Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 7, 1227-1233

In vivo and *in vitro* assessment of the anti-*mycoplasma* activity of curcumin nanoparticles and their impact on health and performance of broiler chickens

Mohamed Shakal[1], Fady S. Youssef[2], Gehad G. Mohamed[3,4], Sameh H. Ismail[5], Amira M. Qoraa[1], Heba M. Salem[1,6*]

[1]*Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, 12211, Giza, Egypt.*
[2]*Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.*
[3]*Chemistry Department, Faculty of Science, Cairo University, 12613 Giza, Egypt.*
[4]*Nanoscience Department, Basic and Applied Sciences Institute, Egypt-Japan University of Science and Technology, New Borg El Arab, Alexandria, 21934, Egypt.*
[5]*Nanotechnology for Postgraduate Studies - Cairo University- Sheikh Zayed Branch Campus, Sheikh Zayed City, Giza PO 12588, Egypt.*
[6]*Department of Diseases of Birds, Rabbits, Fish & their Care & Wildlife, School of Veterinary Medicine, Badr University in Cairo (BUC), Badr City, Cairo, Egypt.*

ARTICLE INFO

Recieved: 03 July 2024

Accepted: 01 October 2024

*Correspondence:

Corresponding author: Heba M. Salem
E-mail address: dr.hebasalem@cu.edu.eg

Keywords:

Avian mycoplasmosis, AST, ALT, Curcumin-NPs, Tilmicosin, FCR, MIC, MDA, Cholesterol.

ABSTRACT

Globally, the poultry production industry is growing at a rapid pace. *Mycoplasma* is a disease that causes an enormous financial loss to the poultry farming industry. Lately, there have been reports of avian mycoplasmosis resistance to multiple antibiotics especially macrolides. Thus, the purpose of this work was to assess the anti-bacterial activity of curcumin nanoparticles (curcumin-NPs) against *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) *in vitro* and *In vivo* as a possible alternative for antibiotics. *In vitro* investigations were used to determine the curcumin-NPs' minimum inhibitory concentration (MIC) versus MG and MS. To conduct *In vivo* research, 216 birds were divided into nine groups, each consisting of 24 birds in triplicate and 8 birds apiece. The groups were as follows: G1 was given an MG challenge; G2 received an MG challenge and 0.5% curcum-in-NPs; G3 received an MG challenge and 1% curcumin-NPs; G4 MG challenged and treated with tilmicosin, G5 challenged with MS; G6 infected with MS and supplied with 0.5% curcumin-NPs; G7 infected with MS and supplied with 1% curcumin-NPs; G8 received an MS challenge and treated with tilmicosin and G9 were the control negative group. The conclusion is that curcumin-NPs demonstrated *in vitro* anti-*Mycoplasma* activities; adding 1% curcumin-NPs to the drinking water for five days was a much more potent treatment than adding 0.5% curcumin-NPs and tilmicosin for the control of MG and MS infections in broiler chickens; the treated birds showed improved lipid profiles, better FCR, body weight gain, and a noticeable decrease in the sternness of clinical manifestations as well as lesions score. Additionally, a notable enhancement in renal function (urea & creatinine), hepatic enzymes (ALT & AST), and antioxidant status (Catalase, GSH, and MDA). There has also been a notable advancement in lipid profile. For five days, the use of 1% curcumin-NPs in the drinking water is advised as a secure and efficient treatment for avian mycoplasmosis in broiler chickens.

