

## In vivo management of *Salmonella gallinarum* infection using CuO and ZnO nanoparticles as antibacterial agents

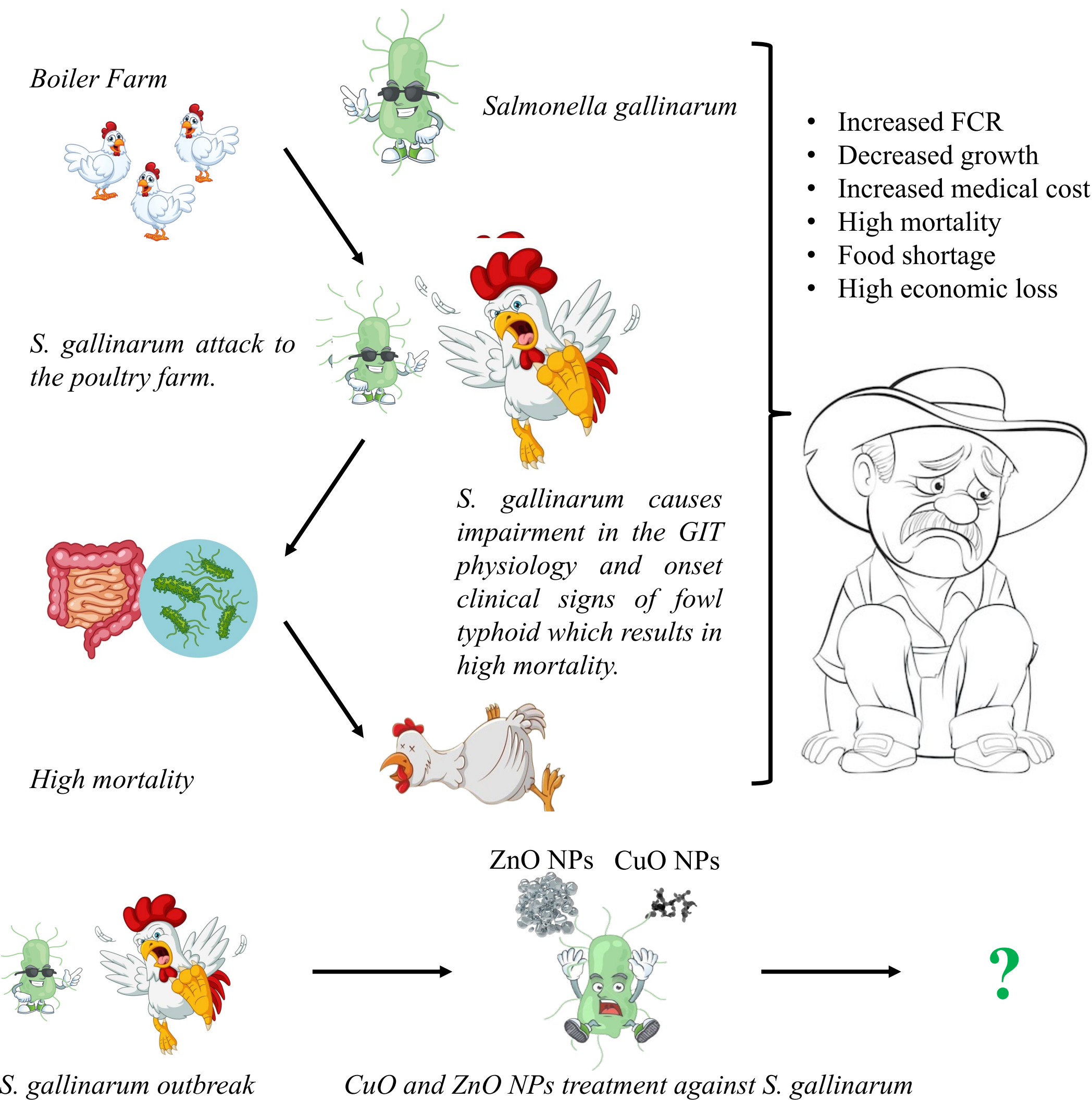
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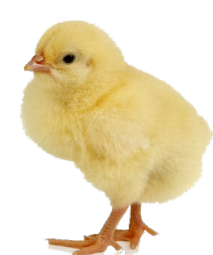
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### INTRODUCTION & AIM

