Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 4, 687-691

Antibacterial efficacy of Zinc oxide nanoparticles against *Escherichia coli* experimental infection in broiler chickens

Mohamed Shakal[1], Hanan S. Khalefa[2], Heba M. Salem[1*]

[1]*Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.*
[2]*Department of veterinary hygiene and management, Faculty of Veterinary Medicine, 12211 Giza, Cairo University, Egypt.*

ARTICLE INFO

Recieved: 06 January 2024

Accepted: 19 March 2024

***Correspondence:**

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

Keywords:

Broiler chicks
E. coli O78
FCR
Immunity
Zinc oxide nanoparticles

ABSTRACT

A serious problem within the poultry sector is avian colibacillosis, which can be found either as a primary or secondary infection, inducing huge financial losses in poultry production and posing a zoonotic threat to humans. *Escherichia coli* (*E. coli*) avian pathogenic strains have recently demonstrated multi-drug resistance. Therefore, this investigation aimed to assess the efficacy of zinc oxide nanoparticles (ZnO-NPs) against avian pathogenic *E. coli* O78 in broiler chickens. Thus, 147 broiler chicks were allocated into seven groups, each with 21 birds with triplicates of seven birds each. In group G1, control birds are negative; in group G2, control-positive infected birds are positive for *E. coli* O87; G3, infected-supplied with 1 mg/L ZnO-NPs in drinking water; G4, infected-treated with 2 mg/L ZnO-NPs in drinking water; G5, infected-treated with antibiotics; G6, supplied for continuous 35 days with 1 mg/L ZnO-NPs; and G7 treated for continuous 35 days with 2 mg/L ZnO-NPs. During 5 weeks observation duration, bird performance, mortalities were monitored, and serum samples were gathered to evaluate the immune system. It was noticed that the birds treated with ZnO-NPs (G6 and G7) gained more weight (P = 0.017) and weighed significantly more than the control-infected birds (G2). On days 1 through 35, there was a significant improvement in the FCR of the infected birds that supplied with ZnO-NPs (G4, G6, and G7) (P =0.034). HI titers did not differ between the groups at 21 and 28 days. In conclusion, in comparison to the non-treated group, treatment with both doses of ZnO-NPs led to reduction in the colonization of *E. coli* in the intestine and cecum, a reduction in the severity of clinical signs, & mortalities, as well as improve birds' performance but no significant differences in humoral immune response against ND vaccine among different groups.

Introduction

Avian pathogenic *E. coli* (APEC) is a widespread bacterial pathogen causing financial losses in the poultry sector (El-Shenawy *et al*., 2023). Avian colibacillosis could be considered as primary or secondary avian pathogen (Ali *et al*., 2020). As it known, *E. coli* could induce septicemic or localized infections including air saculitis, salpingitis, arthritis, sternal bursitis, spondylitis, osteomyelitis, omphalitis, enteritis, pan-ophthalmitis, cellulitis, coli-granuloma, swollen head syndrome, complicated chronic respiratory disease (CCDR) and acute vaginitis in turkey (Swelum *et al*., 2021). Unfortunately, many APEC appeared multi-drug resistant against most common antibiotics (Solà-Ginés *et al*., 2015; Yousef *et al*., 2023) and this is why science has turned to searching for safe alternatives to antibiotics (Abd El-Hack *et al*., 2022a; El-Saadony *et al*., 2022). Nanoparticles (NPs) have proven to be effective therapeutic agents because of their exceptional physicochemical features, attributes, and physically applicable mode of action that is applicable in different fields (Abd El-Ghany *et al*., 2021; Salem *et al*., 2021). Zinc is a crucial microelement that influence birds' performance, meat, and carcass characteristics in addition, broiler under thermal stress supplied with 40 mg/kg dietary zinc revealed improved in birds' productivity and antioxidant status (Rao *et al*., 2016). The majority of NPs' antimicrobial activity comes from their diffusion and penetration through organisms' cell membranes, which causes oxidative stress and the eventual death of the microbial cells (Abd El-Hack *et al*., 2022b). Likewise, ZnO-NPs pass the villi via direct penetration and grant more positive impacts at small levels for birds, further become extremely bioavailable, applying an excellent efficiency, and become more bioactive than bulk formulation of zinc (Abdel-Wareth *et al*., 2022). Thus, the current work goaled to assess the antibacterial efficacy of ZnO-NPs against experimentally induced colibacillosis in broiler chickens and assessment of its impact on birds' performance.

