Effect of zinc oxide nanoparticles on growth performance and absorptive capacity of the intestinal villi in broiler chickens

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Abstract: This study was conducted to investigate the effect of zinc oxide nanoparticles (ZONPs) on performance and absorptive capacity of the intestinal villi in broiler chickens. A total of ninety-one-day old broiler chicks (ROSS 308) were distributed randomly into three treatments (T) with three replicates in each one. T1; basal diet + mineral mixture contains inorganic zinc oxide (40 mg/kg diet), T2; basal diet + mineral mixture contains ZONPs (40 mg/kg diet), T3; basal diet + mineral mixture contains ZONPs (80 mg/kg diet). This study lasted 35d and birds accessed to feed and water ad libitum throughout the experiment. The results showed a significant increase ($p<0.05$) in the body weight gain and feed efficiency of the 40 and 80 ZONP, but feed intake showed non-significant difference. The villus height and crypt depth were significantly increased i is a considerable Zn source for broiler with beneficial effects on the growth performan all parts of the small intestine in the 40 and 80 ZONPS. So, supplementation of ZONP at the dose rate of 40 or 80 mg nce of broiler chickens.

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1. Introduction:

Growth rate and feed efficiency are factors that determine the performance of a broiler flock **(Lesson and Summers 2005**). Zinc (Zn) is the most commonly added trace mineral in broiler chicken feeds. It is important for growth, immune function, metabolism and oxygen free radical scavenging **(Kietzmann and Braun 2006; Feng, Ma et al. 2010; Liu, Lu et al. 2011)**. Previous studies have shown that the effect of zinc from different sources, organic (e.g., zinc lactate, zinc amino acid, zinc chelate) or inorganic (zinc sulfate, zinc oxide) on production performance varies(Baiarzhal, Zilova et al. 2007; Nitrayova, Windisch et al. 2012; Schlegel, Sauvant et al. 2013). The bioavailability of organic zinc is higher than that of inorganic zinc, but the application of organic zinc in animal diets is limited due to its higher cost.

In animal production, to meet the needs of animals, the added concentration of inorganic zinc is 20:30 fold higher than the normal requirement of animals, because of the low utilization rate of inorganic zinc**(Bratz, Gölz et al. 2013**). However, high dietary zinc can lead to excess zinc in the fecal matter which cause environmental pollution**(Poulsen 1995; Broom, Miller et al. 2003)**. Furthermore, high zinc supplementation may affect the balance of other trace elements in the body and reduce the stability of vitamins and other nutrients and long- term application can cause zinc residue in the body**(Case and Carlson 2002; Sundaresan, Anish et al. 2008).**

Recently, nanotechnology and related products had rapid progress in different scientific areas; in fact this branch from science had fundamental affected on the all parts of human life, animal, environment, and industrial life**(Wijnhoven, Peijnenburg et al. 2009).** Recently trace minerals in the form of nanoparticles can be effectively used to fulfill the requirement of minerals in the poultry diets. Due to their extreme small size and unique physical properties, the nanoparticles are likely to be different when compared to their conventional forms.

As a mineral supplement, these are expected to have the advantage of better bioavailability, small dose rate and stable interaction with other components. The nanoparticles can effectively supply the requirement of minerals in the animals, promote growth rate and feed efficiency**(Oberdörster, Oberdörster et al. 2005**). With low use dosage, they can replace antibiotics as growth promoters, eliminate the residue of the antibiotics in the animal products, reduce the environmental contamination and produce pollution-free animal products**(Hett 2004; Schmidt 2009).** our study was aimed to investigate the effect of ZONPs of broiler performance.

2. Materials and Methods:

Zinc oxide Nanoparticle preparations

Zinc oxide nanoparticles (ZONPs) were prepared according to literature with slight modification **(Gusatti, Rosário et al. 2009)**. Briefly, 1.0 M KOH was dissolved under continuous stirring in distilled water under constant temperature 90 ˚C. The appropriate amount of 0.4 M Zinc acetate was added slowly for 60 min into the three-neck glass flask. The white suspension was kept under stirring for two hours at 70 °C. The material formed was filtered and washed several times with deionized water. The washed sample was dried at 65°C in oven for several hours.

Characterization

The structure of the prepared ZONPs was characterized with powder X–ray diffraction (XRD) pattern. The X-ray diffraction spectrum confirms the crystalline and phase formation of the ZONPs that shows the dominance of cubic over the hexagonal phase (Fig. 1). Debye–Scherrer was used to determine the NPs particle size. The average size was calculated as 41.3 nm taking into account that L = 3/4 D, where D is the nanoparticles diameter, in case of small crystallites. Moreover, morphology and size distribution of ZONPs powder was analyzed using transmission electron microscope (TEM), (Fig. 2). The average particle size was calculated as 39.2 nm.

