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Manganese–methionine chelate improves antioxidant activity, immune system and egg manganese enrichment in the aged laying hens

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Abstract

Background: It has been reported that supplementation of manganese (Mn) could alleviate the negative effects of age on egg quality in laying hens. However, limited information is available on compensatory ways in order to reduce the adverse effects of hen age on health and Mn deposition in the body.

Objectives: The objectives were to investigate the effect of organic and inorganic sources of Mn on antioxidant activity, immune system, liver enzymes, shell quality and Mn deposition in the tissues of older laying hens.

Methods: A total of 250, 80-week-old Leghorn laying hens (w36) were allocated into five treatment groups with five replications in a completely randomised design. Treatments were control (without Mn supplementation), 100% Mn sulphate, 75% Mn sulphate + 25% organic Mn chelate, 50% Mn sulphate + 50% organic Mn chelate and 25% Mn sulphate + 75% organic Mn chelate.

Results: The groups fed 50 and 75% organic Mn chelate exhibited the lowest feed conversion ratio, as well as the maximum laying percentage, and egg weight and mass. Except to those fed 75% Mn sulphate, the hens received Mn supplements either as organic or inorganic, had higher immunoglobulin G and M compared with the control ($p < 0.05$). A significant elevation in the values of superoxide dismutase was observed in the hens receiving 50 and 75% organic Mn chelate when compared with the other treatments. The ALP activity decreased with increasing organic Mn chelate. Mn supplementation, either as organic or inorganic, increased Mn deposition in bone, egg yolk and shell, serum and liver.

Conclusion: Dietary supplementation with 50–75% Mn–methionine has the potential to replace Mn-sulphate in laying hens' diet for improving eggshell quality, Mn deposition in the eggshell, antioxidant capacity and immune response, as well as improving laying performance, egg weight and feed conversion ratio.

KEYWORDS
antioxidant properties, immune system, laying hen, liver enzyme, manganese

1 | INTRODUCTION

Trace minerals (TMs) are biologically known as important micronutrients in the laying hen nutrition, which regulate metabolism and have a great effect on poultry performance and health (Sun et al., 2010; Yenice et al., 2015). Manganese (Mn) is among the most abundant metals in the earth and an essential trace element (Gandhi et al., 2022), which is necessary for the normal activity and growth of the body tissues such as bone, as well as improving immune organ weight and performance (Wang et al., 2021). This element acts as an activator and cofactor for superoxide dismutase (SOD), transferases, hydrolases and lyases (Bagga and Patel, 2012) and also plays a role in regulating macromolecule metabolism (carbohydrate, lipid and protein) (Li & Yang, 2018; Barrioni et al., 2019). Mn activates the glycosyl transferases involved in the synthesis of proteoglycans (Xiao et al. 2014) which is important for bone strength and eggshell.

Advancement of the animal's age increases the probability of the occurrence of problems due to oxidative stress, which can affect the whole process of egg formation, as well as compromising egg lipid stability that may be very important for late-phase laying hens (Bree et al. 2002). Insufficient dietary Mn may result in the malfunction of reproduction and negatively affect bone growth (Olgun, 2017). Many efforts have been made to enhance eggshell quality in old laying hens by using dietary inorganic supplements (Esfahani et al., 2021). Minerals are included into the diet in the form of sulphate, oxide, phosphate and carbonate (Seyfori et al., 2018; 2019). Ji et al. (2006) reported an increase in serum Mn content following the use of organic Mn source compared with the Mn sulphate. Micronutrients such as Mn are accumulated in poultry tissues and organs, and their egg contents and shell in different levels depending on several parameters such as their dose and form and physiological factors (Dobrzański et al., 2008).

In recent decades, the effects of Mn supplementation on egg quality and performance of laying hens have been widely investigated. Inorganic Mn supplements are routinely added to conventional laying diets to meet Mn requirements. On the other hand, it is generally accepted that the organic Mn complex or chelates have better absorption or bioefficacy and less environmental contamination than inorganic sources (Ji et al., 2006; Xiao et al., 2014). There is a little information about the effects of combination of different forms of Mn (organic and inorganic) on performance of old laying hens, Mn deposition in the egg and body tissues and immune function, because most of the studies have mainly focused on separate use of different levels of Mn sources. Therefore, the present study aimed to examine the effect of organic and inorganic Mn supplements with different concentrations on performance, antioxidant activity, blood parameters, immune system, liver enzymes, egg shell quality and Mn storage in the egg and other tissues of old Leghorn laying hens (Hy-lineW36).

