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RESEARCH ARTICLE

Dietary Dihydromyricetin Alleviates Lipopolysaccharide-induced Liver and Spleen Injury in Chickens by Enhancing Growth Performance and Immune Function

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ABSTRACT

Dihydromyricetin (DHM), a flavonoid compound present in various plants, exhibits multiple physiological activities including scavenging oxygen free radicals, antioxidant, and anti-inflammatory capabilities. Excessive production of lipopolysaccharide (LPS) by Gram-negative bacteria causes economic losses in the poultry industry by inducing various tissues damage such as liver and spleen. Therefore, we elucidated the protective activities of DHM on LPS-induced liver and spleen injury in chickens by investigating its impacts on growth and immune performance. Results demonstrated that dietary supplementation with 0.025, 0.05, 0.1, and 0.2% DHM increased body weight and average daily gain while enhancing serum biochemical parameters. DHM elevated antioxidant enzymes levels, thereby boosting antioxidant capacity. Regarding immune performance, DHM supplementation improved organ indices of the thymus, *bursa of Fabricius*, and spleen, along with increased IL-2, IFN-γ, and IgG levels. Furthermore, DHM intervention effectively alleviated LPS-induced liver and spleen injury by regulating inflammatory cytokines expression. Histopathological examination and transmission electron microscopy observations corroborated these protective effects. In summary, dietary supplementation with DHM enhances growth performance and immune function in poultry, thereby alleviating the severity of liver and spleen injury.

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INTRODUCTION

The poultry industry is one of the most rapidly growing livestock sectors globally. Poultry meat and eggs have long been recognized as the most crucial sources of dietary protein for the global population (Madacussengua *et al*., 2025). However, alongside the rapid development of the poultry industry, several global challenges affecting poultry production have become increasingly formidable. In recent years, the industry has trended toward highly scaled and intensive production systems, which often lead to overcrowded housing conditions, poor environmental management, and subsequent stress and disease outbreaks in poultry, severely compromising their growth performance. Traditionally, antibiotic growth promoters (AGPs) were widely used to enhance growth and prevent diseases. While, the widespread use of antibiotics has already caused critical issues including drug residues and bacterial resistance, posing significant risks to animal and consumer health (Abou-Jaoudeh *et al*., 2024; Bacanli, 2024). Consequently, to address these pressing challenges, the poultry sector must adopt more sustainable practices to maximize productivity with minimal costs and environmental impact (Zhang *et al*., 2025).

Decades of global practice have demonstrated that adding antibiotics to feed positively enhances the productivity of intensive livestock farming. However, antibiotic residues in livestock products pose food safety risks, soil and water pollution, and particularly the emergence of antimicrobial-resistant microbes, which directly threaten human and animal health (Zackariah *et al*., 2025). Consequently, achieving "antibiotic-free feed, antibiotic alternatives in farming, and antibiotic-free animal products" has become a critical challenge for modern animal husbandry. Identifying alternatives to antibiotics that can improve growth performance in poultry is of paramount significance. Bioactive components from Chinese herbal medicines, long used for disease prevention and treatment, offer advantages such as wide availability, minimal residues, and low toxicity. Recent studies indicate that incorporating herbal ingredients into feed can regulate nutrient metabolism, immune responses, and body health in animals, showing strong potential to replace AGPs (Liu *et al.*, 2024).

Dihydromyricetin (DHM) is mainly present in young stems and leaves of *Ampelopsis grossedentata* (a species from the *Vitaceae* family) and other plants (Chang *et al.*, 2024; Sarkar *et al.*, 2025). DHM exhibits diverse bioactive properties, including free radical scavenging, antioxidant, antithrombotic, anti-inflammatory, and antitumor activities. In recent years, advancements in flavonoid extraction technologies have significantly improved the yield of naturally derived DHM, spurring its growing application in livestock and agricultural research. Studies demonstrate that DHM alleviates intestinal mucosal injury, oxidative stress, and apoptosis while preserving barrier integrity (Chang *et al.*, 2020). Metabolomic analyses reveal that DHM modulates many metabolic pathways, including glycolysis, the tricarboxylic acid cycle, purine metabolism, and the urea cycle (Le *et al.*, 2016). Additionally, DHM inhibits the TLR4/NF-κB pathway and mediates Nrf2 translocation from the cytoplasm to Keap1, thereby activating downstream antioxidant gene expression. This dual mechanism not only enhances intestinal cell proliferation, reduces apoptosis, and mitigates intestinal barrier damage but also strengthens mucosal immunity by stimulating secretory IgA and β-defensin secretion (Xie *et al.*, 2024).

Therefore, this study investigates the capabilities of DHM supplementation on growth and immune performance, biochemical parameters, antioxidant enzyme activity in poultry. Furthermore, it elucidates the intervention ability of DHM on lipopolysaccharide (LPS) caused liver and spleen damage, helping for clinical application and development of DHM in veterinary medicine.

MATERIALS AND METHODS

Ethical statement: The Institutional Animal Care and Use Committee of Northeast Agricultural University (Harbin, Heilongjiang Province) approved the experimental procedures in the present study (Permit Number: NEAUEC20220326).

Animal management and experimental protocols: The DHM had a purity of ≥98%determined by HPLC and was obtained from Winherb Medical Technology Co., Ltd, (Shanghai, China). All chickens were raised at 25±2°C, and in a 12 hours light and dark circulation environment, allowing them to freely feed and drink. The basal diet for chickens was purchased from Harbin Lenong Feed Co., Ltd. (Harbin, China). Its composition and guaranteed analysis values (%) are as follows: crude protein (≥21.0), crude fiber (≤6.0), crude ash (≤8.0), calcium (0.5-2.3), total phosphorus (≥0.3), sodium chloride (0.3-0.8), lysine (≥1.0). The chickens were anesthetized with pentobarbital sodium and sacrificed by cardiac puncture and then serum and tissue were collected.

