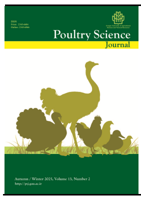

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Optimization of Zinc Hydroxychloride Levels in Quail Diets: Impacts on Performance, Yolk Antioxidant Capacity, Bone Development, and Mineral Excretion

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Abstract
Zinc (Zn) is crucial for animal physiology, yet traditional forms have limitations, prompting the exploration of alternatives such as zinc hydroxychloride (ZnOHCl). This study aims to determine the impact of dietary ZnOHCl on the productive performance, eggshell quality, antioxidant status of yolk, tibia mineralization, and mineral excretion in laying quails. One hundred and twenty-five 10-week-old layer quails were randomly assigned to five experimental groups. The experimental groups received a basal diet containing 34.14 mg/kg Zn from raw materials without additional ZnOHCl supplementation. The other four groups received the same basal diet containing 50, 75, 100, or 125 mg/kg Zn supplemented with ZnOHCl. The feeding trial lasted for 12 weeks. Feed intake was significantly ($P < 0.05$) lower in non-supplemented quails, while the rest of the performance parameters (including final body weight, body weight gain, and feed conversion ratio) remained unaffected ($P > 0.05$), which is similar to those described for egg production parameters (such as hen day egg production, egg weight and egg mass). Dietary ZnOHCl did not significantly ($P > 0.05$) enhance eggshell quality but showed potential to improve antioxidant capacity as evidenced by elevated 2,2-diphenyl-1-picrylhydrazyl values ($P < 0.01$). Zinc excretion in excreta increased with the highest level of ZnOHCl, while another mineral excretion was not affected significantly ($P > 0.05$). Surprisingly, ZnOHCl supplementation did not substantially impact tibia mineral content, indicating that the role of Zn in bone mineralization may involve more complex interactions than previously understood. Present findings indicate that supplementation with ZnOHCl at 100 mg/kg in quail diets may enhance the antioxidant capacity of the yolk without adversely affecting other productive parameters or exacerbating environmental impacts.

Introduction

Zinc (Zn) is a trace element relevant in multiple pathways that ensure optimal animal production by promoting skeletal development, enzyme function, neurobehavioural development, and reproduction (Olgun *et al.,* 2017; Yu *et al.,* 2020; Broom *et al.,* 2021). Zinc also possesses antioxidant properties that influence appropriate immune system functioning, serving as a cofactor for enzymes like superoxide dismutase (SOD) and other metalloenzymes (Ogbuewu and Mbajiorgu, 2022). Additionally, Zn plays a significant role in mineralization processes (Pereira *et al.,* 2020), supporting bone health and eggshell formation (Nguyen *et al.,* 2021; Ogbuewu and Mbajiorgu, 2022).

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Despite the presence of Zn in feedstuffs, supplementary Zn is often included in intensive poultry diets to prevent deficiencies (Esfahani *et al.,* 2021; Ogbuewu and Mbajiorgu, 2022), which can cause economic losses due to increased cracked eggs and leg diseases (Pereira *et al.,* 2020). Conversely, excessive Zn levels can lead to environmental damage through increased Zn excretion (Cufadar *et al.,* 2020). Thus, balancing Zn in poultry diets is crucial. About 30 years ago, the National Research Council (1994) set the requirements for laying quails in 50 mg/kg of Zn. However, significant changes in raw materials, the variety of additives supplemented, and the changes in poultry genetics may necessitate re-evaluating these requirements (Nguyen *et al.,* 2021).

Traditionally, the Zn requirements of poultry have been met using inorganic sources, which are cheaper than organic supplements but have lower bioavailability (Cufadar *et al.,* 2020; Yu *et al.,* 2022). Finding substitute Zn sources in avian feeding has become commonplace to prevent this issue (Olukosi *et al.,* 2018, 2019). Hydroxychloride forms have been reported as an interesting option for avian diets (Jiang *et al.,* 2021; Nguyen *et al.,* 2021; Olgun *et al.,* 2024). The benefit of this inorganic form arises from its crystalline structure, which features robust covalent bonds between hydroxyl groups and chloride ions, unlike the ionic forms that include carbon ligands (Olgun *et al.,* 2024). This structure reduces solubility in neutral solutions and increases solubility in acidic conditions, such as those found in the upper small intestine. This results in delayed release, improved absorption, and decreased environmental excretion (Cemin *et al.,* 2017; Olukosi *et al.,* 2019; Nguyen *et al.,* 2021; Olgun *et al.,* 2024).

