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IMPACT OF DIETARY SUPPLEMENTATION OF Piper betle essential oil ON THE HAEMATO-BIOCHEMICAL AND CAECAL MICROBIAL POPULATION OF BROILER CHICKENS

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Abstract:

The aim of this experiment was to investigate the impact of dietary supplementation of *Piper betle* essential oil on the haemato-biochemical and caecal microbial population of broiler chickens. 240 one-day old Hubbard broiler chicks were used in a 56 days trial. The birds were distributed over 4 groups consisting of 60 birds (4 replicates consisting of 15 birds each) in a completely randomized design. Treatment 1 (T1) basal diet with no *Piper betle* essential oil, T2, T3 and T4 were fed basal diet with 200 mg, 400 mg and 600 mg/kg respectively. Feed and clean water was supplied *ad libitum* throughout the experimental period. Pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentrations, white blood cells, lymphocytes, monocytes, eosinophils and neutrophils values were higher ($P<0.05$) in T2, T3 and T4 relative to T1. Total protein, albumin, globulin, albumin/globulin ratio, cholesterol, aspartate transaminase, alanine transaminase and alanine phosphatase values were significantly ($P<0.05$) influenced in *Piper betle* essential oil supplemented diets whereas urea nitrogen, creatinine, calcium, phosphorus, potassium, sodium and bicarbonate were influenced by the treatments ($P>0.05$). However, all values were within the established ranges for healthy birds. Microbial population of *Escherichia coli*, *Salmonella spp* and *Staphyloccocus aureus* count in the caecum were higher ($P<0.05$) in T1 than in other diets. Conversely, *Lactobacillus spp* count were greater ($P<0.05$) in T2, T3 and T4 relative to T1. Results revealed that *Piper betle* essential oil can be supplemented up to 600 mg/kg without negatively affecting the blood profile and the general health status of broiler chickens.

Keywords: *Piper betle*, heamatology, serum, phytogenics, broiler chicken, food safety

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Introduction

Interest in nutrition and the adoption of antibiotic-free reading systems have been sparked by public concern over potential risks associated with antibiotic resistance to human health (Peter and Hansen, 2021). However, there is mounting evidence that prolonged, non-specific exposure to antibiotics causes bacterial resistance, which has a severe negative influence on the continued effectiveness of these vital medications (Alagbe, 2022; Muritala et al., 2022). As a result, the prophylactic use of antibiotics in animal feed has been limited or prohibited in a number of nations. This increases the need of finding innovative methods to sustain animal performance and safeguard animals from sickness. Additionally, there is opposition to treating animals with antibiotics (Castillo, 2022).

As a result, feed additives that can be utilized as in-feed antibiotic substitutes in chicken feeding regimens have been developed (Singh et al., 2023). In order to protect public health, industry has invested much in research into the relationship between food safety, nutritional value, and quality. The best alternative to antibiotics, essential oils are thought to be free of residue, medication resistance, and harmful side effects (Adewale et al., 2021). Essential oils are plant-based items that don't include any nutrients, minerals, or vitamins but have a favorable impact on birds' performance (Agubosi et al., 2022). They are made from a variety of herbs and spices, and the effects are attributed to the secondary plant components, which are crucial for the plant's defense against pathogenic organisms but have no bearing on the fundamental metabolism of the plant (Musa et al., 2020; Alagbe, 2023).

Studies with essential oils show that the effect is strongly influenced by the quantitatively less significant constituents in addition to the active ingredient (Singh et al., 2021). This indicates that no single chemical, whether separated or even synthesized, can ever be as effective as the sum of all the parts of the plant (Sandra, 2022). Essential oils combine the effects of prebiotics (balanced gut flora and its stabilization) with the effects of antibiotics (antimicrobial activity of certain plant components as Eugenol and carvacrol) (Caroline, 2022). This suggests that using plant extracts can lessen the likelihood of diarrhea or other health challenges in animals (Alagbe et al., 2023). The secondary plant chemicals has shown encouraging antibacterial properties against common livestock diseases in in vitro tests. This suggests the potential for reducing the need for antibiotics by using secondary plant chemicals for prophylaxis and metaphylaxis (Sandra, 2022).