Introduction

Avian mycoplasmosis is a significant risk that reveals severe economic losses in the global avian industry (Marouf *et al*., 2022; Shakal *et al*., 2024a). *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) are the main bacterial pathogens that scare the poultry business decreased growth rate, lower quality of day-old chicks, more expenses for eradication operations, such as site cleaning and depopulation, and higher expenditures for medicine and vaccine (Emam *et al*., 2020; Yadav *et al*., 2022). Infections with MG are commonly referred to as infectious sinusitis in turkeys and chronic respiratory diseases in chickens (Qoraa *et al*., 2023a). It is described by coughing, nasal secretions, respiratory rales, and, oftentimes, sinusitis in turkeys (Limpavithayakul *et al*., 2023). MS infection is commonly referred to as infectious synovitis, this infectious disease affects the synovial membranes of joints as well as tendon sheaths and can range from acute to chronic (Khalifa *et al*., 2013). But in recent years, MS has been linked more often to airsacculitis in hens and occasionally in turkeys rather than synovitis (Yadav *et al*., 2022; Wang *et al*., 2022). Considering *Mycoplasma*s are hard to isolate and MIC tests take a while to provide results, most antimicrobial drugs administered to animals are usually empirical rather than based on hard evidence on susceptibility (Giguère *et al*., 2013; Qoraa *et al*., 2023a,b). Using medicines can be a quick and potent way to minimize financial losses by reducing clinical symptoms and egg transmission (Bastamy *et al*., 2022). For a very long time, flocks of chickens have been using macrolides (tilmicosin, tylosin) as a regular treatment for respiratory disorders related to MS and MG (Awad *et al*., 2022). Veterinarians usually neglect the side effects of synthetic medications and use the macrolides continuously and a prophylactic's dose when prescribing treatment for avian mycoplasmosis; an additional concern is the development of drug resistance to presently available drugs (Grózner *et al*., 2016; Emam *et al*., 2020; Salem *et al*., 2023). Therefore, it is still crucial to monitor MICs in *Mycoplasma*s to detect the development of anti-*Mycoplasma* drug resistance caused by improper use of antimicrobial medications (Bottinelli *et al*., 2022). Therefore, to overcome *Mycoplasma* resistance and reduce the high cost of antimicrobials, it became more imperative to employ novel and alternative treatments (Erfan and Marouf, 2019; Abd El-Hack *et al*., 2022). In the past ten years, the commercial poultry industry has seen a significant transformation thanks to the application of cutting-edge technical solutions and an impressive scientific methodology. In preclinical research, novel nanoparticles (NPs) have many advantages over conventional preparations: they are more stable and soluble, have a high surface area to volume ratio, are physically and chemically active, improve tissue penetration and even allow them to cross cell membranes, improve tissue targeting, and have fewer side effects (Swain *et al*., 2016; Abd El-Ghany *et al*., 2021; Salem *et al*., 2021). *Curcuma longa*, sometimes known as turmeric, is commonly herb utilized in traditional medicine and used as a spice, coloring, and seasoning in a variety of Indian food (El-Saadony *et al*., 2023). Curcuminoids are yellowish turmeric pigments that have a wide range of physiological properties, including anti-oxidative, anti-inflammatory, anti-carcinogenic anti-hepatotoxic, anti-microbial against most gram-positive and gram-negative bacteria, antacid, radioprotective, and hypocholesterolemic effects (El-Saadony *et al*., 2023). Curcumin, bisdemethoxycurcumin, and demethoxycurcumin are three examples of these pigments (Cousins *et al*., 2007; Attia *et al*., 2017; Gernat *et al*., 2021). Although curcumin-NPs were supplemented, the FBW rose and the FCR enhanced (Fathi *et al*., 2024). Curcumin powder's bioavailability is increased by nanoparticles, which have numerous uses in the fields of nutrition and medicine (Rai *et al*., 2015). Also, curcumin-NPs considerably raise titers of avian influenza and ND in broilers (Rajput *et al*., 2013). Also, it had a profoundly favorable effect on lowering total cholesterol, blood triglyceride, LDL-C, and boosting blood HDL-C levels in chickens (Kermanshahi and Riasi, 2006). Additionally, curcumin-NPs decreases lipid peroxidation and enhances the body's natural production of digestive enzymes (Toghyani *et al*., 2011). Through its impact on the expression of genes linked to lipolysis and lipogenesis, curcumin supplementation lowers the accumulation of fat in the abdomen and raises thyroid hormone concentrations (Xie *et al*., 2019). Curcumin demonstrated a significant role in reducing oxidative stress and contributing to the improvement of renal function by reducing damage to renal tissue (Wu *et al*., 2017). Thus, the purpose of this study was to assess curcumin-NPs' antibacterial, antioxidant, and anti-inflammatory properties both *In vivo* and *in vitro* against MS and MG additionally, it was intended to investigate the impacts of curcumin-NPs on the broiler chickens' performance, anti-inflammatory activity, kidney, liver, and blood parameters.

Materials and methods

Ethical approval

The protocol for the study was accepted by The Institutional Animal Care and Use Committee (Vet. CU. IACUC) of the Faculty of Veterinary Medicine, Cairo University, Egypt with number "Vet CU01122022559".

Curcumin nanoparticles preparation, synthesis, and characterization

Synthesis of curcumin nanoparticles using the Sono-chemical method

The sono-chemical method is an effective technique for the synthesis of nanoparticles, including those containing curcumin, a natural polyphenolic compound known for its therapeutic properties. Here's a general approach for the synthesis of curcumin-NPs using the sono-chemical method.

Preparation of the curcumin solution

The curcumin powder (1g) was dissolved in the organic solvent (ethanol 100 ml) to form a curcumin solution of desired concentration.

Sono-chemical Synthesis

In a glass container, the curcumin solution 100ml was combined with the surfactant (Tween 80) 50ml. The ultrasonic probe (sonicator) was immersed into the mixture at condition of applying ultrasonic waves to the mixture for a specific duration and intensity, typically in the range of 60 kHz and 200 W, respectively. The ultrasonic irradiation causes cavitation, which leads to the formation of curcumin nanoparticles. The sono-chemical synthesis method potentially enhances their biological activity or targeting capabilities. However, it's essential to optimize the synthesis parameters, such as sonication time, intensity, and concentration of reagents, to achieve the desired nanoparticle characteristics.

Characterization of the prepared curcumin nanoparticles

Transmission Electron Microscopy (TEM)

TEM was done by using JEOL JEM-2100F Field Emission Electron Microscope. The curcumin nanoparticles was dispersed into deionized water. Drops from the nanoparticle suspension was put onto a carbon-coated copper grid and allowed to dry.
Measurement Conditions: Accelerating voltage: 200 kV, Emission current: 110 µA, magnification range: 50x to 1,500,000x and image acquisition: Digital CCD camera.

