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Effect of dietary black pepper (*Piper nigrum* L.) powder supplementation on growth performance and physiological responses in broiler chickens

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ABSTRACT

This study examined the influence of graded levels of black pepper (*Piper nigrum* L.) fruit powder on growth traits, carcass yields, and physiological profiles in commercial broiler chickens. A total of 160 one-day-old broiler chicks were allocated to four dietary treatments in a completely randomized design. Each treatment comprised four replicate floor pens containing 10 birds each. Birds were maintained on experimental rations across a two-phase rearing cycle lasting 42 days, divided into a starter period (days 1–21) and a finisher period (days 22–42). Dietary regimens consisted of a basal corn-soybean meal control diet without botanical additions (T_1) and three test groups supplemented with dried black pepper powder at 0.25% (T_2), 0.50% (T_3), and 0.75% (T_4) of the total feed. Feed intake and live body weights were documented to calculate body weight gain, average daily gain, and feed conversion ratio. At day 42, biological sampling was executed to determine carcass yields, visceral organ weights, hematological variables, and serum lipid indices. Dietary inclusion of black pepper powder at 0.50% and 0.75% significantly elevated final body weight and improved feed efficiency without negatively impacting cumulative feed intake. Dressed carcass percentage rose significantly in supplemented cohorts relative to control counterparts. Blood analysis demonstrated that black pepper powder lowered serum total cholesterol, packed cell volume, and the heterophil-to-lymphocyte ratio. In summary, dietary supplementation with *Piper nigrum* powder at 0.50% to 0.75% enhances broiler growth performance, reduces physiological stress markers and optimizes lipid profiles, offering a viable natural feed additive for commercial poultry diets.

Keywords: Black pepper, *Piper nigrum*, Broiler chicken, Phytogenic feed additive, Growth performance, Hematology, Lipid profile

1. Introduction

1.1 Background

Intensive poultry production depends on maximizing feed efficiency, supporting gut integrity and achieving optimal carcass yields within short production cycles. In commercial broiler rearing, feeding cost constitutes the largest variable operational expenditure, prompting efforts to lower dietary costs without depressing bird welfare or carcass safety (Gill, 1999; Dickens *et al.*, 2001; Abaza, 2001) [2, 3, 1]. For decades, in-feed antibiotic growth promoters were routinely incorporated into avian rations to improve feed utilization, modulate gut microflora and prevent subclinical digestive illnesses (Damme, 1999; El-Husseiny *et al.*, 2002) [4, 5]. However, escalating global concerns regarding cross-resistance among bacterial pathogens, human health hazards and biological drug residues in tissues have prompted strict bans or regulatory phase-outs of sub-therapeutic antibiotic use (Damme, 1999) [4]. Consequently, poultry nutritionists have directed intensive focus toward natural, non-antibiotic dietary alternatives, including probiotics, prebiotics and phytogenic feed additives (Mohan *et al.*, 1996; Simon, 2005) [6, 7].

1.2 Black pepper as a phytogenic feed additive

Phytogenic feed additives comprise plant-derived materials such as herbs, spices, and non-synthetic secondary metabolites that exert beneficial bioactivities in livestock nutrition (Gill, 1999) [2]. Among these botanical candidates, black pepper (*Piper nigrum* L.), belonging to the family Piperaceae, is a globally recognized culinary spice and traditional medicinal plant valued for its carminative, stimulant, digestive, and antimicrobial attributes (Chopra *et al.*, 1992; Rakesh *et al.*, 2003; Moorthy *et al.*, 2009) [8, 9, 10]. The distinct bioactivity and pungency of black pepper stem predominantly from piperine, an alkaloid found inside the fruit berry, accompanied by functional volatile terpenes such as pinene, sabinene, and limonene (Malini *et*

al., 1999; Nalini *et al.*, 2006) [11, 12]. In poultry, botanical constituents like piperine act directly on digestive physiology by enhancing the flow of gastric juices, stimulating pancreatic lipase and proteases, and accelerating intestinal transit (Moorthy *et al.*, 2009) [10]. In addition, *Piper nigrum* exhibits potent endogenous antioxidant properties, possessing high glutathione peroxidase and glucose-6-phosphate dehydrogenase activities that protect cellular membranes from lipid peroxidation (Karthikeyan *et al.*, 2003; Khalaf *et al.*, 2008) [13, 14].

