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IMPACT OF MANGANESE HYDROXYCHLORIDE ON EGG QUALITY, ANTIOXIDANT CAPACITY, BONE CHARACTERISTICS, AND MINERAL EXCRETION IN LAYING QUAIL

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Abstract

This research investigated the impact of different concentrations of manganese hydroxychloride (MnH) on productive performance, egg quality, antioxidant status, tibia characteristics, and mineral excretion in laying quails. A total of 125 female ten-week-old female quails, were divided into five groups with five subgroups, each containing five quails. The birds were fed isoenergetic and isonitrogenous diets with different levels of MnH (containing 55 g/100 g Mn) at 18.86 mg/kg (basal diet), 40, 60, 80, and 100 mg/kg for twelve weeks. Results indicated that MnH supplementation enhanced egg production and feed intake ($P < 0.05$) compared to the non-supplemented group. The optimal eggshell quality, including shell-breaking strength, thickness, and weight, was observed at 80 mg/kg MnH ($P<0.01$) compared to the basal diet. Regarding the antioxidant capacity in the yolk, the yolk's 2,2- diphenyl- 1- picrylhydrazyl (DPPH) value increased significantly ($P<0.01$) with 100 mg/kg MnH supplementation, while malondialdehyde (MDA) values remained unaffected across all groups. Increasing dietary MnH levels elevated Mn excretion in faeces and reduced copper levels ($P<0.01$). However, MnH addition adversely affected tibia biomechanical properties compared to the basal diet ($P<0.01$), with no significant impact on tibia mineral accumulation ($P>0.05$). These results suggest that including 80 mg/kg MnH in laying quail diets would be adequate to improve certain aspects of production and eggshell quality, although its impact on bone parameters requires further investigation.

Key words*: antioxidant, egg, manganese hydroxychloride, minerals, quail.*

INTRODUCTION

Manganese (Mn) is a crucial trace mineral for avian nutrition, vital for ensuring animal health, egg quality, and performance development (Jasek et al., 2019). This importance stems from Mn's significant role in enzymatic systems responsible for the metabolism of lipids and carbohydrates. For instance, Mn activates glycosyltransferase, an enzyme essential for producing glycosaminoglycans and uronic acid necessary for bone and eggshell growth (Xiao et al., 2014; Khoshbin et al., 2023). Additionally, Mn is a metalloenzymes component that controls mitochondrial oxidative stress by reducing superoxide to peroxide, which is then converted into water (Bozkurt et al., 2015; Noetzold et al., 2020). Thus, a diet deficient in Mn can compromise poultry health, increase production costs, and negatively impact the environment (Gül et al., 2023).

To meet Mn intake needs, both inorganic and organic Mn sources are commonly used in avian feed, alone or in combination (Jasek et al.,

2019). Organic Mn sources generally offer higher bioavailability and stability than inorganic sources like sulphates and oxides (Olgun, 2017; Jasek et al., 2019). Recently, the avian industry has shifted towards using novel mineral additives to enhance performance, improve egg quality, reduce inclusion rates, and minimise environmental damage (Jasek et al., 2020; Jiang et al., 2021; Groff-Urayama et al., 2023; Olgun et al., 2024). In this context, hydroxychloride forms of Mn present a promising alternative as an inorganic source. The advantages of using hydroxychloride trace elements in animal nutrition are their unique crystalline structure, characterised by stronger covalent bonds between hydroxyl groups and chloride ions, unlike ionic forms that contain carbon ligands. This structure results in lower solubility in neutral or water solutions and higher solubility in acidic environments, such as the upper intestine. Consequently, hydroxychloride forms exhibit reduced reactivity with other dietary components compared to ionic sources, leading to delayed release during digestion, enhanced absorption, and decreased environmental excretion (Huang et al., 2013; Jasek et al., 2019; Groff-Urayama et al., 2023; Olgun et al., 2024).

