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Influence of Nanoselenium Supplementation on the Thyroid Hormones and Blood Biochemical Status in Broiler Chicken

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A B S T R A C T

Trace minerals like selenium are regularly added to the diet of broiler chickens as feed supplements to enhance the immune status, antioxidant defence system, meat quality, immune statues and overall growth performance. Selenium is an essential nutritional trace element having various vital role in system mediated selenoproteins. Dietary selenium supplementation in the poultry has been regularly practiced using the inorganic and organic forms. These forms have the limitations of low safety margin and long term accumulative toxicity. Hence, an alternate selenium, hence an alternate form of selenium i.e. nano selenium having greater potential as poultry and livestock feed supplement with higher bioavailability, higher margin of safety and seven fold lower acute toxicity was prepared using starch, ascorbic acid and bovine serum albumin. The nanoselenium (15-40 nm) synthesized were characterized by XRD analysis, transmission electron microscopy and UV spectrophotometry. This nanoselenium was used for the biological trial in broiler chickens owing to simplicity and easy adaptability for large scale production. 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 diet alone. Blood was collected at the end of fourth and sixth weeks and the blood glucose, total cholesterol, triglycerides and thyroid hormone were estimated. The mean plasma glucose, total cholesterol and triglycerides did not differ significantly between treatment and control groups at the end of both fourth and sixth weeks of age. However, these parameters showed an increasing trend from the fourth to sixth week. The triiodothyronine levels and the thyroid hormone activation ratio showed an increasing trend, while thyroxine level exhibited decreasing trend with the level of nanoSe supplementation. The organic and nanoSe supplementation at 0.3 and 0.6 mg/kg might have favoured an efficient conversion of thyroxine to triiodothyronine than inorganic Se and the control group. Hence, it could be inferred that supplementing the nanoselenium (0.3 – 0.6mg/kg diet) did not alter the blood biochemical constituents. The organic and nanoselenium supplementation at 0.3 and 0.6 mg/kg resulted in efficient conversion of thyroxine to triiodothyronine and increased the triiodothyronine level compared to inorganic selenium supplemented and control groups with minimal risk of toxicity and better bioavailability.

Introduction

Modern poultry rearing system impose multitude of stress the broiler chickens such as high stocking density, higher metabolic demand, disease susceptibility, environmental stress and inadequate micronutrients and trace minerals. These stress factors disrupt the delicate critical balance existing between antioxidant defense system and repair mechanisms in tissues, thus generating free radicals leading to tissue damage. Higher level of free radical accumulation in tissues damages the cell membrane and DNA and thereby depresses growth rate (Morrissey and O' Brien, 1998). These stressors are responsible for causing immunosuppression. Incompetency of the immune system increases susceptibility to various infectious diseases resulting in decreased productive performance of the birds.

Glutathione peroxidase, thioredoxin reductase, reduced glutathione and tissue thiols are endogenous antioxidant systems which provide defense against free radicals in tissues. Owing to the faster growth rate in broiler chickens, production of endogenous antioxidant systems is insufficient to scavenge the free radicals. Hence, various synthetic and natural antioxidants are being added in the poultry diet (Godin and Wohaieb, 1988).

Trace minerals like selenium are added to the diet of broiler chickens to enhance the antioxidant, immune statues and overall growth performance. Selenium has a wide range of biological functions in the body (NRC, 1994). 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 (Arner and Holmgren, 2000; Surai, 2000).

Currently, sodium selenite (Na_2SeO_3) and selenate (Na_2SeO_4) as inorganic form, and selenium enriched yeast (SY) as organic form are being used as commercial selenium supplements in animal and poultry diets (FDA, 2002).

