SVU-International Journal of Agricultural Science
Volume 1 Issue (1) pp.:34-45. 2019
Print ISSN 2636-381X | Online ISSN 2636-3801

RESEARCH ARTICLE

Applications of nanoparticles of zinc oxide on productive performance of laying hens

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Abstract: This study was conducted to determine the effects of supplementation of nanoparticles of zinc oxide in laying hen diets on laying performance, egg quality, nutrient digestibility, and zinc retention. A total of one hundred and twenty Bovans Brown laying hens (55-week-old) were assigned to four treatment diets including nanoparticles of zinc oxide at 0, 20, 40, or 60 mg/kg, respectively, for 12 weeks. Each treatment had six replicates with five hens each. The results revealed that feed conversion ratio was significantly improved (linear, $P<0.01$) with increasing levels of nanoparticles of zinc oxide in laying hens diet from 55-59, 59-63, 63–67 and 55-67 weeks of age. Hen day egg production, egg mass were significantly increased ($P<0.05$) by supplementation of nanoparticles of zinc oxide during the experimental periods. Moreover, Haugh unit, shell thickness and eggshell percentage were improved ($P<0.01$) with increasing levels of nanoparticles of zinc oxide. Digestibility of crude protein, ether extract and crude fiber linearly increased ($P<0.001$) with increasing levels of supplementation. Interestingly, the serum biochemical analyses revealed that serum cholesterol, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), urea and somewhat creatinine linearly decreased with increasing Nano-ZnO levels in the diets. In conclusion, inclusion of nanoparticles of zinc oxide at 20, 40, or 60 mg/kg had improved productive performance, Haugh unit, shell quality, nutrient digestibility, cholesterol, liver and kidney functions and can be used as an effective feed additive in laying hens diets.

Key words: Egg production, Egg quality, Laying hens, Nanoparticles, Nutrient digestibility, Serum profile.

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).

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Received: April 29, 2019;
Accepted: May 7, 2019;
Published*:* May 9, 2019.

Nanoparticles of Zinc Oxide (Nano-ZnO) are the specially prepared mineral salt having particle size of 1 to 100 nm (Swain *et al*., 2016) and it may promotes productive performance, modulates the immunity and 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 minerals nanoparticles may

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 may play an active role in productive and reproductive performance of poultry production (Egwurugwu *et al*., 2013). Moreover, zinc can play an important role for the poultry in many aspects such as antioxidant, growth performance, protein synthesis and nutrient metabolism (Salim *et al.*, 2008). Zinc is also necessary for bone development, production, enzyme structure and egg shell formation (Tüzün *et al*., 2018). Furthermore, Nano-ZnO reduced yolk lipids by decreasing biosynthesis and increasing lipid degradation (Zhao *et al*., 2016). Likewise, Abedini *et al.*, (2017) found that supplementation of Nano-ZnO at 80 mg/kg diet enhances the performance and egg quality of laying hens. 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 (Swain *et al*., 2016). Therefore, the purpose of this study was to evaluate the potential of different levels of Nano-ZnO as feed additives in laying hen diets in order to observe its impact on productive performance, nutrient digestibility, egg quality, ammonia emissions and serum metabolic profile.

2. Materials and Methods

2.1. Experimental birds, design and feed preparation

The present study was conducted in the breeding farm of Agricultural Research Centre of the Faculty of Agriculture, South Valley University, Qena, Egypt. A total of one hundred and twenty Bovans Brown laying hens (55-week-old) were assigned to four treatment diets including nanoparticles of zinc oxide at 0, 20, 40, or 60 mg/kg, respectively, for 12 weeks. Each treatment had six replicates with five hens each. The hens were housed in a 60 cm × 60 cm × 40 cm cage. All hens were housed in an environmentally controlled house with temperature maintained at approximately 24◦C. The house had controlled ventilation and lighting (16L:8D) systems. All hens were supplied with feed and water for *ad libitum* consumption. Animal housing and handling procedures during experimentation were approved by the Committee of Ethics of the Animal and Poultry Production, Department of the South Valley University. The hens were fed diets in mash form during the experiment (55–67 weeks of age). The basal diet was formulated (Table 1) according to recommendations of NRC (1994).

