

# Haemato-Biochemical Responses of Growing Pigs to Dietary Inclusion of a *Curcuma longa* and *Nigella sativa* Powder Blend

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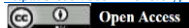
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## ABSTRACT

This study evaluated the safety profile, erythroid stability, and metabolic responses of growing crossbred (Duroc × Landrace) pigs fed dietary levels of a 1:1 mixture of turmeric (*Curcuma longa*) rhizome and black seed (*Nigella sativa*) powder. A 60-day feeding trial was conducted using 50 growing gilts assigned to five dietary treatments in a Completely Randomized Design (5 replicate pens per treatment, 2 pigs per pen). Dietary treatments comprised a corn-soybean meal basal diet supplemented with 0 g/kg (T1, Control), 50 g/kg (T2), 100 g/kg (T3), 150 g/kg (T4), and 200 g/kg (T5) of the 1:1 turmeric/black seed powder blend. At Day 60, blood samples were collected from 5 pigs per treatment (1 pig per pen) to measure hematological parameters (RBC, PCV, Hb, MCV, MCH, MCHC, WBC) and serum biochemical markers (ALT, AST, Uric acid, Creatinine, Total Protein, Albumin, and Globulin). Red blood cell (3.02 – 5.05 ×10<sup>6</sup>/L), Pack cell volume (30.18 – 33.63 %), and Haemoglobin (9.28 – 11.22 g/dL), White blood cell (11.85 – 16.72 (10<sup>9</sup>/L), Mean corpuscular volume (50.63 – 69.05 fL), Mean corpuscular haemoglobin (29.81 – 38.09 pg) and Mean corpuscular haemoglobin concentration (23.48 – 30.96 %) remained within standard reference intervals across all dietary groups (P>0.05), confirming that high inclusion of plant secondary metabolites did not induce anemia or erythrocyte hemolysis. Hepatic injury enzymes (Aspartate Aminotransferase: 140.8 – 149.6 U/L; Alanine Aminotransferase: 28.11 – 29.93 U/L; Alkaline phosphatase: 51.24 – 56.22 U/L) and renal clearance markers (Uric acid: 4.17 – 4.76 mg/dL; Creatinine: 2.26 – 2.35 mg/dL) remained within normal physiological limits (P>0.05), confirming hepatic parenchymal and renal glomerular integrity. Serum Globulin (35.78 – 42.05 g/L) showed an increase (P<0.05) toward T5, elevating Total Protein (68.89 – 82.79 g/L) and improving the Albumin-to-Globulin ratio without causing hepatic metabolic stress. Dietary inclusion of a 1:1 turmeric and black seed powder blended up to 200 g/kg (20% inclusion level) preserves liver and kidney functions, and enhances serum immune proteins in growing swine.

## Introduction

Intensive swine production relies heavily on dietary interventions that maintain gut health, enhance nutrient utilization, and sustain systemic immune stability (Alagbe et al., 2017). Historically, in-feed Antibiotic Growth Promoters (AGPs) were routinely administered to suppress subclinical microbial challenges and improve feed efficiency (Emmanuel and Alagbe, 2025; Shittu et al., 2024). However, global regulatory bans on prophylactic AGP usage spurred by the escalation of antimicrobial resistance and consumer demand for drug-free pork have created a critical need for safe alternative feed additives (Olafadehan et al., 2021). Without prophylactic antibiotics, swine producers face increased risks of subclinical gut inflammation, oxidative stress, impaired nutrient absorption, and elevated systemic vulnerability during the growing phase (Alagbe, 2017; Henandez and Alagbe, 2025). Phytogenic feed additives (PFAs) containing plant secondary metabolites (such as essential oils, polyphenols, flavonoids, and alkaloids) have emerged as sustainable AGP replacements (Omokore and Alagbe, 2019; Singh et al., 2022).

