, nausea, sore throat and vomiting (Vandebroek and Picking, 2020; Noriega et al., 2019). Stem bark addresses skin infection, sexually transmitted disease, pile and eye problems (Ballesteros et al., 2019; Zhiñin et al., 2021). The fruit decoction helps in strengthening circulation of blood, digestion and also serves as tonic for the liver, heart, lungs and kidney (Cuesta et al., 2017). Methanolic extract from *Clinopodium brownei* have been found to exhibit cytotoxic activity (Mestanza-Ramón et al., 2020; Aziz et al., 2018). *Clinopodium brownei* leaves have potential deposit of phyto-compounds which includes, flavonoids, tannins, alkaloids, saponins and phenolic compounds which exhibits potent anti-inflammatory, anti-tumor, anti-arthritic, anti-convulsant, anti-rheumatoid, anti-dysentery, antiviral, gastro-protective, cardio-protective, immune-stimulatory, diuretic, anti-helminthic, anti-paralysis, antimicrobial, antioxidant, antifungal and wound healing properties (Diniz et al., 2020; Bruni et al., 2004).

Previous studies by Shittu et al. (2024) revealed that the supplementation of ginger oil in the diet of birds can accelerate growth, improved feed efficiency, neutralize reactive oxygen species, stabilizes cell membrane, modulate immune activity and coordinate defense against oxidative and inflammatory damage. Obikaonu et al. (2012) also reported that feeding broiler chickens with neem oil at 0.30 mL/kg diet resulted in gut protection by inhibiting pathogenic bacteria and

fungi, relieved chronic inflammation, regulate blood glucose level and cholesterol balance. Essential oils from rosemary, oregano and garlic have been well documented to promote detoxification, protects liver against oxidative stress, improve cardiovascular health, boost the immune system and improve the overall vitality of birds (Nworgu et al., 2007; Agubosi et al., 2022; Alagbe, 2024). However, there is little or no information on the dietary supplementation of *Clinopodium brownei* oil. This research will help to establish an optimum level of *Clinopodium brownei* oil in broiler feed, promote livestock production, sustainability and food safety. It will also help to promote the use of *Clinopodium brownei* oil as natural alternative to antibiotics.

Materials and methods

Study/Experimental area

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Gandhi College of Agriculture, Rajasthan, India between the months of June to August, 2025. The research ethics and guidelines of the Animal Production and Health Department of the institution approved the conduct of the experiment (GN/067H/2025C).

Collection and preparation of *Clinopodium brownei* extracts

Freshly collected leaves of *Clinopodium brownei* from Gandhi College premises was sent to the Taxonomy department of the institution for proper identification and authentication by a certified taxonomist before it was assigned a voucher specimen number GNT/08/005. Collected leaves were sorted, washed with running tap water and dried under shade for 12 days, then grounded into powdered with an electric blender and kept in an airtight containers under room temperature. 500 g of grounded *Clinopodium brownei* was steam-distilled using H-shaped Clavenger apparatus for 2 hours to obtain the essential oil. The essential oil was then dried by anhydrous sodium sulfate and stored in sample bottles and kept in the refrigerator at 4℃ for further analysis.

Chemical analysis of bioactive compounds were carried out using Aludra Quadrupole GC/MS (Model T8050NX). Gas chromatograph was kept at a specification of temperature (15 to 35℃), humidity (25 – 80 %), column head pressure setting of 0 ~ 100 psi, cooling speed of 50 ℃ and maintained at a pressure range of 0 – 999 Kpa once 0.5 mL of *Clinopodium brownei* oil was injected into the machine while the mass spectrometry unit was maintained at mass stability 0.1 amu, maximum scan rate of 10,000 amu, emission current 10 – 350 μA and ionization energy (150 – 320 ℃) before the outcome or results were processed via MS 3200RT software to calculate the percentage concentration and retention time of each compounds as presented in Table 1.

