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Effects of nano-manganese on humoral immune response and oxidative stress in broilers

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Abstract

The objective of the present study was to evaluate the alterations in selected indicators of immune responses and oxidative stress of broilers fed with nano-manganese. One hundred-sixty 1-day-old broiler chicks were randomly assigned into four groups with three replicates. Birds were fed the same basal diet supplemented with nano-manganese oxide, as 0.00 (control group), 50.00, 100, or 150 mg kg^{-1} of diet. The birds were vaccinated against avian influenza (AI), Newcastle disease (ND), infectious bronchitis (IB) and infectious bursal disease (IBD) as the standard vaccination schedule. Blood sample was taken from the brachial vein of birds on 42th day. A significant decrease in antibody titer against sheep RBC was revealed in the nano-manganese 100 and 150 groups compared to the control group. In addition, the antibody titers against IB and ND were significantly lower in the all nano-manganese groups compared to the control group. No significant difference was observed for the antibody titer against AI and oxidative stress indices among the experimental groups. The findings in the present study suggested that nano-manganese at 50.00, 100 and 150 mg kg^{-1} levels might suppress humoral immune response in broilers which should be taken into consideration in supplementation.

Introduction

Among the trace minerals commonly used in poultry, manganese is of particular interest in broilers because of its essential roles in metabolism, skeletal growth, enzyme activities and immune responses[1]. Due to its nutritional significance, NRC recommended 60.00 ppm of manganese for broilers.[2] Manganese is important for sustained activity of superoxide dismutase which is vital for antimicrobial function of immune cells. Therefore, higher levels of manganese are supplemented in diets for enhancing immune response in broiler.[1] However, like all essential trace elements, manganese can be toxic when provided at levels in excess of the biological requirement. Fortification of diets with excess manganese could result in generation of free radicals, inactivation of antioxidant enzymes, impairment of immune response and antagonism affecting other trace elements bioavailability.[3] Thus, using high levels of manganese in diet may mask the advantages of supplementation. Indeed, based on the level of supplementation in the diet, manganese could result in beneficial or detrimental effects on immune function, antioxidative defense, mineral uptake and performance of broiler.[1,4-6]

With the recent development of nanotechnology, nano-trace elements have attracted widespread attention because nanometer particulates exhibit novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability, and low toxicity.[7,8] The high activity and efficiency of nano-trace elements could act as a double-edged sword and nano- trace elements can be toxic when the provided levels exceed biological requirements. For example, it has been reported that a high level of nano-selenium supplementation decreased the immune response and antioxidant activity in broilers. Hence, it is important to investigate the possible effects of nano-trace elements at different levels.[9] On a study performed by Lotfi *et al.* effects of dietary nano- and micro- manganese on growth, performance and bone characteristics of broilers were investigated. Broiler chickens were assigned into different groups, each group were given a diet having a different

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concentration of nano- and micro- manganese (20.00 - 170 mg kg^{-1}) for 35 days. The authors stated that supplementation of nano-manganese could result in better growth and bone characteristics compared to the control and micro-manganese groups.[10] Immunization in birds should be sufficient to mount an effective immune response, however, not too much to avoid pathological side-effects caused by an elevated immune response. One of the most important factors that might modulate the effectiveness of immune response is the oxidative stress that is potentially associated with the immune response itself.[11] Immune cells are vital components of the immune response that kill pathogens by oxygen- dependent and oxygen- independent mechanisms. Oxygen- dependent mechanisms are initiated by the process of phagocytosis or by perturbation of the cell membrane and are dependent on a membrane- bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. This enzyme complex is responsible for the production of reactive oxygen species (ROS) after immune stimulation.[11,12]

ROS play a key role in host defense against pathogens, however, when generated at high levels they can result in oxidative damage.[13,14] An excessive production of ROS during the immune response can result in oxidative stress if not effectively counteracted by the organism's antioxidant defenses. ROS can elicit widespread damage to cells and macromolecules such as enzymes and polyunsaturated membrane lipids. In this regard, administration of antioxidants might be beneficial to limit the oxidative damage.[15-17] Birds exploit a wide range of enzymatic and non-enzymatic substances to cope with oxidants. Micronutrients are antioxidants that have received much attention in birds.[2,3] Trace elements such as zinc, copper, selenium and manganese are also essential components of the body antioxidant defense that play an important role in the prevention of free-radical-induced damage to tissues for maintenance of health and production.[18]

However, no study has been reported to investigate the effects of nano-manganese supplementation on oxidative stress and immune response in broiler chickens. Therefore, the aim of the present study was to evaluate the effect of supplementing nano-manganese at different levels (50.00, 100 and 150 mg kg^{-1}) on immune response and oxidative stress in broilers.

