Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(3): 392-396

Received: 22-03-2020
Accepted: 24-04-2020

P Visha
Associate Professor, Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu, India

P Selvaraj
Professor and Head Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

S Jayachandran
Professor and Head, Department of Veterinary Biochemistry, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Corresponding Author:
P Visha
Associate Professor, Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu, India

Selenoprotein gene (Sel W) expression in the bursa of broiler chickens as influenced by dietary supplementation of nanoselenium

P Visha, P Selvaraj and S Jayachandran

Abstract
Selenium is an essential nutritional trace element having vital role in immunoprotection, antioxidant system and growth. Hence, selenium supplementation in the poultry has been regularly practiced using the inorganic and organic forms. These forms have the limitations of having narrow margin of safety and non specific binding to tissue proteins, hence an alternate form of selenium i.e. nanoselenium having higher bioavailability, higher margin of safety and seven-fold lower acute toxicity was synthesised. The nanoselenium (15-40 nm) synthesized was used for the biological trial in broiler chickens. The treatment groups were supplemented with 0.3 mg/kg sodium selenite (T2), 0.3 mg/kg organic selenium (T3), nanoselenium at three levels viz.0.15 (T4), and 0.3 (T5) and 0.6 mg/kg (T6) and (T1) group was the control, fed with the basal di*et al*one. The Sel W mRNA expression in the bursa of broiler chickens was studied. Supplementing 0.6 mg/kg nanoselenium in chickens resulted in highly increased expression of selenoprotein W in bursa of broiler chickens. As the selenoprotein SelW plays a vital role as antioxidant in both the immune mediating cells and the immune organs, especially during stress, the supplementation of 0.6 mg/kg nanoselenium can enhance the growth and immunity of the broiler chickens.

Keywords: Nanoselenium, broiler chicken, selenoprotein W, bursa of fabricius

1. Introduction
Selenium is a dietary essential trace mineral for poultry. Animals obtain selenium directly by ingestion of the plants or indirectly via intake of selenium containing dietary components of plants or animal origin or by dietary supplementation. Dietary antioxidants such as selenium is important in protecting against the free radical generation, oxidative stress and tissue damage as it is a vital component of selenoenzymes such as glutathione peroxidase, phospholipid peroxidase and thioredoxin reductase. Selenium is pharmacologically active and optimisation of selenium nutrition of poultry will result in increased efficiency of egg and meat Surai (2000) [1].

Selenium participates in various physiological functions, mostly as an integral component of a range of selenoproteins. Selenoprotein W (SelW), the smallest identified selenoprotein, consists of approximately 85-88 amino acids. SelW is ubiquitously expressed in tissues, and its expression is regulated by selenium status and intake. Selenoprotein W mediates T cell immunity through antioxidant mechanisms and thus responds to stress. Se deficiency reduced the expression of SelW and produced statistically significant decreases in SelW mRNA (73%) levels Whanger (2009) [2].

Inspite of these beneficial roles, at higher dietary levels, many selenium compounds can become toxic Spallholz (1994) [3]. The toxicity of selenium mainly depends upon the concentration and the chemical form in which it is present in the diet. Currently, sodium selenite (Na_2SeO_3) and selenate (Na_2SeO_4) as inorganic forms, and selenium enriched yeast as organic form are being used as commercial selenium supplements in livestock and poultry diets.

Compared to organic form of selenium, the inorganic selenium salts have inherent toxicity and low bioavailability. The commercial organic form of selenium has less toxicity and higher bioavailability, but is costlier which limits its usage. Thus, exploring a cost-effective selenium source with high bioavailability and low toxicity is necessary.

Application of nanotechnology in trace mineral nutrition may allow approaches to intervene the absorption into biological system as well as enhance bioavailability and increase retention Hilty *et al.* (2010) [4].

Nanoparticles have high surface activity, many surface-active centres, high catalytic efficacy, strong adsorbing ability and have extensive domino effect. Nanoselenium exhibits novel properties different from other selenium sources and possess equal efficacy compared to organic form of selenium. Similarly, the toxicity of nanoselenium is seven times lower than that of inorganic selenium and three times lower than that of organic selenium. Nanoselenium improved the growth performance, feed conversion efficiency and immune status Cai *et al.* (2012) [5] in broiler chickens. The range between optimal and toxic dietary levels of nanoselenium was wider than that of sodium selenite, and also, nanoselenium was more efficiently retained in the body than sodium selenite Hu *et al.* (2012) [6]. As not much research work has been reported regarding the influence of supplementing nanoselenium on the Sel W mRNA expression in the bursa of broiler chickens, the present work was undertaken.

