International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

CONTEMPLATION UPON NANO RED SELENIUM AND SODIUM SELENITE ON ANTIOXIDANT ENZYMES IN QUAIL UNDER HEAT STRESS

R. Ghazanfarpoor, E. Talebi and A. Abedi

Young Researchers and Elite Club, Darab Branch, Islamic Azad University, Darab, Fars, Iran

ABSTRACT

This study was designed to investigate the effects of Nano-Selenium and Sodium Selenite on some blood biochemical parameters in Japanese quail under heat stress condition. In this investigation, 300 male Japanese quails were used which the chickens were raised to 21 days in same condition. The experiment was performed in a randomized complete block design (RCBD) using 3 treatments and 5 replicates with 20 chicks per each replicate. Quails in the control groups were used the basal diet (without selenium supplement) and the first experimental group admitted basal diet enriched with 0.2 mg/kg Nano-Selenium subsequently the second experimental group employed basal diet with 0.2 mg/kg Sodium Selenite. The chickens daily were included under heat stress condition for 8 h at 34± 0.5^{o}C. The results revealed that glutathione peroxidase and superoxide dismutase activity increased significantly in second experimental group using selenium ($p<0.05$). In the present study, superoxide dismutase activity was significantly increased after adding 0.2 mg sodium selenite and nano-selenium ($p<0.05$).

Keywords: Nano-Selenium, Sodium Selenite, Glutathione Peroxidase, Superoxide Dismutase, Heat stress.

INTRODUCTION

Nanotechnology is regarded as the ability to manipulate measure, manufacture and make predictions at the scale of 1–200 nm. At the nanometer dimension, materials revealed novel properties, different to those of both, the isolated atom and bulk material that the properties depending largely on the size of the particles from which the material is made.

The environmental stress can decrease production and all other important economical traits in animal husbandry. The stress is caused by free radicals and reactive oxygen species. Detrimental effects caused by reactive oxygen species occur as a consequence of an imbalance between the formation and inactivation of these species. Oxidative damage may be involved in the pathogenesis of major diseases such as cancer, atherosclerosis, and certain neurological disorders as well as caused by environmental stresses. The free radicals are atoms or molecules which they are active at the last atomic layer due to their strong affinity with their surrounding molecules (Alexanra *et al.*, 2002). Animals need trace mineral elements in the diet to maintain health and proper essential biochemical and physiological function. The relationship between selenium and energy metabolism, increase feed efficiency, as well as improves reproductive and immune systems (Tamilson *et al.*, 2008).

Selenium is importance to human and animal health and it has many biological functions in living organisms, involved in the regulation of antioxidant mechanisms (Rayman, 2000). Approximately, 40 to 50 percent of the total body selenium exists in glutathione peroxidase. In the form of selenocysteine, the 21st amino acid, Se functions as a redox center of an array of selenoproteins (Copeland, 2003; Driscoll and Copeland, 2003), some of which have important enzymic functions for the redox homoeostasis, such as glutathione peroxidase (GPx) (de Haan *et al*., 2003; Miyamoto *et al*., 2003), phospholipid hydroperoxide glutathione peroxidase (PHGPx) (Imai and Nakagawa, 2003; Nakagawa and Imai, 2000) and thioredoxin reductase (TrxR) (Arner and Holmgren, 2000; Becker *et al*., 2000). Selenium in body increases the amount of enzyme activity about 100 to 1000 times (Burk, 2002). Superoxide anion is the first free radical which derived from oxygen and it is turned and neutral to the oxygen and hydrogen peroxide by superoxide dismutase. Subsequently, hydrogen peroxide converted to water by glutathione peroxidase (Mates, 1999). Glutathione peroxidase, superoxide dismutase and catalase as well as non-enzymatic molecules such as vitamins C, E and A, uric acid, bilirubin protect lipids and nucleic acids from oxidative damage which play an important role in protecting the cell membrane (Michiels *et al*., 1994). The important antioxidant enzymes characteristic is the induction of them in oxidative stress conditions. Thus, cells induction cause by oxidative stress and an increase in the activity of antioxidant enzymes exhibit the cell or tissue adaptation to stress condition (John *et al*., 2001). Several studies also have shown low levels or absence of lipid peroxidation and antioxidant enzymes may reflect a protective effect (Nordberg and Arner, 2001). This study was conducted to examine Nano Red Selenium and Sodium Selenite effects on some antioxidant enzymes in quail under heat stress condition.

