Indian Journal of Science and Technology, Vol 10(32), DOI: 10.17485/ijst/2017/v10i32/106626, August 2017
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Effect of Selenium Nano Particles on Glutathione Peroxidase mRNA Gene Expression in Broiler Chicken

N. Aparna[1*], R. Karunakaran[1] and M. Parthiban[2]

[1]Department of Animal Nutrition, Madras Veterinary College,
Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600007, Tamil Nadu, India;
aparnasaravanan86@gmail.com, Karunakaran.r@tanuvas.ac.in
[2]Department of Animal Biotechnology, Madras Veterinary College,
Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600007, Tamil Nadu, India; drparthiban66@yahoo.com

Abstract

Objectives: To study the effect of Selenium nano particles on liver glutathione peroxidase mRNA(GSHP×1 mRNA) gene expression 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 Nano particles (0.075, 0.1125, 0.1875 and 0.225 mg/kg) along with selenium coarse particle (0.15 mg/kg) as control group. The liver sample from different treatments was analyzed for GSHP×1 mRNA expression. The data were analyzed with Analysis of Variance (ANOVA). **Findings:** The liver GSHP×1 mRNA expression was significantly ($P<0.05$) higher in Selenium nano particles supplemented groups compared to control group with selenium coarse particles found out by doing RT-PCR in 15 birds, with 1 bird selected randomly from each replicate contributing 3 birds per treatment. There was 4.3 fold increases in GSHP×1 mRNA expression in birds supplemented with 0.1875 mg/kg nano selenium. However, when the Selenium nano particles level was increased to 0.225 mg/kg from 0.1875 mg/kg Selenium nano particles, GSHP×1 mRNA expression was reduced to 0.76 fold when compared with control group of 0.15 mg/kg selenium coarse particles (1 fold). It is concluded that dietary level of 0.1875 mg/kg Selenium nano particles increased the expression of liver GSHP×1 mRNA gene in broiler chicken. **Application:** The selenium nanoparticles can be used to improve the glutathione peroxidase gene expression and thus the oxidation resistance in broiler chicken.

Keywords: Broiler Chicken, Gene Expression, GSHP×1 mRNA, Selenium Nano Particles

1. Introduction

Selenium is an integral part of the glutathione peroxidase, which helps to control levels of hydrogen peroxide and lipid peroxides that are produced during normal metabolic activity.

With the recent development of nanotechnology, nano selenium has attracted widespread attention as nanoparticles exhibit large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability, and low toxicity. Moreover it has been found that the nanoparticles exhibited 200 percent more bioavailability than the coarse particles as found in calcium phosphate nanoparticles[1].

GSH-Px contributes significantly to the overall antioxidant defense of muscle in broilers and selenium supplementation of the diet could achieve to decrease tissue susceptibility to lipid peroxidation and increase oxidative stability of skeletal muscle[2]. The birds supplemented with nano selenium showed higher glutathione peroxidase activity in serum and tissue[3].

Available literature shows that only little work seems to have been done to elucidate glutathione peroxidase (GSHPx1) mRNA gene expression in broiler fed supplemental nano selenium. The level of glutathione peroxidase activity is influenced by GSHPx1 gene. Keeping these points in mind, the present research

*Author for correspondence

work was carried out to elucidate the effect of selenium nanoparticles on glutathione peroxidase mRNA gene expression in broiler chicken.

2. Materials and Methods

2.1 Synthesis of Selenium Nanoparticles

Nano selenium of 30 - 60 nm size and spherical shape was synthesized by water phase solution method[4] at laboratory level.

2.2 Selenium Nano Particles Supplementation in Broiler Chicken Diet

To study the effect of graded level of inclusion of Selenium nano particles on the liver glutathione peroxidase gene expression, 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 Nano particles), 50 % less (0.075 mg/kg Selenium Nano particles), 25 % more (0.1875 mg/kg Selenium nano particles) and 50 % more (0.225 mg/kg Selenium Nano particles) of control group. The details of the experimental design 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 Nano particles - 25	Basal diet + 0.1125 mg/kg Selenium Nano particles	30
Selenium Nano particles - 50	Basal diet + 0.075 mg/kg Selenium Nano particles	30
Selenium Nano particles + 25	Basal diet + 0.1875 mg/kg Selenium Nano particles	30
Selenium Nano particles + 50	Basal diet + 0.225 mg/kg Selenium Nano particles	30

CP = Coarse particle Se = selenium

In this experiment 150 day old broiler chicks (COBB - 400) belonging to a single hatch were purchased from a commercial farm. Three replicates of 10 birds were maintained for each treatment. The experimental birds were housed individually in four tiered, well ventilated battery cages provided with artificial lighting. The birds were fed *ad libitum* quantity of their respective experimental rations in separate feed troughs. Clean drinking water was provided *ad libitum*. The required amount of Selenium Nano particles were prepared in the laboratory. The experimental rations were formulated as per specification for broiler chicken[5]. The ingredient composition of basal diet is presented in Table 2.

