Original Article

Effect of Different amounts of Zinc Oxide Nanoparticles on the Performance and Activity of Mucosal Enzymes in Japanese Quail from 1 to 21 Days of Age

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ABSTRACT

This study aimed to investigate the effect of varying quantities of zinc oxide nanoparticles (ZnO NPs) on growth performance and mucosal enzyme activity in Japanese quails at an early age. Using a completely randomized experimental design, 160 one-day-old quail chicks were randomly assigned to 4 experimental treatments and each treatment contained 4 replicate pens of 10 birds. The experimental treatments included T1: control (a basal diet containing 35.2 mg Zn only), T2, T3, and T4 containing basal diet plus 20, 40, and 60 mg ZnO NPs, respectively. Performance characteristics were recorded weekly. After 21 days, one quail was selected and slaughtered from each experimental cage with a body weight equal to the average body weight of quails in the same experimental cage. After slaughtering and opening the abdominal cavity, a 5 cm sample was taken from the jejunum of the small intestine. The jejunum sample was stored at -80°C until the measurement of alkaline phosphatase, amylase, and lipase enzymes. The results showed that live weight was higher in the T3 and T4 groups than in the control group ($P<0.05$). The feed conversion ratio was also lower in birds fed with basal diets supplemented with 40 and 60 mg ZnO NPs/kg (T3 and T4, respectively), compared to control treatments ($P>0.05$). The results showed that amylase and lipase activity increased in the birds fed with 40 and 60 mg ZnO NPs/kg of the basal diet, in comparison to the control treatment; however, they were not significant ($P>0.05$). The results of this study indicated that the addition of 40 or 60 mg ZnO NPs/kg to the basal diet could be used as a supplement to improve performance traits and enhance mucosal enzyme activity in Japanese quail in the starter stage.

Keywords: Enzyme, Japanese quail, Nanoparticles, Performance

1. Introduction

Nanoparticles are the most widely used elements in nanoscience and nanotechnology, and their interesting properties have led them to gain multiple applications in the chemical, medical, pharmaceutical, animal nutrition, and agricultural industries. The use of nanoparticles (e.g., Cu, Se) in different concentrations to improve the health of livestock and poultry was one of the first applications of nanotechnology products (1). Weight gain in farm livestock is the conversion of nutrients in the food into a product, which is meat and other products. To achieve this goal, the following two factors are essential: the health of the animal and the digestive system. This is due to the favorable secretory properties of the different parts of the intestine, especially the jejunum (the main site of nutrient absorption), and the secretion of digestive enzymes to increase the absorption of various nutrients. The creation of such conditions for farm animals may, in particular, improve their production and allow better economic justification of production plans (2). Recently, trace elements in the form of nanoparticles have been used as a new source to meet the needs of poultry and other farm animals (3). The results of previous studies have reported the positive effects of adding different nanoparticles of minerals on the performance characteristics of broiler chickens (3). Recently, researchers have used nanoparticles of minerals, especially micromaterials, to increase the efficiency and production of broiler chickens; however, the scientific reports are insufficient in this regard, particularly in relation to the effect of oxidizing nanoparticles. There are no reports on the source of digestive enzyme activity (1). Researchers have reported that nanoparticles of minerals, especially microelements, increase efficiency and production in feeding broiler chickens (3). There are very few scientific reports in this regard, in particular regarding the effect of zinc oxide nanoparticles (ZnO NPs) on digestive enzyme activity.

Therefore, this study was carried out to investigate the effect of different amounts of ZnO NPs on the growth performance and activity of amylase, lipase, and alkaline phosphatase (ALP) enzymes in the mucosal enzyme activity at the jejunum of Japanese quail from day 1 to 21 of age.