In Southeast Asian and East African countries, *Piper betle*, an evergreen perennial root climber from the family Piperaceae, is widely cultivated (Madhumita et al., 2019, 2020). According to Gu et al. (2004); Pin et al. (2011), Peninsular Malaysia is home to the *P. betle*, a native plant known locally as "daun sirih." It is a strong herbal plant that has both therapeutic and nutritional qualities (Pin et al., 2009; Pu et al., 2010). *P. betle* performs multiple biological roles which includes; antifungal, antioxidant, and antimicrobial properties (Santos et al., 2013; Yang et al., 2010). Eugenol, methyl eugenol, and a few sterols are among the P. betle's known bioactive substances (Sushma et al., 2020; Dalla et al., 2011). Eugenol, isoeugenol, and other phenolic components of *P. betle*, including hydroxychavicol, have antioxidant properties (Suwanphakdee et al., 2016). Eugenol and isoeugenol's antibacterial and radical-scavenging properties have previously been discussed (Santos et al., 2013).

The effect of diets supplemented with *Piper betle* essential oil on production performance and the ideal dosage for regulating the specific pathobiome elements have not been studied. This study will establish a link between in vitro results and field dosages, giving the usage of the oil a solid scientific foundation. Therefore, this

experiment was carried out to examine the impact of dietary supplementation of *Piper betle* essential oil on the haemato-biochemical and caecal microbial population of broiler chickens.

Materials and methods

Study site, ethical consent and extraction of *Piper betle* essential oil (PBO)

The study was carried out at the Poultry unit of Sumitra institute located between 23º 13' N and 72º 41' E. The study was carried out according to the rules and specifications of protocols approved by research ethic committee of Sumitra Research Institute, Gujarat, India.

Fresh *Piper betle* leaves were harvested within Sumitra Teaching and Research farm and taken to the Department of Crop protection for detailed identification and authentication by a qualified taxonomist. To eliminate dirt and other particles, leaves were washed with running tap water followed by distilled water. The samples were then divided into smaller pieces to minimize their surface area before being transported to the laboratory for extraction. Steam distillation technique with clavenger apparatus was employed. Setting up the apparatus requires: a steam generator, round bottom flask, beaker, condenser, safety tube, seperatory funnel and a thermometer. 100 grams of sliced *Piper betle* leaves were immersed into a 500 mL round bottom flask and connected to the steam generator by a delivery tube which has an inlet and outlet. The inlet is connected to a thermometer while the outlet was attached to a condenser and heated to 80 ºC for 30 minutes. The steam that was produced passed through the condenser, and when it was cold, distillate was collected in a beaker. For the purpose of obtaining *Piper betle* essential oil (PBO), distillate is put through a seperatory funnel.

Experimental animals, design and diet

240 one-day old Hubbard broiler chicks were used in the 56 days trial. The birds were purchased from a reputable hatchery in India and distributed over 4 groups consisting of 60 birds (4 replicates consisting of 15 birds each) in a completely randomized design. Chicks were housed in a battery cage measuring (95cm × 60 cm × 45 cm) (length × breath × height) at a height of 80 cm from the ground equipped with automatic feeders and nipple drinkers in a semi-closed pens. On arrival birds were given glucose (5 g to 10 liters of water) and water soluble vitamin (Vitamine®) at 2 g to 5 liters of water. Yellow corn, soya meal with other ingredients were formulated based on Hubbard recommendation for broilers. Other routine management practices and vaccination program weres strictly adhered to.

The experimental groups were as follows:

A control group (standard feed with no additives): Group 1

Piper betle essential oil (PBO) supplemented group (standard feed plus 200 mg, 400 mg and 600 mg/kg PBO) in group 2, 3 and 4 respectively.

