

## Effects of nanoparticles of zinc oxide on productive performance of laying hens. – a review.

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**Abstract:** Nanotechnology has the potential to revolutionize in poultry industry with new tools for the molecular treatment of diseases and enhancing the ability of animal to absorb nutrients and therefore improving productive performance response of poultry. The essential trace elements play important roles such as nutrients metabolism, antioxidant, component of numerous metalloenzymes and protein. Nanoparticles zinc oxide (Nano-ZnO) is the specially prepared mineral salt having particle size of 1 to 100 nm. It promotes growth can act as antibacterial agent, modulates the immunity and production of laying hens. Using of Nano-ZnO in laying hen's nutrition as nanoparticle size will help and improve the egg production sector. Therefore, the purpose of this study is to give an overview on the potential of Nano-ZnO as feed additives in laying hen diets, in order to explore the mechanism of the effects of Nano-ZnO supplementation on laying hen productive performance and to observe their influence on feed intake, ammonia emissions, digestibility, egg production, and egg quality.

**Key words:** Egg production, Egg quality, Laying hens, Mode of action, Nanotechnology

### 1. Introduction

Nanotechnology is concerned with materials whose structures display significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nano scaled size (Wang 2000). Nanoparticles of Zinc Oxide (Nano-ZnO) is the specially prepared mineral salt having particle size of 1 to 100 nm (Swain et al., 2016) it can act as antibacterial, antioxidant and improve reproduction of the poultry. The commonly used grains in basal laying hen diets are rich in phytate content that may reduce availability and inhibit absorption of zinc.

With the use of nanotechnology, nanoparticles can be used as a supplemental source of trace minerals in the diets which have been found to have several novel properties different to those from bulk materials or commercial salts of these minerals (Mohapatra et al., 2014). The feed additives like nanoparticles of trace minerals play an active role in productive and reproductive performance of poultry production (Egwurugwu et al., 2013). Zinc element is an essential trace element and plays an important part in growth performance, carcass traits, and meat quality in broiler (Liu et al., 2011; Rajendran et al., 2013). Moreover, zinc can play an important role for the animals in many aspects such as antioxidant, growth performance, glandular development, protein synthesis, carbohydrate metabolism, nutrition, production performance and function as a cofactor in

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more than 300 metalloenzymes (Salim et al. 2008). It is also necessary for bone development, growth, enzyme structure and egg shell formation (Tüzün et al., 2018). Furthermore, Nano-ZnO reduced yolk lipids by decreasing biosynthesis, increasing lipid degradation (Zhao et al., 2016). Supplementations of 35 mg Zn/kg or 35 mg Zn + 250 mg vitamin C/kg to laying hens diet were significantly improved hen day egg production compared to the control diet (Gerzilov et al., 2015).

Rao et al. (2016) reported that broiler fed dietary supplemented with 40 mg/kg Zn was significantly improved growth performance and antioxidant responses of broiler male chicks exposed to heat stress during 1-21 days of age. Likewise, Sunder et al. (2008) showed that addition of zinc to broiler diets was important for improving immune response and mineral retention. Moreover, Abedini et al. (2017) found that supplementation of Nano-ZnO at 80 mg/kg diet enhances the performance and egg quality of laying hens. Furthermore, Bami et al. (2018) noted that supplementation of Nano-ZnO level 80 ppm during the summer is the best source of zinc compared to inorganic and organic sources of Zn for make better immunity and performance of broiler chickens. Therefore, nanotechnology may serve as a suitable source to replace the minerals in the diet. –These can be used at lower doses and can provide better result than the conventional Zn sources and indirectly prevents environmental contamination also (Swain et al., 2016). The purpose of this study is to give an overview on the potential of nanoparticles of zinc oxide as feed additives in laying hen diets, in order to explore the mode of action and observe its influence on feed intake, ammonia emissions, digestibility, egg production, and egg quality.

## **2. Mode of action of zinc in poultry nutrition**

Zinc can play an important role for human and animals in many aspects such as antioxidant, poultry production, glandular development,