The global population of Japanese quails is increasing, driven by their ease of management, high demand for eggs and meat, rapid production cycles, and economic sustainability (Sarmiento-García et al., 2023). Despite these advantages, there has been relatively little research on their nutritional requirements; instead, information has mostly been based on data published 30 years ago by the National Research Council (1994). While numerous studies have addressed macronutrient needs such as energy, protein, calcium, and phosphorus, there is a notable lack of research on the Mn requirements of quail.

The National Research Council (1994) recommended a Mn requirement of 60 mg/kg for laying quails. In contrast, Gökmen & Bahtiyarca (2018) suggested that certain developmental and egg quality parameters could be enhanced with diets containing 81.56 mg/kg of Mn (including 21.56 mg/kg dietary content plus 60 mg/kg supplementation). However, to our knowledge, this is the only study available on the dietary administration of Mn (whether inorganic or organic) to laying quails, and there is no literature on the supplementation of Mn hydroxychloride (MnH) in quail diets. This research aims to address this gap by examining the effects of increasing levels of MnH supplementation on the performance, egg quality, antioxidant capacity in eggs, mineral excretion, and bone properties of quails. The study seeks to determine the optimal MnH dosage level to support these parameters effectively.

MATERIALS AND METHODS

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.

One hundred twenty-five female quails (*Coturnix coturnix Japonica*) weighing 255.50 ± 16.27 g and ten weeks old have been chosen for this investigation. A local indoor farm in Selçuklu, Konya, Türkiye (38°1′36″, 32°30′45″) employed a fully randomised design for 84 days. There are five treatment trial groups, each comprising five subgroups and five quails. The quails have been aleatorily assigned into five identical battery cages (30 x 45 cm). Each cage has been sterilized, well-ventilated, and cleaned. Each pen had a temperature of 22°C (± 2.0) and a 16-hour lighting regime. For *ad libitum* access to food and water, individual feeders and drinks have been placed in each pen.

The basal diet, consisting of corn and soybean meal, was provided to all quails for 84 days. This diet contains a 200 g/kg crude protein (CP) content and 18.86 mg/kg of Mn, which translates to 2900 kcal of metabolizable energy per kilogram of feed (Table 1). Initially, the basal diet included 0 mg/kg of supplemental MnH with 18.86 mg/kg of inherent Mn. MnH (containing 55 g/100 g of Mn) was subsequently added to the basal diet at doses of 40, 60, 80, and 100 mg/kg to create the experimental diets. The foundational diet, offered in a mash form, was formulated to fulfil the nutritional needs of laying quails as outlined by the National Research Council (1994), except for Mn. The

chemical composition of this basic diet was assessed using the methods specified by the AOAC (2006), and the details are presented in Table 1.

Table 1. Basal diet and its nutrient composition

Ingredients	g/kg	Nutrients	g/kg
Corn	544.00	ME (kcal/kg)	2899.08
Soybean meal (460 g/kg CP)	344.00	Crude protein	200.13
Soybean oil	36.50	Crude fibre	28.30
Limestone	56.00	Crude fat	58.38
Dicalcium phosphate	11.40	Moisture	128.32
Salt	3.50	Lysine	10.90
Premix[1]	2.50	Methionine	4.49
DL-methionine	2.10	Cystine	3.73
Total	1000.00	Calcium	24.98
		Total phosphorus	6.37
		Available phosphorus	3.49
		Manganese (mg/kg)	18.86

[1]Premix provides per kg feed: vitamin A, 8000 IU; vitamin D_3, 3000 IU; vitamin E, 5 mg; vitamin K, 2 mg; folic acid, 1 mg; biotin, 0.1 mg; niacin, 50 mg; pantothenic acid, 15 mg; vitamin B12, 0.02 mg; pyridoxine, 4 mg; thiamin, 3 mg; riboflavin, 10 mg; iodine, 1.0 mg; copper, 10 mg; iron, 50 mg; selenium, 0.42 mg; zinc, 60 mg.