Compared to organic form of selenium, the inorganic selenium salts have inherent toxicity and low bioavailability. Because of these properties, inorganic selenium salts cannot be added in the diet more than the recommended level, though more selenium is required during stress and disease conditions. Hence, the organic selenium in the form of SeMet and selenium enriched yeast is used in nutritional supplements due to their excellent bioavailability and lower acute toxicity among various selenium forms (Schrauzer, 2003). But as SeMet can be nonspecifically incorporated into proteins in place of methionine, concerns have been raised that SeMet could potentially cause accumulation of selenium in tissues to toxic levels (Waschulewski and Sunde, 1988). Thus, exploring a cost effective selenium source with high bioavailability and low toxicity is necessary.

In this regard, nanotechnological approaches in trace mineral nutrition allows intervention in the absorption process in biological system as well as enhances bioavailability (Hilty *et al.,* 2010). Nanoparticles have higher surface activity, high catalytic efficacy, strong adsorbing ability and have extensive domino effect (Xu *et al.,* 2003). 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 (Peng *et al.,* 2007). Currently, nano elemental selenium (nanoSe), is used in nutritional supplements and has promising applications in medical therapy

(Zhang *et al.,* 2001 and Gao *et al.,* 2002). Nanoselenium improved the growth performance, feed conversion efficiency (Zhou and Wang, 2011) and antioxidant enzyme activity (Cai *et al.,* 2012) 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 absorbed in the body than sodium selenite (Hu *et al.,* 2012).

Furthermore, Zhang *et al.,* (2005) reported that nanoSe possessed higher efficiency than selenite, selenomethionine, and methylselenocysteine (Zhang *et al.,* 2008; Wang *et al.,* 2007) in upregulating selenoenzymes in mice and rats and exhibited lesser toxicity (Zhang *et al.,* 2001).

Even though studies have conducted earlier to assess the effect of selenium in poultry, there is only little research done to study the influence of nanoselenium on thyroid hormones and status of blood biochemical constituents in broiler chickens. Consequently, the aim of the present study was to determine the effects of dietary supplementation of nanoselenium on thyroid hormones and biochemical status in broiler chickens and to compare its efficacy with the inorganic and organic forms.

Materials and Methods

Preparation and characterisation of nanoselenium

Nanored selenium particles were synthesized as per the method described by Zhang *et al.,* (2001) with slight modification using sodium selenite, starch, ascorbic acid and bovine serum albumin. The compositional analysis of the samples were studied based on the energy dispersive analysis of X-Rays using PANalytical X-Ray diffractometer (JEOL Model JED-2300). 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.

A biological trial was conducted with one hundred and eighty numbers of day-old straight broiler chicks (Vencobb 400) obtained from commercial hatchery. The birds were wing banded, weighed and randomly allotted to six groups with three replicates of ten chicks each based on the body weight. The birds were reared in cages under uniform standard managemental practices upto six weeks of age.

Sodium selenite and Selplex™(Alltech, USA) were used as inorganic and organic selenium supplement forms in the diets.

The nanoselenium synthesized in the department of Veterinary Physiology, Veterinary College and research Institute Namakkal was used in the experimental diets. The size of the nanoselenium was found to lie in the range of 15-40 nm as characterised by X Ray diffractional analysis and Transmission electron microscopic studies. The diets were formulated according to the standards prescribed in Bureau of Indian Standards (BIS, 1992) and fed to the birds as per the following schedule.

Broiler prestarter, starter and finisher diets were fed *ad libitum* to the birds from 1 to 14, 15 to 28 and 29 to 42 days of age, respectively. At the end of the experiment (42^{nd} day), six birds per treatment group were randomly selected and slaughtered. At the end of the experiment (42^{nd} day), six birds per treatment group were randomly selected and slaughtered by halal method.

Blood collection

At the end of fourth, fifth and sixth weeks of age, blood samples were collected using 24G sterile needles, from wing vein of six birds per treatment.

Plasma samples for estimation of biochemical constituents and hormones were obtained by collecting blood in sterile tubes with ethylene diamine tetraacetic acid (EDTA) as anticoagulant.

