Effects of different levels of zinc-glycine and zinc hydroxide on the performance, carcass quality, immunity and duodenum morphometric of the broiler chickens

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ABSTRACT
This study was conducted to assess the effect of different levels of zinc hydroxide and zinc-glycine (Zn-Gly) on the performance, carcass quality, immunity, and morphometric of the small intestine. A total of 540 1-day-old Ross-308 broiler chicks were randomly assigned to 9 treatments each having 4 replicates of 15 birds in a 3x3 factorial design. They were fed with different amounts of 0, 50 and 100 mg/kg from zinc hydroxide and Zn-Gly. The results showed the significant effect of different levels of zinc hydroxide on performance, carcass quality, immunity and morphometric of the small intestine ($p < .05$) and also a significant effect of different levels of Zn-Gly on feed intake, feed conversion ratio and thymus ($p < .05$). The results also showed that when birds were fed with the diet containing 100 mg/kg of Zn-Gly and without zinc hydroxide had the highest weight gain and feed intake, whereas the control diet showed the lowest weight gain and feed intake with a significant difference ($p < .05$). Overall, the results showed that feeding broilers with a diet containing 100 mg/kg of Zn-Gly had the greatest effect on weight gain, visceral organs, bursa of Fabricius, thymus, spleen, length of the villus and crypt depth. In general, feeding with different levels of organic and inorganic sources of Zn is more effective than the diets without them.

HIGHLIGHTS
- The diet containing Zn-Gly without zinc hydroxide caused a higher increase in weight gain.
- The used organic sources of Zn in poultry diets are more absorbed compared with the inorganic sources.
- The difference in Zn absorption between organic and inorganic sources can affect the small intestine.

ARTICLE HISTORY
Received 23 September 2020
Revised 5 July 2021
Accepted 6 July 2021

KEYWORDS
Broiler; performance; morphometric; immunity; zinc

Introduction
Zinc (Zn) plays a major role in all metabolic processes due to its catalytic effect on the function of more than 200 enzymes. Zn-containing enzymes are involved in the production or breakdown of carbohydrates, lipids, proteins, and nucleic acids, involving all classes of enzymes. Zn deficiency can stop cell division, or in eukaryotes, it can lead to abnormal differentiation and development and also a wide range of abnormalities (Fathi 2016; Navidshad et al. 2016).

Although it has not yet been possible to fully investigate the effects of Zn deficiency in pure or in combination with metal ions-containing enzymes, basic experiments in molecular genetics and biochemistry have provided a basic understanding of the biological activity of Zn (Suttle 2010). Following Zn deprivation in animals, vitamin A, E levels are decreased in plasma and liver, suggesting the role of Zn to absorb fats. In addition, pancreatic secretions containing phospholipase A2 are associated with Zn. This enzyme hydrolyses phosphatidylcholine, which in turn facilitates absorption and formation of chylomicrons (Noh and Koo 2001).

Phosphatidylcholine injection into the duodenum increases the uptake of fat and fat-soluble vitamins (A and E) observed in rats deprived of Zn (Kucuk et al. 2003). Since the role of Zn in the regulation of
appetite has been confirmed, extensive studies have been done in rats, and it has been shown that the acute reduction of Zn in diets causes rats to consume less food (Salim et al. 2008). Zn deficiency increases gene expression for cholecystokinin, an appetite-regulating hormone (Cao et al. 2002). Also, Zn deficiency often results in an increase in leptin and a decrease in pyruvate kinase, which is regulated by insulin (Suttle 2010). Changes in the concentration of neurotransmitters in the brain have been reported to be due to Zn deficiency, which can then lead to decreased appetite (Beattie et al. 2008).

Acute Zn depletion reduces appetite and the lack of carbohydrates, proteins, and fats supply. Zn deficiency is also effective in the metabolism of carbohydrates, proteins, and fats in the body (Sondergaard et al. 2006). The key enzymes required for carbohydrate metabolism are reduced in Zn deficiency due to reduce Zn-dependent mRNA expression. The role of superoxide dismutase in protecting cells against superoxide radicals has been observed in laboratory studies. Zn deficiency increases the sensitivity of endothelial cells to oxidant-dependent stress (Beattie and Kwun 2004). Zinc is a potent inducer of metallothionein, which eliminates free radicals (Beattie et al. 2002). The reduced size in poultry and a reduction in egg-laying have been observed following dietary Zn deficiency (Park et al. 2004).

