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Research Article

Effect of Zinc Oxide Nanoparticles in Drinking Water on Growth Rate, Biochemical Parameters, and Intestinal Histology of Broilers

Arkan B. Mohammed,[1] **Oday K. Hamad**,[1] **and Thamer A. Khttab**[2]

[1]*Department of Animal Production, College of Agriculture, Tikrit University, Tikrit 34001, Iraq*
[2]*Agriculture Research Office, Tikrit 34001, Iraq*

Correspondence should be addressed to Arkan B. Mohammed; dr.arkanmohammed@tu.edu.iq

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The study objectives were to evaluate effects of zinc oxide nanoparticles (ZnO NPs) on growth rate, gut histomorphology, and physiological parameters of broilers. A total 360 birds were randomly assigned into five groups ($n = 72$ birds per group; four replicates per group $n = 18$), the control group drink water without nano zinc, and ZnO NP groups added 6, 8, 10, and 12 mg/liter to drinking water. The results presented that ZnO NP groups showed significant ($p < 0.05$) in growth performance with reduced feed intake in all ZnO NP groups in comparison to the control at 35 and 42 days of age. Villus height and villi to crypt ratio showed significant differences (p<0.05) in all ZnO NP groups compared to the basal control, but the results showed there was no significant difference (p<0.05) between the ZnO NP and control in crypt depth. There was no significant effect of ZnO NP on total protein and albumin in broiler serum compared to the control at 35 and 42 days of age. The data showed that ZnO NP had a positive effect and improved the ratio of serum globulin and globulin to albumin compared to the others of broilers at 35 and 42 days of age. On the other hand, liver enzyme activity was significantly ($p < 0.05$) different in ZnO NP groups compared to the basal control. The inclusion of ZnO NPs in drinking water significantly improved growth performance and gut histomorphology, and it had an impact on antioxidant and biochemical parameters.

1. Introduction

Zinc plays an important role in many biological activities that occur in the body [1] and is considered an important nutrient for poultry, and Zn is commonly added as a supplement to all formulated poultry diets at a rate of 0.012–0.018% [2]. The biological activity of Zn is required for growth and skeletal development [3]. Mohamed et al. [4] and Khajeh Bami et al. [5] noted that Zn aids in the synthesis, catalytic activity, and stability of numerous protein forms. According to Swain et al. [6], Zn can improve performance, reduce feed intake in animals, increase capacity of antioxidants, and interact with different mineral forms in the gut. Zinc plays multiple roles in metabolism, immune response, and antioxidant systems of poultry [7]. Sahin et al. [8] stated that the source of organic Zn improves broiler performance compared to the inorganic source. On the other hand, Chand et al. found that dietary Zn supplementation in the feed increased body weight in broilers. Nanomaterials can contribute to the sustainability of the livestock sector through improving the quantitative and qualitative production of safe, healthy, and functional animal products[9, 10]. Furthermore, Lee et al. [11] and Badwei et al. [12] focused on nano-zinc sources in the poultry diet business to develop low-cost alternatives to zinc sources. Nano-sized mineral properties differ from inorganic sources in general because of their slighter particle size, greater external area, and increased obtainability [9]. According to El-Shenawy et al. [13], there is a scarcity of data that explain the impact of nano-Zn on poultry production and health. On the other hand, Łukasiewicz et al. [1] noted that the replacement of zinc oxide with ZnO nanoparticles had no significant effect on broiler performance. According to Kumar et al. [14], broiler hens' growth performance and gut shape can all be improved by adding Zn to the diet. Then, this study aimed to explore the effect of ZnO nanoparticles (ZnO NPs) added to drinking water on performance, liver enzyme activity, and gut morphology.

2. Materials and Methods

2.1. Study Design. This study was approved by the Committee of Institutional Animal Care, Tikrit University. A total of 360 broiler chicks were randomly selected and assigned to five groups with four replicates ($n = 18$ per replicate).

2.2. Preparation of ZnO NPs. The subject of the study was an aqueous solution of zinc nanocolloids at a concentration of 50 mg/L, and the concentrations of ZnO NPs (60, 80, 100, and 120 ppm/L) were prepared from this solution for the purpose of the study. The zinc nanoparticles were supplied by a patented technology licensed by Nano Technologies Group, Inc. (USA).