2.2. Productive performance parameters

Hen day egg production, egg weight and feed consumption were daily recorded throughout the experimental period. Feed intake was used to calculate feed conversion ratio (FCR), expressed as kg of feed consumed per kg of egg produced (Catli et al., 2012). Hen day egg production (%) was calculated from the total number of eggs collected from a cage divided by the average number of birds multiplied by the number of days (biweekly as a unit), and the result was multiplied by 100 (Pelica et al., 2009). The average egg weight was calculated based on the total egg weight divided by the number of produced eggs. Egg mass was calculated by multiplying egg weight by egg

production/100. The magnitude of production variables such as feed intake and egg production was adjusted for hen mortalities. Deaths were recorded daily as they occurred.

Table 1. Ingredient composition and chemical analysis (g/kg) of the basal diet

Ingredients	g/kg
Yellow Corn	679
Soybean meal (44 %)	178
Soybean oil	8
Corn gluten meal	25
Di-calcium phosphate	19
Limestone	82
Vitamin and mineral mix[a]	2
DL-Methionine	2.5
DL-Lysine	1.0
Sodium chloride	3
Choline	0.5
Determined analysis, g/kg	
Dry matter	890
Ash	113
Crude protein	183
Ether extract	41.9
ADFom	42.2
NDFom	102
Calcium	37.5
Phosphorus	5.93
Starch	376
Sugar	42.1
Lysine	0.96
Methionine	0.39
Gross energy (MJ/kg)	16.2

[a]Provided the following per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 7200 IU; vitamin E, 20 IU; vitamin B_1, 833 IU; vitamin B_2, 2000 IU; vitamin K, 3 mg; vitamin B_{12}, 60 IU; pyridoxine, 0.225 µg; pantothenic acid, 10 mg; niacin, 35 mg; folic acid, 1.5 mg; biotin 125 mg; Mn, 90 mg; Cu, 7.5 mg; Zn, 65 mg; Fe, 50 mg; Se, 0.1 mg.

2.3. Egg quality parameters

The parameters relative to egg quality were evaluated at 63 and 67 weeks of age. Twenty four eggs were randomly collected per treatment (4 egg per replicate) to determine egg quality parameters according to Abdel-Wareth and Lohakare (2014). The collected eggs were weighed. The eggs were broken out individually onto a glass plate surface and allowed to sit for 5 min. The heights of yolk and albumen, and the diameter of yolk were measured using the calipers. Yolks were separated from albumen manually, and both were weighed. The weight of shell, albumen and yolk were divided by whole egg weight and then multiplied by 100 to determine percentage weight. Eggshell thickness (without inner and outer shell membranes) was measured at the middle part of the eggshell using (QCT) shell thickness micrometre (Technical Services and Supplies Ltd., England). Haugh units (HU) were

calculated from the records of albumen height (H) and egg weight (W) using the following formula: HU = 100 log10 (H - 1.7W $^{0.37}$+ 7.56), according to Haugh (1937)**.**

2.4. Nutrient digestibility

At the end of experiment, a total of 48 healthy laying hens were placed in metabolic cages, two birds randomly selected from each replicate, which were used for collecting excreta. Excreta samples of each replicate were collected and immediately stored at -20 °C. Feed, egg and fecal samples were analyzed for moisture by oven drying (930.15), ash by incineration (942.05), protein by Kjeldahl (984.13), and ether extract by Soxhlet fat analysis (954.02), calcium (927.02) and phosphorous (935.59) as described by the AOAC International (2006). Crude fiber was determined by the Weende method (AOAC, 2006). Calcium was measured using atomic absorption spectrometry and phosphorus was analyzed colorimetrically (method 10.6.1;VDLUFA, 2007). Starch and sugar contents of the diets were quantified using official European Union methods (Anonymous, 2009). The contents of neutral detergent fiber (assayed with a heat stable amylase, aNDFom) and acid detergent fibre (ADFom), both expressed exclusive residual ash, were determined sequentially without sodium sulphite (method 6.5.1 for aNDFomand 6.5.2 for ADFom; VDLUFA, 2007). Lysine and methionine (after oxidation) were analyzed using an amino acid analyser after hydrolysis (6 M HCl) of the diets (method 4.11.1; VDLUFA, 2007). The gross energy content of the diets was measured using an adiabatic bomb calorimeter (model C 200; IKA, Heitersheim, Germany). At the end of the experiment, fresh excreta of all chickens were collected and mixed well for each respective cage and then stored at −20°C until analyzed for ammonia. Ammonia-N (mg NH_3-N/30 ml) from both the blank and from the syringes containing substrate (NH_3-N sample) was measured by distillation (Vapodest 50 s carousel; Gerhardt, Königswinter, Germany).

