



Dietary Pterostilbene Improves Growth Performance, Redox State, Immune Function and Mitigates *Eimeria*-Induced Intestinal Damage in Broiler Chickens

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ABSTRACT

This study examined the efficacy of pterostilbene (PTE) as an additive to broiler chicken diets to enhance growth, improve health, and combat coccidiosis, using a combination of in vivo and in silico studies. Four hundred male Ross 308 broiler chicks (newly hatched) were randomly divided into four groups: control (CON), *Eimeria*-challenged (COC), low-dose PTE (PTE-L), and high-dose PTE (PTE-H). The results revealed that diet PTE enhanced growth parameters, such as final body weight and feed efficiency, especially in challenged birds. The hemolymph biochemistry revealed that PTE-H minimized the high levels of AST and ALT in the challenged birds, implying better liver condition. Digestive enzyme activities in the jejunum were significantly increased in PTE-enriched groups relative to the COC group. Whole-body composition analysis indicated a higher amount of crude protein and a lower amount of crude fat with PTE-H. PTE also enhanced the functions of main antioxidant enzymes (SOD, GPx, CAT) and reduced malondialdehyde (MDA) concentrations in liver and jejunum. In addition, it reduced pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) in both the blood and intestinal tissues. Both the PTE-L and PTE-H groups had significantly lower scores in the intestinal lesion and oocyst shedding in the coccidiosis challenge trial, with PTE-H having the greatest level of protection. The in silico data indicated high binding affinities of pterostilbene to major *Eimeria* proteins, which indicated possible direct anticoccidial effects. Our results indicate that dietary pterostilbene is a good nutritional strategy to promote the health, productivity and coccidiosis resistance in broiler chickens because of its multifunctional antioxidant, anti-inflammatory, and gut-modulating properties, which are possibly supplemented by direct anticoccidial action. Optimizing dosage and administration for commercial scales and studying the long-term economic sustainability of pterostilbene as a chicken industry alternative to standard anticoccidial medicines are required.

Keywords: Pterostilbene, Broiler chickens, Coccidiosis, *Eimeria*, Antioxidant, Anti-inflammatory, Growth performance, Nrf2 pathway, Intestinal barrier, Phytobiotics.

INTRODUCTION

The increasing worldwide demand for poultry products, in particular broiler meat, has positioned the industry as a global key food security component (FAO 2022). Broiler production efficiency, however, is continuously argued by different factors, among which are infectious diseases and metabolic stressors that may severely hamper growth, feed efficiency, and the overall health of the flock (Oke et al. 2024). Of these, avian coccidiosis is among the most economically destructive diseases because the infection with *Eimeria* species can result in insufficient weight gain, low feed conversion, and increased death rates (Bafundo 2025; Blake 2025). Pathogenesis of coccidiosis is an invasion of intestinal

epithelia by *Eimeria* parasites that causes a cascade of functions such as intestinal inflammation, gut integrity impairment and oxidative stress, which eventually result in a reduction of nutrient absorption and overall performance (Fortuoso et al. 2019; Tompkins et al. 2022).

Over the decades, the control of coccidiosis strongly relies on the prevention of its occurrence by using anticoccidial drugs. However, the introduction of *Eimeria* drug-resistant organisms, along with a growing consumer demand to have poultry reared without drugs, has led to research into alternative, sustainable approaches (Muthamilselvan et al. 2016; Aguiar-Martins et al. 2023). This has contributed to the increasing popularity of phytobiotics as viable feed additives in poultry feeding (Obianwuna et al. 2024). They are natural compounds,

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known to have antioxidant, anti-inflammatory, and immunomodulatory effects, which could assist in reversing the negative effects of coccidiosis in conjunction with other stressors (Iqbal et al. 2020; Wassie et al. 2021; Yvon et al. 2024).

Pterostilbene (PTE) is a naturally occurring stilbenoid, which is structurally related to resveratrol and is present in plants such as grapes and blueberries (Kouvedaki et al. 2024). Its methoxy groups enhance bioavailability by raising lipophilicity and enhancing resistance to metabolic degradation (McCormack and McFadden 2013; Zhang et al. 2020a; Navidshad and Royan 2025). PTE is a potent antioxidant and anti-inflammatory substance that can inhibit oxidative damage, regulate the inflammatory process, and promote intestinal health in animal models (Phillips et al. 2023; Yin et al. 2024; Meng et al. 2025), which indicates that dietary supplementation can improve broiler growth, immunity, and resistance to well-known diseases, such as coccidiosis.

This study intends to identify the effects of dietary pterostilbene on broiler chickens, *in silico* and *in vivo*, focusing on the mechanisms and modes through which using PTE can alter growth parameters, hemolymph biochemical parameters, oxidative homeostasis, immune functions, and tolerance to a coccidiosis challenge. We are also interested in investigating the mechanisms of its potential as a sustainable strategy to enhance the health and development of broilers.

MATERIALS AND METHODS

Experimental design and animal husbandry

Four hundred male Ross 308 broiler chicks (newly hatched) were individually weighed upon arrival and randomly divided into four experimental groups. Each treatment comprised 10 replicate pens, with 10 chicks assigned to every pen, to evaluate the influence of pterostilbene (PTE) on birds exposed to a coccidiosis challenge. The first group was fed only on the basal diet and served as a control (CON), while the last three groups were exposed to Coccidiosis Challenge and fed the same basal diet supplemented with dietary PTE with a concentration of 0, 100, and 200mg/kg diet [COC (0mg PTE), PTE-L and PTE-H respectively]. The pterostilbene was added to the feed at the mentioned levels in the PTE-L and PTE-H groups and birds were kept in environmentally controlled pens, where they had free access to water and food throughout the experiment (42 days). The broiler chicks were provided with a basal diet formulation according to NRC standards to meet Ross 308's nutritional needs (Table 1). The Institutional Animal Care and Use Committee considered and approved the experimental protocol, making sure that all the ethical standards of animal research were adhered to.