2 | MATERIALS AND METHODS

This experiment was carried out in the Research Poultry Farm for 12 weeks during summer 2020. In this regard, 250 Leghorn laying hens (Hy-lineW36) at 80-week-old (the last laying phase) were randomly allocated in three-story cages and subjected to five experimental treatments with five repetitions (10 hens per replication). Before the experiment, hens were provided with basal diet for 1 week to habituate, followed by freely access to feed and water throughout the period. The five treatments were: basal diet (control) (29.01 mg/kg Mn) and diets 1–4 containing basal diet and 100% Mn sulphate, 75% Mn sulphate + 25% organic Mn chelate, 50% Mn sulphate + 50% organic Mn chelate and 25% Mn sulphate + 75% organic Mn chelate, respectively (containing 90 mg/kg Mn).

Mn sulphate pentahydrate (Mn-Sulph) with 34% Mn was used as inorganic supplement, and Mn–methionine chelate (Mn-MET) with 5% Mn was used as organic Mn supplement (Ariana Co.). Table 1 outlines the constituents of the used basal diet.

2.1 | Performance traits

The average feed intake was approximately 110 g/day based on the guideline of the desired strain (Hyline International, 2015). The daily feed intake (g), feed conversion ratio and egg production (g/hen/day) were recorded throughout the experiment. Regarding each treatment, the feed level remaining at the next day was subtracted from the total feed for the treatment (around 6 kg/day) to obtain daily feed intake. Furthermore, the eggs were weighted on a digital scale with the precision of 0.01. The following equations were used to determine production percentage, egg weight and feed conversion ratio.

$$P_d = (T_e/n) \times 100$$

in which P_d represents daily production percentage, and n and T_e refer to the number of hen and egg in each experimental unit, respectively.

$$EW = EWT/n$$

where EW indicates mean daily egg weight, EWT and n reflect total egg weight and number in each experimental unit, respectively.

$$FCR = FI/Em$$

where FCR indicates feed conversion ratio, FI is feed intake and Em is egg mass (multiplying production percentage by mean egg weight, divided by 100).

2.2 | Antioxidant activity

To assess antioxidant properties, the serum SOD activity was evaluated by using Randsel SOD diagnostic kit (Randox, Crumlin, UK) in Maad laboratory (co-laboratory of Javaneh Khorasan Co.). Furthermore, thiobarbituric acid reactive substance assay was employed to measure malondialdehyde (MDA) as the final product of lipid peroxidation in the liver cells of hen. The free radical scavenging activity was obtained based on the previous studies using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Qiu et al., 2009).

2.3 | Immunological evaluation

Sheep red blood cell (SRBC) antigen was utilised to examine humoral immunity, the immune response against which was assessed through

TABLE 1 Constituents and nutrients of basal diet (dry matter)

Constituent	Percentage
Corn	53
Soybean	25
Calcium carbonate	10
Vegetable oil	1.4
Wheat bran	6
Dicalcium phosphate	2.2
Sodium chloride	0.25
Sodium bicarbonate	0.15
Bentonite	1.2
Vitamin supplement[a]	0.25
Inorganic supplement[b]	0.25
Methionine-DL	0.2
L-lysine	0.1
Calculated (analysed) composition	
Metabolisable energy (kcal/kg)	2810
Crude protein (%)	15.15
Calcium (%)	4.65
Available phosphorous (%)	0.4
Sodium (%)	0.18
Methionine (%)	0.38
Methionine + cystine (%)	0.65
Lysine (%)	0.8
Arginine (%)	0.9
Threonine (%)	0.59
Manganese (mg/kg)[c]	29.01