Management of birds

400 – 1 day old Hubbard broiler of mixed sex was purchased from a commercial hatchery in Rajasthan, India. Birds were cared for according to management recommendation approved by Indian Society of Animal Production. Before arrival of the birds, battery cages, pens, feeding and watering troughs were properly washed and disinfected with Supermax Morigad® with Aquaclean® in the ratio of 1:1. Upon arrival chicks were unboxed and their average initial body weight was measured using a digital sensitive scale before they were randomly distributed into four groups (T1, T2, T3 and T4) of 100 birds, each group was further divided into five replicate consisting of twenty birds. Anti-stress (Glucose + Multivitamins) for 3 days and the experiment lasted for 42 days. Brooding temperature was maintained at 35 ℃ for the first week and it was reduced each week by 2 ℃ until a temperature of 27℃ was achieved. Experimental diet was formulated to meet the nutrient requirements for broilers requirements according to NRC (2012) recommendations as presented in Table 2. Birds had free access to fresh water, feed and were vaccinated according to the vaccination schedule developed by Gandhi College of Agriculture, Rajasthan based on disease prevalence in the area. Strict biosecurity and other management measures were observed throughout the period of the experiment. During data collection periods,

feed consumption was estimated as the difference between feed offered and refusals from the previous day feeding. Body weight gain (g) was calculated as the difference between final body weight and initial body weight. Feed conversion ratio was determined by dividing total feed consumption by body weight gain. Mortality was recorded as it occurs during the experimental period. Proximate composition of experimental diets were analyzed according to AOAC (2016) official methods.

Set of the experiment

Birds were randomly allocated into four groups, each group has five replicate consisting of twenty birds each in a completely randomized design as follows:

Treatment 1 (Negative group/control): Experimental diet without any additive

Treatment 2 (Positive control): Experimental diet with 2.5 g *Adriamycin® /kg diet (*according to the instructions on the package insert)

Treatment 3: Experimental diet with 2.0 mL *Clinopodium brownei* oil per kg diet

Treatment 4: Experimental diet with 4.0 mL *Clinopodium brownei* oil per kg diet

Sampling and analysis of blood constituents

On the last day of the experimental period, 6 mL of blood was collected from the wing vein of ten randomly selected birds per treatment. 3 ml of blood each for haematological evaluation and serum biochemical analysis. Before collection, ice pack was made available to maintain the samples and prevent deterioration. Blood for haematology was collected into bottles containing EDTA (anticoagulant) while those for serum biochemistry was collected into plain sample bottles. MT-Musson Automated –Haemo Analyzer (GH-3300, China) was used to determine: red blood cell count, haematocrit value, haemoglobin and white blood cell count. Kit was maintained at a temperature of 15 to 30 ℃ and humidity (70 – 85 %) after samples were arranged in the metallic collection chamber. Serum samples were analyzed with HBI – Auto chemistry analyzer (Model VB3000C, Taiwan) and maintained at a sample volume of 245 µL, temperature (10 to 35 ℃) and humidity (5 to 85 %).

Statistical analysis adopted

Data collected were subjected to the analysis of variance (ANOVA) using the General Linear Model Procedure of Statistical Analysis System (SAS, version 9.1.3 of 2001). Duncan multiple range test of the same package was used for the post hoc analysis where significant difference occurred at $P < 0.05$.

Results and discussion

Bioactive compounds in *Clinopodium brownei* oil is presented in Table 1. The essential oil has a unique chemistry and its most prominent compound includes, α-Humulene (27.42 %), Camphor (17.92 %), Hexadecanoic acid (15.23 %), α-Terpineol (14.21 %), γ-Terpinene (12.99 %), p-Cymene (12.56 %), Oleic acid (11.24 %), α-Pinene (10.05 %), β-Caryophyellene (8.17 %) and Cis-linalool oxide (5.93 %) which have been previously associated with anti-inflammatory, antioxidant, gastro-protective, anti-tumor and dermato-protective properties (Hernandez and Alagbe, 2025a,b). β-Caryophyellene have been reported to reduce oxidative stress inside the liver, stabilizes cell membrane and boost glutathione (Singh et al., 2022; Ojediran et al., 2024a,b). α-Terpineol and γ-Terpinene have shown to inhibit the activities of some pathogenic bacteria and fungi in the gastro-intestinal tract of birds (Alagbe, 2025; Adewale et al., 2021). These compounds have also been utilized traditionally in the treatment of chronic inflammations, skin infections, urinary tract infections, pyrexia, sexually transmitted diseases, snake bite, tooth ache amongst others (Musa et al., 2020).