MATERIALS AND METHODS

Animals and experimental conditions:

In this investigation, 300 Japanese quail chicks were used and the chickens were raised to 21 days in the same condition. The chickens were kept in cages 50 × 90 cm. The experiment was performed in a randomized complete block design (RCBD) with 3 treatments and 5 replicates using 20 chicks per replicate. The chickens daily were included under heat stress condition for 8 h at 34± 0.5°C.

Preparation of Nano Red Selenium:

The nano red selenium was prepared by Zhang *et al.* (2004) method. Ingredients for preparation of the nano red selenium were including ascorbic acid and SeO_2. The ascorbic acid solution was added into the aqueous solution of SeO_2 to initiate the reaction. After the addition of ascorbic acid, nano red selenium particles began to formation by color changing from colorless to red. The nano red selenium particle size was obtained in range of 80 to 200 nm which determined by Scanning Electron Microscopy. Sodium Selenite was purchased from Sigma Corporation of America.

Ration:

During the first 21 days, quail chickens were fed basal diet without selenium supplement. The basal diet (Table 1) was prepared according to National Research Council (NRC). The treatments included 1: basal diet (without selenium supplement), 2: basal diet + 0.2 mg/kg sodium selenite, 3: basal diet + 0.2 mg of selenium nanoparticles.

Item %		Calculated values	
Corn grain	43.2	(Kcal/kg) ME	3000
Barely grain	0.2	CP %	24.84
Wheat bran	0.2	Lys %	1.40
Soybean meal	47.79	Met %	0.522
Soybean oil	5.44	Met + Cys %	0.916
Oyster shell	1.35	Ca %	0.839
DCP	0.8	Available phosphorus %	0.315
Salt	0.37	Na %	0.158
DL-Met.	0.15		
Vitamin supplement	0.25		
Mineral supplement *	0.25		

Table 1: Feed and chemical composition of basal diet

*The mineral supplement was free of selenium.

Sampling:

Two chicks per pen were weighted close to the average weight per cage. The blood samples were taken under the wing vein. Blood samples collected in tubes containing anticoagulant (heparin). After hematocrit and hemoglobin determination using a kit from Randox (England), superoxide dismutase and glutathione peroxidase activity was measured in red blood cells.

Measurement of Glutathione peroxidase:

Glutathione peroxidase (glutathione peroxidase) catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. With the exception of phospholipid-hydroperoxide glutathione peroxidase, a monomer, all of the glutathione peroxidase enzymes are tetramers of four identical subunits. GPX is catalyzed oxidation of glutathione (GSH) by Komen hydroperoxides. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) immediately reacts with the recovery in output and the oxidation of NADPH into $NADP^+$. For glutathione peroxidase determination, absorbance is measured at 340 nm.

Measurement of superoxide dismutase:

The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress. Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism. In this method, using xanthine oxidase is able to react with 2-(4–nitro phenol) -3-5- phenol tetrazolium chloride to produce red formazan at wavelength of 505 nm. For determination of enzyme activity in presence of SOD enzyme, superoxide radicals converted to hydrogen peroxide and O_2 to produce red formazan and it is inhibited to produce red.

Statistical analysis:

The data were analyzed by following statistical model:

$Y_{ij} = \mu + T_i + R_j + e_{ij}$

Y_{ij}: each observation

μ: overall mean,

T_i: treatment effect,

R_j: block effect (cage floor)

e_{ij}: error

The results were analyzed using SAS software (version 14) through two-way analysis and Duncan test.

Results and Discussion:

According to antioxidant role of selenium and antioxidant enzymes induced in stress conditions, the effect of sodium selenite and nano selenium was compared on antioxidant enzymes activity. The result revealed that the addition of 0.2 mg/kg nano selenium and sodium selenite increased significantly glutathione peroxidase activity than the control group ($p<0.05$) (Figure 1). Nano selenium on the activity of

