



Efficacy of CuO and ZnO Nanoparticles Against *Salmonella* Gallinarum Infection in Broiler Chickens, and Their Impact on Growth and Biochemical Profiles

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ABSTRACT

Fowl typhoid caused by *Salmonella* Gallinarum challenges poultry production and increases the economic burden. The overuse of antibiotics for treating *S. Gallinarum* infections results in antimicrobial resistance and poses a significant threat to public health. The present study evaluated the antimicrobial activity of copper oxide (CuO) and zinc oxide (ZnO) nanoparticles against *S. Gallinarum* infection in broiler chickens. One-day-old chicks (n = 90) were equally divided into six groups, i.e. control negative, control positive, FLOR-A, CZNP-1, CZNP-2, and CZNP-3. On the 19th day, the chicks in all groups except the control negative were challenged with *S. Gallinarum*. After the appearance of clinical signs, antibiotic treatment was administered to group FLOR-A (Florfenicol, 50mg/L) and CuO and ZnO nanoparticles were administered to groups CZNP-1, CZNP-2, and CZNP-3 at different dose levels: 10 + 25, 15 + 37.5, and 20 + 50 mg/kg/day, respectively. The results revealed that the feed conversion ratio, live body weight, and carcass weight were significantly improved in the CZNP-1, CZNP-2, and CZNP-3 groups compared with FLOR-A. The relative organ weight and serum concentrations of ALT, AST, creatinine, and urea were significantly decreased in the CZNP-1, CZNP-2, and CZNP-3 groups compared to FLOR-A. The CuO and ZnO nanoparticles at a dose level of 15 + 37.5 mg/kg/d had optimum efficacy among all treatment groups. These results confirm the antimicrobial activity of CuO and ZnO nanoparticles, suggesting their potential as a substitute for Florfenicol in treating *S. Gallinarum* infection in broiler chickens.

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Key words

Antimicrobial resistance, Broiler growth, Florfenicol, Serum biochemistry, Carcase, FCR

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INTRODUCTION

Fowl typhoid is an acute septicemic disease of avian species caused by the gram-negative bacterium *Salmonella* Gallinarum. The disease is characterized by weakness, poor growth, diarrhea, splenomegaly, hepatomegaly, necrotic foci on internal organs, acute anemia, leukocytosis, heterophilia, and hemorrhage (Shah et al., 2013). The bacteria infect chicks across all age groups,

causing high morbidity with mortality rates exceeding 75% within the flock (Raza *et al.*, 2022). This results in reduced poultry production and increased economic losses, thereby constituting a global concern (Rubio *et al.*, 2021). Therefore, multiple prophylactic and anaphylactic treatment strategies are adopted to overcome the disease occurrence (Gyles, 2008; Nhung *et al.*, 2017; Revollo, 2018). The intensive use of antibiotics has led to the development of antimicrobial resistance (AMR), posing a threat to both poultry and human health (Patil *et al.*, 2023; Zhou *et al.*, 2020). The AMR can lead to the accumulation of pathogenic bacteria and antibiotic residues in animal-origin food products, ultimately transferring to humans, which can result in decreased efficacy of antibiotics and treatment failure (Roth *et al.*, 2019). Therefore, there is an urgent need to develop new therapeutic strategies to address the issue of AMR in animals and humans and to achieve sustainable control of infectious diseases.

Nanotechnology is widely recognized as a potentially transformative technology in the fields of veterinary medicine and animal health in terms of disease diagnosis, treatment, and drug delivery (Manuja *et al.*, 2012). Previous studies have also demonstrated that nanotechnology can be an effective therapeutic approach to overcoming the problem of AMR (Singh and Singh, 2021). Copper oxide (CuO) and zinc oxide (ZnO) nanoparticles exhibit strong antimicrobial activity against both Gram-negative and Gram-positive bacteria. Metal nanoparticles such as silver, zinc, and copper can be used as alternatives to antibiotics in addition to growth promotion in food animals (Eleftheriadou *et al.*, 2021). The ZnO nanoparticles demonstrated a substantial effect on growth promotion and gut health, with no significant toxic effects in broilers (Mahmoud *et al.*, 2020). An earlier study (El-Shenawy *et al.*, 2023) reported that ZnO nanoparticles significantly improved the growth performance and health status of the chicks suffering from different kinds of bacterial infections. Copper, an essential trace mineral, improves the immune status along with better growth performance, feed conversion ratio, and high breast meat yield in broilers (Sharif *et al.*, 2021). The green synthesized CuO nanoparticles exhibited substantial antimicrobial activity against colibacillosis in broilers and improved kidney and liver function (El-Shenawy *et al.*, 2023). Another study (Forouzandeh *et al.*, 2021) reported that supplementation of dietary CuO in broilers improves the gut microbiota which has a positive impact on the broiler's growth performance. The CuO and ZnO nanoparticles can significantly increase the average body weight gain and decrease the feed conversion ratio (FCR) in broilers (Zhao *et al.*, 2014). A recent study revealed that CuO and ZnO nanoparticles showed significant effects on