Bioactive profiling of *Piper betle* essential oil using gas chromatography - mass spectrophotometry

Phyto-constituents in *Piper betle* essential oil was carried out using Agilent 7000B triple quadrupole GC/MS system. It is a high precision machine with very little noise and accurate expression of achievable detection limit with the following technical specification; mode (standard) EI (high sensitivity extraction source), ion source material (noncoated proprietary inert source), ion source temperature (106 to 350 ºC), filaments (dual filaments for EI), electron energy (100 to 300 eV), mass range (10 to 1.050 m/z), dynamic range (> 10^6), scan rate (up to 6.250 u/s), mass axis stability (< ± 0.10 u over 24 hours), collision cell (linear hexapole), collision cell gas (nitrogen with helium quench gas for reduction of metastable helium), collision energy (selectable up to 60eV), detector (triple axis HED-EM with extended life EM and dynamically ramped iris), total gas flow (up to 80 mL / min GC carrier plus another 5 mL/min of methane for Cl operation plus an additional 1-2 mL/min of N_2) and He for the collision cell gases], pumping system (dual stage turbomolecular pump),

pumping system (Agilent mass hunter acquisition, data handling and reporting) and simultaneous MS and GC (can collect 2 GC detector signals while acquiring MS data).

Blood collection

At the end of the investigation blood samples were collected from five randomly selected birds per replicate for haematological and serum biochemical analysis. Blood for haematology was collected into bottles with anticoagulant (ethylene diaamine tetra acetic acid) while those of serum indices were taken into sample bottles without anticoagulant. 2 mL of blood for haematological evaluation was carried out using OM-2206 auto haematology analyzer. Red blood cell, heamoglobin, pack cell volume and white blood cell values were generated via electrical resistance technique. The machine also have the following technical specifications; manual closed and open tube volume at 100 µL each, work station (intel pentium dual core 2.00 GHz 200 W desktop/tower), (3Gb/s 7200 RPM 16 MB Cache hard drive; 2 GB memory module CD-RW) and (11 inch torch screen with LCD monitor).

Serum biochemical analysis was carried out using Pictus 700 automatic analyzer (model F1209-06A, Hungary) with the following technical specifications; photometric module (8 interference filters: 340, 405, 505, 546, 578, 630, 710 and 872 nm), measuring module (25 µL flow cell volume), 15 mm square cuvette, Minimum aspiration volume: 200 µL and analysis mode.

Intestinal microbial count

On the 56th day of the experiment, caecal samples were collected from five randomly selected birds per replicate into sterile bottles for microbial count. The collected samples were well labeled, mixed with peptone solution and transferred to the laboratory for further analysis. Microbial count was carried out using 7000 RMS microbial analyzer with light induced fluorescence and sophisticated algorithms to detect and quantify microbes. The machine has the following general specifications; sample flow rate (30 mL/min), biological detection limit (1 auto fluorescent units), detection size (≥ 0.3 µm), measurement range (0 – 10,000 total counts/mL), data communication (ethernet – standard RJ 45/ Wi-Fi capable) and data report interval (2 seconds/1mL).

Proximate analysis of experimental diet

Analysis of experimental diet was carried out using Perkin Elmer near infra-red (Model DA 7250, England) which analyzes sample in 60 seconds. The machine has the following technical specifications; operating temperature range (5^{o}C to 40^{o}C), wavelength range (900 – 1700 rpm) and wavelength accuracy (<0.05 nm).

