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INFLUENCE OF DIETARY SELENIUM SOURCES ON THYROID HORMONE ACTIVATION, TISSUE SELENIUM DISTRIBUTION AND ANTIOXIDANT ENZYMES STATUS IN BROILER CHICKENS

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ABSTRACT
on thyroid hormone activation, tissue Se distribution and antioxidant enzymes status in broiler chickens. A total of 320 one-day old broiler chicks were randomly attributed to 5 experimental groups and fed iso-energetic and isonitrogenous diets. The basal diet was supplemented with 0 (control) or 0.3 mg/kg Se from sodium selenite (SS), selenomethionine (SM), selenized yeast (SY) or nano elemental selenium (NS). Dietary SY supplementation at 0.3 mg/kg improved both growth performance and feed efficiency compared with the other groups (P<0.01). However, no significant differences of feed consumption were shown (P>0.05) among treatments. At the end of the test, thioredoxin reductase (TR) and glutathione peroxidase (GPX) activities and also Se concentrations in plasma, liver and breast muscle were greater in groups fed Se-supplemented diets, regardless of source, than in those fed the control diet. The highest values in plasma and tissue Se content were measured in the group supplemented with SY, while the groups receiving NS showed the greatest (P<0.01) plasma and tissues GPX activities. Furthermore, dietary selenium supplementation promoted conversion of thyroxine (T4) to triiodothyronine (T3), which consequently resulted in significantly (P<0.01) higher plasma T3 concentration (p<0.01), while serum thyroxine was not significantly influenced by selenium supplementation. It could be concluded that supplementation of Se can improve antioxidative status and Se concentration in blood and tissues of broiler chick. The dietary supplementation of SY and NS could be utilized more effectively when compared to other Se sources.

KEY WORDS: Broiler, Glutathione Peroxidase, Nano Elemental Selenium, Selenium Deposition, Thioredoxin Reductase, Thyroid Hormone.

INTRODUCTION

As structural and functional constituents of numerous selenoproteins and enzymes, selenium is involved in several biological processes of living organisms (Skřivan et al., 2008). The most important and best-known action of this element is its antioxidant effect, which is due to the production of selenocysteine, an integral part of the active center of glutathione peroxidase (GPX;

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Navarro-Alarcon and Lopez-Martinez, 2000). The basic physiological role of GPX is the removal of hydrogen peroxide (H_2O_2) excess from the cell cytoplasm and prevention of formation and autopropagation of free radicals via catalyzing the reduction of peroxides to alcohols (Burk, 1997; Ganther, 1975). The biochemical function of selenium is reflected not only in its antioxidative effect. As, Se affects thyroid hormone metabolism by the synthesis and activity of the iodothyronine deiodinases (ID), selenium-based enzymes, which converts thyroxine (T4) to the more biologically active 3,3'-5 triiodothyronine (T3; Jensen et al., 1986). It has been reported that Se deficiency is associated with impaired synthesis of thyroid hormone (Kohrle et al., 1992). Since thyroid hormones play an important role in growth and protein turnover (Jianhua et al., 2000), therefore, Se deficiency can influence the overall growth performance and feather development in poultry by impairing T3 production (Edens, 2000). It is well recognized that the expression and the activity of Se-dependent enzymes are dependent on proper and adequate alimentary supply of Se. Mounting evidence has shown that in chickens, growth performance parameters, survival and antioxidant protection level are affected by dietary Se status (Avanzo et al., 2002; Golubkina and Papazyan, 2006; Ozkan et al., 2007; Wang, 2009). The National Research Council (NRC) (1994) indicates a Se requirement of 0.15 mg/kg during intensive broiler growth, but the necessity and the efficacy of Se in inducing Se-containing enzymes also depends on its chemical form (Ortuno et al., 1996). Previous studies have shown that the organic form of selenium, selenomethionine, present in plants and Se-enriched yeast, may have higher bioavailability, antioxidant properties and rates of tissue accumulation as well as lower toxicities than inorganic forms (Wang and Lovell, 1997; Mahan et al., 1999; Mahmoud and Edens, 2003). This is due to the fact that organic Se actively transports through intestinal membrane and also actively accumulates in liver and muscle tissue (Surai, 1999), while inorganic selenium is incorporated slightly into selenoproteins, and large part of it is excreted by urine (Jokić et al., 2009). Thus, there is an increasing interest in the use of organic Se forms, such as Se-methionine or selenized yeast, as supplemental sources of Se rather than inorganic Se. Selenium enriched yeast and nano elemental selenium has been recognized as the most appropriate forms of Se for use in animal nutritional supplements. This is because of their higher excellent bioavailability and low toxic effects among various forms of Se (Wang and Xu, 2008; Zhou and Wang, 2011). Payne and Southern, (2005) indicated that selenized yeast (SY) is deposited into the breast muscle of broilers at a much greater rate than sodium selenite (SS). Valcic´ et al. (2001) reported an increase in GPX activity in broiler fed with organic Se compared with those fed with SS diet. Also, Zhou and Wang (2011) demonstrated that elemental Se supplementation significantly increased GPX activity and Se concentrations in plasma and liver of chicks when compared with the control group. In general, many experimental studies have confirmed that organic and nano elemental sources of Se have advantages that are superior to those provided by the inorganic Se form (Mahan, 1999; Choct et al., 2004; Wang, 2009). Therefore, the main objective of the present study was to evaluate the influences of supplemental dietary selenium sources (as sodium selenite, selenomethionine, selenized yeast or nano elemental selenium) on TR and GPX activities, thyroid hormone activation and tissues Se content in broiler chicks. The performance of chicks was monitored only for guidance.