Quails were randomly assigned into various experimental groups to evaluate their performance indicators based on methods described by Olgun et al. (2022) and Sarmiento et al. (2023). The assessment focused on various parameters such as body weight gain, feed intake, feed conversion ratio (FCR), and egg production. Daily feed consumption was measured by the feed given and subtracting the leftover feed in the feeders. Body weight gain was tracked by weighing the quails at the beginning and end of the study period. The feed conversion ratio was computed as the ratio of feed consumed to body weight gained. Egg production was monitored by counting the number of eggs laid by each group throughout the experiment. The egg-laying rate was calculated as the daily number of eggs produced, expressed as a percentage of the total number of quails in each group.

The egg laboratory at Selcuk University, Konya, Türkiye's Faculty of Agriculture conducted the following analyses. All eggs collected over the last three days of the trial have been evaluated for both internal and exterior quality requirements at room temperature. Broken, damaged, and cracked eggs have been counted throughout the experiment and expressed as a percentage of the total number of eggs (n=300). All measurements of eggshell quality have been done using the techniques described by Olgun et al. (2022).

Lipid peroxidation of the yolk in triplicate on 100 fresh eggs was assessed using quantities of malondialdehyde (MDA) and 1- diphenyl- 2-picrylhydrazyl (DPPH). The thiobarbituric acid reactive substances (TBARS) test was conducted following the methods of Sarmiento-García et al. (2021) and Kilic & Richards (2003) to determine the MDA value. The DPPH radical scavenging activity was determined as described by Sacchetti et al. (2005) and Olgun et al. (2022) with slight modifications. And was used to evaluate the antioxidant capacity of the produced hydrolysates.

The mineral contents of faeces and tibia samples have been evaluated using the wet digestion technique. All analysis for faeces and tibia mineral contents were conducted according to the methods described by Gül et al. (2024). On the 84th day, one quail from each subgroup (n = 25) was randomly chosen and euthanized via cervical dislocation. The right tibia was extracted, cleaned, and stored at -20°C until further analysis. Before examination, the samples were brought to room temperature for six hours in a controlled air environment. The biomechanical properties of the bones were evaluated following the methodologies of Wilson & Ruszler (1996), Armstrong et al. (2002), and Gül et al. (2022).

The statistical analysis utilized cage averages as the experimental units for overall comparisons, with a one-way ANOVA conducted via SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). For specific parameters such as egg quality, MDA, and DPPH levels, individual birds served as the experimental units. The findings are reported as means ± standard errors of the mean (SEM). Statistical significance was established at $P<0.05$. The response of each dependent variable to increasing manganese levels was evaluated using linear, quadratic, and cubic regression models.

RESULTS AND DISCUSSIONS

The National Research Council (1994) established the Mn requirements for avian species to be 60 mg/kg. Since this data was

published in 1994, significant advancements in animal production, primarily due to genetic improvements, have occurred. These changes have enhanced production rates and consequently altered the nutritional requirements of animals. Therefore, it is necessary to re-evaluate these nutritional needs to reflect the current state of animal production (Jasek et al., 2019; Gül et al., 2023).

Performance and egg indicators are detailed in Table 2. MnH supplementation appeared to have minimal impact (P>0.05) on final body weight (270.6-274.1 g), body weight change (10.75-20.13 g), egg weight (12.62-13.26 g), and feed conversion ratio (2.73-2.93), as no significant differences were detected between treatments. Egg production was significantly lower (87.19 per egg/100 quails) in the group without MnH supplementation compared to the other experimental diets (89.85-90.92 though the differences between the MnH-supplemented groups were not statistically significant (P>0.05). This pattern was also observed in feed intake, where MnH supplementation led to a significant decrease (P<0.05). However, feed intake values were similar (P>0.05) among all groups that received MnH supplementation. The current research found that MnH supplementation does not affect performance (in terms of final body weight, body weight change, and feed conversion ratio) compared to non-supplemented quails.