Estimation of Blood Biochemical Profile

The blood biochemicals were analyzed in UV-VIS double beam spectrophotometer (SYSTRONICS, Model 2202, India) using commercial kits (Span Diagnostics Ltd., India). Total cholesterol content (Wybenga *et al.*, 1970), glucose (Tietz, 1976), and triglycerides (McGowan *et al.*, 1983) were estimated in the plasma.

Estimation of Thyroid hormones

Triiodothyronine

Triiodothyronine level in plasma samples was estimated in duplicate using commercial radioimmunoassay kit (Immunotech, Czech Republic). Briefly, to the antibody coated tubes, 25 µl of calibrators (0, 0.75, 1.5, 3.0, 6.0 and 12.0 nmol/l), control and plasma samples were added in duplicate. ^{125}I-labeled triiodothyronine tracer (200 µl) was added to all the tubes and mixed well. The tubes were incubated at 20°C for 1 h in orbital shaker.

The contents were aspirated carefully. The radioactivity of the tubes was counted for 1 min in gamma counter (Stratec, Germany). To obtain total counts per min, 200 µl of tracer was added to 2 additional tubes and the radioactivity measured using gamma counter. The hormone level was expressed in nmol/l.

Thyroxine

Thyroxine level in plasma samples was estimated in duplicate using commercial radioimmunoassay kits (Immunotech, Czech Republic). Briefly, to the antibody coated tubes, 20 µl of calibrators (0, 25, 50, 100, 200 and 400 nmol/l), control and plasma samples were added in duplicate. ^{125}I-labeled thyroxine tracer (500 µl) was added to all the tubes and mixed well. The tubes were incubated at 20°C for 1 h in orbital shaker. The contents were aspirated carefully. The radioactivity of the tubes was counted for 1 min in gamma counter. To obtain total counts per min, 500 µl of tracer was added to 2 additional tubes and the radioactivity measured using gamma counter. The hormone level was expressed in nmol/L.

The activation ratio was calculated as T_3/T_4 x 100.

Statistical Methods

The completely randomized design method was followed for the experiment (Snedecor and Cochran, 1994) and the data collected were analysed using SPSS® 20.0 software package. Post-hoc analysis was done by Tukey honestly significance difference test.

Results and Discussion

Blood biochemicals

The influence of selenium supplementation in different forms on the blood biochemical constituents of broiler chickens are presented in Table 1.

Glucose

The mean plasma glucose did not differ significantly between treatment and control groups at the end of both fourth and sixth

weeks of age. However, these parameters showed an increasing trend from the fourth to sixth week.

These results concurred with that of Bobade *et al.,* (2009) who observed that dietary addition of Vitamin E and selenium in the range of 60 µg to 20 mg in the broiler chickens did not cause any significant changes in the plasma glucose levels. Similarly, Fan *et al.,* (2009) found that supplementing either inorganic or organic selenium in the range of 0.1-0.4 mg/kg did not cause any significant changes in the plasma glucose levels in stressed chickens.

However, supplementation of Sel-Plex™ reduced ($p<0.01$) plasma glucose concentrations in calves with a marginal selenium status compared with the control (Ebrahimi *et al.,* 2009).

Total Cholesterol and Triglycerides

The mean total cholesterol and triglycerides levels did not differ significantly between treatment and control groups at the end of both fourth and sixth weeks of age. However, these parameters showed an increasing trend from the fourth to sixth week which was age related in broiler chickens.

Dietary supplementation (0.25 ppm) of inorganic and organic Se had no significant effect on total cholesterol and triglycerides in the serum of Korean goats (Chung *et al.,* 2007).

Thyroid Hormones

The effect of supplementation of inorganic, organic and nanoselenium on the plasma thyroid hormone levels of broiler chickens is presented in Table 2.