2.5. Blood sampling and laboratory analyses

At the end of experimental period, 12 birds from each treatment were randomly selected (2 birds per replicate). The blood was drawn from wing vein using sterilized needles and syringes in vacutainer tubes for serum collection. Feed was not withdrawn from the feeder before blood was collected. The blood was centrifuged for 10 min (3000 × g) at room temperature. Serum was collected in tubes and stored at -20◦C until further analysis. Liver enzymes including glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), as well as kidney function tests including urea and creatinine, and Cholesterol was analyzed colorimetrically using **(**Spectrophotometer Sunostk, SBA733 Plus).

2.6. Statistical analysis

The statistical analysis was performed using a completely randomized design and the general linear model (GLM) procedure of SAS 9.2 (SAS Institute, 2009). The model only included the level of Nano-ZnO supplementation. Pens were the experimental units for all analysis. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of the increasing levels of inclusion and Duncan multiple range test was used to compare means (Duncan,1955). Significance was declared at $P<0.05$; P-

values less than 0.001 are expressed as "<0.001" rather than the actual value.

3. Results

3.1. Productive performance

There was no mortality and the general health status of laying hens was good throughout the experimental period. The effects of dietary Nano-ZnO supplementation on egg weight, egg mass, egg production, feed intake and feed conversion ratio of Bovans laying hens are presented in Tables 2. Incremental dietary Nano-ZnO levels at 20, 40 and 60 mg/kg linearly increased (P<0.01) egg weight, hen day egg production, egg mass and feed intake during the period of from 55-59, 59-63, 63–67 and 55-67 weeks of age. Supplementation of 60 mg/kg Nano-ZnO resulted in the highest productive performance. Moreover, feed conversion ratio was significantly improved (linear, P<0.01) with increasing levels of Nano-ZnO in laying hens diet. However, the addition of the Nano-ZnO (20, 40, or 60 mg/kg of diet) had no significant (P>0.05) effect (linear or quadratic) on feed inatke among all treatments.

Table 2. Effect of Nano-ZnO on productive performance of laying hens

Items	Nano-Zn (mg/kg)				SEM[a]	P-Value	
	0	20	40	60		Lin[b]	Quad[c]
55–59 weeks of age							
Egg weight, (g/hen/day)	63.47	66.47	66.81	66.82	0.800	0.025	0.014
Egg mass, (g/hen/day)	49.03	56.19	56.95	56.73	1.400	0.022	0.020
Hen-day production, (%)	77.20	84.52	84.741	84.88	1.577	0.013	0.086
Feed intake, (g/hen/day)	100.19	106.75	103.74	103.50	1.841	0.698	0.062
Feed conversion ratio	2.048	1.899	1.829	1.825	0.047	0.019	0.146
59–63 weeks of age							
Egg weight, (g/hen/day)	61.58	65.04	65.33	65.81	0.924	0.020	0.316
Egg mass, (g/hen/day)	49.70	56.80	56.70	57.43	1.56	0.021	0.312
Hen-day production, (%)	80.75	87.36	87.47	87.24	2.301	0.016	0.565
Feed intake, (g/hen/day)	99.76	109.14	103.5	99.07	2.678	0.538	0.033
Feed conversion ratio	2.009	1.923	1.829	1.725	0.064	0.002	0.399
63–67 weeks of age							
Egg weight, (g/hen/day)	63.23	66.47	66.64	66.65	1.481	0.363	0.310
Egg mass, (g/hen/day)	49.69	53.37	54.04	54.26	1.339	0.012	0.152
Hen-day production, (%)	78.63	81.50	81.10	81.41	0.754	0.046	0.129
Feed intake, (g/hen/day)	98.92	100.76	101.04	100.5	2.643	0.261	0.077
Feed conversion ratio	1.991	1.888	1.859	1.852	0.054	0.001	0.668
55–67 weeks of age(total)							
Egg weight, (g/hen/day)	62.76	66.29	66.59	66.73	0.818	0.041	0.072
Egg mass, (g/hen/day)	49.47	55.98	56.20	56.31	0.760	0.001	0.008
Hen-day production, (%)	78.86	84.46	84.47	84.51	0.514	0.001	0.059
Feed intake, (g/hen/day)	99.62	101.88	102.09	100.65	1.629	0.663	0.289
Feed conversion ratio	2.016	1.819	1.816	1.786	0.033	0.008	0.081