Table 2. Influence of dietary MnH levels on the performance of laying quails (n = 125)

Parameters	MnH levels (mg/kg)					S.E.M*	*p*-values	Regression		
	18.86	40	60	80	100			L	Q	C
Initial BW (g)	252.2	254.0	262.8	261.6	254.1	2.35	0.518	0.492	0.180	0.405
Final BW (g)	270.6	274.1	273.5	270.9	273.1	3.67	0.964	0.882	0.555	0.819
BWC (g)	18.48	20.13	10.75	14.88	16.75	1.828	0.569	0.515	0.408	0.475
EP (per egg/100 quails)	87.19^{b}	89.85^{a}	90.18^{a}	90.19^{a}	90.92^{a}	0.422	0.037	0.006	0.189	0.276
EW (g)	12.75	12.62	13.26	12.95	13.08	0.129	0.572	0.304	0.724	0.698
EM (g/quail/day)	11.12	11.34	11.95	11.68	11.89	0.125	0.155	0.033	0.392	0.968
FI (g/quail/day)	31.29^{b}	33.18^{a}	32.63^{a}	33.28^{a}	33.45^{a}	1.186	0.012	0.003	0.178	0.172
FCR	2.83	2.93	2.73	2.85	2.82	0.024	0.137	0.496	0.855	0.334

S.E.M.: Standard error of means, L: Linear, Q: Quadratic, C: Cubic, ^{a,b}: At the P < 0.05 level, means in a row with distinct upper letters vary from one another. BW: Body weight, BWC: Body weight change, EP: Egg production, EW: Egg weight, EM: Egg mass, FI: Feed intake, FCR: Feed conversion ratio.

Similar findings have been reported by previous authors in laying hens (Bai et al., 2014; Xiao et al., 2014; Hajjarmanesh et al., 2023) and broilers (Jasek et al., 2019; Saldanha et al., 2020). As described by Jasek et al. (2019), the absence of the noted differences in performance parameters by rising Mn inclusions could suggest: i) the weight increase demand is rather weak and can be met by Mn levels in dietary ingredients, ii) the enzymes influenced by Mn are not related to increased body weight gain, or iii) a combination of both. However, higher feed intake was reported for all supplemented groups compared to the non-supplemented. In contrast, no differences in this parameter have been found in previous studies on broilers (Jasek et al., 2019; Groff-Urayama et al., 2020), while Khoshbin et al. (2023) showed a trend of increased feed intake with the addition of organic Mn chelate to the diet of laying hens, similar to the findings in this study. Jasek et al. (2019) described that the mechanisms regulating appetite might not be related to Mn intake and could depend on the intake of other minerals. This observation differs from what has been noted in our study for laying quails. Since there is limited information on this subject, this parameter should be re-evaluated to confirm possible differences between quails, broilers, and laying hens. In the current research, egg production rose significantly with increased Mn in the diet, similar to findings by previous authors for laying hens (Noetzold et al., 2020; Khosbin et al., 2023) and ducks (Zhang et al., 2022). For example, reductions in circulating progesterone, estradiol, FSH, and LH are suggestive of Mn deficit, which may impair the function of the hypothalamic-pituitary gonadal axis. Additionally, declines in the activity of certain enzymes (such as pyruvate carboxylase) up-regulated by Mn are associated with lower egg production (Noetzold et al., 2020; Zhang et al., 2022). These outputs align with the recommendation of the National Research Council (1994) which proposed that diets for laying quail should contain at least 60 mg/kg Mn to maintain optimal quail developmental and egg production parameters.