Scanning Electron Microscopy (SEM)

SEM was done by using JEOL JSM-7600F Field Emission Scanning Electron Microscope. Curcumin nanoparticles was dispersed into deionized water. Drops from the nanoparticle suspension was put onto a silicon wafer or metal stub and allowed to dry. Sputter-coat the sample with a thin layer of conductive material (e.g., gold or platinum) to enhance conductivity and improve imaging.
Measurement Conditions: Accelerating voltage: 5-15 kV, Emission current: 10 µA, working distance: 8-10 mm, magnification range: 25x to 1,000,000x and Image acquisition: Digital SEM imaging system.

Dynamic Light Scattering (DLS)

DLS was performed via instrument: Malvern Zetasizer Nano ZS. Curcumin nanoparticles was dispersed into deionized water at an appropriate concentration. Filter the nanoparticle suspension was filtered through a 0.45 µm or 0.22 µm filter to remove any large aggregates or impurities.
Measurement Conditions: Temperature: 25°C (or desired temperature), Scattering angle: 173° backscatter, Refractive index: Specify the refractive index of the dispersant and nanoparticle material, Equilibration time: 120 seconds (or as needed) Number of runs: 3-5 runs per measurement and Analysis model: Choose an appropriate analysis model (e.g., General Purpose for nanoparticles).

Strains of Mycoplasma synoviae and Mycoplasma gallisepticum

Previous research by Qoraa *et al*. (2023a, b) provided the strains of MS and MG used in this investigation. After 48 hours of incubation on PPLO broth, the strains' infectious dose was determined and adjusted to 1 McFarland, producing a suspension of about 10^6 CFU/ml. At one day old, the chicks were infected with MG strains using eye and nasal drops and also, 0.5 ml of MS suspension was injected (S/C) into their foot pads. On the fifth day of the birds' life, they received another dose of MG and MS using the same dosage and method.

For five consecutive days, the birds were given drinking water containing 250 mg/ml of tilmicosin phosphate at a dose of 0.3 ml/L. This was done in accordance with the manufacturer's instructions to treat either MG as well as MS as a chemical therapeutic control, in accordance with Shakal *et al*. (2024b) recommendations.

In vitro assessment of antimicrobial substances

Minimum inhibitory concentration (MIC) determination

A range of curcumin-NPs and tilmicoin concentrations (from 1000 to 0.3 µg/ml) were used to treat the MS and MG isolates. The study was planned to find the lowest level that effectively stops MS and MG multiplication, following the methodology of Shakal *et al*. (2024b) with few modifications.

Applying the microdilution technique, the MIC of tilmicoin of MG and MS and curcumin-NPs was determined (Andrews, 2001). To summaries, PPLO broth was used to suspend 48-hour broth cultures of *Mycoplasma*, and the turbidity was adjusted to 1 McFarland. This produced a suspension that contained around 10^6 CFU/ml (Andrews, 2001). Twelve wells of a 96-well microtiter plate were filled with 50 µl of PPLO Broth culture to assess the MIC. 50 µl of the curcumin-NPs or tilmicoin stock solution was applied to the first well.

To obtain different concentrations of curcumin-NPs or tilmicosin in each well, a tow-fold serial dilution was used (1000 to 0.3 µg/ml). Next, each well received 50 µl of the microbial suspension. After that, the microplate was incubated for 72 hours at 37°C in a microaerobic environment.

The MIC was determined by looking for observable microbial growth in the well that contained curcumin-NPs or tilmicosin concentration. A negative control comprised solely inoculated broth, while a positive control consisted of PPLO broth media with assessed bacterial concentrations. to verify the measurements' worth for the tested microorganisms, each one was done three times. After 72 hours of incubation, the MIC of curcumin-NPs or tilmicosin was determined by visually inhibiting the proliferation of the bacteria (Andrews, 2001). By comparing the tubes' visible turbidity prior to and after incubation, the MIC was determined.

In vivo assessment of antimicrobials

Experimental Design

For this experiment, 221 healthy one-day-old broiler chicks had been used. Five of the birds were humanely killed upon arrival, and samples from the trachea, ais sacs, lungs and synovial fluid were pooled. These samples underwent routine bacteriological isolation to guarantee the non-existence of MG & MS natural infections. The remaining birds (216) were housed in a hygienic facility using a deep litter system and received an adequate supply of feed, water, and a commercial diet that was balanced and free of antibiotics and anti-parasites throughout a period of 35 days. At the prescribed time and route, the birds received standard vaccinations against the ND, IB, AI and IBD viruses.

Two hundred and sixteen birds were divided into nine groups, each with 24 birds, and three replicates, each with eight birds, for each group. The following were the groups: G1 was given an MG challenge; G2 received an MG challenge and 0.5% curcumin-NPs; G3 received an MG challenge and 1% curcumin-NPs; G4 MG challenged and treated with tilmicosin, G5 infected with MS; G6 infected with MS and supplied with 0.5% curcumin-NPs ; G7 infected with MS and supplied with 1% curcumin-NPs; G8 received an MS infected and supplied with tilmicosin and G9 were the control negative group. Beginning at the age of 17 days, each group received a different treatment for consecutive five days.

Assessment of chicken Performance

Clinical symptoms, mortality rates, body weight (BW) and feed conversion rate (FCR)

Throughout the trial, daily observations of clinical symptoms and mortality were recorded, and each bird's feed intake and body weight were assessed once a week.

