



Research Article

Molecular and Biochemical Effects of *Alpinia galanga* Supplementation on Growth and Antioxidant Pathways in Broilers

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Abstract | This paper examined the impacts of adding *Alpinia galanga* (galangal) root powder to the diets of broilers in 0.1%, 0.2% and 0.3% inclusion levels. Two hundred and forty (240) male broilers (Ross 308) one-day-old were randomly assigned to four groups for different feeding treatments namely: control (basal diet), and basal diets with 0.1% (G01), 0.2% (G02) and 0.3% (G03) galangal powder each with a population of six replicates of ten birds. Growth performance, serum biochemistry, antioxidant enzyme activities, and mRNA of SOD1, CAT (Cathepsin), IL-6 (Interleukin-6) and TNF- α (Tumor Necrosis Factor- α) were examined after 42 days. Findings showed that 0.1% and 0.2% galangal supplementation had a significant ($p > 0.05$) positive effect on final body weight, weight gain and feed ratio. Another difference was the improvement in serum lipid profiles (lower cholesterol and triglycerides), liver status (reduced ALT and AST), and high levels of antioxidant enzymes (SOD, CAT, GSH-Px, T-AOC). G01 and G02 birds were characterized by a meaningful increase in the expression of antioxidant-related genes, whereby, the levels of SOD1 in the liver, and CAT in the jejunum, were increased by 1.8 and 2.3 respectively. At the same time, the levels of pro-inflammatory cytokines (IL-6, TNF- α) were also reduced considerably. Nonetheless, the 0.3 percent inclusion level led to lower feed consumption and less performance gain. Which may be caused by Decreased palatability to sum up, *A. galanga* root powder 0.1-0.2% inclusion in the diet can be used successfully to improve growth, metabolic health, and antioxidant status of broilers, whereas 0.3 Should be Averted. Or should be avoided.

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Keywords | *Alpinia galanga*, Broiler chicken, Phyto-genic additive, Anti-oxidant, Gene expression



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Introduction

The world poultry industry is experiencing a major challenge in ensuring the health and productivity of the animals after the prohibition on

the use of antibiotic-resistant growth promoters in feed (Debner and Richards, 2005), seasonal changes and environmental differences (Al-Hamdani *et al.*, 2023). The change has increased focus on natural and safe feed additives, especially plant-based compounds

which have demonstrated positive outcomes in various poultry and aquaculture studies because of their broad-spectrum bioactivity (Windsch *et al.*, 2008; Dama *et al.*, 2015; Ameen *et al.*, 2025; Ahmed, *et al.*, 2025). Galangal (*Alpinia galanga* (L.) Willd). is a perennial herb with rhizomes belonging to Zingiberaceae. It is a traditional medicine ingredient and recent scientific studies have mentioned its abundance in bioactive constituents such as phenolics (galangin, kaempferol), flavonoids, terpenoids and volatile oils (1,8-cineole, α -pinene) (Matsuda *et al.*, 2022; Jantan *et al.*, 2022). Such compounds demonstrate high antimicrobial (Hendriks *et al.*, 2021), antioxidant (Park *et al.*, 2021), anti-inflammatory (Lee *et al.*, 2022) and lipid-lowering activities (Prakash *et al.*, 2023). Plant-based additives like galangal have been demonstrated to boost growth performance by increasing the secretion of digestive enzymes, Improving gut morphology, microbial balance, immunity and antioxidant activity (Zhai *et al.*, 2018; Alagawany *et al.*, 2021). This is especially in consideration of the fact that recent broiler strains are very much vulnerable to oxidative stress, which undermines their growth, health, and meat quality (Sahin *et al.*, 2010). Additionally, current results prove that herbal and aromatic plant supplements are useful to enhance the productive efficiency and physiological resilience of birds (Ameen *et al.*, 2025). Although these encouraging results have been realized, the optimum status of galangal powder on the broiler chicken and the molecular processes behind them is not clearly established. Thus, the objective of the current research was to assess the impact of dietary supplementation (0.1, 0.2, and 0.3% galangal root powder) on the growth performance, serum biochemical parameters, antioxidant status, and the expression of the main antioxidant (SOD1, CAT) and pro-inflammatory genes (IL-6, TNF- α).

Materials and Methods

Experimental design and birds

Two hundred and forty male broiler chicks one-day-old male were weighed and randomly assigned to four dietary treatment groups containing six replicates of ten birds each (240) Ross (308). The experiment took 42 days, which was separated into a starter) 1 days) and a grower/finisher (21 days) period. The experiment was carried out at Experimental Poultry Unit/ Technical Agricultural College/Northern Technical University- Mosul, Iraq. Birds were reared in equal environmental and management conditions.