Materials and methods

Zinc oxide nanoparticles

The characters and the in vitro antibacterial activity of ZONPs were previously cited by Shakal *et al*. (2024).

Experimental birds

152, one day old broiler chicks were used in this study. Five chicks at the day of arrival were sacrificed and the internal organs (yolk sac, intestine, spleen, lung, liver, and heart) were cultured to ensure absence of pathogenic *E. coli* infection. Then the rest 147 birds were allotted into the different experimental groups. The birds were kept in separately cleaned and disinfected houses & delivered with feed & water ad libitum during the experimental period. The birds administered all the required vaccination at the recommended time.

Experimental design

147, day old broiler chicks were distributed into seven groups (21 for each divided into triplicates with 7 birds each). G1, control negative birds; G2, control positive infected birds with *E. coli* O87; G3, infected- supplied with 1mg/L ZnO-NPs; G4, infected supplied with 2mg/L ZnO-NPs; G5, infected treated with antibiotic; G6, supplied with 1mg/L ZnO-NPs continuous for 35 days, and G7, supplied with 2mg/L ZnO-NPs continuous for 35 days.

The *E. coli* O78 used for induction of the infection was kindly provided from Microbiology department Faculty of Veterinary Medicine, Cairo University (Yousef *et al*., 2023). At one day old, each chicken in the infected groups was orally supplied with 1 ml of saline including 10^8 colony

ISSN: 2090-6277/2090-6269/

forming unit (CFU) fresh colonies of *E. coli*/ ml for two successive days.

The selection of antibiotics was based on antibiotic sensitivity test of the challenged bacterial strain to different antibiotics. The birds in G5 supplied with 100 mg/L doxycycline 20% in drinking water for 5 continuous days at 3 days post the experimental infection and the start of clinical signs and mortalities.

Post experimental infection, all the birds were observed daily for clinical signs and mortalities. Dead birds were subjected to postmortem (PM) examination.

Post-mortem lesions

After the onset of clinical signs, post-treatment and at the end of the study (3 time points); one bird was slaughtered per replicate (3 birds/group), and freshly dead birds post infection were exposed for PM examination. At necropsy the lungs, liver, air sacs, pericardium, heart, kidney, spleen, and intestinal tract were examined then any changes in these organs were recorded.

Bacterial re-isolation

Samples from liver, heart, lungs, intestine, and spleen were collected from the sacrificed birds, after onset of clinical signs (3dpi, 5days old), post-treatment (10 days old) and at 35 days old for *E. coli* re-isolation and counting on selective EMB agar (Oxoid).

Performance

Body weight and feed conversion rate

Body weight gain (BWG), all birds were weighted individually weekly for 5 weeks. Feed consumption was assessed on the same days as birds weighing. Feed conversion rate (FCR) was calculated via formula (g feed/g live BWG) according to Timmerman *et al.* (2006).

E. coli caecal and intestinal counts

At 10, and 35 days old, one gram from each intestinal and caecal contents were gathered separately from each bird (3birds/group) then serially diluted in sterile PBS to 1:100, 1:1000, & 1:10000 and 0.1 ml of each dilution and streaked on the surface of EMB agar and incubated for 24 hours at 37°C; typical *E. coli* colonies (metallic green) on EMB agar were counted and recorded as colony-forming units (CFU) per gram. The colonies picked & verified by criteria of Carrido *et al.* (2004).

Immunological evaluation

Humoral immunity (HI)

Anti- ND vaccine antibody titers were assessed by gathering blood samples at 21- and 28-days post ND vaccination from wing veins of randomly chosen 5 birds/group then sera were exposed to HI test as described by Swayne *et al.* (1998).

Statistical analysis

A one-way ANOVA and Tukey's multiple comparison post-hoc test was applied to analyze significant differences among the different fish groups using SPSS 18 software. This study presents the data as the mean and standard error of the mean. A P-value of smaller than 0.05 was statistically significant.

Results

Table 1 summarizes the total mortality rates. The greatest cumulative mortality rates were reported in control positive groups G2 (47.62%) and G3 (23.80%), followed by G4, G5, and G7 (19.05%), and finally, G1, G6, and G7 showed the lowest mortality rate (4.7%). The ZnO-NPs-treated groups showed a significant decrease in mortality rates in contrast to the positive control group (G2).