2. Materials and Methods

2.1 Preparation of nanoselenium

Nanoselenium was prepared according to the method described by Zhang *et al*. (2001) [7] using sodium selenite, reduced glutathione and bovine serum albumin.

2.2 Characterisation of nanoselenium

Samples for transmission electron microscopy (TEM) analysis were prepared by drop-coating selenium nanoparticles solution on to carbon-coated copper TEM grids. Transmission electron micrographs were obtained on JEM- 2100F (JEOL Inc., Japan) instrument with an accelerating voltage of 80 kV.

2.3 Biological trial

The nanoselenium (15-35 nm) synthesized was used for the biological trial in broiler chicken. A biological trial with a total of 180 numbers of day-old broiler chickens divided into six treatment groups with three replicates each were used to study influence of nanoselenium on the Sel W mRNA expression in the bursa of broiler chickens. The five treatment groups supplemented with 0.3 mg/kg sodium selenite (T2), 0.3 mg/kg organic selenium (T3), nanoselenium at three graded doses viz.0.15 mg/kg (T4), 0.3 mg/kg (T5) and 0.6 mg/kg along with one group (T1) maintained on only the basal diet served as control with no additional supplementation of selenium. At the end of the experiment (42nd day), six birds per treatment group were randomly selected and slaughtered.

2.4 Selenoprotein W mRNA expression studies

The quantification of selenoprotein W mRNA expression was done by SYBR green based real time PCR Yu *et al*. (2011) [8]. Briefly, approximately 250 mg of tissue was aseptically removed from the bursa and placed into tubes containing ten times the volume of RNA preserve solution (Helini Biomolecules Ltd., India). The tubes were placed in refrigerating temperature (2 – 8 °C) for 24 h and then transferred to -80 °C deep freezer until further processing.

The bursal tissue (100 mg) was weighed in tubes and 1 ml of TRIzol™ reagent (Invitrogen, China) was added. The RNA extraction was done as per the method described by Yu *et al*. (2011) [8]. The RNA pellets were dissolved in 40 µl of nuclease free water by placing in water bath at 50 ˚C for 20 min. The concentration and purity of the extracted total RNA were determined at 260/280 nm using nanodropspectrophotometer (Implen, Germany) and integrity was assessed by the RNA gel electrophoresis. The RNA was stored at -80 ˚C for subsequent use.

First strand cDNA was synthesized from 1 µg of total RNA using high capacity cDNA reverse transcription kit (Applied Biosystems, USA) according to the manufacturer's instructions. Reactions were performed in a 20 µl reaction mixture containing 2 µl of 10x RT buffer, 0.8 µl of 25x dNTP mix, 2 µl of 10x RT random primer, 1 µl of reverse transcriptase, 1 µl of RNasein and 1 µg of RNA. The remaining volume was made up using triple distilled water. The mixture was incubated at 25 ˚C for 10 min. followed by 37 ˚C for 120 min. and 87 °C for 5 min.

Specific primers for expression of SelW and β-Actin (Bioserve Biotechnologies, India) based on the known sequences were used Sun *et al*. (2011) [9]. SYBR green based real time PCR was performed on a Real time PCR machine ABI 7500 (Applied Biosystems, USA). Reactions were performed in a 25 µl reaction mixture containing 12.5 µl of Fast start universal SYBR Green Master mix (ROX) (Roche, Switzerland), 0.75 µl of each primer (300 nM), 2 µl of cDNA, 9 µl of nuclease free water. The PCR conditions for Sel W and β-Actin consisted of 95 ˚C for 10 min. followed by 40 cycles of 95 ˚C for 15 sec., 65 ˚C for 30 sec. and 72 °C for 30 sec.

A melting curve was run for each plate to confirm the production of a single product. The amplification efficiency for each gene was determined using DART-PCR program. The mRNA relative abundance was calculated according to the method of Pfaffl (2001) [10] accounting for gene specific efficiencies and was normalized to the mean expression of β-Actin.

Primers used

Target gene	Gene Bank Accession number	Primer	Sequence (5'–3')	PCR fragment length (bp)
β-Actin	L08165	Forward Reverse	5'-ACCGCAAATGCTCTAAACC-3' 5'-CCAATCTCGTCTTGTTTTATGC-3'	93
Sel W	GQ 919055	Forward Reverse	5′- CTCCGCGTCACCGTGCTC-3′ 5′-CACCGTCACCTCGAACCA TCCC-3′	150

3. Results and Discussion

3.1 Characterisation of nanoselenium

The particle sizes of synthesised spherical nanoselenium (Fig.1) were found to lie in the range of 15-35 nm. This is in agreement with the size of 20-60 nm reported by Zhang *et al.* (2001) [7] who prepared nanoselenium using reduced glutathione and bovine serum albumin. The aggregation of Se^0 atoms was well controlled by the presence of protein (bovine serum albumin) in the redox solution.