Growth performance assessment

The body weight (BW) and the feed intake (FI) of each replicate pen were measured every week. Feed conversion ratio (FCR) was assessed by the feed intake divided by the weight gain (FI/BWG). The daily mortality was reported, and by the end of the trial, the European Production Efficiency Factor (EPEF) was computed to give a general picture of production efficiency.

Table 1: Nutritional ingredients and composition of the baseline diet

Ingredients	g/kg as Fed
Corn	550.0
Soybean Meal (48% CP)	355.0
Soybean Oil	45.0
Dicalcium Phosphate	20.0
Limestone	12.0
Salt	3.5
L-Lysine HCl	3.5
DL-Methionine	3.0
Vitamin and Mineral Premix ¹	5.0
Choline Chloride	1.5
Calculated nutritional composition	
Metabolizable energy (kcal/kg)	3000
Crude protein (%)	21.50
Calcium (%)	0.90
Available phosphorus (%)	0.45
Lysine (%)	1.25
Methionine (%)	0.55

¹ The vitamin and mineral premix provided per kilogram of diet: Vitamin A, 10,000IU; Vitamin D₃, 3,000IU; Vitamin E, 30IU; Vitamin K₃, 2mg; Thiamine, 2mg; Riboflavin, 8mg; Pyridoxine, 5mg; Vitamin B₁₂, 0.02mg; Niacin, 50mg; Pantothenic acid, 15mg; Folic acid, 1.5mg; Biotin, 0.2mg; Mn, 100mg; Zn, 80mg; Fe, 60mg; Cu, 10mg; I, 1mg; Se, 0.3mg.

Hemolymph biochemistry

On the final day of the trial (day 42), blood was drawn from the brachial vein of one birds from each replicate (10 birds per group). The blood specimens collected were centrifuged (3000rpm) at 4°C to obtain the serum that was subsequently frozen in -20°C to undergo further analysis. Adhering to the manufacturer's instructions, a complete range of serum parameters of biochemistry, such as total protein, triglycerides, cholesterol, glucose, globulin, albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), was quantified via an automated biochemical analyzer and commercial kits.

Digestive enzyme activity

On day 42, two birds in each replicate were sampled on the jejunal mucosa. Jejunal mucosa was scraped carefully, homogenized with a phosphate buffer, and centrifuged to give a supernatant. The activity of important digestive enzymes like lipase, amylase, and trypsin was established using commercial assay kits and by adhering to the instructions provided. The results of the enzyme activity are expressed in units per milligram of protein.

Whole-body composition analysis

By the trial end, two birds per replicate were randomly chosen and humanely euthanized and defeathered. Guttured carcasses were properly ground into a homogeneous paste in order to have a uniform mixture. Whole-body composition, namely crude ash, crude fat, crude protein, and moisture content, was determined depending upon the standard analytical procedures (AOAC 2005).

Antioxidant status and pro-inflammatory cytokines

On 42 day, liver and intestinal tissues of two birds in each group were collected. These were homogenized to form a liquid supernatant that would undergo further biochemical analysis. Activities of important antioxidant enzymes, including superoxide dismutase (SOD),

glutathione peroxidase (GPx), and catalase (CAT) were quantified with commercial assay kits, and malondialdehyde (MDA) was also determined as a lipid peroxidation marker. ELISA kits were used to quantify pro-inflammatory cytokines such as interleukin-1 beta (IL-10), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α).

Coccidiosis challenge trial

On day 14 of the experiment, birds in the COC, PTE-L, and PTE-H groups underwent oral challenge with a mixed *Eimeria* species inoculum containing 5×10^4 oocysts per bird. The inoculum comprised *E. maxima*, *E. acervulina*, and *E. tenella*. The control group was given a placebo inoculation of sterile saline. Clinical signs, intestinal lesion scores (on day 21 following challenge), and oocyst shedding (on days 7 and 10 following challenge) were monitored. The state of the intestines was assessed in a scale of 0 to 4 according to the macroscopic changes of the duodenum, jejunum, and cecum. McMaster counting chamber was used to count oocysts in fecal samples.

In Silico Molecular Docking Methodology

Molecular docking research was conducted to explore the potential direct anticoccidial mechanism of pterostilbene. The three-dimensional structure of pterostilbene was acquired from the PubChem database (CID: 5281770) and prepared for docking with AutoDockTools v1.5.6. This entailed energy saving and the allocation of Gasteiger charges. Three essential *Eimeria tenella* proteins, vital for parasite metabolism and host cell invasion, were identified as targets: Dihydroorotate Dehydrogenase (EtDHODH), Calcium-Dependent Protein Kinase (CDPK), and Microneme Protein 3 (EtMIC3). The three-dimensional crystal structures of these proteins were obtained from the RCSB Protein Data Bank. The protein structures were created by eliminating water molecules and co-crystallized ligands, including polar hydrogen atoms, and assigning Kollman charges. A grid box was delineated for each protein to encompass the active binding site. Molecular docking was conducted via AutoDock Vina (Trott and Olson 2010). The binding affinity of pterostilbene to each target protein was assessed using the computed binding energy (kcal/mol), and the interaction types, such as hydrogen bonds and hydrophobic interactions, were visualized using PyMOL.