Severe symptoms of infection were observed in the control group who were positively infected with the *E. coli* O78 challenged group, including a pasty vent with brownish-colored watery droppings and brownish-colored diarrhea (Figure 1).

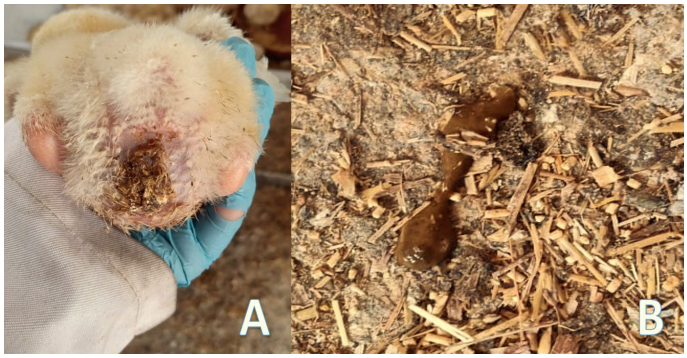

Fig. 1. Clinical signs in control positive *E. coli* O78 challenged group showing A: Pasty vent with brownish color watery droppings, B: Brown color diarrhea in the litter.

Clinical symptoms were less severe in the groups treated with both concentrations of ZnO-NPs & antibiotics, while the control group exhibited no clinical signs.

The severity of PM lesions was less severe in the treated birds than in the control negative group & no PM lesions were observed in the sacrificed birds from control negative group. As shown by Figure 2, some birds with the *E. coli* O78 challenged group were found to have unabsorbed yolk sacs, enteritis, pericarditis, perihepatitis, air saculitis, subcapsular hemorrhage on the liver, nephritis, and distended ureters with urates. Moreover, PM of the control positive *E. coli* O78 challenged group showed that the two blind caeca were filled with frothy gaseous brownish color contents (Figure 3).

Table 2 shows a significant difference in body weight (BW) ($p < 0.05$)

Table 1. Cumulative mortality rate among different experimental groups.

	1st week	2nd week	3rd week	4th week	5th week	Total mortality (total=21)	%
G1	1	0	0	0	0	1	4.76
G2	5	3	2	0	0	10	47.62
G3	2	3	0	0	0	5	23.8
G4	3	1	0	0	0	4	19.05
G5	2	2	0	0	0	4	19.05
G6	0	1	0	0	0	1	4.76
G7	0	0	0	0	1	1	4.76

Table 2. Effect of ZnO-NPs and antibiotic treatments on body weight, body weight gain, feed intake, & feed conversion ratio in broiler infected with (Mean ±SE).

	Different treatment groups							SEM	P value
	G1	G2	G3	G4	G5	G6	G7		
					Body weight (g)				
Day one	51	50.9	50.8	51.01	51.03	51	51	0.00	1
21 days	872^{ab}	834^{b}	852^{ab}	862^{ab}	837^{b}	894^{ab}	933^{a}	0.01	0.03
35 days	2420^{ab}	2356^{b}	2511^{ab}	2554^{ab}	2472^{ab}	2531^{ab}	2671^{a}	0.02	0.02
					Body weight gain (g)				
1–21 days	821^{ab}	783^{b}	801^{ab}	810^{ab}	803^{ab}	842^{ab}	880^{a}	8.7	0.06
22-35 days	1548	1522	1650	1692	1635	1637	1745	23.31	0.14
1–35 days	2369^{ab}	2305^{b}	2460^{ab}	2503^{ab}	2421^{ab}	2480^{ab}	2620^{a}	24.57	0.02
					Feed intake (g)				
1–21 days	1023	1092	1097	1102	1115	1097	1088	3.34	
22-35 days	2820	2710	2822	2827	2837	2801	2757	5.19	
1–35 days	3843	3802	3919	3929	3952	3898	3845	6.11	
					Feed conversion ratio (g feed/g gain)				
1–21 days	1.257^{ab}	1.404^{a}	1.378^{ab}	1.369^{ab}	1.402^{a}	1.311^{ab}	1.237^{b}	0.02	0.01
22-35 days	1.84	1.79	1.74	1.70	1.76	1.72	1.59	0.03	0.21
1–35 days	1.632^{ab}	1.657^{a}	1.606^{ab}	1.579^{ab}	1.640^{a}	1.577^{ab}	1.473^{b}	0.02	0.03