1.3 Previous research and biological actions

A substantial body of research demonstrates that supplementing broiler rations with medicinal spices supports growth and improves dietary utilization (Afifi, 2001; Hassan *et al.*, 2004, 2007; Lewis *et al.*, 2003) [15, 16, 17]. Phytogetic herbs like black pepper, red pepper (*Capsicum annuum* L.), garlic (*Allium sativum*), anise, and fennel have consistently demonstrated growth-promoting, antimicrobial, and digestive properties in broiler chicks (El-Deek *et al.*, 2003; Cavallito *et al.*, 1994; Fayed *et al.*, 2011) [18, 19]. Dietary black pepper powder and related spice blends improve feed consumption patterns and body weight gain across distinct rearing stages (Al-Harathi, 2002a, 2002b; Tollba and Hassan, 2003) [20, 21].

Beyond live performance, herbal supplements actively modulate hematological dynamics, blood glucose concentrations, and serum lipid compositions (Shahverdi *et al.*, 2013; Ghaedi *et al.*, 2014) [23, 22]. Dietary black pepper, cinnamon, and herbal combinations reduce systemic cholesterol and improve circulating lipid fractions (Kwon *et al.*, 2003; Molla *et al.*, 2012) [24, 25]. This hypocholesterolemic response is linked to piperine accelerating energy metabolism, stimulating lipid thermogenesis, and increasing peripheral nutrient absorption (Malini *et al.*, 1999; Khalaf *et al.*, 2008) [11, 14]. Furthermore, dietary herbs alter avian leukocyte profiles, where reductions in the heterophil-to-lymphocyte (H:L) ratio signify attenuated physiological and environmental stress responses (Gross *et al.*, 1983; Avallone *et al.*, 1996; Tollba *et al.*, 2007) [27, 26, 28].

1.4 Research gap and rationale

Despite favorable reports, the literature reveals contradictory findings regarding effective inclusion levels, feed intake trends, and hematological alterations (Ademola *et al.*, 2009; Doley *et al.*, 2009; Hosseini Mansoub, 2011) [29, 30, 31]. Certain studies have observed no significant variation in cumulative feed consumption with spice additions, while others report marked appetite stimulation or localized feed avoidance (Ademola *et al.*, 2009 [29]; Sahes *et al.*, 1403 H.Q.). Furthermore, multiple experiments utilized small pen replicates, very low inclusion micro-doses (0.01% to 0.02%), or multi-herb mixtures that confound the specific, isolated contributions of pure black pepper (Abbas, 2009 [32]; Sahes *et al.*, 1403 H.Q.). Crucially, reports of depressed erythrocyte numbers and hemoglobin concentrations under higher black pepper additions require systematic evaluation under controlled, fully replicated experimental designs (Al-Kassie *et al.*, 2011) [33].

1.5 Study objective

The primary objective of this experiment was to evaluate the dietary effects of graded levels of black pepper (*Piper nigrum* L.) fruit powder (0.00%, 0.25%, 0.50%, and 0.75%) on growth performance traits, carcass yields, hematological variables, and serum lipid indices of commercial broiler chickens reared over a 42-day production period.

2. Materials and Methods

2.1 Ethical approval and experimental facility

Experimental practices and bird management protocols followed standard institutional animal care and welfare guidelines for research poultry. Chicks were housed in an environmentally regulated research facility fitted with partitioned deep-litter floor pens.

2.2 Birds, housing, and management

A total of 160 one-day-old unsexed Ross 308 broiler chicks were purchased from a commercial hatchery. Upon arrival, all chicks were individually weighed to establish baseline weights and distributed randomly across pens measuring 1.5m × 1.5m. Floors were covered with clean dry wood shavings distributed evenly to an average depth of 7 cm to serve as bedding litter.

Brooding temperatures were held at 33 °C to 35 °C during the initial 7 days and lowered incrementally by approximately 3 °C per week until reaching a constant ambient room temperature of 24 °C at day 28. Relative humidity was maintained between 55% and 65%. The illumination schedule provided 23 hours of light and 1 hour of darkness for the initial three days, after which birds received 20 hours of continuous light and 4 hours of darkness daily. Feed and fresh drinking water were available *ad libitum* throughout the 42-day experimental period. Chicks received routine vaccinations against Newcastle Disease (ND) and Infectious Bronchitis (IB) via ocular drop application according to standard schedules.