Materials and Methods

Birds, groups and sampling. A total number of 160 1-day-old broiler chicks (Ross 308) were randomly assigned into four groups with three replicates, 13 or 14 birds per replicate. Birds were fed the same basal diet supplemented with nano-manganese oxide, as 0.00 (control group), 50.00, 100, or 150 mg kg^{-1} of diet. To meet the nutrient requirements of the chickens during the experimental period (1-42 days), typical corn-soybean meal-based diets were formulated to meet NRC requirements (Table 1).[2] The size of the nano-manganese (Merck, Darmstadt, Germany) was measured by Particle Size Analyzer (Vasco3; Cordouan Technologies, Pessac, France). The size of the nano-manganese was 30.00 to 150 nm, and the median size was 76.00 nm. The temperature was maintained at 33.00 ± 1.00 ˚C which was gradually decreased 2.00 ˚C each week until reaching 20.00 ˚C and kept at that level. A 24-hr light regimen was conducted throughout the 42-day trial. The birds were vaccinated against avian influenza (AI), Newcastle disease (ND), infectious bronchitis (IB) and infectious bursal disease (IBD), based on the standard vaccination program. Blood sample was collected from the brachial vein of birds on 42th day. Approximately 5.00 mL of blood without anticoagulant was centrifuged at 1,800 *g* for 15 min. Serum was collected and stored at −20.00 ˚C until analysis.

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad (No. 3/45087, date 10/15/2017).

Immune responses. The effect of supplementing of nano-manganese on humoral immune responses were studied via measuring the antibody titers against AI, ND and IB using ELISA (Idexx, Westbrook, USA) and hemagglutination inhibition (HI) assays. In addition, sheep red blood cells (sRBC), a non-pathogenic antigen, were used for evaluating the humoral immune response in broiler chickens. The broilers from each dietary group were injected intramuscularly with 0.20 mL of 10.00% sRBC on the 28th day and blood samples were collected at the end of the experiment (day 42).

Table 1. Composition and nutrient levels of basal diets.

Items	Starter	Finisher
Ingredient (%)		
Corn	50.54	63.17
Soybean meal	42.00	31.00
Soybean oil	3.00	2.00
Dicalcium phosphate	1.80	1.40
Calcium carbonate	1.36	1.13
NaCl	0.30	0.30
Premix[1]	1.00	1.00
Composition[2] (%)		
Protein (%)	22.50	18.50
Calcium (%)	0.90	0.85
Phosphorus (%)	0.45	0.42
Sodium (%)	0.17	0.17
Lysine	1.25	1.15
Methionine	0.55	0.45
Metabolizable energy (kcal kg^{-1})	3,100	3,050

[1] The premix provided the following per kilogram of diet: Cholecalciferol, 5,000 IU; vitamin A, 24,000 IU; vitamin E, 13.00, IU; vitamin K, 2.50 IU; vitamin B1, 1.00 mg; Riboflavin, 75 mg; Pyridoxine, 3.00 mg; vitamin B12, 15.00 µg; folic acid, 250 µg; nicotinic acid, 17.50 mg; calcium pantothenate, 12.50 mg; Mn, 60.00 mg; Cu, 13 mg; Zn, 40.00 mg; Se, 0.15 mg and Fe, 75.00 mg.
[2] Components are based on calculated values.

Subsequently, microhemagglutination activity of serum was estimated and the antibody titers (log_2) were measured following the standard procedure.[19]

Oxidative stress indices. Malondialdehyde (MDA), as a marker of lipid peroxidation, was measured in serum samples according to Placer *et al.*[20] Dithionitrobenzoic acid method was used for measurement of reduced glutathione (GSH) in the serum samples.[21,22] Ferric reducing ability of plasma (FRAP) assay was used for determination of total antioxidant capacity of the serum samples.[22]

Statistical analysis. SPSS for Windows (version 16.0; IBM Corp., Armonk, USA) was used for statistical analysis with a p value of < 0.05 as statistically significant. One-way ANOVA and Bonferroni tests were used for comparison of oxidative stress indices among the different groups. These data were expressed as mean ± standard error (SE). Non-parametric Kruskal Wallis and Mann Whitney U tests were used for comparison of immunological parameters in the trial groups. Quartiles 1 (25.00%), 2 (50.00%, median), and 3 (75.00%) were the descriptive parameters used for these measures.