protein synthesis, carbohydrate metabolism, nutrition, production performance and function as a cofactor in more than 300 metalloenzymes (Salim, 2008). It is necessary for bone development, growth, enzyme structure and eggshell formation (Tüzün, 2018). Addition of zinc could be reduced stress on the animals and increased the surface area which can enhance the bioavailability of the minerals (Rajendran et al. 2013). Nano-ZnO reduced yolk lipids by decreasing biosynthesis and increasing lipid degradation (Zhao *et al.*, 2016). The thickness of eggshell dropped and easily broken eggs with increased the age of layers, therefore addition of Nano-ZnO can enhance carbonic anhydrase which is playing an important role in the reaction of  $\text{CO}_2 + \text{H}_2\text{O}$  becoming  $\text{HCO}_3^-$  which the main component of eggshells ( $\text{HCO}_3 + \text{Ca}$  incorporation to  $\text{CaCO}_3$ ) (Tsai *et al.*, 2016). Moreover, zinc can play an important role in decreasing the negative impact of both cold and heat stress. For example, adding a combination of zinc and vitamin C in the diet of layer hens decreased the negative impact of both cold and heat stress, thus, there were an improvement in egg production compared to the control group (Gerzilov *et al.*, 2015). Rao *et al.* (2016) reported that supplementation of 40 mg/kg Zn significantly improved growth performance and anti-oxidant responses of broiler male chicks exposed to heat stress during 1-21 days of age. Sunder et al. (2008) showed that addition of zinc to broiler diets was important for improving immune response and mineral retention. Moreover, Abedini et al. (2017) found that supplementation of Nano-ZnO at 80 mg/kg diet enhance the performance and egg quality of laying hen. Therefore, nanotechnology serves as a suitable source to replace the minerals in the diet. Bami et al. (2018) reported that supplementation of Nano-ZnO level at 80 ppm during the summer is the best source of zinc compared to inorganic and organic sources of Zinc for make better immunity and performance of broiler chickens.

## **3. Recommendation levels of zinc in laying hens diets**

The zinc levels in the laying hens' diets vary widely according to dietary ingredients and zinc supplementation, ranging from 30 to 120 mg/kg (NRC, 1994; Abedini, 2017; Martin, 2016). The NRC (1994) recommends 33 mg Zn/kg of feed for diet of Brown Leghorn-Laying hens during the laying period. However, Abedini, et al (2017) reported that added 40, 80, and 120 mg Nano-ZnO/kg to laying diet and found that the diet optimal contained of zinc are 40 to 80 mg Nano-ZnO /kg. Also, Martin (2016) reported that supplementation of the same level of different sources of zinc (Availa zinc or zinc sulfate) at 40, 80, and 120 mg Zn/kg to laying diet, improved productive performance of laying hens without any side effects. Furthermore, Qin et al. (2017) found that supplementation of 30, 60, 90 and 120 mg (ZnSO<sub>4</sub>·7H<sub>2</sub>O)/kg diet and the recommended levels was 72 mg Zn/kg of diet for the brown-shell laying hens during the period from 20 to 40 weeks of age. Idowu et al. (2011) studied the impact of different sources of zinc (zinc oxide, zinc sulphate, zinc carbonate and zinc proteinate) each was added at 140 mg/Kg laying diet and noted that zinc proteinate was the best source.

#### **4. Effect of Nano-ZnO on productive performance of laying hens**

##### **4.1. Feed intake**

Many studies clarified that zinc could improve the laying hens feed intake. For example, Abedini et al. (2018) found that the feed intake was significantly ( $P < 0.05$ ) increased in Hy-Line W36 laying hens consuming 40 and 80 mg Nano-ZnO /kg from compared with 120 mg Nano-ZnO /kg and control group. In addition, Mao and Lien (2017) reported a significant ( $P < 0.001$ ) increase in average daily feed intake of brown layers (Hendrix) fed dietary supplemented with 80 mg/kg nano zinc compared with the control group. Also, Ahmadi et al. (2013) observed that supplementation of 120 mg/kg of Nano-ZnO significantly ( $P < 0.05$ ) increased feed intake. Similar results were observed in broiler according to Huang et al. (2007) who found that supplementation of ZnSO<sub>4</sub>·7H<sub>2</sub>O at 20 up

to 140 mg/kg were significantly increased daily feed intake compared to control group. On the other hand, Abedini et al. (2017) reported that supplementation of 80 mg Nano-ZnO /kg to laying hens diet did not affect feed intake. In addition, Tsai et al. (2016) studied the effect of different sources of zinc on white Leghorn laying hens and observed that 60 mg/kg Nano-ZnO did not affect feed intake. Likewise, Badawi et al. (2017) reported that feed intake of broiler chickens was not significantly affected by supplementation of 40 ppm Nano-ZnO /kg. Also, Zhang et al. (2017) found that the average daily feed intake was not affected in Hy-line layers fed diet supplemented with 35, 70, or 140 mg organic or inorganic zinc per kg diet compared to the control group. Yıldırım (2017) observed that supplementation of 20, 40, 60, 80 and 100 mg Nano-ZnO /kg to super Nick laying hens did not affect the feed intake. Plaimast et al. (2008) found that the amount of feed intake was not affected in hens diet added with 300 and 600 µg Zn/g from inorganic and organic zinc. Toriki et al. (2015) noted that feed intake was non ( $P > 0.05$ ) differences among treatment groups in Lohmann LSL laying hens fed diet supplemented with 40 mg Zn/kg. Also, Qin et al. (2017) reported that there was non-significant effect on feed intake of laying hens fed on dietary supplemented with 30, 60, 90 and 120 mg Zn/kg for 20 weeks. On the onther hand, Tabatabaie et al. (2007) found that feed intake was significantly ( $P < 0.05$ ) decreased in Hy-line layer fed diet supplemented with 50 mg/kg organic zinc compared with control group. Ahmadi et al. (2013) worked on broilers (Ross-308) and observed that supplementation of 120 mg/kg of Nano-ZnO significantly increased feed intake.