The 750 1-day-old Hy-line White female chickens purchased from Xianfeng chicken farm (Harbin, China) after approval of the ethical committee (NEAUEC20220326). After 7 days of adaptive feeding, excluding chickens that died unexpectedly, they were randomly divided into five groups. There were 3 replicates of 50 chickens in each group. At 8 days of age, chickens were fed diets supplemented with 0, 0.025, 0.05, 0.1 and 0.2% (quality percentage) DHM in the basal diet, respectively. According to the conventional compound feed formulation method (Mohamed *et al*., 2023; Xu *et al*., 2025), DHM was thoroughly mixed with a small portion of the basal feed to ensure uniformity. This mixture is then gradually added to the remaining feed ingredients and mixed for a duration to guarantee uniform distribution throughout the feed. The DHM solution is prepared immediately before use, and fresh feed is formulated regularly to prevent deterioration of DHM. Body weight (BW) of chickens at 14, 28 and 42 days was recorded, and serum, thymus, spleen and bursa of chickens at 14, 21, 28, 35 or 42 days were collected.

The 150 1-day-old Hy-line White female chickens were randomly divided into 5 groups with 3 replicates, after 7 days of adaptive feeding, excluding chickens that died unexpectedly. There were 3 replicates of 10 chickens in each group. At 8 days of age, chickens were fed 0.025, 0.05 and 0.1% DHM for 14 days. At 22 days of age, LPS-challenged chickens were intraperitoneally administered LPS solution at a dose of 60mg/kg body weight after a 12 hour fast (Huang *et al*., 2017). After 12 h, serum was collected, and liver and spleen were isolated.

Growth performance: Chickens were weighed at 14, 28 and 42 days of age. The average daily gain (ADG) was calculated for each group.

Biochemical index detection: The levels of total protein (TP, 23501060), albumin (ALB, 23501070), calcium (Ca, 23501230), phosphorus (P, 23501160), lactate dehydrogenase (LDH, 23501140), direct bilirubin (DBIL, 23501270), aspartate aminotransferase (AST, 23501010) and alanine aminotransferase (ALT, 23501000) were measured by biochemical analyser using corresponding kits (Jiangsu Sinnowa Medical Technology Co., Ltd., Nanjing, China).

Antioxidant status: The levels of total superoxide dismutase (T-SOD, A001-1-2), glutathione peroxidase (GSH-Px, A005-1-2), total antioxidant capacity (T-AOC, A015-1-2) and malondialdehyde (MDA, A003-1-2) were measured by corresponding kits (Nanjing Jiancheng Institute of Biotechnology, China).

Immune organ development: At 14, 21 and 42 days, the thymus, spleen and bursal of chickens from each group were removed, and the fresh weight was weighed to calculate the organ index.

Quantitative RT-PCR detection: Total RNA from liver and spleen was isolated and reversely transcribed into cDNA in accordance with the manufacturer's guidelines

(Taylor *et al.*, 2010; Chang *et al.*, 2019). A quantitative RT-PCR reaction was executed and the thermal cycling conditions were as follows: 95°C for 30s; 95°C for 5s, and 60°C for 30s for 45 cycles. Table 1 presents the primers used in this study. Using β-actin as the housekeeping gene, the target genes levels were normalized. Following the standard procedure for quantitative RT-PCR, with triplicate measurements per sample, the relative expression levels of each target mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and compared with the control group.

Table 1: Genes and primers used in this study

Gene	GenBank Accession	Sequences (5' to 3')	Product Size
β-actin	NM_205518.1	forward: CTCTGACTGACCGCGTTACT reverse: TACCAACCATCACACCCTGAT	172 bp
IL-1β	NM_204524.1	forward: GCATCAAGGGCTACAAGCTC reverse: CAGGCGGTAGAAGATGAAGC	131 bp
IL-8	HM179639.1	forward: ACACTCCTAACCATGAACGGCAAG reverse: CTGGCACCGCAGCTCATTCC	114 bp
IL-10	NM_001004414.2	forward: CAGCACCAGTCATCAGCAGAGC reverse: GCAGGTGAAGAAGCGGTGACAG	94 bp
TNF-α	AY765397.1	forward: CTCAGGACAGCCTATGCCAACAAG reverse: GCCACCACACGACAGCCAAG	178 bp

Histological analysis: Histopathological examination of chicken liver and spleen tissues was performed following standard protocols for paraffin sectioning and haematoxylin and eosin (H&E) staining (Li *et al.,* 2025). The liver and spleen tissues were fixed with 10% neutral formalin buffer. After dehydration in an ascending series of ethanol and clearing in dimethylbenzene, the tissues were embedded in paraffin and sectioned transversely at 4μm. After H&E staining, the slides were examined (100X magnifications).

Transmission electron microscopy analysis: The tissues were fixed in glutaraldehyde, post-fixed in osmic acid fixing solution, dehydrated, and embedded in epoxy resin (Du *et al.*, 2017). The tissues were sliced by an ultramicrotome, stained with uranium acetate and lead citrate, observed and photographed by transmission electron microscope.

Statistical Analysis: Statistical analysis was performed using one-way analysis of variance (ANOVA) using SPSS, version 19.0 software. The Duncan multiple comparison test was used to examine the statistical significance (P<0.05 and P<0.01) between groups.

