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Original article

EFFECT OF DIETARY ZINC OXIDE NANOPARTICLES ON GROWTH PERFORMANCE AND *CLOSTRIDIUM PERFRINGENS* INFECTION IN BROILER CHICKENS

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Summary

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The aim of this work was to study how different concentrations of zinc oxide nanoparticles (ZONPs) affected broiler chicken resistance and susceptibility to *C. perfringens* challenge, as well as growth performance and intestinal morphology. A total of 84 Ross-308 broilers, 7 days of age were randomly divided into 7 treatment groups: first group (negative control), second group – basal diet only (positive control), third group (positive control treated with amoxicillin), fourth, fifth, sixth and seventh groups – basal diet supplemented with 10, 20, 30, and 40 mg ZONPs per kg ration, respectively. All treatments were replicated 4 times, with three birds in each pen. All groups were inoculated orally with 5×10^4 sporulated coccidial oocyst, and after 5 days, the same groups were challenged orally with 2 mL broth culture with 1.8×10^8 CFU/bird of *C. perfringens* type A, for 3 successive days. Our findings showed that ZONPs used as a feed additive, inhibited *C. perfringens* proliferation in the intestine. ZONPs supplemented groups had significant ($P<0.05$) improvement in overall body weight gain and feed consumption than the other groups. However, there was no significant difference in feed conversion ratio between all groups. Chicks supplemented with different ZONPs concentrations showed a significant increase ($P<0.05$) in villus height and villus/crypt ratio in small intestine, however, there was no significant difference in crypt depth between all groups ($P>0.05$). Chicks supplemented with ZONPs showed superior disease resistance and superior growth efficiency. Furthermore, 20 and 40 mg/kg ZONPs improved growth performance and intestinal parameters compared to other concentrations. As a result, ZONPs may be used in poultry feed as an alternative to antibiotics.

Key words: *C. perfringens,* growth performance, intestinal morphology, zinc oxide nanoparticles

INTRODUCTION

In poultry, *C. perfringens* is the most common cause of necrotic enteritis (Timbermont *et al.,* 2011). As a result of the alpha-toxin, *C. perfringens* causes necrotic enteritis in chickens, which can result in increased mortality, reduced feed conversion, and slower growth rate (Petit *et al.,*1999).

The use of in-feed antibiotics for farm animals has been banned, restricted or is under consideration due to possible emergence of antibiotic resistant pathogenic microorganisms and residuals in foods (Patra *et al.*, 2019). Nano-feed substances could help in increasing the feed proficiency and production performance, diminishing feed costs, and expanding the yield and value of animal products (Gopi *et al.*, 2017).

Recently, nanoforms or nanoparticles (NPs) of essential minerals have been explored for growth performance, feed utilisation and health status of animals (Patra & Lalhriatpuii, 2020). Nano-minerals have been shown to enhance growth, egg production and quality, immune modulation and antioxidant status, and at the same time economise the production by reducing the supplemental dose of minerals and improving the feed conversion ratio. They seem also to be less toxic than conventional mineral sources (Swain *et al.,* 2021). Therefore, nano-minerals could serve both as antimicrobial feed additives and mineral supplements. Besides nano-essential minerals, nanomaterials may modulate gut microbiome by directly inhibiting the growth of microorganisms, or altering their metabolic functions in the gut (Patra, 2019).

As a result of the small NPs size, their passage is very fast through the walls of the gastrointestinal tract, creating many important effects in various body systems (Hameed, 2021). Also, nano-applications are used in poultry and animal production systems using available tools and techniques without affecting animal health and welfare (Haben *et al.,* 2020).

Zinc oxide nanoparticles (ZONPs) have the advantage of being an antimicrobial growth promoter, a nutritional supplement for humans and animals (Swain *et al.,* 2016). Moreover, when used in small doses, ZONPs can substitute antibiotics as growth promoters, remove antibiotic residues in animal products, and minimise environmental pollution (Schmidt, 2009). The positive effect of ZONPs supplementation on growth may be due to the important role of zinc in the overall performance and physiological processes of poultry, as it is the main component of a large number of metallo enzymes, which are involved in metabolism of energy, nucleic acids, and protein (Attia *et al.* 2019). In addition, ZONPs guard against *Eimeria* infestation (Abd El Megid *et al.,* 2018). Moreover, organic Zn supplementation reduced intestinal permeability and attenuated intestinal inflammation of broilers co-challenged with coccidia and *C. perfringens* (Bortolouzzi *et al.,* 2019).