The first week was kept at a temperature of 33 ± 1 °C and incrementally lowered to 24 °C per week till the end of the experiment. The relative humidity was 65 and there was a 23 h light/1 h dark light/dark program of exposure to the birds. The feed and water were given at will, and all the husbandry procedures were made according to the general broiler management principles. The Institutional Animal Care and Use Committee at the Northern Technical University, Mosul, Iraq approved all the experimental procedures.

Dietary treatments and preparation

Control: Basal corn-soybean meal diet.

- G0.1: Basal diet + 0.1% A. galanga root powder.
- G0.2: Basal diet + 0.2% A. galanga root powder.
- G0.3: Basal diet + 0.3% A. galanga root powder.

A. galanga rhizomes were authenticated, washed, dried in the oven at 40° C, ground to a fine powder (1 mm sieve), and thoroughly combined with the basal diets. Diets were developed to match or even surpass the NRC (1994) nutrient requirements.

Feed formulation, proximate composition, and nutrient levels

The nutrient needs of the broilers were met or exceeded through the basal diet (NRC, 1994). The ingredient composition and nutrient content of the experimental diets are presented in Table 1.

Table 1: Ingredient composition (g/kg diet) of the basal and experimental diets

Ingredient	Con-trol	G0.1 (0.1%)	G0.2 (0.2%)	G0.3 (0.3%)
Corn	550	549	548	547
Soybean meal (44% CP)	360	359	358	357
Vegetable oil	30	30	30	30
Dicalcium phosphate	18	18	18	18
Limestone	10	10	10	10
DL-Methionine	3	3	3	3
Lysine-HCl	2	2	2	2
Vitamin-mineral premix*	5	5	5	5
Salt	2	2	2	2
Alpinia galanga powder	--	1	2	3
Total	1000	1000	1000	1000

*Each kilogram of premix provided: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 15 mg; vitamin K3, 2 mg; vitamin B12, 0.02 mg; riboflavin, 5 mg; niacin, 25 mg; pantothenic acid, 10 mg; manganese, 60 mg; zinc, 50 mg; iron, 30 mg; copper, 10 mg; selenium, 0.2 mg; iodine, 0.5 mg.

Table 2: Proximate composition (%) of experimental diets (as fed basis)

Parameter	Control	G0.1 (0.1%)	G0.2 (0.2%)	G0.3 (0.3%)
Dry matter	89.1	89.2	89.3	89.2
Crude protein	21.3	21.2	21.4	21.3
Ether extract	4.2	4.3	4.3	4.4
Crude fiber	3.8	3.9	3.9	4.0
Ash	6.1	6.0	6.2	6.1
NFE	64.6	64.5	64.2	64.0

Representative samples from each dietary treatment were analyzed for proximate composition according to AOAC (2019) methods. The parameters included dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, and nitrogen-free extract (NFE). Results are presented on a dry matter basis. The analyzed nutrient composition met or slightly exceeded NRC (1994) requirements for broiler chickens. The proximate composition and nutrient levels of the experimental diets are presented in Table 2.

Data and sample collection

At days 1 and 42, each bird was weighed separately, and each replicate's feed intake was noted once a week. One bird per replicate (six per treatment) was chosen at random for sampling on day 42. The wing vein was used to draw blood samples, which were then centrifuged to extract serum and kept at -80°C. For the purpose of analyzing gene expression, liver and jejunum tissues were promptly removed, snap-frozen in liquid nitrogen, and kept at -80°C.

Biochemical and antioxidant assays

Commercial diagnostic kits (Randox Laboratories Ltd., UK) were used to analyze serum biochemical parameters. SOD, CAT, GSH-Px, and T-AOC activities in serum were measured using spectrophotometric techniques as outlined by Benzie and Strain (1996) and Goth (1991) in order to assess antioxidant status.

Gene expression analysis (qRT-PCR)

The TRIzol® reagent (Invitrogen, USA) was used to extract total RNA from the tissues of the liver and jejunum. A High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) was used to synthesize cDNA after RNA purity and concentration were assessed. SYBR Green Master

Mix was used for quantitative real-time PCR on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). Relative gene expression was calculated using (Livak and Schmittgen, method (2001)).

Statistical analysis

One-way ANOVA was used to analyze all of the data in SPSS Statistics version 26.0 (IBM Corp., USA). Tukey's HSD post-hoc test was used to compare means when significant differences were found. The significance level was set at $p < 0.05$, and the results were presented as mean $\pm$ SEM.