As a result of changes in consumer preferences and the demand for alternative tastes, eggs and quail meat are becoming more popular and their production is expanding worldwide (Sarmiento-García *et al.,* 2023, 2024). However, compared to laying hens and broilers, the information about the nutritional requirements of quails is quite limited (Olgun *et al.,* 2022). Recent reports have described that supplementation with Zn hydroxychloride (ZnOHCl) could improve certain parameters of avian production, including eggshell quality and bone health status (Jiang *et al.,* 2021; Nguyen *et al.,* 2021; Yu *et al.,* 2022; Olgun *et al.,* 2024). However, the effect of ZnOHCl, as an inorganic source in the diet, on quail production has not been thoroughly studied and it is not clear what the appropriate level of ZnOHCl should be. Increased mineral bioavailability could decrease the amount of minerals that need to be supplemented in the diet to satisfy nutritional mineral requirements (Esfahani *et al.,* 2021; Olgun *et al.,* 2024). Hence, this study aims to determine the effect of adding different dietary levels of ZnOHCl on the performance and egg quality, antioxidant status of yolk, bone mineralization, and mineral excretion of laying quails.

Materials and methods

Quails' Husbandry

The trial was conducted at a local farm (38°1′36″, 32°30′45″) in Selçuklu, Konya, Türkiye for 84 days. Upon their arrival, 125 female 10-week-old Japanese quails (*Coturnix coturnix Japonica*) were weighed (253.30 ± 9.48 g) and then randomly assigned to five different treatment groups, with each group further divided into five subgroups. All birds have been housed in well-aired, clean, and sanitized cages (30 × 45 cm). The room temperature has been set at 22°C (± 2.0) and a 16-h lighting and 8-h darkness regimen have been delivered. All pens have been fitted with separate feeders and drinkers, enabling *ad libitum* access to feed and water.

Treatment diets

The same basal diet with typical commercial ingredients (soybean meal and corn), with 20% crude protein (CP) and 2900 kcal/kg of metabolizable energy (ME), has been provided for the quails (Table 1). The basal diet (containing 34.14 mg/kg Zn) without ZnOHCl supplementation has been considered the control diet. The basal diet was prepared in the mash form according to NRC (1994) recommendations, except for Zn. The five treatments consisted of a control diet combined with four incremental levels of ZnOHCl (containing 55% of Zn). Zinc Hydroxichloride has been incorporated into the control diet at the expense of corn to reach the concentration of 50, 75, 100, and 125 mg/kg of Zn in the diet.

The AOAC (2005) suggested methods have been replicated to ascertain the chemical makeup of the baseline diet. Drying at 105°C has been used to assess the water content (method 942.05). The Kjeldahl technique (method 990.03) and Soxhlet extraction (method 2003.06) have been used to determine the protein and fat contents, respectively, and incineration has been used to estimate the ash content (method 2001.12). Table 1 contains a list of all those outcomes.

Determination of performance

No mortality or illness symptoms were detected during the trial. Body weight gain was calculated from the difference between the initial and final group weights. Feed intake (g/quail/day) has been measured based on the report of Olgun *et al.* (2022). Hen-Day Egg Production (HDEP) was determined by dividing the number of eggs collected daily by the total number of birds and multiplying by 100. Egg weight, egg mass (g/quail/day), and feed conversion ratio have been assessed according to Sarmiento-García *et al.* (2023).