the health status of broilers suffering from colibacillosis in terms of immunological, histopathological and hematobiochemical parameters (Elbasuni *et al.*, 2024). We have recently reported that a mixture of CuO and ZnO nanoparticles significantly improved the health status of broilers suffering from fowl typhoid in terms of reduced mortality, improved phagocytic activity of macrophages, and serum biochemistry in broilers (Raza *et al.*, 2024). Previous studies showed that the antibacterial efficacy of CuO at dose rate 20, 30 and 40 mg/kg/d without any significant toxic effects (Ahmed *et al.*, 2022) and the antibacterial efficacy of ZnO nanoparticles at dose rate 50, 75 and 100 mg/kg/d without any substantial toxicity in broilers (Bahadar *et al.*, 2024). Besides the growing concern of nanoparticles accumulation in the soil or water, the nanoparticles not only have beneficial impact on the animal health but also have a promising effects in terms of maintaining a healthy environment in the poultry house along with micronutrient availability in the soil for optimal agriculture production (Achari *et al.*, 2018; Rashid *et al.*, 2023). The residues of these nanoparticles killed the microbes by producing reactive oxygen species leading to reduced ammonia production in the poultry house resulting in maintaining the healthy in house environment and preserving the public health by avoiding the antibiotic residues (Zarnab *et al.*, 2022). Regarding the accumulation of ZnO and CuO nanoparticles in the soil, Zn and Cu are among the essential micronutrients for plants. The ZnO and CuO nanoparticles have been explored as an efficient, slow-releasing zinc and copper fertilizer to reduce the widespread Zn and Cu deficiency in agricultural soil (Sun *et al.*, 2021). Previously, we have reported that CuO and ZnO nanoparticles have shown antimicrobial activity against *S. Gallinarum* in broilers in term of reduced mortality, improved hematological parameters, increased lymphoproliferative response of the lymphocytes and phagocytic activity of the macrophages, increased concentration of immunoglobulins, serum proteins and lipids, along with gross and histopathologically of immune organs (Raza *et al.*, 2022, 2024). Although CuO and ZnO nanoparticles have shown numerous advantages in veterinary medicine and animal production, their impact on the growth performance, liver function and kidney function of broilers infected with fowl typhoid remains unexplored.

Given the reported antibacterial and growth-promoting effects of CuO and ZnO nanoparticles, we hypothesised that these nanoparticles could serve as potential therapeutic supplements to improve the health status of the chicks recovering from fowl typhoid. Therefore, this study aimed to (i) compare the therapeutic efficacy of CuO and ZnO nanoparticles with Florfenicol,

and (ii) evaluate their effects on feed conversion ratio (FCR), growth and serum biochemical parameters in broiler chicks affected from fowl typhoid.

MATERIALS AND METHODS

Experimental birds

A total of 90 specific pathogen-free, Habbard broiler *Gallus domesticus* chicks (body weight = 42 ± 2 g, one-day-old) were used in this experiment. They were kept under consistent, suitable management conditions following ethical standards. The chicks were provided with feed and water *ad libitum*.

Preparation of nanoparticles

The CuO and ZnO nanoparticles used in the present study were prepared through the co-precipitation method using copper chloride (CuCl_2), zinc sulphate (ZnSO_4) and sodium hydroxide (NaOH) as precursors. The size of CuO and ZnO nanoparticles was 33.20 nm and 97.50 nm, respectively, while the purity of both nanoparticles was 99.99% (Raza *et al.*, 2024).