Results

Oxidative stress. Although decreased FRAP concentration in all nano-manganese groups and increased MDA concentration in nano-manganese 50.00 and 100 groups were noted when compared to the control group, no significant differences were observed for oxidative stress indices among the trial groups (Table 2).

Immune response. A significant decrease in antibody titer against sRBC was revealed in the nano-manganese 100 and 150 groups compared to the control group (p < 0.01). In addition, the antibody titers against IB and ND were significantly lower in the all nano-manganese groups compared to the control group (p < 0.01). No significant difference was observed for the antibody titer against AI among the trial groups (Table 3).

Discussion

Manganese has been supplemented in broiler diets to improve skeletal growth and enhance immune responses.[1] However, the level of supplementation is too critical since manganese in high levels could result in induction of oxidative stress and impairment of immune response.[3,23] Although, nano-trace elements have attracted widespread attention in broiler nutrition, their higher activity and efficiency could act as a double-edged sword. Similar to micro- trace elements, nano- trace elements can be toxic when the provided levels exceed the biological requirements.[9] Therefore, it is too important to investigate the effects of different levels of nano-trace elements in different biological systems to make sure about their beneficial effects.

Although the effects of dietary nano-manganese (20.00 - 170 mg kg^{-1}) on growth, performance and bone characteristics have been investigated,[10] its effect on oxidative stress status and immune response has not been evaluated in broilers. The results of the present study showed significant decreases in the antibody titer against sRBC in the nano-manganese 100 and 150 groups and in the antibody titers against IB and ND in the all nano-manganese groups compared to the control group (p < 0.01). The effects of micro-manganese on the immune response and oxidative stress status have been reported differently. In the study performed by Yang *et al.* supplementing the diet with 40.00 - 160 mg kg^{-1} manganese had no significant effects on lymphocyte proliferation in peripheral blood, ND antibodies titers and relative weight of the spleen.[4] Gajula *et al.* reported that a basal diet of corn–soybean meal supplemented with manganese at 60.00, 120 or 240 mg kg^{-1} had no significant effect on the antibody titers to sRBC, however, manganese at 120 mg kg^{-1} increased cell-mediated immune response to phytohemagglutinin. It was stated that enhanced cell-mediated immunity might be related to elevated

Table 2. Oxidative stress indices in broilers fed diets containing different levels of nano-manganese (day 42).

Parameters	Control	Nano-manganese (mg kg^{-1} of diet)		
		50.00	100	150
Malondialdehyde (nmol mL^{-1})	12.08 ± 1.68	13.63 ± 2.26	14.06 ± 2.08	11.01 ± 1.78
Reduced glutathione (mmol L^{-1})	1.17 ± 0.06	1.28 ± 0.04	1.18 ± 0.08	1.08 ± 0.04
Ferric reducing ability of plasma (mmol L^{-1})	1.50 ± 0.04	1.45 ± 0.04	1.38 ± 0.04	1.42 ± 0.04

No significant differences were observed between the trial groups (p > 0.05).

Table 3. Immune responses in broilers fed diets containing different levels of nano-manganese (day 42).

Items	Control			Nano-manganese (mg kg^{-1} of diet)								
				50.00			100			150		
	Q1	Q2	Q3	Q1	Q2	Q3	Q1	Q2	Q3	Q1	Q2	Q3
sRBC titer	4.00	5.00[a]	5.00	4.00	5.00[ab]	6.00	3.00	4.00[b]	4.75	3.00	4.00[b]	4.00
ND titer	6.00	6.00[a]	7.00	5.00	6.00[b]	6.00	5.00	5.50[b]	6.00	5.00	6.00[b]	6.25
IB titer	723	941[a]	1237	491	671[b]	1076	399	579[b]	903	478	705[b]	956
AI titer	4.00	5.00	6.00	4.00	5.00	6.00	4.00	5.00	6.00	4.00	5.00	6.00