Table 1. Ingredients and nutrient levels of basal control diets (as-fed basis)

Ingredients	g/kg	Nutrient composition	g/kg
Maize	544.0	Metabolizable energy (kcal/kg)	2900
Soybean-meal	344.0	Crude protein	200.13
Soybean-oil	36.5	Crude fiber	28.30
Limestone	56.0	Crude fat	58.38
Dicalcium phosphate	11.4	Moisture	128.32
Salt	3.5	Lysine	10.90
Premix[1]	2.5	Methionine	4.49
DL-methionine	2.1	Cystine	3.73
Total	1000.0	Calcium	24.98
		Total phosphorus	6.37
		Available phosphorus	3.49
		Zinc, mg/kg	34.14

[1]Nutritive values are expressed as g/1000 g except for Zn concentration. 1 Premix (vitamin-mineral mixture) as contained per kg: 8800 IU Vitamin A, 3000 IU Vitamin D3, 5 mg Vitamin E, 2 mg Vitamin K, 0.02 mg Vitamin B12, 0.1 mg Biotin, 1 mg Folic acid, 50 mg Niacin, 15 mg Pantothenic acid, 4 mg Pyridoxine, 10 mg Riboflavin, 3 mg Thiamine, 10 mg Copper, 1.0 mg Iodine, 50 mg Iron, 60 mg Manganese, 0.42 Selenium.

Determination of eggshell quality parameters

Eggshell quality was assessed using 300 eggs collected during the last three days of the study at the Egg Quality Laboratory, Selcuk University, Türkiye. Cracked, broken, and damaged eggs (n=225) were recorded as a percentage of the total. Shell strength was measured using an Egg Force Reader (Orka), while shell thickness (µm) was averaged across three sections with a micrometer (Mitutoyo). The relative % of shells was calculated by weighing dried, membrane-less shells and dividing them by egg Yolk antioxidant status.

Malondialdehyde (MDA) and 1-diphenyl-2-picrylhydrazyl (DPPH) concentrations were measured in triplicate from 100 fresh eggs to assess yolk lipid peroxidation. MDA levels were quantified using the TBARS test, following Kilic and Richards (2003) and Sarmiento-García *et al.* (2021), with results expressed as µmol MDA/kg yolk. The antioxidant capacity was evaluated via DPPH radical scavenging activity following Sacchetti et al. (2005) and Olgun *et al.* (2022). Absorbance was recorded at 530 nm for MDA and 517 nm for DPPH.

Excreta and tibia mineral concentration assessment

The mineral content was determined in excreta (n = 25) and tibia (n = 25) samples. Initially, excreta and tibia samples were dried at 105 °C for 24 hours and weighed to 0.3 ± 0.01 g. After drying, 3 mL of 70% perchloric acid and 5 mL of nitric acid (63.01 M) were added to each plate. The samples were then digested in a microwave oven (CEM Corp, Matthews, NC, USA) at 190 °C for 40 minutes. Subsequently, 0.1 g of the digested samples were accurately weighed to determine the mineral concentrations using inductively coupled plasma optical emission spectrometry (ICP-OES) (Thermo Scientific 7200 ICP-OES Analyzer, Thermo Fisher Scientific, Waltham, USA).

Statistical analysis

All data underwent one-way ANOVA analysis using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Each pen served as the experimental unit for growth performance measurements, while individual birds were considered the experimental unit for all other measurements. The normality of errors was assessed using the Shapiro-Wilk test. Table results are presented as means ± Standard Error of the Mean (SEM). Statistical significance was defined as $P < 0.05$, with $P < 0.10$ indicating a trend. The ability of linear and quadratic models to explain the relationship between the dependent variable and increasing dietary ZnOHCl levels was assessed through orthogonal polynomial contrasts.

