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# Effect of dietary supplementation of essential oil from *Zingiber officinale* on blood biochemical parameters of broiler chicks

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**Abstract**
The effect of essential oil from *Zingiber officinale* on hemato-biochemical parameters was assessed in a total of ninety six, day old Vencobb 430 broiler chicks which were randomly assigned to one of the three dietary treatments representing: basal diet with no supplements as control ($T_1$), basal diet + essential oil from *Z. officinale* at 100 mg/kg ($T_2$) and basal diet + essential oil from *Z. officinale* at 200 mg/kg ($T_3$), each treatment having four replicates, with eight chicks each. The antioxidant activity of essential oil was analyzed by DPPH assay. The feeding trial was conducted for 42 days and the blood was collected on the 42$^{nd}$ day and subjected to hemato-biochemical assay. The complete blood count and HDL level were statistically similar ($p$>0.05) among treatments while the total cholesterol and LDL level were significantly lower ($p$<0.05) in $T_2$ (97.25 and 32.75 mg/dL) and $T_3$ (98.50 and 34.00 mg/dL) than $T_1$ (136.00 and 48.25 mg/dL).

**Keywords:** Essential oil, *Zingiber officinale*, blood biochemical parameters

## 1. Introduction
Essential oils are volatile aromatic oily substances obtained from different parts of a plant and they are extracted by distillation from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots. Chemically, essential oils are complex and highly variable mixtures of terpenoids (monoterpenes and sesquiterpenes), aromatic compounds (aldehyde, alcohol, phenol and methoxyderivative) and terpenoids (isoprenoids) (Nazarro *et al*., 2013) [15]. Several essential oil components exhibit strong antimicrobial action. Phenols, alcohols, ketones and aldehydes are mainly associated with the antibacterial actions (Nazzaro *et al*., 2013) [15]. European Union has banned the use of antibiotic feed additives in feed because of emergence of antibacterial resistance among human being, hence phytogenic feed additives like essential oils are having a great importance in broiler industry (Abd *et al*., 2018) [1]. Some essential oils promote production of immunoglobulins, enhance lymphocytic activity and boost interferon-γ release (Gopi *et al*., 2014; Krishan and Narang, 2014) [8, 13]. The essential oil from *Zingiber officinale* contains bioactive compounds like gingerol and zingerone, which contribute to its cholesterol-lowering properties (Abd *et al*., 2020) [2]. Lower cholesterol levels can result in leaner meat and lower the risk of cardiovascular disease and related health problems. Hence this study is planned to assess the antioxidant property of essential oil from ginger and to evaluate their effect on blood biochemical parameters inbroilers.

## 2. Materials and Methods
The essential oils from *Zingiber officinale* (Elixir extracts Pvt Ltd, Muvattupuzha, Kerala) was tested for the antioxidant activity by DPPH assay. Four mL samples of various concentrations of the extracts in methanol were prepared and added to one mL solution of DPPH radical in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min after which the absorbance was measured at 517 nm with a spectrophotometer. Inhibition of free radical DPPH as percentage was calculated as (%) = 100 × ($A_{blank}$ -$A_{sample}$) / $A_{blank}$, where $A_{blank}$ is the absorbance of the control (containing all reagents except the test compound) and $A_{sample}$ is the absorbance of the test compound.

### 2.1 Experimental animals, design and feed preparation

Ninety six, day old Vencobb 430 broiler chicks were purchased from commercial hatchery and were grouped randomly into three treatments ($T_1$, $T_2$ and $T_3$), each treatment having four replicates, with eight chicks in each replicate. The birds were reared under standard protocol, vaccinated and fed with the experimental feed for a period of 42 days. The dietary treatments werea maize-soybean meal based basal diet as control ($T_1$), basal diet supplemented with essential oil from *Zingiber officinale* at 100 mg/kg ($T_2$) or 200 mg/kg ($T_3$). Experimental rations were prepared as per BIS (2007) specification for the different classes. The ingredient composition of broiler pre-starter, starter and finisher ration are shown in the Table 1.