Three birds per replicate were ethically blood sampled from wing vein at 17 days old (prior to treatment) and 22 days old (after treatment) from the wing vein using both standard and anticoagulated tubes. to measure the macroscopic lesion score, three randomly chosen birds from each replicate were humanely killed on day twenty-two of their life.

The respiratory manifestation was seen separately in the chickens additionally, Kempf *et al*. (1998) documented and scored the postmortem lesions of MG in the air sacs of dead and slaughtered birds both during and post therapy, whereas Kleven *et al*. (1972, 1975) recorded and scored clinical signs and postmortem manifestations resulting from MS infection.

Blood antioxidant and lipid peroxides

The activity of antioxidants in blood was investigated by measuring the activities of the enzymes catalase (CAT), reduced glutathione (GSH) and lipid peroxidase (MDA). Catalase was determined by using kits from Bio-diagnostic company, Dokki, Egypt, this test was carried out in harmony with Aebi (1984) method for the enzymatic colorimetric assessment of catalase activity. Reduced glutathione was measured using kits from Biodiagnostic company, Egypt, in accordance with the protocol outlined by Beutler *et al*. (1963). Measuring serum malondialdehyde (MDA) level was done based on the principle that thiobarbituric acid reactive product is formed when thiobarbituric acid (TBA) and malondialdehyde (MDA) combine in an acidic medium for 30 minutes at 95˚C. The absorbance of the pink product that results can be detected at 534 nm (Ohkawa *et al*., 1979).

Measurement of cholesterol and triglyceride levels

Cholesterol level was determined by using Biodiagnostic® kits from Cairo, Egypt, blood cholesterol levels were measured in accordance with Richmond (1973) instructions. Triacylglycerol level was measured by using kits from Biodiagnostic®, Cairo, Egypt, the serum triacylglycerol levels was ascertained, following the guidelines provided by Fossati and Prencipe (1982).

Kidney functions

The colorimetric method, which was invented by Fawcett and Scott (1960), was applied to determine the serum urea level. Serum creatinine was determined following Husdan and Rapoport (1968) methodology, which uses a colorimetric method, was used to determine the serum creatinine level.

Liver enzymes

Colorimetric measurement of alanine aminotransferase (ALT) was used to determine the activity of ALT, following Reitman and Frankel's 1957 methodology. Colorimetric analysis of aspartate aminotransferase (AST) was used to determine the activity of AST, following Reitman and Frankel's 1957 methodology.

Statistical analysis

The results are conveyed as mean ± standard deviation (mean ± SE). The results were statistically analyzed via analysis of variance (ANOVA), the difference was regard as significant when ($P < 0.05$) applying SPSS 27 (IBM. NY, USA).

Results

Characterization of curcumin-NPs

Transmission Electron Microscopy (TEM) findings

TEM imaging revealed that the curcumin nanoparticles had a spherical morphology. The nanoparticles appeared well-dispersed and exhibited a narrow size distribution. The average particle size, as decided from the TEM micrographs, was approximately 75 nm.

The spherical shape of the nanoparticles can be attributed to the sono-chemical synthesis method, which tends to produce particles with a spherical morphology due to the cavitation forces and the minimization of surface energy. The uniform size distribution suggests that the synthesis conditions, such as the ratio of curcumin to *Mycoplasma* components, sonication time, and surfactant concentration, were optimized to yield monodisperse nanoparticles (Figure 1A).

Scanning Electron Microscopy (SEM) findings

SEM analysis corroborated the spherical shape and uniform morphology of the curcumin nanoparticles observed in the TEM images. The SEM micrographs provided additional information about the surface topography and revealed that the nanoparticles had a smooth surface texture. The smooth surface texture of the nanoparticles may be advantageous for various applications, such as drug delivery or biomedical imaging, as it can influence the nanoparticles' interaction with biological systems and their stability in physiological environments (Figure 1B).

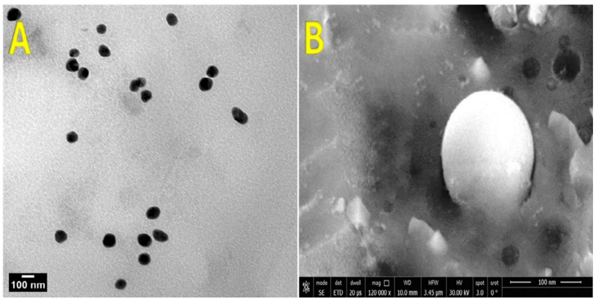

Figure 1. A: TEM image illustrated spherical shape of curcumin nanoparticles; B: SEM image illustrated spherical shape of curcumin nanoparticles.

Results of Dynamic Light Scattering (DLS)

DLS measurements showed a single, narrow peak in the size distribution, indicating a monodisperse population of nanoparticles. The Z-average hydrodynamic diameter of the curcumin nanoparticles was found to be approximately 80 nm, with a low polydispersity index (PDI) value, typically below 0.2, suggesting a narrow size distribution (Figure 2).

The hydrodynamic diameter of 80 nm obtained from DLS analysis is slightly larger than the average size of 75 nm observed in TEM micrographs. This difference can be attributed to the fact that DLS measures the hydrodynamic diameter, which includes the nanoparticle core and any solvation layers or surface coatings, while TEM provides a direct measurement of the core particle size in the dry state. The consistency between the TEM and DLS results, with a difference within the expected range, confirms the successful synthesis of monodisperse curcumin nanoparticles with a narrow size distribution.

