

PROTECTIVE ROLE OF PHYTOGENIC COMPOUNDS AGAINST OXIDATIVE STRESS INDUCED BY MYCOPLASMA GALLISEPTICUM IN POULTRY

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ABSTRACT

Mycoplasma gallisepticum (MG) is a significant respiratory pathogen of poultry which may negatively affect productive performance and cause oxidative and inflammatory damage to tissues. The protective effects of a diet supplemented with curcumin, thymol and quercetin against respiratory injury, pathogen burden, inflammation and oxidative stress associated with the MG in broiler chickens were evaluated in this study. A total of 120 1-day-old male Ross 308 broilers were divided into five dietary treatments with six replicate pens per dietary treatment as follows: non-challenged control (CON), MG-challenged control (MG), MG + curcumin (200 mg/kg), MG + thymol (150 mg/kg), and MG + quercetin (200 mg/kg). Growth performance, oxidative stress biomarkers, inflammatory cytokines, MG DNA abundance and respiratory histopathology were assessed after MG challenge on day 21. Final body weight, body weight gain and feed conversion were significantly depressed for MG challenge birds compared to CON birds. Moreover, the malondialdehyde content was elevated with a simultaneous decline in the levels of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in MG challenge. Levels of pro-inflammatory cytokines were elevated, while anti-inflammatory cytokine levels were decreased (IL-10). Partial recovery of these changes occurred with phytogetic supplementation, which increased antioxidant defenses and decreased inflammatory responses. The treatment also decreased the amount of MG DNA and mitigated the severity of the lesions in the trachea and lungs. Quercetin exhibited the greatest numerical improvement on a number of integrated endpoints. The dietary treatment with curcumin, thymol, and quercetin as a whole reduced several pathological effects caused by the MG challenge, indicating their potential for use as nutrition strategies to enhance the antioxidant system, inflammatory homeostasis and respiratory health of broilers.

Keywords: *Mycoplasma gallisepticum*; phytogetic feed additives; oxidative stress; curcumin; thymol; quercetin; broilers

1. INTRODUCTION

Mycoplasma gallisepticum (MG) is a significant respiratory pathogen of chickens that causes chronic respiratory disease (CRD) in chickens, which encompasses poor feed efficiency, high production expenses, and huge economic losses for the poultry industry (Feberwee et al., 2022; Chaidez-Ibarra et al., 2022; Wu et al., 2024). Through sophisticated immune evasion mechanisms and interactions with the respiratory epithelium, MG can create a persistent infection and maintain inflammation and tissue damage (Zhang et al., 2024). In addition to localised respiratory pathology, experimental evidence shows that MG infection can alter cellular energy metabolism, antioxidant defence and inflammatory signalling pathways, all of which could be seen as important aspects of the pathogenesis of MG (Ishfaq et al., 2020; Hu et al., 2021). It has also been shown that MG infection can also lead to impaired antioxidant activity, oxidative damage, apoptosis and structural damage of immune tissues, (Hu et al., 2021; Ishfaq et al., 2021). During infection, physiological performance and integrity of tissues could be impaired by these interrelated processes. Biosecurity, vaccination, and antimicrobial therapy are the main methods used for current MG control, but there are restrictions on the use of these methods due to the emergence of antimicrobial resistance, non-perfect cover, and persistent infection, which has led to growing interest in complementary nutritional strategies (Rashid et al., 2021; Feberwee et al., 2022; Wu et al., 2024). The antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties of phytogetic feed additives (PFAs) such as constituents of essential oils and polyphenols and flavonoids derived from plants have gained much attention (Abdelli et al., 2021; Righi et al., 2021; Biswas et al., 2024; Wang et al., 2024). Their multimodal biological activity makes them especially relevant in diseases characterised by the presence of a pathogen load, oxidative imbalance and inflammation. There are some candidate

phytogetic compounds that have complementary biological properties such as curcumin, thymol and quercetin. Recent studies suggest that curcumin may also impact gut microbiota, redox regulation, and inflammatory signaling in poultry, which highlights its potential anti-inflammatory properties (Ruan et al., 2022; Zhang et al., 2024; Wu et al., 2024). Thymol is a phenolic monoterpene with antioxidant, antimicrobial and anti-inflammatory properties, and has been studied as a natural feed additive to boost the poultry health (Gabbai-Armelin et al., 2022). The antioxidant and immunomodulatory effect of quercetin may decrease inflammatory cytokine response and oxidative damage in challenged poultry (Sun et al., 2022; Feng et al., 2023). What is noteworthy is that quercetin's direct effects on MG-related oxidative and inflammatory damage have also been demonstrated by modulating the TLR2/MyD88/NF- κ B pathway (Wang et al., 2023). Although significant progress has been made, little comparative data on the protection of phytogetic compound against oxidative stress and inflammatory and respiratory tissue damage induced by MG in broilers is available. Accordingly, in the present study, a model of experimental MG was challenged by supplementation with dietary curcumin, thymol and quercetin and their effects were assessed on growth performance, oxidative stress markers, inflammatory cytokines, MG burden and tracheal and pulmonary histopathology. We hypothesized that phytogetic supplementation would reduce the oxidative and inflammatory damage seen in MG and, as a result, will enhance the integrity of respiratory tissue and productive performance.

2. Materials and Methods

2.1. Birds, houses and experimental design

One-day-old male Ross 308 broiler chicks were obtained from a commercial hatchery and raised under normal broiler chick management conditions for 42 days. When the chicks arrived, each was put on a scale and divided into five experimental groups with six replicate pens

containing four chicks per pen. The growth performance variables were regarded as the experimental units of pens. The birds were kept throughout the experiment on fresh wood-shaving litter in an environmentally controlled house with free choice of feed and drinking water. The temperature and lighting were gradually changed based on the management recommendations for the Ross 308 broilers. The experimental treatments were non-challenged control (CON) with the basal diet, and *Mycoplasma gallisepticum* -challenged control (MG) fed the basal diet, and the birds were fed the basal diet with 200 mg/kg curcumin (MG+CUR), 200 mg/kg thymol (MG+THY), and 200 mg/kg quercetin (MG+QUE). The basal starter (days 1-21) and grower-finisher (days 22-42) diets were designed to provide the nutrient requirements of broiler chickens. The basal feed batch was used to prepare experimental feed diets to minimize variation in nutrient composition. The phytogetic compounds were first added to a small amount of basal diet to create a premix and then gradually added to the basal diet to ensure a uniform distribution in the different diets. The experimental diets did not contain any antibiotic growth promoters. All bird-related methods would be implemented with the relevant animal ethics committee at the institution having approved it and would conform to the institutional guidelines for caring and using animals.