Previous studies evaluating dietary garlic and ginger powders observed improvements in total protein levels and reductions in systemic lipid peroxidation (Adewale et al., 2021; Muritala et al., 2022). However, high inclusion rates (>30 g/kg) occasionally induced mild gastric mucosal irritation, altered blood urea nitrogen levels, or reduced feed intake due to strong pungency (Agubosi et al., 2022). Dietary supplementation with carvacrol- and thymol-rich essential oils enhanced systemic antioxidant capacity and elevated serum globulins (Oluwafemi et al., 2022; Singh et al., 2021). Individual additions of turmeric rhizome powder (*Curcuma longa*) or black seed (*Nigella sativa*) meal have consistently shown positive immunomodulatory and hepatoprotective properties (Ojediran et al., 2024; Daniel et al., 2023). However, relying on a single botanical often targets limited metabolic pathways, requiring higher dose inclusion to achieve desired physiological outcomes (Hernandez and Alagbe, 2025).

Combining botanicals into a polyherbal formulation leverages additive or synergistic interactions between different bioactive compounds. Turmeric contains curcuminoids (predominantly curcumin), which regulate cellular antioxidant defense via the Nrf2-ARE signaling pathway and downregulate pro-inflammatory cytokines (Alagbe, 2014; Ojediran et al., 2024). Black seed contains thymoquinone, thymol, and unsaturated fatty acids, which exert direct lipophilic free-radical scavenging, stimulate gut-associated lymphoid tissues, and enhance immunoglobulin production (Abass, 2010; Ahmadi, 2010). A 1:1 combination of turmeric and black seed powder presents a dual-action botanical complex. However, evaluating novel polyherbal additives at high inclusion levels (0 – 200 g/kg) requires assessing physiological safety. Plant secondary metabolites such as tannins, alkaloids, and saponins can exert anti-nutritional effects, bind dietary iron, or induce metabolic stress on the liver and kidneys if fed in excess (Al-Beitawi et al., 2008). Clinical hematology and serum chemistry profiling provide quantitative measures of immune response, and organ function (Ali and Fateh, 2014).

This study evaluated the effects of incremental dietary inclusion levels (0, 50, 100, 150, and 200 g/kg) of a 1:1 turmeric and black seed polyherbal powder blend on the hematological and serum biochemical parameters of growing crossbred (Duroc × Landrace) pigs.

## **Materials and Methods**

### **Description of Experimental Location**

Vet Etcetera Farms, Kwali, Piggery Unit was used for the Experiment. The site is situated within latitude 8°52'N and longitude 7°00' E with an average annual rainfall ranging from 1,100 mm to 1,600 mm, peaking between July and September. The mean annual ambient temperature ranges from 26.5 °C to 34.5°C, and the relative humidity fluctuates between 45% during the dry season and 82% during the rainy season (Federal Capital Territory Administration [FCTA], 2022).

### **Collection and preparation of Turmeric/Black seed power**

Fresh rhizomes of turmeric (*Curcuma longa*) and seeds of black seed (*Nigella sativa*) were procured from local agricultural markets in Abuja, Nigeria, and processed by washing raw turmeric rhizomes with clean tap water, slicing them into thin 2–3 mm chips, and shade-drying them at 30–32 °C for 10 days alongside black seeds (shade-dried for 5 days) until a constant moisture content below 10% was reached. The dried materials were individually pulverized using a commercial stainless steel hammer mill fitted with a 1.0 mm screen, homogeneously blended in a 1:1 weight-for-weight ratio using a commercial feed mixer to yield the polyherbal phytogenic feed additive (PFA), and stored in airtight polyethylene containers at 4°C until dietary incorporation. 100 g of the blended sample was taken to the laboratory to determine the phytochemical components according to the procedure previously described by Alagbe (2022).