Low level of Zn in food of plant origin will meet by supplementing Zn to poultry diets. In general, many natural foods are relatively poor in Zn. Due to the importance of Zn, it is necessary to evaluate its organic and mineral sources to find their effects on the different functions of Zn. It has noted that organic sources of Zn are more biologically active than mineral sources (zinc oxide and zinc sulphate), and consequently, the organic compounds of Zn have been more used to feed animals (Spears et al. 2004). For example, organic compounds, such as lysine- zinc and methionine- zinc have been more considered due to their higher bioavailability. Supplementation of Zn to broiler diets has shown favourable outcomes, especially at the beginning of the growing season (Sunder et al. 2008). Zn supplementation also has a positive effect on growth rate. Supplementation of Zn to broiler diets reduced using antibiotics and also has shown effective in digestion and absorption of nutrients (Ali et al. 2017). Supplementation of Zn (organic and mineral) to broiler diets improved their performance (Saleh et al. 2018). Although the biological activity of the organic and mineral zinc sources has not been yet identified, some differences have found in poultry. Several investigations have been conducted comparing the effects of organic and mineral sources of Zn on broiler diets. Regarding organic sources, unlike methionine, Zn-Gly has less been considered. Also, regarding mineral sources, zinc sulphate, zinc carbonate, zinc chloride and zinc oxide as the best absorbable forms of Zn have been more evaluated. Zinc hydroxide is also an absorbable mineral that can be easily used by poultry and its OH ion is removed in the intestine acting as a base in some reactions and the released Zn can play its role. Therefore, we used zinc hydroxide as an inorganic source and Zn-Gly as an organic source of Zn that based on the obtained favourable results can be added to other organic and inorganic sources of Zn inorder to be more effective. Accordingly, this study aimed at comparing the biological activity of zinc hydroxide with its organic source (Zn-Gly) added to the broiler diet on the performance, carcass quality, immunity, and morphology of the small intestine.

Materials and methods

Dietary treatments

A total of 540 one-day-old Ross-308 broiler chicks were randomly assigned to 9 treatments each having 4 replicates of 15 birds in a 3 x 3 factorial arrangement (36 samples). The chicks of each replicate were kept in a separate pen with automatic drinkers and manual feeders.

Three levels of zinc hydroxide as a mineral source of Zn (0, 50 and 100 mg/kg diet) and 3 levels of Zn-Gly as organic source (0, 50 and 100 mg/kg diet) were used. Zn-Gly 17% and Zinc hydroxide 55% were obtained from the ORFFA® Co. Ltd, Netherlands. The rations were similar in energy and protein levels and were considered according to the guidelines by the Ross Nutrition Specifications 2019. Other conditions, such as temperature, light, humidity, ventilation and vaccination were similar in all treatments according to the breeding standards for Ross-308 broiler chicks. The experiment lasted for 42 days, though which the primary, growth, and final rations were used (Table 1).

Performance measurement

Body weight gain and feed intake were measured throughout each treatment and the whole period (at the end of 42nd days of age). The considered diet of each sample was provided and at the end of each
treatment, the amount of unused diet was deduced obtaining the amount of food intake. The feed conversion ratio was calculated as follows:

\[ \text{Feed conversion} = \frac{\text{Feed intake}}{\text{Bodyweight gain}} \]

The European Performance Efficiency Index (EPEI) was also obtained at the end of 42nd days of age according to the following formula:

\[ \frac{\text{Mean weight (kg)}}{\text{shelf life}^2} \times \frac{\text{Number of breeding days}}{\text{feed conversion ratio}} \]

Carcass quality measurement

At the end of 42nd days of age, two chicks with the average weight close to the average weight of the treatment were selected from each replicate and after slaughtering and removal of feathers and skin, through a longitudinal abdominal incision, breasts, thighs, viscera, visceral fat were isolated and weighed.

Immunity measurement

At the end of 42nd days of age, two chicks with the average weight close to the average weight of the treatment were selected from each replicate and after slaughtering and removal of feathers and skin, through longitudinal abdominal incision, the lymph nodes, including spleen, thymus, and bursa of fabricius were removed and weighed.