ZONPs could be used to supplement broilers to improve both performance and digestibility with a limited impact on bone strength (Alkhtib *et al.,* 2020). In addition, supplementation of ZONPs to the diets was reported to have no harmful effect on birds' health status and could be used instead of the traditional zinc sources in broiler diets (Abd El-Haliem *et al.,* 2020). ZONPs have the ability to cross the gastrointestinal tract and from there, are further distributed in the blood and target organs, which in turn increases immune response and resistance to infection (Yusof *et al.,* 2019).

Wang *et al.* (2016) demonstrated that long term exposure to 50 and 500 mg/kg nano-ZnO diets showed minimal toxicity. However, excessive amounts of ZONPs can produce toxic effects, thereby inhibiting the growth of broilers (Zhao *et al.,* 2014). Zinc nanoparticles supplementation at 60, 45 or 30 ppm improved growth performance while a lower level (15 ppm) significantly reduced broiler performance and feed efficiency parameters (El-Katcha *et al.,* 2017). In addition, broiler chickens fed 100 mg/kg ZONPs exhibited lower feed intake and feed conversion ratio than controls (Ramiah *et al.*, 2019).

The aim of our study was to assess the effects of dietary zinc oxide nanoparticles as an alternative to antibiotics on *C. perfringens* infection in broilers and evaluate the impact of dietary ZONPs on broiler growth performance and the small intestine morphology.

MATERIALS AND METHODS

Isolation and identification of C. perfringens

A total of 100 intestinal samples were obtained aseptically from recently dead and diseased chickens (22–40 days of age) from commercial broiler farms in Assiut governorate (with a history of necrotic enteritis outbreaks).

The crude cultures were streaked for isolation in duplicate on Reinforced Clostridium agar (Oxoid) plates and incubated anaerobically at 37 °C for 48 hours using an anaerobic atmosphere generation system. The following biochemical tests were performed on pure cultures of isolated *C. perfringens:* lecithinase, motility, and skim milk coagulation (stormy reaction) (Quinn *et al.,* 2002). The sequences of the oligonucleotide primers were constructed using the alpha toxin sequence as a guide (Yoo *et al.,* 1997). Table 1 shows the nucleotide sequence of each primer pair as well as the size of the PCR element.

Synthesis of zinc oxide nanoparticles

ZONPs were synthesised using the co-precipitation method. Two solutions were prepared in deionised water: 0.1 M Zn acetate dihydrate (solution 1) and 0.2 M NaOH (solution 2). The two solutions were then combined in one beaker and stirred at 700 rpm for 2 hours at 70 ^{o}C. After 2 hours, the initially clear solution has turned white and milky. A white substance precipitated after centrifugation at 4,200 rpm for 3 min. After that, the precipitated liquid was washed with deionised water before being treated with acetone. ZONPs in powder form were obtained by drying the sample in a laboratory oven for 6 h at 75 °C (Othman *et al.,* 2017; 2018).

X-ray diffraction (XRD) was used to describe ZONPs in order to learn more about their structural properties (Fig. 1). Transmission electron microscope (TEM) was used to examine the morphology and size distribution of ZONPs powder (Fig. 2). The average particle size was found to be 61.2 nm.

Design of the experiment

The experiment was carried out at the Poultry Diseases Department, Veterinary Medicine, Assiut University.

In our experiment, a total of 84 Ross-308 broilers, 7 days of age were randomly divided into 7 treatment groups. All treatments were replicated 4 times, with three birds in each pen. The groups were as followed: first group (negative control), second group – basal diet only (positive control), third group (positive control treated with amoxicillin), fourth, fifth,

Table 1. List of primers used for PCR assay*

Toxin	Primer	Sequence	Amplified product
Alpha toxin	F R	GTTGATAGCGCAGGACATGTTAAG CATGTAGTCATCTGTTCCAGCATC	402 bp

* Yoo *et al.* (1997).