the enzyme was more effective compare to sodium selenite, but it was not significant (p>0.05). Payen and Southern (2005) reported that selenium has positive effect on blood glutathione peroxidase activity in poultry. Increased blood levels of glutathione peroxidase in broiler when there is a sufficient amount of selenium in diet play an important protective response against oxidative damage which caused by heat stress (Altan *et al*., 2003). Zeinali *et al.* (1388) and Ramazani *et al.* (1390) exhibited that addition of 0.3 mg/kg selenium to diet can increase glutathione peroxidase activity under heat stress condition. Camel *et al.* (2003) showed positive effects of selenium supplement on glutathione peroxidase activity in chicken's blood under heat stress conditions. Recent research has also reported that using of Nano selenium in broiler diet caused a significant increase in glutathione peroxidase activity and free radical scavenging (Cai *et al*., 2012). Paton *et al*. (2002) reported that addition of selenium to diet with selenium deficiency can increase GPx activity. Addition of selenium to the diet is caused in higher retention of vitamin E and vitamin E in plasma also reduce production of Hydro peroxidase which decreased glutathione to protect cells by reducing the need and this could be the reason for increased glutathione peroxidase (Lin *et al.*, 2006). Contor *et al.* (1975) reported that usage of control diet and diets containing organic or inorganic selenium sources in amount of 0.2 mg/kg were no significant effect on glutathione peroxidase activity. The comparison between nano red selenium and sodium selenite on glutathione peroxidase activity and antioxidant systems demonstrated that nano red selenium is suitable for increasing glutathione peroxidase activity in quail under heat stress. Xu *et al.* (2003) showed that the most appropriate form of selenium is nano-selenium, selenomethionine and sodium selenite.

Another part of the defensive system of enzymatic antioxidant is defense system of cells against oxidative stress. The superoxide dismutase is a rapid removal of free radicals. Kucuk *et al*. (2003) stated that superoxide dismutase is the first line of cellular defense against toxic free radicals caused by free radicals. Thus, superoxide dismutase is an important enzyme for prevention of cell lipid peroxidation and production of free radicals such as O_2 and HO in blood and tissues. Antioxidant such as selenium, vit E *etc*. increase antioxidant enzyme levels in the blood of chickens under stress condition.

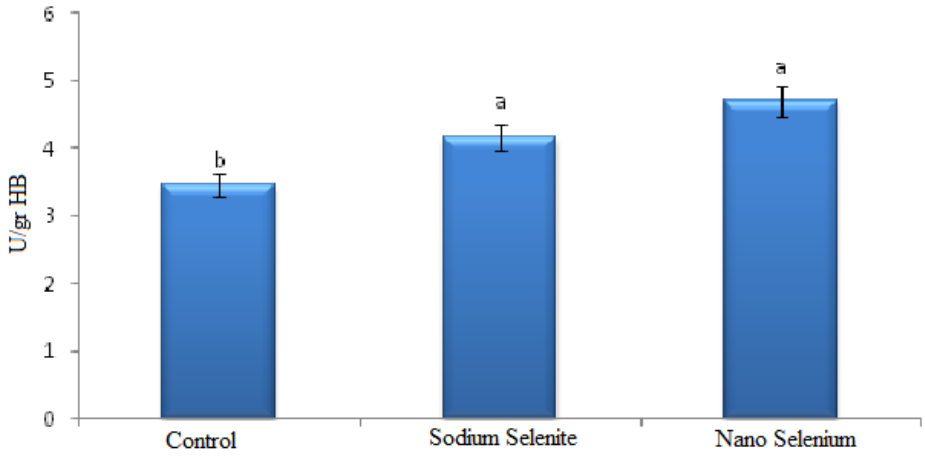

Figure 1: Effect of Nano Selenium and Sodium Selenite on GPx in quial under heat stress condition

In the present study, superoxide dismutase activity was significantly increased after adding 0.2 mg sodium selenite and nano-selenium ($p<0.05$) (Figure 2). Zeinali *et al.* (2009) and Ramazani *et al.* (2011) also reported significant effect of selenium supplementation on serum superoxide dismutase levels in broilers under heat stress. Angkananporn and kijiparkorn (2003) revealed that chickens fed with selenium supplementation showed significantly higher level of SOD (superoxide dismutase activity) compared to the control group, which is consistent with the results of this study. Furthermore, Pirseljein *et al*. (2006) reported that dietary supplementation of organic selenium had different effect on superoxide dismutase activity in broiler. Researchers have shown that superoxide anion is the first oxygen-derived free radicals to oxygen and hydrogen peroxide by SOD. Subsequently, hydrogen peroxide converts to water by glutathione peroxidase (Mates, 1999).