Measurement of the morphometric traits of the duodenum

At the end of 42nd days of age, one chick was selected from each replicate and after slaughtering, the small intestine was removed and three slices were prepared from the duodenum. The intestinal slices were fixed in 10% formalin buffer solution and immersed in paraffin. Then, using a microtome, four 5-µm slices were prepared from each slice and stained with hematoxyline. They were placed on the slide and observed using an Olympus IX70 microscope and the length and width of the villus and the depth of the crypt gland were measured by stereological image software. Also, the villus/crypt ratio was also calculated (Levkut et al. 2017).

Statistical analysis

The study was based on a 3 × 3 factorial, completely randomised design using the following statistical model:

\[ Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk} \]

Data were analysed by SPSS software (SPSS, 2015) and Duncan’s method (p < .05) considering the significance level of 5%.

Results

Performance traits

Main effect:

The effect of different levels of zinc hydroxide on weight gain, feed intake, feed conversion ratio and EPEI was significant (p < .05). The highest and lowest effects were found at the levels of 100 and 0 mg/kg, however, regarding EPEI, the levels of 50 and 0 mg/kg showed the highest and lowest effects, respectively. The effect of Zn-Gly levels on feed intake and conversion ratio was significant (p < .05) and the highest and the lowest effects were found at the levels of 100 and 0 mg/kg, respectively. Zn supplementation as Zn-Gly (100 mg/kg) increased weight gain and feed intake during the whole period and also conversion ratio in broiler chickens. The EPEI showed the best results by adding Zn-Gly (50 mg/kg) (Table 2).

Interaction effect:

The feed intake and FCR showed the best results after 50 mg/kg zinc hydroxide without Zn-Gly.

Carcass quality

Main effect:

Different levels of zinc hydroxide showed a significant effect on viscera and visceral fat (p < .05). The highest

### Table 1. Ingredients and chemical composition of the basal rations.

| Ingredients, % | Starter period (1–10 days) | Grower period (11–24 days) | Finisher period (25–42 days) |
|----------------|---------------------------|---------------------------|-----------------------------|
| Corn           | 580.770                   | 590.950                   | 650.330                     |
| Soybean meal   | 360.620                   | 330.620                   | 280.310                     |
| Soybean oil    | 10.440                    | 20.960                    | 20.810                      |
| Di calcium-phosphate | 10.740         | 10.500                    | 10.560                      |
| CaCO3          | 10.340                    | 10.110                    | 10.150                      |
| NaCl           | 0.20                      | 0.200                     | 0.200                       |
| Vitamin premix | 0.250                     | 0.250                     | 0.250                       |
| Mineral premix | 0.250                     | 0.250                     | 0.250                       |
| L-lysine       | 0.150                     | –                        | 0.200                       |
| DL-methionine  | 0.240                     | 0.160                     | 0.140                       |
| Energy (ME, kcal/kg) | 3000                     | 3100                      | 3200                        |
| Protein, %     | 210.120                   | 20.160                    | 180.280                     |
| Calcium, %     | 10.008                    | 0.860                     | 0.870                       |
| Available phosphorus, % | 0.480               | 0.430                     | 0.430                       |
| Lysine, %      | 10.210                    | 10.060                    | 0.930                       |
| Methionine, %  | 0.560                     | 0.470                     | 0.420                       |
| Methionine + cysteine | 0.900                   | 0.800                     | 0.730                       |
| Threonine      | 0.780                     | 0.750                     | 0.670                       |

*Each kilogram contains: 3,500,000 IU vitE, 1,000 IU vitD3, 9000 IU vitA, 1,000 mg vitK3, 900 mg vitB1, 5000 mg vitB6, 3,150 mg vitB8, 800 mg vitB12, 25000 mg choline and 500 mg Biotin.*

*Each kilogram contains: 120 mg Mn, 20 mg Fe, 16 mg Cu, 1.25 mg I, 0.3 mg Se, 0 mg Zn.

ME: Metabolizable Energy.
and lowest effects on relative weight (%) viscera and visceral fat were observed at 100 and 0 mg/kg levels, respectively. Different levels of zinc glycine showed not significant effect on viscera and visceral fat. Different levels of zinc hydroxide and zinc glycine showed not significant effect on thigh and breast.

**Interaction effect:**
Different treatments of zinc hydroxide and zinc glycine showed not significant effect on carcass quality. The viscera and visceral fat showed the highest weight after treatment with 50 mg/kg of zinc hydroxide and with no zinc glycine (Table 3).