The variations in results of previous studies could be due to feed intake depends on several factors like genotype, housing, hygienic conditions, management, feeding system and diet attributes. Also, this is due to the lack of standardization in varying parts of experimental research. Nevertheless, it has to be kept in mind that there is still insufficient significant evidence on zinc as mineral supplement on laying hens.

#### 4.2. Feed conversion ratio

The feed conversion ratio (FCR) describes the hens' overall efficiency in converting ingested feed mass into egg mass over a specific period of time (Bozkurt et al., 2014). Abedini et al. (2017) found that FCR of laying hens was not affected by adding 80 mg Nano-ZnO /kg diet. In addition to, Tabatabaie et al. (2007) observed that FCR of Hy-line layer was not affected by 25 and 50 ppm organic and inorganic zinc. Abedini et al. (2018) found that adding the diets with 40, 80, and 120 mg Nano-ZnO /kg had no significant effect on FCR of laying hens. Also, Badawi et al. (2017) found that FCR of Cobb broiler chicks was significant ( $P < 0.05$ ) increased in group fed diet with 40 ppm Nano-ZnO/kg compared with control group. Mao and Lien (2017) supplementation of laying hens diets with 80 mg/kg Nano-ZnO had no beneficial influence on FCR. Zhang et al. (2017) found that FCR was not affected in Hy-line layers consuming 35, 70, or 140 mg organic or inorganic zinc per kg diet compared to the control group. In addition to, Yıldırım (2017) added 20, 40, 60, 80 and 100 mg Nano-ZnO /kg diet of Super Nick laying hens observed that FCR was non ( $P > 0.05$ ) differences between treatment groups. Idowu et al. (2011) reported that added 140 mg/kg diet of layer from different sources of zinc (Zinc Oxide, Zinc Sulphate, Zinc Carbonate and Zinc Proteinates) and observed that FCR was not affected by treatment groups. Furthermore, Plaimast et al. (2008) found that FCR was non ( $P > 0.05$ ) differences among treatment groups in laying consumed diet supplemented with 300 or 600  $\mu\text{g}$  Zn/g from organic and inorganic zinc. Qin et al. (2017) noted that FCR of layer was not affected by supplemented 30, 60, 90 and 120 mg Zn/kg diet. On the other hand, few studies reported that Zinc addition can reduce (improve) FCR. such as, Torki et al. (2015) noted that FCR of in Lohmann LSL laying hens fed diet supplemented with 40 mg/kg was significantly ( $P < 0.05$ ) decreased compared with control group. This indicates that FCR of laying hens in response to the same zinc may differ under

various experimental procedures or environmental conditions

#### 4.3. Egg production

The egg production was affected by zinc supplementation according to Abedini et al. (2018) reported that the egg production was significantly ( $P < 0.05$ ) increased in hens fed 80 mg Nano-ZnO/kg compared with 120 mg Nano-ZnO/kg and control group. Abedini et al. (2017) reported that egg production significantly ( $P < 0.05$ ) increased by supplementation of 80 mg Nano-ZnO/kg compared with control group. In addition to, Torki et al. (2015) found that that egg production significantly ( $P < 0.05$ ) increased in Lohmann LSL laying hens consumed diet supplemented with 40 mg/kg. Idowu et al. (2011) who added different sources of zinc and observed that egg production was significantly ( $P < 0.05$ ) increased in hens fed diet supplemented with 140 mg/kg ZnSO<sub>4</sub> and Zn proteinates compared with same level of ZnO, ZnCO and control group. Moreover, El-katcha, (2018) observed a significant increase in egg production in hens consumed diet supplemented with 30 and 60 ppm.