Results

Growth performance

Growth performance was significantly impacted by the addition of galangal powder to the diet (Table 3). In comparison to the control and G0.3 groups, the G0.1 and G0.2 groups had significantly higher final body weights and body weight gains ($p < 0.05$). The G0.1 and G0.2 groups showed a significant improvement in FCR. Even though there was no discernible difference in the groups' total feed intake, the G0.3 group's performance significantly declined and their FCR was higher than the control's.

Table 3: Effects of *Alpinia galanga* supplementation on growth performance of broiler chickens (1–42 days).

Parameter	Control	G0.1	G0.2	G0.3	SEM	p-value
Initial BW(body weight) (g)	45.2	45.3	45.1	45.4	0.18	NS
Final BW (body weight) (g)	2380 ^b	2505 ^a	2538 ^a	2310 ^c	22.4	0.031
BWG(body weight growth) (g)	2335 ^b	2460 ^a	2493 ^a	2265 ^c	20.8	0.029
Feed Intake (g)	3905	3920	3880	3985	35.1	NS
FCR) Feed Conversion Ratio(	1.67 ^b	1.59 ^a	1.55 ^a	1.76 ^c	0.02	0.025

^a, ^b, ^c Means within a row with different superscripts differ significantly ($p < 0.05$).

Serum biochemical parameters

Serum biochemical profiles were significantly improved by galangal supplementation (Table 4). All

galangal-supplemented groups showed significantly lower serum cholesterol and triglyceride levels ($p < 0.05$) than the control group, with the G0.2 group showing the strongest effect. Indicating better hepatic health, the G0.1 and G0.2 groups showed a significant decrease in the activities of the liver enzymes ALT and AST. There was no discernible change in serum glucose or total protein.

Table 4: Effects of *Alpinia galanga* supplementation on serum biochemical parameters of broilers at 42 days.

Parameter	Con-trol	G0.1	G0.2	G0.3	SEM	p-val-ue
Glucose (mg/dL)	229	226	224	232	3.1	NS
Cholesterol (mg/dL)	167 ^a	142 ^b	135 ^b	150 ^b	2.9	0.018
Triglycerides (mg/dL)	121 ^a	100 ^b	95 ^b	110 ^b	2.2	0.020
Total Pro-tein (g/dL)	4.1	4.4	4.5	4.2	0.08	NS
ALT (U/L)	25.8 ^a	23.1 ^b	22.4 ^b	26.9 ^a	0.9	0.034
AST (U/L)	143 ^a	132 ^b	129 ^b	140 ^a	3.3	0.041

^{a, b} Means within a row with different superscripts differ significantly ($p < 0.05$).

Antioxidant enzyme activities

Supplementing broilers with galangal significantly improved their antioxidant status (Table 5). Serum levels of SOD, CAT, GSH-Px, and T-AOC were significantly ($p < 0.05$) higher in the G0.1 and G0.2 groups than in the control and G0.3 groups.

Table 5: Effects of *Alpinia galanga* supplementation on serum antioxidant enzyme activities in broilers at 42 days.

Parameter	Con-trol	G0.1	G0.2	G0.3	SEM	p-val-ue
SOD (U/mL)	52.3 ^b	60.4 ^a	62.8 ^a	55.6 ^b	1.1	0.012
CAT (U/mL)	23.1 ^b	27.4 ^a	29.1 ^a	24.0 ^b	0.8	0.018
GSH-Px (U/ mL)	75.2 ^b	85.3 ^a	87.6 ^a	79.1 ^b	1.7	0.016
T-AOC (mmol/L)	3.18 ^b	3.86 ^a	3.94 ^a	3.32 ^b	0.09	0.015

^{a, b} Means within a row with different superscripts differ significantly ($p < 0.05$).

Relative gene expression

Significant molecular modulation was found by gene expression analysis (Table 6). The G0.1 and G0.2 groups showed a significant (1.7–2.3-fold) increase

in the expression of antioxidant genes SOD1 and CAT in the liver and jejunum when compared to the control. The pro-inflammatory genes TNF- α and IL-6, on the other hand, showed markedly decreased expression in these groups. Comparing the G0.3 group to the control, there were either negligible or no discernible changes in gene expression.

Table 6: Effects of *Alpinia galanga* supplementation on relative mRNA expression of antioxidant and pro-inflammatory genes in liver and jejunum tissues of broilers at 42 days.