### **Pen Preparation Prior to Trial Commencement**

Two weeks before animal placement, open-sided concrete pens were thoroughly scrubbed with high-pressure water jets and disinfected using a broad-spectrum mixture of Glutaraldehyde (15% w/v) + Didecyltrimethylammonium chloride (10% w/v) (TH4+®, Sogeval, France) applied at a 0.5% v/v dilution (5 mL/L water). Feeders and automatic nipple drinkers were dismantled, descaled, disinfected, and reinstalled. The facility was locked and rested for 7 days post-disinfection.

### **Management of Experimental Animals and Design**

Fifty growing crossbred gilts (Duroc × Landrace) were procured from a reputable breeding farms in Bwari, Abuja in the month of August 2026. On arrival animals were quarantined and acclimatized for 14 days prior to trial commencement. During quarantine, animals received prophylactic health

management. Subcutaneous administration of Ivermectin (Ivomec®) at 0.3 mg/kg body weight (1 mL/33 kg BW). Water-soluble multivitamins (Stressmat®) was also provided *ad libitum* in drinking water for 3 consecutive days post-arrival. Pigs were housed in individual concrete-floored pens (2.0 m×1.5 m) equipped with nipple drinkers and concrete troughs, receiving feed and clean water *ad libitum* and cared for according to the method proposed by Nigerian Institute of Animal Science. At the end of the adjustment period, weights of the pigs were balanced and randomly assigned to the five dietary groups (10 gilts /group) in a feeding trial that lasted for 60 days excluding the 2 weeks acclimatization period. Basal diet was formulated to meet the nutritional requirements of swine according to NRC (2012). The group consists:

T1 (Control): Basal diet without turmeric/black seed powder blend

T2: Basal diet + 50 g/kg (5 %) 1:1 turmeric/black seed powder blend

T3: Basal diet + 100 g/kg (10 %) 1:1 turmeric/black seed powder blend

T4: Basal diet + 150 g/kg (15 %) 1:1 turmeric/black seed powder blend

T5: Basal diet + 200 g/kg (20 %) 1:1 turmeric/black seed powder blend

The experimental diets were offered to the pigs twice daily (8am and 1:00 pm). Daily feed consumption was recorded and the feed leftover and/or wastage were weighed daily before supplying fresh feed. Fresh clean water was also provided always throughout the experiment. Records of average daily feed intake and weekly body weight gain were taken. The daily feed intake was calculated by deducting feed leftover and waste from the total daily feed supplied. Proximate composition of experimental diet was analyzed using Near Infra-Red Feed Analyzer (Model DS300A, Netherlands).

#### Blood Sampling, Hematology, and Serum Biochemistry

At Day 60 of the experimental feeding period, 5 pigs per treatment (1 pig per replicate pen, randomly selected) were randomly sampled. Blood sample was collected using 21-gauge needles. 3 mL of blood was collected into tubes containing Ethylenediaminetetraacetic acid dipotassium salt (K2 EDTA) for haematological studies and placed in an ice pack. Red Blood Cell count (RBC), White Blood Cell count (WBC), Hemoglobin (Hb), and Packed Cell Volume (PCV) were analyzed within 2 hours using an Automated Hematology Analyzer (Mindray BC-2800 Vet, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). 2.5 mL of blood was collected into non-coagulant tubes, allowed to clot at room temperature, and centrifuged at 3,000×g for 15 minutes at 4°C using a refrigerated tabletop centrifuge (Eppendorf Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany). Clear serum was harvested and stored at -20°C. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Uric acid, and Creatinine were measured spectrophotometrically using commercial diagnostic kits (Randox Laboratories Ltd., Antrim, UK) on an Automated Clinical Chemistry Analyzer (Mindray BS-200, Shenzhen, China).