Data Analysis

All data obtained were subjected to statistical analysis of variance (ANOVA) using Statistical Analytical System (SAS, 2003). Treatment means were compared using Duncan multiple range test of the same software. The Statistical model used is shown below:

$Yi = \mu + Tij + eij$

Where: Yi = the effect of the jth observation in the ith treatment µ = general mean of the population Ti = the effect of the ith treatment where i = 4 eij = random error associated with the jth observation in the ith treatment

Table 1: Ingredient and chemical composition of experimental diet (% DM)

Ingredients	Starters' mash (0-21days)	Growers mash (22 – 35 days)	Finishers' mash (36 – 56 days)
Yellow maize	51.00	47.00	55.00
Wheat offal	2.00	10.00	5.00
Soy bean meal	32.00	27.00	30.00

Fish meal (72 %)	2.00	1.00	2.00
Groundnut meal	8.00	6.50	3.00
Oyster shell	1.50	2.00	2.40
Bone meal	3.00	4.00	5.00
Lysine	0.20	0.20	0.20
Methionine	0.25	0.25	0.20
*Premix	0.25	0.25	0.25
Salt	0.30	0.30	0.40
Toxin binder	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein	22.87	19.10	20.61
Crude fibre	4.18	5.02	5.13
Ether extract	4.50	4.66	4.75
Calcium	1.57	1.71	1.87
Phosphorus	0.60	0.85	0.91
Lysine	1.50	1.52	1.67
Meth +Cysteine	0.91	0.93	0.95
Energy (Kcal/kg)	3002.7	2887.5	3168.6
Determined analysis			
Crude protein	23.41	19.33	20.95
Crude fibre	4.00	4.43	4.40
Ether extract	4.67	4.42	4.51
Calcium	1.66	1.68	1.81
Phosphorus	0.74	0.72	0.81
Lysine	1.96	1.90	1.98
Meth +Cysteine	1.10	1.23	1.32
Energy (Kcal/kg)	3017.6	2910.3	3200.3

*****Starter premix: Min/vitamin premix supplied per kg diet: - vitamin A, 10,000 I.U; vitamin E, 28.0 mg; vitamin D 4,000I.U, vitamin K, 5.00mg; vitamin B2, 5.0mg; Niacin, 80 mg; vitamin B12, 25 mg; choline chloride, 100 mg; Manganese, 10.0 mg; Zinc, 40.1mg; Copper, 8.0g; folic acid, 4.5mg; Iron, 5.1g; pantothenic acid, 30mg; biotin, 31.5g; antioxidant, 70mg

**Growers premix: Min/vitamin premix supplied per kg diet: - vitamin A, 6,000 I.U; vitamin E, 15.0 mg; vitamin D 2,000I.U, vitamin K, 5.00mg; vitamin B2, 5.0mg; Niacin, 65 mg; vitamin B12, 20 mg; choline chloride, 70 mg; Manganese, 3.0 mg; Zinc, 35.1mg; Copper, 2.0g; folic acid, 2.5mg; Iron, 7.1g; pantothenic acid, 18mg; biotin, 35.5g; antioxidant, 60mg

***Finisher premix: Min/vitamin premix supplied per kg diet: - vitamin A, 7,800 I.U; vitamin E, 20.0 mg; vitamin D 2,500I.U, vitamin K, 10.00mg; vitamin B2, 8.0mg; Niacin, 80 mg; vitamin B12, 30 mg; choline chloride, 80 mg; Manganese, 3.5 mg; Zinc, 30.2mg; Copper, 5.0g; folic acid, 2.0mg; Iron, 5.2g; pantothenic acid, 20 mg; biotin, 30.0g; antioxidant, 65mg

RESULTS AND DISCUSSION

Bioactive profiling of *Piper betle* essential oil with gas chromatography- mass spectrometry