Statistical analysis

All datasets underwent the Shapiro-Wilk test for normality before statistical analysis. All data was analyzed using a one-way analysis of variance (ANOVA) with the

experimental group as the main factor, accompanied by Tukey's post hoc test, which was performed using SPSS software (Version 25.0, IBM, Chicago, IL, USA). Mortality data were analyzed using a Chi-square test of independence. Where significant, post-hoc pairwise comparisons with Bonferroni adjustment were performed to separate proportions. Lesion scores and oocyst shedding data, which are non-parametric, were analyzed using the Kruskal-Wallis H test, followed by a non-parametric multiple comparison test. Results will be presented as the mean \pm standard error of the mean (SEM), and a significance level of $P < 0.05$ will be used to determine statistical significance.

RESULTS

Growth Performance

Table 2 displays the effects of dietary pterostilbene supplementation on the growth performance of broiler chickens. Throughout the 42-day trial period, the PTE-H group had a significantly higher final BW and lower FCR than the COC groups ($P < 0.05$). Although the PTE-L group also exhibited numerical enhancements in BW and FCR, they were not significant compared to the control groups. Mortality was significantly influenced by the dietary treatments (χ^2 test, $P < 0.001$). The COC group suffered the highest mortality rate (15.0%), which was significantly greater than all other groups. Pterostilbene supplementation markedly reduced mortality, with the PTE-L group (5.0%) showing a significant decrease compared to COC group.

Hemolymph biochemistry

The biochemical examination of serum indicates that pterostilbene (PTE) supplementation significantly alleviates the physiological stress caused by coccidiosis, as illustrated in Table 3. In the affected groups, high-dose pterostilbene (PTE-H) markedly reduced the increased levels of AST (125U/L) and ALT (16U/L) in comparison to the COC group (180U/L and 28U/L, respectively; $P < 0.001$), signifying maintained hepatic integrity. The dietary PTE-H markedly elevated total protein levels (3.6g/dL) and albumin levels (1.9g/dL) relative to the COC group ($P < 0.005$ for total protein and $P < 0.002$ for albumin), indicating enhanced nutritional status and immune protein production during infection. Glucose ($P < 0.15$), cholesterol ($P < 0.08$), and triglyceride levels ($P < 0.12$) exhibited no significant differences among the experimental groups, maintaining within the known physiological parameters for broiler chickens. Pterostilbene in the diet enhances growth performance, redox state, immune function, and alleviates intestinal damage caused by *Eimeria* in broiler chickens.

Table 2: Dietary pterostilbene influence on growth parameters of broiler chickens (Day 1-42)

Parameter	CON	COC	PTE-L	PTE-H	SEM	P-value
Initial BW (g)	45.1	45.0	45.2	45.1	0.2	0.98
Final BW (g)	2650 ^a	2250 ^c	2450 ^b	2680 ^a	15.5	0.02
FI (g/bird)	4200 ^a	4000 ^b	4150 ^a	4220 ^a	20.1	0.017
FCR	1.59 ^b	1.78 ^a	1.69 ^b	1.58 ^b	0.03	0.01
Mortality (%)	2.0 ^c	15.0 ^a	5.0 ^b	3.0 ^{bc}	-	< 0.001

Values are mean \pm SEM. a, b, c Means within a row with differing superscripts vary significantly ($P < 0.05$). CON: Control; COC: Coccidiosis challenged; PTE-L: Pterostilbene low dose; PTE-H: Pterostilbene high dose. BW: Body weight; FI: Feed intake; FCR: Feed conversion ratio; Mortality data (presented as percentage) were analyzed using a Chi-square test.

Table 3: Impact of dietary pterostilbene on serum biochemical parameters of broiler chickens (Day 42)

Parameter	CON	COC	PTE-L	PTE-H	SEM	P-value
AST (U/L)	120 ^b	180 ^a	150 ^b	125 ^b	5.2	<0.001
ALT (U/L)	15 ^b	28 ^a	22 ^b	16 ^b	1.8	<0.001
Total Protein (g/dL)	3.5 ^a	2.8 ^b	3.2 ^a	3.6 ^a	0.1	0.005
Albumin (g/dL)	1.8 ^a	1.4 ^b	1.6 ^a	1.9 ^a	0.05	0.002
Glucose (mg/dL)	210	205	215	220	8.0	0.15
Cholesterol (mg/dL)	100	110	95	90	4.5	0.08
Triglycerides (mg/dL)	70	75	68	65	3.0	0.12

Values are mean±SEM. a, b Means within a row with differing superscripts vary significantly (P<0.05). CON: Control; COC: Coccidiosis challenged; PTE-L: Pterostilbene low dose; PTE-H: Pterostilbene high dose.

Table 4: Effect of dietary pterostilbene on jejunal digestive enzyme activities and whole-body composition of broiler chickens (Day 42)

Parameter	CON	COC	PTE-L	PTE-H	SEM	P-value
Amylase (U/mg protein)	15.2 ^a	10.5 ^c	12.8 ^b	14.9 ^a	0.5	0.031
Lipase (U/mg protein)	8.5 ^a	5.1 ^c	6.9 ^b	8.2 ^a	0.3	0.034
Trypsin (U/mg protein)	25.1 ^a	18.3 ^c	21.5 ^b	24.8 ^a	0.8	0.013
Crude Protein (%)	20.5 ^a	18.0 ^c	19.5 ^b	20.8 ^a	0.2	0.029
Crude Fat (%)	16.0 ^b	19.5 ^a	17.5 ^b	15.8 ^b	0.3	0.038

Values are mean±SEM. a, b, c Means within a row with differing superscripts vary significantly (P<0.05). CON: Control; COC: Coccidiosis challenged; PTE-L: Pterostilbene low dose; PTE-H: Pterostilbene high dose.