2.3 Experimental design and dietary treatments

The experiment was conducted using a Completely Randomized Design (CRD) with four distinct dietary treatments:

- **Treatment 1 (T_1 - Control):** Basal diet without botanical additions (0.00% black pepper).
- **Treatment 2 (T_2):** Basal diet supplemented with 0.25% (2.5 g/kg) black pepper powder.
- **Treatment 3 (T_3):** Basal diet supplemented with 0.50% (5.0 g/kg) black pepper powder.
- **Treatment 4 (T_4):** Basal diet supplemented with 0.75% (7.5 g/kg) black pepper powder.

Each treatment comprised four replicate floor pens, with 10 chicks per pen, yielding 40 birds per dietary treatment.

2.4 Preparation of black pepper powder

High-grade dried black pepper (*Piper nigrum* L.) fruits were commercially procured. The dried corns were mechanically cleaned, sorted to remove dirt and extraneous matter, and pulverized into a fine, homogeneous powder using an electric mill. The milled material was passed through a 1.0-mm mesh screen and maintained in sealed, airtight containers protected from moisture and light. To ensure uniform dispersion throughout the mash feed, the botanical powder was incorporated using stepwise geometric pre-mixing with the vitamin-mineral carrier before mechanical blending into the primary basal diet batches.

2.5 Experimental diets

Rations were formulated to meet or exceed the nutritional guidelines established by the National Research Council (NRC, 1994) [34]. Birds were fed according to a two-phase nutritional program consisting of a starter diet supplied from day 1 to day 21, followed by a finisher diet provided from day 22 to day 42. All dietary treatments within each feeding phase were compounded from identical batches of corn and soybean meal (Table 1).

2.6 Performance measurements

Chicks were weighed individually on an analytical balance at placement (day 1), at the conclusion of the starter phase (day 21), and at the end of the trial (day 42). Feed delivered to each replicate pen and unconsumed orts were weighed weekly to record pen-level feed intake. Body weight gain (BWG) was computed as the difference between final and initial live body weights across each phase. Average daily gain (ADG) and average daily feed intake (ADFI) were calculated for the starter (days 1–21), finisher (days 22–42), and cumulative feeding intervals (days 1–42). Feed conversion ratio (FCR) was calculated as cumulative feed consumed divided by cumulative live body weight gain per pen. Mortality was monitored daily to adjust feed efficiency calculations.

2.7 Carcass and organ evaluation

At 42 days of age, two broilers reflecting the median body weight of each replicate pen (8 birds per treatment; 32 birds total) were fasted for 8 hours with drinking water available. Individual pre-slaughter live body weights were recorded. Birds were humanely slaughtered

by severing the jugular vein, completely bled, scalded, and defeathered. Heads, shanks, and internal visceral organs were excised. Carcasses were drained and chilled at 4 °C for 2 hours to determine eviscerated chilled dressed weights (Zuidhof, 2005) [35]. Dressing percentage was computed relative to live pre-slaughter body weight:

$$\text{Dressing Percentage (\%)} = \left(\frac{\text{Dressed Carcass Weight}}{\text{Pre-slaughter Live Weight}} \right) \times 100$$

Internal edible giblet organs (heart, liver, and emptied muscular gizzard) were excised, dissected free of surrounding fat, and weighed. Organ weights were expressed as proportional percentages of live pre-slaughter body weight.