2.2. Phytogetic compounds

The experimental phytogetic compounds used were curcumin, thymol and quercetin of analytical/feed-grade purity (95%+). Curcumin and Quercetin were directly added to their diets as pre-mix, while thymol was added to the diet after mixing it with a suitable carrier so that it is evenly distributed throughout the diet. Experimental diets were formulated weekly and kept under cool, dry conditions, away from direct light in sealed containers until used. The phytogetic supplementation started on day 1 and was continued during the 42-day experimental period. The concentrations were chosen to be biologically relevant and to allow direct comparisons between

the three phytogetic strategies that have antioxidant and anti-inflammatory activity.

2.3. *Mycoplasma gallisepticum* challenge and confirmation of infection

On the 21st day, birds from the four challenged treatments were subjected to a characterized strain of *M. gallisepticum* via an institutionally approved experiment-infection protocol. Control birds were handled in the same way but without exposure to MG. For non-challenged birds, biosecurity conditions were provided and challenged birds were kept separately; there was no cross-contamination between the two groups. Birds were observed for respiratory symptoms, such as coughing, sneezing, nasal discharge, respiratory difficulty, conjunctival changes, depression, and decrease in activity after challenge daily for five days. The infection status was confirmed afterwards by quantitative PCR (qPCR) specific for MG, using the samples collected from the trachea during post-challenge sampling. Any procedures involving propagation, preparation and administration of the challenge organism will be carried out in an appropriately equipped veterinary microbiology facility, under institutional biosafety and animal-welfare approval.

2.4. Growth performance

Body weight was recorded on a pen basis on days 1, 21, 28, 35, and 42. Feed offered and residual feed were kept for each pen for the respective periods. Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) were determined for days 1-21, 22-42 and 1-42. Weight gain of body was calculated as the difference between the final and initial body weight and FCR was calculated as feed intake / weight gain of the body. When necessary, feed intake and BWG were adjusted for mortalities. The daily mortality was noted during the experiment.

2.5. Clinical assessment

Responses to the clinical challenge with MG were assessed daily. Respiratory signs were evaluated by means of a semi-quantitative scoring system that was predetermined and was based on the clinical

severity of the signs. Respiratory symptoms such as sneezing, coughing, nasal discharge, difficulty breathing and depression were included in the assessment and severity of these symptoms. When possible, the clinical scoring was done on the assumption that the results of biochemical or molecular analysis were not known to the evaluator and the identity of the treatment was not known to the evaluator.

2.6. Sample collection

Biological samples were taken on days 28 and 42 from 2 birds in each replicate pen, who in each pen had bodyweights that most closely approached the pen mean. Blood was placed in sterile tubes and left to clot at room temperature, and then centrifuged at $3,000 \times g$ for 15 min at 4°C . Aliquots of serum were stored at -80°C for biochemical and cytokine analyses. Selected birds were humanely euthanized following institutional animal-welfare procedures at the final sampling. The tissues in the trachea and lungs were taken immediately. Samples for oxidative-stress analysis were rinsed with ice-cold physiological saline, snap frozen and stored at -80°C ; those to be used for molecular detection were collected aseptically in sterile containers; and tissues for histopathological examination were immediately placed in 10% neutral-buffered formalin.

2.7. Determination of oxidative-stress and antioxidant biomarkers

The oxidative status was determined in the serum and homogenates of lung tissue by measuring the levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH-Px) peroxidase. MDA was used as a marker of lipid peroxidation while SOD, CAT and GSH-Px were used to assess endogenous enzymatic antioxidant defense. Lung tissue was homogenized in phosphate buffered saline in an ice cold extraction buffer in a suitable ratio and centrifuged at a cold temperature. The supernatant was used for biochemical analysis. Protein concentration was determined to be able to normalize tissue antioxidant activity, if necessary. The concentration of MDA was measured via thiobarbituric acid-reactive

substances (TBARS), and the activities of SOD, CAT and GSH-Px were measured through colorimetric assay procedures validated. Analysis was performed in duplicate and all the treatment groups were split between analytical batches to minimize batch related bias.

2.8. Determination of inflammatory cytokines

Serum levels of interleukin- 1β (IL- 1β), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and interleukin-10 (IL-10) were used to assess the systemic inflammatory response. Chicken-specific sandwich enzyme-linked immunosorbent assays (ELISA) were used for the quantification of cytokines. All samples and standards were tested twice using the assay procedure. A microplate spectrophotometer was used to measure absorbance, and four-parameter logistic standard curves were used to calculate the concentrations of cytokines. pg/mL were used for the concentrations. Interleukin- 1β (IL- 1β), Interleukin-6 (IL-6) and Tumor necrosis factor- α (TNF- α) were selected to represent the inflammatory response associated with MG, while Interleukin-10 (IL-10) was included as a representative of the counter-regulatory anti-inflammatory response.

2.9. DNA extraction and quantitative detection of *M. gallisepticum*

The genomic DNA was isolated from tracheal samples with silica column DNA extraction kit. Prior to the amplification, DNA concentration and purity were determined spectrophotometrically. A validated species-specific qPCR assay was used to quantify MG DNA. Positive controls, negative extraction controls, and no-template controls were used with each amplification run. Amplification specificity was confirmed per the requirements of the assay and analyses were performed in duplicate. The abundance of MG was normalized with the amount of biological material analysed and presented as log-transformed MG DNA copies per unit of sample. In addition to modulating host oxidative and inflammatory responses, the molecular data were used to see if phytogetic

supplementation also affected the pathogen burden.