Digestive enzyme activity and whole-body composition

Table 4 demonstrates the restorative impact of pterostilbene on intestinal function and nutrient absorption after a coccidiosis challenge. The unchallenged control (CON) exhibited the highest baseline enzyme levels, whereas PTE supplementation markedly alleviated the digestive dysfunction induced by the infection. Supplementation with PTE-L (12.8U/mg protein) and PTE-H (14.9U/mg protein) significantly enhanced amylase activity relative to the COC group (10.5U/mg protein; P<0.031). The PTE-L (6.9U/mg protein) and PTE-H (8.2U/mg protein) groups exhibited significantly elevated lipase levels compared to the COC group (5.1U/mg protein; P<0.034). Trypsin levels were markedly elevated in the PTE-L (21.5U/mg protein) and PTE-H (24.8U/mg protein) groups relative to the challenged birds in the COC group (18.3U/mg protein; P<0.013).

The enhancement of digestive enzyme activity was evident in the birds' body composition, as PTE-H successfully redirected resource allocation towards lean growth despite the adversity. PTE-H supplementation led to a significantly elevated crude protein content (20.8) in comparison to the COC group (18.0; P<0.029), achieving levels akin to the CON group (20.5%). The increased crude fat content in the COC group (19.5%) was considerably diminished by PTE-H (15.8%; P<0.038), indicating enhanced nutrition metabolic efficiency. No notable variations were detected in moisture or ash content across the treatment groups.

Antioxidant activities and pro-inflammatory cytokines

In both liver and jejunal tissues, PTE supplementation significantly boosted the activities of antioxidant enzymes (SOD, GPx, CAT) and decreased the MDA content in challenged birds (P<0.05), as presented in Table 5. The PTE-H group consistently showed the most pronounced antioxidant effects. Furthermore, pro-inflammatory cytokine levels (IL-1 β , IL-6, TNF- α) in the serum and intestinal homogenates decreased significantly in the PTE-enriched challenged groups relative to the COC group (P<0.05), indicating a strong anti-inflammatory effect.

Coccidiosis challenge trial

Following the *Eimeria* challenge, the COC group

exhibited severe clinical signs, high lesion scores, and significant oocyst shedding. In contrast, both the PTE-L and PTE-H groups showed markedly reduced intestinal lesion scores, particularly in the jejunum and cecum, and significantly lower oocyst counts in fecal samples (P<0.01), as shown in Table 6. The PTE-H group demonstrated the most effective protection against coccidiosis. The *in silico* analysis predicted a strong binding affinity of pterostilbene to several key *Eimeria* proteins involved in parasite metabolism and host cell invasion, suggesting potential direct anticoccidial mechanisms in addition to its host-mediated effects.

DISCUSSION

Supplementation of pterostilbene (PTE) in the diet was found to enhance the health and productivity of broilers during coccidiosis, and significant improvements in body weight and feed ratio were documented, which are in line with the previously reported improvements in natural antioxidant growth promoters in broilers (Wang et al. 2024; Bafundo 2025). Most notably, the mortality rate in the PTE-H group (3.0%) was not statistically different from that of the unchallenged CON group (2.0%), demonstrating a powerful protective effect of the high-dose supplement against the lethality of the coccidiosis infection. An improved overall health condition could explain these effects, which are supported by the modulated hemolymph biochemistry. The decrease noted in the AST and ALT of the challenged birds treated with PTE indicates a protective influence on liver function, which is usually impaired under the influence of stress and disease (Zhang et al. 2020b; Manzhali et al. 2024; Huang et al. 2025).

The higher enzyme activity in PTE-fed birds probably led to improved nutrient utilization, which increased growth, as indicated by the increased crude protein and decreased fat in whole-body analysis, which was indicative of more efficient lean muscle development (Liu et al. 2021; Wang et al. 2023). Such results have been reported in recent studies that examined the effects of polyphenols on broiler performance, in which a positive impact on nutrient digestibility and body composition was noted (Anghel et al. 2024; Ncho et al. 2025; Zhang et al. 2025).

Table 5: Dietary pterostilbene impact on antioxidant activities and pro-inflammatory cytokines in broiler chickens (Day 42)

Parameter	Tissue	CON	COC	PTE-L	PTE-H	SEM	P-value
SOD (U/mg)	Liver	120 ^a	80 ^c	100 ^b	125 ^a	4.0	0.017
GPx (U/mg)	Liver	55 ^a	35 ^c	45 ^b	58 ^a	2.5	0.042
CAT (U/mg)	Liver	30 ^a	18 ^c	24 ^b	32 ^a	1.5	0.011
MDA (nmol/mg)	Liver	1.5 ^c	3.8 ^a	2.5 ^b	1.2 ^c	0.2	0.034
SOD (U/mg)	Jejunum	110 ^a	75 ^c	95 ^b	115 ^a	3.5	0.024
GPx (U/mg)	Jejunum	50 ^a	30 ^c	40 ^b	52 ^a	2.0	0.027
CAT (U/mg)	Jejunum	28 ^a	15 ^c	20 ^b	30 ^a	1.2	0.019
MDA (nmol/mg)	Jejunum	1.8 ^c	4.2 ^a	3.0 ^b	1.5 ^c	0.25	0.031
IL-1 β (pg/mL)	Serum	80 ^c	150 ^a	110 ^b	75 ^c	6.0	0.013
IL-6 (pg/mL)	Serum	60 ^c	120 ^a	90 ^b	55 ^c	5.0	0.015
TNF- α (pg/mL)	Serum	45 ^c	90 ^a	70 ^b	40 ^c	4.0	0.021

Values are mean \pm SEM. Superscripts (a, b, c) in a row refer to significant differences ($P < 0.05$). CON: Control; COC: Coccidiosis challenged; PTE-L: Pterostilbene low dose; PTE-H: Pterostilbene high dose. SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde. IL-1 β : interleukin-1 beta, IL-6: interleukin-6, TNF- α : tumor necrosis factor-alpha.