The mean triiodothyronine levels were higher in all the selenium supplemented groups than the control at sixth weeks of age. The triiodothyronine levels did not vary significantly between the nanoselenium groups (T4, T5 and T6) however, the 0.6 mg/kg nanoselenium supplemented chickens had significantly highest plasma triiodothyronine level at the end of fourth week. At the end of sixth week, the triiodothyronine level showed an increasing trend with all the nanoselenium group (T4, T5 and T6) having significantly ($p<0.05$) higher plasma triiodothyronine level than the control. The triiodothyronine level did not differ significantly ($p<0.05$) between organic and nanoselenium supplemented chickens. Increasing the nanoselenium supplementation from 0.3 to 0.6 mg/kg did not significantly increase the mean triiodothyronine level at the end of sixth week of age.

The mean plasma thyroxine levels at the end of sixth week were significantly lower in organic and nanoselenium supplemented groups compared to the control. Thyroxine level showed age related decrease in all the groups from fourth to sixth week.

The mean thyroid hormone activation ratio at the end of both fourth and sixth was significantly ($p<0.05$) higher in the T5 and T6 groups when compared to the control. However at the end of sixth week increasing the nanoselenium supplementation from 0.3 to 0.6 mg/kg did not cause a significant rise in the activation ratio.

The results of the present study were in agreement with the findings of Upton *et al.,* (2008), Fan *et al.,* (2009) and Valcic *et al.,* (2011) who reported significantly ($p<0.05$) decreased T_3 levels and the activation ratio of thyroid hormones in control group compared with the birds fed 0.2 ppm of either inorganic or organic selenium in the diet, and the serum thyroxine level was higher in birds receiving diets without selenium as compared to the

birds supplemented with inorganic or organic selenium in the diet. They also reported that the conversion of thyroxine to triiodothyronine was better when organic selenium was used as the source of selenium in broiler.

Hence, there exists a positive correlation between selenium level in the diet and plasma T_3 concentration and thyroid hormone activation ratio and a negative correlation between selenium level in the diet and plasma T_4 concentration as reported by Edens (2001). Moreover, Hassanin *et al.,* (2013) reported that feeding nanoselenium (3 - 20 nm) at 0.5 mg/kg body weight to male rats restored serum free T_3 and T_4 concentrations as well as thyroid antioxidant activity by returning the GSH, malondialdehyde concentration, catalase and SOD activities to nearly normal levels after a chromium induced thyrotoxicity.

Selenoproteins in thyroid gland, such as glutathione peroxidase and thioredoxin reductase were able to remove excessive peroxides generated in the thyroid follicles by stress and thus, maintained the integrity of the structure and function of thyroid gland (Ekholm and Bjorkman, 1997). Type III 5'deiodinase, another selenoprotein, is responsible for conversion of thyroxine to triiodothyronine in the body. Moreover, the extra-thyroidal conversion of thyroxine to triiodothyronine was mediated by the hepatic Se dependent type I, 5'-iodothyronine deiodinase enzyme (Edens, 2001). Thus supplementation of selenium elevated deiodinase activity and increased the production of biologically active triiodothyronine, thereby finally promoting body growth (Arthur *et al.,* 1990).

Triiodothyronine is the prime hormone that regulates animal growth by controlling the body's energy and protein anabolism. Selenium deficiency can cause the reduction of triiodothyronine synthesis and growth inhibition (Schmidt and Reavill, 2008). In the present study, the triiodothyronine level and the thyroid hormone activation ratio showed an increasing trend with the nanoselenium group (T4, T5 and T6) having significantly ($p<0.05$) higher plasma triiodothyronine level and T5 and T6 groups having higher activation ratio and lower thyroxine levels than the control group. The organic and nanoselenium supplementation at 0.3 and 0.6 mg/kg resulted in efficient conversion of thyroxine to triiodothyronine and increased the triiodothyronine level compared to inorganic selenium supplemented and control groups.