On the other hand, some studies noted that zinc supplementation did not affect egg production. Mao and Lien (2017) found that basal diet supplemented with 80 mg Nano-ZnO/kg had not affect egg production of laying hens. Moreover, Zhang et al. (2017) found that the egg production was not affected in Hy-line layers fed diet supplemented with 35, 70, or 140 mg organic or inorganic zinc per kg diet compared to the control group. Also, Yıldırım (2017) reported that egg production was not significantly affected by supplementation of 20, 40, 60, 80 and 100 mg Nano-ZnO /kg diet of Super Nick laying hens. Also, Tsai et al. (2016) found that egg production was not affected by 60 mg/kg different sources of zinc (ZnO –Nano-ZnO and Zn-methionine). Same result was observed by Tabatabaie et al. (2007) who found that egg production of Hy-line layer was not affected by (25 and 50 ppm) organic or inorganic zinc. Plaimast et al. (2008) found that dietary supplemented with 300 and 600  $\mu\text{g}$  Zn/g laying diet did not affect

in egg production. Moreover, Qin et al. (2017) found that supplemented Zinc with 30, 60, 90, or 120 mg Zn/kg diet of layer had non-significant effects. In contrast, few studies reported a significant decrease in egg production. Such as Zhao et al. (2016) observed that hen day egg production was significantly ( $P < 0.05$ ) decreased in layer fed diet with Nano-ZnO at 10, 25, 50, and 100 mg/kg during the period from 18 to 22 weeks of age compared with control group.

#### **4.4. Egg weight and egg mass**

It has been reported that the egg weight was significantly increased when hens fed diet supplemented with different level of Zn. Torki et al. (2015) reported that dietary supplementation of 40 mg Zn/kg to Lohmann LSL laying hens increased egg weight and egg mass ( $P < 0.05$ ) compared to control group. Amem and Al-Daraji (2011) observed that egg weight increased ( $P < 0.05$ ) by addition 50, 75, 100 mg/kg Zn to Cobb 500 broiler breeder hens.

On the other hand, Abedini et al. (2018) reported that supplementation of 40, 80, and 120 mg Nano-ZnO/kg to Hy-Line W36 laying hens diet resulted in the egg weight was not affected by treatment groups. Also, Qin et al. (2017) found that added of zinc levels from 30 to 120 mg Zn/kg diet of layer did not affected on the egg weight. Same result was observed by Mao and Lien (2017) found that addition of laying hens diets with 80 mg/kg nano zinc had no significant influence on egg weight. Moreover, Zhang et al. (2017) noted that egg weight was not affected in Hy-line layers fed diet with 35, 70, or 140 mg organic or inorganic zinc per kg diet compared to the control group. Abedini et al. (2017) reported that supplementation of 80 mg Nano-ZnO/kg to laying hens had not affect the egg weight. In addition to, Tsai et al. (2016) reported that egg weight was not affected by 60 mg Nano-ZnO/kg ( $P > 0.05$ ). Furthermore, Tabatabaie et al. (2007) observed that addition of 25 and 50 ppm organic and inorganic zinc did not significantly affect egg weight of hi-line layer. Yıldırım (2017) found that egg weight was not affected by added different sources of zinc

level at 20, 40, 60, 80 and 100 mg/kg diet (zinc oxide, zinc-proteinate and nano zinc-oxide powder). Also, Idowu et al. (2011) found that egg weight was not affected by added 140 mg/kg diet from different sources of zinc (zinc oxide, zinc sulphate, zinc carbonate and zinc proteinate). Plaimast et al. (2008) found that egg weight was not affected in layer fed diet added with 300 and 600  $\mu\text{g}$  Zn/g. The results of many studies showed that using different levels of Zinc caused a significant increased in egg mass. Such as, Abedini et al. (2018) reported that egg mass was significantly ( $P < 0.05$ ) increased in hens fed diets with 40 and 80mg Nano-ZnO/kg compared with 120 mg /kg and control group. Abedini et al. (2017) they Observed that egg mass was significantly ( $P < 0.05$ ) increased in laying fed with 80 mg Nano-ZnO/kg compared with control group. On the other hand, Mao and Lien (2017) found that supplementation of 80 mg Nano-ZnO/kg diet did not significant effect on egg mass. Yıldırım (2017) supplementation different level of Nano-ZnO at 20, 40, 60, 80 and 100 mg /kg to laying hen diets did not affect egg mass. Qin et al. (2017) indicated that egg mass was not affected in layer consumed diet supplemented with 30, 60, 90 and 120 mg Zn/kg diet.

