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Clinicopathological Studies on the Effect of Nano Selenium Particles in Broilers

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Abstract

The reason for this research was to analyze the potential immune-stimulating and antioxidant properties of nano-selenium (nano-Se) in broiler chickens. The study utilized 150 one-day-old Cobb broiler chickens, which were arbitrarily allocated to six groups of 25 chickens every: G1 (control), G2 (0.3 ml nano-selenium/L water), G3 (0.5 ml nano-selenium/L water), G4 (*E. coli,* 2 x 10^7 cfu), G5 (0.3 ml nano-selenium/L water and *E. coli*), and G6 (0.5 ml nano-selenium/L water and *E. coli*). Various immune response, antioxidant, and oxidative stress parameters were evaluated. The results revealed that infected chickens had significantly lower levels of immunoglobulins (IgG, IgM, IgA), glutathione peroxidase (GPX), superoxide dismutase (SOD), and interleukin-4 (IL-4) compared to the control group. Conversely, the infected chickens revealed a marked higher in interleukin-2 (IL-2), interferon-gamma (IF-γ), and malondialdehyde (MDA). In contrast, infected and nano-se treated chickens exhibited a rise in IgG, IgM, IgA, GSH, GPX, SOD, and IL-4 with a notable decline in IF-γ, IL-2, and MDA relative to the infected group. These findings suggest that nano-se may play a significant role in immune response, antioxidant activity, and control and prevention of *E. coli* infections in broiler chickens. These results imply that nano-se may have a substantial role in strengthening the immune response, antioxidant activity, and management and prevention of *E. coli* infections in broiler chickens.

KEYWORDS
E. coli, nano-selenium, MDA, SOD, GSH, GPX, IgM and IgG

INTRODUCTION

Selenium is an essential constituent of several compounds that augment the immune system's reactivity, either by modulating the production of specific cytokines or by fortifying the defense cells to counteract oxidative stress (Habibian *et al.*, 2014). Nano-selenium appears to be the favored frame in poultry sustenance. It has been detailed that, nano-selenium, a normal bioactive molecule, has the potential to make strides have insusceptibility, protect cellular working against the oxidative push and lipid peroxidation and in some way boost development performance (Ibrahim *et al.*, 2020; Selim *et al.*, 2015).

Numerous challenges are confronting the poultry industry, within the bleeding edge of the course, avian colibacillosis, which is an irresistible malady that influences birds and is caused by *E. coli*. It is a significant factor responsible for causing boredom, mortality, and severe economic losses in the poultry sector due to its affiliation with many other illnesses as an essential pathogen or an auxiliary pathogen (Kabir, 2010).

The goal of this research was to delineate the protocols of varied concentrations of Nano-Selenium (0.3ml Nano-Selenium/L water and 0.5ml Nano-Selenium/L water) in both in vivo and in vitro settings using *E. coli*, to assess multiple facets encompassing: 1) Immunological parameters such as IgG, IgM, IgA, IL-2, IL-4, and IF-γ. 2) Antioxidant and oxidative stress parameters including GSH, GPX, SOD, and MD.

MATERIALS AND METHODS

150 Cobb broiler chicks, aged one day old and weighing between 45-50 g on average, were obtained from Ismailia/Misr Poultry Company located in Ismailia, Egypt. These chicks were reared in floor pens for 35 days and randomly allocated into six groups of 25 birds each, for a study duration of 5 weeks. They were provided with ad libitum access to water and feed throughout the study. The chicks were fed a commercial broiler starter diet from the day of hatch until 3 weeks of age, and subsequently given a producer finisher diet until the completion of the study at 5 weeks of age. The diets were developed to fulfill the suggested nutritional standards (NRC, 1994). The birds were subjected to ocular administration of vaccines against Newcastle disease (ND), Gumboro disease, and infectious bursal disease (IBD) (Giambrone and Ronald, 1986). The preparation of nano-selenium was carried out following the method described by Ali *et al.* (2020).

Experimental design

A total of 150 clinically healthy chicks were subjected to random division into six groups, with each group consisting of 25

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chicks raised for 5 weeks. The groups were categorized as follows: Group 1 (G1) was kept as a control group; Group 2 (G2) was orally administered with 0.3ml of nano-selenium per liter of water; Group 3 (G3) was orally administered with 0.5ml of Nano-Selenium per liter of water; Group 4 (G4) was infected with *E. coli* only; Group 5 (G5) was infected with *E. coli* and administered with 0.3ml of nano-selenium per liter of water; and Group 6 (G6) was infected with *E. coli* and administered with 0.5ml of nano-selenium per liter of water. The chicks in groups 4, 5, and 6 were intranasally inoculated with 0.5 ml saline containing 2×10^7 C.F.U. of *E. coli* at 14 days of age (Peighambari *et al*., 2000).