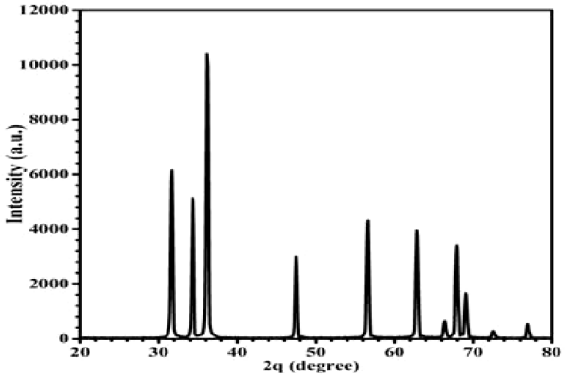

Fig. 1. XRD pattern of the synthesised ZnO NPs.

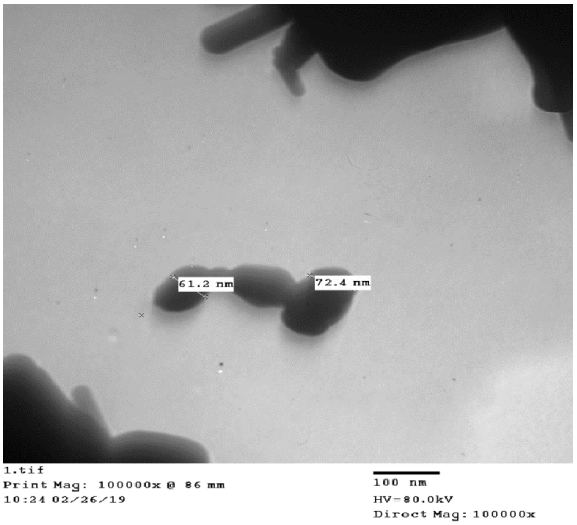

Fig. 2. TEM graph of the synthesised ZnO NPs.

sixth and seventh groups – basal diet supplemented with 10, 20, 30, and 40 mg ZONPs per kg ration, respectively. The basal diet was formulated to maintain the nutritional requirements of broilers during the starter and finisher periods (Table 2).

All groups were inoculated orally with 5×10^4 sporulated oocysts of coccidia at 14

Table 2. Feed composition

Ingredients (%)	Starter (0 to 21 days)	Grower (22 to 30 days)
Corn grain	50.30	61.20
Soybean meal (45% protein)	36.00	28.00
Corn gluten meal	3.40	3.00
Soybean oil	6.00	4.50
Limestone powder	2.14	1.85
Sodium phosphate monobasic	1.45	0.85
Common salt	0.30	0.30
Methionine	0.11	0.00
Premix*	0.30	0.30
Total	100	100
Calculated analysis (%)		
Crude protein (%)	23.02	20.21
Lysine (%)	1.31	1.01
Methionine (%)	0.50	0.38
Calcium (%)	1.00	0.90
Non-phytate phosphorus (%)	0.45	0.30
Total zinc (mg/kg)	74.21	75.74
Crude fibre (%)	3.46	3.27
ME (kcal/kg)	3193	3220

*Provided per 2.5 kg: Vit. A, 1200000 IU; Vit. D3, 300,000 IU; Vit. E, 700mg; Vit.K3, 500 mg; Vit. B1, 500 mg; Vit. B2, 200 mg; Vit. B6, 600 mg; Vit. B12, 3 mg; Vit. C, 450 mg; Niacin, 3000 mg; Methionine, 3000 mg; Pantothenic acid, 670 mg; Folic acid 300 mg; Biotin, 6 mg; Choline chloride, 10,000 mg; Magnesium sulfate, 3000 mg; Copper sulfate, 3000 mg; Iron sulfate, 10,000 mg; Zinc, 400 mg; Cobalt sulfate, 300 mg.

days of age, with the exception of the control negative group (Hofacre *et al.,* 1998). After 5 days, the same groups were challenged orally with a toxigenic *C. perfringens* type A strain identified by Gholamiandehkordi *et al.* (2007) by inoculating 2 mL broth culture (1.8×10^8 CFU) per bird daily for 3 days (19^{th}, 21^{st} and 23^{th} days of age). Feed conversion ratio (FCR) was determined on the basis of the live body weight (BW) and feed intake (FI) which were assessed weekly.