Table 1: Prominent Bioactive compounds in *Clinopodium brownei* oil by GC-MS analysis

Bioactive compounds	% Area	Retention time (min)
γ-Terpinene	10.94	12.99
α-Terpineol	6.03	14.21
β-Caryophyellene	15.05	8.17
α-Humulene	7.75	27.42
α-Pinene	12.37	10.05
p-Cymene	10.04	12.56
Cis-linalool oxide	8.74	5.93
Camphor	7.93	17.95
Hexadecanoic acid	11.46	15.23
Oleic acid	9.22	11.24

Table 2: Percentage composition of experimental diet for starter phase (0-21d) and finisher phase (22-42d)

Ingredients	Starter (%)	Finisher (%)
Maize	50.18	55.02
Wheat bran	4.01	5.63
Soymeal	35.06	30.0
Fish meal	5.00	2.00
Mono Calcium Phosphate (MCP)	3.00	4.00
Calcium bicarbonate	1.50	2.00
Lysine	0.20	0.25
Methionine	0.25	0.25
Premix (Vitamin and Mineral)	0.25	0.25
Salt	0.30	0.35
Toxin binder	0.25	0.25
Total	100	100
Analyzed Nutrients (Percentage)		
Crude protein	23.44	21.09
Ether extract	4.02	3.94
Crude fibre	3.47	3.83
Calcium	1.18	1.21
Phosphorus	0.58	0.61
ME (kcal/kg)	3015.3	3218.2

Each 2.5 kg consists of: Vit A 12000, 000 IU; Vit D3, 2000, 000 IU; Vit. E. 10g; Vit k3 2 g; Vit B1, 1000 mg ; Vit B2, 49g ; Vit B6, 105 g; Vit B12, 10 mg; Pantothenic acid, 10 g; Niacin, 20 g , Folic acid , 1000 mg ; Biotin, 50 g; Choline Chloride, 500 mg, Fe, 30 g; Mn, 40 g; Cu, 3 g; Co, 200 mg; Si, 100 mg and Zn , 45 g

Growth performance of Hubbard broilers fed diet supplemented with *Clinopodium brownei* oil is presented in Table 3. In the starter, finisher and overall result obtained showed that birds fed diet supplemented with *Clinopodium brownei* oil in treatment 3 and treatment 4 had the highest ($p<0.05$) body weight gain and feed consumption followed by the antibiotics group (treatment 2) with the non-supplemented group showing the least figures. Body weight result suggests that the presence of bioactive compounds in *Clinopodium brownei* oil especially α-Humulene can protect the stomach lining from pathogenic organisms to allow smooth digestion and absorption of nutrients which translates to a better weight gain (Shittu and Alagbe, 2020). These compounds

also have no withdrawal period and are generally regarded as safe (Hernandez and Alagbe, 2025). Previous studies have also shown that α-Humulene can shut down inflammatory messenger and protects tissues from oxidative stress (Zubairu et al., 2025). Though birds fed with antibiotics also show an appreciable body weight gain because synthetic antibiotics also interfere with the activities of some pathogens in the gut, however, its continuous use can cause the deposit of toxic residues in animal products, environment as well as antimicrobial resistance (Omokore and Alagbe, 2019). The result obtained in this study aligns with the report of Agubosi et al. (2022) who supplemented sunflower essential oil in the diet of broiler chickens. John et al. (2024), also recorded a body weight gain of 2205 – 2607.1 g/b in birds fed diet supplemented with phytogenics. Increase in feed consumption especially among birds in Treatment 3 and 4 indicates that *Clinopodium brownei* oil improved the aroma and taste of feed compared to the other treatments. These result is in agreement with the report of Muritala et al. (2022) who reported a higher feed intake in broilers fed diet supplemented with *Polyalthia longfolia* leaf. Overall total feed consumption value which varied from 4743.2 – 5203.9 g/b was higher than 4800.2 – 5106.7 g/b reported by Zubairu et al. (2025) when *Sphenocentrum jollyanum* oil was fed to broiler chickens. Variation in result could be attributed to the differences in the chemical compounds of essential oils, breed, geographical location as well as concentration or dosage administered (Ojediran et al., 2024). Highest mortality was recorded in the non-supplemented treatment followed by the antibiotic group while none was reported in treatment 3 and 4. The presence of γ-Terpinene, Camphor and Hexadecanoic acid ignites an antimicrobial impact in the tightening, protection of the gut lining and inhibits the activities of bacteria and fungi (Alagbe et al., 2022). The overall mortality rate (1.00 %) recorded in antibiotic growth suggests that possibility of resistance by some of the pathogens in the gut causing gastro-intestinal disturbance and also possibly interfering with the absorption of nutrients (Hernandez and Alagbe, 2025). The best feed conversion was recorded among birds in treatment 3 and 4 compared to the other group. This result is in consonance with the report of Adewale et al. (2021); Muritala et al. (2022).