Results and Discussion

Productive Performance

There were no differences ($P > 0.05$) in final body weight, body weight gain, HDEP, egg weight, egg mass, and feed conversion ratio among experimental diets (Table 2). Regarding performance parameters, only feed intake was affected quadratically ($P < 0.05$) by ZnOHCl levels. Quails fed a diet enriched with 100 mg/kg of ZnOHCl exhibited the highest level of feed intake in contrast to quails received non-supplemented diet and those on a diet containing 50 mg/kg of Zn. Zinc is involved in several enzymatic and metabolic processes, ensuring optimal growth and animal production (Nguyen *et al.,* 2021; Ogbuewu and Mbajiorgu*,* 2022; Yu *et al.,* 2020). Contrary to expectations, present results showed that most performance and egg production parameters were not impaired by the ZnOHCl levels supplied, demonstrating similar values to the control group, which had no supplementation. These findings partially align with previous research (Abd El-Hack *et al.,* 2018; Cufadar *et al.,* 2020; Esfahani *et al.,* 2021; Niknia *et al.,* 2022). In the present study, significant differences ($P < 0.05$) were observed in feed intake, with the control group showing the lowest feed intake. This result aligns with Niknia *et al.* (2022), who reported increased feed intake with the addition of inorganic or organic Zn forms (15-45 mg/kg Zn) to the basal diet (63.58 mg/kg) in laying hens, consistent with the findings of Abd El-Hack *et al.* (2018). They observed an increase in feed intake could be due to Zn's impact on the appetite mechanism, potentially leading to anorexia if Zn levels are insufficient (Esfahani *et al.,* 2021). On the opposite, several previous research pointed out that dietary Zn has no effects on feed intake (Olgun and Yıldız, 2017; Cufadar *et al.,* 2020; Han *et al.,* 2020; Yu *et al.,* 2020). The Zn source, basal diet concentration, specific quail strain, and the age of the birds could explain the absence of significant differences in these parameters (Esfahani *et al.,* 2021; Ogbuewu and Mbajiorgu, 2022). These factors could have masked the potential benefits of ZnOHCl supplementation, suggesting that a longer study period or different experimental conditions might yield different results.

Table 2. Effect of supplementation with increasing levels of ZnOHCl on performance (n = 125) in laying quails.

Parameters	Zn concentration, mg/kg					*SEM*	*p - values*		
	34.14	50	75	100	125		Anova	L	Q
Initial body weight, g	250.75	252.25	254.38	257.38	251.75	2.732	0.956	0.750	0.553
Final body weight, g	268.38	270.50	273.25	279.50	270.50	2.559	0.719	0.507	0.350
Body weight change, g	17.63	18.25	18.88	22.13	18.75	1.901	0.963	0.683	0.711
Hen-Day Egg Production, per egg/100 quails	91.99	89.97	93.36	91.27	91.07	0.501	0.300	0.906	0.512
Egg weight, g	12.84	12.80	12.92	13.04	12.89	0.101	0.964	0.661	0.734
Egg mass, g/quail/day	11.82	11.54	12.07	11.91	11.74	0.135	0.818	0.819	0.598
Feed intake, g/quail/day	30.96^{c}	31.40^{bc}	33.12^{ab}	34.03^{a}	32.43^{abc}	0.346	**0.017**	**0.013**	**0.025**
Feed conversion ratio	2.62	2.75	2.75	2.86	2.77	0.038	0.413	0.155	0.290

SEM: Standard error of means, L: Linear effect, Q: Quadratic effect, ^{a,b,c}: Means with different upper letters in the same row are different at the $P < 0.05$ level.

Eggshell quality

According to Table 3, all eggshell quality parameters evaluated remained constant ($P > 0.05$) regardless of the ZnOHCl concentration in the diet of laying quail. Given that subpar eggshell quality results in significant economic setbacks for poultry farming, it continues to pose a substantial challenge in the industry (Gül *et al.,* 2023). Contrary to our expectations, including dietary ZnOHCl levels did not influence any of this study's measured eggshell quality parameters. This lack of significant impact is consistent with findings from previous research by Olgun and Yıldız (2017), Cufadar *et al.* (2020), Han *et al.* (2020), Yu *et al.* (2020), and Kannan *et al.* (2022), which also reported that varying dietary Zn levels did not substantially affect most eggshell quality metrics in laying hens. However, the present results contrast with those of Aghaei *et al.* (2017), Zhang *et al.* (2017), and Abd El-Hack *et al.* (2018), who observed improvements in some eggshell attributes with the inclusion of organic Zn in the diet. Zhang *et al.* (2017) suggested that increased Zn content can affect the eggshell ultrastructure and organic matrix, enhancing the elastic modulus and fracture resistance. The lack of significant improvements in eggshell quality with ZnOHCl supplementation in our study may be due to various factors. Despite Zn's role in the carbonic anhydrase enzyme, other elements such as dietary balance, the bioavailability of the Zn source, and the overall health and age of the birds might play more pivotal roles in determining eggshell quality (Nguyen *et al.,* 2021; Ogbuewu and Mbajiorgu, 2022). For example, Esfahani *et al.* (2021) found that Zn supplementation had a favorable effect on eggshell quality, particularly in older groups of hens.