Experimental design

During the first nine days post-hatch, all the chicks were housed together to allow for environmental adaptation. On the 10th day, the chicks were equally divided into six groups (15 chicks in each group) viz., control negative, control positive, FLOR-A (Florfenicol), CZNP-1, CZNP-2, and CZNP-3. At the 19th day of age, the five groups, control, positive, FLOR-A, CZNP-1, CZNP-2, and CZNP-3, were challenged with *S. Gallinarum* (1×10^8 colony-forming units/mL) orally, as reported previously (Raza *et al.*, 2024). After the appearance of clinical signs, the CuO and ZnO nanoparticles were given to groups, CZNP-1 (CuO 10 + ZnO 25 mg/kg/d), CZNP-2 (CuO 15 + ZnO 37.5 mg/kg/d), CZNP-3 (CuO 20 + ZnO 50 mg/kg/d) via the oral route and Florfenicol (FLOR-A®) at 50 mg/ L of drinking water. The chicks were sacrificed humanely on days 26 and 30 of the age. Blood samples were collected directly from the jugular vein in 5 ml sterile syringes; serum was separated and collected in the 1.5 ml Eppendorf tubes and stored at -20°C for further analysis (liver and kidney function tests).

Growth parameters

The direct antimicrobial activity of CuO and ZnO nanoparticles against *S. Gallinarum* has been reported previously (Raza *et al.*, 2022, 2024). In the present study, we aimed to evaluate the therapeutic efficacy of CuO and ZnO nanoparticles against *S. Gallinarum* induced infection in broilers by measuring growth performance

(FCR, live body weight, carcass weight), and relative organ weight (spleen, thymus, bursa of Fabricius, lungs, heart, kidney, liver, gizzard, proventriculus, and intestine), as these parameters reflect the clinical recovery of infected birds following nanoparticles treatment. The FCR and relative organ weight were calculated using the following equations reported by previous studies (Balijepalli *et al.*, 2015; Jeremiah *et al.*, 2015).

Weight gain (g) = Final body weight(g) – Initial body weight(g)

$$\text{FCR} = \frac{\text{Average feed intake (g)}}{\text{Average body weight gain (g)}}$$

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Average body weight (g)}} \times 100$$

Serum biochemical parameters

Similarly, to assess the safety and therapeutic effect of CuO and ZnO nanoparticles on liver and kidney functions, serum samples were analysed to measure alanine transaminase (ALT), aspartate aminotransferase (AST), urea, and creatinine. Commercially available kits for ALT (Cat # 5.17531.0001; Merck), AST (Cat # 5.17521.0001; Merck), urea (Cat # 5.17611.0001; Merck), and creatinine (Cat # 5.17551.0001; Merck) were used as per the manufacturer's guidelines.

Statistical analysis

Data were analyzed using a completely randomized design with analysis of variance (ANOVA). Differences among group means were assessed using Tukey's post hoc test. All statistical analyses were conducted using IBM SPSS Statistics, Version 25 (IBM Corporation, USA).

RESULTS

FCR, live body weight, and carcass weight

The FCR, live body weight, and carcass of all treatment groups, including FLOR-A, CZNP-1, CZNP-2, and CZNP-3, control negative and control positive groups, were determined to evaluate the effect of CuO and ZnO nanoparticles on *S. Gallinarum* infection in chicks. The antimicrobial effect of CuO and ZnO nanoparticles and florfenicol in *S. Gallinarum* infected chicks on the 7th and 11th days post-infection was measured in terms of improved FCR, live body weight and carcass weight is comprehensively outlined in Table I.

Briefly, on the 7th and 11th days post-infection, the FCR of the control positive group increased ($p < 0.001$), while the live body weight and carcass weight decreased ($p < 0.001$) compared to those of the control negative group. After the treatment of Florfenicol and a mixture of CuO and ZnO nanoparticles, FCR was decreased ($p < 0.001$), and live body weight and carcass weight were

increased ($p < 0.001$) in FLOR-A, CZNP-1, CZNP-2, and CZNP-3 groups compared to that of the control positive group (Table 1). The CZNP-2 group had the lowest FCR and maximum live body weight and carcass weight among all treatment groups.

Relative organ weight

The relative organ weight (immune and viscerals) of all treatment groups including FLOR-A, CZNP-1, CZNP-2 and CZNP-3, control negative and control positive groups, were determined on the 7th and 11th days post-infection.

Table 1. Effect of CuO and ZnO nanoparticles and Florfenicol on *S. Gallinarum* infected broiler chicks in terms of feed conversion ratio, live body weight and carcass weight, weight of immune organs (spleen, thymus, and bursa of Fabricius) and relative organ weight of visceral organs (lungs, heart, kidney, liver, gizzard, proventriculus and intestine).