All clinical symptoms, postmortem lesions, mortalities, and the rate of inoculated organism re-isolation were reported. Duodenum, jejunum, ileum and caecum segments were collected for histological examinations. Tissues were fixed in 10% formalin buffer solution, stained with haematoxylin and eosin and examined microscopically. The analysed morphometric variables included: villus length, crypts depth and villus height /crypts depth ratio (Uni *et al.,* 1999).

Statistical analysis

The obtained data were statistically analysed using the SAS System's general linear model (Steel & Torrie, 1980), The effect of different treatments on *C. perfringens*-infected broiler chickens was investigated using analysis of variance (ANOVA). Duncan's Multiple Range test was used to assess the statistical significance of the disparity in group means. All variables were considered significant at $P<0.05$.

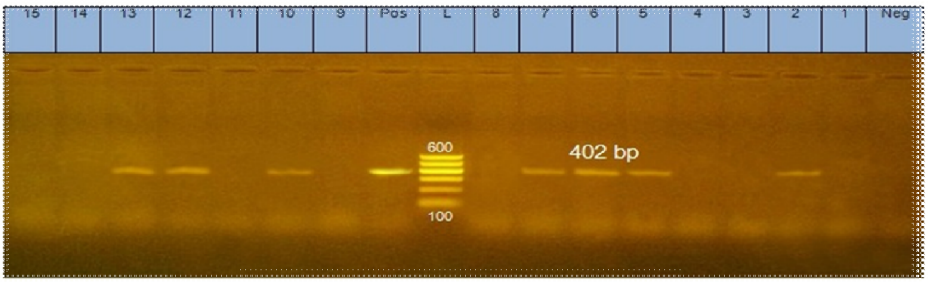

Fig. 3. Uniplex PCR for toxin typing of *C.perfringens* field isolates. L: 100 bp DNA ladder, lanes 1–15 : samples (lanes 2, 5, 6, 7, 10, 12 and 13 *C. perfringens* type A); Pos.: positive control; Neg.: negative control.

RESULTS

The average occurrence of *C. perfringens* strains in the current study was 37 out of 100 analysed samples (37%). Postmortem inspection of freshly killed broiler chickens revealed significant dehydration, enteritis and friable ballooning of the small intestine.

PCR results (Fig. 3) revealed that all of the tested isolates were type A and carried the alpha toxin gene (*cpa*).

In comparison to other groups, ZONPs-supplemented groups were successful in alleviating diarrhoea and pathological necrotic lesions of *C. perfringens* in the intestines after challenge but control groups exhibited extreme brown to bloody diarrhoea, ballooning in the intestine, and pathological necrotic lesions, with one case of mortality.

Microscopic examination of intestine after *C. perfringens* infection in the positive control group showed severe haemorrhage, presence of developmental stages of *Eimeria* spp. in the epithelium of intestinal villi, hyperplasia of intestinal cleft, haemorrhages and intestinal hyperplasia of some villi (Fig. 4, 5 & 6).

Under challenge conditions, the addition of various levels of ZONPs to broiler diets provided the chicks with a beneficial level of defense against *C. perfringens* infection. The overall growth performance results (Table 3) revealed beneficial effects of ZONPs supplementation. There were significant ($P<0.05$) differences in the overall body weight gain (BWG) and feed intake (FI) of the experimental groups compared with control group. No significantly different ($P>0.05$) feed conversion ratio (FCR) was detected among the experimental groups.

Histomorphometric measurements of duodenum, jejunum, ileum and caecum presented in Table 4, revealed beneficial effects of ZONPs supplementation.

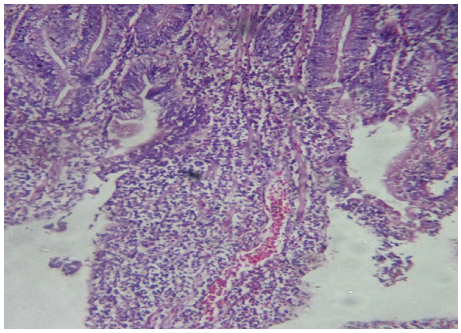

Fig. 4. Photomicrograph of ileum of a chicken from group II (positive control) showing hyperplasia of intestinal cleft (H&E, ×100).