[a]The vitamin supplement existing in 1 kg of diet consists of 3,200,000 IU of vitamin A, 1,320,000 iU of vitamin D3, 8000 IU of vitamin E, 1000 mg/kg of vitamin K3, 1000 mg/kg of vitamin B1, 2200 mg/kg of vitamin B2, 3200 mg/kg of vitamin calpan, 12,000 mg/kg of niacin, 1600 mg/kg of B6, 360 mg/kg of B9, 9 mg/kg of B12, 30 mg/kg of biotin, 44,000 mg/kg of choline and 3000 mg/kg of antioxidant.
[b]Manganese-free inorganic supplement contains 32,000 mg of zinc, 32,000 mg of copper, 480 mg of iodine, 88 mg of selenium and 16,000 mg of iron per kg of basal diet.
[c]Determined by the analysis of duplicate samples.

measuring serum immunoglobulins (Ig). Two weeks before slaughtering, two hens were selected from each repetition and injected with the intended solution. Then, the hens were blood sampled (10 ml) to determine Ig level on the slaughtering day.

2.4 | Liver enzymes activity

In each replication, two blood samples were taken during slaughter, the serum of which was analysed by Maad laboratory to specify the effects of adding organic and inorganic Mn supplements on liver function and enzymes (aminotransferase [AST], alkaline phosphatase [ALP] and alanine aminotransferase [ALT]) (Moharreri et al., 2022).

2.5 | Mn storages in egg and other tissues

At the end of the experimental period, two hens were selected from each replication to collect their blood samples (10 ml) during slaughtering. All experimental hens were fasted for 10 h before slaughtering to enhance measurement accuracy and precision. In addition, the serum was obtained by centrifuging at 3000×*g* for 10 min and held at −20°C until evaluating its Mn value. To determine bone Mn concentration, right tibia was separated, followed by removing soft and cartilaginous parts, drying in an oven for 24 h and placing into a 550°C furnace to prepare ash. Then, atomic absorption spectroscopy was applied to obtain Mn content in bone, egg yolk and liver. Serum Mn was measured by using inductively coupled plasma optical emission spectrometry (Varian) in the Parto Azmoon Javaneh Khorasan (AOAC, 1995).

2.6 | Egg quality measurements

At the end of the trial (week 92), a sample of 10 eggs per replication (50 eggs/treatment) was used to evaluate the quality of eggs. The thickness of shells was measured using a thickness gauge (OSK13469) with an accuracy of 0.01 by measuring three points (two ends and centre in millimetres). In order to evaluate the fracture resistance of the shell, the digital egg shell force Gauge model-II was used in terms of the kilogram of force required to break the shell in a cross section of one square centimetre (Mohiti Asli et al., 2007).

2.7 | Data analysis

Data were collected and transferred to Excel software and categorised. After checking the normality of data, the General Linear Model (GLM) procedure of SAS software (version 9.2) was used to analyse data for a completely randomised design. The means of treatment were compared using Tukey Kramer test with a probability level of 0.05.

3 | RESULTS

One week before the trial period, all laying hens were fed the basal diet, and they showed normal performance (the data not shown). The results related to the effects of organic and inorganic Mn supplement at different levels on production performance of old laying hens are provided in Table 2. As shown, the diets containing 50 and 75% organic Mn significantly improved feed conversion ratio, laying percentage and egg weight and mass without any significant effect on feed intake ($p < 0.05$). Additionally, all Mn contained-diets were led to a greater egg weight compared with the control ($p < 0.05$).

TABLE 2 Effects of organic and inorganic manganese supplements on production performance of old laying hens

	Experimental diets† Mn-Sulph 4:Mn-MET (%)						
Production performance index	0 (Basal diet)	100:0	75:25	50:50	25:75	SEM	*p* Value
Feed intake (g)	107.30	107.57	107.64	107.79	107.95	0.148	0.71
Laying percentage	62.03[b]	63.33[b]	64.32[b]	69.00[a]	71.67[a]	1.108	<0.001
Egg weight (g)	65.17[c]	66.46[b]	66.42[b]	67.09[a,b]	68.09[a]	0.275	<0.001
Egg mass (g)	40.62[b]	42.10[b]	42.73[b]	46.29[a]	48.83[a]	0.804	<0.001
Feed Conversion ratio	2.64[a]	2.56[a]	2.52[a]	2.34[b]	2.23[b]	0.043	<0.001

†Basal diet, no manganese supplement; diet 1, basal diet + 100% manganese sulphate; diet 2, basal diet + 75% manganese sulphate + 25% organic manganese chelate; diet 3, basal diet + 50% manganese sulphate + 50% organic manganese chelate; diet 4, basal diet + 25% manganese sulphate + 75% organic manganese chelate.
[a,b,c] In each row, a statistically significant difference is found among the means with various letters ($p < 0.05$).
SEM, standard error of the mean.