Bird management:

The experiment was conducted in the poultry farm at Faculty of Veterinary medicine, Kafrelsheikh, Egypt. A total number of ninety unsexed one-day old Ross 308 broiler chicks were weighed and distributed randomly into three dietary treatments with three replicates of ten chicks in each replicate. The dietary treatments were T1(basal diet contains 40 mg inorganic Zn/kg diet), T2 (basal diet contains 40 mg Nano Zn/kg diet), T3 (basal diet contains 80 mg Nano Zn). The broilers were reared in deep litter system, under standard conditions of lighting with a 23:1 light: dark cycle, environmental temperature of 33 ˚C decreased weekly by 3 C till stabilized at 25 ±2 ˚C and ventilation. The experimental birds had ad libitum feed and water for five weeks.

All the chicks were vaccinated against New-Castle disease on 7th day and infectious bursal disease on 14th day. Basaldiets for broiler starter, grower and finisher were formulated according to**(NRC 1994)**. The ingredient and proximate composition of the experimental basal diet for the birds are presented in Table 1.

Growth performance:

Feed intake and body weight gain were recorded weekly and cumulated at the end of the experiment and the feed conversion rate (FCR) was calculated (feed intake to body weight gain)

Histo-morphometric parameters of small intestine:

After the end of the experiment three birds from each replicate were randomly selected and killed by slaughtering. About 2.5 cm of the duodenum, jejunum and ileum were sectioned and fixed in 10% neutral-buffered formalin for 3 days for tissue fixation. The samples were dehydrated and rinsed in several times in absolute alcohol, and then embedded in paraffin. Serial 5-µm longitudinal sections were cut on Leica Rotary Microtome (RM 2145, Leica Microsystems, and Wentzler, Germany) and mounted on glass slides. Then, slides routinely stained with hematoxylin and eosin (H & E). For histo-morphometric analysis was performed using Image J analysis software (National Institutes of Health, MD, USA), whereas the entire mucosal length, villus height (measured from the tip of the villus to the villus- crypt junction), villus width from the mid of the villus and crypt depth (measured from the crypt-villus junction to the base of the crypt) similar to that reported by **(De Los Santos, Donoghue et al. 2007).**

3. Results:

1-Effect of zinc oxide nanoparticles on growth performance:

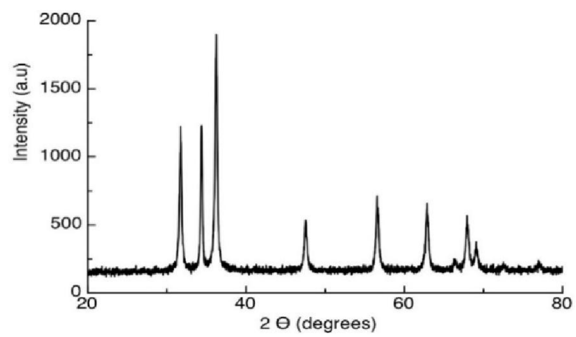

Fig. 1: XRD pattern of the synthesized zinc oxide nanoparticles.

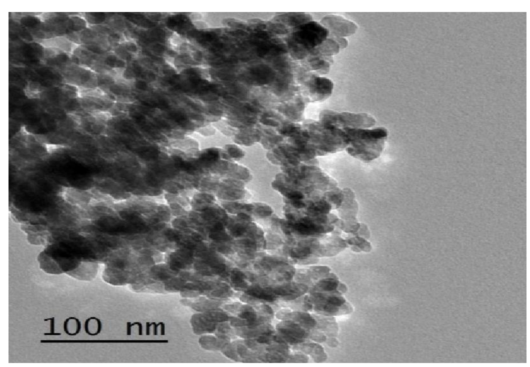

Fig. 2: TEM graph of the synthesizeze4`d ZnO NPs

Our results showed that there was a significant increase in the body weight gain in birds fed a diet with 40 and 80 mg Nano Zn /kg diet ($P<0.05$) throughout the experiment compared with that of the control group (Table 2). However, there was no significant effect on the feed intake between all groups. Subsequently, there was a significant decrease in the feed conversion ratio of broilers fed Nano Zn containing diets.

2- The absorptive capacity of the intestinal villi in broiler chicks:

Supplementation of zinc either in inorganic or Nano forms didn't show any pathological alterations, most intestinal segments examined were within normal limits. Interestingly, supplementation of Nanozinc increased the intestinal absorptive capacity parameters in dose-related manner. The entire mucosal length, villi length, width and crypt depth were markedly increased ($p<0.05$) in birds supplemented with Nano-Zn at dose 40 and 80 ppm in comparison with the control group (Table 5).