Bioactive profiling of *Piper betle* essential oil with gas chromatography- mass spectrometry shown in Table 2. The result indicates that *Piper betle* essential oil contains infinite variety of active ingredients of ethno-pharmacological uses. A total of 18 compounds were recognized based on their retention time as well as percentage areas. The major compounds are; phenol 2 methoxy-3-(2 propenyl) (30.80 %), 1,6-cyclodecadiene-1-methyl -5-methylene (21.67 %), aromadendrene (12.33 %), chavibelol (10.92 %), eugenol (5.10 %) and anethole (4.76 %). The other bioactive compounds were less than 1 %. Phenol 2 methoxy-3-(2 propenyl) and 1,6- cyclodecadiene-1-methyl -5-methylene shows several biological activities including; antioxidant, anti-inflammatory, anti-nociceptive, anxiolytic, anticancer and demonstrating antimicrobial uses (Pradhan and Suri, 2013). Chavibelol have been extensively studied for their antioxidant and scavenging free radicals properties (Vandana and Shalini, 2014). Anethole and eugenol reduces the risk of cardiovascular diseases, improves blood circulation by strengthening capillaries and treatment of inflamed tissues (Laskhmi et al., 2005). Estragole and α- eudesmol possess anti-bacterial, analgesic and anti-plasmodic properties (Urmila and Sensei, 2012). Singh et al. (2023) reported that phentermine are polyphenols which possess antimicrobial, antioxidant, anti-fibrotic, immune-stimulatory and hepato-protective properties. According to Alagbe (2023), cultivars, extraction techniques, age of plant amongst others are factors which influence the chemical composition of essential oils. However, the result obtained in this analysis corroborates with the findings of Urmila and Sensei (2012). Scientific studies have shown that a synergy in these bioactive compounds could be absorbents that binds to and eliminate undesirable constituents in the intestine of livestock (Adewale et al., 2021), they can also serve as co-factors for enzymatic reactions as well as substrate for biochemical reactions (Singh et al., 2021; Muritala et al., 2022).

Table 2: Bioactive profiling of *Piper betle* essential oil with gas chromatography- mass spectrometry

Compounds	RT (minutes)	M.W (g/mol)	Molecular formula	% Area
β-Elemenone	6.211	218	$C_{15}H_{22}O$	0.33
butyl 8-methylnonyl benzene 1,2-dicarboxyl	8.004	362	$C_{22}H_{34}O_4$	0.56
1-Methylcyclopropanemethanol	8.713	86	$C_5H_{10}O$	0.88
1,2-Benzenedicarboxylic acid	10.231	278	$C_{16}H_{22}O_4$	0.92
2,4-Dimethylheptanedioic acid dimethyl ester	10.882	216	$C_{11}H_{20}O_4$	0.44
Pentadecanoic acid,14-methyl-,methyl ester	11.304	270	$C_{19}H_{34}O_2$	0.31
Anethole	11.609	306	$C_{18}H_{26}O_4$	4.76
heptanoic acid, docosyl ester	13.440	438	$C_{29}H_{58}O_2$	0.05
Eugenol	15.116	164	$C_{10}H_{12}O_2$	5.10
Tridecanoic acid	15.660	214	$C_{13}H_{26}O_2$	0.19
Estragole	15.916	234	$C_{13}H_{14}O_s$	0.65
α- eudesmol	19.671	242	$C_{15}H_{30}O_2$	0.44
Phentermine	19.770	149	$C_{10}H_{15}N$	0.12
α- terpenyl acetate	21.009	230	$C_{12}H_{22}O_4$	0.06
Phenol 2 methoxy-3-(2 propenyl)	23.445	212	$C_{13}H_{24}O_2$	30.80
1,6- cyclodecadiene-1-methyl -5-methylene	24.006	152	$C_{10}H_{16}O$	21.67

Aromadendrene	24.117	200	$C_{12}H_{24}O_2$	12.33
Chavibelol	28.006	256	$C_{16}H_{32}O_2$	10.92

Haematological parameters of broiler chicken fed diet supplemented with *Piper betle* essential oil