TABLE 3 Effects of organic and inorganic manganese supplement with various concentrations on the antioxidant activity in old laying hens

	Experimental diets† Mn-Sulph:Mn-MET (%)						
Parameter	0 (Basal diet)	100:0	75:25	50:50	25:75	SEM	*p* Value
SOD (U/ml)	284.3[c]	201.7[e]	216.8[d]	341.9[b]	363.5[a]	9.24	0.0001
MDA§ (%)	100.0[a]	96.56[a]	93.80[a]	87.63[b]	85.30[b]	6.12	0.0001
DPPH scavenging activity (%)	71.50[b]	76.33[b]	82.50[a]	87.50[a]	88.33[a]	7.24	0.0001

†Basal diet, no manganese supplement; diet 1, basal diet + 100% manganese sulphate; diet 2, basal diet + 75% manganese sulphate + 25% organic manganese chelate; diet 3, basal diet + 50% manganese sulphate + 50% organic manganese chelate; diet 4, basal diet + 25% manganese sulphate + 75% organic manganese chelate.
[a,b,c,d] In each row, a statistically significant difference is found among the means with various letters ($p < 0.05$).
§MDA relative to the control (%)
SEM, standard error of the mean.

Based on the results about antioxidant properties of organic and inorganic Mn supplement in old laying hens (Table 3), MDA, DPPH scavenging activity and SOD levels were significantly influenced by the treatments ($p < 0.05$). Compared with the control, the hens receiving 50 and 75% organic Mn had lower MDA level ($p < 0.05$). The diets containing organic Mn resulted in significant increase in DPPH scavenging percentage compared with the control and 100% inorganic Mn based diet ($p < 0.05$). A significant higher SOD values were also detected in hens fed with 50 and 75% organic Mn compared with the other treatments ($p < 0.05$).

Table 4 summarises the immunological effects of organic and inorganic Mn supplement on old laying hens, by demonstrating a significant higher IgG and IgM level after receiving the experimental treatments, especially 100% Mn sulphate, compared with the control ($p < 0.05$).

The effects of using various concentrations of organic and inorganic Mn supplements on serum liver enzyme levels in old laying hens are presented in Table 5. The results revealed the effectiveness of the experimental treatments on aspartate AST, ALT and ALP ($p < 0.05$). Feeding 100% Mn sulphate and also 75% Mn chelate was led to the lowest AST value, while the minimum ALT concentration was observed in birds fed 50% organic + 50% inorganic Mn. However, supplementing diets with Mn either as inorganic or organic form, significantly reduced ALP level compared with the control ($p < 0.05$). The serum ALP values were also decreased with increasing the level of organic Mn chelate in the diet ($p < 0.05$).

Table 6 represents tissue Mn storage in the old laying hens fed the various levels of organic and inorganic Mn supplements. As demonstrated, a significant greater Mn content was found in the tibia of those receiving organic Mn chelate compared with other treatments ($p < 0.05$). Although the highest serum Mn concentration was detected in 75% organic Mn fed hens, more Mn was deposited in yolk and egg shell of hens fed the diet containing 50% organic Mn and 100% inorganic Mn, respectively. The Mn storage was significantly elevated in the liver of hens fed with organic and inorganic Mn supplements compared with the control ($p < 0.05$).

Table 7 shows the effect of Mn form on shell weight, thickness and breaking strength. The results showed that using Mn-Sulph and Mn-MET did not have a significant effect on shell weight, thickness and breaking strength ($p > 0.05$). However, as compared with the control group and 100% Mn sulphate-fed hens, a numerical increase was observed in the 75% organic supplement treatment ($p < 0.1$).