**Table 1:** Ingredient composition of experimental pre-starter, starter and finisher feed (%).

| Ingredients | Pre-starter | Starter | Finisher |
|---|---|---|---|
| Maize | 51.90 | 52.80 | 56.80 |
| SBM | 41.50 | 39.20 | 34.10 |
| DCP | 1.80 | 1.80 | 1.90 |
| Calcite | 1.40 | 1.40 | 1.40 |
| Salt | 0.38 | 0.38 | 0.38 |
| Vegetable oil | 2.64 | 4.10 | 5.21 |
| Trace mineral mix[1] | 0.10 | 0.10 | 0.10 |
| Vitamin premix[2] | 0.08 | 0.08 | 0.08 |
| Lysine[3] | 0.06 | 0.18 | 0.06 |
| Methionine[4] | 0.20 | 0.20 | 0.20 |
| Choline[5] | 0.10 | 0.10 | 0.10 |
| Toxin binder[6] | 0.10 | 0.10 | 0.10 |
| Liver Powder | 0.03 | 0.03 | 0.03 |
| Coccidiostat[7] | 0.05 | 0.05 | 0.05 |

1. Each kilo gram of mineral mixture contains- Manganese-100 g, Zinc-85 g, Iron- 90 g, Copper-15 g, Iodine-1.8 g, Selenium-0.45 g, Organic chromium- 0.15 g.
2. Each kilo gram of vitamin premix supplement contains Vitamin A - 82,500 IU, Vitamin D 3 – 12000 IU, Vitamin B 2 – 50 mg, Vitamin K – 10 mg, Vitamin B 1 – 4.0 mg, Vitamin B6 – 8.0 mg, Vitamin B 12 – 40 mcg, Niacin 60 mg, Calcium pantothenate – 40 mg, Vitamin E – 40 mg
3. L-Lysine mono-hydrochloride 98.5%. (Feed grade)
4. DL-Methionine 99%. (Feed grade)
5. Choline chloride 60%. (Feed grade)
6. Toxin binder containing blend of Hydrated Sodium Aluminosilicate, organic acids, activated charcoal and natural herbal Ingredients
7. Coccidiostat- Diclazuril-(0.5%)-each kg preparation containing 5 g of diclazuril

### 2.2 Estimation of complete blood count

On the 42nd day trial, two birds from each replicate of three treatment groups were randomly selected and blood samples (2 ml) were collected from jugular vein of each bird in separate vials for whole blood and serum and taken for hematological analysis. The complete blood counts were analysed using three-part fully automated haematology analyser (MindrayBC-2800 Vet). The hematocrit (HCT), RBC count, MCV and MCHC were assessed. HCT is determined by centrifugation of a microhematocrit tube and determination of the percentage of RBCs per volume of blood and calculated by HCT = MCV*RBC/10. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) indicate average cell size, average cell hemoglobin content and average cell hemoglobin concentration, respectively. The RDW (Red cell distribution width) measures the difference in the volume and size of the red blood cells. The hemoglobin (HGB) represents the oxygen carrying capacity of the RBC and is reported as grams of hemoglobin per deciliter of blood. The MCHC is calculated by MCHC = (HGB/HCT)*100 (Jones and Allison, 2007) [12]. Analysis of the WBCs was done by flow cytometry within the cell counter (Silverman, 2019) [18]. Concerning the platelet parameter, the three important parameters are plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW), which can be yielded from analysis. PCT is the measurement derived from the platelet count and the mean platelet volume; and PDW is the measurement derived from direct flow cytometric measurement of platelet cell volume (Wiwanitkit, 2004) [20].

### 2.3 Serological analysis

Serum was separated by centrifugation of whole blood at 3000 rpm for 10 minutes and analysed for total cholesterol by CHOD-PAP method (Lie *et al*., 1976) [14] and LDL and HDL (Burtis & Ashwood, 1999) [6] using standard kits (Agappe) in Semi-automated clinical biochemical analyser (Microlab 300, Merck, France).

## 3. Results & Discussion

### 3.1 Anti-oxidant activity of essential oil

The antioxidant activity at various concentrations of *Zingiber officinale* essential oil, assessed by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method is presented in Table 2.