As shown in Table 3, haematological parameters of broiler chicken fed diet supplemented with *Piper betle* essential oil. All the parameters examined will influenced ($P<0.05$) by the treatments. However, values were within the established ranges for healthy birds reported by Livingston et al. (2020); Nanbol et al. (2016) suggesting that the health status of the birds were not compromised by dietary supplementation of *Piper betle* essential oil. Pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, lymphocytes, neutrophils, monocytes and eosinophils values were higher ($P<0.05$) in T2, T3 and T4 than T1. Red blood cell range [9.12 – 12.90 ($\times10^6$/L)] reported in this study indicates the absence of erythrocytosis, this gives room for oxygen sufficiency in the system of the birds (Shittu and Alagbe, 2021). Pack cell volume and heamoglobin values varied from (34.18 – 41.00 %) and 100.6 – 131.6 (g/L) a sign of no blood shortage (aneamia) in the animals. Vitamin deficiencies, auto-immune disorders, hepatic diseases and enlarged spleen have been identified as causes of low white blood counts in livestock (Bilkova et al., 2017; Alagbe, et al., 2022). According to Meluzzi et al. (2018), monocytes travel through the blood to the tissues in the body where they become macrophages and dendritic cells which are important immune cells that fights infections and boost immune responses. Lymphocytes are natural killer cells that are responsible for antibody production, destruction of infected cells and regulation of immune response (Alagbe, 2022). Neutrophils destroys invading bacteria and other pathogens by engulfing them while eosinophils are involved in allergic inflammation (Ross, 1979).

Table 3: Haematological parameters of broiler chicken fed diet supplemented with *Piper betle* essential oil

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	Ref. Range
Pack cell volume (%)	34.18^b	40.70^a	40.92^a	41.00^a	1.67	31- 51
RBC ($\times10^6$/L)	9.12^b	12.33^a	12.85^a	12.90^a	0.55	8 – 18
Haemoglobin (g/L)	100.6^b	127.6^a	129.5^a	131.6^a	5.68	100 – 180
MCH (pg)	19.35^b	27.71^a	28.83^a	29.96^a	0.84	16 – 30
MCV (fl)	49.50^b	59.40^a	60.85^a	61.23^a	1.73	30 – 60
MCHC (%)	35.01^b	41.57^a	43.08^a	44.26^a	1.56	29 – 50
WBC ($\times10^9$/L)	8.42^b	11.55^a	12.50^a	12.58^a	0.41	5 – 21
Lymphocytes (%)	51.48^b	67.62^a	67.95^a	70.10^a	1.94	20 – 85
Neutrophils (%)	23.70^b	32.18^a	33.04^a	33.86^a	1.40	21 – 50
Monocytes (%)	1.77^b	2.03^a	2.19^a	2.85^a	0.01	1 – 5
Eosinophils (%)	1.45^b	2.99^a	3.02^a	3.08^a	0.01	1 – 4

[a,b,c]Means in a row without a similar superscripts differ (P<0.05); SEM: standard error of the mean; Standard feed with no additives: Group 1; *Piper betle* essential oil (PBO) supplemented group (standard feed plus 200 mg, 400 mg and 600 mg/kg PBO) in group 2, 3 and 4 respectively.

Serum biochemical indices of broiler chicken fed diets supplemented with *Piper betle* essential oil