Table 3. Effect of supplementation with increasing levels of ZnOHCl on eggshell quality (n = 300) in laying quails

Parameters	Zn concentration, mg/kg					*SEM*	*p - values*		
	34.14	50	75	100	125		Anova	L	Q
Damaged egg, per egg/100 eggs	0.34	1.52	1.26	0.34	0.17	0.288	0.483	0.404	0.247
Eggshell-breaking strength, N	13.29	12.78	11.78	12.67	12.64	0.213	0.273	0.389	0.103
Relative eggshell weight, g shell/ 100 g egg	8.81	8.44	8.40	8.90	8.64	0.095	0.371	0.784	0.449
Eggshell thickness, µm	232.34	227.96	223.99	233.41	227.86	1.292	0.128	0.759	0.343

SEM: Standard error of means, L: Linear effect, Q: Quadratic effect

Yolk antioxidant status

Table 4 presents data on the impact of varying Zn levels on the antioxidant capacity of the yolk, measured through DPPH and MDA assays. Significant differences ($P < 0.01$) were observed among the experimental groups in terms of yolk DPPH levels. The group receiving 125 mg/kg Zn in their diet exhibited the highest DPPH value, significantly exceeding those of both the other supplemented groups and the control group. In contrast, MDA values were not statistically affected (*P* > 0.05) by dietary ZnOHCl levels, although a numerical increase in MDA concentration was noted as dietary ZnOHCl levels increased. This difference between DPPH and MDA results may reflect the distinct mechanisms each assay measures. The DPPH assay directly assesses the radical scavenging activity of antioxidants, providing a general estimate of the antioxidant capacity. On the other hand, MDA is a marker of lipid peroxidation, which may not immediately reflect changes in antioxidant capacity but rather the extent of oxidative damage. The lack of a significant reduction in MDA levels, despite the increase in DPPH values, could suggest that the dietary ZnOHCl was more effective in boosting the quail's overall antioxidant defense system (as indicated by DPPH) than in directly mitigating lipid peroxidation during the experimental period. Factors such as the duration of Zn supplementation or the birds' age may play a role in this discrepancy, as lipid peroxidation might take longer to respond to dietary interventions compared to general antioxidant capacity (Zhang *et al.,* 2017; Yu *et al.,* 2020).

While our study is the first, to our knowledge, to specifically investigate the impact of varying ZnOHCl levels on yolk antioxidant capacity, it aligns partially with existing literature. For instance, Yu *et al.* (2020) observed improved serum Cu-ZnSOD levels with Zn supplementation (organic and inorganic forms at 70 mg/kg) in laying hens. Li *et al.* (2019) similarly reported enhanced serum GSH-Px and T-AOC activities, along with reduced serum MDA levels, when dietary Zn was increased (Zn-Met at 20–100 mg/kg) in hens. Zhang *et al.* (2017) demonstrated that Zn supplementation (80 mg Zn/kg as Zn-Gly) improved antioxidant enzyme activities and reduced MDA in 39-week-old breeding broilers, compared to 80 mg Zn/kg as $ZnSO_4$. Niknia *et al.* (2022) further highlighted that both organic and inorganic Zn form elevated serum SOD levels in aged hens, using treatments that ranged from a basal diet with 30 mg/kg zinc sulfate to up to 45 mg/kg organic zinc. Overall, these findings suggest that ZnOHCl supplementation may enhance the antioxidant capacity of quail egg yolks by improving radical scavenging activity, potentially through its role as a cofactor of Cu-ZnSOD. However, its impact on reducing lipid peroxidation may require longer periods or may vary with the age of the birds. Future studies should further explore the potential time-dependent effects of Zn supplementation on both general antioxidant capacity and lipid peroxidation markers.