Means with different lowercase letters in the same row indicate significant differences at P < 0.05. SE: standard error, 3 replicates/group (21birds/group).

between groups from the third week until 35 days old. BW was the highest reported in G7 (2671 g), followed by G6 & G4 that were treated with ZnO-NPs or infected and administered with ZnO-NPs. The infected group (G2) recorded 2420 g, and the infected group with antibiotic treatment recorded 2560 g. BWG was significant (p< 0.05) for all groups in the experiment. In general, the birds treated with ZnO-NPs (G6 and G7) showed significantly higher body weights (P =0.018) & BWG (P =0.017) than the control birds (G2).

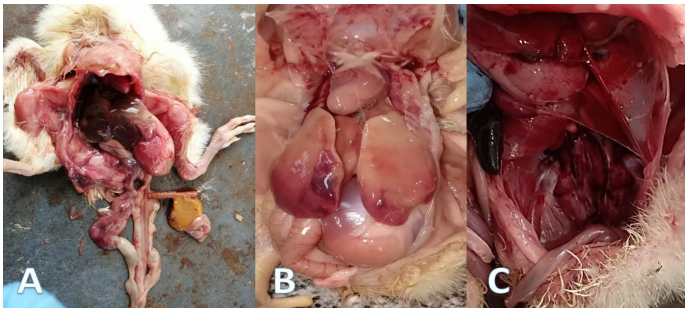

Fig. 2. Postmortem of control positive *E. coli* O78 challenged group showing A: unabsorbed yolk sac and congested liver, B: subcapsular hemorrhage on liver, C: nephritis and ureters distended with urates.

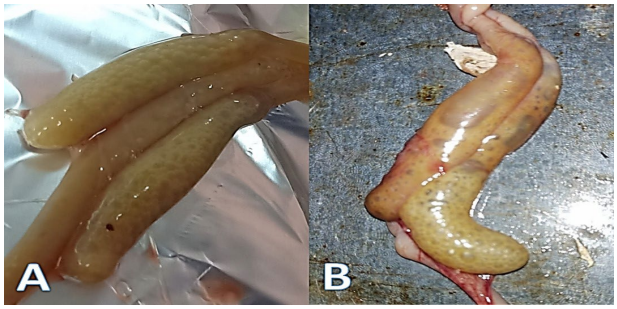

Fig. 3. Postmortem of control positive *E. coli* O78 challenged group showing A and B: the two blind caeca filled with frothy gaseous brownish color contents.

The FCR of the infected birds receiving ZONPs (G3 and G4) improved significantly between days 1 and 35 (P =0.034).

Table 3 illustrates the intestinal and cecal loads of *E. coli* at 10 and 35 days. In comparison to infected birds in G2, G6 & G7 had significantly lesser counts (P = 0.001). Additionally, the intestinal *E. coli* counts of G3, G4, G6, and G7 were significantly lesser than those of G1 & G2 (P <0.05).

Table 3. Effect of ZnO-NPs on broiler chicken cecal and intestinal *E. coli* counts after at 10-, & 35-day post infection (Mean± SE).

Groups	Cecal		Intestinal	
	10 days	35 days	10 days	35 days
G1	9.14^{b}	9.33^{ab}	8.7947^{b}	8.71
G2	9.80^{b}	9.75^{a}	9.7860^{c}	9.80
G3	7.43^{a}	9.31^{ab}	7.1754^{ab}	8.69
G4	7.28^{a}	9.30^{ab}	6.7195^{a}	8.66
G5	7.29^{a}	9.27^{aab}	6.7347^{a}	8.62
G6	7.42^{a}	7.40^{b}	6.9248^{a}	6.83
G7	7.39a	7.33^{b}	6.7518^{a}	6.68
SEM	0.28	0.25	0.33	0.32
P value	0.02	0.01	0.03	0.05

Means with different lowercase letters in the same row indicate significant differences at P < 0.05. SE: standard error, 3 replicates/group (21birds/group).

Figure 4 illustrates that *E. coli* re-isolation and number were significantly lower in infected treated G4 than in G2.