## **5. Effect of zinc on egg quality parameters**

### **5.1. Physical egg quality**

Abedini et al. (2017) supplementation of 80 mg Nano-ZnO/kg to laying hen diet significantly increased shell thickness and Haugh unit compared with control group. Tsai et al (2016) reported that eggshell thickness was significantly ( $P < 0.05$ ) increased supplementation of 60 mg Nano-ZnO/kg compared with control group. Also, Torki et al. (2015) indicated that the egg shell thickness was significantly ( $P < 0.05$ ) increased in layer fed diet supplemented with 40 mg Zn/kg compared to control group. However, Tabatabaie et al. (2007) found that the yolk weight, shell thickness, shell weight were not affected in Hy-line layer fed diet supplemented with 25 and 50 ppm organic and inorganic zinc. Abedini et al. (2018) found that

supplementation of 40, 80, and 120 mg Nano-ZnO/kg to Hy-Line laying hens diet did not change the egg shell weight, yolk weight, albumen weight and height. Moreover, Mao and Lien (2017) found that eggshell strength and Haugh unit of laying hens were not significantly affected by 80 mg Nano-ZnO/supplementation, but albumen width was significantly ( $p < 0.005$ ) decreased compared with control group. Idowu *et al.* (2011) found that egg length, shell index, egg shell thickness, egg shell weight, yolk weight, albumen weight and height were not affected in layer fed diet supplemented with 140 mg Zn/kg.

### 5.2. Zinc concentration of egg

Abedini *et al.* (2017) found that supplementation of 80 mg Nano-ZnO /kg significantly ( $P < 0.01$ ) increased eggs yolk Zn concentration compared to control group. Also, Abedini *et al.* (2018) found that adding the diets with 40, 80, and 120 mg Nano-ZnO/kg had significantly ( $P < 0.01$ ) increased eggs yolk Zn concentration compared with control group. Likewise, Plaimast *et al.* (2008) observed that the Zn content of egg yolk was significantly ( $p < 0.005$ ) increased in layer consumed diet supplemented with 600  $\mu\text{g}$  Zn/g compared with control group. Mao and Lien (2017) found that the eggshell zinc content was significant ( $P < 0.005$ ) increase in layer fed diet with 80 mg Nano-ZnO/kg compared with the control group.

### 6. Effect of zinc on blood metabolic profile

Some blood parameter has a significant change due to Nano-ZnO supplementation to laying hens' diet. Yang *et al.* (2012) found that estrogen and thyroxine (T4) hormone concentrations were not significantly different in layer fed diet supplemented with 30 mg/kg Zn, 65 mg/kg Zn+30 mg/kg Mn and 100 mg/kg Zn+60 mg/kg Mn, but 30 mg/kg Zn treatment significantly ( $P < 0.005$ ) decreased triiodothyronine (T3) compared with control group. A lot of studies indicated that supplementation of Nano-ZnO to layer diet

significantly increased blood zinc content. For example, Abedini *et al.* (2018) found that supplementation of laying hen diet with 40, 80, and 120 mg Nano-ZnO/kg had significantly ( $P < 0.05$ ) increased serum Zn concentration compared with control group. Mao and Lien (2017) found that supplementation of Nano-ZnO at 80 mg/kg to layer diet significantly ( $P < 0.01$ ) increased serum Zn concentration compared with control group. Also, Idowu *et al.* (2011) found that plasma Zn concentration was significantly ( $P < 0.05$ ) increased in layer consumed diet with 140 mg/kg from different sources of zinc (zinc oxide, zinc sulphate, zinc carbonate and zinc proteinate). Moreover, Torki *et al.* (2015) found that plasma Zn concentration of the layer was significantly ( $P < 0.05$ ) increased by Zn diet supplementation compared with control group. However, Abedini *et al.* (2017) observed that supplementation of 80 mg Nano-ZnO kg to layer diet did not have an effect on plasma Zn concentration. Badawi *et al.* (2017) found that adding 40 ppm Nano-ZnO/kg to Cobb broiler chicks' diet had not affect total cholesterol and LDL cholesterol, however HDL cholesterol was significantly ( $P < 0.05$ ) increased compared with control group. Also, Zhao *et al.* (2016) found that total cholesterol of laying hens was significantly decreased ( $P < 0.05$ ) by supplementation of the Nano-ZnO at 100 and 200 mg/kg compared to control group. However, Torki *et al.* (2015) found that serum cholesterol was not affected in layer fed diet with 40 mg Zn/kg diet.

### CONCLUSION

Generally, it can be concluded that Nano-ZnO has the potential to be considered as an alternative to other sources and improving productive performance and health status of laying hens. Nevertheless, there is still further research under standardized conditions needed to evaluate the exact mechanism of action and to determine the optimal dietary inclusion level in order to optimize egg production, nutrient retentions and maintain healthy birds.

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