Table 4. Effect of supplementation with increasing levels of ZnOHCl on antioxidant status of the yolk (n =100) expressed as DPPH and MDA values

Parameters	**Zn concentration, mg/kg**					***SEM***	***p - values***		
	34.14	**50**	**75**	**100**	**125**		**Anova**	**L**	**Q**
DPPH, % reducing	6.23^{b}	6.90^{b}	6.59^{b}	7.15^{b}	10.14^{a}	0.277	<0.001	<0.001	<0.001
MDA value, µmol MDA/kg	2.00	2.28	2.15	2.28	2.53	0.091	0.461	0.113	0.771

DPPH: 2,2-diphenyl-1-picrylhydrazyl, MDA: Malondialdehyde, *SEM*.: Standard error of means, L: Linear effect, Q: Quadratic effect, a,b: Means with different upper letters in the same row are different at the *P* < 0.01 level.

Mineral excretion

Table 5 demonstrates the effect of dietary supplementation with increasing levels of ZnOHCl on mineral excretion. Interestingly, excreta Zn concentration increased linearly from the non-supplemented quails′ diet to those that received the highest level of ZnOHCl. Nevertheless, significant differences (*P* < 0.01) have been only observed when the highest level of ZnOHCl is compared with the rest of the experimental diets. Overall, the rest of the mineral concentrations (including copper, manganese, calcium and phosphorus) assessed in the excreta have not been affected (*P* > 0.05) by the rising levels of dietary ZnOHCl, without differences between the experimental diets and the control group. However, a linear (*P* < 0.05) effect has been noted for the concentration of excreta copper. As shown in Table 5, excreta copper content decreases as the concentration of ZnOHCl in the diet increases, except for the control diet. An opposite trend is observed for excreta phosphorus, which increases linearly (*P* < 0.01) from the control group to the group that received the maximum concentration of ZnOHCl with the diet.

In this study, it was shown that only the highest level of ZnOHCl in the diet resulted in a significantly higher value of Zn excreted. The rest of the groups showed similar values of excreted Zn as the control group, with no significant effects on the excretion of other minerals observed with different dietary ZnOHCl levels. Similar findings were reported by Villagómez-Estrada *et al.* (2021) in pigs, where increasing dietary Zn levels led to higher Zn excretion. Cufadar *et al.* (2020) also noted increased Zn excretion but decreased calcium and phosphorus excretion in laying hens fed diets supplemented with varying Zn levels (20-100 mg/kg The strict homoeostatic regulation of Zn absorption and excretion means that excess dietary Zn is either not absorbed or is endogenously released and eliminated through excreta (Goff, 2018). Consequently, higher levels of Zn in poultry diets lead to increased Zn excretion, which raises environmental concerns. However, evidence indicates that ZnOHCl, compared to traditional inorganic Zn sources, results in lower mineral excretion rates. This finding suggests that using ZnOHCl could be a more environmentally sustainable approach for incorporating Zn into quail diets while minimising mineral residues.

Table 5. Effect of supplementation with increasing levels of ZnOHCl on mineral contents of excreta (n = 25) in laying quails

Parameters	Zn concentration, mg/kg					*SEM*	*p - values*		
	34.14	**50**	**75**	**100**	**125**		**Anova**	**L**	**Q**
Zn, mg/kg	139.00^{b}	151.77^{b}	179.20^{b}	180.46^{b}	237.88^{a}	9.416	**0.002**	**<0.001**	0.381
Copper, mg/kg	23.22	24.37	22.87	21.66	21.54	0.391	0.111	**0.022**	0.687
Manganese, mg/kg	333.01	331.79	319.52	300.46	329.77	5.474	0.303	0.341	0.167
Calcium, %	4.93	5.52	5.26	5.15	5.67	0.135	0.467	0.282	0.870
Phosphorus, %	1.90	1.92	2.14	2.00	2.08	0.035	0.129	0.068	0.323

SEM: Standard error of means, L: Linear effect, Q: Quadratic effect, ^{a,b}: Means with different upper letters in the same row are different at the *P* < 0.01 level