Hematological specimens were collected via the brachial vein, and the extracted serum was stored at a frigid temperature of -20°C to facilitate subsequent analysis of immunological, antioxidant, and oxidative stress parameters.

Immunological parameters were assessed using ELISA. IgG, IgM, and IgA were measured with an ELISA Kit. IL-2 and IL-4 were determined with a commercial ELISA Kit, while IFN-γ was analyzed using a commercial ELISA Kit.

Antioxidant and oxidative stress parameters were measured by analyzing hepatic antioxidant enzyme activities (Zhang *et al*., 2016), MDA content, and the activities of CAT, GSH-Px, and SOD using specialized kits and a semi-automated spectrophotometer (Erba-Chem7, Germany).

Statistical analysis

The statistical analysis involved using SPSS (10) software (Snedecor and Cochran, 1989). To ensure a comprehensive analysis of the data, a one-way ANOVA with Duncan multiple comparison tests was used to assess differences between the means of the different groups.

RESULTS AND DISCUSSION

Immunoglobulin, produced by B cells, is critical in regulating humoral immunity. IgG is the most versatile immunoglobulin and dominates in the extravascular spaces (Smith, 1988). IgM is a potent complement-fixing immunoglobulin that efficiently lyses microorganisms and agglutinates them, facilitating their removal (Rhodes and Pflanzer, 2002). Mucosal secretions contain a high abundance of IgA, which is resistant to degradation by host proteases. (Konkel and Chen, 2011). Its main functions include preventing macromolecule absorption and allergen binding, Suppressing the inflammatory effects of immunoglobulin., and neutralizing bacterial toxins, serving as the first line of defense against various infections (Kamada and Nunez, 2014; Zhao *et al*., 2016; Davison *et al*., 2008). A significant reduction in levels of IgG, IgM, and IgA was observed in the infected group (G4) at the 3rd and 5th weeks compared to the control group, according to the results of the immunoglobulin analysis (Tables 1 and 2). These findings align with the observations of Madian *et al*. (2008), noted lower concentrations of immunoglobulin in sera from *E. coli* infected birds compared to controls. Wang *et al*. (2017) also reported that *E. coli*-infected broilers had significantly lower ileal mucosal IgA concentrations than broilers in the control group. These outcomes are attributed to the immunosuppressive effects of *E. coli*. In chickens, the initiation of humoral-related antibodies in the immune system depends on the efficacy of the bursa of Fabricius (Glick, 1970) and the thymus for cellularly related antibodies (Cooper *et al*., 1966). Changes in the cellular structure of these tissues caused by infection can result in weakened immune responses (Glick, 1967). In contrast, infected and treated groups (G5 and G6) demonstrated a significant increase in levels of IgG, IgM, and IgA compared to the infected group (G4) at the 3rd and 5th weeks (Tables 1 and 2). The enhancement in serum immunoglobulin levels could be attributed to the vital biological function of nano-selenium in increasing T helper cells and promoting the secretion of cytokines, which are required for initiating humoral

Table 1. Effect of different levels of nano-selenium on immunological parameters on healthy and *E. coli* experimentally infected groups at 3 weeks of age.

Groups	IgG (ng/ml)	IgM (ng/ml)	IgA (ng/ml)	IL2 (pg/ml)	IL4 (pg/ml)	IF-γ (pg/ml)
G1	437.0 ±8.7^{a}	474.6±11.9^{a}	698.5±14.5^{a}	1.75±0.02^{c}	87.29±1.6^{a}	37.83±0.93^{b}
G2	441.6±10.1^{a}	532.9±14.1^{a}	458.5±17.5^{b}	2.11±0.05^{b}	91.5±0.87^{a}	40.92±0.51^{b}
G3	453.0±10.2^{a}	491.0±15.3^{a}	679.25±17.3^{a}	2.15±0.07^{b}	92.06±1.1^{a}	40.78±0.62^{b}
G4	53.25±8.92^{c}	221.63±6.6^{c}	246.5±18.5^{d}	2.57±0.05^{a}	52.43±1.4^{c}	44.61±0.84^{a}
G5	222.50± 9.8^{b}	298.3±8.5^{b}	371.3±10.7^{c}	2.10±0.01^{b}	62.16±1.1^{b}	36.65±0.87^{b}
G6	230.00±0.4^{b}	356.13±7.1^{b}	364.75±11.8^{c}	2.17±0.06^{b}	62.45±1.1^{b}	38.93±0.58^{b}

Table 2. Effect of nano-selenium on immunological parameters on healthy and *E. coli* experimentally infected groups at 5 weeks of age.