Poultry industry is a major contributor to global food security, providing a huge amount of dietary protein. Its rapid expansion has played a crucial role in addressing food shortages worldwide. However, infectious diseases remain a significant challenge in the poultry industry, leading to reduced production and increased economic burden. Antibiotics are widely used to overcome the problem of infectious diseases that leads to antimicrobial resistance. Developing new antimicrobial drugs is crucial to combat antimicrobial resistance. The CuO and ZnO nanoparticles exhibit promising antimicrobial activity against bacteria. Present study aimed to assess the antimicrobial activity of CuO and ZnO nanoparticles against *Salmonella gallinarum*.



### METHOD



**Day-1** Arrival of chicks (n = 90, Age = 1 day, weight = 42 ± 2 g).

**Day-10** Division of Chicks into 6 individual groups (n = 15) i.e. Control negative, control positive, FLOR-A, CZNP-1, CZNP-2, CZNP-3.

**Day-19** Challenge of *S. gallinarum* to birds of all groups except control negative group.

**Day-22-25** Treatment of Florfenicol to group FLOR-A and Mixture of CuO and ZnO nanoparticles to groups CZNP-1, CZNP-2 and CZNP-3.

**Day-26** Sampling-1, Human sacrifice of birds and collection of serum samples and determination of live weight, carcass weight, relative organ weight (Immune organs and visceral organs).

**Day-26-29** Treatment of Florfenicol to group FLOR-A and Mixture of CuO and ZnO nanoparticles to groups CZNP-1, CZNP-2 and CZNP-3.

**Day-30** Sampling-2, Human sacrifice of birds and collection of serum samples and determination of live weight, carcass weight, relative organ weight (Immune organs and visceral organs).

### RESULTS & DISCUSSION

**Table 1.** Antimicrobial activity of CuO and ZnO nanoparticles and Florfenicol in *S. gallinarum* infected broilers in terms of feed conversion ratio, live body weight and carcass weight.

Growth Parameters		Treatments						p-value
		Control Negative	Control Positive	Florfenicol mg/L FLOR-A (50)	Nanoparticle levels of CuO and ZnO mg/kg/d CZNP-1 (10 + 25)	CZNP-2 (15 + 37.5)	CZNP-3 (20 + 50)	
Feed Conversion Ratio	S-1	1.37 ± 0.05 <sup>a</sup>	2.08 ± 0.05 <sup>b</sup>	1.54 ± 0.02 <sup>cd</sup>	1.54 ± 0.05 <sup>cd</sup>	1.46 ± 0.03 <sup>cd</sup>	1.62 ± 0.03 <sup>c</sup>	0.000
	S-2	1.46 ± 0.04 <sup>a</sup>	2.25 ± 0.04 <sup>b</sup>	1.81 ± 0.04 <sup>c</sup>	1.60 ± 0.03 <sup>cd</sup>	1.53 ± 0.02 <sup>cd</sup>	1.65 ± 0.05 <sup>d</sup>	0.000
Live Body Weight (g)	S-1	1408.75 ± 59.53 <sup>a</sup>	937.80 ± 24.93 <sup>b</sup>	1248.00 ± 17.95 <sup>c</sup>	1249.80 ± 41.67 <sup>c</sup>	1322.00 ± 27.56 <sup>d</sup>	1191.67 ± 24.33 <sup>c</sup>	0.000
	S-2	1821.25 ± 41.34 <sup>a</sup>	1202.25 ± 22.16 <sup>b</sup>	1612.00 ± 32.01 <sup>c</sup>	1668.20 ± 31.59 <sup>cd</sup>	1738.33 ± 46.74 <sup>d</sup>	1475.00 ± 30.62 <sup>c</sup>	0.000
Carcass Weight (g)	S-1	808.50 ± 69.54 <sup>a</sup>	558.20 ± 19.28 <sup>b</sup>	703.75 ± 13.23 <sup>cd</sup>	668.01 ± 23.07 <sup>d</sup>	766.75 ± 35.34 <sup>de</sup>	628.34 ± 32.71 <sup>cd</sup>	0.000
	S-2	1064.25 ± 52.34 <sup>a</sup>	690.25 ± 63.81 <sup>b</sup>	985.01 ± 40.62 <sup>c</sup>	1014.65 ± 27.71 <sup>cd</sup>	1008.67 ± 36.58 <sup>cd</sup>	959.50 ± 28.15 <sup>c</sup>	0.000