Tibia mineralization

Data presented in Table 6 revealed the effects of supplementation with increasing levels of ZnOHCl on tibia mineralization. In the study, tibia Zn, copper, calcium, and phosphorus levels have been determined at the range of 305.03-350.01 mg/kg, 2.81-3.14 mg/kg, 23.62-24.83%, and 17.17-18.08%, respectively, and, contrary to the expected, no statistical difference has been observed for any of them (*P* > 0.05). Only differences have been described for the concentration of manganese, which linearly decreased with incremental dietary ZnOHCl levels (*P* < 0.01). The manganese concentration was reduced (*P* < 0.01) in the experimental group with the highest level of ZnOHCl (125 mg/kg) compared to the control and the lowest ZnOHCl levels (50 mg/kg). Similarly, a numerical decrease in Zn level has been observed as the level of ZnOHCl in the diet increased from the control group (350.01 mg/kg) to the highest level of ZnOHCl in the diet. Bones serve as a crucial reservoir of zinc (Zn), and the mineral content of the tibia is often used as an indicator of dietary Zn levels in poultry (Ma *et al.,* 2018).

Table 6. Effect of supplementation with increasing levels of ZnOHCl on mineral contents of the tibia (n = 25) in laying quails

Parameters	Zn concentration, mg/kg					*SEM*	*p - values*		
	34.14	**50**	**75**	**100**	**125**		**Anova**	**L**	**Q**
Zn, mg/kg	350.01	308.85	311.24	305.03	317.78	6.538	0.181	0.169	0.075
Copper, mg/kg	2.81	2.78	2.95	2.95	3.14	0.081	0.684	0.173	0.775
Manganese, mg/kg	15.26^{a}	14.54^{ab}	13.78^{abc}	12.84^{bc}	11.58^{c}	0.397	**0.016**	**0.001**	0.788
Calcium, %	24.83	24.40	24.73	23.62	24.58	0.296	0.746	0.580	0.570
Phosphorus, %	17.17	17.14	17.47	17.21	18.08	0.185	0.489	0.160	0.476

SEM: Standard error of means, L: Linear effect, Q: Quadratic effect, ^{a,b,c}: Means with different upper letters in the same row are different at the *P* < 0.05 level.

In the present study, dietary Zn levels only affected the manganese (Mn) content of tibia minerals, with no significant impact observed on other assessed minerals, including Zn itself. These findings partially align with Cufadar *et al.* (2020), who reported that bone Zn content remained unaffected by dietary Zn levels, while calcium and phosphorus levels in the tibia were reduced. Similarly, Olgun and Yıldız (2017) and Nguyen *et al.* (2021) found no significant effects of dietary Zn levels on tibia calcium and phosphorus levels in laying hens and broilers, respectively. Contrary to our findings, several studies have reported increases in bone Zn levels with higher dietary Zn in various avian species, including quails (Aghaei *et al.,* 2017), laying hens (Olgun and Yıldız, 2017; Niknia *et al.,* 2022), and broilers (Nguyen *et al.,* 2021; Yu *et al.,* 2022). These discrepancies may be attributed to differences in the Zn sources used. Perez *et al.* (2017) suggested that hydroxychlorinated forms of Zn have lower interactions with other minerals and vitamins in feed, such as vitamin A, which may enhance Zn absorption. This improved absorption could explain the absence of significant differences in bone mineral content observed in our study. The minimal effects of dietary Zn observed on tibia mineral content in the present study highlight the complexities involved in Zn metabolism and its interaction with other minerals in poultry. These findings suggest that the choice of Zn source in poultry diets could significantly influence mineral absorption and bone mineralization. Understanding these interactions is crucial for optimizing dietary strategies to meet Zn requirements effectively without compromising bone health or mineral balance in poultry. Hence, further investigations are warranted to elucidate the mechanisms underlying Zn absorption and utilization in poultry bones.

Conclusion

Overall, this study underscores ZnOHCl's potential as a viable alternative to traditional zinc sources in poultry diets. However, future research should prioritize optimizing ZnOHCl dosage and investigating its interactions with other nutrients to maximize its benefits for avian health and productivity.

Ethical Approval

There are no particular restrictions for keeping experimental animals because the following investigation has been conducted with farm animals. Nevertheless, the standards outlined in the European Animal Protection Policy (EPCEU, 2010), as well as the principles reported in the 1964 Declaration of Helsinki were met throughout the experimental study.

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