Table 1: The ingredient and proximate composition of the experimental basal diet for the birds

Ingredients	Starter	Grower	Finisher
corn grains %	56.5	64.3	68.6
Soybean meal48 %	31.5	23.7	18.7
Corn gluten meal60 %	6	6	6
Soya oil %	2	2.5	3.5
Di-calcium phosphate %	1.8	1.3	1.07
Limestone %	1.3	1.4	1.3
Lysine %	0	0.097	0.076
Methionine %	0.1	0.021	0
Salt %	0.5	0.38	0.45
Premix %	0.3	0.3	0.3
Total	100	100	100
Kcal ME/kg diet	3054.6	3168.1	3287.4
Crude protein%	23.05	20.09	18.13
Lysine %	1.16	1.04	0.88
Methionine %	0.51	0.39	0.34
Crude fat %	4.62	5.14	6.24
Calcium %	1.001	0.907	0.805
Available phosphorus %	0.465	0.36	0.31
Zinc %	31.3	27.92	25.7

Provided vitamins per kg of the feed: vitamin A, 1500 IU, vitamin D3, 200 ICU, vitamin E, 10 IU, vitamin k, 0.5 mg, vitamin B1, 1.8 mg, vitamin B2, 3.6 mg; pyridoxine, 3.5 mg; pantothenic acid 10 mg; niacin 35 mg; folacin 0.55 mg; choline 1300 mg; biotin 0.15 mg; vitamin B12 0.01 mg. supplied minerals per kg of the feed; Cu; 8 mg, I; 0.35mg, Fe 80 mg, Mn, 60mg, Se, 0.15 mg, Zn 40 mg.

Table 2: Growth performance of broiler chicken

Groups / **Parameters**	**Control**	**Nano-zinc 40**	**Nano-zinc 80**
Initial body weight	42.13^{a}±0.6464	41.23^{a}±0.4464	41.57^{a}±0.4665
Final body weight	1332^{a}±33.08	1437^{b}±37.08	1552^{b}±36.06
Body weight gain	1290^{a}±33.22	1408^{b}±35.88	1521^{c}±32.44
Feed intake	2405^{a}±7.397	2408^{a}±12.16	2408^{a}±24.93
Feed conversion ratio	1.896^{a}±0.04457	1.757^{b}±0.04307	1.615^{c}±0.03324

The data are presented as a mean ± SE, ^{a}represents non-significant value, ^{b}represents a significant value, while ^{c} a highly significant value

Table 3: Effect of zinc oxide nanoparticles on the absorptive capacity of the intestinal villi in broiler chicks

	Control	**Nano- zinc 40**	**Nano-zinc 80**
duodenum villi length	1085^{a}±96.21	1500 ^{b}±75.55	1635^{b}±74.48
duodenum Villi width	177.8 ^{a}± 27.84	249.6^{a}± 14.64	266.6^{a}± 48.33
duodenum Crypt depth	102^{a} ± 0.7113	183.7^{b}± 17.75	236.7^{b}± 12.30
Duodenum crypt/villi ratio	0.09548^{a}± 0.008372	0.1121^{a}± 0.02040	0.1449^{a}± 0.005633
jejunum villi length	1290^{a}±38.49	1844^{b}±184.0	2008^{b}±73.78
jejunum Villi width	165.9^{a}±26.12	174.0^{a}±12.75	203.6^{a}±15.90
jejunum Crypt depth	150.2^{a}± 12.33	252.6^{b}±30.29	296.2^{b}±14.84
jejunum crypt/villi ratio	0.1165^{a}±0.009132	0.1424^{a}±0.02769	0.1478^{a}±0.008730
Ilium villi length	435.0 ^{a}±52.74	671.3^{b}±29.46	779.8^{b} ±21.68
Ilium Villi width	61.08 ^{a}±4.292	168.2 ^{b}±21.49	186.4 ^{b}±6.959
Ilium Crypt depth	102.9 ^{a}±13.70	182.1 ^{b}±10.44	208.8 ^{b}±14.65
Ilium crypt/villi ratio	0.2467 ^{a}±0.05517	0.2713^{a}±0.009290	0.2684^{a}±0.02194

Mean ± standard errors in the same row with different superscript are significantly different