Eggshell quality remains a critical issue for avian production due to its significant economic implications for producers (Gül et al., 2022; Hajjarmanesh et al., 2023). Table 3 illustrates the impact of varying dietary MnH levels on eggshell quality. The incidence of damaged eggs (ranging from 0.16 to 2.21 per 100 eggs) did not show significant variation with different MnH supplementation levels (P>0.05). However, quails receiving 80 mg/kg (13.25 N) and 100 mg/kg (13.03 N) Mn exhibited significantly greater eggshell strength (P<0.01) compared to those in the non-supplemented group and those receiving 40 mg/kg and 60 mg/kg Mn (11.78 N, 11.62 N, and 11.60 N, respectively). In terms of relative eggshell weight, the group supplemented with 80 mg/kg MnH had the highest value (9.22 g shell/100 g egg), significantly surpassing the non-supplemented group and those supplemented with 40 and 60 mg/kg (8.19, 8.43, and 8.15 g shell/100 g egg, respectively (P<0.01). Additionally, eggshell thickness was notably higher (P<0.01) in the group receiving 80 mg/kg Mn (244.8 µm) compared to the control groups and the group receiving 60 mg/kg MnH (229.9 µm and 232.7 µm, respectively). In this study, the thickness, breaking strength, and weight of eggshells have significantly been enhanced at levels of 80 mg/kg MnH compared to non-supplemented quails. These findings align with those of Noetzold et al. (2020) and Hajjarmanesh et al. (2023), who reported similar improvements when manganese was added to the diets of laying hens. Cui et al. (2019) observed that adding 21.95 mg/kg of Mn to a basal diet enhanced eggshell thickness and breaking strength, and supplementing with 40 mg/kg Mn was sufficient for optimal shell quality. In another study, Xiao et al. (2014) described that a dietary addition of 100 mg/kg Mn to the diet enhanced shell thickness and breaking strength.

Table 3. Influence of dietary MnH levels on the eggshell quality (n = 300) of laying quails

Parameters	MnH levels (mg/kg)					S.E.M*	*p*-values	Regression		
	18.86	40	60	80	100			L	Q	C
Damaged egg rate (per egg/100 eggs)	0.89	1.01	0.17	2.21	0.16	0.275	0.101	0.883	0.515	0.099
Eggshell-breaking strength (kg)	11.78^{b}	11.62^{b}	11.60^{b}	13.25^{a}	13.03^{a}	0.204	0.003	0.001	0.207	0.084
Relative eggshell weight (g shell/ 100g egg)	8.19^{c}	8.43^{bc}	8.15^{c}	9.22^{a}	8.75^{ab}	0.104	0.001	0.001	0.972	0.065
Eggshell thickness (µm)	229.9^{b}	238.2^{ab}	232.7^{b}	244.8^{a}	241.7^{a}	1.62	0.008	0.003	0.649	0.897

S.E.M.: Standard error of means, L: Linear, Q: Quadratic, C: Cubic, ^{a,b}: At the P < 0.05 level, means in a row with distinct upper letters vary from one another.

Zarghi et al. (2023) observed that shell weight increased in laying hens receiving Mn at a level of 0-90 mg/kg, while other eggshell quality criteria have been unaffected. The results of this study suggest that the MnH level required to improve eggshell quality parameters is higher than the level needed to maintain overall performance and productivity in laying quails. The observed improvements in eggshell quality (increased breaking strength, thickness, and relative weight) as dietary Mn increased are likely due to the enhancement of the palisade and mammillary layers, along with the shell membrane. Mn is essential for the synthesis of glycosaminoglycans and glycoproteins, as it regulates glycosyltransferase, an enzyme involved in the production of proteoglycans, which are key components of the eggshell matrix. Consequently, Mn supplementation has been associated with increased membrane glycosaminoglycan content, which contributes to improved shell morphology (Noetzold et al., 2020).

Mn is an integral part of manganese superoxide dismutase (Mn-SOD), the primary antioxidant enzyme that protects cells from oxidative stress, neutralizes reactive oxygen species, reduces lipid peroxidation, and enhances overall antioxidant capacity (Zhu et al., 2017). According to the data in Table 4, the yolk TBARS value (1.414-2.230 µmol MDA/kg) has been unaffected by dietary MnH levels (P>0.05). However, according to these results, the DPPH value could be more sensitive to MnH rose in comparison to those described for TBARS value. The highest dose of MnH (at a level of 100 mg/kg Mn) resulted in a significant rise (P<0.01) in the DPPH value compared to the rest of the experimental groups.