TABLE 4 Effects of organic and inorganic manganese supplement with different levels on IgG and IgM after injection SRBC in old laying hens

	Experimental diets† Mn-Sulph:Mn-MET (%)						
Immunological parameters	0 (Basal diet)	100:0	75:25	50:50	25:75	SEM	*p* Value
IgG (ng/ml)	162.6[d]	206.5[a]	157.3[e]	194.8[b]	177.3[c]	1.090	0.0001
IgM (ng/ml)	64.10[c]	83.60[a]	65.70[c]	81.80[a]	75.40[b]	0.779	0.0001

†Basal diet, no manganese supplement; diet 1, basal diet + 100% manganese sulphate; diet 2, basal diet + 75% manganese sulphate + 25% organic manganese chelate; diet 3, basal diet + 50% manganese sulphate + 50% organic manganese chelate; diet 4, basal diet + 25% manganese sulphate + 75% organic manganese chelate.

[a,b,c,d,e] In each row, a statistically significant difference is found among the means with various letters ($p < 0.05$).

SEM, standard error of the mean.

TABLE 5 Effects of the various concentrations of organic and inorganic manganese supplement on the serum liver enzyme levels of old laying hens

	Experimental diets† Mn-Sulph:Mn-MET (%)						
Parameter	0 (Basal diet)	100:0	75:25	50:50	25:75	SEM	*p* Value
AST (U/L)	224.60[c]	195.60[d]	252.40[a]	244.70[b]	196.80[d]	3.40	0.0001
ALT (U/L)	31.40[b]	42.00[a]	41.50[a]	26.90[c]	32.40[b]	0.871	0.0001
ALP (U/L)	1260.50[a]	981.40[b]	764.30[c]	436.90[d]	370.10[e]	47.68	0.0001

†Basal diet, no manganese supplement; diet 1, basal diet + 100% manganese sulphate; diet 2, basal diet + 75% manganese sulphate + 25% organic manganese chelate; diet 3, basal diet + 50% manganese sulphate + 50% organic manganese chelate; diet 4, basal diet + 25% manganese sulphate + 75% organic manganese chelate.

[a,b,c,d] In each row, a statistically significant difference is found among the means with various letters ($p < 0.05$).

SEM, standard error of the mean.

TABLE 6 Tissue manganese storage in the old laying hens fed organic and inorganic manganese supplement with different levels

	Experimental diets† Mn-Sulph:Mn-MET (%)						
Parameter	0 (Basal diet)	100:0	75:25	50:50	25:75	SEM	*p* Value
Bone Mn (mg/kg)	5.907[b]	6.391[b]	7.626[a]	8.117[a]	8.255[a]	0.160	<0.001
Yolk Mn mg/kg	0.472[c]	0.736[b]	0.532[c]	1.061[a]	0.692[b]	0.033	<0.001
Shell Mn (mg/kg)	0.937[c]	1.758[a]	1.003[c]	1.588[a,b]	1.323[b]	0.066	<0.001
Serum Mn (mg/dl)	0.546[d]	0.895[c]	1.506[b]	0.798[c]	1.795[a]	0.070	<0.001
Liver Mn (mg/kg)	1.935[c]	3.243[b]	3.956[a,b]	4.340[a]	4.264[a]	0.181	<0.001

†Basal diet, no manganese supplement; diet 1, basal diet + 100% manganese sulphate; diet 2, basal diet + 75% manganese sulphate + 25% organic manganese chelate; diet 3, basal diet + 50% manganese sulphate + 50% organic manganese chelate; diet 4, basal diet + 25% manganese sulphate + 75% organic manganese chelate.

[a,b,c,d] In each column, a statistically significant difference is found among the means with various letters ($p < 0.05$).

SEM, standard error of the mean.

4 | DISCUSSION

The results of the present study indicated that egg weight and feed conversion ratio were improved after adding Mn compared with the control diet. Ao et al. (2009) reported that inorganic supplement can maintain or increase laying hen performance, which is consistent with the results herein. Some researchers found that growth and feed conversion ratio were improved as an effect of Mn intake (Jasek et al., 2019). In general, the dietary requirement for Mn depends on the physiological state of the bird (Gheisari et al., 2011). In our experiment, the basal diet contained 29.01 mg/kg Mn which is approximately higher than those considered to cause a deficiency recommended by NRC (1994) for laying hens (17–23 mg/kg).