**Immunity**

**Main effect:**
Different levels of zinc hydroxide showed a significant effect on relative weight of spleen, thymus, and bursa of fabricius (p < .05). The highest and lowest effects on spleen were observed at 100 and 0 mg/kg levels, respectively. Regarding bursa of fabricius, the highest and lowest effects were found at the levels of 50 and 0 mg/kg, whereas the levels of 0 and 100 mg/kg showed the highest and lowest effects on thymus, respectively. Different levels of Zn-Gly showed a significant effect on thymus (p < .05). At the end of 42nd days of age, a significant difference was found in the weight of thymus, spleen, and bursa of fabricius (p < .05). The spleen weight showed the highest weight after treatment with Zn-Gly without zinc hydroxide (100 mg/kg). The bursa of fabricius showed higher weight after the control treatment than the other treatments and also thymus was found with the highest weight after treatment with Zn-Gly and zinc hydroxide (50 mg/kg) (Table 4).

**Interaction effect:**
Regarding the interaction effects significant differences were found in the weight of thymus, spleen, and bursa of Fabricius (p < .05). The spleen showed the highest weight after treatment with the diet without Zn-Gly and zinc hydroxide (The control group). The bursa of Fabricius showed higher weight after 100 mg/kg treatment of zinc hydroxide without zinc glycine than the other treatments and also thymus was found with the highest weight after treatment with 50 mg/kg Zn-Gly and zinc hydroxide (Table 4).

**Morphometry of the duodenum**

**Main effect:**
Different levels of zinc hydroxide showed significant effects on the height of the villus, crypt depth and villus/crypt ratio (p < .05). The highest and lowest effects were observed at the levels of 100 and 0 mg/kg, respectively.

**Interaction effect:**
Different levels of Zn-Gly had no significant effect on small intestinal morphology (p > .05).
of the breeding, a significant difference was observed in height of the villus and depth of the crypt and villus/crypt ratio in all treatments. Treatment with 100 mg/kg Zn-Gly without zinc hydroxide was more effective (p < .05). No significant difference was observed in the width of the villus (p > .05). The villus/crypt ratio showed the highest rate after treatment with Zn-Gly (100 mg/kg) and zinc hydroxide (50 mg/kg) (Table 5).

### Table 3. Comparison of mean relative weight of carcass parts in chicks fed with different levels of glycine-zinc and zinc-hydroxide.

| Treatment | Thigh, % | Breast, % | Viscera*, % | Abdominal fat, % |
|-----------|----------|-----------|-------------|------------------|
| Zn-H, mg/kg diet |          |          |             |                  |
| 0         | 21.9800  | 43.9900  | 9.1900b     | 1.8500c          |
| 50        | 24.2000  | 44.2300  | 9.2800a     | 1.8600b          |
| 100       | 24.2200  | 44.4300  | 9.2900a     | 1.8700a          |
| p-value   | .3000    | .3700    | p < .0010   | p < .0010        |
| SEM       | 0.0900   | 1.6680   | 0.3400      | 0.0703           |
| Zn-Gly, mg/kg diet |        |          |             |                  |
| 0         | 23.4200  | 44.5600  | 9.2000     | 1.8600           |
| 50        | 24.1100  | 44.2400  | 9.2400     | 1.8600           |
| 100       | 22.0200  | 44.0300  | 9.2000     | 1.8600           |
| p-value   | .3900    | .3800    | .4900      | .4800            |
| SEM       | 0.4812   | 0.8822   | 0.1846     | 0.3723           |

Viscera include oesophagus, crop, gizzard, pancreas, liver, gall bladder, small and large intestine.

Means without superscript letters or without superscript letters within the same column do not differ (p ≥ .05).

SEM: Standard error of means, Zn-H: Zinc hydroxide, Zn-Gly: Zinc-glycine.