<sup>a, b, c, d, e</sup> Mean ± SD in rows with different superscripts are significantly different, n = 3, S-1 and S-2 represent the sampling at 7<sup>th</sup> day and 11<sup>th</sup> 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 nanoparticles treatment at dose 10 + 25 mg/kg/d), CZNP-2 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 15 + 37.5 mg/kg/d), CZNP-3 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 20 + 50 mg/kg/d).

**Table 2.** Antimicrobial activity of CuO and ZnO nanoparticles and Florfenicol in *S. gallinarum* infected broilers in terms of relative organ weight of immune organs (spleen, thymus, and bursa of Fabricius).

Relative Organ Weight (Immune organs)		Treatments						p-value
		Control Negative	Control Positive	Florfenicol mg/L FLOR-A (50)	Nanoparticle levels of CuO and ZnO mg/kg/d CZNP-1 (10 + 25)	CZNP-2 (15 + 37.5)	CZNP-3 (20 + 50)	
Relative Spleen Weight (g)	S-1	0.13 ± 0.008 <sup>a</sup>	0.31 ± 0.03 <sup>b</sup>	0.17 ± 0.007 <sup>a</sup>	0.18 ± 0.06 <sup>a</sup>	0.15 ± 0.03 <sup>a</sup>	0.17 ± 0.009 <sup>a</sup>	0.000
	S-2	0.19 ± 0.01 <sup>a</sup>	0.27 ± 0.02 <sup>b</sup>	0.14 ± 0.005 <sup>a</sup>	0.15 ± 0.01 <sup>ac</sup>	0.17 ± 0.05 <sup>ac</sup>	0.15 ± 0.01 <sup>ac</sup>	0.000
Relative Thymus Weight (g)	S-1	0.45 ± 0.06 <sup>a</sup>	0.42 ± 0.12 <sup>b</sup>	0.30 ± 0.15 <sup>a</sup>	0.47 ± 0.09 <sup>a</sup>	0.36 ± 0.24 <sup>a</sup>	0.39 ± 0.03 <sup>a</sup>	0.000
	S-2	0.48 ± 0.14 <sup>a</sup>	0.27 ± 0.11 <sup>b</sup>	0.31 ± 0.05 <sup>a</sup>	0.44 ± 0.22 <sup>a</sup>	0.49 ± 0.07 <sup>a</sup>	0.42 ± 0.52 <sup>a</sup>	0.000
Relative Bursa Weight (g)	S-1	0.12 ± 0.01 <sup>a</sup>	0.29 ± 0.04 <sup>b</sup>	0.13 ± 0.02 <sup>a</sup>	0.13 ± 0.01 <sup>a</sup>	0.12 ± 0.03 <sup>a</sup>	0.15 ± 0.02 <sup>a</sup>	0.000
	S-2	0.11 ± 0.01 <sup>a</sup>	0.24 ± 0.06 <sup>b</sup>	0.12 ± 0.01 <sup>a</sup>	0.12 ± 0.02 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.11 ± 0.01 <sup>a</sup>	0.451

<sup>a, b, c, d</sup> Mean ± SD in rows with different superscripts are significantly different, n = 3, S-1 and S-2 represent the sampling at 7<sup>th</sup> day and 11<sup>th</sup> 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 nanoparticles treatment at dose 10 + 25 mg/kg/d), CZNP-2 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 15 + 37.5 mg/kg/d), CZNP-3 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 20 + 50 mg/kg/d).