2.10. Histopathological examination

The ends of the trachea and lungs were fixed in 10% neutral buffered formalin, processed in graded ethanol, cleared, embedded in paraffin, sectioned at about 4 μ m and stained with hematoxylin and eosin. The sections of the trachea were analysed for epithelial degeneration, epithelial desquamation, thickening of the mucosa, congestion and infiltration of inflammatory cells. The lung sections were examined for congestion, pulmonary oedema, inflammatory infiltration and abnormality of the normal pulmonary architecture. Histopathological lesions were evaluated on a semi-quantitative basis from 0 to 4, 0 being no visible lesions, 1 being minimal lesions, 2 mild lesions, 3 moderate lesions and 4 severe tissue damage. Each section was analysed from a number of non-overlapping microscopic fields, and the mean lesion score was determined for each bird sampled. The evaluator blinded to treatment allocation performed histological evaluation. The same magnification and image-acquisition parameters were used for the same number of representative images in each treatment group.

2.11. Statistical analysis

All analyses were conducted in R (version 4.5.2) with appropriate linear/mixed-effects models, Tukey adjusted post-hoc comparisons and nonparametric analysis for ordinal outcomes. All values are given as mean $\pm$ SEM or 95% CI; correlations were analyzed by Spearman's method and P value < 0.05 was used for statistical significance.

3. Results

3.1. Phytogetic supplementation improved growth performance following *M. gallisepticum* challenge

The initial weights of all the experimental groups were similar (Table 1) which resulted in comparable initial body weight conditions. BWs in the MG group were also significantly lower than in the non-challenged group ($P < 0.001$) after *Mycoplasma gallisepticum* (MG) challenge. The final body weight (BW) and body-weight gain (BWG) of the MG group were also significantly reduced in comparison to the non-challenged control group ($P < 0.001$) after challenge with *Mycoplasma gallisepticum* (MG). In addition, the MG challenge was related to a decreased feed consumption and increased FCR. The adverse effects of MG challenge were partially mitigated by curcumin, thymol, or quercetin supplementation. Compared with the MG group, all the phytogetic treated groups showed significantly higher final body weight, body-weight gain and lower FCR. The MG+QUE group had the highest numerical gains in final body weight and FCR compared to the other two phytogetic groups, but there was no statistical difference found between phytogetic groups. Figure 1 depicts the time course of changes in body weight. In all groups, body weight increased gradually over the period of the experiment but in the MG-challenged birds, a significant difference from the non-challenged control was found after challenge. The phytogetic groups had higher body weights than the untreated group (MG) throughout the post challenge phase

Table 1. Effects of phytogetic supplementation on growth performance of broilers challenged with *Mycoplasma gallisepticum*

Parameter	CON	MG	MG+CUR	MG+THY	MG+QUE	P-value
Initial BW (g)	44.8 $\pm$ 0.6	44.7 $\pm$ 0.7	44.9 $\pm$ 0.6	44.6 $\pm$ 0.7	44.8 $\pm$ 0.6	0.982
Final BW (g)	2386 $\pm$ 38 ^a	2118 $\pm$ 42 ^c	2264 $\pm$ 35 ^b	2281 $\pm$ 39 ^b	2310 $\pm$ 37 ^b	<0.001
BW gain (g)	2341 $\pm$ 38 ^a	2073 $\pm$ 42 ^c	2219 $\pm$ 35 ^b	2237 $\pm$ 39 ^b	2265 $\pm$ 37 ^b	<0.001
Feed intake (g)	3524 $\pm$ 46 ^a	3328 $\pm$ 51 ^b	3439 $\pm$ 48 ^{ab}	3452 $\pm$ 44 ^{ab}	3471 $\pm$ 47 ^{ab}	0.018
FCR	1.51 $\pm$ 0.03 ^a	1.61 $\pm$ 0.04 ^c	1.55 $\pm$ 0.03 ^{ab}	1.54 $\pm$ 0.03 ^{ab}	1.53 $\pm$ 0.03 ^{ab}	0.021
Mortality (%)	0	8.3	4.2	4.2	4.2	0.214

Values are presented as mean $\pm$ SEM. Different superscript letters within a row indicate significant differences following Tukey adjustment ($P < 0.05$). CON, non-challenged control; MG, MG-challenged control; CUR, curcumin; THY, thymol; QUE, quercetin; BW, body weight; BWG, body-weight gain; FCR, feed conversion ratio.