3.2 Selenoprotein W mRNA expression studies

The relative fold change in SelW mRNA expression in the bursa of broiler chicken fed with different selenium sources is presented in (Fig. 2). As compared with the control, the SelW mRNA expression in the bursa was found to increase by 0.146, 0.151, 0.366, 0.757 and 1.095 folds in the birds fed

with 0.3 mg/kg sodium selenite, 0.3 mg/kg organic selenium, 0.15 mg, 0.3 mg and 0.6 mg of nanoselenium/kg diet, respectively. Thus, a source and dose related increase of the SelW mRNA expression was observed in the bursa of Fabricius. It could be inferred that supplementing 0.6 mg/kg nanoselenium in chickens resulted in highly increased expression of selenoprotein W in bursa. These results concurred with Yu *et al*. (2011) [8] who reported that the SelW mRNA expression in the bursa of Fabricius of chickens was highest (9.76 folds) in birds fed 5 mg/kg sodium selenite when compared with the control group. Increasing the dietary content of sodium selenite to >3 mg/kg diet, SelW levels decreased in the thymus and, in contrast, increased in the bursa of Fabricius.

Similarly, SelW mRNA abundance in the spleen of rats fed the diets containing 0.1–4.0 mg/kg Se dose dependently increased with increasing dietary Se content Yeh *et al*.(1997a) [11]. The level of SelW mRNA in spleen was significantly elevated in sheep fed Se-supplemented diets. SelW mRNA levels were found to be significantly lower in the spleen of sheep fed a deficient diet Yeh *et al*. (1997b) [12]. These data suggest that SelW mRNA expression is generally sensitive to Se dietary supplementation in this tissue in mammals.

The biological function of Se is primarily elicited through selenoproteins Stadtman (2000) [13], a group of proteins that contain the amino acid selenocysteine (Sec) as an integral part of their polypeptide chain Yu *et al*. (2011) [8]. In mammals, approximately 30 families of selenoproteins have been identified Kryukov *et al.* (2003) [14]. Many of the Se-containing enzymes and proteins are essential for the normal growth, development, functioning and metabolism of organisms Castellano *et al*. (2005) [15]. Selenoproteins influence inflammatory responses by regulating the oxidative state of immune cells McCord (2000) [16]. Mice with a T-cell-specific deletion in tRNASec, resulting in knockout of all T-cell selenoproteins, have significantly diminished numbers of functional T cells and exhibited moderate to severe atrophy of the thymus, spleen, and lymph nodes. Furthermore, the mice have reduced antigen-specific production of immunoglobulins *in vivo*, implying a dysfunctional adaptive immune response Shrimali *et al.* (2008) [17]. A complete lack of selenoproteins in T cells led to decreased pools of mature T cells and atrophy of the thymus, spleen, and lymph nodes Shrimali *et al* (2008) [17]. The thymus, bursa of Fabricius, and spleen have selenium affinity and they are site for production and maturation of T cells and B cells Kiremidjian *et al*. (1994) [18].

SelW is a small selenoprotein, Yeh *et al*. (1997a) [11] and in which selenocysteine is located in the N-terminal portion of a relatively short functional domain. Cells that overexpress SelW have been found to be more resistant to oxidative stress. It has been suggested that the expression of selenoprotein W plays an important role in protection of cells from oxidative stress in the cellular defense system Wang *et al*. (2010) [19]. Se and SelW play an important role in the immune organs of birds and during the diseases of immune system. SelW is an antioxidant protein, and it plays an important role in cellular defense against oxidative damage and Se metabolic pathways in the immune system of birds. The SelW gene was widely expressed in the spleen in rats Yeh *et al*. (1997) [11] and sheep Yeh *et al*. (1997b) [12], and its expression level was generally sensitive to Se status in these animals. Se exerts its biological activity as the amino acid Sec and is incorporated into selenoproteins in all Archaea, Bacteria, and Eukaryas. The incorporation of Se as Sec into a selenoprotein requires a specific mechanism to decode the UGA codon in mRNA, which normally operates in translation termination Gu *et al.* (2000) [20]. The Sec insertion into the selenoproteins is specified by a UGA codon, which is normally read as a stop signal. The translation of selenoprotein mRNAs into selenoproteins requires a cis-acting SECIS element, which is a hairpin structure present in the 3'-untranslated regions (3'-UTRs) of selenoprotein mRNAs in eukaryotes Li *et al*. (2010a) [21], and several transacting factors Li *et al.* (2010b) [22]. Thus, selenoprotein expression depends on dietary Se intake. Initially, the dietary selenium undergoes reductive metabolisms yielding hydrogen selenide (H_2Se) which is converted to Sec Butlet *et al*. (1999) [23]. Hydrogen selenide must be first transformed to Sec, for which H_2Se has to be metabolized into selenophosphate after catalysis by the SEPHS2 Butlet *et al*. (1999) [23]. Selenophosphate reacts with the tRNA specific for serine Ser-tRNASec via the enzyme Sec-tRNA synthase to form Sec bound tRNA (Sec-tRNASec), from which Sec is incorporated into selenoproteins by the Sec specific UGA codon Yeh *et al*. (1995) [24]. Thus, the influence of Se content was not limited to the regulation of SelW expression but extends to components of the selenoprotein biosynthesis machinery.