2. Materials and Methods

2.1. Birds and experimental diets

This study was conducted at the research center of Islamic Azad University, Sanandaj Branch, Kurdistan, Iran, over 21 days. A total of 160 one-day-old quail were purchased from a quail farm near Sanandaj city, and then by using a completely randomized experimental design were assigned to 4 experimental treatments and each treatment contained 4 replicate pens of 10 birds. The experimental treatments consisted of four groups: T1 (control, basal diet without ZnO NPs, content 35.2 Zn), T2 (basal diet plus 20 mg ZnO NPs/kg), T3 (basal diet plus 40 mg ZnO NPs/kg), and T4 (basal diet plus 50 mg ZnO NPs/kg). Birds were raised in a deep litter system (the deep litter system is one of the poultry breeding methods) with identical housing and management conditions for each treatment. All conditions, such as temperature, brooder room ventilation, light regime, and inoculation, were the same for all experimental treatments during the experimental period, and the only factor that differed between treatments was the amount of ZnO NPs added to the basal diet. Corn seed and soybean meal were used as the main ingredients of the basic ration; the diet was formulated using UFFDA software based on the nutritional recommendations of the National Research Committee (4). Birds had access to feed and water throughout the experimental period. Ethical approval for the experiment was obtained from the Institutional Animal Ethics Committee of the Research Centre of Islamic Azad University, Sanandaj Branch. The composition and analysis of the basal diet are presented in table 1. The amount of zinc in the basic diet and experimental treatments is calculated in table 2.

2.2. Characteristics of ZnO NPs used for the experimental treatments

The physical form of ZnO NPs used in this study

Table 1. Ingredient and chemical composition of the basal diet (As fed) from 1 to 21 days of age

Feed ingredients	%
Corn grain	56.23
Soybean meal (44%)	38.00
Soybean oil	2.08
DL-met	0.22
L-lysine, HCl	0.31
Limestone	0.35
Di-calcium phosphate	1.73
Salt (NaCl)	0.22
Mineral and Vitamin premix[1]	0.60
Calculated analysis	100
ME (kcal/kg)	2900
CP (%)	21.15
Total P (%)	0.61
Available P (%)	0.47
Ca (%)	1.06
Lys (%)	1.21
Met (%)	0.52
Met+Cys (%)	0.88
Zinc (mg/kg)	39.02

[1]Per kilogram of starter diet from 1 to 21 d contained: Ca, 210 g; P, 85.7 g; Fe, 1,250 mg; Cu, 250 mg; I, 15 mg; Se, 8.2 mg; vitamin A, 250,000 IU; vitamin D3, 50,000 IU; vitamin E, 275 mg; vitamin K3, 42.5 mg; vitamin B1, 45 mg; vitamin B2, 150 mg; vitamin B6, 62.5 mg; vitamin B12, 300 mcg; niacin, 1,000 mg; folic acid, 27 mg; pantothenic acid, 400 mg; choline, 12.5 g; biotin, 2mg; Met, 45 g and Zn, 36.29 mg/kg.

was powder and was purchased from Nonmaterial Co, Huston, Texas, USA. The results of the transmission electron microscope showed that the mean particle size of ZnO NPs was 20±3 nm and the purity was 99.99%. The shape of ZnO NPs was almost spherical.

2.3. Preparation of the experimental diets

To prepare an experimental diet and add ZnO NPs uniformly, the amount of ZnO NPs required for each experimental treatment was first separated from the main source (99.99% pure) and added gradually at 1, 2, and 3 kg. The basic ration was completely mixed and then blended with the total diet ration prepared for the whole experimental period (21 days) and administered to the quail chicks.

2.4. Recording of performance traits

Feed intake and live weight were recorded weekly, and then the feed conversion ratio was calculated using the data obtained throughout the experimental period (21 days). Daily mortality for each pen was recorded and used to correct the performance traits using appropriate equations.

2.5. Sampling for mucosal enzyme activity

At 21 days of age, 12 h before sampling for the measurement of the activity of the enzymes amylase, lipase, and ALP in the mucosal layer, a section (5 cm) was taken from the middle of the jejunum, cut lengthwise, washed with double distilled water and buffer solution, quickly placed in aluminum foil, and then stored in the laboratory at -80°C until the measurement of the above enzymes. A volume of 0.5 g of the mucosal surface of the jejunum was separated from a slide, diluted, and homogenized after adding 200 µl of phosphate buffer (pH=7.4). After centrifugation (Sonoplas model at 900 rpm for 10 min) of the mixture, the clear liquid above the solution was separated and poured into microtubes (Eppendorf) and stored at -80°C until enzyme activity was measured. Subsequently, ALP, amylase, and lipase were measured using an automated analyzer (Mindray BS -200, China) and special kits from Pars Azmun (Iran). To express the activity of the enzymes, the total protein of the test samples was measured, and the activity of the enzymes was calculated based on the international unit in grams of protein (IU/g protein).

