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Effect of Different Dietary Supplementation Forms of Zinc and Manganese on their Concentration and Some Parameters of Tibia and Liver of Laying Hens

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Abstract

This study was carried out to determine the effect of different forms of Zn and Mn on mineral concentrations and some parameters of tibia and liver in laying hens. 162 LSL laying hens 30-weeks old were randomly allocated to nine dietary treatments, each of six replicates (3 birds each). Birds were distributed in a factorial design of (3 zinc forms × 3 manganese forms: inorganic, organic, and nanoparticles), birds were fed until the age of 46 weeks. Inorganic forms of Zn and Mn were supplemented at 60 mg/kg and 100 mg/kg, respectively, while, organic and nano forms were supplemented at 50% of the inorganic level. The results indicated that the main effect of dietary Zn-NPs reduced tibia weight ($P< 0.01$), and tibia Zn concentration ($P< 0.01$), while Zn concentration was higher ($P< 0.05$) in the liver when birds were fed Zn-NPs. The main effect of Mn forms had no effect on all tibia and liver parameters. However, the interaction between Zn and Mn forms showed that feeding Zn-Met with Mn-NPs significantly reduced ($P< 0.01$) tibia Zn content. Also, feeding Zn-NPs with Mn-oxide or Zn-NPs with Mn-Met led to a significant increase ($P< 0.05$) in liver Zn content. The interactions had no effect on tibia length or tibia ash percent. However, the interaction had a significant effect on tibia weight ($P< 0.05$). In conclusion, replacing the inorganic form of dietary Zn and Mn with Nano forms at 50% of the inorganic form can highly increase Zn concentration in the liver.

Keywords: Zinc, Manganese, Inorganic, Nanoparticles, Organic, Tibia, Liver, laying hen

INTRODUCTION

Before the 20th century, the widespread use of mineral supplements was not common (Schaible, 1941). Extensive researches on poultry's trace mineral requirements ensued, and it became evident that understanding the absolute amounts of minerals was insufficient. Consequently, studies were conducted to explore potential interrelationships among minerals, their absorption percentage in the body, and their impact on other biological functions. This led to significant advancements in the field of trace elements in poultry nutrition.

Two main types of sources can be supplemented to enhance trace elements: inorganic and organic sources (AAFCO, 1998). Inorganic minerals may potentially interact with various substances in the gastrointestinal tract, such as fiber, tannin, phytate, oxalate, silicates, or other minerals, leading to potential absorption interference. The enhanced bioavailability of organic minerals is attributed to their protection against such interactions. Binding minerals to chelating agents like amino acids or hydrolyzed proteins renders them more stable and less reactive within the digestive tract. In addition, the primary advantage of employing organic minerals stems from their enhanced absorption, utilizing the same absorption pathways as the amino acids to which they are attached. This reduces competition for binding sites with inorganic trace minerals, consequently decreasing the excretion of these minerals through bile and feces (Stefanello *et al*., 2014; Singh *et al*., 2015). Consequently, the widespread adoption of organic minerals at relatively low levels in poultry diets is notable, driven by their ecological and physiological benefits.

On the other hand, nanomineral forms exhibit elevated surface activity and possess the capacity to infiltrate cells, actively affecting intracellular metabolism by stimulating diverse processes (Nikonov *et al*., 2012). This characteristic holds significance as it enhances the absorption of trace minerals, especially those with low bioavailability. Furthermore, minerals in nanoparticle form alleviate intestinal mineral antagonism, consequently diminishing excretion and mitigating environmental pollution (Marappan *et al*., 2017). Nevertheless, the effect of nanoparticles on health and the environment is yet another field of concern. Davies (2009) elucidated that the implementation of nanotechnology in animal nutrition necessitates careful consideration in terms of risk analysis, regulatory policies, and oversight.

Cufadar *et al*., (2019) concluded that no significant effects on tibia Zn content and tibia weight by Zn sources, however, liver Zn content was increased significantly by feeding on nano Zn compared to those fed inorganic or organic Zn. Also, Abedini *et al*., (2018) found that Zn content of tibia and liver was higher when the birds fed Zn-methionine and ZnO Nanoparticles as compared to the Zn-oxide group. On the contrary, studies of Tüzün *et al*., (2018) on Japanese laying quail and Olgun and Yildiz, (2017) on Lohmann LSL-Lite laying hens, found that regardless of the other Zn forms, nano Zn had no effect on bones' Zn content. Tsai et al. (2016) observed a noteworthy increase in zinc retention in both the nano-Zn and organic-Zn groups compared to the control and ZnO groups.