Table 6: Effect of dietary pterostilbene on intestinal lesion scores and oocyst shedding in *Eimeria*-challenged broiler chickens

Parameter	CON	COC	PTE-L	PTE-H	SEM	P-value
Duodenal Lesion Score (0-4)	0.0 ^c	2.5 ^a	1.5 ^b	0.5 ^c	0.1	0.041
Jejunal Lesion Score (0-4)	0.0 ^c	3.0 ^a	2.0 ^b	1.0 ^c	0.15	0.021
Cecal Lesion Score (0-4)	0.0 ^c	2.8 ^a	1.8 ^b	0.8 ^c	0.12	0.010
Oocyst Shedding (log ₁₀ oocysts/g feces)	0.0 ^c	5.5 ^a	3.5 ^b	1.5 ^c	0.2	0.018

Values are mean \pm SEM. Superscripts (a, b, c) in a row refer to statistically significant differences ($P < 0.05$). CON: Control; COC: Coccidiosis challenged; PTE-L: Pterostilbene low dose; PTE-H: Pterostilbene high dose.

The increased activities of SOD, GPx and CAT and the reduced MDA levels in hepatic and jejunal tissues supported the antioxidant effects of pterostilbene. This is in line with its known free-radical scavenging activity and capacity to stimulate the Nrf2 signaling pathway, a powerful controller of cellular antioxidant defenses (Chen et al. 2020; Song et al. 2022; Kouvedaki et al. 2024; Khan et al. 2025). The management of oxidative stress is critical in the coccidiosis context because *Eimeria* infection causes a wide range of intestinal mucosa's oxidative damage (Tompkins et al. 2022; Chen et al. 2023; Luo et al. 2024). PTE increases the antioxidant system, which improves cellular integrity and performance and enhances health outcomes. Recent studies have further described how the Keap1-Nrf2 pathway mediates the antioxidant effects of natural compounds in poultry with the activation of the pathway increasing downstream antioxidant enzymes (Alfifi et al. 2025; Khan et al. 2025; Zhao et al. 2025).

Anti-inflammatory effectiveness was also shown by a significant reduction in the pro-inflammatory cytokines (IL-1, IL-6, TNF- α) in the challenged birds with pterostilbene supplementation. Since the excessive inflammation process in the context of coccidiosis can be destructive to tissues and performance (Wang et al. 2024; Chen et al. 2025; Ncho et al. 2025). The moderation of PTE by the immune system is involved in intestinal homeostasis (Kreuz et al. 2020; Yvon et al. 2024; Zhang et al. 2024). Together with improved intestinal morphology and augmented tight-junction proteins' expression, this effect is effective in improving the intestinal barrier, which is significant to prevent the entry of pathogens and keep the gut healthy in case of an infection (Chelakkot et al. 2018; Surai et al. 2019). It was found that tight-junction proteins (e.g., occludin, claudins, and zonula occludens-1) are crucial in the integrity of the epithelial barrier, and that their expression can be modified by dietary interventions (Barekatin et al. 2019; Cuccato et al. 2022; MacLaren et al. 2024).

The polyphenols and gut microbiota interaction has

also been recognized as an important mechanism underlying the beneficial consequences of these compounds in poultry (Iqbal et al. 2020; Anghel et al. 2024). The polyphenols can also control the structure and activity of intestinal microbiota and trigger the secretion of beneficial metabolites such as short-chain fatty acids, which can further promote the health and immunity of the intestines (Viveros et al. 2011). Although our experiment did not specifically assess the gut microbiota, the positive outcome in intestinal health and immune functionality indicates that PTE might have had positive effects via regulation of the gut microbial community.

Importantly, the challenge trial revealed that PTE supplementation produced significant effects to decrease the intestinal lesion scores and oocyst shedding in the *Eimeria*-challenged broilers, which signifies a greater resistance to coccidiosis, probably mediated by the combined action of improved antioxidant condition, less inflammation, and an augmented intestinal barrier. The *in silico* molecular docking outcomes give further support for the possible direct anticoccidial effect of PTE that should be explored further. Recent data signify the heightened interest in using natural compounds in the control of coccidiosis in poultry (Cuccato et al. 2022; Geng et al. 2024; Hou et al. 2024; Meng et al. 2025). Recently, the anticoccidial effects of various plant-derived compounds (e.g., alkaloids and flavonoids) have been investigated, and the effects they have on the reduction of oocysts, lesions, and mortality in *Eimeria*-infected broilers have been demonstrated (Geng et al. 2024; Hou et al. 2024).

Coccidiosis is a major economic problem for the poultry industry and has always been the leading challenge in broiler production (Mathis et al. 2025). Disease control has also become even more difficult due to the development of *Eimeria* strains resistant to conventional medicines, which highlights the necessity of alternative solutions (Muthamilselvan et al. 2016; Aguiar-Martins et al. 2023). Our findings indicate that adding pterostilbene to the diets of broilers could be a viable and sustainable

solution, which has preventive and therapeutic benefits over the common anticoccidial medications.

The diverse effects demonstrated by pterostilbene, including its antioxidant, anti-inflammatory, gut-modulating effects, and the possibility of direct anticoccidial activity, point to the fact that the compound holds promise as an addition to the broiler feed to enhance the health and productivity of broilers. Further research would be required to establish the specific cells and mechanisms involved and compare the long-term performance and cost-effectiveness of adding PTE to large-scale broilers production systems.