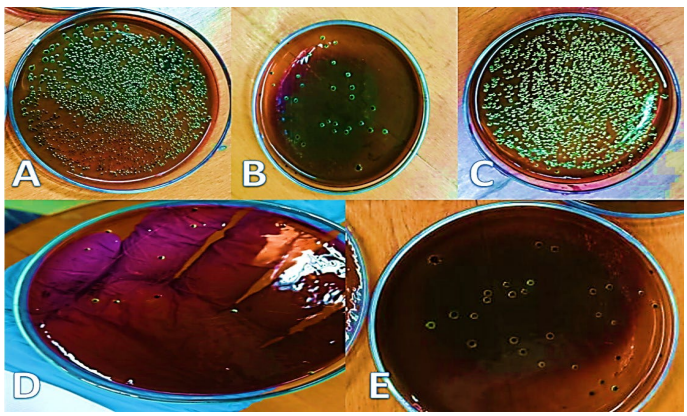

Fig. 4. Microbiological *E. coli* count from the caecal content at the end of the treatment for the same dilution (1x 106) in all experimental groups: A: Group 2, control positive birds showing uncountable colonies, B: Group 4, infected nanoparticle treated showing countable colonies (2mg/L), C: Group 5, infected antibiotic treated showing countable colonies, D: Group 7, nanoparticles treated all over the observation period showing countable colonies, E: Group 1, control negative birds showing countable colonies.

Figure 5 shows HI titer values post ND vaccination at 21 or 28 days, there were no significant differences in HI titers between different groups.

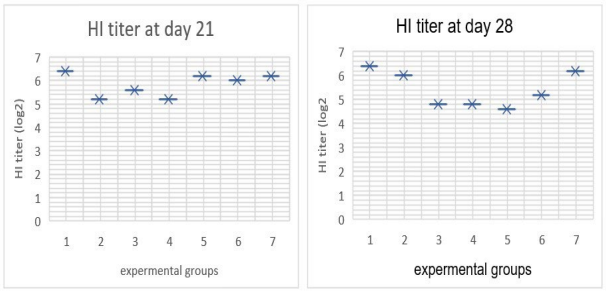

Fig. 5. Effect of ZnO-NPs on broiler chicken HI titer against NDV.

Discussion

Recent technologies are being widely applied to poultry sector like nanotechnology (Nabi *et al*., 2020; Reda *et al*., 2020). ZnO-NPs have a dose-dependent effect on bird performance and physiological state of poultry and livestock (Mahmoud *et al*., 2021). Like other metal oxides, ZnO-NPs exhibit antibacterial activity against a wide variety of bacteria. This study demonstrated that ZnO-NPs could exhibit potent anti-*E. coli* activities.

As an outcome of the use of ZnO-NPs, *in vivo* efficacy was demonstrated through a reduction of cumulative mortalities (Table 1) in either group infected or treated with ZnO-NPs. The evidence indicates that ZnO-NPs are highly effective antibacterial agents. Also, it has been demonstrated that ZnO-NPs stop the growth of bacteria like *E. coli* and Staphylococcus aureus; during the interaction between ZnO-NPs and microbes, Zn^{2+} ions are released through channels, destroying bacterial viability (Brayner *et al*., 2006).

In this investigation, clinical signs included ruffled feathers, general depression, and pasty vents with brownish color diarrhea as observed in control positive *E. coli* O78 challenged birds (Figure 1), birds of G3 and G4 exhibited similar findings (varying degrees of diarrhea). According to Ibrahim *et al.* (2019), the observed signs of naturally infected chicks with *E. coli* are unthriftines, appetite loss, decreased BWG, respiratory distress, dropped wings, closed eyes, and general congestion. The PM examination of the freshly dead control positive *E. coli* O78 challenged group in Figures 2 and 3 revealed the presence of un-absorbed yolk sac, subcapsular hemorrhage on the liver, enteritis, pericarditis, perihepatitis, air sacculitis, nephritis, and ureters distended with urates in some birds, and the two blind caeca filled with frothy gaseous brownish color contents. Our findings were in concur with Kemmett *et al*. (2014) who noticed similar PM lesions (pericarditis, perihepatitis, air sacculitis, and hepatic lesions) in chickens due to avian pathogenic *E. coli* infection. Also, Singh *et al*. (2018) recorded similar PM lesions in chickens due to *E. coli* infection.