Parameters		Negative control	Positive control	Treatments				p value
				FLOR-A florfenicol (mg/L) (50)	Nanoparticle levels of CuO and ZnO (mg/kg/d)			
					CZNP-1 (10 + 25)	CZNP-2 (15 + 37.5)	CZNP-3 (20 + 50)	
Growth parameters								
Feed conversion ratio	S-1	1.37±0.05 ^a	2.08±0.05 ^b	1.54±0.02 ^{cd}	1.54±0.05 ^{cd}	1.46±0.03 ^{ad}	1.62±0.03 ^c	0.000
	S-2	1.46±0.04 ^a	2.25±0.04 ^b	1.81±0.04 ^c	1.60±0.03 ^{de}	1.53±0.02 ^{ae}	1.65±0.05 ^d	0.000
Live body weight (g)	S-1	1408.75±59.53 ^a	937.80±24.93 ^b	1248.00±17.95 ^c	1249.80±41.67 ^c	1322.00±27.56 ^d	1191.67±24.33 ^c	0.000
	S-2	1821.25±41.34 ^a	1202.25±22.16 ^b	1612.00±32.01 ^c	1668.20±31.59 ^{de}	1738.33±46.74 ^d	1475.00±30.62 ^c	0.000
Carcass weight (g)	S-1	808.50±69.54 ^a	558.20±19.28 ^b	703.75±13.23 ^{cd}	668.01±23.07 ^d	766.75±35.34 ^{ac}	628.34±32.71 ^{bd}	0.000
	S-2	1064.25±52.34 ^a	690.25±63.81 ^b	985.01±40.62 ^c	1014.65±27.71 ^{ac}	1008.67±36.58 ^{ac}	959.50±28.15 ^c	0.000
Relative immune organ weight								
Spleen weight (g)	S-1	0.13±0.008 ^a	0.31±0.03 ^b	0.17±0.007 ^a	0.18±0.06 ^a	0.15±0.03 ^a	0.17±0.009 ^a	0.000
	S-2	0.19±0.01 ^a	0.27±0.02 ^b	0.14±0.005 ^c	0.15±0.01 ^{ac}	0.17±0.05 ^{ac}	0.15±0.01 ^{ac}	0.000
Thymus weight (g)	S-1	0.45±0.06 ^a	0.42±0.12 ^b	0.30±0.15 ^a	0.47±0.09 ^a	0.36±0.24 ^a	0.39±0.03 ^a	0.000
	S-2	0.48±0.14 ^a	0.27±0.11 ^b	0.31±0.05 ^a	0.44±0.22 ^a	0.49±0.07 ^a	0.42±0.52 ^a	0.000
Bursa weight (g)	S-1	0.12±0.01 ^a	0.29±0.04 ^b	0.13±0.02 ^a	0.13±0.01 ^a	0.12±0.03 ^a	0.15±0.02 ^a	0.000
	S-2	0.11±0.01 ^a	0.24±0.06 ^a	0.12±0.01 ^a	0.12±0.02 ^a	0.10±0.01 ^a	0.11±0.01 ^a	0.451
Relative weight visceral organs								
Lungs weight (g)	S-1	0.47±0.03 ^a	0.85±0.04 ^b	0.49±0.03 ^a	0.47±0.03 ^a	0.50±0.04 ^a	0.49±0.03 ^a	0.000
	S-2	0.46±0.02 ^a	0.91±0.01 ^b	0.46±0.02 ^a	0.47±0.03 ^a	0.49±0.03 ^a	0.48±0.02 ^a	0.000
Heart weight (g)	S-1	0.49±0.02 ^a	0.56±0.01 ^{ab}	0.49±0.05 ^{ab}	0.51±0.06 ^{ab}	0.50±0.02 ^{ab}	0.57±0.01 ^b	0.013
	S-2	0.67±0.03 ^a	0.78±0.02 ^b	0.59±0.02 ^c	0.57±0.02 ^{cd}	0.56±0.05 ^{cd}	0.54±0.01 ^{cd}	0.000
Kidney weight (g)	S-1	0.71±0.02 ^a	1.22±0.19 ^b	0.89±0.017 ^c	0.91±0.02 ^c	0.77±0.02 ^a	0.92±0.19 ^c	0.000
	S-2	0.70±0.03 ^a	1.10±0.02 ^b	0.73±0.01 ^{ac}	0.79±0.09 ^c	0.68±0.04 ^a	0.85±0.05 ^{ac}	0.000
Liver weight (g)	S-1	4.15±0.07 ^a	7.23±0.36 ^b	5.09±0.11 ^{cd}	4.86±0.14 ^d	5.00±0.15 ^d	5.43±0.054 ^c	0.000
	S-2	3.81±0.14 ^a	6.69±0.49 ^b	4.44±0.34 ^{cd}	4.76±0.51 ^a	3.40±0.21 ^{ac}	4.98±0.53 ^d	0.000
Gizzard weight (g)	S-1	1.47±0.089 ^a	2.14±0.18 ^b	1.71±0.07 ^{ac}	1.72±0.14 ^c	1.63±0.04 ^{ac}	1.69±0.04 ^{ac}	0.000
	S-2	1.82±0.06 ^a	2.01±0.16 ^a	2.07±0.12 ^a	1.93±0.15 ^a	1.98±0.03 ^a	2.13±0.18 ^a	0.131
Proventriculus weight (g)	S-1	0.44±0.02 ^a	0.65±0.05 ^b	0.48±0.02 ^{ac}	0.47±0.03 ^{ac}	0.51±0.02 ^c	0.49±0.02 ^{ac}	0.000
	S-2	0.48±0.01 ^a	0.51±0.02 ^a	0.46±0.01 ^a	0.47±0.01 ^a	0.48±0.06 ^a	0.52±0.03 ^a	0.058
Intestine weight (g)	S-1	5.58±0.13 ^a	8.43±0.31 ^b	5.89±0.27 ^{ac}	5.91±0.42 ^{ac}	5.76±0.19 ^b	6.69±0.27 ^b	0.000
	S-2	5.17±0.14 ^a	7.93±0.24 ^b	5.68±0.16 ^{ac}	5.58±0.04 ^{ac}	5.47±0.36 ^{ac}	6.33±0.33 ^c	0.000