RESULTS

DHM improves growth performance: The addition of 0.05% DHM increased the BW of 28-day-old chickens, while 0.025-0.2% DHM supplementation led to an elevation in BW at 42 days of age (Fig. 1). Dietary inclusion of 0.025-0.2% DHM improved the ADG during the 0-42 days period (P<0.05 or P<0.01). However, no changes in ADG were observed in the 0-14, 15-28, or 29-42 days phases. These results indicate that DHM exhibits growth-promoting effects in poultry.

Effects of DHM on biochemical indices and antioxidant status: As illustrated in Fig. 2, at 14 days of age, 0.025 and 0.05% DHM supplementation reduced serum LDH activity. The addition of 0.1% DHM decreased serum ALT, LDH, and DBIL levels, while 0.2% DHM reduced DBIL content. At 21 days of age, 0.025 and 0.05% DHM supplementation lowered LDH activity. By 28 days of age, 0.05, 0.1, and 0.2% DHM reduced LDH activity (P<0.05 or P<0.01). Meanwhile, DHM showed no significant effects on other serum parameters.

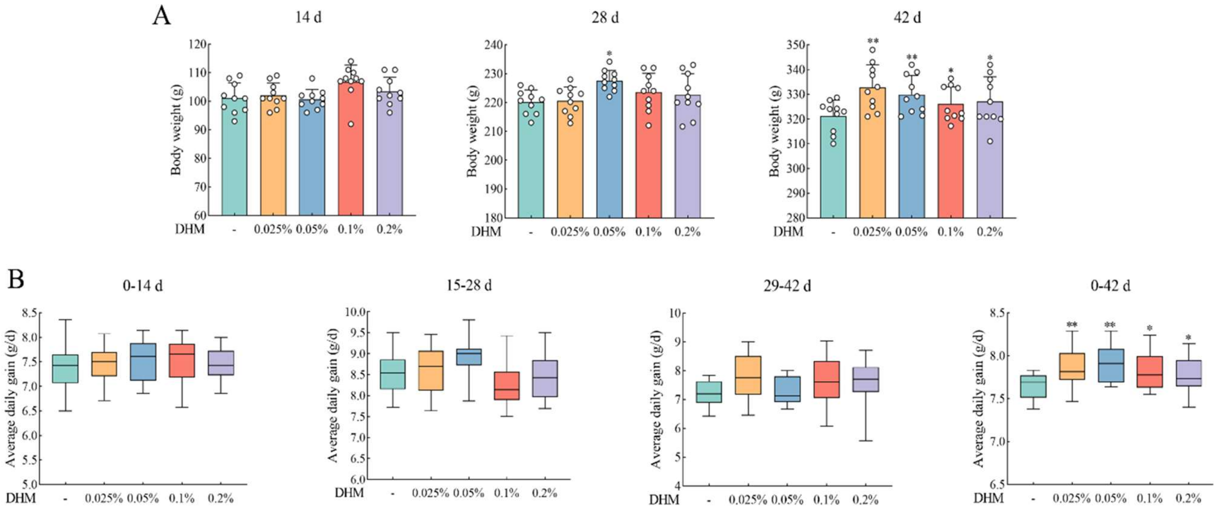

Fig. 1: Effects of dietary supplementation with DHM on growth performance of chickens. **(A)** Chicken weight at 14, 28 and 42 days of age. **(B)** chicken ADG at 0-14, 15-28, 29-42 and 0-42 days. Values are expressed as the mean±SD for each group (n=10). *represented P<0.05 and **represented P<0.01 compared with control group.

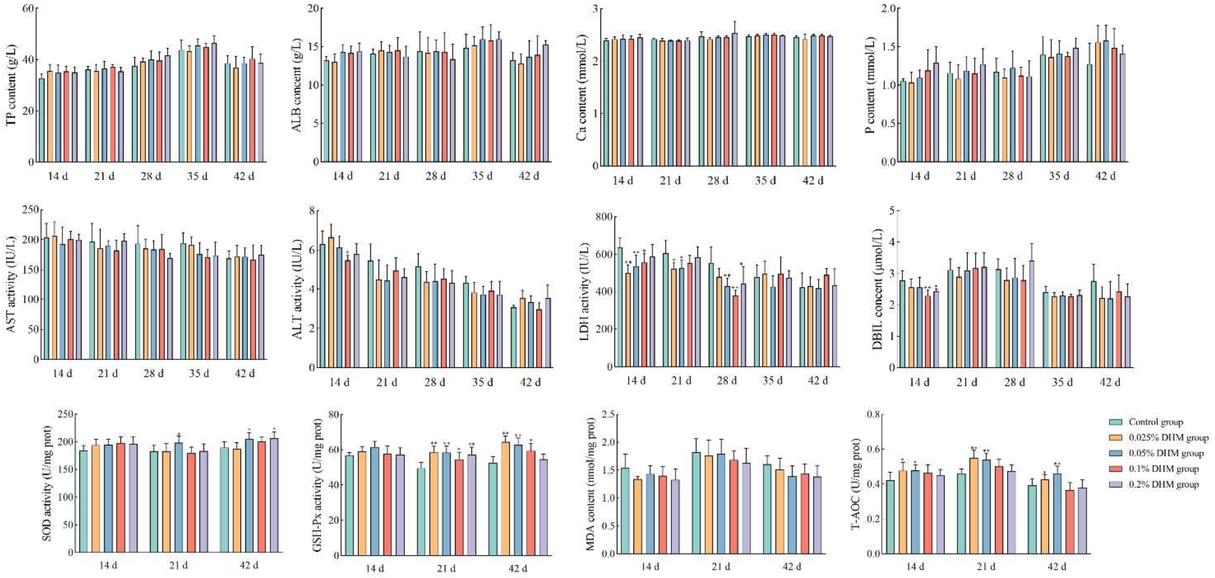

Fig. 2: Effects of dietary supplementation with DHM on biochemical indices and antioxidant status of chickens. Biochemical indices include serum TP, ALB, Ca, P, AST, ALT, LDH and DBIL level. Antioxidant status indices include liver SOD, GSH-Px, SOD and T-AOC level. Values are expressed as the mean ± SD for each group (n = 10). *represented P<0.05 and **represented P<0.01 compared with control group.