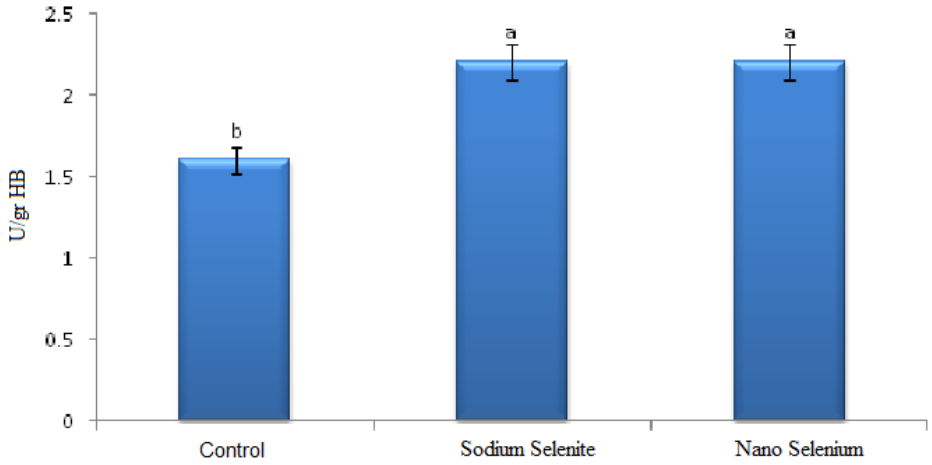

Figure 2: Effect of Nano Selenium and Sodium Selenite on SOD in quial under heat stress condition

Glutathione peroxidase and superoxide dismutase activity is measured in whole blood and red blood cells and the enzyme compared with hematocrit and hemoglobin and discussion on hematocrit and hemoglobin is important for this type research. Experiments showed that selenium deficiency is associated with anemia due to reduced hemoglobin. Selenium protects the hemoglobin and iron absorption from the intestine is inhibited (Pavlik *et al*., 2012). The results of this study indicated that the usage of selenium supplements increase blood hematocrit compare to control group (Figure 3), but this increase was not significant ($p>0.05$). Between the two groups using sodium selenite and Nano-Selenium was also no difference in percentage of hematocrit.

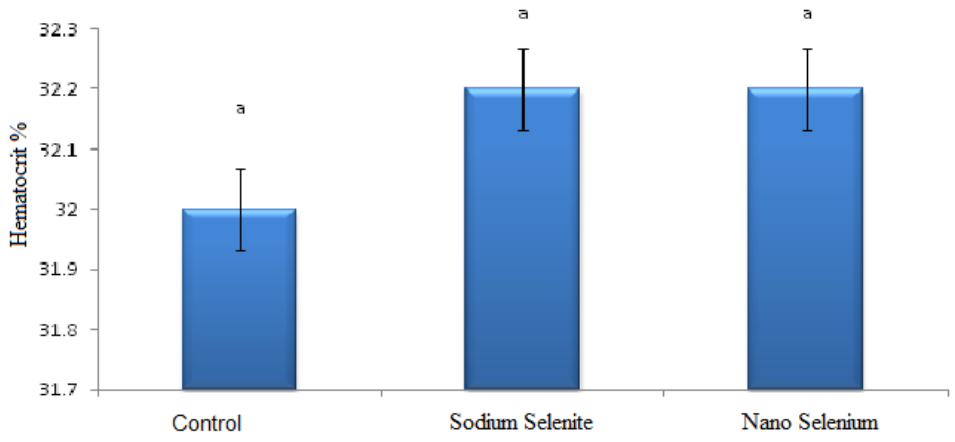

Figure 3: Effect of Nano Selenium and Sodium Selenite on hematocrit percentage in quial under heat stress condition

The Selenium supplements increased significantly hemoglobin compared to control group ($p<0.05$) (Figure 4) and the use of nano-selenium treatment compare to sodium selenite exhibited higher hemoglobin but the difference was not significant. Mohri *et al*. (2005) reported that cows injected with selenium were significantly increased hematocrit, hemoglobin and white blood cells in calves which are consistent with the results of our study. According to antioxidant protective effect of selenium reduced excretion of electrolytes such as iron which is the main component of hemoglobin under heat stress condition and subsequently hemoglobin will be increase. Therefore, selenium is increased hematocrit and hemoglobin that increase in hemoglobin and hematocrit levels of selenium can be used to protect cell membranes and intracellular tissues by antioxidant effects of this element. This is likely to increase the life span of red blood cells.