2.3 Glutathione Peroxidase mRNA Expression in Liver Sample of Broiler Chicken

On 35th day of feeding experiment, birds were slaughtered by Halal method and liver samples were collected from 15 birds with 1 bird randomly selected from each replicate contributing 3 birds per treatment and immediately processed for RNA isolation.

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_3 200 IU, Vitamin E 10 IU, Vitamin K_3, Vitamin B_{12} 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.

2.4 Protocol Followed for RNA Isolation for PCR

RNA isolation was done according to the procedure outlined by[6]. Liver sample was collected and homogenised

in mortar and pestle and mixed with 1ml Trizol reagent by pipetting out for 10 -15 times. It was incubated for 5-10 min at room temperature. 200 µl of chloroform was added and shaken vigorously by hand for 30 seconds. It was incubated at room temperature for 5 min. The mixture was centrifuged in ice at 12000 RPM for 15 min at 4 °C. The aqueous phase was transferred to a clean tube and 500 µl of isopropanol was added to it and slightly inverted followed by incubation at –20 °C for 30 min. It was then centrifuged at 12000 RPM for 20 min at 4 °C and the supernatant was discarded and to the pellet, 1 ml of 70 % ethanol was added. Then it was centrifuged at 12000 RPM for 15 min at 4 °C the supernatant was discarded. The pellet was air dried and about 20 µl of Nuclease Free Water was added into it and it was processed for cDNA synthesis immediately.

Complementary DNA (cDNA) was synthesised from the isolated RNA by using the cDNA synthesis kit using random hexa primers following the procedure outlined by[7]. Template RNA (8 µl), Random Hexa Primer (1µl), Nuclease Free Water (3 µl) were taken in eppendorf tubes and centrifuged briefly and then incubated at 65 °C for 5 min. The mixture was kept in – 20 °C. Reaction Buffer (3.5×) (4 µl), Ribolock RNase Inhibitor (1 µl), 10 mM dNTP mix (2 µl), Revert Aid H minus (1 µl) and M-MuLv Reverse Transcriptase (12 µl) were mixed gently and centrifuged. Random hexa primer was incubated for 5 min at 25 °C followed by 60 min at 42 °C. The reaction was terminated by heating at 70 °C for 5 min. then; synthesised cDNA was stored at 20 °C until further study.

The Polymerase Chain Reaction (PCR) on synthesised cDNA was carried out according to[7]. Master mix (12.5 µl), Forward primer (1 µl), Reverse primer (1 µl), cDNA (2 µl) and Nuclease Free Water (8.5 µL) were mixed and run on 2 % agarose gel for electrophoresis with ethidium bromide in 1x Tris acetate EDTA buffer by following the reaction conditions of 60 cycles at 95 °C for 10 seconds followed by 95 °C for 5 seconds and 60 °C for 31 seconds

Table 3. The sense and antisense primer sequences of glutathione peroxidase and reference genes

Gene	Primers position	Primers sequences (5 3)	Amplicon size (bp)	Reference
Glutathione peroxidise	Forward	TTGTAAACATCAGGGGCAAA	140	[14]
	Reverse	TGGGCCAAGATCTTTCTGTAA		
Gluceraldehyde -3phosphate dehydrogenase	Forward	CCTCTCTGGCAAAGTCCAAG	180	[14]
	Reverse	CAACATCAAATGGGCAGATG		
β – actin	Forward	TGCGTGACATCAAGGAGAAG	244	[15]
	Reverse	TGCCAGGGTACATTGTGGTA		

Figure 3. The relative expression of mRNA of glutathione peroxidase gene in liver as a result of nano selenium supplementation in broiler chicken

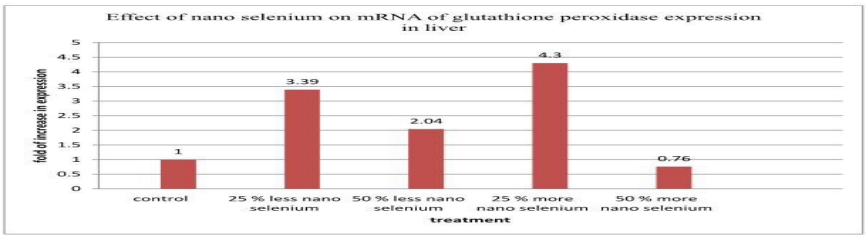

Figure 1. The relative expression of mRNA of glutathione peroxidase gene in liver as a result of nano selenium supplementation in broiler chicken.

and then run at 4 °C for final extension as prescribed by[8]. Finally, the gel documentation was done to document the presence of bands to verify the PCR product.