^{a, b, c, d, e} Mean ± SD in rows with different superscripts are significantly different, n = 3, S-1 and S-2 represent the sampling at 7th day and 11th day post-infection respectively. Groups: Control negative (no *S. Gallinarum* infection, no treatment), Control positive (*S. Gallinarum* infection, no treatment), FLOR-A (*S. Gallinarum* infection, florfenicol treatment at dose 50 mg/L drinking water), CZNP-1 (*S. Gallinarum* infection, CuO + ZnO NPs treatment at dose 10 + 25 mg/kg/d), CZNP-2 (*S. Gallinarum* infection, CuO + ZnO NPs treatment at dose 15 + 37.5 mg/kg/d), CZNP-3 (*S. Gallinarum* infection, CuO + ZnO NPs treatment at dose 20 + 50 mg/kg/d).

Immune organs

The relative weight of immune organs (spleen, thymus, and bursa of Fabricius) in all treatment groups, including FLOR-A, CZNP-1, CZNP-2, and CZNP-3, control negative and control positive groups, was determined to evaluate the effect of CuO and ZnO nanoparticles on *S. Gallinarum* infection in chicks. The antimicrobial effect of CuO and ZnO nanoparticles and florfenicol in *S. Gallinarum* infected chicks on the 7th and 11th days post-infection on the relative organ weight of immune organs (spleen, thymus and bursa of Fabricius) is comprehensively outlined in [Table I](#).

Briefly, on the 7th day post-infection, the relative organ weights of the spleen, thymus, and bursa of Fabricius were increased ($p < 0.001$) in the positive control group compared to the negative control group. However, after the treatment of Florfenicol and a mixture of CuO and ZnO nanoparticles, the relative organ weight of the spleen, thymus and bursa of Fabricius was decreased ($p < 0.001$) in groups FLOR-A, CZNP-1, CZNP-2, and CZNP-3 in comparison to that of the positive control group and comparable to that of negative control group. Likewise, on the 11th day post-infection, the relative weights of the spleen, thymus, and bursa of Fabricius were increased in the positive control group compared to the negative control group. However, after the treatment of Florfenicol and a mixture of CuO and ZnO nanoparticles, the relative organ weight of the spleen and thymus was decreased ($p < 0.001$) in groups FLOR-A, CZNP-1, CZNP-2, and CZNP-3 in comparison to that of the positive control group and comparable to that of control negative group. However, no significant difference was observed in the relative weight of the bursa of Fabricius among all groups.