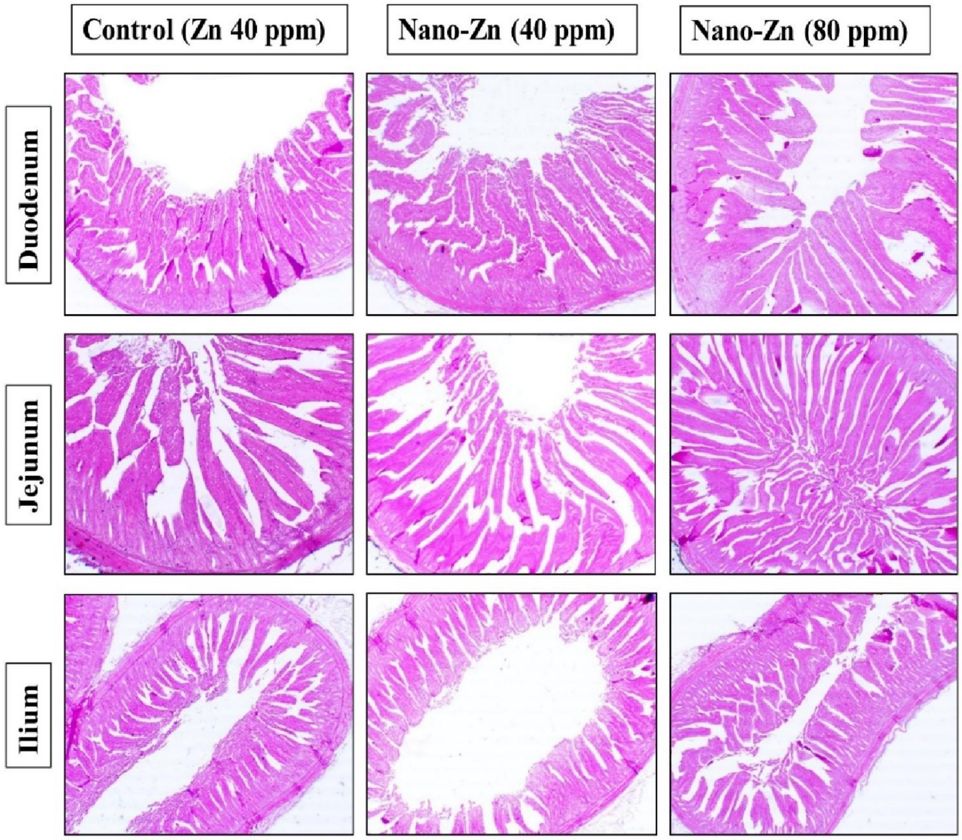

Fig (3). Photomicrograph of different intestinal sections including duodenum, jejunum and ilium from different birds supplemented with different forms of zinc

4. Discussion:

Nanotechnology has been successfully used to improve the utilization efficiency of trace elements in diets. Compared with conventional ZnO, nano-ZnO has many desirable properties, such as strong chemical activity, oxidation reactions, and permeability. Compared with ZnO, nano-ZnO has a stronger chemical activity and participates in oxidation reactions with a variety of organic compounds. In addition, the permeability of nano-ZnO can also help prevent adverse gastrointestinal reactions and improve the absorption of medicine (**Zhao et al., 2014)**. In the present study it was observed that appropriate levels of ZONPs (40 and 80 mg/kg diet) could enhance body weight gain and achieve a better feed conversion rate when compared with the control group (40 mg/kg diet), but the feed intake was non-significant in all groups. Our results are matched with **Swain et al. (2014)** who said that ZONPs has been reported to enhance growth performance, improve feed utility and provide economic benefits in poultry. Also, (**Zhao, Tan et al. 2014**) found that appropriate levels of zinc oxide nanoparticles (20 and 60 mg/kg) could enhance body weight gain and achieve a better feed conversion ratio compared with60 mg zinc oxide/kg diet. Also **Lina, FengHua et al. 2009** reported that zinc oxide nanoparticles improve the production performance of broilers on 42 days of feeding at the level of 40 mg/kg in the diet. In contrast (Pimentel, Cook et al. 1991) reported that zinc supplement in broilers diets had no significant effect on feed intake, total body weight and feed conversion ratio.

In the current study, the obtained results showed that Nano-zinc oxide 40- and 80- had increased the length and crypt depth in duodenum, jejunum and ilium, but the width and the villus: crypt ratio were non-significant in all groups. **(Mocchegiani, Costarelli et al. 2013)** demonstrated that zinc has effects such as increasing intestinal crypt-cell proliferation, improving epithelial cell turnover and repair, and maintaining the structure and function of the intestinal barrier. Our results are in agreement with **(Ali, Masood et al. 2017)** who found that villus height (VH) and villus surface area (VSA) in all parts of small intestine of 40 ZONP supplemented birds was significantly increased. In jejunum, the VH, VW, VSA and VH: CD were also increased in 80 ZONP. Also our results were matched with **(Ahmadi and Rahimi**

2011) who showed that birds supplemented with 60 and 90mg of ZONP/kg have increased histological parameters in jejunum during the starter phase in chicken.

5. Conclusion:

Our conclusion for this research indicated that zinc oxide nanoparticles at the dose rate of 40 or 80 mg is a considerable zinc source for broiler chicken with beneficial effects on the growth performance of broiler chicken.

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