In Table 2, we summarized the impacts of ZnO-NPs supplementation on broiler chick body weight at various experimental levels (alone in different concentrations or combination with infected *E. coli* groups). In all dietary treatments, the birds had approximately the same initial BW at the beginning of the experiment. In comparison with other groups at all experimental weeks, chicks supplemented with ZnO-NPs at both levels (1 mg & 2 mg) exhibited the heaviest live body weight. The enhancement in chicken performance was noted during the following 14 days (from the 14th to the 28th days) after administration of ZnO-NPs over 5 days (14th to 19th day).

ZnO-NPs supplementation affects FC & FCR; because of these treatments, the amount of FC per chick per week was changed. In infected broiler chickens (G3 and G4), the addition of ZnO-NPs resulted in numerically higher weekly and cumulative FC than in controls. Regarding FCR, Table 2 indicates that G4 and G4 had the highest FCRs (1.473 and 1.579), respectively, compared to G1 and G2. According to previous studies published in the literature, ZnO-NPs at 20–60 mg/kg of diet can enhance BWG and FCR of broiler chickens (Mahmoud *et al*., 2020). As reported by Hafez *et al*. (2017), ZnO-NPs are known to have a unique property that increases intestinal absorption capacity by increasing villi length, width, mucosal length, and crypt depth. Therefore, the higher absorption efficiency of ZnO-NPs results in advanced Zn bioavailability (Abedini *et al*., 2018).

In poultry farms, APEC is the main etiology of colibacillosis (Swelum *et al*., 2021). This organism can cause enteric and extraintestinal infections in a syndrome that is characterized by diarrhea and/or enteritis (Singh *et al*., 2018). APEC can colonize chickens' respiratory and intestinal tracts. According to Hussain *et al*. (2022), APEC isolates were found in the feces of broilers that had signs of colibacillosis. Saha *et al*. (2020) noted that APEC isolates were found in broiler droppings at the highest level (33.33%). The cecal and intestinal loads of *E. coli* in the G6 and G7 groups at 10- and 30-days post-infection were significantly lower (P = 0.001) than in G2.

Additionally, intestinal *E. coli* counts were significantly lower in G3, G4, G6, and G7 when compared with G1 and G2. The results of this study are regular with those of a prior study in which ZONPs showed antimicrobial effect against *E. coli* in vitro, the antibacterial effects of metal nanoparticles may be caused by disruption & penetration of the microorganism's cell membrane, resulting in injury to the cell wall and cytoplasmic leakage (Abd El-Ghany *et al*., 2021). Additionally, Hassan *et al*. (2016) found that ZnO-NPs penetrate intestinal cells directly and exert more beneficial effects at a lower dose than zinc in bulk formulation, as well as being highly bioavailable, exerting greater value, and become more bioactive than zinc in bulk formulation (Hussain *et al*, 2018). Among their many applications are their antimicrobial properties against certain pathogens (Hussain *et al*, 2018).

As mentioned in Figure 5, chickens treated with ZnO-NPs did not exhibit any significant changes in their immune response against ND vaccine. Also, there was no significant difference among the different groups in terms of HI titers at 21 & 28 days. Our results disagreed with Khajeh *et al.* (2018) who found that the administration of 50 mg ZnO-NPs /kg feed in combination with probiotic (10^{10}CFU/kg) improve the chickens' humoral immunity and these variations may be contributed to the usage of probiotics parallel with ZnO-NPs or due to the usage of different dose and route of admiration (50 mg ZnO-NPs /kg diet).

Conclusion

Treatment with both 1 mg/L & 2 mg/L ZnO-NPs in drinking water decrease mortalities and improve birds' performance and reduce the colonization of *E. coli* in the intestine and cecum, but it has no significant differences on humoral immune response against ND vaccine among different groups.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abd El-Ghany, W.A., Shaalan, M., Salem H.M., 2021. Nanoparticles applications in poultry production: An updated review. Worlds Poult. Sci. J. 77, 001-1025.

Abd El-Hack, M.E., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Soliman, M.M., Youssef, G.B., Taha, A.E., Soliman, S.M., Ahmed, A.E., El-Kott, A.F., Al Syaad, K.M., Swelum, A.A., 2022a. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult. Sci. 101, 101696.

