

# Effect of Selenium Nanoparticles Supplementation on Oxidation Resistance of Broiler Chicken

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## Abstract

**Objectives:** This study was undertaken with the objective of studying the effect of selenium nanoparticles on oxidation resistance in broiler chicken. **Methods:** An *in vivo* feeding experiment was conducted in 150 broiler chicks. three replicates of 10 birds were maintained for each treatment with various levels of selenium nanoparticles (0.075, 0.1125, 0.1875 and 0.225 mg/kg ) along with selenium coarse particles (0.15 mg/kg) as control group. The serum samples obtained from different treatments were subjected to analysis of antioxidant enzymes. The data obtained were analysed with ANalysis Of VAriance (ANOVA). **Findings:** Birds supplemented with 0.1875 mg/kg selenium nano-particles showed a significant ( $p < 0.05$ ), increase in serum superoxide dismutase activity and glutathione peroxidase activity and decrease in malondialdehyde concentration. But, when the dietary level of Selenium Nanoparticles was increased to 0.225 mg/kg, level of superoxide dismutase and glutathione peroxidase activities in serum got significantly ( $p < 0.05$ ) reduced and the concentration of malondialdehyde increased when the levels were compared to the control group and remaining treatment groups. So it is concluded that selenium nanoparticles of level 0.1875 mg/kg feed improved the activities of antioxidant enzymes and thus the oxidation resistance in broiler chicken but when the selenium nanoparticles were supplemented at a level of 0.225 mg/kg feed it reduced the level of antioxidative enzymes in broiler chicken. **Application:** The selenium nanoparticles can be used to improve the oxidation resistance in broiler chicken.

**Keywords:** Antioxidant Enzymes, Broiler Chicken, Oxidative Resistance, Selenium Nanoparticles

## 1. Introduction

Selenium is a part of the enzyme glutathione peroxidase which plays an important part in controlling the level of hydrogen peroxide and lipid peroxides which are produced during normal metabolic activity<sup>1</sup>. A concentration of 0.15 mg of selenium/kg of diet is recommended<sup>2</sup> for broiler chickens throughout the growth period. However, there is widespread concern that the selenium minimum recommendation<sup>2</sup>

is not sufficient to prevent production losses due to selenium deficiency and therefore there is continued research into alternative selenium sources and alternative selenium supplementation levels. Nano selenium shows better properties such as increased surface activity, catalytic efficiency, large surface area but decreased toxicity<sup>3</sup>. Hence, in order to avert toxicity while supplying optimum and maximum dose of selenium, supplementation of nano selenium is proposed. Moreover it has been found that the

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nanoparticles exhibited 200 percent more bioavailability than the coarse particles as found in calcium phosphate nanoparticles<sup>4</sup>.

The excessive cellular damage resulting from oxidation may be the reason for the drip loss in broiler meat<sup>5</sup>. Glutathione peroxidase contributes significantly to the overall antioxidant defence of muscle in broilers and selenium supplementation of the diet could decrease tissue susceptibility to lipid peroxidation and increase oxidative stability of skeletal muscle<sup>6</sup>. The birds supplemented with nano selenium showed an increase in glutathione peroxidase activity in serum and tissue, decrease in drip loss and also an increase in the keeping quality of meat<sup>7</sup>. This study was undertaken with the objective of elucidating the effect of Selenium Nanoparticles supplementation on oxidation resistance in broiler chicken.

## 2. Materials and Methods

### 2.1 Synthesis and Characterization of Selenium Nanoparticles

Selenium Nanoparticles of size 30 – 60 nm was synthesised by water phase solution method<sup>8</sup> and characterized by particle size analyzer.

### 3. Optimising the Level of Selenium Nanoparticles Supplementation in Broiler Chicken Diet

To study the effect of graded level of inclusion of Selenium Nanoparticles on the antioxidant enzymes, a feeding trial was conducted for 35 days. The feeding trial had five experimental groups with a control group (0.15 mg/kg selenium coarse particles) and four treatment groups with 25% less (0.1125 mg/kg Selenium Nanoparticles), 50% less (0.075 mg/kg Selenium Nanoparticles), 25% more (0.1875 mg/kg Selenium Nanoparticles) and 50% more (0.225 mg/kg Selenium Nanoparticles) of control group. The details of the experimental design used in this feeding trial are furnished in Table 1.