**Table 3.** Antimicrobial activity of CuO and ZnO nanoparticles and Florfenicol in *S. gallinarum* infected broilers in terms of relative organ weight of visceral organs (lungs, heart, kidney, liver, gizzard, proventriculus and intestine).

Relative Organ Weight (Visceral Organs)		Treatments						p-value
		Control Negative	Control Positive	Florfenicol mg/L FLOR-A (50)	Nanoparticle levels of CuO and ZnO mg/kg/d CZNP-1 (10 + 25)	CZNP-2 (15 + 37.5)	CZNP-3 (20 + 50)	
Relative Lungs Weight (g)	S-1	0.47 ± 0.03 <sup>a</sup>	0.85 ± 0.04 <sup>b</sup>	0.49 ± 0.03 <sup>a</sup>	0.47 ± 0.03 <sup>a</sup>	0.50 ± 0.04 <sup>a</sup>	0.49 ± 0.03 <sup>a</sup>	0.000
	S-2	0.46 ± 0.02 <sup>a</sup>	0.91 ± 0.01 <sup>b</sup>	0.46 ± 0.02 <sup>a</sup>	0.47 ± 0.03 <sup>a</sup>	0.49 ± 0.03 <sup>a</sup>	0.48 ± 0.02 <sup>a</sup>	0.000
Relative Heart Weight (g)	S-1	0.49 ± 0.02 <sup>a</sup>	0.56 ± 0.01 <sup>ab</sup>	0.49 ± 0.05 <sup>ab</sup>	0.51 ± 0.06 <sup>ab</sup>	0.50 ± 0.02 <sup>ab</sup>	0.57 ± 0.01 <sup>b</sup>	0.013
	S-2	0.67 ± 0.03 <sup>a</sup>	0.78 ± 0.02 <sup>b</sup>	0.59 ± 0.02 <sup>a</sup>	0.57 ± 0.02 <sup>cd</sup>	0.56 ± 0.05 <sup>cd</sup>	0.54 ± 0.01 <sup>cd</sup>	0.000
Relative Kidney Weight (g)	S-1	0.71 ± 0.02 <sup>a</sup>	1.22 ± 0.19 <sup>b</sup>	0.89 ± 0.017 <sup>a</sup>	0.91 ± 0.02 <sup>a</sup>	0.77 ± 0.02 <sup>a</sup>	0.92 ± 0.19 <sup>a</sup>	0.000
	S-2	0.70 ± 0.03 <sup>a</sup>	1.10 ± 0.02 <sup>b</sup>	0.73 ± 0.01 <sup>ac</sup>	0.79 ± 0.09 <sup>a</sup>	0.68 ± 0.04 <sup>a</sup>	0.85 ± 0.05 <sup>ac</sup>	0.000
Relative Liver Weight (g)	S-1	4.15 ± 0.07 <sup>a</sup>	7.23 ± 0.36 <sup>b</sup>	5.09 ± 0.11 <sup>cd</sup>	4.86 ± 0.14 <sup>d</sup>	5.00 ± 0.15 <sup>d</sup>	5.43 ± 0.054 <sup>e</sup>	0.000
	S-2	3.81 ± 0.14 <sup>a</sup>	6.69 ± 0.49 <sup>b</sup>	4.44 ± 0.34 <sup>cd</sup>	4.76 ± 0.51 <sup>d</sup>	3.40 ± 0.21 <sup>ac</sup>	4.98 ± 0.53 <sup>d</sup>	0.000
Relative Gizzard Weight (g)	S-1	1.47 ± 0.089 <sup>a</sup>	2.14 ± 0.18 <sup>b</sup>	1.71 ± 0.07 <sup>ac</sup>	1.72 ± 0.14 <sup>d</sup>	1.63 ± 0.04 <sup>ac</sup>	1.69 ± 0.04 <sup>ac</sup>	0.000
	S-2	1.82 ± 0.06 <sup>a</sup>	2.01 ± 0.16 <sup>b</sup>	2.07 ± 0.12 <sup>a</sup>	1.93 ± 0.15 <sup>a</sup>	1.98 ± 0.03 <sup>a</sup>	2.13 ± 0.18 <sup>a</sup>	0.131
Relative Proventriculus Weight (g)	S-1	0.44 ± 0.02 <sup>a</sup>	0.65 ± 0.05 <sup>b</sup>	0.48 ± 0.02 <sup>ac</sup>	0.47 ± 0.03 <sup>ac</sup>	0.51 ± 0.02 <sup>a</sup>	0.49 ± 0.02 <sup>ac</sup>	0.000
	S-2	0.48 ± 0.01 <sup>a</sup>	0.51 ± 0.02 <sup>a</sup>	0.46 ± 0.01 <sup>a</sup>	0.47 ± 0.01 <sup>a</sup>	0.48 ± 0.06 <sup>a</sup>	0.52 ± 0.03 <sup>a</sup>	0.058
Relative Intestine Weight (g)	S-1	5.58 ± 0.13 <sup>a</sup>	8.43 ± 0.31 <sup>b</sup>	5.89 ± 0.27 <sup>ac</sup>	5.91 ± 0.42 <sup>ac</sup>	5.76 ± 0.19 <sup>b</sup>	6.69 ± 0.27 <sup>b</sup>	0.000
	S-2	5.17 ± 0.14 <sup>a</sup>	7.93 ± 0.24 <sup>b</sup>	5.68 ± 0.16 <sup>ac</sup>	5.58 ± 0.04 <sup>ac</sup>	5.47 ± 0.36 <sup>ac</sup>	6.33 ± 0.33 <sup>a</sup>	0.000