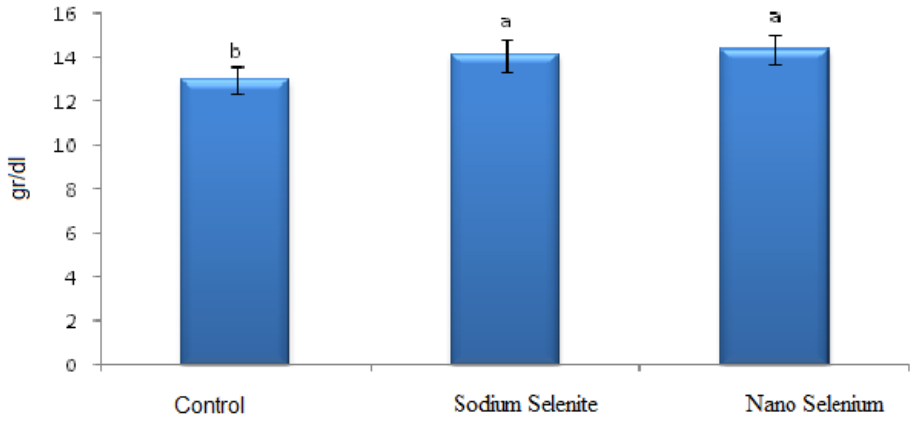

Figure 4: Effect of Nano Selenium and Sodium Selenite on hemoglobin in quial under heat stress condition

It is clear from the results that nano-selenium supplementation increases glutathione peroxidase enzyme and hemoglobin compare to sodium selenite. This is probably due to less toxicity, bioavailability, higher absorption and catalytic efficiency of selenium nanoparticles (Zhang *et al*., 2001).

REFERENCES

1. Alexandra K, Adams, MD, Thomas M. Best MD. The role of antioxidants in exercise and disease prevention. *The physician and sports medicine*, 2002; **30** (5): 37-44.
2. Altan O, Pabuccuoglu A, Altan A, Konyalioglu S, Bayraktar H. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Br. Poult. Science*, 2003; **44**: 545-550.
3. Angkanaporn K, Kijparkorn S. Effect of selenium supplementation on growth performance, thyroid hormone (T_3) levels, antioxidant enzyme and disaccharidase activities in broiler chicks. Veterinary thesis, The Chulalongkorn University Faculty of Veterinary Science. Thailand, (2003).
4. Arner ESJ, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur. J. Biochem*, 20002; **267**: 6102–6109.
5. Becker K, Gromer S, Schirmer RH, Muller S. Thioredoxin reductase as a pathophysiological factor and drug target. *European Journal of Biochemistry*, 2000; **267**: 6118–6125.
6. Burk RF. Selenium, an antioxidant nutrient. Nutrition in clinical Care, 2002; 2: 75-79.
7. 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. *Poult Sci*, 2012; **91**: 2532-2539.
8. Cantor AH, Langevin ML, Noguchi T, Scott ML. Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. *J. Nutrition*, 1975; **105**: 106-111.
9. Copeland PR. Regulation of gene expression by stop codon recoding: selenocysteine. *Gene*, 2003; **312**: 17–25.
10. De Haan JB, Crack PJ, Flentjar N, Iannello RC. Hertzog, P.J., Kola, I. An imbalance in antioxidant defense affects cellular function: the pathophysiological consequences of a reduction in antioxidant defense in the glutathione peroxidase-1 (Gpx1) knockout mouse. *Redox Report*, 2003; **8**: 69–79.
11. Driscoll DM, Copeland PR. Mechanism and regulation of selenoprotein synthesis. *Annu. Rev. Nutr*., 2003; **23**: 17–40.
12. Imai H, Nakagawa Y. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic. Biol. Med*., 2003; **34**: 145–169.
13. John S, Kale M, Rathore N, Bhatnagar D. Protective effect of vitamine E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J. Nut Biochem*, 2001; **12**: 500-504.
14. Kucuk O, Sahin N, Sahin K, Gursu MF, Gulcu F, Ozcelic M, Issi M. Egg production, egg quality and lipid peroxidation status in laying hens maintained at a low ambient temperature (6°C) and fed a vitamin C and vitamin E-supplemented diet. *Vet. Med-Czech*, 2003; **48**: 33-40.
15. Lin H, Sui SJ, Jiao HC, Buyse J, Decuypere E. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. *Comp. Biochem. and Physio*, 2006; **143**: 400-405.
16. Mates JM, Perez-Gomez C, Decastro IN. Antioxidant enzymes and human diseases. *Clin Biochem*, 1999; **32**:595-603.