### Table 4. Comparison of mean relative weight of immunity organs in chicks fed with different levels of glycine-zinc and zinc-hydroxide.

| Treatment | Bursa of fabricus, % | Spleen, % | Thymus, % |
|-----------|----------------------|-----------|-----------|
| Zn-H, mg/kg diet |          |          |           |
| 0         | 0.14000a            | 0.17000a  | 0.35000a  |
| 50        | 0.13000b            | 0.18000a  | 0.34000a  |
| 100       | 0.12000c            | 0.19000a  | 0.31000b  |
| p-value   | p < .0100           | p < .0100 | p < .0100 |
| SEM       | 0.00086             | 0.00750   | 0.00750   |
| Zn-Gly, mg/kg diet |        |          |           |
| 0         | 0.13000             | 0.18000   | 0.34000a  |
| 50        | 0.13000             | 0.18000   | 0.34000a  |
| 100       | 0.13000             | 0.18000   | 0.31000a  |
| p-value   | .23000              | .37000    | .37000    |
| SEM       | 0.00045             | 0.00390   | 0.00390   |

Means without superscript letters or without superscript letters within the same column do not differ (p ≥ .05).

SEM: Standard error of means, Zn-H: Zinc hydroxide, Zn-Gly: Zinc-glycine.
Table 5. Comparison of mean small intestine morphometry in chicks fed with different levels of glycine-zinc and zinc-hydroxide.

| Treatment | Villus height, μm | Villus width, μm | Crypt depth, μm | Villus height: crypt depth |
|-----------|------------------|-----------------|----------------|---------------------------|
| Zn-H, mg/kg diet |                   |                 |                |                           |
| 0         | 1282.1000        | 135.9800        | 307.4500       | 4.2600                    |
| 50        | 1882.2200        | 134.0300        | 351.6500       | 5.4900                    |
| 100       | 2011.9800        | 148.2500        | 373.6800       | 5.6500                    |
| p-value   | p < .0010        | 5900            | .0400          | p < .0010                 |
| SEM       | 143.7150         | 147.5200        | 28.3750        | 0.5040                    |
| Zn-Gly, mg/kg diet |               |                 |                |                           |
| 0         | 1698.2900        | 137.3200        | 360.1400       | 4.7500                    |
| 50        | 1712.6400        | 136.4300        | 328.9900       | 5.4200                    |
| 100       | 1756.2700        | 144.5100        | 343.6500       | 5.2300                    |
| p-value   | .3700            | .8400           | .5100          | .3000                     |
| SEM       | 49.2120          | 145.882         | 26.3950        | 0.4646                    |
| Zn-H (0 mg/kg diet)- Zn-Gly (0 mg/kg diet) | 1063.5000b | 132.1000        | 314.2000       | 3.4000                    |
| Zn-H (50 mg/kg diet)- Zn-Gly (0 mg/kg diet) | 1326.6000b   | 153.2000        | 312.1000       | 4.3000                    |
| Zn-H (100 mg/kg diet)- Zn-Gly (0 mg/kg diet) | 1455.8000a     | 122.5000        | 295.9000       | 5.0000                    |
| Zn-H (0 mg/kg diet)- Zn-Gly (50 mg/kg diet) | 1932.4000bc    | 136.8000        | 339.1000       | 5.8000                    |
| Zn-H (50 mg/kg diet)- Zn-Gly (50 mg/kg diet) | 1841.4000bc    | 119.5000        | 337.1000       | 5.0000                    |
| Zn-H (100 mg/kg diet)- Zn-Gly (50 mg/kg diet) | 1872.6000bc     | 145.7000        | 336.6000       | 5.6000                    |
| Zn-H (0 mg/kg diet)- Zn-Gly (100 mg/kg diet) | 2098.8000a     | 142.9000        | 427.0000       | 5.0000                    |
| Zn-H (50 mg/kg diet)- Zn-Gly (100 mg/kg diet) | 1969.8000b   | 136.5000        | 295.6000       | 6.8000                    |
| Zn-H (100 mg/kg diet)- Zn-Gly (100 mg/kg diet) | 1967.3000b     | 165.2000        | 398.3000       | 5.0000                    |
| p-value   | p < .0010        | .7600           | .0300          | p < .0010                 |
| SEM       | 57.1700          | 5.8800          | 11.4500        | 0.2000                    |

Means without superscript letters or without superscript letters within the same column do not differ (p ≥ .05).
SEM: Standard error of means, Zn-H: Zinc hydroxide, Zn-Gly: Zinc-glycine.