Gene	Tis-sue	Con-trol	G0.1 (0.1%)	G0.2 (0.2%)	G0.3 (0.3%)	SEM	p-val-ue
SOD1	Liver	1.00 ^c	1.75 ^a	2.10 ^a	1.15 ^{bc}	0.08	0.010
CAT	Jeju-num	1.00 ^c	1.65 ^b	2.25 ^a	1.08 ^c	0.11	0.005
IL-6	Liver	1.00 ^a	0.68 ^b	0.55 ^b	0.92 ^{ab}	0.06	0.022
TNF- α	Jeju-num	1.00 ^a	0.75 ^b	0.52 ^c	0.88 ^{ab}	0.05	0.015

^{a, b, c} Means within a row with different superscripts differ significantly ($p < 0.05$).

Discussion

The present findings demonstrate that dietary supplementation with 0.1–0.2% *Alpinia galanga* root powder improves growth performance and feed efficiency in broiler chickens, consistent with previous reports on phytogetic feed additives (Greathead, 2003; Hashemi and Davoodi, 2011; Windisch *et al.*, 2008). The improvement in FCR without significant changes in feed intake suggests enhanced nutrient utilization efficiency, likely mediated by bioactive compounds such as 1,8-cineole and galangin, which are known to stimulate digestive enzyme secretion and improve intestinal function (Platel and Srinivasan, 2004; Matsuda *et al.*, 2022; Prakash *et al.*, 2023). The reduction in serum cholesterol and triglycerides, along with decreased ALT and AST activities, indicates improved lipid metabolism and hepatoprotective effects of *A. galanga*. These effects may be attributed to the antioxidant and anti-inflammatory properties of its phenolic and flavonoid constituents (Park *et al.*, 2021; Wu *et al.*, 2021; Prakash *et al.*, 2023). Enhanced antioxidant enzyme activities (SOD, CAT, GSH-Px, and T-AOC) further support the role of *A. galanga* in mitigating oxidative stress in broilers, in line with previous findings on phytogetic additives and natural antioxidants in poultry nutrition (Sahin *et al.*, 2010; Dhama *et al.*, 2015; Matsuda *et*

al., 2022). Notably, the absence of significant changes in serum glucose and total protein suggests that *A. galanga* supplementation improved metabolic and hepatic status without adversely affecting general protein metabolism or energy homeostasis. Moreover, the more pronounced responses observed at the 0.2% inclusion level compared with 0.1% in several parameters indicate a dose-dependent biological efficacy within the optimal range. At the molecular level, the upregulation of SOD1 and CAT and downregulation of IL-6 and TNF- α in the G0.1 and G0.2 groups suggest activation of endogenous antioxidant defense pathways and attenuation of inflammatory responses. A plausible mechanism involves activation of the Nrf2/ARE signaling pathway, whereby phytochemicals in *A. galanga* facilitate nuclear translocation of Nrf2, leading to increased transcription of antioxidant genes and suppression of pro-inflammatory cytokines (Mao *et al.*, 2019; Lee *et al.*, 2022; Wu *et al.*, 2021). In contrast, the 0.3% inclusion level adversely affected performance, which may be due to reduced palatability and potential inhibitory effects of high concentrations of essential oils on feed intake and nutrient utilization (Franz and Novak, 2010; Windisch *et al.*, 2008; Zhai *et al.*, 2018; Tiwari *et al.*, 2023; Zeng *et al.*, 2022).

Conclusions and Recommendations

Dietary supplementation with *Alpinia galanga* root powder at 0.1–0.2% significantly enhances growth performance, improves feed efficiency, modulates lipid metabolism, strengthens antioxidant defenses, and regulates inflammatory gene expression in broiler chickens. However, supplementation at 0.3% negatively affects performance, likely due to reduced palatability. Therefore, 0.1–0.2% inclusion levels are recommended for practical application as a phytogetic feed additive in antibiotic-free poultry production systems.

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Novelty Statement

This research highlights the novel molecular and biochemical mechanisms through which *Alpinia galan-*

ga supplementation improves growth and antioxidant status in broiler chickens.

Author Contributions

Haneen Mowfak Ahmed: Conceptualization of the study, experimental design, and supervision of broiler rearing.

Ghassan Fathi Mohammed: Preparation of *Alpinia galanga* supplements, data collection on growth performance, serum biochemical parameters, and antioxidant assays.

AbdulRahman basil awad: Gene expression analysis, statistical analysis, and writing the original draft of the manuscript. All authors have read, critically reviewed, and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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