Gene expression was measured by real-time PCR analysis using the Real Time PCR system ABI PRISM 7300 (Applied Biosystems, CA, USA) with SYBR Premix Ex Taq™ (Perfect Real Time, Takara, Shiga, Japan). The thermal cycle was as follows: 1 cycle at 95 °C for 10 seconds, and 60 cycles at 95 °C for 5 seconds and 60 °C for 31 seconds as denoted by[8]. The primers used are given in the Table 3.

3. Statistical Analysis

Data were analysed with analysis of variance (ANOVA) as per the procedure of statistical analysis system (SPSS)[9].

4. Results

4.1 Liver Glutathione Peroxidase mRNA Expression in Liver Sample of Broiler Chicken

The cycle threshold (C_t) values obtained as a result of real time PCR for the target gene (glutathione peroxidase) and reference gene (glyceraldehyde phosphate dehydrogenase (GAPDH)) were used to obtain the relative quantification (R) using the formula outlined by[10]. The cycle threshold is the value which gives the number of cycles needed to achieve amplification. The low ct indicates only low numbers of cycles are needed for the amplification indicating better expression. So C_t value is inversely proportional to the fold of gene expression. The relative quantification of gene expression is calculated as

$R = 2^{-\Delta\Delta ct}$

Where,

Δct is obtained by subtracting the ct (reference) from ct (target). ΔΔct is obtained by subtracting the Δct of control from Δct which was obtained from the calculation. This is delta-delta approximation method. After getting the R (Relative gene expression) value, the values are plotted in the form of column diagram. Each (R) value denoted for different treatments in the column diagram denotes the number of fold increase in gene expression compared to control group whose expression is denoted as 1. The relative expression of mRNA of glutathione peroxidase gene in liver as a result of Selenium Nano particles supplementation in broiler chicken is depicted in Figure 1.

The fold change in GSHPx1 Mrna gene expression in Selenium Nano particles treatment was compared against control group (0.15 mg/kg selenium coarse particles) where fold of expression was considered as 1. Birds supplemented with nano selenium had significant ($p < 0.05$) level of liver GSHPx1 mRNA gene expression compared to control group. At a dietary nano selenium level of 25 % more (0.1875 mg/kg), there was 4.3 fold increases in GSHPx1 gene expression. However, when the supplemental Selenium nano particles level was elevated to 0.225 mg/kg, increase was just 0.76 fold.

5. Discussion

Many studies have shown that expression of glutathione peroxidise is regulated by dietary selenium level[11]. However, there was no appreciation of the expression of the GPx1 gene affected by nano selenium in the liver of broiler chicken. In the results of this study, the liver GSHPx1 mRNA expression was significantly higher in Selenium nano particles supplemented groups compared to control group. There was 4.3 fold increases in GSHPx1 mRNA expression in birds supplemented with 0.1875 mg/kg Selenium nano particles. However, when the Selenium nano particles level was increased to 0.225 mg/kg from 0.1875 mg/kg nano selenium, GPx1 mRNA expression was reduced to 0.76 fold, indicating that at higher level Selenium Nano particles reduced the expression of GPx1 gene.

A dose-dependent effect of supplemental Selenium was found on the expression level of GSH-Px1[12]. An optimal level of Selenium can increase GSH-Px1 mRNA expression, while higher doses of selenium inhibit its expression. One explanation for this phenomenon is the toxicity of selenium, which maybe partly due to the formation of the selenite ion free radical. Excessive selenite radical ions, together with abundant glutathione, can generate superoxide anions and produce other types of reactive oxygen species. Abundant reactive oxygen species could lead to fragmentation or degeneration of DNA, RNA, proteins and other biological macromolecules, and cause cellular and bodily injury[13]. Therefore, selenium has a dual role in animals. The role selenium plays depends on the amount and form of selenium supplemented in the diet. In this study, the highest expression level of GSH-Px1 mRNA in the liver was observed in broiler chicken fed with the diet of 0.1875 mg/kg nano selenium.

When the Selenium nano particles level was increased to 0.225 mg/kg from 0.1875 mg/kg Selenium Nano particles, GSHPx1 mRNA expression was reduced to 0.76 fold when compared to the control group with 0.15 mg/kg selenium coarse particles (1 fold), indicating that at higher level Selenium nano particles reduced the expression of GSHPx1 gene.