Groups	IgG (ng/ml)	IgM (ng/ml)	IgA (ng/ml)	IL2 (pg/ml)	IL4 (pg/ml)	IF-γ (pg/ml)
G1	80.58±0.16^{a}	79.34±6.84^{a}	66.30±5.83^{b}	1.60±0.036^{c}	94.13±1.66^{a}	30.02±0.59^{c}
G2	81.51±8.55^{a}	82.32±8.36^{a}	83.45±4.13^{a}	1.91±0.016^{b}	96.77±1.86^{a}	30.85±0.60^{c}
G3	87.35±6.38^{a}	8.65±4.42^{a}	63.85±7.59 ^{b}	1.98±0.01^{a}	93.70±1.91^{a}	30.46±1.27^{c}
G4	46.30±2.14^{c}	51.80±3.35^{b}	40.20±2.42 ^{c}	2.21±0.02^{a}	63.58±0.82^{b}	52.08 ±0.96^{a}
G5	62.50±1.44^{b}	84.20±4.27^{a}	71.10±1.21^{ab}	1.94±0.006^{b}	90.97±1.17^{a}	39.29 ±0.89^{b}
G6	64.84±1.94^{b}	83.24±2.81^{a}	70.85±5.28^{ab}	1.96±0.013^{b}	91.04±1.29^{a}	40.37±1.31^{b}

Table 3. Effect of different levels of nano-selenium on antioxidant parameters on healthy and *E. coli* experimentally infected groups at 3 weeks of age.

Groups	GSH (ng/ml)	GPX (U/mL)	SOD (U/mL)	MDA (nmol/mL)
G1	117.00±6.3^{a}	122.95±3.2^{a}	111.26±0.6^{a}	3.78±0.04^{c}
G2	116.00±7.51^{a}	120.05±4.07^{a}	114.49±1.84^{a}	3.68±0.25^{c}
G3	114.75±3.67^{a}	120.50±5.14^{a}	116.85±0.78^{a}	3.57±0.11^{c}
G4	51.61±1.95^{c}	32.55±2.77^{d}	50.16±4.59 ^{d}	7.09±0.28^{a}
G5	95.60±3.58^{b}	53.85±4.30^{c}	63.85±6.15 ^{c}	4.79±0.41^{b}
G6	88.67±4.32^{b}	82.95±3.96^{b}	90.40±1.27 ^{b}	4.76±0.36^{b}

Table 4. The effect of different levels of nano-selenium on antioxidant parameters on healthy and *E. coli* experimentally infected groups at 5 weeks of age.

Groups	GSH (ng/ml)	GPX (U/mL)	SOD (U/mL)	MDA (nmol/mL)
G1	103.25 ± 1.6^{bc}	135.21 ± 2.66^{b}	139.80 ± 5.6^{b}	3.37 ± 0.081^{d}
G2	118.1 ± 1.3^{b}	146.9 ± 4.2^{ab}	146.55 ± 8.5^{b}	3.22 ± 0.035^{d}
G3	115.6 ± 1.1^{a}	148.3 ± 3.4^{a}	183.85 ± 9.4^{a}	3.12 ± 0.12^{d}
G4	51.61 ± 1.95^{d}	84.37 ± 1.66^{d}	63.3 ± 7.8^{d}	7.07 ± 0.05^{a}
G5	90.6 ± 1.69^{c}	99.31 ± 1.20^{c}	85.30 ± 2.7^{c}	4.53 ± 0.018^{b}
G6	88.67 ± 4.32^{c}	103.15 ± 1.82^{c}	89.75 ± 2.45^{c}	4.21 ± 0.026^{c}

immunity and specialization of B cell lymphocytes for immunoglobulin production (Abbas *et al*., 2015; Shabani *et al*., 2019).

In terms of immunoglobulin levels, the groups treated with nano-se (G2 and G3) did not show any changes in IgG and IgM levels. However, elevation in IgA was observed in G2, while G3 showed no difference when match up to the control group (G1) in the 5th week. These results are steady with Abdel-Moneim *et al*. (2022), who reported higher levels of IgA in birds fed with nano-se supplemented diets compared to the control group. Similarly, Alagawany *et al*. (2021) found no differences in IgM and IgA levels between the group that received nano-se and the control group.

Cytokines are essential small proteins involved in regulating and coordinating the immune response. They are involved in the immune responses, and any dysregulation in cytokine production can lead to various pathological disorders (Tayal and Kalra, 2008). When exposed to antigenic stimulation, naive and Th2 cells release Interleukin-2 and IL-4, respectively (Zhang *et al*., 2013). IL-2 is involved in the inflammatory process and can have both pro-inflammatory and regulatory features (Granucci *et al*., 2004). Interleukin-4 is an anti-inflammatory cytokine that primarily works by reducing the activity of pro-inflammatory molecules. (Min *et al*., 2004; Gregory *et al*., 2006). Interferon-γ is a pro-inflammatory cytokine that has been shown to cause immunopathology in several models of inflammation (van Holten *et al*., 2004).