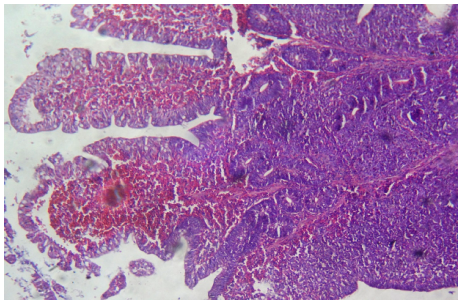

Fig. 5. Photomicrograph of jejunum of a chicken from group II (positive control) showing sever hemorrhage (H&E, ×100).

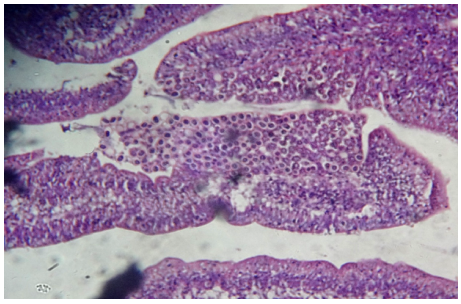

Fig. 6. Photomicrograph of duodenum of a chicken from group II (positive control) showing *Eimeria* stages in enterocytes of intestinal villi (H&E, ×200).

In challenged birds, there were significant ($P<0.05$) differences in the villus height (VH) and villus/crypt ratio (v/c) in all segments of intestine of the experimental groups when compared with other groups. Crypt depth (CD) did not differ considerably ($P>0.05$) among the experimental groups.

DISCUSSION

In poultry, *C. perfringens* type A causes necrotic enteritis (NE), which leads to economic losses due to mortality, morbidity, weight loss, a low feed conversion ratio, and poor performance (Sarkar *et al.,* 2013). The present results indicated that *C. perfringens* was prevalent in affected broilers chickens (37%). Our results are in agreement with those of Abdelazem & Wael (2015), who found a high prevalence of *C. perfringens* (57.6%) in reported cases of necrotic enteritis.

Because of its high sensitivity, PCR has been commonly used to recognise *C. perfringens* toxin genes. The alpha toxin gene was found in all suspected isolates, according to our findings. The key viru-

Table 3. Effects of dietary addition ZONPs on growth performance in chickens from different groups from 7 to 30 days of age. I: negative control; II: basal diet (positive control); III (positive control + amoxicillin): IV, V, VI and VII: basal diet + 10, 20, 30, and 40 mg ZONPs per kg ration.

Groups	Body weight gain /kg	Daily feed intake /kg	Feed conversion rate kg feed/kg gain
I	0.062 ± 0.0013^{ABC}	0.098 ± 0.0029^{AB}	1.658 ± 0.0459
II	0.058 ± 0.0016^{CD}	0.103 ± 0.0067^{AB}	1.829 ± 0.1130
III	0.057 ± 0.0017^{D}	0.096 ± 0.0020^{B}	1.727 ± 0.0664
IV	0.059 ± 0.0007^{BCD}	0.102 ± 0.0027^{AB}	1.717 ± 0.0239
V	0.064 ± 0.0012^{A}	0.103 ± 0.0030^{A}	1.733 ± 0.0371
VI	0.062 ± 0.0020^{ABC}	0.101 ± 0.0042^{AB}	1.679 ± 0.0651
VII	0.064 ± 0.0017^{AB}	0.101 ± 0.0033^{A}	1.717 ± 0.0409
P value	0.000	0.010	0.615

Means bearing different superscripts within the column are significantly different ($P<0.05$).

Table 4. Effects of dietary addition ZONPs on intestinal morphology in chickens from different groups from 7 to 30 days of age. I: negative control; II: basal diet (positive control); III (positive control + amoxicillin): IV, V, VI and VII: basal diet + 10, 20, 30, and 40 mg ZONPs per kg ration