17. Michiels C, Raes M, Toussaint O, Remacle J. Importance of Se glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress. *Free Radical Biology and Medicine*, 1994; **17**: 235-248.
18. Miyamoto Y, Koh YH, Park YS, Fujiwara N, Sakiyama H, Misonou Y, Ookawara T, Suzuki K, Honke K, Taniguchi N. Oxidative stress caused by inactivation of glutathione peroxidase and adaptive responses*. Biol. Chem*. 2003; **384**: 567–574.
19. Mohri M, Seifi HA, Khodadadi J. Effects of preweaning parenteral supplementation of vitamin E and selenium on hematology, serum proteins, and weight gain in dairy calves. *Comp Clin Pathol*., 2005; **14**: 149-154.
20. Nakagawa Y, Imai H. Novel functions of mitochondrial phospholipid hydroperoxide glutathione peroxidase (PHGPx) as an anti-apoptotic factor. *J. Health Science*, 2000; **46**: 414–417.
21. Nordberg J, Arner E. Reactive oxygen species, antioxidant, and the mammalian thioredoxin system. *Free Radic Biol Med*, 2001; **31**: 1287-312.
22. Paton ND, Cantor AH, Pescatore AJ, Ford MJ, Smith CA. The effect of dietary selenium source and level on the uptake of selenium by developing chick embryos. *Poult. Sci*, 2002; **81**: 1548-1555.
23. Pavlik A, Slama P, Lichovnikova M, Havlicek Z. Effect of dietary selenium forms on selenium blood concertration in laying hens. Journal of Microbiology, *Biotechnology and Food Sciences,* 2012; **1**: 1082-1089.
24. Payne RL, Southern LL. Changes in glutathione peroxidase and tissue selenium concentrations of broiler after consuming a diet adequate in selenium. *J. Poult Sci*, 2005; **84**: 1268-1276.
25. Pirsljin J, Milinkovic Tur S, Beer Ljubic B, Zdelar-Tuk M, Poljicak-Milas N. Influence of organic selenium food supplements on age-related changes on antioxidant system in thigh muscle of broiler chicken. *Biochemical Journal*, 2006; **21**: 539-541.
26. Ramezani S, Riasi A, Afzali N, Fathi Nasari MA. Effect of selenium and sodium bicarbonate supplementation diets on blood biochemical properties, growth performance and carcass traits of broilers in heat stress condition. *Veterinary Journal*, 1390; **90**: 13-22.
27. Rayman MP. The importance of selenium to human health. *Lancet*, 2000; **356**: 233–241.
28. Sahin K, Kucuk O. Effects of Vit. E and selenium on performance, digestibility of nutrients and carcass characteristics of Japanese quails reared under heat stress. J. Ainm. Physiol. *Anim. Nutr*., 2001; **85(11-12)**: 342-348.
29. Tomlinson DJ, Socha MT, De Frain JM. Role of trace minerals in the immune system, Penn State Dairy Cattle Nutrition Workshop, 2008; 39-52.
30. Xu, BH, Xu ZL, Xia MS. Effect of nano red elemental selenium on GPx activity of broiler chick kidney cells in vitro. Wuhan Univ. *J. Nat. Sci.,*2003; **8 (4)**: 1167-1172.
31. Zainali A, Riasi A, Kermanshahi H, Farhangfar H, Ziaie H. Effect of Sodium Selenite and Turmeric Powder on Growth Performance, Carcass Quality and Blood Antioxidant Metabolites of Heat Stressed

Broiler Chickens. *Journal of Animal Science*, 2006; **19(2)**: 69-85.

32. Zhang JS, Gao XY, Zhang LD, Bao YP. Biological effects of a nano red elemental selenium. *Biofactors*, 2001; **15**: 27-38.
33. Zhang Y, Zhang J, Wang HY, Chen HY. Synthesis of selenium nanoparticles in the presence of polysaccharides. *Materials Letters*, 2004; **58**: 2590-2594.