The selenoprotein SelW plays a vital role as antioxidant in both the immune mediating cells and the immune organs as a whole, apart from mediating T cell immunity, especially during stress Whanger (2011) [2]. SelW downregulated the mRNA expressions of inflammatory factors (iNOS, COX-2, NF-κB, PTGEs, and TNF-α) in the chicken immune organs (spleen, thymus and bursa of Fabricius). SelW played an important role in the protection of immune organs of birds from inflammatory injury by the regulations of inflammation-related genes Yu *et al*. (2014) [25].

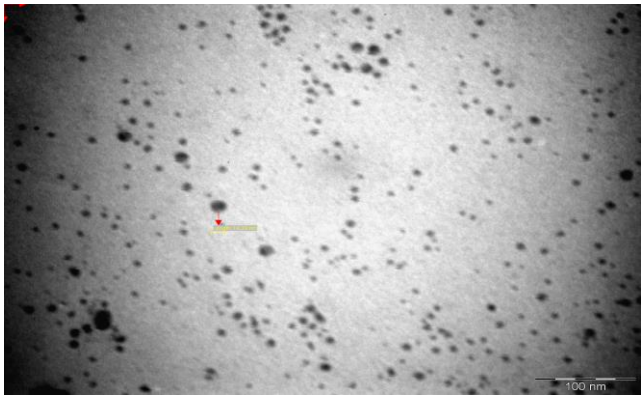

Fig 1: TEM image of nanoselenium

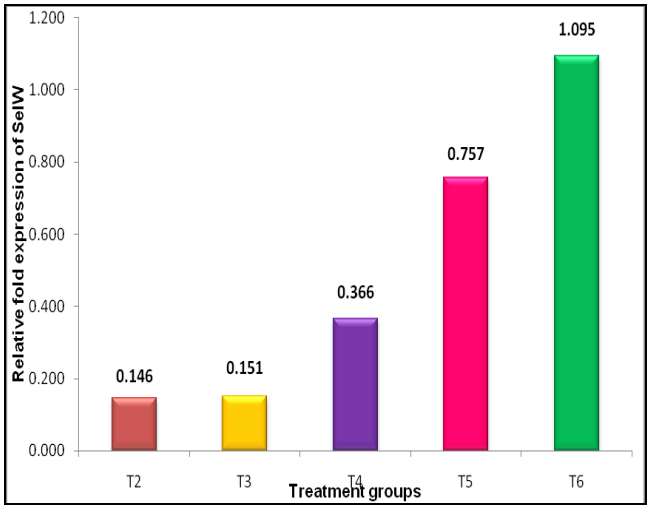

Fig 2: Relative fold expression of Sel W mRNA in bursa of Fabricius at sixth week in broiler chicken fed inorganic, organic and nanoselenium

4. Conclusion

Selenium plays an important role in the exhibition of immune response through the selenoproteins. Hence, it could be concluded that selenium supplementation increased the SelW mRNA expression in the bursa of chicken in a source and dose dependent manner. An increased SelW mRNA expression in the bursa of nanoselenium supplemented groups enhanced the immunocompetance in these birds. Thus, the nanoselenium supplemented (0.6 mg/kg) birds had higher level of immunity and hence higher body weight.