Table 4. Influence of dietary MnH levels on the yolk (n = 100) DPPH and MDA values of laying quails

Parameters	MnH levels (mg/kg)							Regression		
	18.86	40	60	80	100	S.E.M*	*p* -values	L	Q	C
DPPH (% reducing)	6.764[b]	6.857[b]	6.154[b]	6.936[b]	9.125[a]	0.2017	<0.001	<0.001	<0.001	0.029
MDA (µmol MDA/kg)	1.604	2.230	1.958	1.782	1.414	0.1349	0.368	0.408	0.091	0.481

S.E.M.: Standard error of means, L: Linear, Q: Quadratic, C: Cubic, [a,b]: At the P < 0.05 level, means in a row with distinct upper letters vary from one another. TBARS: thiobarbituric acid reactive substances, DPPH: 2,2-diphenyl-1-picrylhydrazyl.

In this study, the reduction percentage of DPPH in the yolk increased significantly at a dietary level of 100 mg/kg MnH, while other groups, including the non-supplemented group, exhibited similar DPPH values. These outputs suggest an adequate bioavailability of Mn at doses of 100 mg/kg that would be transported and deposited into the yolk to exert its antioxidant capacity. This is linked to the rise in Mn-SOD activity and the decrease in lipoprotein lipase activity with higher Mn supplementation. Mn-SOD increases antioxidant capacity to scavenge reactive oxygen species and decline lipid peroxidation as can be observed in this study (Cui et al., 2019; Khosbin et al., 2013). Nevertheless, the yolk MDA value has been unaffected by MnH supplementation. To our knowledge, there are no studies that jointly examine the effect of MnH on DPPH and TBARS values in egg yolk. Nevertheless, numerous studies have demonstrated the antioxidant benefits of dietary Mn supplements in various tissues. Khoshbin et al. (2023) clarified that the Mn addition to the diet at a level of 90 mg/kg enhanced the serum DPPH and declined MDA values in laying hens. Similar findings have been obtained in broilers, Bozkurt et al. (2015), described a decrease in serum MDA levels with dietary Mn ranging from 6.25 to 50 mg/kg. Additionally, Mn supplementation has been shown to effectively reduce lipid oxidation in broilers (Shokri et al., 2021) and duck meat (Yang et al., 2021).

Mn that is not absorbed in the intestine is excreted in the bile or faeces (Zhang et al., 2022), so as expected, as the dietary Mn level increased, the faecal Mn content also increased. Table 5 summarises the impact of different dietary levels of MnH on the mineral content in quail faeces. There was a significant positive correlation (P<0.001) between the levels of MnH in the diet and the concentration of Mn in the faeces Specifically, quails on an Mn-deficient diet exhibited the lowest faecal Mn levels, while those receiving higher Mn supplements showed increased excretion. Interestingly, the dietary Mn level did not significantly influence faecal zinc content (P>0.05), though a quadratic regression trend (P<0.05) was noted. Conversely, faecal copper content declined linearly (P<0.01) with increasing Mn supplementation, dropping from 27.26 mg/kg in the non-supplemented group to 21.94 mg/kg in the group receiving 100 mg/kg of Mn. Calcium and phosphorus excretion patterns also responded to Mn supplementation.

Table 5. Influence of dietary MnH levels on the faeces mineral composition (n = 25) of laying quails

Parameters	MnH levels (mg/kg)							Regression		
	18.86	40	60	80	100	S.E.M*	*p*-values	L	Q	C
Manganese (mg/kg)	199.8[d]	212.9[cd]	223.9[c]	249.4[b]	269.0[a]	5.57	<0.001	<0.001	0.147	0.809
Zinc (mg/kg)	313.3	324.7	327.1	320.0	297.3	4.13	0.144	0.205	0.024	0.762
Copper (mg/kg)	27.26[a]	25.07[b]	23.40[bc]	24.62[b]	21.94[c]	0.457	<0.001	<0.001	0.486	0.067
Calcium (g/100g)	5.04	6.27	4.90	5.18	5.26	0.173	0.080	0.582	0.561	0.038
Phosphorus (g/100g)	1.56	1.85	1.79	1.87	1.93	0.045	0.063	0.012	0.344	0.248

S.E.M.: Standard error of means, L: Linear, Q: Quadratic, C: Cubic, [a,b]: At the P < 0.05 level, means in a row with distinct upper letters vary from one another.