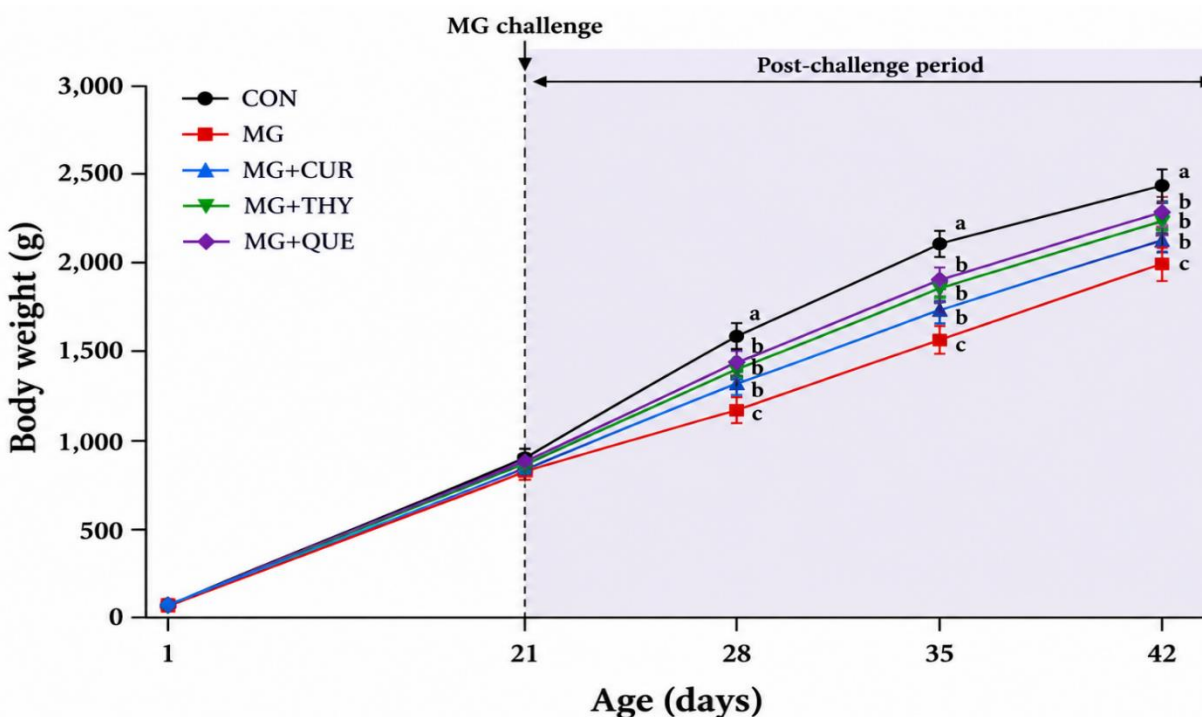


Figure 1. Growth trajectory of broilers subjected to *M. gallisepticum* challenge and phyto-genic supplementation.

3.2. Phyto-genic supplementation attenuated MG-associated oxidative stress

MG challenge resulted in a significant change in systemic and tissue oxidative status (Table 2; Figure 2). MDA concentration was found to be significantly elevated in MG-challenged birds, while the activities of SOD, CAT and GSH-Px were significantly decreased ($P < 0.001$ for all comparisons) compared to the CON group. The level of increase of MDA was about 44% in the MG group in comparison to non-challenged control group, showing significant lipid peroxidation after MG challenge. Both phyto-genic interventions were effective in inhibiting oxidative modifications associated with MG. All three

phyto-genic interventions significantly inhibited the MG associated oxidative modifications. The concentrations of MDA were found to be lower in MG+CUR, MG+THY and MG+QUE groups when compared to the untreated MG group; however, the activities of SOD, CAT and GSH-Px were significantly elevated. The lowest concentration of MDA was observed in Quercetin group and the highest numerical increase in SOD and GSH-Px activity was observed in Curcumin group among the challenged groups. However, it was not significant for most oxidative-stress endpoints in the differences among the three phyto-genic treatments.

Table 2. Effects of phytogetic supplementation on oxidative-stress and antioxidant biomarkers in MG-challenged broilers

Biomarker	CON	MG	MG+CUR	MG+THY	MG+QUE	P-value
MDA (nmol/mL)	2.84 ± 0.12 ^a	4.09 ± 0.16 ^c	3.31 ± 0.13 ^b	3.38 ± 0.14 ^b	3.20 ± 0.12 ^b	<0.001
SOD (U/mL)	148.2 ± 4.8 ^a	112.6 ± 4.5 ^c	137.4 ± 4.2 ^b	134.8 ± 4.6 ^b	139.1 ± 4.4 ^b	<0.001
CAT (U/mL)	61.4 ± 2.1 ^a	45.7 ± 1.9 ^c	56.8 ± 2.0 ^b	55.9 ± 1.8 ^b	57.6 ± 2.0 ^b	<0.001
GSH-Px (U/mL)	82.6 ± 2.9 ^a	61.3 ± 2.6 ^c	76.8 ± 2.7 ^b	74.9 ± 2.5 ^b	77.5 ± 2.8 ^b	<0.001

Values are presented as mean ± SEM. Different superscript letters indicate significant differences following Tukey adjustment ($P < 0.05$). MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase.

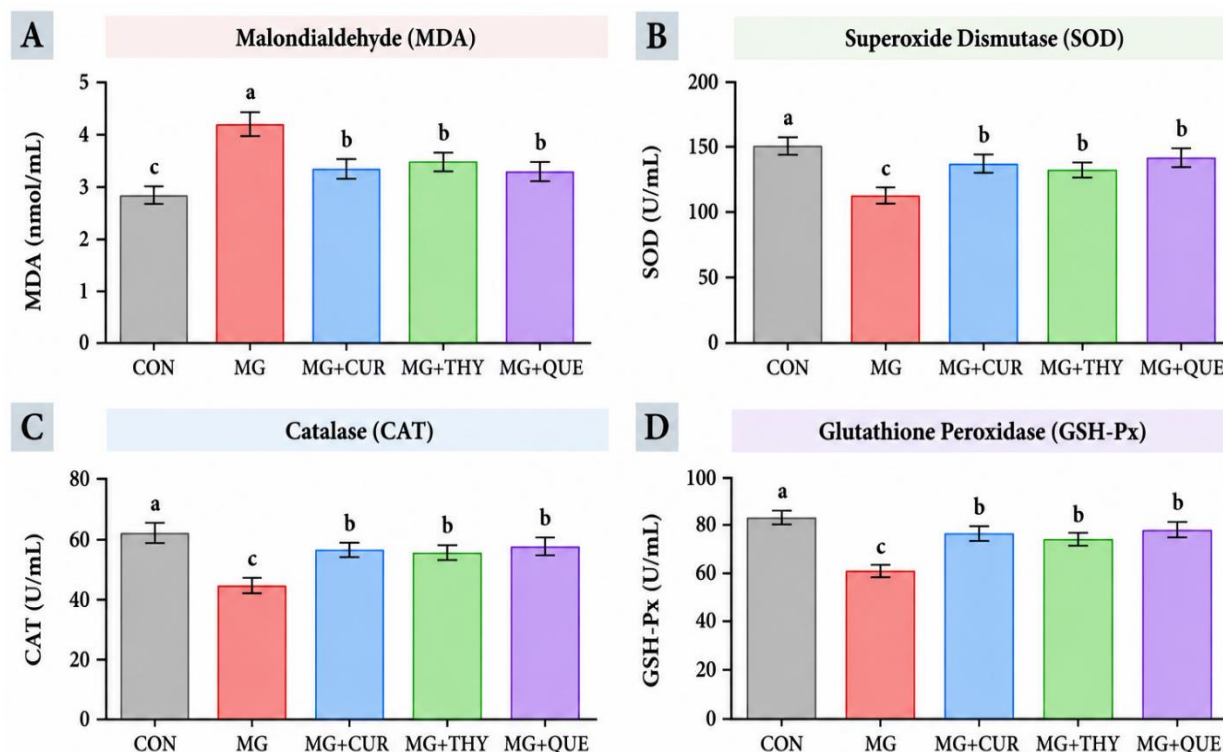


Figure 2. Effects of phytogetic supplementation on oxidative-stress and antioxidant biomarkers.