Values in each row are means of 6 replicates for each treatment (5 birds/replicate).

[a] Standard error of the means

[b] Linear responses to dietary inclusion levels.

[c] Quadratic responses to dietary inclusion levels.

3.2. Nutrient digestibility

The results of nutrient digestibility as affected by feeding different levels of Nano–ZnO in laying hens are given in Table 3. Supplementation of Nano-ZnO in hen diet at levels of 20,40 and 60 mg Zn/kg for 12 weeks had a linearly increase in dry matter, ether extract, crude protein and crud fiber digestibility compared to control diet.

Moreover, results indicated that ammonia production was linearly ($p<0.011$) decreased in hens consumed diet supplemented at 20, 40 and 60 mg/kg compared with control group.

Table 3. Effect of Nano-ZnO on nutrient digestibility and ammonia emission (g/100g of DM excreta) of laying hens

Items	Nano-Zn (mg/kg)				SEM[a]	P-Value	
	0	20	40	60		Lin[b]	Quad[c]
Dry Matter %	65.49	68.36	68.53	69.90	1.611	0.017	0.858
Crude Protein %	62.403	68.152	69.135	70.03	1.352	0.005	0. 202
Ether Extract %	80.076	89.829	89.861	89.867	2.598	0.004	0.089
Crude Fiber %	38.907	43.816	44.744	44.602	2.813	0.005	0.321
Ammonia, g/100g	6.368	4.143	3.979	3.689	0.537	0.001	0.109

Values in each row are means of 12 replicates for each treatment.
[a] Standard error of the means
[b] Linear responses to dietary inclusion levels.
[c] Quadratic responses to dietary inclusion levels.

3.3. Egg quality

Egg quality criteria were greater for hens fed the Nano-ZnO diet than those fed the control diet at 67 weeks of age (Table 4 and 5). The Haugh unit was higher ($P<0.01$) for laying hens fed Nano-ZnO dietary treatments than those fed control diet. Dietary addition of Nano-ZnO (20, 40, or 60 mg/kg) to laying hens for 12 weeks resulted in a linear increase ($P<0.01$) in egg shell percentage, and eggshell thickness ($P<0.01$) in a dose-dependent manner. However, dietary Nano-ZnO had no significant effect on albumen and yolk percentages ($P\geq0.05$) in the laying hens at 67 weeks of age. Results of chemical composition of egg are presented in Table 5. Egg ether extract content was not significantly ($P\geq0.05$) influenced in laying hens fed diets supplemented with Nano-ZnO. On the other hand, the Nano-ZnO supplementations resulted in a linear increase in egg contents of crude protein ($P=0.001$) and dry matter ($P=0.017$) with the increasing levels in the diets.

3.4. *Serum biochemical parameters*

The influences of dietary supplementation with Nano-ZnO on some serum biochemical analyses of laying hens are shown in Table 6. Interestingly, the serum biochemical analyses revealed that serum cholesterol ($P=0.013$) linearly decreased with increasing Nano-ZnO levels in the diets. Likewise, supplementation

of Nano-ZnO to laying hens diet linearly decreased serum GOT (P=0.006), GPT (P=0.002) and urea (P=0.011) compared to control group. However, serum creatinine was quadratically decreased (P=0.014) with increasing supplementation of Nano-ZnO.