**Table 2:** The antioxidant activity of essential oil (percentage)

| | Concentration of essential oil from *Z. officinale* (%) | | | |
|---|---|---|---|---|
| | 1 | 2 | 5 | 10 |
| Percentage of inhibition (%) | 78.33 | 63.33 | 53.33 | 43.33 |

The antioxidant activity of *Z. officinale* essential oil found to be increasing linearly with the increasing concentration of EO. Amiri (2010) [21] assessed the antioxidant activity of essential oil from *Teucrium orientale* sp. and said that in DPPH method, the antioxidants react with the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration and degree of discoloration indicates the free radical scavenging activities (RSA) of the sample/antioxidant. The values were comparable to the results of Amiri (2010) [21]. The antioxidant action of ginger extract estimated through (RSA), using rapid 1,1-diphenyl-2- picrylhydrazyl (DPPH) technique, indicated maximum antioxidative action associated at 250 mg/Litre concentration in drinking water.

### 3.2 Complete blood count

The complete blood count (Table 3) of broiler chicks fed on diet with different levels of ginger essential oil for 42 days showed statistically similar ($p$>0.05) level in all the parameters assessed and were found to be in the normal range for chicken. The WBC count (*$10^3$/µL) ranged from 252.55 to 257.40, RBC (*$10^6$/µL) count ranged from 2.65 to 2.83, HGB (g/L) count was from 13.85 to 15.53 and HCT level was from 34.25 to 35.1%. The characteristics of RBC ie MCV (fL) was from 128.22 to 130.27, MCH (pL) was from 57.27 to 58.22 and MCHC (g/dL) was from 45.22 to 46.15. The platelet

parameters viz. PCT (%) was from 0.03 to 0.04, PDW was from 14.75 to 15.92 and MPV (fL) was from 6.00 to 6.02.

Adegoke *et al*. (2018) reported statically similar values of red blood cell count (RBC) (2.97 and 3.14*$10^{12}$/L), Haemoglobin (HGB) (11.35 and 11.80 g/dL) level mean corpuscular haemoglobin (MCH) (38.35 and 37.63 pg) and mean corpuscular hemoglobin concentration (MCHC) (33.38 and 33.24 g/dL) in broilers fed with turmeric and pepper powder compared to control and white blood cell (WBC) (6.55 and 5.70 *$10^3$/L) count was significantly higher ($p$<0.05) in treatment supplemented with both turmeric (400 g/kg) and pepper powder (200 g/kg) compared to control. Al-Khalaifah *et al*. (2022) [5] reported significantly higher ($p$<0.05) WBC count in treatments supplemented with ginger powder at 5, 10 and 15 g/kg ranged from 74.22 to 79.6*$10^3$/ µL than control (47.9) and significantly higher heterophils count (33.6%) in treatments supplemented with ginger powder at 15 g/kg than control (13.1%). Chung *et al*. (2021) [7] also reported that the leukocyte values increased with an increase in the concentration of essential oil from *Zingiber officinale* (EOZO) in the diet, indicating that EOZO (0, 1, 1.5 and 2%) improved the immune system of the nile tilapia fish. They also observed an increase in erythrocytes, haemoglobin and haematocrit values indicating improvement in the transport of oxygen to the body tissues, thus avoiding fish anaemia.

**Table 3:** Complete blood count of broiler chicks

| Parameter | $T_1$ | $T_2$ | $T_3$ | F value (P value) |
|---|---|---|---|---|
| WBC ($10^3$/µL) | 254.56±0.58 | 252.55±2.11 | 257.40±2.77 | 1.429 (0.289 ns) |
| RBC ($10^6$/µl) | 2.81±0.25 | 2.83±0.13 | 2.65±0.36 | 0.148 (0.864 ns) |
| HGB (g/L) | 15.53±0.49 | 15.30±0.36 | 13.85±0.681 | 2.976 (0.102 ns) |
| HCT (%) | 34.85±0.69 | 35.15±0.64 | 34.25±0.83 | 0.396 (0.684 ns) |
| MCV (fL) | 128.22±1.28 | 130.27±3.55 | 128.52±2.57 | 0.176 (0.841 ns) |
| MCH (pL) | 57.27±1.15 | 58.225±1.04 | 57.85±1.01 | 0.201 (0.821 ns) |
| MCHC (g/dL) | 46.15±0.59 | 45.22±0.66 | 45.95±0.624 | 0.608 (0.565 ns) |
| RDW (%) | 7.95±0.64 | 8.00±0.50 | 7.80±0.49 | 0.036 (0.965 ns) |
| PLT ($10^3$/µL) | 70.25±3.19 | 70.75±2.84 | 67.25±2.46 | 0.441 (0.656 ns) |
| MPV (fL) | 6.00±0.15 | 6.00±0.09 | 6.02±0.131 | 0.013 (0.987 ns) |
| PDW | 14.75±0.48 | 15.92±0.30 | 15.62±0.21 | 3.088 (0.095 ns) |
| PCT (%) | 0.04±0.01 | 0.04±0.01 | 0.03±0.01 | 0.111 (0.896 ns) |