Additionally, this study assessed the SOD, MDA, GSH-Px, and T-AOC levels in the liver of chickens at 14, 21, and 42 days of age (shown in Fig. 2). At 14 days of age, 0.025 and 0.05% DHM supplementation increased hepatic T-AOC activity. By 21 days of age, 0.025% DHM elevated GSH-Px and T-AOC levels, while 0.05% DHM enhanced SOD, GSH-Px, and T-AOC levels. Higher doses (0.1 and 0.2% DHM) increased GSH-Px activity. At 42 days of age, 0.025% DHM boosted GSH-Px and T-AOC, 0.05% DHM improved SOD, GSH-Px, and T-AOC, 0.1% DHM elevated GSH-Px, and 0.2% DHM enhanced SOD activity (P<0.05 or P<0.01). No effects on hepatic MDA content were observed across all DHM doses. These findings indicate that DHM exerts no apparent toxicity in poultry and effectively enhances systemic antioxidant capacity.

DHM enhances the immune performance of chickens: This study evaluated the immune organ indices of chickens across experimental groups, as depicted in Fig. 3A. The 0.025% DHM supplementation exhibited an increased spleen index at 14 days of age, while the 0.05% DHM supplementation showed elevated spleen, thymus, and *bursa of Fabricius* indices at 14 days of age. At 21 days of age, 0.025% DHM supplementation enhanced spleen and thymus indices, and 0.1% DHM increased the bursa index. By 42 days of age, 0.05, 0.1, and 0.2% DHM all improved the spleen index (P<0.05 or P<0.01).

Subsequently, the serum contents of IL-2, IFN-γ and IgG were measured in each group. As seen in Fig. 3B, at 14 days of age, serum IL-2, IFN-γ and IgG contents were increased by 0.025, 0.05 and 0.1% DHM supplementation, and IL-2 content was increased by 0.2% DHM. At 21 days of age, 0.025% DHM increased IFN-γ content, 0.05% DHM increased IL-2, IFN-γ and IgG contents, 0.1 and 0.2% DHM increased IL-2 content. At 42 days of age, 0.1 and 0.2% DHM increased IL-2 and IFN-γ contents (P<0.05 or P<0.01). These results show that DHM could promote immune performance of chickens.

DHM alleviates LPS-induced liver injury: To investigate whether DHM exerts protective effects on LPS-induced liver damage, serum AST, ALT, LDH, and DBIL, and hepatic IL-1β, IL-8, IL-10, and TNF-α expression, were measured following intervention with 0.025, 0.05, and 0.1% DHM (Fig. 4A and B). Notably, LPS exposure altered the levels of all aforementioned biomarkers. In contrast, 0.05% DHM suppressed LPS-induced elevation of all markers except DBIL content and IL-8 expression. Moreover, 0.1% DHM effectively mitigated LPS-driven abnormalities in AST, DBIL, IL-1β, IL-8, and IL-10 (P<0.05 or P<0.01).

The histopathological micrographs of liver tissues showed that the control hepatocytes exhibited normal structure with deeply stained cytoplasm (Fig. 4C). The hepatic lobule architecture remained intact, displaying orderly hepatocyte arrangement centred around the central vein. The hepatocytes were regularly shaped, with clear boundaries and dense, uniform alignment, showing no signs of cellular degeneration or abnormalities. In the LPS group, the chicken liver tissues exhibited swelling, disorganized hepatic cord structures, and necrosis with lymphocyte infiltration around the central vein. In contrast, under DHM intervention, the hepatic lobule structure in chickens was restored to normal, accompanied by reduced lymphocyte infiltration.

The liver tissues observed under transmission electron microscopy are shown in Fig. 4D. The results demonstrated that the control group exhibited intact cellular morphology and organelle structures. Following LPS-induced injury, hepatocytes developed numerous vacuoles, and mitochondrial structures appeared blurred. In contrast, DHM alleviated the extent of hepatocellular damage. With increasing doses of DHM, the ultrastructure of organelles gradually normalized. These findings indicate that DHM shows a mitigating effect on LPS-induced liver injury.