There was a significant difference between experimental and basal diets in case of egg weight ($p < 0.05$). Manangi et al. (2015) showed that birds fed chelated minerals at 40–10–40 (Zn–Cu–Mn) had a lower egg weight than those fed the chelates at 20–5–20 ($p < 0.05$). Similar variations in egg weight with organic TMs have been reported

TABLE 7 Effect of feeding supplemental inorganic and organic manganese on eggshell quality in old laying hens

Parameter	Experimental diets Mn-Sulph:Mn-MET (%)					SEM	*p* Value
	0 (Basal diet)	100:0	75:25	50:50	25:75		
Egg weight (g)	65.16[c]	66.46[b]	66.41[b]	67.09[a,b]	68.09[a]	0.275	<0.001
Shell thickness (mm)	0.25	0.26	0.27	0.27	0.29	0.011	0.09
Shell strength (k/cm^2)	2.11	1.93	2.56	1.94	2.86	0.319	0.19

Basal diet, no manganese supplement; diet 1, basal diet + 100% manganese sulphate; diet 2, basal diet + 75% manganese sulphate + 25% organic manganese chelate; diet 3, basal diet + 50% manganese sulphate + 50% organic manganese chelate; diet 4, basal diet + 25% manganese sulphate + 75% organic manganese chelate.

[a,b,c]Means in each row with different superscripts are significantly different ($p < 0.05$).

SEM, standard error of means.

previously (Lim and Paik, 2003; Mabe et al, 2003; Lim and Paik, 2006). The inclusion of organic Mn supplement into laying hens diet increased their egg and body weight during 49–61 weeks of age (Yıldız et al., 2011). Ghalesefidi et al. (2019) reported a significant rise in egg mass following the treatment with 70 and 90 mg of Mn hydroxychloride. In addition, the hens fed organic zinc and Mn sources increased feed intake, and egg weight and shape index compared with those received inorganic sources (Darvishi et al., 2020). According to Cui et al. (2019), the dietary supplementation with amino acid-complexed Mn leads to a linear and quadratic improvement in the egg mass of 23–46-week-old laying hens. Furthermore, feeding 40 and 80 mg/kg Mn resulted in enhancing egg production and declining feed conversion ratio, while no benefit was obtained at the values above 120 mg/kg (Cui et al., 2019).

The addition of Mn supplement into poultry diet diminishes the content of lipid peroxidation products and promotes antioxidant enzyme activity (Bai et al., 2014; Bozkurt et al., 2015). Zhang et al. (2020) reported that the use of Mn supplement is linearly and quadratically associated with decreasing MDA levels in laying duck ($p < 0.001$). The poultry received a diet containing 100–400 mg/kg of Mn sulphate supplement exhibit a diminution in lipoprotein lipase activity and an elevation in the Mn SOD (MnSOD) activity, and consequently a decline in MDA value (Lu et al., 2006). Cui et al. (2019) reported that dietary supplementation of 40 or 80 mg/kg Mn increased total SOD activity in serum of laying hens ($p < 0.05$). In addition, Mn is a component of the MnSOD which is a primary antioxidant enzyme protecting cells against oxidative stress, and increasing antioxidant ability to remove reactive oxygen species and reduce lipid peroxidation in poultry (Zhu et al., 2017).

The results of the present study are in line with those demonstrated an improved immune response of broilers at 35 days of age after adding Mn with 120 ppm concentration into diet (Gajula et al., 2011). Some researchers confirmed the important role of Mn in supporting the normal functions of poultry immune system (e.g., Lu et al., 2006; Gajula et al., 2011; Junior et al., 2019). Additionally, Mn supplementation results in promoting antibody titre to SRBC and improving the cell-mediated immunity of basophil sensitivity to plant lectins (Gajula et al., 2011; Pan et al., 2018). This improvement in immune-competence may be ascribed to Mn contribution to MnSOD activity (Luo et al., 2007), which is vital for macrophage integrity (Ghosh et al., 2016). Moreover, this element elevates phagocytic ability (Son et al., 2007). In general, Mn can be described as a mineral related to immunity or immunity-supporting functions (Kidd, 2004). Similar to our results, Zhang et al. (2021) reported that IgG and IgM concentrations in serum were not significantly different between 100% inorganic TMs (Cu, Zn, Mn and Fe in sulphate form) and 50% organic minerals (MET chelated form) in aged laying hens, while 20 and 30% organic treatments decreased the content of Igs G and M. However, other studies have shown an increase in IgG response in laying hens (Manangi et al., 2015) and male turkeys (Ferket and Qureshi, 1992) fed chelates when compared with birds fed inorganic mineral supplement sources. The results from the current study provide further evidence as to the importance of Mn in ensuring the health and productivity of laying hens and also the impact of mineral source on this process.