#### Statistical 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.

#### Results and Discussion

Phytochemical composition of turmeric/black seed powder is combined in a 1:1 ratio (Table 1) reveals the presence of phenols (894.4 mg/g), flavonoids (609.1 mg/g), steroids (208.5 mg/g), saponins (186.5 mg/g), tannins (103.2 mg/g), and alkaloids (56.2 mg/g). These compounds have therapeutic or medicinal properties: anti-inflammatory, antioxidant, immune-stimulatory, analgesics, gastro-protective, anti-tumor, anti-cancer, antidiarrheal, anti-helminthic, hepato-protective amongst others (Ojediran et al., 2024; Hernandez and Alagbe, 2025; Singh et al., 2022).

Haematological indices of growing pigs fed diet supplemented with turmeric/black seed powder blend is presented in Table 3. Pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and white blood cell concentration were more in T2 - T5 than T1 ( $p < 0.05$ ). However, all values were within the standard reference range for healthy growing pigs (Harvey, 2012; Chmielowiec et al., 2012). Haematological values could serve as baseline or reference information for comparison in conditions of nutrient deficiency, physiology and health status of farm animals (Hernandez and Alagbe, 2025; Daniel et al., 2023). The pack cell volume, haemoglobin and red blood cell concentration were within the normal

ranges (22.00–36.00 %), (7.00–13.00 g/dL) and ( $1.58–5.28 \times 10^6/\text{L}$ ) reported by Mpetile et al. (2015); Klem et al. (2010) and Lingaas et al. (1992). High pack cell volume, haemoglobin and red blood cell concentration in T2 – T5 indicates oxygen sufficiency in the tissues giving room for efficient nutrient availability in pigs (Friendship et al., 1984; Omokore and Alagbe, 2019). Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were within 45.09 – 65.00 fl, 25.00 – 50.00 pg and 18.00 – 85.00 % recorded by Czech et al. (2017); Abeni et al. (2018) and Štukelj et al. (2013). The mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration recorded in this study suggests the absence of anaemia (Jain et al. (2011); Ježek et al, 2018). White blood cells are responsible for the production of antibodies which fights infection in the body (Emmanuel and Alagbe, 2025). White blood cell value was within 10.00 – 25.00 ( $10^9/\text{L}$ ) reported by Jain et al. (2011). This result indicates that the animals were healthy as a result of their strengthened immune system when fed diet supplemented with turmeric/black seed blend (Daniel et al., 2023).

As revealed in Table 4, serum biochemical indices of growing pigs fed diet supplemented with turmeric/black seed powder blend. Total protein, albumin, globulin values were influenced ( $p < 0.05$ ) by the treatment except uric acid, creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase ( $p > 0.05$ ). However, all values were within the reference range for clinical healthy growing pigs (Buzzard et al., 2013). Total protein, albumin and globulin values were within 56.00 – 80.00 g/L, 25.00 – 45.00 g/L and 25.0 – 40.00 g/L reported by Perri et al. (2017); Lingas et al. (1992) and Stevančević et al. (2014) respectively. High concentration of total protein in T2 – T5 indicates sufficient dietary across the group (Omokore and Alagbe, 2019). The concentration of turmeric/black seed powder blend in the diet did not hinder the absorption of nutrient in the blood. Elevated uric acid and creatinine concentration indicates renal dysfunction (Emmanuel and Alagbe, 2025). The urea and creatinine concentrations were within 0.88 – 5.00 mg/dL and 0.02 – 2.20 mg/dL cited by Stevančević et al. (2019). Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase concentrations which varied from 140.9 – 149.6 (U/L), 28.11 – 29.93 (U/L) and 51.24 – 55.66 (U/L) respectively. The aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were not affected suggesting that turmeric/black seed powder was non-toxic to pigs. Elevated concentration of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase suggests hepatic damage (Yu et al., 2019; Casas et al., 2015).