Q1, Q2 and Q3= quartiles 25.00, 50.00 (median) and 75.00%, respectively.
sRBC= Sheep red blood cells; ND= Newcastle disease; IB= Infectious bronchitis; AI= Avian influenza.
[ab] Medians within rows lacking a common superscript letter differ significantly at p < 0.05.

production of interleukin-2 and increased function of superoxide dismutase, which is vital for the integrity of macrophages and heterophils.[1] Sunder *et al*. concluded that the supplementation of manganese at 100 mg kg^{-1} level was essential for skeletal growth and optimum immune response. Manganese supplementation at 100 mg kg^{-1} level was as efficient as higher levels (up to 800 ppm). Higher levels of manganese (1,600 mg kg^{-1} and above) had negative effect on antibody titers against sRBC and cutaneous basophil hypersensitivity to phyto-hemagglutinin in broilers.[3] Similarly in a study performed by Liu *et al*., it was demonstrated that manganese-supplemented diet containing 600, 900, and 1,800 mg kg^{-1} decreased iron, zinc and calcium contents in immune organs. In addition, IL-1β and IL-2 mRNA levels in immune organs and IL-1β and IL-2 concentrations in blood serum were decreased following manganese supplementation. The authors stated that magnesium at above mentioned levels can disturb the balance of trace elements in immune organs and induce immune suppression in the molecular level.[5] The results of the present study showed that supplementing nano-manganese at 50.00, 100 and 150 mg kg^{-1} levels exhibited a similar effect to the supplementation of high levels of micro-manganese in terms of suppressed immune function.

Enhanced oxidative stress, as decreased antioxidant capacity and increased lipid peroxidation, was noted in the broilers supplemented with nano-manganese, although it was statistically insignificant ($p > 0.05$). The observed findings in the present study suggested that supplementation of nano-manganese at 50.00, 100 and 150 mg kg^{-1} levels not only did not reveal antioxidant properties, but resulted in mild oxidative stress. Micro-manganese at 20.00 - 100 mg kg^{-1} levels revealed antioxidative features and diminished superoxide anions (O^{-2}), and increased antioxidant activity were noted in shrimps fed manganese- supplemented diets.[24] However, higher levels of manganese showed oxidative properties. In a study performed by Liu *et al*. cocks were fed either a commercial diet or a manganese-supplemented diet containing 600, 900, and 1,800 mg kg^{-1} manganese chloride. Diets supplemented with manganese increased MDA concentration but decreased antioxidant enzymes activities (superoxide dismutase, glutathione peroxidase) in blood serum and immune organs (spleen, thymus and bursa of Fabricius). DNA single strand break and DNA–protein crosslink revealed time and dosage effect in lymphocytes of immune organs. It was concluded that manganese supplementation at high levels, above 600 mg kg^{-1}, resulted in oxidative damage of immune system by altering antioxidant defense system, lipid peroxidation and apoptosis that might be responsible to some extent in immune suppression.[6] Several studies have reported that manganese-induced cytotoxicity of immune cells is related to oxidative stress.[23,25] The overproduction of ROS and alterations in antioxidant defense system are the possible mechanisms of the oxidative stress induced by manganese supplementation at high levels. The degree of oxidative stress was sensitive to the manganese concentration.[6] The increase of manganese nanoparticles from 10.00 to 100 mg kg^{-1} resulted in a decrease in the plasma IgM level, however, caused an increase in plasma MDA concentration in young turkeys.[26] Consequently, although it has a vital role in the antioxidant defense system, a high level of manganese, especially in the form of manganese nanoparticles may induce oxidative stress which may exacerbate apoptosis. Similarly, excessive manganese nanoparticles can alter the balance of trace elements in the immune organs and induce immune suppression such as reduction in the IgM level which has a major function in the primary immune response following exposure to a pathogen.[26]

In conclusion, the present study was the first research in which nano-manganese effects on oxidative stress status and immune response was evaluated in broiler chickens. The observed findings suggested that nano-manganese at 50.00, 100 and 150 mg kg^{-1} levels suppressed humoral immune response in broilers which could be taken into consideration in supplementation. The mechanism of this effect remains to be further studied. In addition, the effects of nano-manganese lower than 50.00 mg kg^{-1} on immune response and oxidative stress should also be further investigated.

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Conflict of interest

The authors declare there is no conflict of interests.

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