There is a scarcity of literature addressing the potential impacts of manganese nanoparticles on Zn retention in the body of laying hens, even studies conducted on other species observed that Mn content of turkeys' liver, breast, and skin (Jankowski *et al*., 2019) and broilers bones (Lotfi *et al*., 2014) were not affected by dietary Mn nanoparticles. While, Lotfi *et al*., (2014) indicated that tibia length increased significantly by feeding on nano manganese sulfate compared to micro-Mn form in broilers, they added that a comparison of dry tibia weight indicated that the bone weight increased significantly by feeding nano Mn supplementation instead of micro-Mn. Recently, Matuszewski *et al*., (2020) noted that the length, diameter, and weight-breaking strength of the femur did not differ between Mn forms treatments in Ross broiler chicks.

Therefore, the aim of this study was to determine the effects of different Zn and Mn forms on mineral concentrations and some parameters of tibia and liver in laying hens.

MATERIALS AND METHODS

1. Birds

One hundred and sixty-two laying hens 30-weeks old with almost equal body weight (1674 ± 16 gram) were assigned randomly to nine dietary

treatments, with each comprising six replicates, and each replicate of three birds. Birds were housed in three-tiered three-dimensional cages 45*50*45 cm (3 birds/cage) and were fed the nine treatments diets until the age of 46 weeks.
Birds were reared during the experimental period (16 weeks) under standard conditions according to the conventional standards of LSL classic strain with respect to temperature, humidity, ventilation, lighting, hygiene, feeding and drinking systems.

2. Diets and experimental design

The control treatment (T1) was fed a basal (corn-soybean meal-based diet). The diet formulated according to the recommendations of NRC (1994) to meet the daily nutrient requirement of laying hens at their peak period. The basal diet was supplemented with minerals and vitamins premix free of zinc and manganese. The composition and nutrient levels of the basal diet are outlined in Table 1.

The birds were allocated in a factorial design of 3 zinc forms by 3 manganese forms, and were fed the nine treatments until the age of 46 weeks. Zinc and Manganese supplementation forms were:(1) inorganic form (ZnO and Mn_2O_3). According to NRC (1994), ZnO and Mn_2O_3were supplemented to the basal diet at 60 mg/kg, and 100 mg/kg, respectively. (2) Organic form (Zn-methionine and Mn-methionine). (3) Nano sized minerals form (ZnO nanoparticles and Mn2O3 nanoparticles). Organic and nano forms of Zn and Mn were supplemented to the basal diet at 50% of the inorganic level (50 mg Mn/kg and 30 mg Zn/kg). The nine treatments were as shown in (Table 2).

Table 1. Composition of the basal diet.

Ingredients	%	Calculated analysis	%
Corn	55	ME (Kcal/Kg)	2750
Soybean meal (46%)	16.2	Crude protein %	16.49
Gluten	4.5	Crude fiber %	3.45
Wheat bran	9.54	Ether Extract %	2.62
Limestone	9.5	Lysine %	0.70
Mono-calcium phosphate	1.5	Methionine %	0.37
Soybean oil	2.9	Methionine + Cysteine %	0.65
NaCl	0.3	Calcium %	3.93
DL-Methionine	0.08	Total Phosphorus %	0.70
L-Lysine	0.02	Non phaytate phosphorus %	0.43
Choline chloride	0.09	Zinc (mg/kg)	24
Anti-Mycotoxin	0.05	Manganese (mg/kg)	30
Vitamins and minerals premix1	0.32		
Total	100		

[1]The premix free of zinc and manganese provided the following per 3 kg of the diet: vitamin A, 10,000,000 IU; vitamin E, 10,000 mg; vitamin D3, 3,000,000 IU; vitamin K3, 2000 mg; vitamin B2, 5,000 mg; vitamin B1, 1,000 mg; vitamin B12, 10 mg; vitamin B6, 1,500 mg; Biotin, 50 mg; Niacin, 30,000 mg; pantothenic acid, 10,000 mg; folic acid, 1,000 mg; iron, 30,000 mg; copper, 4,000 mg; iodine, 1,000 mg; sulfur, 100 mg; selenium, 100 mg.