Discussion

**Performance traits (BW gain, feed intake, feed conversion ratio)**

The results showed that Zn hydroxide increased body weight, and at 100 mg/kg level had the best result. Zn deficiency reduces the growth rate in animals and also reduces the function of the growth hormone receptor and the attached protein. Zn deficiency damages nucleic acid biosynthesis and amino acid consumption or protein synthesis (O’Dell and Reeves 1989). Zn plays an important role in protein metabolism due to its presence in enzymes, such as aminopeptidase and also as a cofactor. Tomaszewska et al. (2017) reported that Zn-Gly increases the concentration of insulin-like growth factors in the blood. The results obtained in this study were consistent with the results reported by Bartlett and Smith (2003), Huang et al. (2007), Feng et al. (2010), Kwiecien et al. (2016), Tomaszewska et al. (2017), Abd El-Hack et al. (2017), Olukosi et al. (2018), and Dukare et al. (2018), whereas they were not consistent with those announced by Sunder et al. (2008), Yogesh et al. (2013), Badawi et al. (2017), Zakaria et al. (2017) and Zhang et al. (2018). Hosseini et al. (2018) reported enhanced body weight gain following treatment with zinc methionine compared with Zn-Gly. The inconsistency in the obtained results can be due to the used level of Zn, so that in these studies, the level of used Zn in the diet was more than the bird requirement. In general, organic in comparison with inorganic sources of Zn can lead to weight gain in broilers (Ao et al. 2009).

Zn-hydroxide at 50 mg/kg level and treatment with 50 mg/kg of Zn hydroxide without Zn-Gly showed the best results in feed intake.

Since the role of Zn in the regulation of appetite has been extensively studied, it has been shown that the acute reduction of Zn in diets reduces food intake (Salim et al. 2008). Zn deficiency increases gene expression for cholecystokinin, an appetite-regulating hormone (Cao et al. 2002). Also, Zn deficiency often results in an increase in leptin and a decrease in pyruvate kinase, which is regulated by insulin (Suttle 2010). Changes in the concentration of neurotransmitters in the brain have been reported to be due to Zn depletion, which can then lead to decreased appetite and food intake (Beattie et al. 2008). Acute Zn depletion reduces appetite and which led to the lack of carbohydrates, proteins, and fats supply. Carbohydrate metabolism is reduced in Zn deficiency due to decreased Zn-dependent mRNA expression (Sondergaard et al. 2006). In this study, Zn showed a significant effect on feed intake throughout the treatment period and treatments with highest level of Zn-Gly were found with more feed intake, whereas those containing lowest level of Zn-Gly caused lower feed intake (p < .05). 

These results are consistent with those reported by Batal et al. (2001), Bartlett and Smith (2003), Huang et al. (2007), Sunder et al. (2008) and Feng et al. (2010), whereas they were not consistent with those
announced by Jahanian et al. (2008), Yogesh et al. (2013) Hosseini et al. (2018), and Zhang et al. (2018). Different results can be due to the level of Zn in the basal diet or its amount and source. In addition, dietary ligands, such as phytate forming an insoluble compound with Zn preventing Zn absorption as well as high levels of dietary calcium, which increase Zn bonding with phytate, are involved in Zn absorption (Rossi et al. 2007).

Feed conversion ratio Zn-hydroxide at 0 mg/kg level and treatment with 50 mg/kg of Zn hydroxide without Zn-Gly showed the best results.

The obtained results were consistent with the results of Feng et al. (2010) Badawi et al. (2017), whereas they were not consistent with those reported by Batal et al. (2001), Huang et al. (2007), Sunder et al. (2008), Yogesh et al. (2013), Ivanisinova et al. (2016) and Zhang et al. (2018). Also, Saleh et al. (2018) reported that using organic sources of Zn can improve the feed conversion ratio. Hosseini et al. (2018) reported that zinc methionine is more effective on feed conversion ratio than Zn-Gly which can be due to fact that bioavailability of these compounds are affected by several factors, of which the type and composition of the organic compound combined with Zn and also chemical reactions associated with the digestion of organic binders (Gropper et al. 2009).

**Carcass quality**

Zn hydroxide at 100 mg/kg level showed the best results on partial weight of viscera and visceral fat. Zhang et al. (2018) found that inorganic Zn (100 mg/kg) is effective in breast weight gain. Abd El-Hack et al. (2017) also obtained similar results. The studied treatments were not found significantly effective on thigh and breast weight gain ($p < .05$), which was not consistent with the results of Jahanian et al. (2008). Hosseini et al. (2018) announced a significant difference in breast weight, which was not consistent with our results, whereas regarding thigh weight, their results were in line with ours and there was no significant difference. Kwiecień et al. (2016) also reported that Zn-Gly increased breast weight; however, it was not significantly different from the control group, which is consistent with our findings. The results of Zakaria et al. (2017) studies were in line with the present study. Different obtained results can be due to several factors, such as the source of Zn (organic or inorganic), the concentration of Zn in the diet, and breeding and environmental conditions (Zakaria et al. 2017).