Table 2. Concentration of Zn and ZnO-nano in the experimental diets[1] (by analysis)

Experimental diets	Level of Zinc in Experimental diets(mg/kg basal diet)[2]	
	Zinc (mg/kg)	ZnO-nano
T1-Control	39.02	0
T2	39.02	20
T3	39.02	40
T4	39.02	60

[1]Zinc source was zinc oxide (Purity = 72%) and zinc oxide nanoparticles (Purity= 99.99%)
[2]The basal diet zinc was calculated based on NRC (1994).

2.6. Statistical analysis

Data were analyzed in a completely randomized design using the general linear model procedure of SAS for Windows version 9.1 (5). Significant differences were determined using the LSD test at the 0.05 significance level, and the results were reported as means ± standard errors of the means. The statistical model used in this study was:

$Yij = \mu + Ti + eij$

where Yij is the value of the individual observation in the experiment, μ is the average of the attributes, Ti is the effect of experimental treatments, and eij is experimental error.

3. Results and Discussion

3.1. Growth performance

The results related to the effect of different ZnO NPs on growth performance are displayed in figures 1 to 3. The results showed that live weight was higher in T3 and T4 groups than in the control group (*P*>0.05). The feed conversion ratio was also lower in birds fed with the basal diet supplemented with 40 and 60 mg ZnO NPs/kg (T3 and T4, respectively) than in the control group (*P*<0.05). Researchers have reported that ZnO NPs have unique properties, such as reducing particle size and increasing surface area relative to particle volume compared to other sources. Therefore, these properties may affect the zinc absorption rate and ultimately the nutritional effects. This may be one of the reasons for the positive effects on growth performance and the rise in mucosal enzyme activity in this study (6). Another report indicated that ZnO NPs in the diet (20 mg/kg) improved growth performance and antioxidant power (7). Turkeys fed on diets treated with Se and ZnO NPs also showed a significant (*P*≤0.05) improvement in feed conversion, in comparison to untreated animals (8). A diet containing ZnO NPs has the potential to be considered an alternative to other sources and can improve the productive performance and health status of laying hens (9). The growth performance of the animals was reportedly improved by the addition of ZnO NPs to the diet. Broilers being fed with ZnO NPs at doses of 20 and 60 mg/kg gained more body weight and had a better feed conversion ratio than when conventional Zn was used (Zhao et al., 2014). At an optimal dosage of 20 mg/kg, biologically active ZnO NPs improved the growth performance of broilers (10).

3.2. Activity of mucosal enzymes

The results related to the effect of different ZnO NPs dosages on the activity of enzymes in the mucous layer of the jejunum of the small intestine are