Table 4. Effect of Nano-ZnO on egg quality of laying hens at 67 weeks of age

Items	Nano-Zn (mg/kg)				SEM[a]	P-Value	
	0	20	40	60		Lin[b]	Quad[c]
Haugh unit, score	80.107	87.666	88.503	88.792	3.009	0.001	0. 452
Shell thickness (mm)	0.331	0.364	0.365	0.367	0.021	0.012	0.087
Shell (%)	11.775	12.008	12.443	12.777	0.220	0.008	0.825
Albumen (%)	65.168	65.276	64.298	64.066	0.713	0.216	0.818
Yolk (%)	23.056	22.716	23.259	23.157	0.710	0.797	0.870

Values in each row are means of 24 replicates for each treatment (6 eggs /replicate).
[a] Standard error of the means.
[b] Linear responses to dietary inclusion levels.
[c] Quadratic responses to dietary inclusion levels.

Table 5. Effect of Nano-ZnO on egg chemical compositions of laying hens at 67 weeks of age

Items	Nano-Zn (mg/kg)				SEM[a]	P-Value	
	0	20	40	60		Lin[b]	Quad[c]
Dry matter	59.928	62.928	64.827	65.948	1.480	0.017	0.543
Ether extract	34.488	34.064	37.952	37.314	2.736	0.342	0.969
Crude protein	49.893	53.826	57.331	57.906	1.209	0.001	0.202

Values in each row are means of 24 replicates for each treatment (6 eggs /replicate).
[a] Standard error of the means
[b] Linear responses to dietary inclusion levels.
[c] Quadratic responses to dietary inclusion levels.

Table 6. Effect of Nano-ZnO on blood parameters

Items	Nano-Zn (mg/kg)				SEM[a]	P-Value	
	0	20	40	60		Lin[b]	Quad[c]
Cholesterol (mg/dl)	440	333	325	280	19.338	0.013	0.313
GOT(u/l)	417	363	361	360	8.58	0.006	0.405
GPT (u/l)	95.690	77.50	74.94	74.18	6.150	0.002	0.161
Urea (mg/l)	16.097	10.52	8.61	7.33	1.055	0.011	0.102
Creatinine (mg/dl)	3.520	2.333	1.477	2.857	0.410	0.159	0.014

Values in each row are means of 12 replicates for each treatment (2 birds /replicate).
[a] Standard error of the means
[b] Linear responses to dietary inclusion levels.
[c] Quadratic responses to dietary inclusion levels

4. Discussion

In the current study, the health status of laying hens was good during the experimental period (55 to 67 weeks of age) which could be related to the controlled house system.

Due to scarcity of available information on effect of Nano-ZnO on laying hens, comparison was done with other studies that used other Zn sources. In current study, supplementation of Nano-ZnO at 20,40 and 60 mg/kg improved hen day egg production, egg weight, egg mass and feed conversion ratio, which was mainly due to improving the conversion of digested feed into eggs showing linear effects. These results are agree with Torki *et al.* (2015) who found that supplementation of 40 mg Zn/kg significantly improved egg production, egg mass and egg weight, but feed intake of laying hens was not affected. Also, Supplementation of (80 mg/kg) Nano-ZnO to laying hens diet significantly increased egg production and egg mass without any significant effect on feed intake (Abedini *et al.*, 2017). 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 supplementing the diets with 40, 80, and 120 mg Nano-ZnO /kg had no significant effect on FCR of laying hens. Idowu *et al.* (2011) 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 ZnSO4 and Zn proteinate compared with same level of ZnO, $ZnCO_3$ and control group. Moreover, El-katcha et al. (2018) observed a significant increase in egg production in hens consumed diet supplemented with 30 and 60 ppm of Nano-ZnO/kg diet.