Table 1. Ingredient composition and calculated nutrient profiles of experimental basal diets

Ingredient (%)	Starter Phase (Days 1–21)	Finisher Phase (Days 22–42)
Yellow corn	51.00	53.30
Soybean meal (45% CP)	30.00	25.00
Wheat	13.80	15.00
Vegetable oil	1.00	2.50
Mineral and vitamin premix*	2.50	2.50
Dicalcium phosphate	1.20	1.20
Common salt (NaCl)	0.30	0.30
DL-Methionine	0.10	0.10
L-Lysine HCl	0.10	0.10
Total	100.00	100.00
Calculated Nutrient Composition		
Metabolizable energy (kcal/kg)	3,000	3,086
Crude protein (%)	21.30	19.50
Calcium (%)	0.69	0.52
Available phosphorus (%)	0.74	0.69
Lysine (%)	1.19	1.08
Methionine (%)	0.33	0.31

*Premix provided per kilogram of finished feed: Vitamin A, 367,500 IU; Vitamin D3, 133,500 IU; Vitamin E, 1,920 mg; Vitamin K3, 83.4 mg; Vitamin B1, 50 mg; Vitamin B2, 150 mg; Vitamin B3, 500 mg; Vitamin B6, 1,775 mg; Vitamin B12, 0.8 mg; Folic acid, 600 mg; Biotin, 27 mg; Choline, 5,767.5 mg; Fe, 2,667 mg; Cu, 333.75 mg; Mn, 3,334 mg; Co, 203 mg; Zn, 2,334 mg; Ca, 100.75 mg; Se, 10 mg; P, 65,446 mg; DL-Methionine, 36,667 mg; Ethoxyquin, 200 mg; Flavophospholipol, 50 mg; fish meal, 30 g; wheat bran carrier, 1,800 g.

2.8 Hematological and biochemical profiling

Immediately prior to slaughter on day 42, blood was drawn from the brachial wing vein of sampled birds using sterile 21-gauge needles. A 2-mL portion was transferred into tubes coated with dipotassium ethylenediaminetetraacetic acid (K₂EDTA) for hematological analyses, and a 3-mL portion was transferred into untreated collection tubes for serum isolation.

- **Hematological Parameters:** Anticoagulated blood was processed within two hours of sampling. Red blood cell (RBC) and white blood cell (WBC) counts were performed using a calibrated hemocytometer. Packed cell volume (PCV) was measured using micro-hematocrit capillary tubes spun at 12,000 × g for 5 minutes. Hemoglobin (Hb) concentration was determined via the standard cyanmethemoglobin procedure. Fresh blood smears were prepared on glass slides, air-dried, fixed with absolute methanol, and stained with Giemsa stain; 100 leukocytes were differentiated under an optical microscope to calculate the heterophil-to-lymphocyte (H:L) ratio (Gross *et al.*, 1983) [27].
- **Serum Biochemical Indices:** Clotted tubes were centrifuged at 2,500 × g for 15 minutes to isolate clear serum. Serum total cholesterol and fasting glucose concentrations were quantified photometrically using standardized commercial diagnostic test kits (Zlatkis *et al.*, 1993; Ellefson and Caraway, 1967; George, 2001) [36, 37].

2.9 Statistical analysis

Data were evaluated by One-Way Analysis of Variance (ANOVA) in a Completely Randomized Design utilizing the General Linear Model (GLM) procedure of SAS (1996) [38]. The pen replicate represented the experimental unit for feed intake, body weight gain, and FCR, whereas the individual bird served as the experimental unit for carcass parameters, hematological variables, and serum biochemical markers. The mathematical model was:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where:

- Y_{ij} = observed response variable
- μ = overall population mean
- T_i = fixed dietary treatment effect ($i = 1, 2, 3, 4$)
- ϵ_{ij} = random experimental error ($\epsilon_{ij} \sim N(0, \sigma^2)$)

Differences between treatment means were separated using Duncan's Multiple Range Test, with statistical significance declared at $P < 0.05$.

3. Results

3.1 Growth performance

The effects of dietary black pepper powder on live weight, weight gain, feed intake, and feed conversion efficiency are summarized in Table 2.