2.3. Bird Management. The experimental diets were fed in the pelleted diet. The diets were prepared according to the procedure in [15]. Broiler Ross 308 were fed with (2950 kcal/kg) metabolizable energy and (22.23%) crude protein in the first phase (1–21 days), and in the second phase (22–42 days), (3050 kcal/kg) metabolizable energy and (21.22%) crude protein.

Nano-ZnO was added to drinking water at 60, 80, 100, and 120 ppm/L in the ZnO groups, and regular drinking water was used in the control group. The birds were reared under uniform conditions up to 42 days. The body weight of broilers, feed intake, and feed conversion ratio were recorded in two phases throughout the study period.

2.4. Blood Samples. At 35 and 42 days of age, the samples were collected from four birds randomly selected per group. About five ml were taken from the wing veins and transferred into nonheparinized tubes. The coagulated samples were centrifuged at 4000 rpm for 15 minutes, then clear serum got separated and stored in a −20°C freezer for the upcoming biochemical analysis. Total protein, albumin, and globulin were determined using a kit (Biolabo/French). Liver enzyme activity (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were determined by using spectrophotometric methods using a kit (Biolabo/French).

2.5. Histology Samples and Measurements. Duodenum samples (two samples each group) were prepared according to [16]. Villus height was measured in triplicate for each intestinal cross section. Crypt depth was defined as the depth of the invagination between two villi, villi width was measured at the midpoint of the villus, and villi height was measured from the villus-crypt junction to the margin of the villi. All morphological features were measured using an image processing and analysis system (crypt depth or villus height) by an Olympus microscope equipped with digital screen.

2.6. Statistical Analysis. Data were analyzed using ANOVA of Statistical Package for SAS [17], and comparison of means was done using Duncan's multiple range test [18], and significance was considered at $p < 0.05$.

$$Y_{ij} = \mu + T_i + e_{ij}, \tag{1}$$

where Y_{ij} is the value of j for group i, μ is the mean of parameters, T_i is the effect of group i (ZnO NP concentrations in drinking water), ande_{ij}normally scattered with an normal of 0 and a modification of$\sigma^2 e$.

3. Results and Discussion

3.1. Broiler Performance. The effect of ZnO NP added in drinking water on chicken growthperformance is shown in Tables 1 and 2. During day 35 ofage, the growth rate was increased in groups ZnO NP (60ppm/L) and (120 ppm/L) (p < 0.05) in comparison to the control group. Birds drinking water including ZnO NP wassignificantly increased by ($p <$ 0.05) in the total body weightand total body weight gain on day 35 (Table 1). On the other hand, the same result applies on the total body weight andtotal body weight gain on day 42; there was significant (p > 0.05) difference in comparison to the control group, asshown in Table 2. Feed intake was significantly ($p < 0.05$) lower on days 35 (Table 1) and 42 (Table 2) due to birds drinking water which included 60, 80, 100, and 120 ppm/L of ZnO NP compared to the control. Also, total feed conversionimproved in ZnO NP groups compared to the control group,especially in birds that were added 60 and 120 ppm/L to their drinking water of ZnO NP.

Many studies showed that Zn isimportant for many biological activities in poultry [19–21]and is required for skeletal system and improved immunityresponses [3]. Therefore, deficiency of Zn in the poultry dietcauses many issues and affects the feed intake and rategrowth in broilers [13, 22, 23]. From previous studies, Zn isimportant in cell functions, protein, and carbohydratemetabolism [22]. In the present study, birds supplementedwith different Zn NP concentrations in drinking water hada positive significance affect to growth parameters. These results agreed with Mohammadi et al. [24], Swain et al. [6],and Kumar et al. [14] who reported that broiler-fed dietsupplement with nano-Zn enhanced growth, and reducedproduction cost. The same results showed that nano-Znimproved the broiler weight and immune system organs [5, 25]. This result does not agree with Hasan [26] and Łukasiewicz et al. [1] who showed that replacing ZnO withZnO nanoparticles had no beneficial effect on broiler performance.Numerous studies on broilers have shown thatnano-Zn has higher bioavailability compared to the traditionalZn source, suggesting that measuring the concentrationof minerals in organs could allow a more accurateassessment of nanoparticle bioavailability [23, 24, 27].