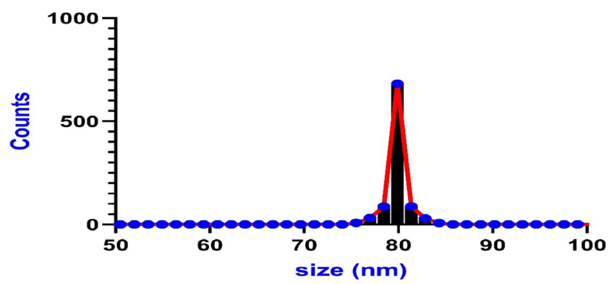

Figure 2. DLS curve of curcumin nanoparticles.

In vitro assessment of antimicrobials

The findings showed that the MIC of curcumin-NPs against MG and MS was 0.0156 µg/ml and 0.0312 µg/ml, respectively, while the MIC of Tilmicosin against MG and MS was 0.00781 µg/ml and 0.0625 µg/ml, respectively.

In vivo assessment of antimicrobials

Mortalities during the experiments

Results showed that the control positive groups, G1 20.8% (5/24) and G5 16.6% (4/24) had the highest cumulative mortality rates, while G2 and G6 showed the same mortality rate of 12.5% (3/24) and G7 and G8 4.1% (1/24). In contrast, G3, G4, and G9 showed no mortalities throughout the experiment. The MG infected and treated group showed no mortalities, whereas the MS group showed a lowered mortality rate. These findings were observed in the groups supplied with 1% curcumin-NPs.

Clinical signs score

Figure 3 demonstrated that, among MG challenged groups, G1 exhibited a significantly greater increase in the degree of clinical signs, followed by G2. On the other hand, birds treated with 1% curcumin-NPs in G3 and G4 and treated with tilmicosin demonstrated a significantly lower severity of clinical signs than G1. In contrast, birds treated with 1% curcumin-NPs in group G7 exhibited a substantial decrease in the sternness of clinical manifestations, in comparison to G5, whereas G5 displayed a larger significant elevation in the sternness of clinical manifestations between MS challenged groups, subsequent by G6. When compared to G9, all experimental groups exhibited a statistically significant increase in the severity of clinical symptoms (p ≤0.05).

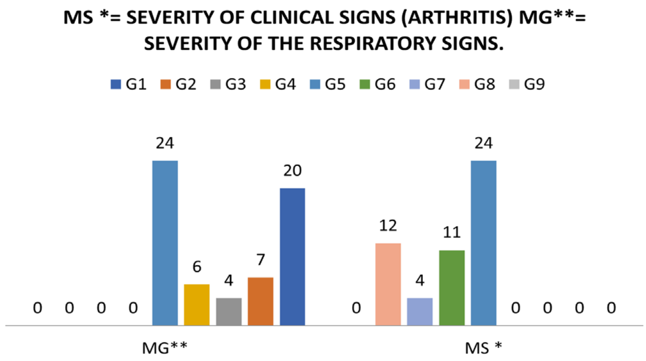

Figure 3. Clinical signs score in all experimental groups.
MS *: Severity of clinical signs (arthritis); MG**: Severity of the respiratory signs

Macroscopic lesions score

The degree of sternal bursitis shown a substantial rise in its lesion scoring in G1, followed by G2, in groups infected with MG. Compared to G1, birds treated with 1% curcumin-NPs in G3 and G4 and given tilmicosin showed a substantial decrease in lesion score. The air saculitis lesion score showed that G1 had the highest elevation, followed by G2, and that G3 and G4 had the lowest lesion scores. When compared to G1, the lung lesion scoring in G1 showed a substantial rise, subsequent by that of G2 but G3, and G4, which displayed the smallest lung macroscopic lesion score.b

The severity of arthritis significantly increased the lesion score in G1, G2, and G4 groups with MS challenges. Compared to G1, birds treated with 1% curcumin-NPs showed a substantial decrease in the lesion scoring. The sternal bursitis lesion score showed that G1 had the highest elevation, after G2, G4. G3 had the lowest lesion scoring. In G1, G2, and G4, the air saculitis lesion score increased significantly, while no air saculitis lesion score was seen in G3.

When compared to G9, all challenged birds, whether they had MG or MS, showed a marked rise in the various macroscopic lesions scores.

Evaluation of birds' performance

As shown in Figure 4, The FBW (g) showed a significant increase in control negative birds in G9 (2100), while challenged birds with MG revealed that G3 show the highest body weight (1900), followed by G2 (1810) then G4 (1750) when compared with G1 (1300) at p-value ≤0.05 that clear the negative impact of MG challenge on bird performance that expressed in a significant decrease in FBW in G1 (1300) when compared with G9 (2100) at p-value ≤0.05.

In birds challenged with MS, birds in G6 showed a significant increase in FBW (1920) followed by G7 (1900) then G8 (1450). Conversely, G5 showed a significant decrease in FBW (1390) when compared with other experimental groups and G9 (2100) at p-value ≤0.05.