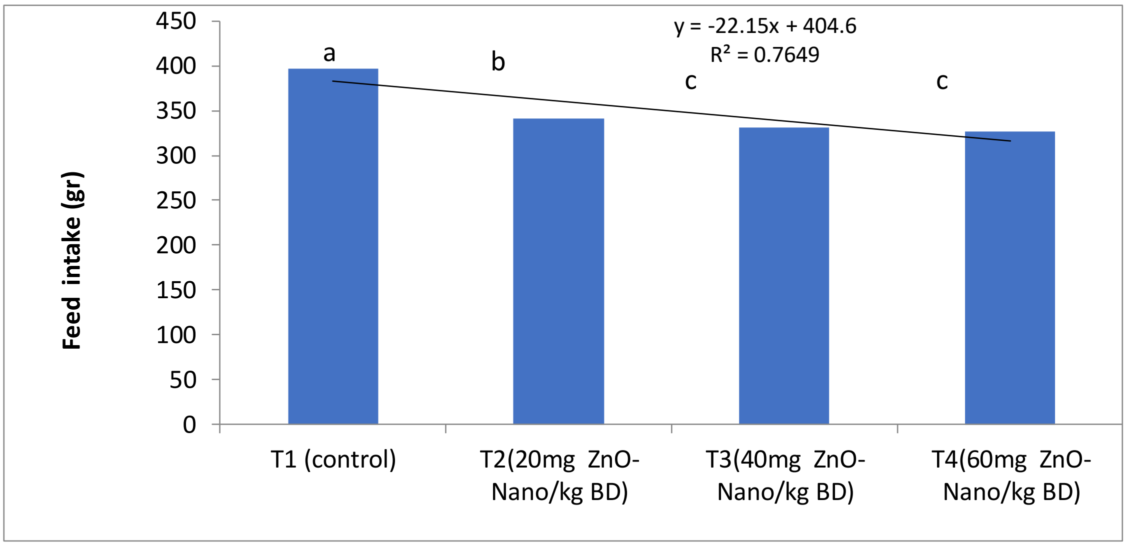

Figure1. The effect of experimental treatments on the mean feed intake

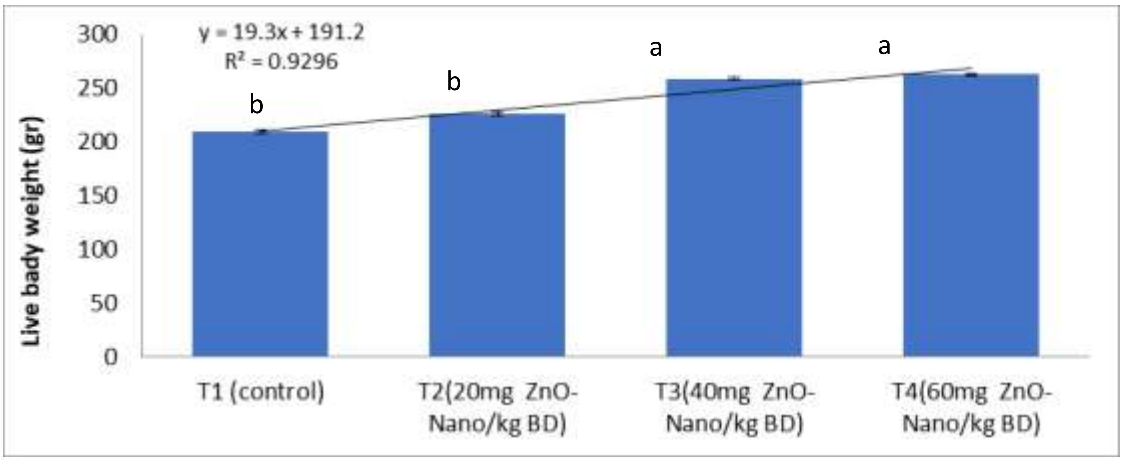

Figure 2. The effect of experimental treatments on the mean live body weight

summarized in table 3. The highest activity of amylase and lipase enzymes was observed in birds fed with the basal diet containing 60 mg of ZnO NPs, while the lowest activity was seen in the control treatment (without ZnO NPs). The results demonstrated that the activity of lipase and amylase enzymes significantly increased in quails fed with 40 and 60 mg ZnO NPs, compared to the control treatment at 21 days of age (P<0.05). The obtained data revealed that the ALP (which is an enzyme that helps fight bad bacteria in the gut and improves digestion) enzyme activity at 21 days of age was not affected by the addition of ZnO NPs to the basal diet (P>0.05). However, an increasing trend of ALP enzyme activity was observed in the experimental treatments, compared to the control. Research has shown that Zn has an important and significant role in more than 300 enzymes. Therefore, the presence of a significant difference in the activity of amylase and lipase enzymes associated with an increase in Zn supplementation of the basic diet, compared to the control group, was not far from expectations (6).

Some researchers have reported that the addition of 100 mg of ZnO per kg of basal diet significantly increases the activity of the enzyme leucine aminopeptidase in the small intestine of broiler chickens (2). It has also been reported that the addition of 2,000 to 2,500 mg of Zn per kilogram of feed (in the form of ZnO) to weaned pigs improves and increases the activity of some digestive enzymes in the pancreas and small intestine (11). Other researchers reported that Zn supplementation in