Table 2. The experimental design

Treatment	Zinc		Manganese	
T1 (Control)	60 mg/kg	Zn oxide	100 mg/kg	Mn oxide
T2	60 mg/kg	Zn oxide	50 mg/kg	Mn methionine
T3	60 mg/kg	Zn oxide	50 mg/kg	Mn nano
T4	30 mg/kg	Zn methionine	100 mg/kg	Mn oxide
T5	30 mg/kg	Zn methionine	50 mg/kg	Mn methionine
T6	30 mg/kg	Zn methionine	50 mg/kg	Mn nano
T7	30 mg/kg	Zn nano	100 mg/kg	Mn oxide
T8	30 mg/kg	Zn nano	50 mg/kg	Mn methionine
T9	30 mg/kg	Zn nano	50 mg/kg	Mn nano

3. Criteria Studied

At the end of the experiment (46 weeks), one bird from each replicate (54 birds) were slaughtered. Liver and right tibia were separated, weighed, measured, and then tibia was manually cleaned from adhering tissue with a stainless blade. Liver was frozen (−20°C) until analysis.

Tibia and liver samples weighed and dried at 70 °C for 72h and dried weights were recorded. Then the samples were milled and burned at 600°C for 2 h and ash weights were recorded.

Ash samples were solubilized (0.2 gram of liver or tibia) in 20 ml HCl (37%) on heater 200 °C and gradually increased to 300 °C for 1 hour. The solution was transferred after filtration to a volumetric flask and it was completed to 100 ml with deionized water. Zn and Mn concentration were measured by atomic absorption spectrophotometry (model pinnacle AAS 900t).

4. Statistical analysis:

Data were statistically analyzed by the completely randomized design using the general linear model's procedure of SAS (1998). A factorial design 3x3 was used, considering the Zn forms and Mn forms as the main effects, as follows:

$$Yijk = \mu + Si + Lj + (S*L)\ ij + eijk.$$

Where, Yijk = an observation; μ = general mean; Si = fixed effect of ith Zn forms, i = 1 & 2 & 3 (oxide, organic or nanoparticles); Lj = fixed effect of jth Mn forms,j = 1 & 2 & 3(oxide, organic and nanoparticles); S*Lij = interaction effect of ith Zn forms and jth Mn forms; eijk = error of the model, which included all the other effects not specified in the mixed model.

Differences among means of the experimental groups were tested for significance by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Zn and Mn concentration in liver and tibia bone

The effects of different dietary forms of Zn and Mn and their interaction on their concentration in liver and tibia of layers after 16 weeks of feeding trial are presented in Table (3).

1.1. Zinc concentration

There were significant effects among Zn forms on Zn content of tibia and liver. Zinc concentration was significantly higher (P< 0.01) in the liver when birds were fed diets supplemented with Zn-NPs compared to those fed Zn oxide or Zn-Met. This may be because the active ingredients of feed additives in nanoform have high surface activity and cell penetration ability and can effectively influence intracellular metabolism by stimulating various processes (Nikonov *et al*., 2012). This finding is in agreement with the findings of (Cufadar *et al*., 2019; Abedini *et al*., 2018; Zhao *et al*., 2016) who reported that a significant increase in Zn concentration of liver due to feeding Zn-NPs compared to other forms in laying hens (Cufadar *et al*., 2019; Abedini *et al*., 2018; Zhao *et al*., 2016). While, in other studies, Zn forms had no effect on Zn content of liver (Ramiah *et al*., 2019; Ibrahim *et al*., 2017).

However, it was noticeable in the current study that tibia Zn content was higher (P< 0.01) in the treatment that fed Zn oxide (68.7 mg/kg) followed by those fed Zn-NPS (52.6 mg/kg) compared to those fed Zn-Met (41.5 mg/kg). This may be due to lowering the dietary level of the organic forms and nano forms to 50% of the level of the inorganic form. On the contrary, other studies reported that dietary Zn forms did not have a significant effect on tibia Zn content in laying hens (Cufadar *et al*., 2019; Olgun and Yildiz, 2017), while in broilers, Attia, (2020) found that low levels of Zn-NPs increased significantly Zn tibia content compared to high levels of Zn-NPs and control group (100 mg Zn oxide). Also, Ibrahim *et al*., (2017) and Mohammadi *et al*., (2015) found that feeding Zn-NPs, organic Zn or mixing them up significantly increased tibia Zn content compared to inorganic Zn.