Collectively, it was observed that the relative weight of the spleen, thymus and bursa of Fabricius was increased in the chicks with *S. Gallinarum*. In contrast, treatment of CuO and ZnO nanoparticles decreased the relative weight of the spleen, thymus and bursa of Fabricius in FLOR-A, CZNP-1, CZNP-2, and CZNP-3 treated groups. However, the group CZNP-2 had the lowest relative weight of the spleen, thymus, and bursa of Fabricius among all treatment groups, viz., FLOR-A, CZNP-1, and CZNP-3.

Visceral organs

The relative organ weight of visceral organs (lungs, heart, kidney, gizzard, proventriculus, and intestine) of all treatment groups including FLOR-A, CZNP-1, CZNP-2 and CZNP-3, control negative and control positive groups, were determined to evaluate the effect of CuO and ZnO nanoparticles on *S. gallinarum* infected chicks. The antimicrobial effect of CuO and ZnO nanoparticles

and florfenicol in *S. Gallinarum*-infected chicks on the 7th and 11th days post-infection on the relative organ weight of lungs, heart, kidney, gizzard, proventriculus, and intestine is presented in [Table I](#).

On the 7th and 11th days post-infection, the relative organ weights of the lungs, kidneys, liver, and intestine were significantly increased in the positive control group compared to the negative control group. However, treatment with CuO and ZnO nanoparticles resulted in a significant decrease in the relative organ weights ($p < 0.001$) in the nanoparticle-treated groups CZNP-1, CZNP-2, and CZNP-3 on both observation days.

On the 7th day post-infection, the relative organ weights of the gizzard and proventriculus increased significantly ($p < 0.001$) in the positive control group compared to the negative control group. In contrast, the relative heart weight remained unchanged across all treatment groups (FLOR-A, CZNP-1, CZNP-2) and control groups, except for CZNP-3. By the 11th day post-infection, there were no significant differences in the relative weights of the gizzard and proventriculus across all treatment groups (FLOR-A, CZNP-1, CZNP-2, and CZNP-3). However, the relative heart weight increased significantly ($p < 0.001$) in the positive control group compared to the negative control group.

Collectively, the treatment with CuO and ZnO nanoparticles effectively reduced the relative weights of the visceral organs, bringing them back to normal ranges observed in healthy controls.

Serum biochemical parameters

The serum concentration of ALT, AST, urea, and creatinine was determined in all treatment groups including FLOR-A, CZNP-1, CZNP-2, CZNP-3, control negative, and control positive groups. The serum concentration of ALT, AST, urea, and creatinine in *S. Gallinarum*-infected broilers treated with CuO and ZnO nanoparticles on the 7th and 11th days post-infection is mentioned in [Table II](#).

On both the 7th and 11th days post-infection, the concentrations of ALT, AST, urea, and creatinine were significantly increased ($p < 0.001$) in the positive control group compared to the negative control group. However, treatments with florfenicol and a combination of CuO and ZnO nanoparticles significantly reduced these concentrations ($p < 0.001$) in groups FLOR-A, CZNP-1, CZNP-2, and CZNP-3 on both measurement days. Notably, among nanoparticle-treated groups, CZNP-2 showed reductions in ALT, AST, urea, and creatinine levels that were comparable to those observed in the florfenicol-treated group FLOR-A and the negative control group.

Table II. Effect of CuO and ZnO nanoparticles and Florfenicol in *S. Gallinarum* infected broiler chicks in terms of alanine transaminase (ALT), aspartate aminotransferase (AST), urea and creatinine.