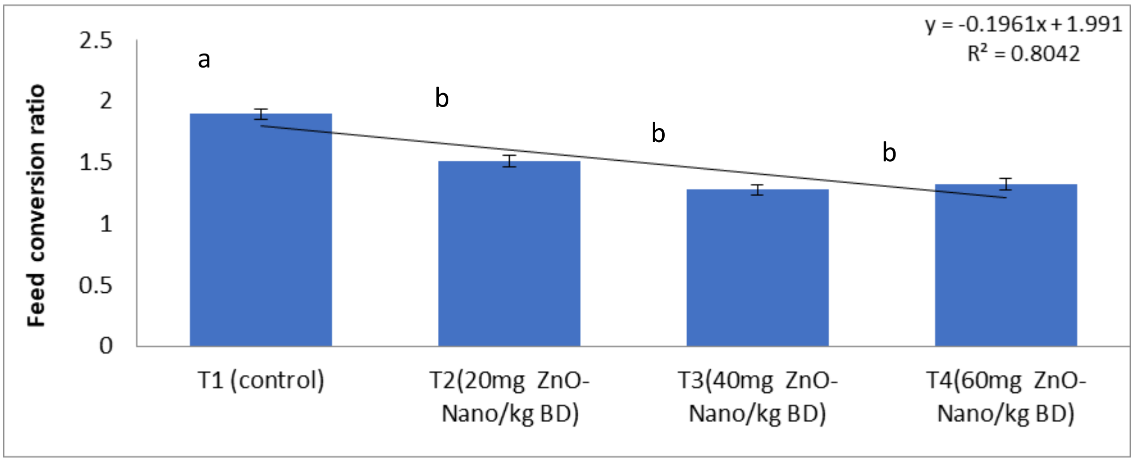

Figure 3. The effect of experimental treatments on the mean feed conversion ratio

Table 3. The effect of different levels of ZnO-nano on the activity of mucous enzymes of the *quail's* jejunum (at the age of 21 days)

Experimental diets	Enzyme activity (IU/gr of mucosal total protein)		
	Lipase (LIP)	**Amylase (AMY)**	**alkaline phosphatase (ALP)**
T1 (control, without ZnO-nano)	3.21[b]	135.9[b]	1209.7
T2 (20mg ZnO-Nano/kg BD[2])	3.30[b]	137.2[b]	1215.1
T3 (40mg ZnO-Nano/kg BD)	4.2[a]	143.6[a]	1225.3
T2 (50mg ZnO-Nano/kg BD)	4.5[a]	150.1[a]	12488
SEM	0.18	4.27	124.5
P-Value	0.037	0.021	0.172
Significant	*	*	Ns[3]
Linear equation	Y=0.477x+2.61; R^2=0.91	Y=4.9x+129.5; R^2=0.94	Y=0.03x+1193.2; R^2=0.91

[1]Means with different superscripts in the same column differ (P<0.05)
[2]BD= Basal diet
[3]No significant

chelated form boosted the activity of digestive enzymes in the small intestine, in comparison to the control group. This report confirms that the type of Zn source can probably have a different effect on digestive enzymes. Therefore, ZnO NPs as a new source (due to the unique properties of nanoparticles) might have caused a change in the production and activity of quail intestinal mucosal enzymes (12). The first factor that affects the activity of enzymes in the digestive tract is the hydrogen concentration, and we know perfectly well that this concentration is different in various parts of the intestine and consequently causes differences in the activity of digestive enzymes (13). The results of studies have indicated that a pH of 6.9 to 9 is the most favorable pH for the activity of the enzyme ALP. The addition of Zn to the diet of broiler chickens creates the desired pH. Therefore, it is possible that the Zn ion released from the ZnO NPs combines with chlorine ions and ultimately causes a decrease in pH or an increase in free hydrogen ions in the intestine, creating a suitable environment for the activity of ALP and other digestive enzymes (13).

The element Zn is involved in numerous vital functions of the body and is added to the animal’s ration as an organic or supplemental supplement. This element plays a widespread and all-encompassing role in the biochemical processes of the body by participating in a catalytic site of over 300 enzymes (14). By disrupting the structure and action of enzymes, it plays a part in complex biochemical processes, such as the synthesis and degradation of proteins, energy metabolism, and the synthesis of hereditary molecules (15). A larger surface area per mass compared to larger particles of the same chemistry makes nanoparticles more biologically active (16). Therefore, the enhanced properties of nanoparticles may have adverse effects when used in different biological systems (16, 17).

The findings of the current study demonstrated that the addition of ZnO NPs to the diets of Japanese quail could improve the parameters of growth performance and increase mucosal enzymes for better digestion of nutrients and better performance.

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Authors' Contribution

Research project proposal: A. F.Authors' Contribution

Research project proposal: A. F.
Review of research sources and background: R. F.
The practical part of research: K. KH.
Analysis of raw research data: A.F.

Data interpretation: A.F.

Ethics

This research was conducted based on the protocols approved by Islamic Azad University and the research ethics charter.

Conflict of Interest

The authors declare that they have no conflict of interest.

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