Table 2. Growth performance traits of broiler chickens fed diets supplemented with black pepper (*Piper nigrum* L.) powder

Parameter	T1 (Control)	T2 (0.25% BP)	T3 (0.50% BP)	T4 (0.75% BP)	SEM	P-value
Initial Body Weight (g)	42.10	42.35	42.20	42.15	0.45	0.962
Day 21 Body Weight (g)	785.40 ^b	808.20 ^{ab}	832.50 ^a	838.10 ^a	9.85	0.018
Day 42 Body Weight (g)	2,618.50 ^c	2,674.30 ^{bc}	2,750.80 ^b	2,829.40 ^a	28.60	0.001
Starter Body Weight Gain (g)	743.30 ^b	765.85 ^{ab}	790.30 ^a	795.95 ^a	9.60	0.021
Finisher Body Weight Gain (g)	1,833.10 ^c	1,866.10 ^{bc}	1,918.30 ^b	1,991.30 ^a	21.40	0.002
Cumulative Body Weight Gain (g)	2,576.40 ^c	2,631.95 ^{bc}	2,708.60 ^b	2,787.25 ^a	28.50	0.001
Starter Feed Intake (g/bird)	1,180.20	1,202.50	1,215.40	1,210.80	14.30	0.380
Finisher Feed Intake (g/bird)	3,688.40	3,560.10	3,656.20	3,694.50	42.10	0.195
Cumulative Feed Intake (g/bird)	4,868.60	4,762.60	4,871.60	4,905.30	48.20	0.224
Starter FCR (g feed:g gain)	1.59 ^a	1.57 ^{ab}	1.54 ^b	1.52 ^b	0.02	0.041
Finisher FCR (g feed:g gain)	2.01 ^a	1.91 ^b	1.90 ^b	1.85 ^b	0.03	0.008
Cumulative FCR (g feed:g gain)	1.89 ^a	1.81 ^b	1.80 ^b	1.76 ^b	0.02	0.004

^{a, b, c} Means within a row bearing different superscript letters differ significantly ($P < 0.05$). SEM: Standard error of the mean; BP: Black pepper; FCR: Feed conversion ratio.

Dietary supplementation with black pepper powder produced noticeable improvements in live body weight and body weight gain. At the end of the starter phase (day 21), broilers fed 0.50% (T_3) and 0.75% (T_4) black pepper exhibited significantly greater live weights ($P = 0.018$) than unsupplemented control birds (T_1). At day 42, the highest body weights were recorded in birds receiving 0.75% black pepper (2,829.40 g), followed by the 0.50% cohort (2,750.80 g), both

of which were significantly heavier ($P = 0.001$) than the control group (2,618.50 g).

Cumulative feed consumption over the 42-day cycle showed no significant differences among treatments ($P = 0.224$). Because live weight gain improved while feed consumption remained constant, cumulative FCR improved significantly ($P = 0.004$) across all supplemented groups (T_2 : 1.81, T_3 : 1.80, and T_4 : 1.76) relative to control broilers (T_1 : 1.89).

3.2 Carcass traits and relative internal organ weights

The carcass yields and relative organ proportions recorded at 42 days of age are shown in Table 3.

Table 3. Carcass yield characteristics and relative internal organ weights of broilers fed black pepper (*Piper nigrum* L.) powder

Parameter	T1 (Control)	T2 (0.25% BP)	T3 (0.50% BP)	T4 (0.75% BP)	SEM	P-value
Pre-slaughter Live Weight (g)	2,624.10 ^c	2,668.50 ^{bc}	2,748.20 ^b	2,831.00 ^a	29.10	0.001
Dressing Percentage (%)	73.25 ^b	74.80 ^{ab}	75.90 ^a	76.85 ^a	0.58	0.012
Relative Liver Weight (%)	2.62	2.78	2.34	2.68	0.11	0.315
Relative Heart Weight (%)	0.67	0.71	0.64	0.69	0.02	0.442
Relative Gizzard Weight (%)	2.92	3.08	2.82	2.84	0.12	0.518

^{a, b, c} Means within a row bearing different superscript letters differ significantly ($P < 0.05$). SEM: Standard error of the mean; BP: Black pepper.

Dressed carcass percentage increased significantly ($P = 0.012$) in broilers supplemented with black pepper, reaching 75.90% in T_3 and

76.85% in T_4 compared to 73.25% in the control group. The relative weights of internal organs (liver, heart, and gizzard) showed no significant variations among treatments ($P > 0.05$), indicating normal organ development without signs of internal toxicity.

3.3 Hematological profiles and biochemical indicators

Table 4 presents the hematological indicators and serum biochemical concentrations measured at day 42.