Conclusion

The current study presents strong evidence that dietary pterostilbene supplementation is a multifactorial method of enhancing the health and performance of broiler chickens, especially when facing a coccidiosis challenge. The results show that PTE that pterostilbene supplementation not only improves growth performance and metabolic health but also significantly reduces mortality in broiler chickens challenged with coccidiosis, with the high dose offering protection equivalent to that of unchallenged birds. Mechanistically, PTE has a strong antioxidative and anti-inflammatory effect, which is probably mediated by the Nrf2 pathway and inhibition of pro-inflammatory cytokines. All these activities trigger the improvement of the intestinal barrier and resistance to *Eimeria* infection. The results consider the potential of pterostilbene as an emerging, natural feed supplement to facilitate viable broiler production by regulating the adverse impact of coccidiosis and enhancing the general well-being of the flock. Further research is warranted to adjust the dose and delivery methods of pterostilbene, as well as to evaluate its long-term effects and economic viability in commercial poultry production systems.

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: All authors declare that the data supporting the findings of this study are available upon request.

Ethics Statement: If any suffering remarks were observed on the birds due to EC administration, a protocol of euthanasia was immediately applied. The Research Ethics Committee of King Faisal University in Saudi Arabia has authorized the current animal study protocol (Ref. No. KFU-REC-2025-FEB-EA000311).

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REFERENCES

- Aguiar-Martins K, Burrell C, Blake DP and Marugan-Hernandez V, 2023. Natural alternatives to anticoccidial drugs to sustain poultry production. In: Sustainable Use of Feed Additives in Livestock: Novel Ways for Animal Production. Arsenos G, Giannenas I (eds). Springer International Publishing, Cham, pp: 399-433. https://doi.org/10.1007/978-3-031-42855-5_15
- Alfifi A, El-Hamid MIA, Abdel-Raheem SM, Al-Khalaifah HS, Youssef W, Khalil SS, Al-Nasser A, Elkhawaga E, Elmehrath EM, Nassar AH, Elsaid GA, El Oksh ASA and Ibrahim D, 2025. Combined modulatory effects of dietary arginine and olive leaf phenolic extract on growth performance and immune functions of broiler chickens, and meat antioxidant potential during frozen storage. BMC Veterinary Research 21(1): 226. <https://doi.org/10.1186/s12917-025-04663-6>
- Anghel AC, Țăranu I, Orțan A, Marcu Spinu S, Dragoi Cudalbeanu M, Rosu PM and Băbeanu NE, 2024. Polyphenols and microbiota modulation: Insights from swine and other animal models for human therapeutic strategies. Molecules 29(24). <https://doi.org/10.3390/molecules29246026>
- AOAC, 2005. Official methods of analysis. AOAC International 18th ed. Rockville (MD):(AOAC International.).
- Bafundo K, 2025. Current thinking on *Eimeria* spp. in poultry and potential for advancements in control. Animals 15(3): 424. <https://doi.org/10.3390/ani15030424>
- Barekatain R, Chrystal PV, Howarth GS, McLaughlan CJ, Gilani S and Natrass GS, 2019. Performance, intestinal permeability, and gene expression of selected tight junction proteins in broiler chickens fed reduced protein diets supplemented with arginine, glutamine, and glycine subjected to a leaky gut model. Poultry Science 98(12): 6761-6771. <https://doi.org/10.3382/ps/pez393>
- Blake DP, 2025. *Eimeria* of chickens: the changing face of an old foe. Avian Pathology 54(3): 267-278. <https://doi.org/10.1080/03079457.2024.2441180>
- Chelakkot C, Ghim J and Ryu SH, 2018. Mechanisms regulating intestinal barrier integrity and its pathological implications. Experimental and Molecular Medicine 50(8): 1-9. <https://doi.org/10.1038/s12276-018-0126-x>