Serum biochemistry parameters	Treatments							p value
	Negative control	Positive control	FLOR-A Florfenicol (mg/L) (50)	Nanoparticle levels of CuO and ZnO mg/kg/d				
				CZNP-1 (10 + 25)	CZNP-2 (15 + 37.5)	CZNP-3 (20 + 50)		
ALT (IU/L)	S-1	12.60 ± 0.21 ^a	30.67 ± 1.68 ^b	14.43 ± 0.38 ^c	16.76 ± 2.28 ^d	14.12 ± 1.38 ^{ac}	23.76 ± 2.41 ^c	0.000
	S-2	12.18 ± 1.17 ^a	29.76 ± 1.16 ^b	14.58 ± 1.38 ^c	16.27 ± 0.95 ^d	13.57 ± 0.53 ^c	23.96 ± 2.54 ^c	0.000
AST (IU/L)	S-1	126.01 ± 3.46 ^a	189.61 ± 2.37 ^b	160.24 ± 2.44 ^c	158.41 ± 4.98 ^c	134.23 ± 10.39 ^a	180.66 ± 3.06 ^b	0.000
	S-2	110.55 ± 5.04 ^a	205.01 ± 10.35 ^b	149.52 ± 6.56 ^c	170.19 ± 5.89 ^d	133.32 ± 8.17 ^c	187.59 ± 11.83 ^f	0.000
Urea (mg/dL)	S-1	23.66 ± 1.33 ^a	36.82 ± 2.26 ^b	25.58 ± 1.23 ^c	29.94 ± 0.73 ^d	25.61 ± 1.46 ^c	27.81 ± 2.75 ^c	0.000
	S-2	24.18 ± 1.32 ^a	38.61 ± 4.71 ^b	26.33 ± 2.27 ^{cd}	27.56 ± 1.64 ^{cc}	26.03 ± 0.74 ^d	28.16 ± 0.66 ^c	0.000
Creatinine (mg/dL)	S-1	1.01 ± 0.058 ^a	2.19 ± 0.11 ^b	1.67 ± 0.061 ^c	1.71 ± 0.075 ^c	1.18 ± 0.059 ^d	1.82 ± 0.071 ^c	0.000
	S-2	0.99 ± 0.12 ^a	2.74 ± 0.23 ^b	1.36 ± 0.16 ^c	1.86 ± 0.093 ^d	1.24 ± 0.08 ^{ac}	1.76 ± 0.04 ^d	0.000

For statistical details of treatments and details of groups, see Table I.

DISCUSSION

The development of optimal alternatives to antibiotics is critically needed to address the issue of antimicrobial resistance in poultry and public health. Metal oxide nanoparticles have been reported to possess antibacterial properties, exhibiting optimum efficacy against both Gram-positive and Gram-negative bacteria. Therefore, in the present study, we investigated the antibacterial effect of CuO and ZnO nanoparticles against *S. Gallinarum* infection, as well as their growth-promoting effects in broiler chicks.

The findings of the present study have substantially supported the antibacterial and growth-promoting activities of CuO and ZnO nanoparticles. After the challenge with *S. Gallinarum* infection, the chicks exhibited clinical signs and post-mortem lesions, including dehydration, a pale and shrunken comb, diarrhea, fecal adherence to the vent, ruffled feathers, weakness, anorexia, a bronze-coloured liver, necrotic foci on internal organs, hepatomegaly, and splenomegaly. These clinical signs might be attributed to the rapid dissemination of induced infection of *S. Gallinarum*. However, after treatment with CuO and ZnO nanoparticles, the hydration status, fecal consistency, and feed intake were improved in the nanoparticle-treated groups compared to the positive control group. The recovery from diarrhea and improved hydration status of the chicks suffering from *S. gallinarum* induced infection may be attributed to the antidiarrheal activity of ZnO nanoparticles, which is also supported by a recent study (Kanmani *et al.*, 2023).

In our study, *S. Gallinarum* infection caused a significant increase in the FCR; however, following

treatment with CuO and ZnO nanoparticles, the FCR was significantly decreased in the nanoparticle-treated groups, namely CZNP-1, CZNP-2, and CZNP-3. In addition, after the treatment with CuO and ZnO nanoparticles, the treated groups, viz; CZNP-1, CZNP-2 and CZNP-3 exhibited a substantially increased body weight and carcass weight. However, group CZNP-2 had a significantly increased body weight and decreased FCR as compared to group CZNP-3, which might be associated with the growth-promoting activity of copper at low doses, enhancing feed conversion and nutrient absorption (El-Kassab *et al.*, 2019). The ZnO nanoparticles play a vital role in enhancing the absorption capacity of the gut by increasing the length and width of the intestinal villi, which may correlate with increased nutrient absorption, ultimately leading to increased body weight. Furthermore, Zn may have contributed to the increase in the body weight of chicks provided with CuO and ZnO nanoparticles since it is an essential part of various enzymes involved in the protein and energy metabolism of broilers via boosting the activity of insulin-like growth (Abdel-Wareth *et al.*, 2022). However, the decreased body weight of group CZNP-3 might be attributed to the high-dose cytotoxic effects of Cu²⁺ ions, which hinder the enzymes and cause tissue damage and oxidative stress in the muscle (Morsy *et al.*, 2021). Similarly, high doses of ZnO nanoparticles may also have toxic effects on the immune status and growth performance in broilers (Dosoky *et al.*, 2022).