On the other hand, Tsai *et al.* (2016) found that 60 mg/kg Nano-ZnO did not affect feed intake, egg production, egg weight and egg mass of white Leghorn laying hens. In addition to, Mao and Lien (2017) observed that egg mass, egg production, egg weight and feed conversion ratio were not affected, but feed intake was significantly increased when layer fed diet supplemented with 80 mg Nano-ZnO /kg. Furthermore, in current study, birds fed diets supplemented with 20, 40 and 60 mg/kg Nano-ZnO linearly increased nutrient digestibility of dry matter, crude protein, crude fiber and ether extract (Table 3). These findings might be directly associated with improvements in laying performance. The results agree with Saleh *et al.* (2018) who reported that supplementation of zinc at 25, 50 and 100 mg/kg to broiler diets improved dry matter digestibility and crude protein utilization compared to control diet. In this connection, supplementation of zinc at 30 and 60 mg/kg linearly increased digestibility of dry matter, organic matter, crude protein and ether extract compared to control diet (Sahin and Kucuk, 2003; Sahin *et al.*, 2009). On the other hand, Tsai *et al.* (2016) found that supplementation 60 mg/kg of different sources of zinc (ZnO, Zn-methionine and Nano-ZnO) had no significant ($P > 0.05$) effect on dry matter, crude ash, crude protein and crude fat. Shinde *et al.* (2006) observed that supplemented 20 mg/kg of zinc did not significantly affect the nutrient digestibility compared to the control group. The present study showed that eggshell percentage, eggshell thickness, and Haugh unit as well as dry matter and crude protein composition of eggs were linearly increased in laying hens. It could be supposed that Nanoparticles of zinc oxide may provide a site of calcium deposition in uterus and consequently increase shell weight and shell thickness in

comparison to control group. Our findings are in agreement with Tsai *et al*. (2016) who found that supplementation of Nano-ZnO at 60 mg/kg to layer diet significantly (P<0.05) increased eggshell thickness. Also, Abedini *et al.* (2017) showed that layer fed diet containing 80 mg/kg Nano-ZnO significantly (P<0.05) increased shell thickness and Haugh unit compared with control group. Likewise, Abedini *et al.* (2018) reported that supplementation of Nano-ZnO at 40, 80, and 120 mg Zn/kg improved Haugh unit and shell thickness of Hy-Line W36 laying hens. However, Zhao *et al.,* (2016) reported that the egg protein and water contents were not affected in layer fed diet with Nano-ZnO at 10, 25, 50, 100 and 200 mg/kg. Only limited systematic research is available, which handicaps full comprehension of physiological responses of Nano-ZnO. Interestingly, Nano-ZnO linearly decreased the serum cholesterol, GOT, GPT, urea and creatinine levels in laying hens in our study. The values are within the normal physiological range (Akbari *et al.,* 2016; Azzam *et al.,* 2011; Saleh *et al.,* 2018; El-katcha *et al.,* 2018). In general, serum GOT and GPT were considered as an indices for liver damage as well as urea and creatinine were considered as an indices for kidney damage. Therefore, results of the present study may provide evidences for occurrence of no toxicity of feeding Nano-ZnO in laying hens during 55 to 67 weeks of age. These results agree with El-katcha *et al.* (2018) who reported that serum liver enzymes (GOT and GPT) activities and cholesterol concentrations were reduced in laying hens supplemented with 30 mg of Nano-ZnO/kg diet. In current study, serum cholesterol concentrations were decreased with increasing Nano-ZnO supplementations. The decrease in cholesterol may be due to that Zn prevent cholesterol from absorption in gastro intestinal tract (Tizard et al., 1989; Wang et al., 2011) and may promote the growth and activity of lactic acid bacteria, which reduces the cholesterol level by producing enzymes disintegrating bile salts and making them unconjugated (Gibson and Roberfroid, 1995). Furthermore, Aksu et al. (2011) reported that supplementation of organic complexes of Zn to chicken diets decreased total cholesterol and LDL-Cholesterol compared to control. Zhao *et al.* (2016) found that serum 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. Abd-El-Samee *et al.* (2013) reported that serum cholesterol level was decreased by supplementation of Zn to broiler diets. Likewise, Uyanik *et al.* (2001) found that supplementation of Zn to broiler diets decreased serum cholesterol concentrations. However, Torki et al. (2015) found that serum cholesterol of laying hens was not affected by feeding 40 mg Zn/kg diet.

5. Conclusion

In conclusion, Nano-ZnO could be incorporated into laying hen's diet at inclusion levels up to 60 mg/kg for 12 weeks, with improvements in egg production, egg weight, egg mass, feed conversion ratio, Haugh unit, eggshell and serum cholesterol. Inclusion of Nano-ZnO to laying hens diet increased the digestibility of crude protein, ether extract, and crude fiber without any toxicity of liver or kidney of laying hens.