On the other hand, Mn forms did not have any significant effect (P> 0.05) on Zn content or Mn content in liver and tibia.

The interactions between Zn forms and Mn forms showed significant differences in Zn concentration of tibia and liver. Feeding Zn-Met with Mn-NPs significantly reduced (P< 0.01) tibia Zn content compared to feeding Zn oxide with Mn-NPs.

Referring to liver Zn content, it was noticeable that feeding Zn-NPs with Mn oxide or Zn-NPs with Mn-Met resulted in a significant increase ($P< 0.05$) in Zn content compared to the other treatments.

1.2. Manganese concentration

The current study showed that Mn concentration in liver and tibia bone was not affected by Mn forms or Zn forms. This result reveals the superiority of the organic or nano form over the inorganic form since they were used at only 50% of the level of the inorganic form. Similar results were recorded that Mn forms did not affect Mn and Zn content in the liver of turkeys (Jankowski *et al*., 2019) and broilers (Matuszewski *et al*., 2020). Moreover, Lotfi *et al*. (2014) stated that Mn different forms (Nano or Micro) didn't significantly affect Mn content of tibia in broilers. Interaction between Zn forms and Mn forms, in the current study, showed that feeding a combination of Zn-NPs with Mn oxide led to a significant increase of Mn content in the liver compared to feeding Zn-Met with -Mn-NPs. while interaction had no effect on Mn content in the tibia bone.

Table 3. Zinc and manganese concentration in liver and tibia bone as affected by different dietary supplementation forms of Zn and Mn and their interaction

Concentration Treatment	Zn concentration		Mn concentration	
	Tibia	Liver	Tibia	Liver
	mg/kg	mg/kg	mg/kg	mg/kg
Effect of Zinc				
ZnO	68.7^{a}	41.9^{b}	41.4	20.6
Zn-Met	41.5^{c}	38.2^{b}	53.6	16.6
Zn-NPs	52.6^{b}	100.7^{a}	43.9	26.1
SEM ±	4.2	± 7.79	±5.5	± 3.0
P-Value	**	**	NS	NS
Effect of Manganese				
MnO	51.5	68.8	48.4	26.1
Mn-Met	52.3	60.0	36.2	21.2
Mn-NPs	59.1	52.1	54.3	15.9
SEM ±	4.2	± 7.79	5.5	± 3.0
P-Value	NS	NS	NS	NS
Interaction				
ZnO×MnO	57.3^{bcd}	56.6^{b}	30.7	20.5^{bc}
ZnO×MnMet	70.1^{abc}	28.9^{b}	47.2	16.5^{bc}
ZnO×MnNPs	78.6^{a}	40.1^{b}	46.2	24.7^{b}
ZnMet×MnO	51.6^{cd}	27.1^{b}	61.4	16.9^{bc}
ZnMet×MnMet	43.7^{de}	37.2^{b}	35.2	22.9^{bc}
ZnMet×MnNPs	29.2^{e}	50.3^{b}	64.2	9.91^{c}
ZnNPs×MnO	45.4^{de}	122.7^{a}	53.3	41.0^{a}
ZnNPs×MnMet	42.9^{de}	113.7^{a}	26.0	24.2^{bc}
ZnNPs×MnNPs	69.6^{abc}	65.9^{b}	52.4	13.2^{bc}
SEM ±	± 7.5	± 13.48	± 8.7	± 5.03
P-Value	**	*	NS	*

^{abcde} Means within the same column with distinct superscripts are considered significantly different at the 0.05 level or 0.01 level of significance.

2. Tibia bone and liver parameters

Table (4) represents the effect of feeding different forms of Zn and Mn and their interaction on tibia length, tibia and liver weights (g) and ash percent (%) after 16 weeks of starting the feeding trial.