As shown in Table 4, serum biochemical indices of broiler chicken fed diets supplemented with *Piper betle* essential oil. Total protein values varied from 4.55 – 5.58 (g/dL) which is comparable to the values reported by Abaxis (2005). Albumin, globulin and albumin/globulin ratio values were higher (P<0.05) in T3 and T4 relative to the other groups. Though these values were significantly (P<0.05) affected by the dietary supplementation of *Piper betle* essential oil. However, all serum biochemical values were within the optimum range for birds reported by Merck veterinary manual (201 0). The albumin, albumin/globulin ratio and globulin values were within the normal range from 2.70 – 4.00 g/dL, 0.50 – 1.80 g/dL and 1.50 – 5.00 g/dL respectively for a healthy chicken (Riddell, 2011) indicating absence of metabolic disorder (Olafadehan et al., 2011). According to Alagbe et al. (2023); Omokore and Alagbe (2019), albumin is the most abundant protein in the body tasked with the responsibility of maintaining oncotic pressure in the blood thereby preventing fluids from leaking out of the blood vessels. Albumin and globulin ratio as well as total protein reflects the absence of liver damage, inflammatory response and nephrotic syndrome in birds (Mitruka and Rawnsley, 1977). The value of cholesterol (65.28 – 66.16 mmol/L) recorded in this study was lower than 88.05 – 100.5 mmol/L reported by Sobayo et al. (2013) suggesting that cellular processes in birds were not hindered. Cholesterol are precursors for the production of bile, steroid hormone and vitamin D (Oluwafemi et al., 2021). The results on creatinine and uric acid values indicates uncompromised liver and kidney damage (Olafadehan et al., 2021). Aspartate transaminase, alanine transaminase and alanine phosphatase were not significantly (P>0.05) affected by dietary supplementation of *Piper betle* essential oil. However, all values were within the reference ranges for birds revealing the absence of hepatic or liver, bile duct, kidney, skeletal and heart muscle damage in birds. Drug toxicity, auto-immune hepatitis amongst others are factors that could lead to liver damage (Agubosi et al., 2022).

Table 4: Serum biochemical indices of broiler chicken fed diets supplemented with *Piper betle* essential oil

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	Reference values
Total protein (g/dL)	4.55[b]	4.58[b]	5.00[a]	5.58[a]	0.02	2.00 – 7.00
Albumin (g/dL)	2.41[b]	2.41[b]	2.50[a]	2.50[a]	0.01	2.70 – 4.00
Globulin (g/dL)	2.14[b]	2.17[b]	2.50[a]	2.58[a]	0.01	1.50 – 5.00
Albumin/globulin ratio	0.95[b]	1.02[a]	1.03[a]	1.01[a]	0.01	0.50 – 1.80
Cholesterol (mmol/L)	66.16	65.40	65.28	65.77	1.69	25.0 – 80.0
Urea nitrogen (mmol/L)	3.10	3.17	3.20	3.15	0.01	1.77 – 6.00
Creatinine (mmol/L)	1.25	1.11	1.05	1.21	0.01	0.50 – 4.00
AST (U/L)	110.5	101.6	108.2	102.3	5.30	50.0 – 130.0

Alanine transaminase (U/L)	62.50	60.87	60.55	60.27	1.80	25.0 – 100.0
Alanine phosphatase (U/L)	44.87	48.02	44.83	44.60	1.70	12.0 – 90.0

AST: Aspartate transaminase; [a,b,c]Means in a row without a similar superscripts differ (P<0.05); SEM: standard error of mean; Standard feed with no additives: Group 1; *Piper betle* essential oil (PBO) supplemented group (standard feed plus 200 mg, 400 mg and 600 mg/kg PBO) in group 2, 3 and 4 respectively.

Serum minerals of broiler chicken fed diet supplemented with *Piper betle* essential oil

As shown in Table 5, serum minerals of broiler chicken fed diet supplemented with *Piper betle* essential oil. Calcium, potassium, sodium, bicarbonate, phosphorus and magnesium concentrations were not altered (P>0.05) by the treatments. However, values were within the normal ranges for birds (Albokhadaim, 2012; Abdi-Hachesoo et al. (2011) suggesting the absence of cardiovascular diseases, dehydration, liver and kidney diseases (Harr, 2002). Sodium are required for proper function of kidney and nerve as well as acid/base (pH) level (Avram, 2004). Calcium aids to stabilize blood pressure, secretes hormones and enzymes, blood vessel expansion and contraction (Albokhadaim, 2012). Normal bicarbonate, potassium, magnesium and phosphorus values implies the absence of kidney dis-functions and other metabolic disorders (Thrall, 2007).