* Significant at 0.05 level; ns non-significant

Means having different letter as superscript differ significantly within a row

### 3.3 Serum biochemical parameters

**Table 4:** Serum parameters (mg/dL)

| Parameter | $T_1$ | $T_2$ | $T_3$ | F (p value) |
|---|---|---|---|---|
| Total cholesterol | 136.00$^a$±5.12 | 97.25 $^b$±4.37 | 98.50 $^b$±4.79 | 21.347 (0.000*) |
| LDL | 48.25$^a$±5.34 | 32.75 $^b$±4.49 | 34.00 $^b$±3.11 | 5.138 (0.032*) |
| HDL | 97.50±4.98 | 96.75±7.68 | 94.50±7.12 | 0.054 (0.947 ns) |

* Significant at 0.05 level; ns non-significant

Means having different letter as superscript differ significantly within a row

The serum parameters of broiler chicks fed on two levels of essential oil presented in Table 4 showed essential oil supplemented groups ($T_2$ and $T_3$) had significantly lower ($p$<0.05) total cholesterol (97.25 and 98.50 mg/dL) and LDL (32.75 and 34.00 mg/dL) than control (136.00 and 48.25 mg/dL) group and there was no significant difference ($p$>0.05) in HDL level among treatments (97.50, 96.75 and 94.50 mg/dL). These findings were similar with ginger extract supplementation in broilers (Saeid *et al*., 2010) [22], ginger essential oil supplementation in Japanese quails (Herve *et al*., 2019) [10] and ginger powder supplelementation in broilers (Al-Khalaifah *et al*., 2022) [5]. The anti-hypercholesterolemic and hypolipidemic properties of ginger could be due to inhibition of the enzyme hydroxylmethyl-glutaryl-coenzyme-A reductase (HMG-CoA) or by increased excretion of bile acid and cholesterol in faeces (Al-Khalaifah *et al*., 2022) [5]. Similar to our findings Samant *et al*., 2021 [17] reorted that incorporation of lemon grass essential oil and turmeric rhizome powder in the diet of commercial broiler chickens had lower level of serum cholesterol.

In contrast to our findings Habibi *et al*. (2014) [9] reported that there was no significant difference ($p$>0.05) in LDL, HDL and total cholesterol among treatments supplemented with *Z. officinale* essential oil at 75 and 150 mg/kg in broilers. Wen *et al*. (2020) [19] observed significantly higher ($p$<0.05) total cholesterol in treatment supplemented with ginger extract than control in broilers.

The hypocholesterolemic effect of essential oil is mainly through inhibiting the enzymes involved in the synthesis of cholesterol. One of the mechanisms is development of insoluble saponin-cholesterol complexes in the gastrointestinal tract of chickens, which results in prevention of absorption of both endogenous and exogenous cholesterol (Abdulkarimi *et al*., 2011) [3]. Jazi *et al*. (2018) [11] reported that essential oils exhibit hypocholesterolemic property by inhibiting pentose phosphate pathway. Adding essential oils to the broiler feed lowers blood levels of cholesterol and LDL and improving the lipid profile and thus would improve the quality of meat in broilers.

## 4. Conclusion

The present study demonstrated that dietary supplementation of essential oil from *Z. officinale* at both levels had hypolipidemic effect in broilers.

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