3.3. Phytogetic compounds modulated the inflammatory response to MG challenge

The pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α were significantly elevated in the MG group relative to CON, with the greatest increase seen for IL-6 (Figure 3). The concentrations of IL-1 β , IL-6 and TNF- α rose by about 68%, 74% and 61%, respectively, after MG challenge ($P < 0.001$). All 3 pro-inflammatory cytokines were significantly lower in phytogetic supplemented animals compared to MG group. Both curcumin and quercetin resulted in similar reductions in IL-

1 β and TNF- α and thymol resulted in a slightly higher numerical reduction in IL-6. However, no differences were observed between the three phytogetic treatments despite the numerical differences. Concentrations of IL-10 were decreased in the MG group compared to the CON group ($P = 0.004$), however. The supplementation of curcumin, thymol and quercetin resulted in higher levels of IL-10 than the untreated MG group, the highest being in the quercetin treated birds.

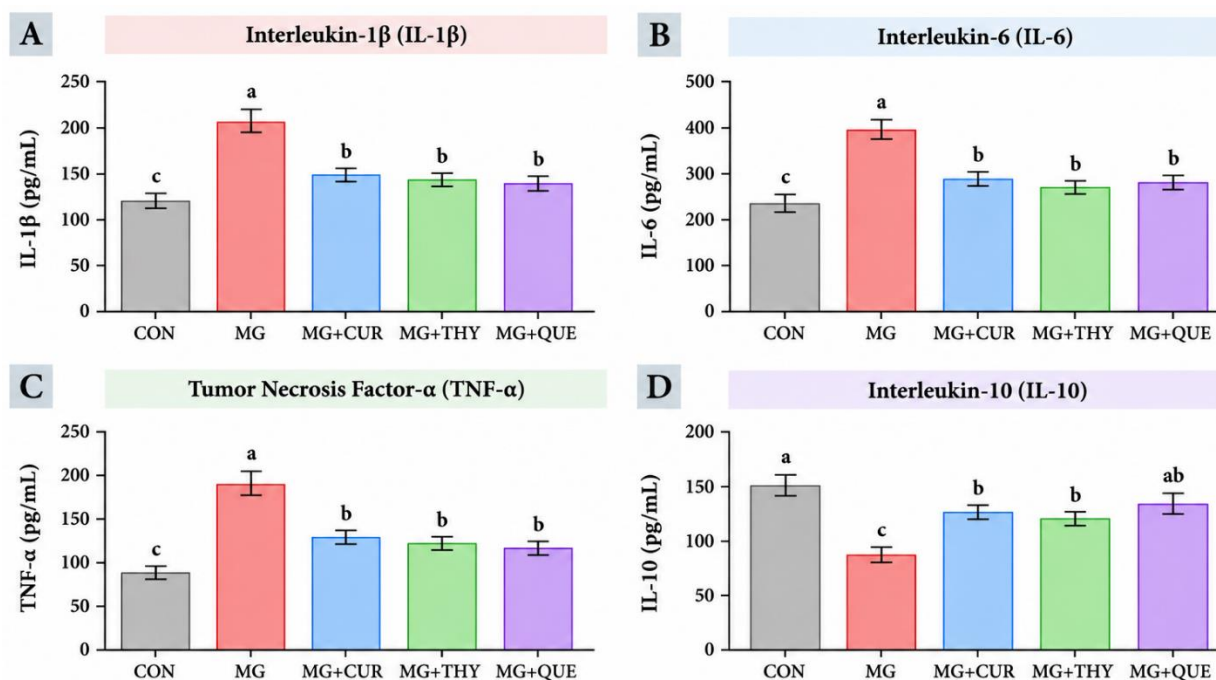


Figure 3. Modulation of inflammatory cytokines by phytogetic supplementation in MG-challenged broilers. (A) IL-1 β , (B) IL-6, (C) TNF- α , and (D) IL-10. Values are presented as mean $\pm$ SEM. Different letters indicate significant differences among treatments ($P < 0.05$).

3.4. Phytogetic supplementation reduced MG-associated respiratory injury

Table 3 shows the presence of MG DNA in the challenged birds, whereas no MG DNA was detected in the non-challenged control group, using specific qPCR. Birds treated with curcumin, thymol and quercetin showed lower MG burden compared to the untreated MG group but the decrease was significant only in MG+QUE group ($P = 0.018$). There was a correlation with the severity of the clinical and histopathological findings for the MG challenge. Birds in the MG group had significantly higher clinical scores (respiratory) and lesion scores (trachea and lungs) than the CON group. Histologically, there was

disruption of the epithelium, inflammatory-cell infiltration, mucosal thickening, pulmonary congestion and inflammatory changes in MG-challenged birds. There was a significant difference between the clinical and histopathological severity of phytogetic supplementation group and MG group. Quercetin and curcumin followed with the lowest tracheal and lung lesion scores followed by thymol. Comparatively good preservation of the respiratory tissue architecture was seen in phytogetic treated birds as compared to untreated birds challenged with MG during representative histological examination (Figure 4).

Table 3. Effects of phytogetic supplementation on MG burden, clinical response, and respiratory histopathology

Parameter	CON	MG	MG+CUR	MG+THY	MG+QUE	P-value
MG load (log10 copies)	ND	5.84 $\pm$ 0.21 ^a	5.31 $\pm$ 0.19 ^{ab}	5.24 $\pm$ 0.18 ^{ab}	4.96 $\pm$ 0.17 ^b	0.018
Clinical score	0.12 $\pm$ 0.05 ^c	2.41 $\pm$ 0.18 ^a	1.48 $\pm$ 0.14 ^b	1.39 $\pm$ 0.13 ^b	1.21 $\pm$ 0.12 ^b	<0.001

Tracheal lesion score	0.18 ± 0.06 ^c	2.87 ± 0.19 ^a	1.72 ± 0.15 ^b	1.65 ± 0.16 ^b	1.43 ± 0.14 ^b	<0.001
Lung lesion score	0.15 ± 0.05 ^c	2.54 ± 0.17 ^a	1.61 ± 0.14 ^b	1.56 ± 0.15 ^b	1.32 ± 0.13 ^b	<0.001

Values are presented as mean ± SEM. Different superscript letters indicate significant differences following Tukey-adjusted comparisons ($P < 0.05$). ND, not detected.