Table.1

Treatment groups	Diets
T1(control)	Standard diet with no selenium supplementation
T2	Standard diet + 0.3mg sodium selenite/kg feed
T3	Standard diet + 0.3mg organic selenium (Selplex™)/kg feed
T4	Standard diet + 0.15mg nanoselenium/kg feed
T5	Standard diet + 0.3mg nanoselenium/kg feed
T6	Standard diet + 0.6mg nanoselenium/kg feed

Table.2

Mean (±SE) plasma biochemical profile (mg/dl) of broiler chickens fed inorganic, organic and nanoselenium						
Treatment groups	**Glucose**		**Cholesterol**		**Triglycerides**	
	IV Week	**VI Week**	**IV Week**	**VI Week**	**IV Week**	**VI Week**
T1 - standard diet	154.25±2.20	184.89±2.63	173.57±2.97	187.24±3.41	71.23±2.27	75.55±1.56
T2 - standard diet + 0.3mg inorganic Se/kg	160.63±2.15	183.66±5.32	162.78±3.47	185.79±4.11	70.40±3.04	80.00±3.64
T3 - standard diet + 0.3mg organic Se /kg	165.68±2.84	187.20±2.61	170.81±4.93	180.67±4.08	72.84±2.55	81.18±1.01
T4 - standard diet + 0.15mg nanoSe /kg	164.63±3.41	196.77±2.88	171.99±1.29	184.35±2.20	70.24±2.12	74.58±1.73
T5 - standard diet + 0.3mg nanoSe /kg	162.67±5.02	190.97±4.84	172.91±3.20	183.95±3.32	71.87±1.98	80.52±1.44
T6 - standard diet + 0.6 mg nanoSe /kg	161.80±1.11	194.19 ±3.11	169.23±4.45	180.93±4.08	69.75±2.81	78.63±2.55
Means within the same column bearing different superscripts did not differ significantly (p>0.05)						

Table.3

Mean (±SE) plasma triiodothyronine and thyroxine levels (nmol/L) in broiler chickens fed inorganic, organic and nanoselenium

Treatment groups	Triiodothyronine		Thyroxine		Activation ratio (T_3/T_4 x100)	
	IV week	VI week	IV week	VI week	IV week	VI week
T1 - standard diet	$1.11^{a} \pm 0.15$	$1.38^{a} \pm 0.27$	$32.50^{c} \pm 1.33$	$22.74^{c} \pm 1.67$	$2.93^{a} \pm 0.47$	$6.34^{a} \pm 0.91$
T2 - standard diet + 0.3mg inorganic	$1.19^{a} \pm 0.07$	$1.81^{b} \pm 0.16$	$30.33^{bc} \pm 2.52$	$20.83^{bc} \pm 1.53$	$4.05^{ab} \pm 0.35$	$8.95^{ab} \pm 1.25$
T3 - standard diet + 0.3mg organic Se /kg	$1.27^{bc} \pm 0.06$	$2.21^{bc} \pm 0.17$	$29.87^{bc} \pm 2.52$	$18.51^{ab} \pm 0.65$	$4.39^{ab} \pm 0.39$	$11.92^{bc} \pm 0.70$
T4 - standard diet + 0.15mg nanoSe /kg	$1.35^{bc} \pm 0.11$	$2.28^{c} \pm 0.17$	$25.29^{ab} \pm 1.31$	$17.47^{ab} \pm 1.11$	$5.36^{bc} \pm 0.38$	$13.55^{cd} \pm 1.90$
T5 - standard diet + 0.3mg nanoSe /kg	$1.45^{bc} \pm 0.10$	$2.59^{c} \pm 0.14$	$23.36^{a} \pm 2.18$	$16.35^{a} \pm 1.08$	$6.42^{cd} \pm 0.64$	$16.01^{d} \pm 0.82$
T6 - standard diet + 0.6 mg nanoSe /kg	$1.57^{d} \pm 0.12$	$2.60^{c} \pm 0.08$	$22.26^{a} \pm 2.40$	$16.00^{a} \pm 0.36$	$7.47^{d} \pm 0.99$	$16.34^{d} \pm 0.77$

Means within the same column bearing different superscripts differ significantly ($p<0.05$)

Selenium activates a key protein involved in the insulin signal cascade (Stapleton, 2000) and causes partial restoration of mRNA levels of glucokinase and pyruvate kinase enzymes. It also decreases the elevated level of mRNA and the activity of phosphoenol pyruvate carboxy kinase enzyme (Becker *et al.,* 1996). Additionally, an insulin-like effect for selenate has been attributed.The insulin like actions of selenium include stimulation of glucose uptake and regulation of metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis and pentose phosphate pathway (Becker *et al.,* 1996; Stapleton, 2000). Selenium has the ability to maintain the expression of the lipogenic enzymes, glucose-6-phosphate dehydrogenase (G_6PDH) and fatty acid synthase (FAS) (Berg *et al.*, 1995).