6. Conclusion

The expression of liver glutathione peroxidase gene was found out by using qRT – PCR and the relative fold of expression in treatment groups compared to the control group indicated that 25 % (0.1875 mg/kg0.1875 mg/kg nano selenium) more Selenium nano particles 0.1875 mg/kg showed 4.3 fold of increase in glutathione peroxidase gene expression compared to the control group. When the Selenium nano particles level was increased to 0.225 mg/kg from 0.1875 mg/kg Selenium Nano particles, GSHP×1 mRNA expression was reduced to 0.76 fold when compared to the control group with 0.15 mg/kg selenium coarse particles, indicating that at higher level Selenium Nano particles reduced the expression of GSHP×1 gene.

Therefore, it is concluded that a dietary level of 0.1875 mg/kg Selenium Nano particles increased the expression of liver GSHP×1 mRNA gene which is important in improving the oxidation resistance in broiler chicken.

7. Acknowledgement

The authors are thankful to Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, Tamil Nadu, India - 600051 for providing necessary facilities to conduct the research work at the Department of Animal Nutrition, Madras Veterinary College, Chennai - 600 007.

8. References

1. Vijayakumar MP, Balakrishnan V. Evaluating the Bioavailability of Calcium Phosphate Nanoparticles as Mineral Supplement in Broiler Chicken. Indian Journal of Science and Technology. 2014; 7(10):1475-80.
2. Surai PF. Nottingham University Press: Natural antioxidants in avian nutrition and reproduction. 2002.
3. Cai SJ, Wu CX, Gong LM, Song T, Wu H, Zhang LY. Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. Poultry Science. 2012; 91:2532-9. Crossref. PMid:22991539.
4. Razi KM, Maamoury RS, Banihashemi S. Preparation of nano selenium particles by water solution phase method from industrial dust. International Journal of Nano Dimension. 2011; 1(4):261-7.
5. BIS (Bureau of Indian Standards). New Delhi: Indian Standard Poultry Feeds-Specification. 5th edn. 2007.
6. Choczynski P, Sacchi N. Single-step method of RNA isolation. Analytical Biochemistry. 1987; 162:156-9. Crossref, Crossref
7. Seal BS, King DJ, Bennett JD. Characterization of Newcastle disease virus isolates by reverse transcription PCR coupled to direct nucleotide sequencing and development of sequence database for epidemiological pathotype prediction and molecular analysis. Journal of Clinical Microbiology. 1995; 33(10):2624-30. PMid:8567895 PMCid:PMC228544.
8. Eid Y. Antioxidative effects of canolol in broiler chickens. Egyptian Poultry Science. 2013; 33:99-106.
9. SPSS, Statistical Package for the Social Sciences. Version 17.0 software for windows. https://en.wikipedia.org/wiki/SPSS,03/12/2016.
10. Pfaffl MW. A new mathematical model for relative quantification in real time RT-PCR. Nucleic Acids Research. 2001; 29:2002-7. Crossref.
11. Christensen MJ, Janghorbani M, Steinke FH, Istfan N,Young VR. Simultaneous determination of absorption of selenium from poultry meat and selenite in young men: application of a triple stable-isotope method. British Journal of Nutrition. 1983; 50:43-50. Crossref. PMid:6882731.
12. Zhang Y, Suzhen Z, Xuepeng W, Chunyang W, Fuchang I. The effect of dietary selenium levels on growth performance, antioxidant capacity and glutathione peroxidase 1 (GSHPx1) mRNA expression in growing meat rabbits. Animal Feed Science and Technology. 2011; 169:259-64. Crossref.
13. Seko Y, Saito Y, Kitahara J, Imura N. Active oxygen generation by the reaction of selenite with reduced glutathione in vitro. Wendel, A. (Ed.), Springer: Berlin: Selenium in Biology and Medicine. 1989; p. 70-3. Crossref.
14. Li Y, Li QGM, Zhio H, Hua W, Guo-Xiang W, Zhang DJY Ji C. Effects of lipoic acid on immune function, the antioxidant defence system and inflammation related genes expression of broiler chickens fed aflatoxin contaminated diets. International Journal of Molecular Sciences. 2014; 15:5649-62. Crossref. PMid:24699046 PMCid:PMC4013587.
15. Yarru LP, Settivari RS, Gowda NKS, Antoniou E, Ledoux DR, Rottinghaus G. Effect of turmeric (Curcuma longa) on the expression of hepatic genes associated with biotransformation, antioxidant and immune system in broiler chicks fed aflatoxin. Poultry Science. 2009; 88:2620-7. Crossref. PMid:19903961.