The findings showed an increased relative organ weight of the liver, intestine, spleen, thymus, and bursa of Fabricius in *S. Gallinarum* infected chicks, which were substantially decreased after treatment with the CuO and ZnO nanoparticles. The *S. Gallinarum* was inoculated

orally to the chicks, which led to a marked increase in bacterial growth in the liver and intestine. The liver and intestine are the primary sites for the colonization of the *S. Gallinarum*, which might lead to the inflammatory response and increased relative organ weight; however, a decreased relative organ weight of the liver, spleen, thymus, bursa of Fabricius and intestine indicate recovery of chicks from *S. Gallinarum* infection after treatment with the CuO and ZnO nanoparticles. A previous study by [Vadalasetty et al. \(2018\)](#) also documented similar results after the treatment of silver nanoparticles against *Campylobacter jejuni* and CuO nanoparticles against *Escherichia coli* infection, respectively, in broilers. A substantial increase in the relative organ weight of the liver and kidney was observed in chicks from the positive control group, which might be attributed to severe hepatitis and nephritis due to *S. Gallinarum* infection ([Abbas, 2021](#)). However, after treatment with the CuO and ZnO nanoparticles, the relative organ weight of the liver and kidney was decreased. The decreased relative organ weight of the kidney and liver might be correlated with the recovery from nephritis and hepatitis due to the anti-inflammatory activity of CuO and ZnO nanoparticles ([Al-Radadi et al., 2022](#); [Singh et al., 2023](#)).

Furthermore, the serum concentration of ALT and AST were considered as the indices of liver damage, while urea and creatinine levels were considered as indices of kidney damage. The increased concentration of ALT, AST, urea, and creatinine in the positive control group indicates severe damage to the liver and kidneys ([Dar et al., 2018](#)). The increased serum concentration of ALT and AST may be correlated with hepatocellular damage, characterized by increased cell membrane permeability and leakage of cytoplasmic ALT into blood ([Han et al., 2019](#); [Liaqat et al., 2023](#)). However, a significant decrease in the serum concentration of ALT, AST, urea, and creatinine was observed in the CuO and ZnO nanoparticles-treated groups, which is consistent with the findings of a previous study ([El-Maddawy et al., 2022](#)). These lower levels of ALT, AST, urea, and creatinine may indicate the antioxidant activity of ZnO nanoparticles, thereby improving the health status of the chicks ([Mohammed et al., 2023](#)). On the other hand, [Ibrahim et al. \(2017\)](#) endorsed that ZnO nanoparticles have no significant effect on ALT and AST levels in broilers. This difference in ALT and AST levels might be linked with the *S. Gallinarum* infection and therapeutic efficacy of ZnO nanoparticles in our study.

CONCLUSION

Based on the results of the current study, supplementation of CuO and ZnO nanoparticles at all

tested doses demonstrated a therapeutic effect against *S. Gallinarum* infection in broilers, with the dose level of 15 + 37.5 mg/kg/d showing optimum efficacy. Supplementation improved FCR, growth performance, and health status, as reflected by enhanced liver and kidney function. However, the present study did not evaluate the cost-effectiveness of CuO and ZnO nanoparticles compared to Florfenicol. Therefore, while these nanoparticles show therapeutic potential as alternatives to antibiotics, further studies are needed to assess MIC₅₀, bacterial load and their economic feasibility before they can be recommended as practical substitutes in poultry production.

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Ethical statement

The experimental chicks were provided with an appropriate environment to express their natural behaviour and freedom from hunger, thirst, pain, and injury during the whole experiment period. The animal care and experimental protocols used in the present study were approved by the Graduate Study Research Board in adherence to the guidelines set forth by the Institution of Animal Care and Use Committee at the University of Agriculture Faisalabad, Pakistan (approval number: DGS/7045-48).

Data availability

Available from the corresponding author upon reasonable request.

Generative AI and AI assisted technology statement

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

Statement of conflict of interest

The authors have declared no conflict of interest.

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