Table 3: Growth performance of Hubbard broilers fed diet supplemented with *Clinopodium brownei* oil

Parameters	T1	T2	T3	T4	SEM
Starter phase (0 to 21 d)					
Initial body weight (g/b)	53.11	52.96	53.11	53.12	0.31
Final body weight (g/b)	663.7c	702.8b	755.4a	760.9a	33.17
Body weight gain (g/b)	610.59c	649.84b	702.29a	707.78a	21.06
Total feed consumption (g/b)	1442.6c	1511.7b	1600.7a	1605.2a	67.92
Feed conversion ratio	2.36a	2.32b	2.27c	2.26c	0.21
Mortality (%)	1.00	1.00	-	-	0.03
Finisher phase (22 to 42 d)					
Body weight gain (g/b)	1509.5c	1771.8b	1866.1a	1868.9a	73.61
Total feed consumption (g/b)	3300.6c	3508.6b	3591.2a	3598.7a	102.8
Feed conversion ratio	2.19a	2.00b	1.92c	1.92c	0.03
Mortality (%)	1.50	-	-	-	0.02
Overall (1 to 42 days)					
Body weight gain (g/b)	2173.2c	2474.6b	2621.5a	2629.8a	88.76
Average daily weight gain (g/b)	51.74c	58.92b	62.42a	62.61a	0.57
Total feed consumption (g/b)	4743.2c	5020.3b	5191.9a	5203.9a	151.2

Average daily feed consumption (g/b)	112.9c	119.5b	123.6a	123.9a	2.86
Feed conversion ratio	2.18a	2.03b	2.00c	2.00c	0.03
Mortality (%)	2.50a	1.00b	-	-	0.01

Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; T1: Experimental diet without oil (control); T2: experimental diet + 2.5 g Adriamycin® /kg diet; T3: experimental diet + 2.0 mL *Clinopodium brownei* oil/kg diet; T4: experimental diet + 2.0 mL *Clinopodium brownei* oil/kg diet

Haematological parameters of Hubbard broilers fed diet supplemented with *Clinopodium brownei* oil is presented in Table 4. Pack cell volume, red blood cell and white blood cell values were higher ($p<0.05$) in treatment 2, 3 and 4 compared to treatment 1. Hemoglobin value was higher in treatment 3 and 4, intermediate in treatment 2 and lowest in treatment 1 ($p<0.05$). The pack cell volume recorded in this study was within 25.09 – 36.00 % range cited by Musa et al. (2020). Haemoglobin value was within 7.50 – 20.00 g/dL referenced by Agubosi et al. (2022). These values point to the presence of iron sufficiency in the blood (John, 2024c). Red blood cell values were within the values 1.90 – 3.08 (10^{12}/L) recorded by Musa et al. (2020); Oluwafemi et al. (2022) who supplemented turmeric oil in the diet of broiler chickens. However, values were lower than 2.02 – 3.50 (10^{12}/L) reported by John (2024c). This result suggests sufficient oxygen in the tissue to drive absorbed nutrient round the body of birds to ensure proper functioning of the system (John, 2024d). The values of white blood cell in this experiment were within the normal range 9.66 – 26.00 (10^{9}/L) reported by Shittu and Alagbe (2020) who fed broilers with *Sida acuta* leaf extract. Omokore and Alagbe (2019) reported that high concentration of white blood cell in the serum triggers the production of antibodies to prevent the entry and proliferation of infection in the body.