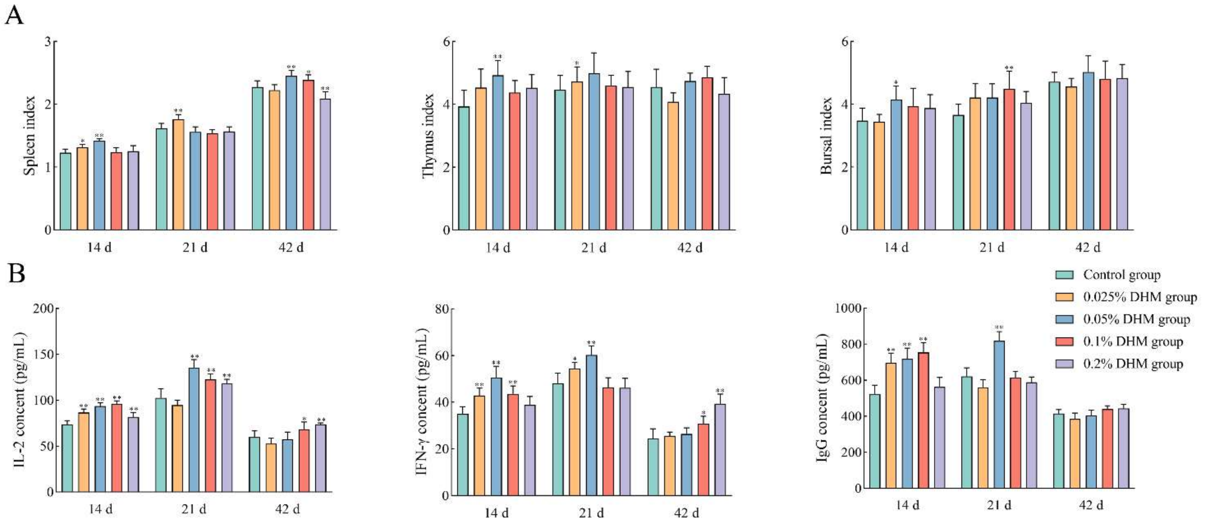

Fig. 3: Effects of DHM on immune organ index and cytokine levels of chickens at different doses. **(A)** Changes of spleen, thymus and bursa index of chickens in different DHM groups. **(B)** Effects of DHM on serum IL-2, IFN-γ and IgG contents in chickens. Values are expressed as the mean±SD for each group (n = 10). *represented P<0.05 and **represented P<0.01 compared with control group.

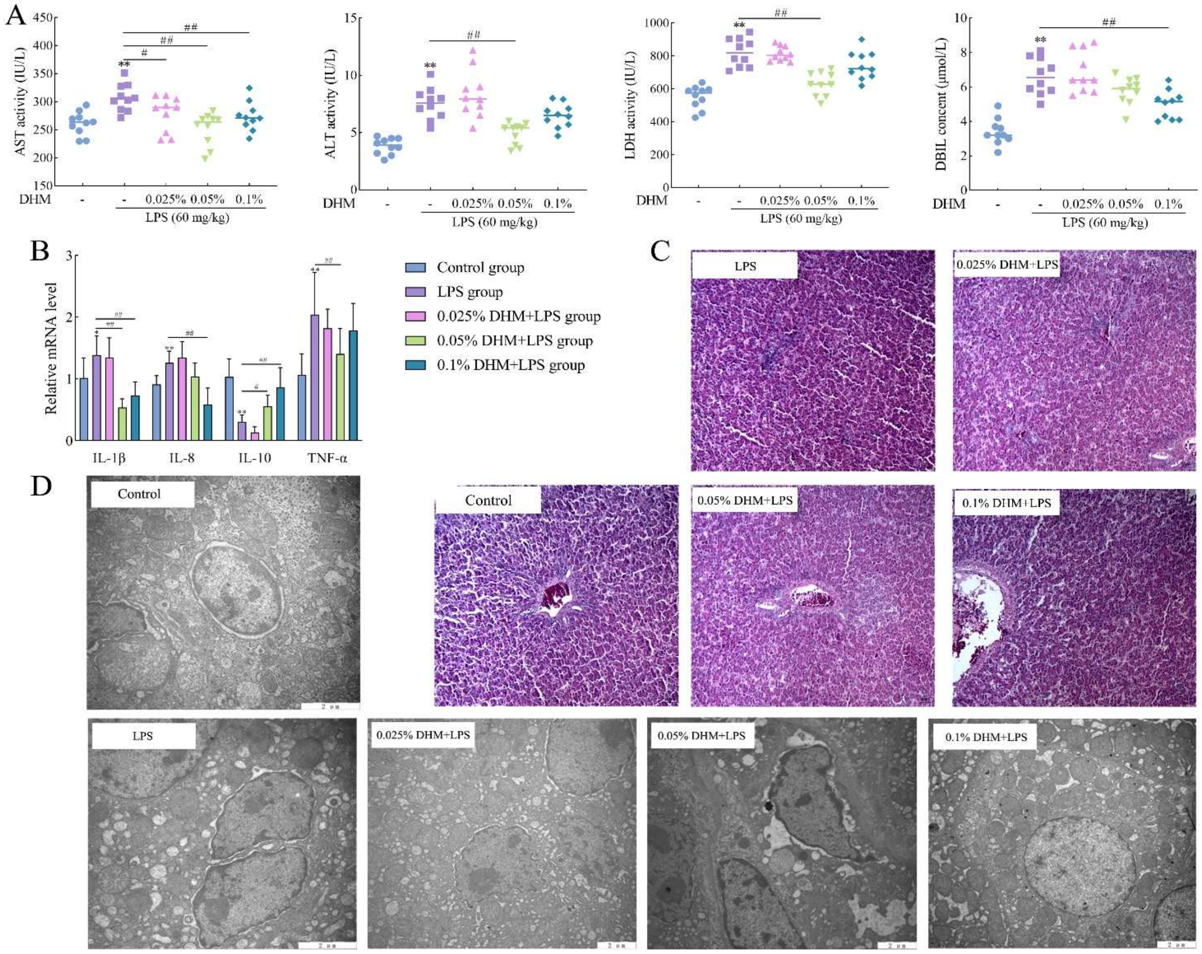

Fig. 4: Effects of 0.025, 0.05 and 0.1% DHM on LPS-induced liver injury. Changes of AST, ALT, LDH and DBIL level in serum **(A)**, and IL-1β, IL-8, IL-10 and TNF-α mRNA expression in liver **(B)** after treatment with 60mg/kg LPS for 12 h, 0.025, 0.05 and 0.1% DHM for 14 days. **(C)** Chicken liver pathology. Liver sections were stained by H&E. All the liver sections were examined by light microscopy and the images were displayed at 100X the original magnification. **(D)** Transmission electron microscope observation. Transmission electron microscope observation at 20000X the original magnification for liver after 60mg/kg LPS exposure for 12h followed by 14 days of 0.025, 0.05 and 0.1% DHM treatment. Values were expressed as the mean±SD of each group (n=10). **represented P<0.01 compared with control group. # represented P<0.05 and ## represented P<0.01 compared with LPS group.