Comparing G2 (challenged with MG) and G5 (challenged with MS) both revealed a significant reduction in FBW when compared to G9 that declare the negative impact of avian mycoplasmosis in birds' performance.

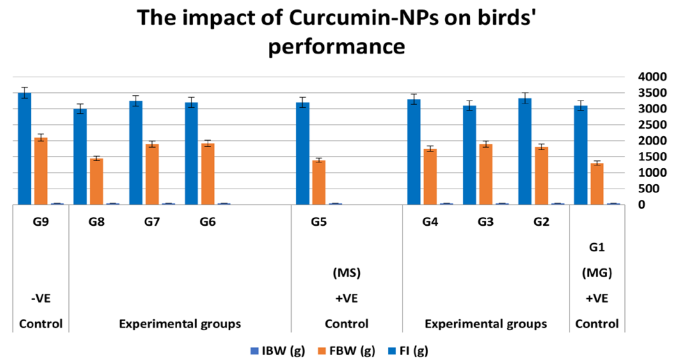

Figure 4. The impact of curcumin-NPs on birds' performance. Initial body weight (IBW), Final body weight (FBW), Feed intake (FI), Data are presented mean ±SD, p-value ≤0.05 indicate significant difference.

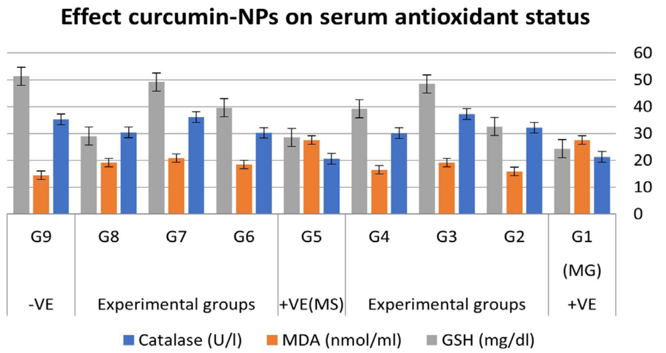

Figure 6. Effect of curcumin-NPs on serum antioxidant status. Malondialdehyde (MDA), Glutathione (GSH). Data are presented mean ±SD, p-value ≤0.05 indicate significant difference.

As presented in Figure 5 , regarding FCR, G9 showed a significant improvement (1.63) followed by G7 (1.752) then G3 (1.850) when matched with G1 (2.47) and G5 (2.379) at p-value ≤0.05. The birds supplied with 1% curcumin-NPs showed the best improvement in birds' performance among all challenged treated birds with either 1% curcumin-NPs or tilmicosin with birds. No significant difference was recorded among all the experimental groups in FI.

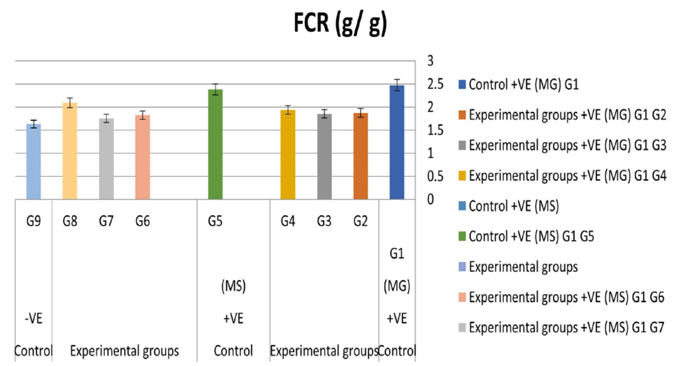

Figure 5. The impact of curcumin-NPs on FCR. FCR (feed conversion ratio), Data are presented mean ±SD, p-value ≤0.05 indicate significant difference.

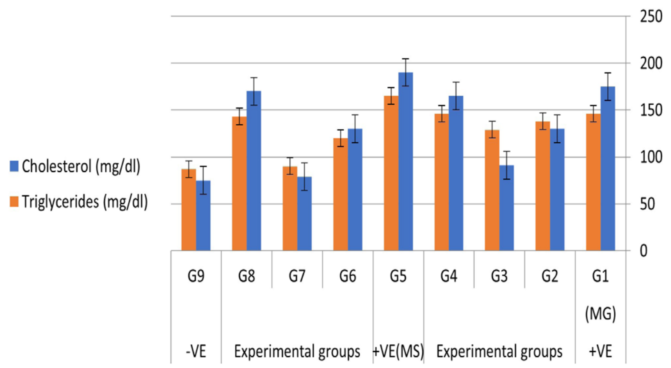

Figure 7. Effect of curcumin-NPs on blood cholesterol and triglycerides levels. Data are presented mean ±SD, p-value ≤0.05 indicate significant difference.

Effect curcumin-NPs on serum antioxidant status

As seen in Figure 6, the antioxidant status of the birds revealed that the catalase level (U/ml) showed a significant increase in G3 (37.2) and G7 (36.1) when contrasted to G1 (21.3) and G5 (20.6), respectively at p-value ≤0.05. MDA level (Ng/ml) exhibited a significant increase in G1 (27.5) and G5 (27.6) when compared with birds in G2 (15.9) ang G6 (18.4). The GSH level (mg/dl) showed a significant increase in G7 (49.2) then G3 (48.5) and G6 (39.6) when compared with birds in G1 (24.3) and G5 (28.5) at p-value ≤0.05. As noticed in Figure 6, the antioxidant status expressing GSH, MDA and catalase levels showed a significant improvement in G9, G7 and G3 in comparison with G1 and G5.