Table 5: Serum minerals of broiler chicken fed diet supplemented with *Piper betle* essential oil

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	Ref. values
Calcium (mmol/L)	2.07	2.11	2.15	2.18	0.01	1.0 – 4.00
Potassium (mmol/L)	1.40	1.52	1.56	1.60	0.01	1.00 – 3.00
Sodium (mmol/L)	100.2	104.6	108.7	110.1	2.93	90.0 – 141
Bicarbonate (mmol/L)	30.19	33.51	33.62	33.68	1.26	10 – 50
Phosphorus (mmol/L)	1.72	1.79	1.81	1.85	0.01	1.0 – 3.0
Magnesium (mmol/L)	10.56	10.85	10.92	10.98	0.04	8.00 – 15.00

SEM: standard error of mean; standard feed with no additives: Group 1; *Piper betle* essential oil (PBO) supplemented group (standard feed plus 200 mg, 400 mg and 600 mg/kg PBO) in group 2, 3 and 4 respectively.

Caecal microbial population of broiler chicken fed diet supplemented with *Piper betle* essential oil

As presented in Table 6, caecal microbial population of broiler chicken fed diet supplemented with *Piper betle* essential oil. *Escherichia coli*, *Staphyllococcus aureus* and *Salmonella spp* counts were higher (P<0.05) in T1 relative to the other treatments. Conversely, *Lactobacillus spp* count were higher (P<0.05) in T2, T3 and T4 than in T1. The higher values in pathogenic organisms (*Escherichia coli*, *Staphyllococcus aureus* and *Salmonella spp*) implies that *Piper betle* essential oil are selective inhibitors of deleterious intestinal bacteria and also acts as absorbents that binds to and eliminate undesirable constituents in the intestine (Oluwafemi et al., 2022; Musa et al., 2020). They could also act as substrate for beneficial bacteria's (*Lactobacillus spp*), these activities of the essential oil could be attributed to the efficacy of phyto-constituents in the sample. Dysbacteriosis occurs when there is a microbial imbalance or maladaptation between the beneficial bacterial microbiota and potential disease causing organisms (Anne and Eckel, 2022). Lactobacilli produce lactic acid which lowers the pH in the gut against pathogens (Basharat, 2023). So by stimulating the *Lactobacillus spp* with *Piper betle* essential oil will create an optimal environment for their proliferation and suppression of pathogenic bacteria. These results corroborated with the findings of Kirkpinar et al. (2011) where a decrease

in pathogenic organisms were recorded in broiler chicken fed garlic essential oil at 800 mg/kg. Similar observation was made by Jamroz et al. (2003) who reported a significant (P<0.05) increase in *Lactobacillus spp* count of broiler chicks fed phytogenic extracts.

Table 6: Caecal microbial population of broiler chicken fed diet supplemented with *Piper betle* essential oil

Parameters (Cfu/g)	Group 1	Group 2	Group 3	Group 4	SEM	Ref. values
E.coli	8.10^{a}	6.17^{b}	6.06^{b}	6.00^{b}	0.03	Not defined
Lactobacillus spp	11.23^{b}	17.18^{a}	18.03^{a}	18.24^{a}	0.41	Not defined
Staphyllococcus aureus	3.08^{a}	1.93^{b}	1.88^{b}	1.57^{b}	0.01	Not referenced
Salmonella spp	5.77^{a}	4.00^{b}	3.92^{b}	3.90^{b}	0.01	Not defined

^{a,b,c}Means in a row without a similar superscripts differ (*P*<0.05); SEM: standard error of mean; Standard feed with no additives: Group 1; *Piper betle* essential oil (PBO) supplemented group (standard feed plus 200 mg, 400 mg and 600 mg/kg PBO) in group 2, 3 and 4 respectively.

Conclusion

In conclusion, the use of essential oil most especially *Piper betle* essential oil is considered as one of the most potential antibiotic potential with no residue, no drug resistance and no toxic effects. The outcome of this experiment revealed that the dietary supplementation of *Piper betle* essential oil has no harm to bird's blood profile and microbial count in the caecum. Thus, *Piper betle* essential oil can be fed to broilers up to 600 mg/kg without compromising the blood parameters and health status of broiler chickens.

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