3.2. Serum Protein. The result of zinc oxide NP on serum protein on broiler at 35 days of age is shown in Table 3. Results showed that there was no remarkable difference ($p > 0.05$) in total protein and albumin between groups; on

Table 1: Effect of zinc oxide nanoparticles (ZnO NPs) on broiler production at 35 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
IBW	42.40	42.70	41.77	42.84	41.74	0.722
TBW	1655.05^{b}	1760.36^{a}	1706.54^{ab}	1719.02^{ab}	1735.56^{a}	0.049
TBWG	1612.64^{b}	1717.65^{a}	1664.76^{ab}	1676.18^{ab}	1693.82^{a}	0.051
TFI	3191.25^{a}	2938.02^{b}	3180.63^{a}	3185.31^{a}	3002.66^{b}	<0.0001
TFCR	1.98^{a}	1.71^{c}	1.91^{a}	1.90^{a}	1.89^{b}	0.001

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). IBW = initial body weight, TBW = total body weight, TBWG = total body weight gain, TFI = total feed intake, TFCR = total feed conversion ratio, and NDW = normal drinking water.

Table 2: Effect of zinc oxide nanoparticles (ZnO NPs) on broiler production at 42 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
IBW	42.40	42.70	41.77	42.84	41.74	0.722
TBW	2224.83^{b}	2467.29^{a}	2461.85^{a}	2518.88^{a}	2472.08^{a}	0.006
TBWG	2182.43^{b}	2424.58^{a}	2420.08^{a}	2476.04^{a}	2430.34^{a}	0.006
TFI	4388.36^{a}	4043.71^{c}	4204.63^{ab}	4364.44^{a}	4147.00^{b}	0.005
TFCR	2.01^{a}	1.65^{c}	1.73^{a}	1.76^{a}	1.70^{b}	0.002

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). IBW = initial body weight, TBW = total body weight, TBWG = total body weight gain, TFI = total feed intake, TFCR = total feed convenes ratio, andNDW = normal drinking water.

Table 3: Effect of zinc oxide nanoparticles (ZnO NPs) on total protein, albumin, globulin, and globulin to albumin ratio (%) at 35 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
Total protein (g/dL)	2.87	3.04	3.15	3.37	3.20	0.258
Albumin (g/dL)	1.31	1.47	1.41	1.40	1.39	0.305
Globulin (g/dL)	1.56^{b}	1.57^{b}	1.74^{a}	1.97^{a}	1.80^{a}	0.002
G/A ratio	1.19^{b}	1.09^{b}	1.32^{a}	1.40^{a}	1.29^{a}	0.005

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). NDW: normal drinking water.

the other hand, globulin concentration and globulin to albumin ratio were higher in the serum of broiler with added ZnO NP (80, 100, and 120 ppm/L) to drinking water than in the serum of broiler in the control group and ZnO NP (60 ppm/L) group at 35 days of age. The same results are shown in Table 4 (42 d), and there was a significant effect to adding zinc oxide nanoparticles to drinking water compared to the control group in globulin concentration, and globulin to albumin ratio except for total protein and albumin results showed there were no significant differences between the groups.

Our results showed that there was no negative effect of ZnO NP on total protein and albumin in broiler serum compared to control, meaning that these were within the normal range of broilers at 35 and 42 days of age. On the other hand, the data showed that when ZnO NP was added to the drinking water (80, 100, and 120 ppm/L), the ratio of serum globulin and globulin to albumin had a positive effect and improved compared to the others; perhaps this is because blood can quickly absorb and disseminate nanoparticles with a large active surface area and high catalytic effectiveness [6]. Nanoparticles can therefore be delivered directly to the intended organs as dietary Zn intake. In contrast, Bartlett and Smith [28] found that increasing the Zn level in the diet did not affect the plasma Zn concentration. The results agree with the findings of Ahmadi et al. [2] and Akash et al. [29] who recognized some effects of ZnO NP on some biochemical parameters; however, these data are inconsistent with [30, 31].

3.3. Liver Enzyme Activity. Liver enzyme activity (aspartate aminotransferase and alanine aminotransferase) is shown in Tables 5 and 6. The data in Table 5 show that adding ZnO NPs to drinking water improved the aspartate aminotransferase and alanine aminotransferase levels compared to others in broiler serum at 35 days of age. We found the same results at 42 days of age, and there was an improvement in aspartate aminotransferase and alanine aminotransferase levels when compared with the control group.