5. References

1. Surai PF. Organic selenium: benefits to animals and humans, a biochemist's view. In: T.P. Lyons and K.A. Jacques (Eds.) Biotechnology in the Feed industry. Proc. of Alltech's 16th Annual Symposium. Nottingham University Press, Nottingham, UK, 2000, 205-260.
2. Whanger PD. Selenoprotein expression and function-Selenoprotein W. Biochimica et Biophysica Acta., 2011; 1790:1448-1452.
3. Spallholz JE. On the nature of selenium toxicity and carcinostatic activity. Free Radical Biology and Medicine. 1994; 17:45-64.
4. Hilty FM, Arnold M, Hilbe M, Teleki A, Knijnenburg JTN, Ehrensperger F *et al*. Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nature Nanotechnology. 2010; 5:374-380.
5. Cai SJ, Wu CX, Gong LM, Song T, Wu H, Zhang LY. Effects of nanoselenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. Poultry Science. 2012; 91:2532-2539.
6. Hu CH, Li YL, Xiong L, Zhang HM, Song J, Xia MS. Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. Animal Feed Science and Technology. 2012; 177:204-210.
7. Zhang JS, Gao XY, Zhang LD, Bao YP. Biological effects of a nano red elemental selenium. Biofactors. 2001; 15:27-38.
8. Yu D, Li JL, Zhang JL, Gao XJ, Xu S. Effects of dietary selenium on selenoprotein W gene expression in the chicken immune organs. Biological Trace Element Research. 2011; 144:678-687.
9. Sun B, Wang R, Li J, Jiang Z, Xu S. Dietary selenium affects selenoprotein W gene expression in the liver of chicken. Biological Trace Element Research. 2011; 143:1516-1523.9.
10. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research. 2001; 29:e45
11. Yeh JY, Vendeland SC, Gu Q, Butler JA, Ou BR, Whanger PD. Dietary selenium increases Selenoprotein W levels in rat tissues. Journal of Nutrition. 1997a; 127:2165-2172
12. Yeh JY, Gu QP, Ma B, Forsberg NE, Whanger PD. Selenium influences tissue levels of selenoprotein W in sheep. Journal of Nutrition. 1997b; 127:394-402.
13. Stadtman TC. Selenium biochemistry. Mammalian selenoenzymes. Annals of the New York Academy of Sciences. 2000; 899:399-402.
14. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R *et al* Characterization of mammalian selenoproteomes. Science. 2003; 300(5624):1439-1443.
15. Castellano S, Lobanov AV, Chapple C, Novoselov S, Albrecht M, Hua D *et al* Diversity and functional plasticity of eukaryotic selenoproteins: identification and characterization of the Sel J family. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:16188-16193.
16. McCord JM. The evolution of free radicals and oxidative stress. American Journal of Medicine. 2000; 108:652-659.
17. Shrimali RK, Irons RD, Carlson BA, Sano Y, Gladyshev VN, Park JM *et al*. Selenoproteins mediate T cell immunity through an antioxidant mechanism. Journal of Biological Chemistry.2008; 283:20181-20185.
18. Kiremidjian-Schumacher L, Roy M, Wishe HI, Cohen MW, Stotzky G. Supplementation with selenium and human immune cell functions, II: effect on cytotoxic lymphocytes and natural killer cells. Biological trace element research. 1994; 41:115-127.
19. Wang XL, Yang CP, Xu K, Qin OJ. Selenoprotein W depletion *in vitro* might indicate that its main function is not as an antioxidative enzyme. Biochemistry (Mosc). 2010; 75(2):201-207.
20. Gu QP, Sun Y, Ream LW, Whanger PD. Selenoprotein W accumulates primarily in primate skeletal muscle, heart, brain and tongue. Mol Cell Biochem. 2000; 204:49-56.
21. Li JL, Ruan HF, Li HX, Li S, Xu SW, Tang ZX. Molecular cloning, characterization and mRNA expression analysis of a novel selenoprotein: avian selenoprotein W from chicken. Molecular Biology Reports PMID. 2010a; 21132387.
22. Li JL, Li HX, Li S, Jiang ZH, Xu SW, Tang ZX. Selenoprotein W gene expression in the gastrointestinal tract of chicken is affected by dietary selenium. Bio Metals. 2010b; 24:291-299.
23. Butler JA, Xia Y, Zhou YY, Sun Y, Whanger PD. Selenium, cellular glutathione peroxidase and selenoprotein W in fetal tissues from deficient, adequate and excessive selenium areas of China. FASEB Journal. 1999; 13:A248.
24. Yeh JY, Beilstein MA, Andrews JS, Whanger PD. Tissue distribution and influence of selenium status on levels of selenoprotein W. FASEB Journal. 1995; 9(5):392-396

25. Yu D, Zhang Z, Yao H, Li S, Xu S. The role of selenoprotein W in inflammatory injury in chicken immune tissues and cultured splenic lymphocyte. Biometals. 2014; 28:75-87.