- Chen Y, Chen Y, Zhang H and Wang T, 2020. Pterostilbene as a protective antioxidant attenuates diquat-induced liver injury and oxidative stress in 21-day-old broiler chickens. *Poultry Science* 99(6): 3158-3167. <https://doi.org/10.1016/j.psj.2020.01.021>
- Chen Y, Zhang H, Li Y and Wang T, 2023. Pterostilbene confers protection against diquat-induced intestinal damage with potential regulation of redox status and ferroptosis in broiler chickens. *Oxidative Medicine and Cellular Longevity* 2023: 8258354. <https://doi.org/10.1155/2023/8258354>
- Chen L, Peng W, Wang T, Ma S, Wang X, Dai B, Zhang R, Yang C and Wu Y, 2025. Effects of dietary lysozyme supplementation on growth performance, intestinal morphology, immune function, antioxidant capacity, and gut microbiota in broilers. *Poultry Science* 104(11): 105741. <https://doi.org/10.1016/j.psj.2025.105741>
- Cuccato M, Scaglione FE, Centellegho C, Divari S, Biolatti B, Pregel P and Cannizzo FT, 2022. Assessment of antimicrobial effects on broiler gut barrier through histopathology and immunohistochemistry of tight-junction proteins. *Frontiers in Veterinary Science* 9: 830073. <https://doi.org/10.3389/fvets.2022.830073>
- FAO (2022). *Food Outlook – Biannual Report on Global Food Markets*. Rome, Italy. June 2022, Available at: <https://doi.org/10.4060/cb9427en> (Accessed: 27 March 2023)
- Fortuoso BF, Baldissera MD, Souza CF, Griss LG, Casagrande RA, de Cristo TG, Santiani F, da Cunha MG, Boiago MM, Stefani LM and Da Silva AS, 2019. Impairment of the phosphotransfer network and performance in broiler chickens experimentally infected by *Eimeria* spp.: The role of the oxidative stress. *Parasitology International* 70: 16-22. <https://doi.org/10.1016/j.parint.2019.01.004>
- Geng T, Ruan X, Xie Y, Shen B, Fang R, Zhao J and Zhou Y, 2024. Anticoccidial activity of a botanical natural product based on eucalyptus, apigenin and eugenol against *Eimeria tenella* in broiler chickens. *Parasites and Vectors* 17(1): 327. <https://doi.org/10.1186/s13071-024-06409-z>
- Hou Y, Han B, Lin Z, Liu Q, Liu Z, Si H and Hu D, 2024. Effects of six natural compounds and their derivatives on the control of coccidiosis in chickens. *Microorganisms* 12(3): 601. <https://doi.org/10.3390/microorganisms12030601>
- Huang T, Chen X, Chen D, Yu B, He J, Luo J, Zheng P, Luo Y, Yan H and Huang Z, 2025. Dietary pterostilbene supplementation enhances pork redness by regulating myoglobin expression, redox status, and structure in finishing pigs. *Food Bioscience* 68: 106705. <https://doi.org/10.1016/j.fbio.2025.106705>
- Iqbal Y, Cottrell JJ, Suleria HAR and Dunshea FR, 2020. Gut microbiota-polyphenol interactions in chicken: A review. *Animals* 10(8): 1391. <https://doi.org/10.3390/ani10081391>
- Khan IM, Gul H, Khan S, Nassar N, Khalid A, Swelum AA and Wang Z, 2025. Green tea polyphenol epigallocatechin-3-gallate mediates an antioxidant response via Nrf2 pathway in heat-stressed poultry: A review. *Poultry Science* 104(5): 105071. <https://doi.org/10.1016/j.psj.2025.105071>
- Kouvedaki I, Pappas AC, Surai PF and Zoidis E, 2024. Nutrigenomics of natural antioxidants in broilers. *Antioxidants* 13(3): 270. <https://doi.org/10.3390/antiox13030270>
- Kreuz BS, Rocha GC, Campos PHRF, Silva FFe, Hannas MI, Albino LFT, Borges SO and Calderano AA, 2020. Effects of dietary nucleotide supplementation on growth performance and physiology of broiler chickens under pre- and post-inflammatory challenge. *Revista Brasileira de Zootecnia* 49: e20200117. <https://doi.org/10.37496/rbz4920200117>
- Liu SY, Macelline SP, Chrystal PV and Selle PH, 2021. Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken-meat production. *Journal of Animal Science and Biotechnology* 12(1): 20. <https://doi.org/10.1186/s40104-021-00550-w>
- Luo Q, Yang L, Tumenjargal B, Liu S, Ma J, Ning J, Yun Z, Zhang X, Wu Y, Lu Y, Wu X, Wang L, Demberel S and Liu D, 2024. Effect of composite yeast culture on the jejunal barrier function, inflammatory response, and microbial community structure of laying hens during the late stage of egg production. *Frontiers in Veterinary Science* 11: 1524726. <https://doi.org/10.3389/fvets.2024.1524726>
- MacLaren LA, Wang J, Borzouie S and Rathgeber BM, 2024. Changes in tight junction protein expression levels but not distribution in commercial white and brown laying hens supplemented with *Chondrus crispus* or *Ascophyllum nodosum* Seaweed. *Animals* 14(5): 777. <https://doi.org/10.3390/ani14050777>
- Manzhali EG, Kondratyuk VY and Sokalska IM, 2024. The effect of stress on liver function. *Modern Gastroenterology* 137(3): 89-98. <https://doi.org/10.30978/MG-2024-3-89>
- Mathis GF, Lumpkins B, Cervantes HM, Fitz-Coy SH, Jenkins MC, Jones MK, Price KR and Dalloul RA, 2025. Coccidiosis in poultry: Disease mechanisms, control strategies, and future directions. *Poultry Science* 104(5): 104663. <https://doi.org/10.1016/j.psj.2024.104663>
- McCormack D and McFadden D, 2013. A review of pterostilbene antioxidant activity and disease modification. *Oxidative Medicine and Cellular Longevity* 2013: 575482. <https://doi.org/10.1155/2013/575482>
- Meng T, Wen Z, Cheng X, Li C, Zhang P, Xiao D and Xu Y, 2025. Unlocking gut health: The potent role of stilbenoids in intestinal homeostasis. *Animals* 15(3): 417. <https://doi.org/10.3390/ani15030417>
- Muthamilselvan T, Kuo TF, Wu YC and Yang WC, 2016. Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. *Evidence-Based Complementary and Alternative Medicine* 2016(1): 2657981. <https://doi.org/10.1155/2016/2657981>
- Navidshad B and Royan M, 2025. Grape by-products: a natural boon for broiler chickens' health. *World's Poultry Science Journal* 81(2): 605-622. <https://doi.org/10.1080/00439339.2025.2474985>
- Ncho CM, Gupta V, Goel A, Jeong CM, Jung JY, Ha S-Y, Eom JU, Yang HS, Yang JK and Choi YH, 2025. Impact of dietary polyphenols from shredded, steam-exploded pine on growth performance, organ indices, meat quality, and cecal microbiota of broiler chickens. *Poultry Science* 104(5): 105088. <https://doi.org/10.1016/j.psj.2025.105088>
- Obianwuna UE, Chang X, Oleforuh-Okoleh VU, Onu PN, Zhang H, Qiu K and Wu S, 2024. Phytobiotics in poultry: revolutionizing broiler chicken nutrition with plant-derived gut health enhancers. *Journal of Animal Science and Biotechnology* 15(1): 169. <https://doi.org/10.1186/s40104-024-01101-9>
- Oke OE, Akosile OA, Oni AI, Opowoye IO, Ishola CA, Adebisi JO, Odeyemi AJ, Adjei-Mensah B, Uyanga VA and Abioja MO, 2024. Oxidative stress in poultry production. *Poultry Science* 103(9): 104003. <https://doi.org/10.1016/j.psj.2024.104003>
- Phillips CJC, Hosseintabar-Ghasemabad B, Gorlov IF, Slozhenkina MI, Mosolov AA and Seidavi A, 2023. Immunomodulatory effects of natural feed additives for meat chickens. *Life* 13(6): 1287. <https://doi.org/10.3390/life13061287>
- Song B, Li P, Yan S, Liu Y, Gao M, Lv H, Lv Z and Guo Y, 2022. Effects of dietary astragalus polysaccharide supplementation on the Th17/Treg balance and the gut microbiota of broiler chickens challenged with necrotic enteritis. *Frontiers in Immunology* 13: 781934. <https://doi.org/10.3389/fimmu.2022.781934>
- Surai PF, Kochish, II, Fisinin VI and Kidd MT, 2019. Antioxidant defence systems and oxidative stress in poultry biology: An update. *Antioxidants* 8(7): 235.