Regarding the blood lipid profile, Figure 7 clears that cholesterol level (mg/dl) showed a significant decrease in G7 (79) and G3 (91) while G2 and G6 revealed the same cholesterol level (130) on the other hand both G1 (175) and G5 (190) showed a significant increase in cholesterol level when compared with G9 at p-value ≤0.05. Triglycerides level (mg/dl) revealed a significant decrease in G7 (90), G6 (120) and G3 (148) when compared with G1 (146) and G5 (165) at p-value ≤0.05.

As seen in Figure 8, in relation to kidney function, creatinine level (mg/dl) showed a significant decrease in G7 (0.25) and G3 (0.27) when compared with birds in G1 (0.82) and G5 (0.44) at p-value ≤0.05. Also, urea level (mg/dl) showed a significant rise in G7 (11) and G3 (11) when compared with G1 (17) at p-value ≤0.05. G4 and G8 showed the same urea level (14).

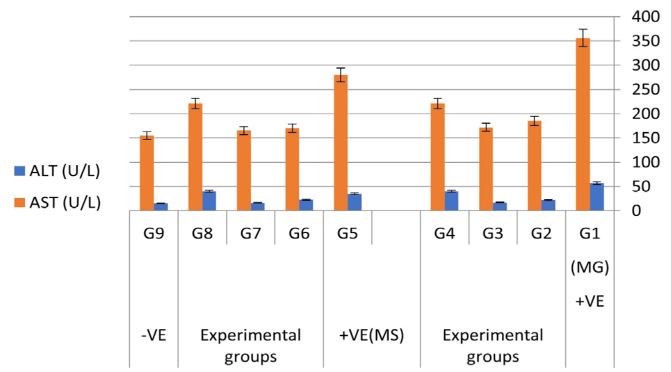

Figure 8. Effect of curcumin-NPs on liver enzymes activities. Alanine transaminase (ALT), Aspartate aminotransferase (AST). Data are presented mean ±SD, p-value ≤0.05 indicate significant difference.

As noticed in Figure 9, in relation to liver function, ALT level (U/L) showed a significant decrease in G7 (16) followed by G3 (17) when compared with G1 (57) and G8 (40) at p-value ≤0.05. AST activity (U/L) revealed a significant reduction in G7 (165), G6 (170) and G3 (172) when compared with G1 (356) and G5 (280) at p-value ≤0.05. Also, birds treated with tilmicosin showed an elevated activity of AST G4 (221) and G8 (223) when compared with G3 (172) and G7 (165) at p-value ≤0.05, respectively.

Discussion

The poultry industry suffers significant losses due to the most harmful avian *Mycoplasma* (Shakal *et al.*, 2024b). However, avian *Mycoplasma* remains to adapt to the avian industry and can elude the bird immune response that permits its continued presence, particularly in highly pop ulated poultry zones, despite the deployment of biosecurity measures alongside vaccination programmes (Bottinelli *et al.*, 2022). While long-term use of antimicrobial drugs might lead to the development of anti-*Mycoplasma* drug resistance, antibiotics may be helpful in some cases when controlling an outbreak (Taiyari *et al.*, 2021). Since ancient times,

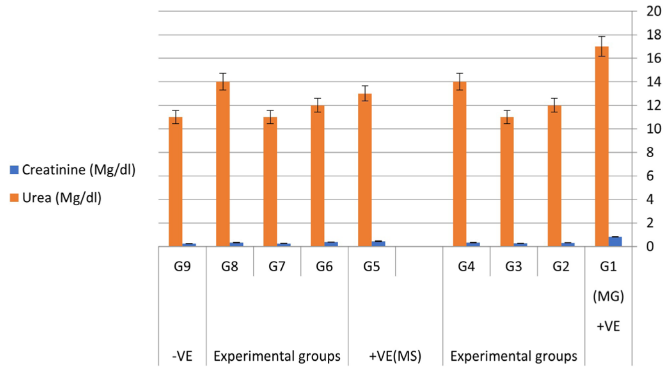

Figure 9. Effect of curcumin-NPs on kidney function. Data are presented mean ±SD, p-value ≤0.05 indicate significant difference.