In the current study, with only 50% of the level of the inorganic form, as either organic or nano forms, the main effect of Zn forms showed that dietary Zn-NPs significantly increased (P< 0.01) tibia weight compared to Zn-Met or Zn oxide. In addition, tibia ash percent was increased significantly (P< 0.01) by feeding hens either Zn-NPs or Zn-Met compared to Zn oxide. However, Zn forms did not affect tibia length. On the contrary, Cufadar *et al*, (2019) found that Zn forms (Zn oxide, Zn proteinate or Zn-NPs) did not affect the tibia weight in H&N Super Nick laying hens.

Moreover, no significant effect (P> 0.05) was detected for Mn forms on tibia weight, length or ash percent.

The interactions between Zn and Mn forms did not show significant differences (P> 0.05) in both tibia length or tibia ash percent. However, tibia weights were significantly influenced by the interactions between Zn and Mn forms (P<0.05). Feeding hens Zn-Met with Mn-Met, Zn-Met with Mn oxide or Zn oxide with Mn-Met recorded higher (P< 0.05) tibia weights (6.25, 6.13 and 6.12 g; respectively) compared to feeding Zn-NPs with Mn oxide (5.16 g). Swiatkiewicz and Koreleski, (2008) concluded that Zn and Mn oxide substitution with amino acid complexes of Zn and Mn had no effect on geometrical parameters of tibia and their ash content. Few studies have been carried out on the effect of dietary forms of Zn and Mn on tibia parameters in laying hens.

Referring to liver parameters, the main effect of Zn and Mn forms and their interaction did not show any significant influence (P> 0.05) on liver weight or ash percent. The difference between studies may be attributed to the difference in dietary Zn and Mn levels, their sources or periods of feeding.

Table 4. Tibia and liver weights (g), ash content (%) and tibia length (mm) as affected by different forms of Zn and Mn and their interaction

Concentration Treatment	Tibia bone			Liver	
	Weight (g)	Length (mm)	Ash %	Weight (g)	Ash %
Effect of Zinc					
ZnO	6.04^{a}	116.57	44.72^{b}	32.04	11.15
Zn-Met	5.98^{a}	117.50	49.14^{a}	31.42	11.98
Zn-NPs	5.54^{b}	116.22	48.71^{a}	31.98	11.88
SEM ±	0.11	0.76	±0.87	±1.117	±0.26
P-Value	**	NS	**	NS	NS
Effect of Manganese					
MnO	5.96	117.17	47.35	32.04	12.02
Mn-Met	5.85	116.94	48.03	31.42	11.79
Mn-NPs	5.74	116.17	47.19	31.98	11.21
SEM ±	±0.11	±0.69	±0.84	±1.117	±0.26
P-Value	NS	NS	NS	NS	NS
Interaction					
ZnO×MnO	5.97^{ab}	117.23	44.02	33.7	11.95
ZnO×MnMet	6.12^{a}	116.61	43.86	30.47	11.55
ZnO×MnNPs	6.04^{ab}	115.86	46.28	32.0	9.96
ZnMet×MnO	6.13^{a}	118.22	49.01	30.03	11.84
ZnMet×MnMet	6.25^{a}	117.67	50.04	30.43	12.05
ZnMet×MnNPs	5.57^{bc}	116.62	48.38	33.78	12.06
ZnNPs×MnO	5.16^{c}	116.05	49.01	32.8	12.28
ZnNPs×MnMet	5.52^{bc}	116.56	50.2	29.87	11.77
ZnNPs×MnNPs	5.95^{ab}	116.04	46.92	33.28	11.60
SEM ±	±0.19	±1.38	±1.15	±1.935	±0.43
P-Value	*	NS	NS	NS	NS

^{abcde} Means within the same column with distinct superscripts are considered significantly different at the 0.05 level or 0.01 level of significance.

CONCLUSIONS

Replacing the inorganic form of dietary Zn and Mn with Nano forms and lowering the level of the nano forms to 50% of the level of the inorganic form can highly increase Zn concentration in the liver and significantly decrease its concentration in tibia bone, and reduce tibia weight. However, Mn forms did not affect the characteristics of the liver and tibia bone. Furthermore, the interaction between Zn forms and Mn forms did not show consistent trends on the studied parameters of the liver and tibia bone. Thus, the implementation of nanotechnology in poultry nutrition necessitates careful consideration in terms of risk analysis, regulatory policies, and oversight.

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