DHM interferes with spleen injury induced by LPS: To investigate whether DHM exerts protective abilities on LPS-induced splenic injury, the IL-1β, IL-8, IL-10, and TNF-α expression in spleen tissues were measured after intervention with 0.025, 0.05, and 0.1% DHM (Fig. 5A). Notably, LPS exposure upregulated the IL-1β and IL-8 expressions, but downregulated the IL-10 expression. Both 0.05 and 0.1% DHM suppressed the changes of these three cytokines ($P<0.05$ or $P<0.01$) but not TNF-α.

As shown in the histopathological micrographs of spleen tissue via H&E staining (Fig. 5B), the control group exhibited normal splenic architecture with clearly demarcated boundaries between white pulp and red pulp. Following 12 hours of LPS stimulation, the distinction between white and red pulp became blurred. In contrast, the splenic structure in the DHM intervention group displayed a restoration toward normal morphology.

Transmission electron microscopy images of splenic tissue are presented in Fig. 5C. The results revealed intact cellular morphology and organelle structures in the control group. After LPS stimulation, a small number of vacuoles formed within splenic cells. DHM alleviated the severity of splenic cell damage, and with increasing DHM doses, the organelle structures progressively normalized. These findings indicate that DHM exhibits a mitigatory effect on LPS-induced splenic injury.

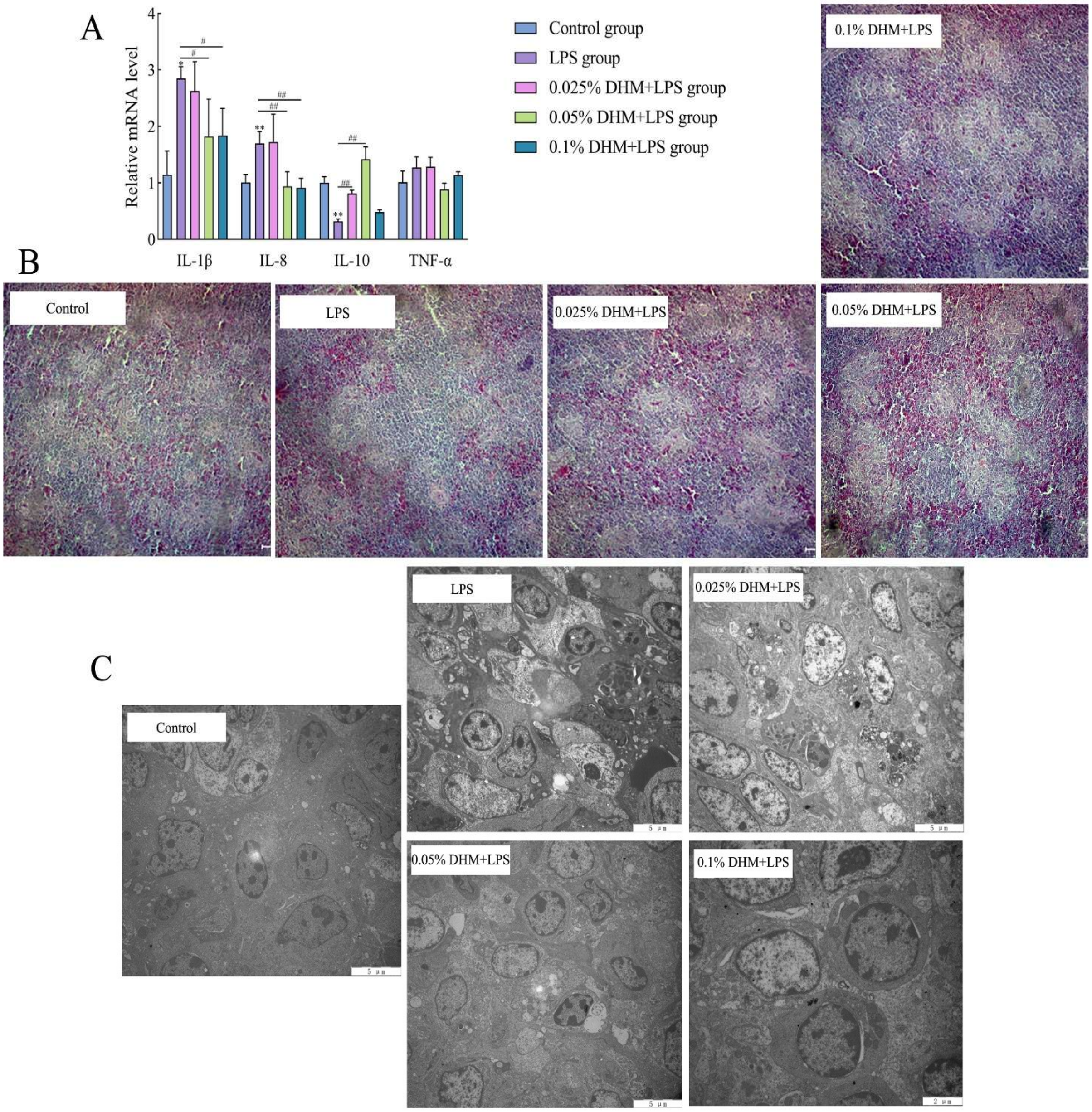

Fig. 5: Effects of 0.025, 0.05 and 0.1% DHM on LPS-induced spleen injury. **(A)** Changes of IL-1β, IL-8, IL-10 and TNF-α mRNA expression in spleen after treatment with 60mg/kg LPS for 12h followed by 14 days of 0.025, 0.05 and 0.1% DHM treatment. **(B)** Chicken spleen pathology. Spleen sections were stained by H&E. All the spleen sections were examined by light microscopy and the images were displayed at 100X the original magnification. **(C)** Transmission electron microscope observation. Transmission electron microscope observation at 20000X the original magnification for spleen after 60 mg/kg LPS exposure for 12 h followed by 14 days of 0.025, 0.05 and 0.1% DHM treatment. Values were expressed as the mean±SD of each group (n=10). *represented $P<0.05$ and **represented $P<0.01$ compared with control group. # represented $P<0.05$ and ## represented $P<0.01$ compared with LPS group.