traditional medicine has utilised turmeric, a spice that is widely available in many nations, for its antibacterial properties (Aderemi and Alabi, 2023). Using nanotechnology, curcumin's bioavailability can be enhanced, leading to the potential for C-NPs to be utilised as a natural and safe feed supplement to enhance performance (Jyotirmayee & Mahalik, 2022). According to our findings, curcumin-NPs had an antibacterial effect against MS and MG both *in vitro* and *In vivo*. In the current study, curcumin-NPs, particularly at a dose of 1%, demonstrated *In vivo* efficacy as seen by a decrease in cumulative mortalities in both MG and MS challenged groups. Additionally, groups treated with 1% curcumin-NPs showed a substantial decrease in clinical symptoms and macroscopic lesion score. Furthermore, Handharyani *et al*. (2020) discovered that the nano extracts of garlic, zedoary, and turmeric exhibit antibacterial activity against MG and E. coli, and they may be utilized to manage chronic respiratory disease (CRD) in chickens. Additionally, curcumin-NPs demonstrated antibacterial action against the majority of gram- positive and gram-negative bacteria, as noted by Reda *et al*. (2020). Curcumin-NPs minimum inhibitory concentration (MIC) against MS and MG in this investigation was 0.0312 µg/ml & 0.0156 µg/ml, respectively. In a related investigation, Bhawana *et al*. (2011) found that the minimum inhibitory concentrations (MICs) of curcumin-NPs for S. aureus, B. subtilis, E. coli, and P. aeruginosa were, in that order, 100, 75, 250, and 200 µg/mL. Recently, the use of nano-curcumin in chicken feed is attracting attention as a potential means of enhancing the physiological function, production, and general health of chickens (Reda *et al*., 2020). In this investigation, the use of 1% curcumin-NPs in G3 and G7 improved the growth performance of the birds compared to other groups. Our results support those of Handharyani *et al*. (2020), who discovered that the treatment of birds with turmeric, zedoary, and garlic nano extracts led to improvements in their body weight and growth performance. Also, Rahmani *et al*. (2018) noticed that when curcumin or curcumin-NPs were supplemented, the birds' BW and WG increased and their FCR decreased in comparison to the control group. Furthermore, Reda *et al*. (2020) discovered that adding curcumin-NPs to quail feed improved the birds' growth rate, lipid profile, blood indices, antioxidant status, and immune response and it also increased the quantity of lactic acid bacteria and decreased the number of pathogenic bacteria. Also, our findings agreed with those of Guo *et al*. (2023), who discovered that while curcumin and pueraria extract were added to the broiler diet for a 28-day trial, the broilers' gastrointestinal health and antioxidant status were improved instead by increasing the activities of antioxidant enzymes and improving intestinal morphology. Curcumin, when used as a functional molecule, can help poultry develop and perform better by functioning as a potent natural antioxidant, which may be one reason for the good influence of curcumin-NPs on birds' growth performance (Reda *et al*., 2020). Furthermore, consuming curcumin stimulates the release of bile acids and the enzymes lipase, amylase, trypsin, and chymotrypsin (Platel *et al*., 2002). Increased production of these enzymes may be the reason for curcumin's favourable benefits on broiler growth (Chattopadhyay *et al*., 2004; El-Saadony *et al*., 2023). The observed boost in growth in birds fed diets containing curcumin may be attributed to modifications in the intestinal morphology of the birds (Chattopadhyay *et al*., 2004). According to our observations, the lipid profile (levels of triglycerides and cholesterol) of the birds treated with 1% curcumin-NPs significantly decreased also, 0.5% curcumin-NPs show positive impact on lipid profile. Our findings corroborated those of Reda *et al*. (2020), who discovered that adding curcumin-NPs to quail meal reduced the serum lipid profile. The findings in the group that received 1% curcumin-NPs are consistent with studies by Malekizadeh *et al*. (2012) in laying hens, Saraswati *et al*. (2013) in Japanese quails, and Fallah and Mirzaei (2016) in broiler chicks given varying amounts of turmeric supplementation. Furthermore, curcumin was demonstrated to lower total cholesterol, presumably through blocking the function of the hepatic 3-hydroxyl-3-methylglutaryl CoA-reductase (HMGCR) enzyme, which oversees generating total cholesterol in the liver's tissues (Kumari *et al*., 2007; AMA, 2011). According to Nouzarian *et al*. (2011), hens' development and health depend on their ability to maintain antioxidant levels. According to Sahin *et al*. (2012), curcumin, the primary antioxidant found in *Curcuma longa*, is a powerful oxygen species scavenger and the use of curcumin-NPs in the current investigation enhanced the treated birds' antioxidant status. Our findings in concur with those of Reda *et al*. (2020), who observed that curcumin-NPs enhanced the quails' antioxidant state. These results may also be explained by the fact that curcumin scavenges free radicals, inhibits oxidative enzymes, and promotes de novo glutathione, all of which help cells maintain their antioxidant status (El-Agamy, 2010). Additionally, the malondialdehyde level was decreased and the enzymatic activities of SOD and GSH-PX were improved by the turmeric rhizome extract (Wang *et al*., 2015). Our research indicates that using curcumin-NPs enhanced renal and hepatic function. According to these findings, which corroborated those of Kim *et al*. (2013), adding curcumin and curcumin-NPs to broiler diets improved hepatic function by raising serum TP and lowering AST and ALT activity. According to these arguments, 1% curcumin-NPs can be used as a substitute for antibiotics to treat MG and MS infections without having an adverse effect on the economically and productive characteristics of broilers.

Conclusion

To prevent the development of anti-*Mycoplasma* drug resistance, the MIC test should be used routinely to determine the best anti-*Mycoplasma* drug. With best results at concentrations of 1%, curcumin-NPs had a substantial anti-*Mycoplasma* activity. The use of 1% curcumin-NPs enhances the antioxidant status of the broiler chickens, improves the liver and kidney functions, and enhances the growth performance of birds.

Conflict of interest

The authors have no conflict of interest to declare.

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