A cubic regression (P<0.05) was evident for calcium, with the highest excretion noted in the 40 mg/kg Mn group. Phosphorus excretion showed a linear regression (P<0.05), peaking at the 100 mg/kg Mn supplementation level. Similar to the current study, Mwangi et al. (2019), Matuszewski et al. (2020), de Carvalho et al. (2021), Xia et al. (2022) and Zhang et al. (2022) reported that the Mn level excreted through faeces increased with the increasing Mn in the diet in avian. Nevertheless, it is important to point out that regardless of the doses, the current findings reveal a lower mineral excretion than those reported when inorganic Mn has been

added to the avian´s diet. This is due to the increased absorption of organic forms such as hydroxychloride, reducing the excretion through the faeces and contributing to environmental protection (Jasek et al., 2020; Jiang et al., 2021; Groff-Urayama et al., 2023; Olgun et al., 2024). Table 6 shows the impact of different levels of dietary MnH on the biomechanical properties of the tibia in laying quails.

Cortical bone thickness, shear force, cortical bone cross-sectional area, and shear stress have significantly been affected by the treatments ($P<0.01$). Cortical bone cross-sectional area and cortical bone thickness showed similar behaviour, with a high correlation with increasing concentration of MnH ($P<0.01$). Contrary to the expected, both parameters decreased significantly as the concentration of MnH rose (from 0.476 to 0.331 mm and from 1.79 to 1.27 mm^2, respectively). Shear force and shear stress parameters decreased with the addition of MnH to the diet (from 187.2 to 115.5 N and from 106.57 to 80.45 N/mm^2, respectively).

The shear force recorded the lowest values with a dietary dose of Mn of 60 mg/kg, while shear stress showed the lowest values in the groups that had received 40 and 60 mg/kg of MnH in the diet. It has been described that Mn regulates the formation of proteoglycans, which are involved in bone development (Jasek et al., 2019). Contrary to those expected, the outputs of the current research do not seem clear. The biomechanical traits of the tibia in laying quails that had been supplemented with MnH have been observed to be worse compared to the non-supplemented group. Similar findings have been reported by Jasek et al. (2019) who found inconsistent findings with the supplementation of high doses of Mn. Those authors considered that the outputs are inconclusive and proposed that the Mn levels evaluated are not sufficient to improve bone parameters, and it would be necessary to use a larger number of birds in future experiments to evaluate these parameters. According to the previous literature, studies assessing bone parameters are scarce and contradictory. Cui et al. (2019) demonstrated that the administration of Mn (0-800 mg/kg) to the diet does not affect the tibia-breaking strength, similar to those reported by Bozkurt et al. (2015) and Shokri et al. (2021), who described that the inorganic or organic Mn addition had no impact on the tibia biomechanical properties of broilers. The duration of the study, the age of the animals and the species used may be responsible for the variations observed between previous studies. Future research would be necessary to confirm the findings obtained for the laying quails.

The tibia mineral level of laying quails is reflected in Table 7. Tibia mineral content has not been seen to be sensitive to MnH supplementation. No differences ($P > 0.05$) in the tibia mineral content have been described amongst experimental groups for any of the minerals assessed. The minimum and maximum values of the tibia mineral levels are as follows: Mn (12.78-10.22 mg/kg), zinc (311.6-289.8 mg/kg), copper (3.67-2.83 mg/kg), calcium (25.11-22.78 g/100 g) and phosphorus (18.32-16.11 g/100 g). Contrary to expectations, but following the outputs obtained in the tibia parameters, MnH supplementation does not affect the mineral concentration of the tibia, which is consistent with those proposed by Xiao et al. (2015). Similarly, Bai et al. (2014) explained that the supplementation of 60 and 300 mg/kg Mn to the basal diet (24.35 mg/kg Mn) in hens had no effect on tibia zinc and copper content but raised tibia Mn level (300 mg/kg Mn). On the opposite, Yıldız et al. (2011) reported that the Mn addition to the basal diet in laying hens improved the tibia Mn, calcium, and phosphorus however caused a decrease in zinc and copper.