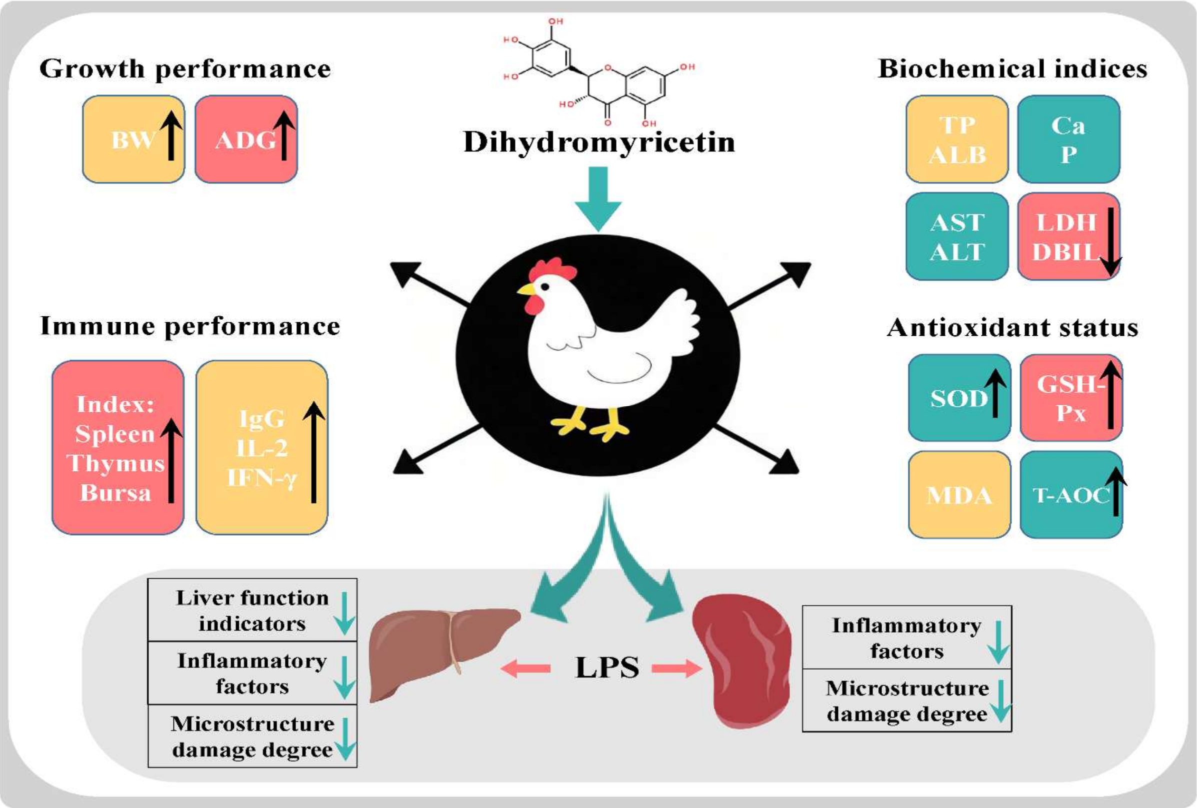

Fig. 6: A brief diagram of this study shows that DHM enhances the growth performance, immune performance and antioxidant capacity of chickens, and maintains the levels of serum biochemical indicators, thereby alleviating the liver and spleen damage caused by LPS in chickens.

DISCUSSION

With the gradual decrease in the use of antibiotics in animal husbandry, it is imperative to identify viable alternatives to enhance immune function and prevent pathogenic microbial infections in livestock (Mudasir Ahmad *et al.*, 2024). Currently, immunomodulators used in conjunction with vaccination are gaining widespread application in the farming industry, particularly demonstrating broad prospects in poultry production (Jiao *et al.*, 2025). DHM, a flavonoid compound containing multiple phenolic hydroxyl groups, exhibits diverse physiological activities such as scavenging oxygen free radicals, antioxidation, and anti-inflammatory effects. Advances in flavonoid extraction technologies and significant improvements in the yield of monomeric compounds have facilitated the expanding utilization of DHM in animal husbandry. Therefore, this study elucidated the impact of DHM on growth and immune performance in Hy-Line White chickens, and its ameliorative effects on liver and spleen injury.

The growth and development of poultry are influenced by multiple factors, including genetics, environmental conditions, and nutritional levels. Enhancing poultry growth can improve production efficiency and economic benefits in poultry farming. BW and ADG are critical metrics for evaluating growth performance (Guo *et al.*, 2023). Studies indicate that flavonoids enhance activity of digestive enzymes, promote feed digestion and conversion, modulate intestinal environments, regulate metabolic processes, and improve growth performance in animals. Recent studies have shown that dietary supplementation of quercetin, rutin, and baicalin can promote growth performance and antioxidant function in chickens (Abdel-Latif *et al.*, 2021; Li *et al.*, 2023; Liu *et al.*, 2025). In this study, DHM was administered to 7-day-old chickens and continuously supplemented until 42 days of age. The BW of DHM-treated chickens was higher than that of the control. All DHM doses increased the ADG from 0 to 42 days. In contrast, among these three flavonoid compounds, quercetin and rutin failed to demonstrate significant improvement in BW and ADG, while only baicalin showed a certain increase of BW and ADG in chicken. Therefore, compared to them, DHM exhibited a strong growth-promoting effect.