<sup>a, b, c, d</sup> Mean ± SD in rows with different superscripts are significantly different, n = 3, S-1 and S-2 represent the sampling at 7<sup>th</sup> day and 11<sup>th</sup> 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 nanoparticles treatment at dose 10 + 25 mg/kg/d), CZNP-2 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 15 + 37.5 mg/kg/d), CZNP-3 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 20 + 50 mg/kg/d).

**Table 4.** Antimicrobial activity of CuO and ZnO nanoparticles and Florfenicol in *S. gallinarum* infected broilers in terms of alanine transaminase, alanine aminotransferase, Urea and Creatinine.

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

<sup>a, b, c, d, e, f</sup> Mean ± SD in rows with different superscripts are significantly different, n = 3, S-1 and S-2 represent the sampling at 7<sup>th</sup> day and 11<sup>th</sup> 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 nanoparticles treatment at dose 10 + 25 mg/kg/d), CZNP-2 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 15 + 37.5 mg/kg/d), CZNP-3 (*S. gallinarum* infection, CuO + ZnO nanoparticles treatment at dose 20 + 50 mg/kg/d).

### CONCLUSION

Based on the findings of the present study, it was concluded that the supplementation of CuO and ZnO nanoparticles at any dose has a therapeutic effect against *S. gallinarum* infection in broilers and can be used as substitute of Florfenicol, however the CuO and ZnO nanoparticles at dose level 15 + 37.5 mg/kg/d have optimum efficacy among all other dose level. Moreover, the supplementation of CuO and ZnO nanoparticles to the chicks suffering from fowl typhoid can improve the FCR, growth and the health status by enhancing the liver and kidney function.

### FUTURE WORK / REFERENCES

The CuO and ZnO nanoparticles are currently water insoluble which limits their commercial use in poultry industry. Therefore, further studies are suggested to find an effective excipient which makes CuO and ZnO nanoparticles water soluble to make their commercial use possible.

- Raza, M. A., Eungyung, K., Shakeel, M., Fiaz, M., Ma, L., Kim, H., Kim, C.Y., Kim, D., Park, K., Javed, M.T. and Kim, M., 2023. Evaluation of zinc oxide and copper oxide nanoparticles as potential alternatives to antibiotics for managing fowl typhoid in broilers. *Journal of Animal Science and Technology*.