In another study, Cui et al. (2019) stated that Mn addition to the diet enhanced the Mn level while not affecting the tibia calcium content, similar to those described by Jasek et al. (2019). Several explanations may be responsible for the differences found among the different studies. For example, Junchang & Ruangpanit (2023), who examined the concentration of some minerals in bones from old hens, reported that old hens are less sensitive to Mn administration, which could justify the findings of the current research. In addition, the species used could be responsible for the differences. Regardless, more investigation is required to reassess these findings.

Table 6. Influence of dietary MnH levels on the tibia biomechanical traits (n = 25) of laying quails

Parameters	MnH levels (mg/kg)					S.E.M*	*p*-values	Regression		
	18.86	40	60	80	100			L	Q	C
Cortical bone thickness (mm)	0.476^{a}	0.410^{bc}	0.383^{cd}	0.455^{ab}	0.331^{d}	0.0134	<0.001	0.001	0.865	0.002
Cortical bone cross-sectional area (mm^2)	1.79^{a}	1.55^{b}	1.43^{c}	1.56^{b}	1.27^{c}	0.046	0.001	<0.001	0.615	0.035
Shear force (N)	187.2^{a}	131.0^{bc}	115.5^{c}	150.9^{b}	129.7^{bc}	6.15	<0.001	0.002	0.001	0.003
Shear stress (N/mm^2)	106.57^{a}	84.67^{c}	80.45^{c}	96.75^{ab}	102.11^{a}	2.876	0.005	0.906	0.001	0.109

S.E.M.: Standard error of means, L: Linear, Q: Quadratic, C: Cubic, ^{a,b}: At the P < 0.05 level, means in a row with distinct upper letters vary from one another.

Table 7. Influence of dietary Mn levels on the tibia mineral composition of laying quails (n = 25)

Parameters	Mn levels (mg/kg)					S.E.M*	*p*-values	Regression		
	18.86	40	60	80	100			L	Q	C
Manganese (mg/kg)	12.78	10.22	11.48	10.51	11.33	1.538	0.058	0.170	0.056	0.314
Zinc (mg/kg)	311.6	289.9	294.0	297.6	289.8	7.62	0.910	0.531	0.687	0.536
Copper (mg/kg)	3.18	3.32	2.83	2.90	3.67	0.135	0.280	0.566	0.117	0.145
Calcium (g/100g)	24.67	23.71	25.11	24.91	22.78	1.793	0.191	0.262	0.215	0.076
Phosphorus (g/100g)	17.75	16.11	17.37	18.32	17.09	0.355	0.389	0.739	0.809	0.055

S.E.M.: Standard error of means, L: Linear, Q: Quadratic, C: Cubic, ^{a,b}: At the P < 0.05 level, means in a row with distinct upper letters vary from one another.

CONCLUSIONS

This study offers a significant advancement in understanding the effects of MnH supplementation in laying quails. The findings indicate that MnH supplementation is beneficial for enhancing certain performance indicators, egg production, and egg quality in laying quails, with 80 mg/kg MnH identified as a particularly effective dose. However, the effects on bone integrity remain unclear. Regarding environmental impact, while increased MnH doses resulted in higher Mn excretion, the levels were still lower than those recorded for other sources of organic Mn. This suggests that MnH is a more environmentally friendly option compared to other Mn sources. Nevertheless, the beneficial effects on bone integrity are not clear. Further research is necessary to fully understand the implications of MnH supplementation on avian bone health and to explore the long-term effects of MnH supplementation and its interaction with other dietary components.

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