Under normal physiological conditions, the components of blood are maintained within specific ranges. Consequently, serum biochemical parameters act as indicators of growth performance, metabolic status, and overall health. Variations in serum TP, ALB, Ca, and P levels can reflect the metabolic function and physiological state of poultry (Cowieson *et al.*, 2024). Similar to other flavonoid compounds, in our findings, the serum TP, ALB, and P levels in chickens exhibited an upward trend with increasing DHM supplementation, while blood Ca concentrations remained relatively stable across different age groups.

When hepatocytes are damaged, ALT and AST are released into the bloodstream, thereby elevating the activity of these enzymes in serum (Shi *et al.*, 2022). Bilirubin, the primary metabolic product of iron-porphyrin compounds in the body, is a critical clinical indicator for evaluating liver function (Hamoud *et al.*, 2018). LDH, a zinc-containing glycolytic enzyme, is present in the heart, liver and kidneys (Ferriero *et al.*, 2018). MDA, T-AOC, T-SOD, and GSH-Px levels are vital biomarkers reflecting antioxidant capacity and are closely associated with overall health (Xu *et al.*, 2023). Previous studies on quercetin, rutin, and baicalin have demonstrated that these flavonoid compounds exhibit significant antioxidant capabilities. Similarly, in this study, supplementation of chicken feed with varying DHM doses reduced transaminases, LDH and DBIL levels, while enhancing hepatic SOD and GSH-Px activities, thereby strengthening total hepatic antioxidant capacity. Although MDA content in the liver of DHM-treated chickens showed no alteration, a declining trend was observed with higher DHM doses. These findings demonstrate that DHM exhibits no overt toxicity to chicken growth and enhances antioxidant capacity by promoting hepatic antioxidant enzymes expression.

In terms of immune performance, one of the most common indicators in poultry is the weight of immune organs. These organs serve as the primary sites for immune responses and provide the material foundation for immune function execution. The status of immune organ indices can reflect the immunological state of animals to a certain extent (Cai *et al.*, 2022). Their development directly influences immune competence, and their growth status can be used to evaluate the immunological condition of birds. IL-2, a multifunctional cytokine produced by antigen-activated T cells, regulates leukocyte activity in the immune system and participates in the proliferation of T, NK, and B cells (Liao *et al.*, 2013; Spolski *et al.*, 2017). IFN-γ exhibits high species specificity and possesses antiviral and antiproliferative activities. It modulates cellular immunity by activating macrophages to enhance phagocytic function, promoting the maturation and activation of cytotoxic T lymphocytes (CTLs), and facilitating B cell differentiation and antibody production. IgG, the predominant antibody generated during immune responses, is widely distributed in circulation and constitutes 75% of total circulating immunoglobulins (Bournazos and Ravetch, 2017). Yang *et al.* (2020) demonstrated that quercetin enhances immune organs development and improves immune function in chickens by upregulating cytokines such as IFN-γ and immunoglobulins like IgA expression. Our findings revealed that DHM promotes the maturation of immune organs in chickens, enhances the synthesis of cytokines and antibodies, and exerts immunostimulatory effects on poultry immunity.

In recent years, avian colibacillosis has remained a major bacterial disease threatening the poultry industry. *Escherichia coli* possesses numerous serotypes and can spread through various routes, including feed, drinking water, feces, and equipment. As an opportunistic pathogen, it causes infections when stress factors compromise host resistance, leading to significant economic losses in poultry production. LPS, a key component of the *Escherichia coli* cell wall, induces the production of TNF-α, IL-1β, IL-6 to trigger inflammatory responses and stimulates excessive reactive oxygen species generation (Meng *et al.*, 2024; Peng *et al.*, 2024). The anti-inflammatory and antioxidant properties of flavonoid compounds make them commonly used to mitigate inflammation-related damage. Xia *et al.* (2025) demonstrated that quercetin alleviates LPS-induced liver injury in chickens by regulating mitochondrial dynamics and metabolic abnormalities in hepatocytes, thereby reducing necroptosis and apoptosis. Cheng *et al.* (2017) revealed that baicalin exerts protective effects against LPS-induced hepatic inflammation in chickens by downregulating TLR4/NF-κB-mediated inflammatory pathway. In alignment with these findings, we evaluated the preventive potential of DHM against bacterial diseases in chickens by administering varying DHM doses to mitigate LPS-induced hepatic and splenic injury. The findings demonstrate that DHM reduces serum transaminase and LDH activities, suppresses pro-inflammatory cytokine expression in the liver and spleen, and alleviates LPS-induced histopathological and ultrastructural damage in these organs, indicating its therapeutic potential against poultry bacterial diseases. Fig. 6 shows a schematic diagram illustrating the promotion of functional development and intervention effects on liver and spleen damage by DHM in chickens.

Conclusions: Dietary supplementation with DHM enhances growth performance and immune function in chickens, improves serum biochemical parameters, boosts antioxidant capacity, and effectively alleviates LPS-induced hepatic and splenic damage. This study is conducive to DHM application in the poultry industry to promote growth, maintain health, and prevent inflammatory injuries in poultry.

Competing interests: The authors declare that they have no competing interests.

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Authors contribution: YC and FL: conceptualization and methodology. WC, MG, YL and ZJ: investigation, data curation, and formal analysis. YC: writing – original draft and preparation. XJ, SW, YR and RL: writing – review and editing. CL and FL: visualization and supervision. All authors know and approved the final manuscript.

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