**Table 1.** Experimental design for in vivo feeding experiment in broiler chicken.

Groups	Dietary Treatment	Chicks / Treatment
Control - Se CP	Basal diet + 0.15 mg/kg selenium coarse particles	30
Selenium Nanoparticles - 25	Basal diet + 0.1125 mg/kg Selenium Nanoparticles	30
Selenium Nanoparticles - 50	Basal diet + 0.075 mg/kg Selenium Nanoparticles	30
Selenium Nanoparticles + 25	Basal diet + 0.1875 mg/kg Selenium Nanoparticles	30
Selenium Nanoparticles + 50	Basal diet + 0.225 mg/kg Selenium Nanoparticles	30

CP = Coarse particle; Se = selenium

In these experiment 150 numbers of one - day old COBB - 400 broiler chicks were purchased from a commercial farm. Each treatment had three replicates with 10 birds per replicate. The experimental birds were maintained in four tiered battery cages which had good ventilation and artificial lighting. An ad libitum quantity of feed and drinking water were provided for all the birds in different treatments. The required amount of nano selenium was prepared in the laboratory. The experimental rations were formulated as per specification for broiler chicken<sup>9</sup>. The ingredient composition of basal diet is presented in Table 2.

**Table 2.** The ingredient composition of basal diets.

Ingredients, g/kg	Starter, 0 – 21 days	Finisher, 22– 35 days
Maize	588	599.2
Bajra	-	33
Vegetable oil	24	35
Soya bean meal	269	222
Fish meal	97	90
Lysine	1	-
Methionine	1	0.8
Vitamin-mineral mixture	20	20

Provided per kilogram of diet: Vitamin A - 1500 IU, Vitamin D<sub>3</sub> - 200 IU, Vitamin E - 10 IU, Vitamin K<sub>3</sub>, Vitamin B<sub>12</sub> 0.01 mg, Biotin 0.15 mg, Choline 1100 mg, Folic acid 0.55 mg, Niacin 30 mg, Pantothenic acid 10 mg, Pyridoxine 3.5 mg, Riboflavin 3.5 mg, Thiamine 1.8 mg, Copper 8 mg, Iodine 0.35 mg, Iron 80 mg, Manganese 60 mg, Zinc 40 mg.

### 3.1 Effect of Nano Selenium on Antioxidant Enzymes Status of Broilers of Five Weeks of Age

At the end of 35th day of experiment the whole blood was collected from the wing vein of 30 birds (2 birds per replicate contributing to 6 birds per treatment). Then the serum was collected and used to analyze the anti oxidative enzyme levels.

### 3.2 Superoxide Dismutase Activity

The activity of Super-Oxide Dismutase (SOD) was measured by following the procedure outlined by<sup>10</sup>, using SOD determination kit obtained from Sigma Aldrich (catalog number-19160). The sample and blank were run in triplicates.

The SOD activity (inhibition rate %) was calculated using the following formula as given in kit manufacturer's manual.

$$\left[ \frac{(A \text{ blank1} - A \text{ blank3}) - (A \text{ sample} - A \text{ blank2})}{(A \text{ blank1} - A \text{ blank3})} \times 100 \right]$$

A= Absorbance at 450 nm.

### 3.3 Glutathione Peroxidase Activity

Glutathione peroxidase (GSPHx1) activity was measured by following the procedure outlined by<sup>11</sup>, using the assay kit obtained from Sigma-Aldrich (catalog number CGP1). The samples and blank were run in triplicates. The amount of enzyme in the sample was calculated using the formula as given in the kit manufacturer's manual with modification in the extinction coefficient for NADPH, as for the 96 well flat bottomed plate the extinction coefficient was 3.73. Activity per extract (units/ml) =  $(\Delta A_{340} \times DF \times 0.2) / (3.73 \times V)$ ,

$$\Delta A_{340} = A_{340}/\text{min}_{(\text{blank})} - A_{340}/\text{min}_{(\text{sample})}$$

3.73 =  $\epsilon$  <sup>mM</sup> for NADPH

DF= dilution factor of sample before adding to reaction

V= sample volume in ml, 0.2= total volume in one well

### 3.4 Lipid Peroxidation (Malondialdehyde) Assay

Lipid Peroxidation (LP) was determined as per the procedure outlined by<sup>12</sup>, using the lipid peroxidation kit (Sigma-Aldrich catalog number MAK089). The samples were run in triplicate.

The malondialdehyde concentration was found out by following the formula as given in kit manufacturer's manual.

$$(Sa/Sv) \times 4 \times D = C$$

where,

Sa is the amount of Malondialdehyde in unknown sample (nmole), Sv is the sample volume (ml), C is the concentration of Malondialdehyde in sample, D is the Dilution factor and 4 is the Correction factor.

## 4. Statistical Analysis

The data obtained from experiment was analyzed using SPSS<sup>13</sup> with Analysis of Variance (ANOVA).

## 5. Results

### 5.1 Effect of Selenium Nanoparticles on Antioxidant Enzymes Status of Broilers of Five Weeks of Age

The results of antioxidant enzyme assays are given in Table 3.