Table 4: Haematological parameters of Hubbard broilers fed diet supplemented with *Clinopodium brownei* oil

Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**
Pack cell volume (%)	28.16b	30.96a	32.18a	33.04a	0.37
Hemoglobin (g/dL)	8.42c	10.07b	13.59a	14.02a	0.06
Red blood cell (10^{12}/L)	2.15b	2.28a	2.53a	2.55a	0.02
White blood cell (10^{9}/L)	10.03c	13.72b	16.32a	16.84a	0.03

Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; T1: Experimental diet without oil (control); T2: experimental diet + 2.5 g Adriamycin® /kg diet; T3: experimental diet + 2.0 mL *Clinopodium brownei* oil/kg diet; T4: experimental diet + 2.0 mL *Clinopodium brownei* oil/kg diet

Serum biochemical indices of Hubbard broilers fed diet supplemented with *Clinopodium brownei* oil is presented in Table 5. Total protein concentration in treatment 1 (4.40 g/dL) were lower ($p<0.05$) than treatment 2 (4.85 g/dL), treatment 3 (5.40 g/dL) and treatment 4 (5.46 g/dL). Albumin and globulin values which varied from 2.09 – 2.61 g/dL and 2.31 – 2.81 g/dL were influenced ($p<0.05$) by the treatment. Total protein values reported in this study were within the normal range 4.00 – 7.51 g/dL reported by Abudabos et al. (2018) when phytogenics was supplemented in the diet of broiler chickens. However, values were lower than 5.00 – 7.08 g/dL recorded by Shittu et al. (2024). Result obtained suggests that the birds were well nourished (John, 2024a). Albumin and globulin values were within the values 2.00 – 4.00 g/dL and 2.00 – 5.00 g/dL reported by Daniel et al. (2023) when papaya essential oil was supplemented in the diet of broiler chicks. Serum globulin plays a significant role in the fight against infections (Musa et al., 2020). Cholesterol values was within the normal range 98.11 – 220 mg/dL reported

by AMTL (2001). Cholesterol serum concentration significantly decreased ($p<0.05$) as the level of *Clinopodium brownei* oil increased across the treatments. This outcome indicates that *Clinopodium brownei* oil possess hypolipidemic properties and can prevent exposure to cardio-vascular disease (Alagbe, 2022). Alanine amino transferase was higher in T1 (30.21 IU/L) than in T2 (26.92 IU/L), T3 (25.06 IU/L) and T4 (24.81 IU/L) ($p<0.05$) while aspartate transaminase was lower ($p<0.05$) in T2 (45.75 IU/L), T3 (45.02 IU/L) and T4 (45.10 IU/L) than in T1 (57.83 IU/L). Alanine amino transferase and aspartate transaminase recorded in this study was within the normal range 20.08 – 47.00 IU/L and 36.00 – 71.00 IU/L reported by Oleforuh-Okoleh et al. (2015) when grounded ginger and garlic were fed to broiler chickens.

Table 5: Serum biochemical indices of Hubbard broilers fed diet supplemented with *Clinopodium brownei* oil

Parameters	T1	T2	T3	T4	SEM
Total protein (g/dL)	4.40c	4.85b	5.40a	5.46a	0.03
Albumin (g/dL)	2.09c	2.31b	2.57a	2.61a	0.02
Globulin (g/dL)	2.31a	2.54a	2.83a	2.85a	0.01
Cholesterol (mg/dL)	102.4a	100.5a	87.76b	85.01b	0.19
Alanine amino transferase (IU/L)	30.21a	26.92b	25.06b	24.81b	0.58
Aspartate transaminase (IU/L)	57.83a	45.75b	45.02b	45.10b	0.44

Means within a row with different letters and significantly different ($p< 0.05$); SEM Standard error; T1: Experimental diet without oil (control); T2: experimental diet + 2.5 g Adriamycin® /kg diet; T3: experimental diet + 2.0 mL *Clinopodium brownei* oil/kg diet; T4: experimental diet + 2.0 mL *Clinopodium brownei* oil/kg diet

Conclusion

It was concluded that *Clinopodium brownei* oil has measurable pharmacological properties. Its bioactive compounds forms an integrated biochemical system that regulates inflammation, protects tissues and promotes organ function. Dietary supplementation of *Clinopodium brownei* oil up to 4 mL/kg diet improved the growth performance, feed efficiency and blood constituents without compromising the health status of birds.

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