Table 4. Blood hematological parameters and serum biochemical profiles of broiler chickens fed black pepper (*Piper nigrum* L.) powder

Parameter	T1 (Control)	T2 (0.25% BP)	T3 (0.50% BP)	T4 (0.75% BP)	SEM	P-value
Hemoglobin (g/100 mL)	8.92 ^a	8.12 ^b	8.04 ^b	7.63 ^c	0.18	0.006
Packed Cell Volume (%)	27.15 ^a	26.78 ^a	24.32 ^b	24.18 ^b	0.42	0.001
Red Blood Cells (10 ⁶ /mm ³)	3.42 ^a	3.12 ^b	3.01 ^b	2.92 ^b	0.06	0.002
White Blood Cells (10 ³ /mm ³)	13.72	13.61	14.08	13.98	0.16	0.354
Heterophil-to-Lymphocyte Ratio	0.41 ^a	0.40 ^a	0.33 ^b	0.32 ^b	0.02	0.008
Total Cholesterol (mg/100 mL)	132.50 ^a	128.40 ^a	107.40 ^b	109.50 ^b	3.45	0.001
Serum Glucose (mg/100 mL)	212.40	218.60	224.50	221.80	4.10	0.282

^{a, b, c} Means within a row bearing different superscript letters differ significantly ($P < 0.05$). SEM: Standard error of the mean; BP: Black pepper.

Dietary black pepper powder altered several hematological values. Broilers receiving 0.50% (T_3) and 0.75% (T_4) black pepper exhibited significantly lower ($P < 0.01$) erythrocyte counts, hemoglobin concentrations, and packed cell volumes compared to unsupplemented controls. Circulating white blood cell concentrations did not differ significantly among diets. The heterophil-to-lymphocyte (H:L) ratio was significantly lower ($P = 0.008$) in the T_3 (0.33) and T_4 (0.32) groups compared to the control group (0.41).

Serum biochemical analysis revealed that black pepper supplementation at 0.50% and 0.75% significantly reduced ($P = 0.001$) total cholesterol concentrations (107.40 mg/100 mL in T_3 and 109.50 mg/100 mL in T_4) relative to the control group (132.50 mg/100 mL). Fasting blood glucose values showed a minor, non-significant numerical elevation in supplemented birds ($P = 0.282$).

4. Discussion

4.1 Effects on growth dynamics and feed efficiency

The observed improvements in body weight gain and feed efficiency confirm the value of black pepper powder as a functional botanical feed additive. Rations supplemented with 0.50% and 0.75% black pepper supported superior live weight gain throughout both the starter and finisher intervals. These results agree with Al-Kassie *et al.* (2011) [33], who documented enhanced body weight gain and improved conversion ratios in broilers fed 0.50% to 1.00% *Piper nigrum*. These findings are further supported by Sahes *et al.* (1403 H.Q.), who reported that adding ground black pepper to broiler rations supported higher weight gain compared to control diets. Similarly, Ghazalah *et al.* (2007) [39] and Tollba *et al.* (2007) [28] reported that herbal additions enhanced digestion and improved growth performance across broiler production cycles.

The primary mechanism behind this growth response is attributed to piperine, the key active alkaloid in *Piper nigrum*. Piperine stimulates the flow of gastric juices, increases digestive secretion, and enhances the activities of pancreatic amylase, trypsin, and lipase (Moorthy *et al.*, 2009) [10]. These digestive actions accelerate nutrient breakdown and support greater absorptive capacity across the small intestine (Mohan *et al.*, 1996) [6]. Furthermore, Ahmad (2005) [40] and Al-Harthi (2002b) [21] noted that active herbal constituents enhance nitrogen retention and improve overall nutrient utilization, allowing birds to convert dietary energy and protein into body tissue more efficiently.

Cumulative feed intake remained statistically uniform across all treatments. These results match those of Ademola *et al.* (2009) [29] and Doley *et al.* (2009) [30], who reported that dietary black pepper did not alter feed consumption in broilers fed isocaloric and isonitrogenous rations. Moderate additions of black pepper powder up to 0.75%

provide functional phytochemical metabolites without imparting excessive pungency that might restrict dietary intake.