Acknowledgements

Financial support provided by Research Management Fund of South Valley University, Egypt is gratefully acknowledged.

Conflict of interest

The authors hereby declare that no competing and conflict of interests exist.

References

Abd-El-Samee D.L., El-Wardany I., Nematallah G.A. and Abo-El-Azab O.M. (2013) 'Effect of dietary organic Zn and prebiotic on productive performance and immune response of growing quails', *Iranian J Applied Anim Sci., 3:761-767.*

Abdel-Wareth, A.A.A. and Lohakare J.D. (2014) 'Effect of dietary supplementation of peppermint on performance, egg quality, and serum metabolic profile of Hy-Line Brown hens during the late laying period. Anim', *Feed Sci. Technol. 197,114-120.*

Abedini M., Shariatmadari F., Torshizi M.A.K. and Ahmadi H.(2018) ' Effects of zinc oxide nanoparticles on performance, egg quality, tissue zinc content, bone parameters, and antioxidative status in laying hens', *Biolog. Trace Ele.Res., 184:259-267.*

Abedini M., Shariatmadari F., Torshizi M.K. and Ahmadi H. (2017) 'Effects of a dietary supplementation with zinc oxide nanoparticles, compared to zinc oxide and zinc methionine, on performance, egg quality and zinc status of laying hens', *Livestock Science*, 203: 30-36.

Akbari, M., Torki, M., and Kaviani, K. (2016) ' Single and combined effects of peppermint and thyme essential oils on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition (6.8±3 °C)', *Int. J. Biometeorol., 60:447-454.*

Aksu T, Aksu MI, Yoruk M.A.and Karaoglu M. (2011) 'Effects of organically-complexed minerals on meat quality in chickens', *Br. Poult. Sci., 52:558–563.*

AOAC. (2006) 'Official methods of analysis 17th edition', Assoc. Off' Anal. Chem, *Arlington, Virginia, USA.*

Azzam, M.M.M., Dong, X.Y., Xie, P., Wang, C. and Zou, X.T. (2011) ' The effect of supplemental l-threonine on laying performance, serum free amino acids, and immune function of laying hens under high-temperature and high-humidity environmental climates. *J. Appl. Poult. Res., 20 : 361–370.*

Catli, A. U., Bozkurt, M. K., Kuçukyılmaz, M., Ginar, E., Bintas, F. and Atik, H. (2012) 'Performance and egg quality of aged laying hens fed diets supplemented with meat and bone meal or oyster shell meal', *South African Journal of Animal Science, 42(1), 74–82.*

Duncan, DB. (1955) 'Multiple ranges and multiple T-test', *Biometrics, 11, 1–42*

Egwurugwu, J.N., Ifedi, C.U., Uchefuna, R.C., Ezeokafor, E.N. and Alagwu E.A. (2013) 'Effects of zinc on male sex hormones and semen quality in rats', *Niger. J. Physiol. Sci., 28: 017-022.*

El-katcha M., M.A., Soltan, M.M. Arafa, k. El-Naggar and Kawarei E.R. (2018) ' Impact of dietary replacement of inorganic zinc by organic or nano sources on productive performance, immune response and some blood biochemical constituents of laying hens', *Alexandria J of Veterinary Sci, 59:48-59.*

Gibson G.R. and Roberfroid M.B. (1995) ' Dietary modulation of the human colonic microbiota. Introducing the

concept of prebiotics', *J Nutrit*. ,*125:1401–1412.*

Haugh, R. R.(1937) 'The Haugh unit for measuring egg quality. *US Egg Poultry Magazine 43:522–555, 572–573*.