Abd El-Hack, M.E., El-Saadony, M. T., Saad, A. M., Salem, H. M., Ashry, N.M., Abo Ghanima, M. M., Shukry, M., Swelum, A. A., Taha, A. E., El-Tahan, A. M., AbuQamar, S. F., El Tarabily, K. A., 2022b. Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. Poult. Sci. 101, 101584.

Abdel-Wareth, A.A., Hussein, K.R., Ismail, Z.S., Lohakare, J., 2022. Effects of zinc oxide nanoparticles on the performance of broiler chickens under hot climatic conditions. Biol. Trace Elem. Res. 200, 5218-5225.

Abedini, M., Shariatmadari, F., Torshizi, M.A.K., Ahmadi, H., 2018. Effects of zinc oxide nanoparticles on performance, egg quality, tissue zinc content, bone parameters, and antioxidative status in laying hens. Biol. Trace Elem. Res. 184, 259–267.

Ali, S.S., Sonbol, F.I., Sun, J., Hussein, M.A., Hafez, A.E.E., Abdelkarim, E.A., Kornaros, M., Ali, A. Azab, M., 2020. Molecular characterization of virulence and drug resistance genes-producing *Escherichia coli* isolated from chicken meat: Metal oxide nanoparticles as novel antibacterial agents. Microb. Pathog. 143, 104164.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M.F., Fiévet, F., 2006. Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. 6, 866–870.

Carrido, M.N., Skjerheim, M., Oppegaard H., Sqrum, H., 2004. Acidified litter benefits the intestinal flora balance of broiler chickens. Appl. Environ. Microbiol. 70, 5208-5215.

El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Abd El-Mageed, T.A., Soliman, S.M., Khafaga, A.F., Swelum, A.A., Ahmed, A.E., Alshammari, F.A., Abd El-Hack, M.E., 2022. The control of poultry salmonellosis using organic agents: an updated overview. Poult. Sci. 101, 101716.

El-Shenawy, F.A., El-Sherbeny, E.M.E., Kassem, S., 2023. Efficacy of zinc oxide and copper oxide nanoparticles on virulence genes of avian pathogenic *E. coli* (APEC) in broilers. BMC Vet. Res. 19, 108.

Hafez, A., Hegazi, S.M., Bakr, A.A., Shishtawy, H.E., 2017. Effect of zinc oxide nanoparticles on growth performance and absorptive capacity of the intestinal villi in broiler chickens. Life Sci. J. 14, 67-72.

Hassan, A.A., Mansour, M.K., Oraby, N.H., Mohamed, A.M., 2016. The efficiency of using silver nanoparticles singly and in combination with traditional antimicrobial agents in control of some fungal and bacterial affection of buffaloes. Int. J. Curr. Res. 8, 29758–29770.

Hussain, H.L., Lqbal, Z., Lqbal, M., Kuang, X., Wang, Y., Yang, L., Lhsan, A., Aqib, A.I., Kaleem, Q.M., Gu, Y., Hau, H., 2022. Coexistence of virulence and β-lactamase genes in avian pathogenic *Escherichia coli*. Microb. Pathog.163, 105389.

Hussain, Z., Khan, J.A., Anwar, H., Andleeb, N., Murtaza, S., Ashar, A., Arif, I., 2018. characterization, and pharmacological evaluation of zinc oxide nanoparticles formulation. Toxicol. Ind. Health 34,753–763

Ibrahim, R.A., Cryer, T.L., Lafi, S.Q., Abu Basha, E., Good, L., Tarazi, Y.H., 2019. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Vet. Res. 15, 159.

Kemmett, K., Williams, N.J., Chaloner, G., Humphrey, S., Wigley, P., Humphrey, T., 2014. The contribution of systemic *Escherichia coli* infection to the early mortalities of commercial broiler chickens. Avian Pathol. 43, 37-42.