According to Bahar et al. (2017), ALT and AST level significantly raised in rat serum after feeding Mn. Excess Mn accumulated in the liver and caused liver damage which consequently increased AST and ALT values in serum of Mn exposed hens (1800 mg/kg) (Jiang et al., 2020). In agreement with our results, Banakar Moghadam et al. (2019) reported a significant reduction in the liver enzyme level of broilers by applying the diet containing organic Mn supplement (100 mg/kg) compared with the other treatments such as those with inorganic one. The activities of AST and ALT were increased in hens fed 100 and 75% inorganic Mn supplementation which could represent a higher load on the liver cells; however, these values were within the normal range for Leghorn (Guerrini et al., 2022) and Backyard hens (Apruzzese ei al., 2018). On the other hand, laying intensity can significantly affect liver function as Goncalves et al. (2010) reported higher AST concentration during the peak of egg production compared with the pre-peak. Therefore, our results may point out that in spite of higher laying percentage and liver Mn deposition, inclusion of 75% organic Mn can mediate liver function. Bai et al. (2012) found higher uptake of organic Mn sources than the inorganic ones. Furthermore, bone and liver Mn concentrations are more sensitive to the nutritional changes in Mn, which can be utilised to analyse bioavailability (Berta et al. 2004). Another study revealed that the 8-week-old laying chicks receiving 80 ppm of organic Mn supplement + 80 ppm of Mn–MET chelate have a significant greater tibial Mn content compared with the other treatments (Das et al., 2014).

Mwangi et al. (2019) referred to an enhanced Mn value in tibia and liver following Mn supplementation to diet, regardless of the applied supplement source (sulphate and protein).

A trend ($p < 0.1$) for thicker eggshell in laying hens received the diet supplemented with 75% Mn-AA compared with those received Mn-Sulph may indicate higher bioavailability of Mn to be used as a component of metalloenzymes responsible for the synthesis of mucopolysaccharides that play an important role in eggshell formation (Swiatkiewicz and Koreleski 2008). In addition, the better intestinal absorption of organic TMs complexed to amino acid via epithelium transporters (Yi et al. 2007) would mediated the Mn uptake by late-phase laying hens. Sun et al. (2010) also observed an improvement in the thickness of the eggshell with the addition of organic Mn. The Mn supplementation can improve the expression of genes encoding proteoglycans and glycoproteins in the eggshell gland, thus increasing the mammillary-knob density during the initial deposition stage of shell formation (Zhang et al., 2018). The improvement in eggshell quality after organic zinc and/or Mn supplementation has already been reported by Xiao et al. (2014) and Zhang et al. (2017). In experiments with late-phase laying hens of 80 weeks of age, Nascimento et al. (2014) found improvement in eggshell quality in birds fed diets supplemented with vitamin D3. Wang et al. (2021) also observed an increase in the thickness of the eggshell when laying hens housed at a high density were supplemented with 25-OH-D3.

5 | CONCLUSION

The results of the present study indicated that, compared with the control group, addition of Mn supplement either as inorganic or organic compounds into the diet improved egg weight, immune response and Mn tissue deposition. However, compared with Mn-Sulph supplement, inclusion of 50 and 75% Mn–MET complex improved production performance, and feed conversion ratio along with improving in antioxidant activity, immune system and liver function. These positive effects was most marked in hens fed 75% organic Mn. In general, organic Mn is suggested as a dietary supplement in old laying hens.

AUTHOR CONTRIBUTION

Study concept and design, *drafting of the manuscript*, *critical revision of the manuscript for important intellectual content*, *study supervision and administrative*, *technical and material support*: Reza Vakili. *Acquisition of data and statistical analysis*: Mohammad Reza Khoshbin. *Analysis and interpretation of data*: Mansour Tahmasbi.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the author initials, upon reasonable request.

ETHICS STATEMENT

We hereby declare all ethical standards have been respected in preparation of the submitted article.

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