4.2 Effects on carcass metrics and visceral yields

Eviscerated dressing percentage increased significantly in broilers supplemented with 0.50% and 0.75% black pepper powder. This response reflects superior live weight gains and enhanced nutrient assimilation during the finisher phase. Similar improvements in carcass dressing yield were documented by Al-Kassie *et al.* (2011) [33] with black pepper, while Al-Kaisse (2009) [41] observed variations across other herbal additions.

The relative weights of edible giblets (liver, heart, and gizzard) remained unaffected by dietary treatments. The stability of relative liver weights indicates that black pepper supplementation up to 0.75% did not induce hepatic swelling or physiological strain. These findings align with earlier poultry trials evaluating non-toxic levels of medicinal plant additives (Hassan *et al.*, 2004; Fayed *et al.*, 2011) [16, 19].

4.3 Effects on hematological profiles and stress biomarkers

Dietary inclusion of black pepper produced discernible changes in blood cellular indices. Erythrocyte counts, hemoglobin concentrations, and packed cell volumes declined in birds receiving 0.50% and 0.75% black pepper. These reductions are consistent with the patterns reported by Al-Kassie *et al.* (2011) [33]. As highlighted by Sturkie (1976) [42], circulating estrogenic activity can downregulate avian erythrocyte formation and decrease packed cell volumes, suggesting that secondary metabolites in black pepper may interact mildly with steroid hormone pathways. Because blood values remained within normal clinical thresholds for healthy broilers and performance was maintained, these adjustments do not indicate pathological anemia.

Total white blood cell counts were unaffected by dietary treatment, indicating no broad inflammatory reactions. However, broilers fed 0.50% and 0.75% black pepper showed a significant decrease in the heterophil-to-lymphocyte (H:L) ratio. In poultry physiology, the H:L ratio serves as a reliable biomarker of systemic and physiological stress (Gross *et al.*, 1983; Avallone *et al.*, 1996) [27, 26]. During physical or environmental stress, elevated adrenocorticotrophic hormone (ACTH) release stimulates corticosterone secretion, typically increasing heterophil numbers while depleting lymphocytes (Gross *et al.*, 1983) [27]. Bioactive compounds in black pepper may modulate the neuroendocrine axis, dampening excessive adrenal stimulation and mitigating physiological stress. In addition, the high antioxidant potential of black pepper, driven by glutathione peroxidase and protective terpenes, protects avian tissues from oxidative damage, contributing to improved cellular resilience (Karthikeyan *et al.*, 2003; Khalaf *et al.*, 2008) [13, 14].

4.4 Effects on lipid profile and circulating cholesterol

Supplementation with 0.50% and 0.75% black pepper led to significant reductions in total serum cholesterol. This hypocholesterolemic response matches the findings of Al-Kassie *et al.* (2011) [33], who observed reduced blood cholesterol in broilers fed black pepper. Similar

lipid-lowering effects from phytogetic additives have been documented in broilers and other animal models (Mohan *et al.*, 1996; Nalini *et al.*, 2006; Kwon *et al.*, 2003) [6, 12, 24].

This hypocholesterolemic effect is driven largely by piperine, which accelerates lipid thermogenesis and enhances energy metabolism (Malini *et al.*, 1999) [11]. In addition, bioactive botanical constituents help suppress hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, the rate-limiting enzyme in endogenous cholesterol biosynthesis, while stimulating bile acid secretion to facilitate fecal cholesterol clearance (Nalini *et al.*, 2006) [12]. By lowering circulating cholesterol without disrupting metabolic stability, dietary black pepper powder supports healthy lipid regulation in commercial broilers.

5. Conclusions

Dietary supplementation with black pepper (*Piper nigrum* L.) fruit powder at inclusion rates of 0.50% and 0.75% significantly improved body weight gain and feed conversion efficiency in broiler chickens over a 42-day rearing cycle. The botanical additive increased dressing percentage without altering relative internal organ proportions. Black pepper supplementation also lowered serum total cholesterol and reduced the heterophil-to-lymphocyte ratio, indicating improved lipid metabolism and reduced systemic stress. Although red blood cell indices decreased slightly at higher inclusion rates, values remained within standard clinical ranges and did not impair bird health or growth.

Incorporating 0.50% to 0.75% black pepper powder into commercial broiler diets represents an effective, natural feeding strategy to improve feed conversion and support bird performance in sustainable poultry production systems.

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