**Immunity**

Zn hydroxide at 50 mg/kg level showed the best effect on increasing partial weight of bursa of Fabricius and at 100 mg/kg on spleen. Treatment with 50 mg/kg of Zn hydroxide without Zn-Gly showed the most increasing partial weight of bursa of Fabricius. Treatment with 100 mg/kg of Zn hydroxide without Zn-Gly showed the most increasing partial weight of spleen. Treatment with 50 mg/kg Zn hydroxide and 50 mg/kg Zn-Gly showed the best increasing partial weight of thymus.

Zn plays an important role in the antioxidant defense system (Prasad and Kucuk 2002). It is also effective in the immune system (Huang et al. 2007). In addition, Zn plays a role in the weight of organs associated with the bird’s immune system, such as the spleen, thymus bursa of Fabricius (Sahraei et al. 2012). Cui et al. (2004) reported that poultry treated with diets without Zn had a significant decrease in the weight of spleen, thymus bursa of Fabricius. Chand et al. (2014) stated supplementation of Zn to broiler diets improves the weight of bursa of Fabricius, spleen and thymus. Rasooli et al. (2018) found the increased levels of Zn in the diet caused an increase in the weight of immune organs, including bursa of Fabricius, spleen and thymus.

**Morphometric of the duodenum**

Zn hydroxide at 100 mg/kg level showed the best result on increasing villus height, crypt depth and villus height to crypt depth ratio. De Grande et al. (2020) found supplementation with ZnAA increased villus length and villus length to crypt depth ratio. Ma et al. (2011) revealed that Zn-Gly didn’t significantly increase the length of the villus, which is consistent with our results; however, it was not consistent regarding villus depth and reported that adding 90 mg/kg Zn-Gly increased the length of a villus in the duodenum, which is consistent with the present study. Levkut et al. (2017) found Zn-Gly more effective than zinc methionine on intestinal villi as it increased villi length, which does not consistent with the results of the present study. Zn can affect the morphology of the small intestine and increase its absorption capacity and growth performance (Feng et al. 2010). In addition, Zn is essential for cell proliferation and differentiation, particularly the regulation of DNA synthesis and
mitosis division (Beyersmann and Haase 2001). Zn deficiency is also associated with a decrease in the villus height (Southon et al. 1986). On the other hand, 42-day-old chicks treated with Zn-Gly (90 mg/kg) showed an increase in their villus height. Average villi surface area of the duodenum has shown a similar pattern and Zn supplementation can affect the villi height and surface (Lonnerdal 2000). The used organic sources of Zn in poultry diets are more absorbed compared with the inorganic sources. The difference in Zn absorption between organic and inorganic sources can affect the growth of intestinal villi (Levkut et al. 2017). Movement of cells from crypts to the villus tip is the cause of renewing, which makes them ready for absorption. Length increment of villus is associated with enzyme increment suitable for digestion and absorption, which is caused by supplements with ZnAA (De Grande et al. 2020).

Conclusion
The results showed significant effects on performance of broilers among different levels (0, 50 and 100 mg/kg) of Zn-hydroxide but there were no significant effect among different levels of Zn-glycine. The obtained results showed that the diet containing Zn-Gly (100 mg/kg) without zinc hydroxide caused a higher increase in weight gain and feed conversion ratio. Also, the diet containing Zn-Gly (100 mg/kg) without zinc hydroxide was found with a more appropriate effect on the crypt depth and the diet containing Zn-Gly (100 mg/kg) and zinc hydroxide (100 mg/kg) associated with immunity organs (spleen, thymus and bursa of fabricius).

Acknowledgements
This manuscript is prepared based on PhD thesis of first author at the Science and Research Branch, Islamic Azad University, Tehran, Iran. We are grateful to the Science and Research Branch, Islamic Azad University, Tehran, Iran for support.

Ethical approval
The experimental protocol was approved by the Animal Ethic Committee of the Science and Research Branch, Islamic Azad University, Tehran, Iran.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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