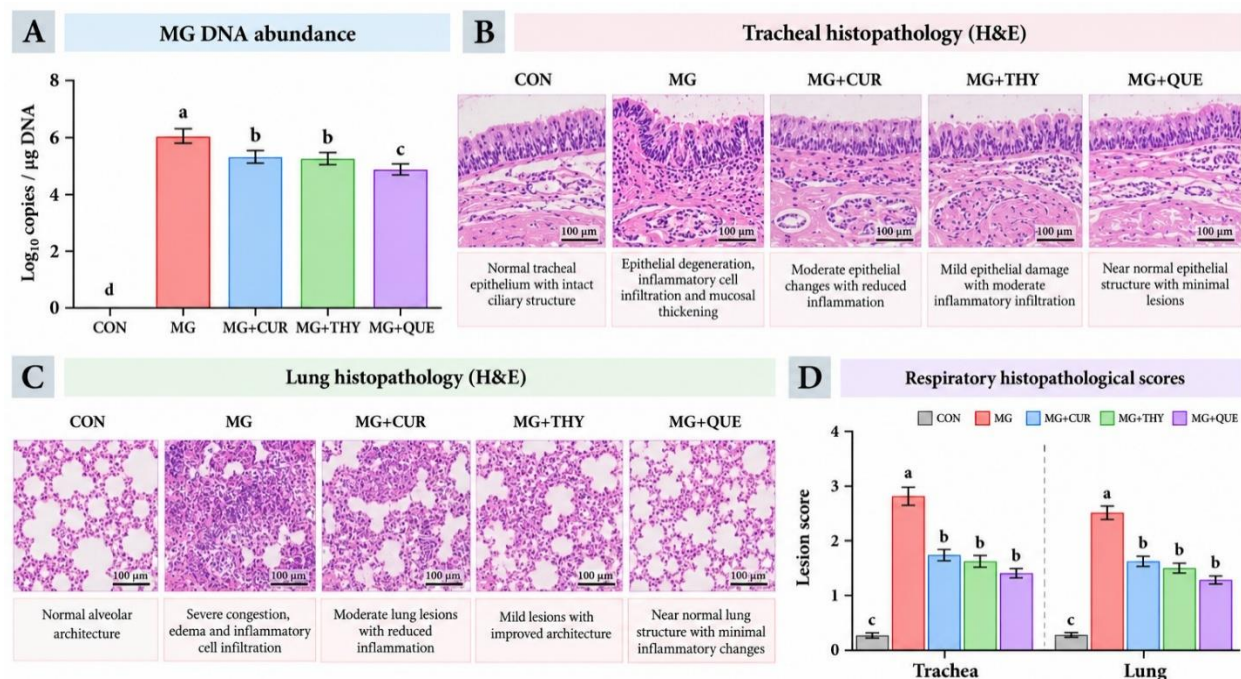


Figure 4. Effects of phytogetic supplementation on MG-associated respiratory injury.

(A) MG burden determined by qPCR; (B) representative tracheal sections; (C) representative lung sections; and (D) quantitative histopathological scores. Histological images were obtained at identical magnification. Scale bar = 100 µm.

3.5. Associations among oxidative stress, inflammation, MG burden, and tissue injury

Exploratory correlation analysis revealed positive correlation between MG burden and severity of the histopathological examination, MDA concentration, and inflammatory cytokine concentrations, while antioxidant enzyme activities were inversely correlated with MDA and inflammatory cytokine concentrations. MDA had positive correlation with IL-1 β , IL-6 and TNF- α and negative correlation with SOD and GSH-Px. The highest correlation was found between the MDA concentration and the tracheal lesion score,

which indicates the higher the oxidative damage the greater the tissue damage to the respiratory tract. These relationships were interpreted as associations and not evidence of a causal relationship.

4. Discussion

The results of the present study suggest that MG challenge resulted in a coordinated decrease in growth parameters, redox balance, inflammatory balance, pathogen burden, and respiratory tissue integrity, which were partly ameliorated by the supplementation of curcumin, thymol, and quercetin in the diet. Losing of final body weight, body weight gain and feed conversion in MG challenged birds is consistent with systemic metabolic cost associated with chronic respiratory disease. In the current study, the reduced productive performance observed was related to the alteration of tissue injury, energy metabolism,

oxidative imbalance and pulmonary inflammation, which have been reported earlier to be associated with MG infection (Ishfaq et al., 2020; Hu et al., 2021). Nutrient interventions were also shown to have an impact on MG infection, with similar performance and health impairments reported in experimentally infected broilers fed phytogetic supplements, which encourages the application of nutritional measures to reduce production losses caused by MG infection (Hashem et al., 2022). One of the main findings was the significant oxidative imbalance after MG challenge, as reflected in the increased activity of MDA and decreased activity of SOD, CAT and GSH-Px. This pattern indicates a greater amount of lipid peroxidation with reduced endogenous antioxidants. Both MG infection and vaccination exposure have been shown to cause measurable changes in systemic oxidative-status biomarkers, and previous investigations showed that MG infection could cause oxidative stress and apoptosis in immune tissues (Hu et al., 2021; Iemmi et al., 2023). The present results support the idea that oxidative injury is a secondary effect of the MG infection and that it may be a significant part of MG-induced tissue damage. All three phytogetic compounds were able to improve antioxidant profile, having lower MDA and higher levels of SOD, CAT and GSH-Px than MG group. This is in line with poultry literature showing positive effects of plant-based feed additives on antioxidant defenses and oxidative damage, though effects may differ depending on feed additive, dose, formulation, tissue, and duration of supplementation (Righi et al., 2021; Pitino et al., 2021; Abdelli et al., 2021). Curcumin exhibits especially high experimental evidence of antioxidant pathway regulating activity in poultry. In recent years, the addition of curcumin to broiler diets has been demonstrated to decrease the levels of MDA and inflammatory mediators and increase antioxidant defenses, partly via Nrf2/ARE pathways (Wu et al., 2024; Zhang et al., 2024). The inflammatory findings further support an integrated oxidative-inflammatory mechanism. The increase in IL-1 β , IL-6, and TNF- α , and the decrease in IL-10 resulted in a shift toward a pro-inflammatory phenotype in MG challenge.