### Conclusion

Dietary supplementation with a 1:1 of turmeric (*Curcuma longa*) and black seed (*Nigella sativa*) powder up to 200 g/kg (20% inclusion level, T5) maintains all primary hematological parameters (RBC, Hb, PCV, MCV, MCH, MCHC and WBC) and serum biochemical parameters (ALT, AST, BUN, Creatinine, Total Protein) were within standard physiological reference ranges for growing crossbred pigs. The 1:1 turmeric/black seed powder blend supports erythrocyte stability, prevents anemia, preserves liver and kidney functions, and increases serum globulin fractions without causing metabolic strain or physiological toxicity. High-level inclusion of this turmeric/black seed powder blend (200 g/kg) is physiologically safe for growing swine.

**Figure 1: Phytochemical components of Turmeric/Black seed powder blend**

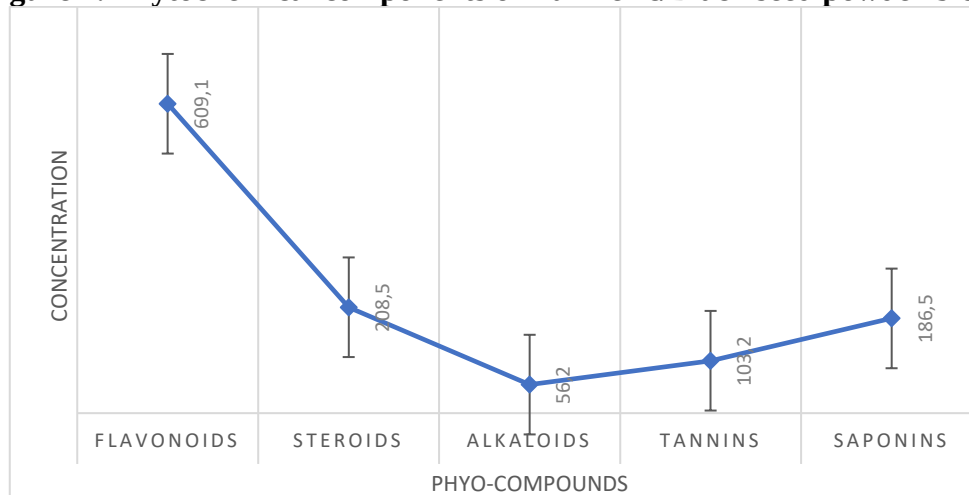


**Table 2: Ingredient and chemical composition of experimental diet (% DM)**

| Ingredients             | % DM   |
|-------------------------|--------|
| Maize                   | 55.50  |
| Wheat bran              | 5.49   |
| Fish meal               | 6.03   |
| Soybean meal            | 30.01  |
| Di-calcium phosphate    | 1.92   |
| Methionine              | 0.25   |
| Lysine                  | 0.25   |
| *Mineral-Vitamin Premix | 0.25   |
| Salt                    | 0.30   |
| Total                   | 100.0  |
| Chemical composition    |        |
| Dry matter              | 87.93  |
| Crude protein           | 16.85  |
| Crude fibre             | 3.77   |
| Ether extract           | 4.13   |
| Ash                     | 7.57   |
| Energy (Kcal/kg)        | 2505.1 |

Each 2.5 kg of vitamins and minerals combination contains: Vit. A 6000000 IU, Vit.B1 2000mg, Vit.B24000mg, Vit.D3 900.000 IU, Vit E 40.000mg, Vit. K3 2000 mg, Pantothenic acid 10.000mg; Nicotinic acid, 50.000g; Vit. B6 2000 mg; Vit. B12 10 mg, Folic acid 3.0g, Biotin 50 mg, Cu 5g, choline 250.000mg, Mn 60g, Fe 50g, , Co 0.1 g, Se 0.1 g, Zn 50 g and Iodine 0.2 g