**Table 3.** The effect of Selenium Nanoparticles on superoxide dismutase (SOD), glutathione peroxidase (GSPHx) and malondialdehyde activity in serum of broilers of five weeks of age (Mean\*±SE)

Treatment	Sod (Inhibition Rate %)	Gshpx (Units/ Ml)	Malondialdehyde (Nmole/ µl)
Control - Se CP	41.73 <sup>b</sup> ± 0.88	1096.33 <sup>b</sup> ± 0.17	3.64 <sup>b</sup> ± 0.05
Selenium Nanoparticles - 25	43.43 <sup>b</sup> ± 0.98	1209.67 <sup>c</sup> ± 0.18	3.62 <sup>b</sup> ± 0.03
Selenium Nanoparticles - 50	43.91 <sup>b</sup> ± 1.26	1130.00 <sup>b</sup> ± 0.25	3.64 <sup>b</sup> ± 0.17
Selenium Nanoparticles + 25	48.11 <sup>c</sup> ± 1.00	1283.33 <sup>d</sup> ± 0.27	3.10 <sup>a</sup> ± 0.09
Selenium Nanoparticles + 50	37.76 <sup>a</sup> ± 1.16	1096.33 <sup>ab</sup> ± 0.13	4.09 <sup>c</sup> ± 0.11

1 Unit = µmol/min<sup>a,b,c</sup> means with different superscripts in a column differ (p ≤ 0.05) \*mean of 6 birds, CP = Coarse particle Se = selenium

A significant ( $p < 0.05$ ) increase in superoxide dismutase activity and glutathione peroxidase cellular activity was observed in birds fed with 25% more (0.1875 mg/kg) Selenium Nanoparticles when compared with control group and other remaining treatment groups. And birds which were fed with% less (0.075 mg/kg) Selenium Nanoparticles showed comparable results to the control (0.15 mg/kg selenium coarse particles). Comparable results were found for glutathione peroxidase activity and oxidation resistance in broilers in the experiment on the effects of nano selenium on the oxidation resistance in broilers<sup>7</sup>. This indicates that 50% (0.075 mg/kg) less Selenium Nanoparticles can be used in place of control group (0.15 mg/kg selenium coarse particles) to produce same activity level of superoxide dismutase in broiler chicks.

Selenium Nanoparticles supplementation at 25% more (0.1875 mg/kg) showed significantly ( $p < 0.05$ ) low level of malondialdehyde concentration when compared to other treatments and control. Selenium Nanoparticles supplementation at 50% less (0.075 mg/kg) showed a comparable result to that of control. Comparable results were found for malondialdehyde analysis in the experiment on the effects of nano selenium on the oxidation resistance in broilers<sup>6</sup>.

The results were consistent with some other research findings<sup>14-17</sup>. It was showed that the selenium nanoparticles supplementation (0.3 mg/kg) increased the glutathione peroxidase activity compared to control birds<sup>7</sup>. It was further reported that activity of glutathione peroxidase and malondialdehyde increased and decreased respectively when nano selenium supplementation was increased to 2 mg/kg<sup>7</sup>.

## 6. Discussion

In the present study, birds supplemented with 0.1875 mg/kg selenium nanoparticles showed a significant ( $p < 0.05$ ) increase in superoxide dismutase activity and glutathione peroxidase activity and decreased malondialdehyde concentration were noticed in birds supplemented with 0.1875 mg/kg nano selenium. However, when the dietary level of Selenium Nanoparticles was increased to 0.225 mg/kg, serum SOD activity and glutathione peroxidase activity

significantly ( $p < 0.05$ ) reduced and the concentration of malondialdehyde increased when compared to the control group and other treatment groups. The current study indicates that feeding Selenium Nanoparticles could improve the antioxidant enzymes and thus improve oxidation resistance when given at the level of 0.1875 mg/kg feed but at the same time when given at the level of 0.225 mg/kg feed, selenium nanoparticles decreased the antioxidant enzymes when compared to the control and other treatment groups. This is due to different levels of oxidative stress with different levels of nano materials<sup>18</sup>. The current results indicate that at the level of 0.225 mg/kg Selenium Nanoparticles, destroys the cell membrane integrity due to oxidative stress resulting in low level of anti oxidant status.

## 7. Conclusion

The current study indicates that feeding Selenium Nanoparticles at the level of 0.1875 mg/kg broiler feed could improve the oxidation resistance by improving the antioxidant enzymes which was found out by the increased level of superoxide dismutase and glutathione peroxidase and decreased level of malondialdehyde concentration in this study. It indicates better disposal of the oxidation products and maintenance of cell membrane integrity. At the same time when increased to the level of 0.225 mg/kg feed, selenium nanoparticles showed decrease in the antioxidant enzymes.

Therefore it is concluded that the selenium nanoparticles at the level of 0.1875 mg/kg of broiler chicken diet could improve the oxidation resistance in broiler chicken.

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