Importantly, the phytogetic treatments tempered these responses. This observation is consistent with the evidence that MG stimulates innate inflammatory signaling and phytogetic interventions have anti-inflammatory effects in dampening unnecessary inflammatory responses. In particular, the work on the experimental models of MG has revealed the ability of quercetin to modulate directly the oxidative stress and the responses of IL-1, IL-6 and TNF- α , associated with pro-inflammatory mechanisms, and to enhance the responses of IL-10, associated with anti-inflammatory mechanisms, by inhibiting the TLR2/MyD88/NF- κ B pathway (Wang et al., 2023). Other studies of poultry, which are independent of the current study, also indicate that quercetin can reduce inflammatory damage and oxidative stress and enhance antioxidant activity and intestinal barrier function (Feng et al., 2023). The results of the improvement in respiratory pathology and decrease in MG DNA abundance give an additional dimension to the results. The phytogetic compounds were not only systemic antioxidants, they also correlated with decreased pathogen burden and decreased tracheal and pulmonary lesion scores. This is especially true of quercetin, which has been recently found to decrease MG colonization and restore respiratory mucosal barrier function by regulating immunological responses, such as macrophage polarization and promoting Th2 response (Wang et al., 2024). Likewise, an essential-oil-based plant medicine has been shown to enhance clinical symptoms, immune responses, bacterial counts and production of broilers with MG (Hashem et al., 2022). Thymol also has established antioxidant, antimicrobial and anti-inflammatory properties, which may explain its protective effects in the current model (Gabbai-Armelin et al., 2022). In summary, these results define a multifactorial protective model, where phytogetic compounds act on different mechanisms in parallel: oxidative stress, inflammatory signaling, damage by pathogens and damage to the respiratory tissue. The significance of such multimodal activity is important, since the use of phytogetic feed additives is considered an ideal alternative to antibiotics and is being

increasingly discussed as such rather than merely as an antimicrobial agent (Wang et al., 2024). Recent studies also suggest that formulation and bioavailability are significant factors affecting their efficacy, and new technologies like nanoformulation have shown potential to improve their antioxidant and immunomodulatory properties (Abdel-Moneim et al., 2025; Wang et al., 2025). A significant restriction is that the current data set does not directly define the molecular pathways that cause these effects. While the Nrf2/ARE and TLR2/MyD88/NF- κ B signaling pathways are biologically plausible candidates, pathway-specific measurements would be required to confirm the mechanisms. Combining transcriptomics or targeted qPCR/protein analysis, respiratory microbiota profiling, dose-response experiments, and pharmacokinetic assessment should therefore be considered in future studies. There is also recent systematic evidence suggesting that quercetin responses might be dose-dependent and hence, an optimal level of inclusion of quercetin needs to be optimized, not assuming one optimal dose for all purposes (Hartanto et al., 2026). Based on the data presented, it is inferred that curcumin and thymol and the present data set the best effect in combination quercetin can mitigate a number of pathological outcomes of the MG challenge. The combined effect of antioxidant, anti-inflammatory, tissue-protective and pathogen-associated responses gives a compelling argument for further controlled testing of phytogetic products as nutritional interventions in respiratory disease in poultry associated with MG.

Conclusion

Phytogetic supplementation had a beneficial effect on the main physiological responses to *M. gallisepticum* challenge in broiler chickens. Curcumin, thymol and quercetin positively influenced growth performance, lipid peroxidation, antioxidant status, inflammatory cytokine response, and tissue injury in the respiratory tract. The simultaneous effect on all four types of endpoints, oxidative, inflammatory, pathological and pathogen, indicates a wide spectrum of protective properties for these

compounds. In the current database, quercetin elicited the highest number of positive responses in various outcomes. Based on these findings, more research is needed on the optimization of dose and further mechanistic studies in order to confirm the use of phytogetic compounds as a viable nutritional approach to control respiratory disease associated with MG in poultry.

REFERENCES

- Abdel-Moneim, A. M. E., Mesalam, N. M., Yang, B., & Elsadek, M. F. (2025). Dietary incorporation of biological curcumin nanoparticles improved growth performance, ileal architecture, antioxidative status, serum lipid profile, and humoral immune response of heat-stressed broiler chickens. *Poultry science*, 104(2), 104740.
- Abdelli, N., Solà-Oriol, D., & Pérez, J. F. (2021). Phytogetic feed additives in poultry: achievements, prospective and challenges. *Animals*, 11(12), 3471.
- Feng, J., Li, Z., Ma, H., Yue, Y., Hao, K., Li, J., ... & Min, Y. (2023). Quercetin alleviates intestinal inflammation and improves intestinal functions via modulating gut microbiota composition in LPS-challenged laying hens. *Poultry Science*, 102(3), 102433.
- Flees, J. J., Ganguly, B., & Dridi, S. (2021). Phytogetic feed additives improve broiler feed efficiency via modulation of intermediary lipid and protein metabolism-related signaling pathways. *Poultry science*, 100(3), 100963.
- Gabbai-Armelin, P. R., Sales, L. S., Ferrisse, T. M., De Oliveira, A. B., De Oliveira, J. R., Giro, E. M. A., & Brighenti, F. L. (2022). A systematic review and meta-analysis of the effect of thymol as an anti-inflammatory and wound healing agent: A review of thymol effect on inflammation and wound healing. *Phytotherapy research*, 36(9), 3415-3443.