Group	Jejunum histomorphology			Caecum histomorphology		
	VH, µm	CD, µm	V/C	VH, µm	CD, µm	V/C
I	986±1.0^{A}	169±.8	5.8 ^{A}	792±.6^{A}	148±.5	5.3^{A}
II	821±.2^{E}	204±.2	4.0E	633±.5^{F}	161±.3	4.0^{C}
III	932±.5^{BC}	170±.5	5.7^{AB}	735±.3^{D}	150±.5	5.0^{AB}
IV	933±.3^{BC}	178±.5	5.3^{BCD}	711±.5^{E}	153±.1	4.7^{B}
V	948±.5^{B}	171±.1	5.6^{ABC}	753±35^{BC}	151±.8	5.0^{AB}
VI	907±.5^{D}	181±.6	4.9^{D}	745±.4^{CD}	159±.8	5.0^{AB}
VII	917±.03^{CD}	176±.4	5.2^{CD}	773±.1^{AB}	154±.9	5.1^{AB}
P value	.000	.021	.00	.000	.847	.004
Group	Duodenum histomorphology			Ileum histomorphology		
	VH, µm	CD, µm	V/C	VH, µm	CD, µm	V/C
I	1321±.8^{C}	190±6	7.0^{B}	921±.8^{A}	160±.7	1321±.8^{C}
II	1103±.1^{D}	192±.3	8.0^{A}	899±.3^{B}	170±.6	1103±.1^{D}
III	1190±.5^{D}	190±3	7.3^{AB}	879±.7^{C}	163±.8	1190±.5^{D}
IV	1310±.2^{C}	190±.3	6.9^{BC}	903±.9^{AB}	169±.7	1310±.2^{C}
V	1389±.8^{C}	189±.3	7.0^{B}	919±.6^{A}	172±.7	1389±.8^{C}
VI	1317±.5^{B}	188±.4	6.3^{CD}	915±.8^{AB}	178±.9	1317±.5^{B}
VII	1499±.4^{A}	193±.6	6.2^{D}	905±.6^{AB}	174±.5	1499±.4^{A}
P value	.000	.999	.001	.001	.461	.000

Means bearing different superscripts within the column are significantly different (P<0.05).

lence factor implicated in necrotic enteritis in chickens is alpha toxin, which is produced by all forms of *C. perfringens* (Baba *et al.,* 1997).

ZnONP was found to be effective against Gram-positive and Gram-negative bacteria (Patra, 2019) and spores resistant to high temperature and pressure (Rosi & Mirkin, 2005).

Furthermore, the electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is critical for the bactericidal behaviour of nanoparticles. In addition, ZONPs have a protective role against *Eimeria* infestation (Abd El Megid *et al.,* 2018) and against coccidiosis caused by *E. papillata* (Dkhil *et al.,* 2015). The dietary supplementation with a low dose of ZONPs enhanced growth and intestinal morphology while reducing diarrhoea and intestinal inflammatory responses (Long *et al.,* 2017). The ability of ZONPs to pass through the gastrointestinal tract and then be further distributed in the blood and target organs, led to an increased immune response and resistance to infection (Yusof *et al.,* 2019).

As result, ZONPs can be used as an important feed additive in poultry diets (Handy *et al.,* 2008). This could be due to the fact that ZONPs as a feed additive improved the intestinal morphology and immune response of chicks (El-Katcha *et al.,* 2017) before coccidian and *C. perfringens* challenge. In addition, Bortoluzzi *et al.* (2019) reported that organic zinc can improve growth performance, intestinal barrier function and regulate cytokine expression in chickens under the attack of coccidia plus *C. perfringes*. In the same

context, ZONPs can be developed as alternative to antibiotics in poultry production and open up new possibilities for the control of microbial pathogens (Yusof *et al.,* 2021).

According to present findings, ZONPs as feed additives in broiler rations had a positive impact and significantly ($P<0.05$) increased weight gain and feed intake compared to the other groups. However, ZONPs demonstrated no significant difference ($P<0.05$) with respect to feed conversion ratio (FCR).

The present results are in agreement with previous report (Sahoo *et al.,* 2016; Ibrahim *et al.,* 2017) who showed that dietary supplementation of ZONPs to broiler diets had significant ($P<0.05$) effects on growth performance and provide economic benefits in poultry. Also, Zhao *et al.* (2014) and Swain *et al.* (2015) claimed that acceptable levels of ZONPs (20 and 60 mg/kg) will boost growth efficiency and FCR in the broilers diet. Similarly, ZONPs increased broiler growth performance after 42 days of feeding at a dose of 40 mg/kg in the diet (Jianyang *et al.,* 2009). Hussan *et al.* (2021) concluded that supplementation of ZONPs at 2.5 ppm improved the body weight and feed efficiency of broilers.