Khajeh Bami, M., Afsharmanesh, M., Salarmoini, M., Tavakoli, H., 2018. Effect of zinc oxide nanoparticles and Bacillus coagulans as probiotic on growth, histomorphology of intestine, and im-

mune parameters in broiler chickens. Comp. Clin. Path. 27, 399-406.
Mahmoud, M.A., Yahia, D., Abdel-Magiud, D.S., Darwish, M.H., Abd-Elkareem, M., Mahmoud, U.T., 2021. Broiler welfare is preserved by long-term low-dose oral exposure to zinc oxide nanoparticles: Preliminary study. Nanotoxicology 15, 605–620.
Mahmoud, U.T., Abdel-Mohsein, H.S., Mahmoud, M.A., Amen, O.A., Hassan, R.I., Abd-El-Malek, A.M., Rageb, S.M., Waly, H.S., Othman, A.A., Osman, M.A., 2020. Effect of zinc oxide nanoparticles on broilers' performance and health status. Trop. Anim. Health Prod. 52, 2043-2054.
Nabi, F., Arain, M.A., Hassan, F., Umar, M., Rajput, N., Alagawany, M., Liu, J., 2020. Nutraceutical role of selenium nanoparticles in poultry nutrition: A review. World Poult. Sci. J. 76, 459–471.
Rao, S., Prakash, R., Raju, B., Panda, M., Kumari, A.R., Reddy, E.P.K., 2016. Effect of supplementing organic forms of zinc, selenium and chromium on performance, antioxidant and immune responses in broiler chicken reared in tropical summer. Biol Trace Elem. Res. 172, 511–520.
Reda, F.M., El-Saadony, M.T., Elnesr, S.S., Alagawany, M., Tufarelli, V., 2020. Effect of dietary supplementation of biological curcumin nanoparticles on growth and carcass traits, antioxidant status, immunity and caecal microbiota of Japanese quails. Animals 10, 754.
Saha, O., Hoque, N.M., Islam, O. K., Rahaman, M.M., Sultana, M., Hossain, A.M., 2020. Multi-drug-Resistant Avian Pathogenic *Escherichia coli* Strains and Association of Their Virulence Genes in Bangladesh. Microorganisms J. 8, 1135.
Salem, H.M., Ismael, E., Shaalan, M., 2021. Evaluation of the Effects of Silver Nanoparticles Against Experimentally Induced Necrotic Enteritis in Broiler Chickens. Int. J. Nanomedicine 16, 6783–6796.
Shakal, M., Youssef, F.S., Mohamed, G.G., Ismail, S.H., Salem, H.M., 2024. Evaluation of antibacterial activity of zinc oxide nanoparticles against avian mycoplasmosis with assessment of its impact on broiler chickens' performance and health. J. Adv. Vet. Res. 14, 37-43.
Singh, G.K., Niyogi, D., Tripathi, K.K., Joshi, N., Vaish, A., Mishra, A., 2018. Pathomorphological changes in broiler chickens due to spontaneous *E. coli* infection. J. pharmacogn. phytochem. 7, 798-801.
Solà-Ginés, M., Cameron-Veas, K., Badiola, I., Dolz, R., Majó, N., Dahbi, G., Viso, S., Mora, A., Blanco, J., Piedra-Carrasco, N., Migura-Garcia, L., 2015. Diversity of multi-drug resistant avian pathogenic *Escherichia coli* (APEC) causing outbreaks of colibacillosis in broilers during 2012 in Spain. PloS one 10, e0143191.
Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E., Reed, W.M., 1998. A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 4th ed. American Association of Avian Pathologists. Inc., Kennett Square, Pennsylvania, USA.
Swelum, A. A., Elbestawy, A.R., El-Saadony, M.T., Hussein, E.O., Alhotan, R., Suliman, G.M., Abd El-Hack, M.E., 2021. Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: an updated overview. Poult. Sci. 100, 101039.
Timmerman, H.M., Veldman, A., van den Elsen, E., Rombouts, F.M., Beynen, A.C., 2006. Mortality and Growth Performance of Broilers Given Drinking Water Supplemented with Chicken-Specific Probiotics. Poult. Sci. 85, 1383–1388, https://doi.org/10.1093/ps/85.8.1383.
Yousef, H.M., Hashad, M.E., Osman, K.M., Alatfeehy, N.M., Hassan, W.M., Elebeedy, L.A., Salem, H.M., Shami, A., Al-Saeed, F.A., El-Saadony, M.T., El-Tarabily, K.A., Marouf, S., 2023. Surveillance of *Escherichia coli* in different types of chicken and duck hatcheries: One health outlook. Poult. Sci. 102, 103108.