Table 4: Effect of zinc oxide nanoparticles (ZnO NPs) on total protein, albumin, globulin, and globulin to albumin ratio (%) at 42 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
Total protein (g/dL)	2.92	3.26	3.22	3.36	3.32	0.360
Albumin (g/dL)	1.56	1.46	1.44	1.47	1.47	0.411
Globulin (g/dL)	1.36^b	1.80^a	1.78^a	1.89^a	1.85^a	0.001
G/A ratio	0.87^b	1.23^a	1.24^a	1.28^a	1.25^a	0.001

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). NDW: normal drinking water.

Table 5: Effect of zinc oxide nanoparticles (ZnO NPs) on liver enzyme activity at 35 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
ALT (UI)	46.31^a	39.29^b	32.86^c	38.00^b	40.06^b	0.007
AST (UI)	162.39^a	155.68^b	146.77^b	156.05^b	135.48^c	0.005

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). NDW: normal drinking water.

Table 6: Effect of zinc oxide nanoparticles (ZnO NPs) on liver enzyme activity at 42 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
ALT (UI)	48.34^a	41.32^b	34.89^c	40.03^{bc}	43.48^{ab}	0.002
AST (UI)	168.73^a	162.02^{ab}	153.11^{bc}	162.39^{ab}	140.58^c	0.004

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). NDW: normal drinking water.

Table 7: Effect of zinc oxide nanoparticles (ZnO NPs) on duodenum morphology of broilers at 42 days of age.

Items	Groups					p value
	Control NDW	NDW with 60 ppm/L ZnO NP	NDW with 80 ppm/L ZnO NP	NDW with 100 ppm/L ZnO NP	NDW with 120 ppm/L ZnO NP	
VH (μm)	757.44^b	894.68^a	915.74^a	902.72^a	915.50^a	<0.0001
CV (μm)	150.92	151.72	143.69	153.84	146.41	0.281
V/C	5.01^b	5.92^a	6.37^a	5.88^a	6.25^a	0.004

^{a,b,c}Means in the same column differ significantly ($p < 0.05$). VH: villus height; CV: crypt depth.

These results agree with previous studies. Prasad et al. [32], Burmana et al. [33], and Zhao et al. [23] indicated that Zn plays a central role as antioxidants and stability of biomembranes and protein, and Fathi et al. [30] showed that Zn NP may reduce Cu, Zn, and SOD. Mondal et al. [31] reported that the ZnO NP dose improved the health status of broilers and increased liver enzyme activity indicating the effectiveness of Zn as an antioxidant.

3.4. Duodenum Morphology. Effect Zinc oxide NP on the histologically measured parametersof duodenum of broiler is shown in (Table 7).Electron microscopy indicated that increased villus height(VL) and ratio (VL : CD) in birds added ZnO NP todrinking water compared to the control, but the resultsshowed there was no significant ($p < 0.05$) differencebetween the ZnO NP and control in crypt depth (CD).

In our study, the resultsshowed that birds drinking water with ZnO NP (6, 8, 10,and 12 mg/L) had a positive villus height than the control.These results illustrate the role of Zn in repairing theepithelial cell and leads to improved villus height inbroilers [27, 39]. Lee et al. [12] demonstrated that nano-Zn plays an important role in modifying tight junctionsand enhancing the barrier functions of gut epithelialmonolayers and maintaining epithelial barrier integrityand function. According to Shao et al. [27], the zinc canrepair the gut injury by reducing the death of ileal epithelialcells, enhancing villus height and crypt depth.

4. Conclusion

Based on the results obtained, ZnO NP could practically be used in the drinking water of broilers at doses of 60, 80, 100, and 120 ppm/l, showing beneficial improved performance, liver function, and duodenum morphology of the broilers. In addition, ZnO NPs (120 ppm/L) have the potential to be used in drinking water without any negative impact on the growth rate of broilers and it helped to improve the health of the birds.

Data Availability

The data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Arkan Baraa Mohammed designed the experiment, measured biochemistry parameters, and performed statistical analysis. Thamer Katab and Oday Hamid measured growth performance parameters. All authors approved the final draft of the manuscript.

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