Our findings are consistent with those of Fathi *et al.* (2016), who found that adding 20 mg/kg ZONPs to broiler feed increased daily weight gain. In broiler chickens, ZONPs can improve growth performance, particularly at doses of 30 mg/kg diet (Ahmadi *et al.,* 2013; El-Katcha *et al.,* 2017). Moreover, ZONPs at a level of 40 mg/kg diet had no harmful effect on health status (Abd El-Haliem *et al.,* 2020), and no negative effects on chicken growth (Łukasiewicz *et al*., 2020) so could be used instead of the traditional zinc sources in broiler diets. Subsequently, nanocomposites can be used to improve the digestion and absorption in poultry as raw materials or new feed additives, improving the quality of nutrients (Hameed, 2021).

In contrast to our results, Bami *et al*. (2019) reported that ZONPs at 25 and 50 mg/kg as well as conventional ZnO at 100 mg/kg did not affect feed intake, body weight gain, feed efficiency. Also, Tsai *et al.* (2016) stated that feeding dietary ZONPs to laying hens had no effect on weight gain or growth performance. Our findings contradict those of Pimental *et al.* (1991), who found that ZONPs additive had no effect on feed consumption, feed conversion ratio or live body weight of broiler chickens. The positive effect of ZONPs supplementation on growth may be due to the important effect of zinc on overall performance and physiological processes of poultry (Attia *et al.*, 2019). The pathogenic microbial load in poultry intestines is harmful because it reduces growth rate, feed efficiency and increases mortality (Swain *et al.,* 2021). The high efficiency of ZONPs in inhibiting the growth of a wide range of pathogens made it a promising substitute for conventional antibiotics (Soni *et al*., 2014).

According to the present findings, ZONPs had significantly increased ($P<0.05$) villus height (VH) and villus crypt ratio (V/C) in all parts of the small intestine. However, there was no significant difference in crypt depth (CD) between all groups. Our findings are consistent with others (Ali *et al.,* 2017) who affirmed that birds supplemented with 40 mg ZONPs/ kg ration had significantly increased VH and villus surface area (VSA) in all parts of the small intestine. In addition, Hafez *et al.* (2017) found that ZONPs (40–80 mg/kg) increased villus length and crypt depth in the duodenum,

jejunum and ileum, but not the width or the V/C ratio.

Some studies found that birds supplemented with 60 and 90 mg of ZONP/kg had increased villus height and V/C ratio in the jejunum during the starter period in chickens (Ahmadi *et al.,* 2013), which is consistent with our findings.

Therefore, increases in VH and V/C ratio leads to increased digestion and absorption (Awad *et al.,* 2008) and increased epithelial cell turnover (Lei *et al.,* 2014). The most commonly used indicator of mucosal integrity and intestinal function is the villus height/crypt ratio (Clarke *et al.,* 2006). ZONPs improve intestinal villi length, width and crypt depth of broilers indicating increased nutrient absorption ability and improved feed quality (El-Katcha *et al.,* 2017). ZONPs have immunostimulating efficiency, they improved growth performance, intestinal villi width, length, crypt depth and goblet cells (El Sawy *et al.,* 2021). Higher villus height may be due to higher ZONPs bioavailability, allowing epithelial barrier integrity and function to be maintained (Ali *et al.,* 2017). Nanoparticles are also involved in improving the internal environment of poultry by increasing the number of goblet cells, characterised by the secretion of mucus, which forms a barrier that protects the intestinal walls (Hameed, 2021).

The findings of the current study revealed that in chickens supplemented with various concentrations of ZONPs, the crypt depth (CD) in the duodenum, jejunum, ilium and caecum were not significantly ($P<0.05$) different. According to Tsirtsikos *et al.* (2012) the increase in crypt depth of chicken supplemented with different concentrations of ZONPs/kg could provide more surface area for nutrient absorption by increasing enterocyte proliferation and intestinal mucin secretion since mucin-producing goblet cells are present primarily in the crypts. Moreover, supplementation of ZONPs to the diets had no harmful effect on birds' health (Abd El-Haliem *et al.,* 2020).

In conclusion under challenge conditions and via improved intestinal morphology, ZONPs supplemented chicks showed superior disease resistance and growth efficiency. In addition, when compared to other concentrations, the required amounts of ZONPs (20 & 40 mg/kg) improved growth performance and intestinal parameters. As a result, zinc oxide nanoparticles may be used in poultry feed as an alternative to antibiotics.

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