Idowu O., Ajuwon R., Oso A. and Akinloye O. (2011) 'Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens' *International J of Poultry Sci, 10: 225-230.*

Mao S. Y. and Lien T. F. (2017) 'Effects of nanosized zinc oxide and γ-polyglutamic acid on eggshell quality and serum parameters of aged laying hens', *Archives of animal nutrition 71, 373-83.*

Mohapatra, P., Swain, R.K., Mishra, S.K., Behera. T., Swain, P., Behura. N.C., Sahoo, G., Sethy, K., Bhol, B.P. and Dhama, K. (2014) 'Effects of dietary nano-selenium supplementation on the performance of laying grower birds', *Asian J. Anim. Vet. Adv., 9: 641-652.*

NRC [National Research Council]. (1994) 'Nutrient requirements of poultry' 9th revised ed., *National Academie Press, Washington, D. C., 155 pp.*

Pelica, K., Garcia, E.A., Silva, A.P., Berto, D.A., Molino, A.B., Vercese, F.(2009) 'Calcium and available phosphorus for laying hens in second production cycle', *Brazilian Journal of Poultry Science, 11, 39–49.*

SAS, Institute. (2009) 'User's Guide: Statistics. Version 9.2. SAS Institute, *Inc., Cary, NC, USA*

Sahin K. and Kucuk O. (2003) 'Zinc supplementation alleviates heat stress in laying. Japanese quail', *J Nutrit., 33, 2808–2811.*

Sahin K, Sahin N, Kucuk O, Hayirli A, Prasad A.S.(2009) ' Role of dietary zinc in heat stressed poultry: a review', *Poult Sci., 88, 2176–2183.*

Saleh A.A., M.M. Ragab, E.A.M. Ahmed, A.M. Abudabos and Ebeid T.A. (2018) ' Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature', *J of Applied Animal Research*, 46: 820-827.

Salim H., Jo C. and Lee B. (2008) ' Zinc in broiler feeding and nutrition', *Avian Biology Research, 1: 5-18.*

Shinde P., Dass R.S., Garg A.K., Chaturvedi V.K. and Kumar R.(2006) ' Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile, and immune response of male Guinea pigs', *Biological trace element research, 112, 247-62.*

Swain P.S. Somu B.N. Rao, D.R., Dominic G. and Selvaraju S.(2016) ' Nano zinc, an alternative to conventional zinc as animal feed supplement- a review', *Animal Nutrition 2 , 134e141.*

Tabatabaie M., Aliarabi H., Saki A., Ahmadi A. and Siyar S. (2007) 'Effect of different sources and levels of zinc on egg quality and laying hen performance', *Pakistan J of Biological Sci., 10: 3476-8.*

Tizard I.R., Carpenter R.H.,McAnalley B.H. and Kemp M.C. (1989) ' The biological activities of mannans and related` complex carbohydrates', *Mol Biother., 1:290–296.*

Torki M., Akbari M. and Kaviani K. (2015) ' Single and combined effects of zinc and cinnamon essential oil in diet on

productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition' *International J of Biometeorology*, *59: 1169-77*.

Tsai Y., Mao S., Li M., Huang J. and Lien T. (2016) 'Effects of nanosize zinc oxide on zinc retention, eggshell quality, immune response and serum parameters of aged laying hens', *Animal Feed Science and Technology*, *213: 99-107*.

Tüzün A.E., Olgun O. and Yildiz A.Ö. (2018) 'The Effect of High-Level Dietary Supplementation with Different Zinc Sources on Performance, Eggshell Quality and Bone Characteristics in Layer Quails' *In: "Agriculture for Life, Life for Agriculture" Conference Proceedings*, *pp. 176-82. Sciendo*

Uyanik F., Eren M., Atasever A., Tunçoku G.and Kolsuz A.H. (2001) 'Changes in some biochemical parameters and organs of broilers exposed to cadmium and effect of zinc on cadmium induced alterations', *Israel J. Vet. Med.*, *56:128–134.*

VDLUFA [Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten]. (2007) 'andbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln', *Darmstadt: VDLUFA-Verlag*.

Wang Z.L. (2000) 'Characterization of nanophase material (*Weinheim: Wiley-VCH Verlag GmbH), p*. 1.

Wang J.H., Wu C.C. and Feng J. (2011) 'Effect of dietary antibacterial peptide and zinc-methionine on performance and serum biochemical parameters in piglets', *Czech J Anim Sci., 56:30–36.*

Zhao Y., Li L., Zhang P.F., Liu X.Q., Zhang W.D., Ding Z.P., Wang S.W., Shen W., Min L.J. and Hao Z.H. (2016) 'Regulation of egg quality and lipids metabolism by Zinc Oxide Nanoparticles', *Poultry Sci., 95: 920-33.*