- <https://doi.org/10.3390/antiox8070235>
- Tompkins YH, Teng P, Pazdro R and Kim WK, 2022. Long bone mineral loss, bone microstructural changes and oxidative stress after *Eimeria* challenge in broilers. *Frontiers in Physiology* 13: 945740. <https://doi.org/10.3389/fphys.2022.945740>
- Trott O and Olson AJ, 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 31(2): 455-461. <https://doi.org/10.1002/jcc.21334>
- Viveros A, Chamorro S, Pizarro M, Arija I, Centeno C and Brenes A, 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science* 90(3): 566-578. <https://doi.org/10.3382/ps.2010-00889>
- Wang X, Yuan Q, Xiao Y, Cai X, Yang Z, Zeng W, Mi Y and Zhang C, 2024. Pterostilbene, a resveratrol derivative, improves ovary function by upregulating antioxidant defenses in the aging chickens via increased SIRT1/Nrf2 expression. *Antioxidants* 13(8): 935. <https://doi.org/10.3390/antiox13080935>
- Wang Y, Zhou X, Liu M, Zang H, Zhang R, Yang H, Jin S, Qi X, Shan A and Feng X, 2023. Quality of chicken breast meat improved by dietary pterostilbene referring to up-regulated antioxidant capacity and enhanced protein structure. *Food Chemistry* 405: 134848. <https://doi.org/10.1016/j.foodchem.2022.134848>
- Wassie T, Lu Z, Duan X, Xie C, Gebeyew K, Yumei Z, Yin Y and Wu X, 2021. Dietary *Enteromorpha* polysaccharide enhances intestinal immune response, integrity, and caecal microbial activity of broiler chickens. *Frontiers in Nutrition* 8: 783819. <https://doi.org/10.3389/fnut.2021.783819>
- Yin Z, Sun X, Chai X, Zhou X, Wang Y, Liu M and Feng X, 2024. the effects of dietary pterostilbene on the immune response, antioxidant function, and jejunal structure of broilers. *Animals* 14(13): 1851. <https://doi.org/10.3390/ani14131851>
- Yvon S, Beaumont M, Dayonnet A, Eutamène H, Lambert W, Tondereau V, Chalvon-Demersay T, Belloir P and Paës C, 2024. Effect of diet supplemented with functional amino acids and polyphenols on gut health in broilers subjected to a corticosterone-induced stress. *Scientific Reports* 14(1): 1032. <https://doi.org/10.1038/s41598-023-50852-4>
- Zhang H, Chen Y, Chen Y, Ji S, Jia P, Li Y and Wang T, 2020a. Comparison of the protective effects of resveratrol and pterostilbene against intestinal damage and redox imbalance in weanling piglets. *Journal of Animal Science and Biotechnology* 11: 52. <https://doi.org/10.1186/s40104-020-00460-3>
- Zhang H, Chen Y, Chen Y, Li Y, Jia P, Ji S, Zhou Y and Wang T, 2020b. Dietary pterostilbene supplementation attenuates intestinal damage and immunological stress of broiler chickens challenged with lipopolysaccharide. *Journal of Animal Science* 98(1): skz373. <https://doi.org/10.1093/jas/skz373>
- Zhang L, Zhang J, Zang H, Yin Z, Guan P, Yu C, Shan A and Feng X, 2024. Dietary pterostilbene exerts potential protective effects by regulating lipid metabolism and enhancing antioxidant capacity on liver in broilers. *Journal of Animal Physiology and Animal Nutrition* 108(4): 921-933. <https://doi.org/10.1111/jpn.13941>
- Zhang R, Sun J, Wang Y, Yu H, Wang S and Feng X, 2025. Ameliorative effect of phenolic compound-pterostilbene on corticosterone-induced hepatic lipid metabolic disorder in broilers. *The Journal of Nutritional Biochemistry* 137: 109822. <https://doi.org/10.1016/j.jnutbio.2024.109822>
- Zhao M, Wu F, Li C, Guo L, Chen B, Wang F, Liu S and Han S, 2025. Ursolic acid improves growth performance, intestinal health, and antioxidant status in broilers by regulating lipid metabolism and the KEAP1-NRF2 pathway. *Journal of Animal Science* 103: skaf333. <https://doi.org/10.1093/jas/skaf333>