**Table 3: Haematological indices of growing pigs fed diet supplemented with turmeric/black seed powder blend**

| Items                                 | T1                 | T2                 | T3                 | T4                 | T5                 | SEM  |
|---------------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| Pack cell volume (%)                  | 30.18 <sup>b</sup> | 31.19 <sup>a</sup> | 32.07 <sup>a</sup> | 32.46 <sup>a</sup> | 33.63 <sup>a</sup> | 0.94 |
| Red blood cell (10 <sup>6</sup> /L)   | 3.02 <sup>b</sup>  | 4.29 <sup>a</sup>  | 4.52 <sup>a</sup>  | 4.92 <sup>a</sup>  | 5.05 <sup>a</sup>  | 0.02 |
| Haemoglobin (g/dL)                    | 9.28 <sup>b</sup>  | 11.56 <sup>a</sup> | 11.73 <sup>a</sup> | 11.87 <sup>a</sup> | 11.22 <sup>a</sup> | 0.02 |
| Mean corpuscular volume (fl)          | 50.63 <sup>b</sup> | 62.18 <sup>a</sup> | 63.02 <sup>a</sup> | 68.67 <sup>a</sup> | 69.05 <sup>a</sup> | 1.25 |
| Mean corpuscular haemoglobin (pg)     | 29.81 <sup>b</sup> | 35.21 <sup>a</sup> | 35.52 <sup>a</sup> | 36.18 <sup>a</sup> | 38.09 <sup>a</sup> | 0.63 |
| MCHC (%)                              | 23.48 <sup>b</sup> | 30.17 <sup>a</sup> | 30.28 <sup>a</sup> | 30.91 <sup>a</sup> | 30.96 <sup>a</sup> | 0.31 |
| White blood cell (10 <sup>9</sup> /L) | 11.85 <sup>b</sup> | 15.75 <sup>a</sup> | 15.91 <sup>a</sup> | 16.61 <sup>a</sup> | 16.72 <sup>a</sup> | 0.03 |

<sup>a,b</sup>: Means in the same row with different superscript are significantly (P< 0.05) different; Mean; T1 (Control): Basal diet without supplementation.

T2: Basal diet + 50 g/kg turmeric/black seed powder blend

T3: Basal diet + 100 g/kg turmeric/black seed powder blend

T4: Basal diet + 150 g/kg turmeric/black seed powder blend

T5: Basal diet + 200 g/kg turmeric/black seed powder blend

**Table 4: Serum biochemical indices of growing pigs fed diet supplemented with turmeric/black seed powder blend**

| Items                            | T1                 | T2     | T3     | T4     | T5     | SEM  |
|----------------------------------|--------------------|--------|--------|--------|--------|------|
| Total protein (g/L)              | 68.89 <sup>b</sup> | 80.53a | 82.11a | 82.30a | 82.79a | 1.46 |
| Albumin (g/L)                    | 33.11b             | 39.98a | 40.06a | 41.58a | 42.06a | 0.88 |
| Globulin (g/L)                   | 35.78b             | 40.55a | 42.05a | 40.72a | 40.73a | 0.61 |
| Uric acid (mg/dL)                | 4.55               | 4.33   | 4.17   | 4.76   | 4.51   | 0.02 |
| Creatinine (mg/dL)               | 2.26               | 2.41   | 2.36   | 2.32   | 2.35   | 0.02 |
| Aspartate Aminotransferase (U/L) | 140.9              | 145.7  | 140.8  | 148.5  | 149.6  | 7.55 |
| Alanine Aminotransferase (U/L)   | 29.51              | 28.11  | 29.09  | 29.02  | 29.93  | 0.76 |
| Alkaline phosphatase (U/L)       | 56.22              | 51.24  | 53.93  | 52.19  | 55.66  | 0.51 |

<sup>a,b</sup>: Means in the same row with different superscript are significantly ( $P < 0.05$ ) different; Mean; T1 (Control): Basal diet without supplementation.

T2: Basal diet + 50 g/kg turmeric/black seed powder blend

T3: Basal diet + 100 g/kg turmeric/black seed powder blend

T4: Basal diet + 150 g/kg turmeric/black seed powder blend

T5: Basal diet + 200 g/kg turmeric/black seed powder blend.

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