The results of this study showed that infected chickens (G4) revealed elevated IL-2 and IFN-γ levels, with a reduction in IL-4 levels compared to the control group (G1) at the 3rd and 5th weeks. These results were parallel with Zhang *et al*. (2013), who reported an increase in IL-2 and IL-4 levels in piglet ileum following *E. coli* challenge. Wang *et al*. (2017) also found that the mRNA expression of IL-2 and IFN-γ in *E. coli*-infected broilers was higher than that in the control group.

In the 3rd and 5th weeks, the infected and treated groups (G5 and G6) exhibited elevated IL-4 levels and decreased levels of IL-2 and IFN-γ. The authors attributed these findings to the anti-inflammatory properties of nano-selenium. Selenium compounds are known to have anti-inflammatory effects by scavenging reactive oxygen species (ROS). Consequently, selenium-containing enzymes can prevent the initiation and activation of pro-inflammatory signaling pathways by eliminating ROS from the cell (McKenzie and Arthur, 2002).

The nano-selenium treated groups (G2 and G3) showed an increase in IL-2 levels, at the 3rd and 5th weeks. These findings coincide with Mahmoud *et al*. (2016), who found that feeding nano-selenium to broilers increased the mRNA expression of the cytokine gene interleukin 2. The researchers proposed that the impact of selenium on immune cells could be attributed to its capacity to enhance the IL-2 receptor expression in these cells.

Antioxidant enzymes and MDA concentration analysis are commonly used techniques to determine oxidative stress levels (Przybylska *et al*., 2007). GSH acts as a scavenger for singlet oxygen and hydroxyl radicals (Diplock, 1994). GPX, a selenoenzyme antioxidant, plays a crucial role in protecting organisms from oxidative stress by reducing hydroperoxides using GSH (Stadtman, 1991; Ursini, 1994). SOD and GPX are the primary enzymatic defenses against harmful oxygen metabolites, and they are crucial for regulating free radicals (Maestro, 1991). MDA is the major final product of lipid peroxidation and is frequently familiar with determining oxidative break levels, which are indicated by a higher level of MDA (Ciftci *et al*., 2010).

In broilers infected with *E. coli*, a consistent decrease in serum GSH and GPX levels was observed (Table 3), which is in line with previous studies by Kilany *et al*. (2018) and El-Tahawy *et al*. (2022), respectively. This decline is believed to be caused by lipid peroxidation, which occurs due to the oxidation of long-chain fatty acids in cell membranes during inflammation. This process can inhibit the activity of antioxidant molecules like GPX, resulting in oxidative stress, as reported by Wang *et al*. (2002); Rinaldi *et al*. (2007) and Roberts *et al*. (2009).

The serum SOD level decreased value in the *E. coli* infected group, which was the same result as Fadl *et al*. (2020) and El-Tahawy *et al*. (2022). Additionally, the serum MDA level was significantly increased in the broilers infected with *E. coli* at the 3rd and 5th weeks (Tables 3 and 4), which is in harmony with Hashem *et al*. (2021) finding of significantly increased MDA levels in broilers infected with *E. coli*. This increase may be due to the extensive damage induced by bacterial endotoxin (LPS) to various organs, including the liver, through the production of reactive oxygen intermediates and subsequent lipid peroxidation (Matsuda *et al*., 1998; Kono *et al*., 2003). On the other hand, the groups that were infected and treated demonstrated an increase in the levels of antioxidant enzymes such as GSH, GPX, and SOD, as well as a decrease in the level of MDA, observed during the 3rd and 5th weeks. The antioxidant properties of nano-se can be regarded as a contributing factor to these findings. Selenium has an antioxidant effect (Levander and Burk, 1994). It forms the active center of GPX, which plays a crucial role as an antioxidant (Brown and Jessup, 1999).

CONCLUSION

The study provides evidence that nano-selenium has both immunostimulatory and antioxidant effects in broiler chickens. The infected groups treated with nano-se exhibited increased levels of GSH, GPX, and SOD, and decreased levels of MDA, indicating that nano-se can mitigate oxidative stress induced by *E. coli* infection. The underlying mechanism for these effects is related to the antioxidant activity of nano-se, as selenium's primary function is to act as an antioxidant by forming the active center of the GPX enzyme. Overall, these findings suggest that nano-se may be a useful supplement for improving the immune response and reducing oxidative stress in broiler chickens, particularly during *E. coli* infections. However, further research is needed to determine the optimal dose and duration of nano-se supplementation in poultry diets.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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