Selenium participates in various physiological functions, mostly as an integral component of a range of selenoproteins. Selenoproteins play an important role in thyroid hormone metabolism. The thyroid gland is characterized by a high concentration of selenium, which is incorporated into selenoproteins.

Some of these selenoproteins have an important antioxidant activity, contributing to the antioxidant defense in the thyroid by removing oxygen free radicals generated during the production of thyroid hormones.

Being incorporated into iodothyronine deiodinases, selenium plays also an essential role in the metabolism of thyroid hormones. Iodothyronine deiodinases (ID) are selenoenzymes responsible for converting the inactive thyroxine (T4) into an active form - 3,5,3′-triiodothyronine (T3) (Arthur *et al.,* 1990).

A strong correlation exists between the amount of Se in the diet and thyroid hormone synthesis. Se-deficiency altered both the synthesis of T3 from T4 in the thyroid gland and the activity of 5′-ID activity in rat tissues (Kohrle *et al.,*1992). Hepatic 5′-ID activity declined ten-fold and plasma T3 was significantly decreased in Se-deficient rats as compared to the Se supplemented rats (Beckett and Arthur, 2005).

Thyroid hormones play an important role in growth and protein turnover. Impaired T3 production could account for an impaired growth rate of animals with Se-deficiency. A lack of both selenium and iodine in rats resulted in severe hypothyroidism and goitre. Selenium deficiency increased hypothyroid stress associated with iodine deficiency (Hayashi, 1993).

The thyrocyte is continually exposed to potentially toxic concentration of peroxides (H_2O_2) and lipid hydroperoxides. The cytotoxic effects of H_2O_2 on thyroid cells induced apoptosis. Selenium is essential for the optimum functioning of the antioxidant system. Selenium is a component of glutathione peroxidase enzyme (GSH-Px) (Rotruck *et al.,* 1973), which is an antioxidant enzyme that catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides and thereby destroys the free radicals produced during normal metabolic activity. In selenium deficiency, the apoptotic response to H_2O_2 was increased (Beckett and Arthur, 2005). Glutathione peroxidases are responsible for glandular protection, since they remove the excess of oxygen free radicals produced during normal synthesis of the thyroid hormones.

Thus, the significantly increased body weight in the nanoselenium supplemented broiler chickens in the present study could be related to the higher T_3 hormone and increased T_3/T_4 activation ratio in these groups compared to the control.

Selenium is an essential trace mineral having multiple vital biological functions and optimisation of selenium nutrition of poultry will result in increased efficiency of egg and meat (Surai, 2000). The bioavailability and the functionality is influenced by the source of selenium. Selenium nanoparticles show special characteristics of transport, exhibit higher absorption efficiencies, enhanced bioactivity and have low toxicity potential.

Thus, it could be concluded that the broiler chicken supplemented with nanoSe at 0.15, 0.3 and 0.6 mg/kg did have any difference in the blood glucose, total cholesterol and triglycerides in comparison to the groups supplemented with other forms of selenium and control group. The triiodothyronine level and the thyroid hormone activation ratio showed an increasing trend with the nanoselenium group having significantly ($p<0.05$) higher plasma triiodothyronine level and the chicken supplemented with nanoselenium at 0.3mg/kg and 0.6 mg/kg of nanoselenium exhibited higher activation ratio and lower thyroxine levels than the control group as evident in the growth pattern of the birds.

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