- Hashem, Y. M., Abd El-Hamid, M. I., Awad, N. F., Ibrahim, D., Elshater, N. S., El-Malt, R. M., ... & Elnahriry, S. S. (2022). Insights into growth-promoting, anti-inflammatory, immunostimulant, and antibacterial activities of Toldin CRD as a novel phytobiotic in broiler chickens experimentally infected with *Mycoplasma gallisepticum*. *Poultry Science*, 101(11), 102154.
- Hartanto, S., Wardono, H. P., Kurnianto, H., Hantoro, F. R. P., Prasetyo, A., Haryanto, B., ... & Sugiharto, S. (2026). Dose-dependent effects of dietary quercetin on performance, egg quality, metabolic health, and antioxidant defense in laying hens: A systematic review and meta-analysis. *Veterinary World*, 19(1), 149.
- Hu, W., Zhang, W., Shah, S. W. A., Ishfaq, M., & Li, J. (2021). *Mycoplasma gallisepticum* infection triggered histopathological changes, oxidative stress and apoptosis in chicken thymus and spleen. *Developmental & Comparative Immunology*, 114, 103832.
- Iemmi, T., Basini, G., Ramoni, R., Bussolati, S., Heredero, A. C., Grasselli, F., ... & Bertini, S. (2023). Research Note: Oxidative stress and immune response following the administration of live attenuated *Mycoplasma gallisepticum* vaccination in backyard chicken. *Poultry Science*, 102(2), 102400.
- Ishfaq, M., Zhang, W., Shah, S. W. A., Wu, Z., Wang, J., Ding, L., & Li, J. (2020). The effect of *Mycoplasma gallisepticum* infection on energy metabolism in chicken lungs: through oxidative stress and inflammation. *Microbial Pathogenesis*, 138, 103848.
- Pitino, R., De Marchi, M., Manuelian, C. L., Johnson, M., Simoni, M., Righi, F., & Tsiplakou, E. (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on yield, quality, and oxidative status of poultry products: A review of the literature of the last 20 years. *Antioxidants*, 10(5), 757.
- Righi, F., Pitino, R., Manuelian, C. L., Simoni, M., Quarantelli, A., De Marchi, M., & Tsiplakou, E. (2021). Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on poultry performances, health, and oxidative status: A review of the literature in the last 20 years. *Antioxidants*, 10(5), 659.
- Ruan, D., Wu, S., Fouad, A. M., Zhu, Y., Huang, W., Chen, Z., ... & Jiang, S. (2022). Curcumin alleviates LPS-induced intestinal homeostatic imbalance through reshaping gut microbiota structure and regulating group 3 innate lymphoid cells in chickens. *Food & Function*, 13(22), 11811-11824.
- Wang, J., Deng, L., Chen, M., Che, Y., Li, L., Zhu, L., ... & Feng, T. (2024). Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. *Animal Nutrition*, 17, 244-264.
- Wang, S., Jin, X., Chen, H., Han, M., Bao, J., Niu, D., ... & Li, J. (2023). Quercetin alleviates *Mycoplasma gallisepticum*-induced inflammatory damage and oxidative stress through inhibition of TLR2/MyD88/NF- κ B pathway in vivo and in vitro. *Microbial pathogenesis*, 176, 106006.
- Wang, S., Guo, L., Gu, F., Bao, J., Guo, Y., Zhang, Y., ... & Li, J. (2024). Quercetin restores respiratory mucosal barrier dysfunction in *Mycoplasma gallisepticum*-infected-infected chicks by enhancing Th2 immune response. *PHYTOMEDICINE*, 133.
- Wu, F., Zhao, M., Tang, Z., Wang, F., Han, S., Liu, S., & Chen, B. (2024). Curcumin alleviates cecal oxidative injury in diquat-induced broilers by regulating the Nrf2/ARE pathway and microflora. *Poultry Science*, 103(5), 103651.
- Zhang, J., Zhang, R., Jin, S., & Feng, X. (2024). Curcumin, a plant polyphenol with multiple physiological functions of improving antioxidation, anti-inflammation, immunomodulation and its application in poultry production. *Journal of Animal Physiology and Animal Nutrition*, 108(6), 1890-1905.

- Behboodi, H. R., Samadi, F., Riasi, A., Najafi, M., Ansari, M., & Ebadi, M. (2024). A comparative study between curcumin and curcumin nanoparticles on reproductive performance and antioxidant system of aged roosters. *Poultry science*, 103(10), 104030.
- Khairunnesa, M., Kumar, A., Wu, S. B., Akter, S., Barekatin, R., Palanisamy, K., & Naseri, K. G. (2026). Phytogenic feed additive mitigates necrotic enteritis-associated gut damage and performance loss in broilers. *Poultry Science*, 106592.
- Biswas, S., Ahn, J. M., & Kim, I. H. (2024). Assessing the potential of phytogenic feed additives: A comprehensive review on their effectiveness as a potent dietary enhancement for nonruminant in swine and poultry. *Journal of animal physiology and animal nutrition*, 108(3), 711-723.
- Chaidez-Ibarra, M. A., Velazquez, D. Z., Enriquez-Verdugo, I., Castro Del Campo, N., Rodriguez-Gaxiola, M. A., Montero-Pardo, A., ... & Gaxiola, S. M. (2022). Pooled molecular occurrence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in poultry: A systematic review and meta-analysis. *Transboundary and emerging diseases*, 69(5), 2499-2511.
- El-Sabrou, K., Khalifah, A., & Mishra, B. (2023). Application of botanical products as nutraceutical feed additives for improving poultry health and production. *Veterinary world*, 16(2), 369.
- Feberwee, A., de Wit, S., & Dijkman, R. (2022). Clinical expression, epidemiology, and monitoring of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*: an update. *Avian pathology*, 51(1), 2-18.
- Sun, L., Guo, L., Xu, G., Li, Z., Appiah, M. O., Yang, L., & Lu, W. (2022). Quercetin reduces inflammation and protects gut microbiota in broilers. *Molecules*, 27(10), 3269.
- Wu, S., Wang, M., Yang, X., Zhao, L., Lan, Z., & Sun, S. (2024). Research progress in the development of vaccines against *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Microorganisms*, 12(8), 1699.