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MATERIALS AND METHODS

Birds, Diets, and Experimental Design

All animal care was conducted according to the University approved methods, and Institutional Animal Ethical Committee approved this experiment. Three hundred and twenty 1-d old broiler chicks (Cobb 500) were assigned randomly to five dietary treatments. Each of the five treatments was replicated 4 times with 16 chicks per each. Birds were housed in deep litter pens (1×2 m). Environmental temperature was set at 32°C on d-1 and lowered stepwise to 23-24°C for the rest of the experiment. Relative humidity and ventilation were arranged under standard conditions. Birds were fed with the experimental diets from day 1 until 28 d with two-time periods included: the starting and growing periods (1 to 14 days and 15 to 28 days), respectively. Diets were formulated according to the recommendations of the National Research Council (1994). The chickens were allowed *ad libitum* access to diets and water. Five different treatments were supplied to the chicks as follows: control: maize-soybean meal basal diet with no supplemental Se, SS: basal diet supplemented with 0.3 mg/kg from sodium selenite[1] (Na_2Se0_3), SM: basal diet supplemented with selenomethionine[2], SY: basal diet supplemented with selenized yeast[3], NS: basal diet supplemented with nano elemental selenium[4] (Table 1). The background selenium content of the control diets were 0.18 and 0.17 mg/kg, in the starter and grower diets, respectively (Table 1). Body weight of all chicks and feed consumption of each group were recorded weekly starting from one day of Age. Growth performance was evaluated in terms of weight gain and feed conversion ratio (FCR) at the end of each feeding period.

Sample Collection and preparation

Blood samples were collected at the end of 2rd and 4th week via the wing vein in sterile heparinized test tubes. Plasma was separated by centrifugation and used for the serum GPX and TR assays. Moreover, at days 28, 2 chicks from each replicate (8 birds per treatment) were sacrificed, and samples of liver and breast muscle were collected for determination of the activity of GPX, TR and Se content in these tissues. The GPX activity was determined by the method of Lawrence and Burke, 1976. Thioredoxin reductase (TR) activity was also measured according to the method described by Luthman and Holmgren (1982). The enzyme activity of GPX and TR was expressed as units per milligram of protein (U/mg prot) in tissues and as units per milliliter (U/ml) in plasma, respectively. All enzymatic assays were conducted within 24 h after extraction.
Furthermore, the diets, plasma, and tissue samples were analyzed for Se concentration by the methods of Tinggi, (1999) by hydride generation atomic absorption spectrophotometry (model AA6501, Shimadzu Ltd.).

Thyroid Hormones

[1] - Sigma-Aldrich Chemical Co., St. Louis, MO, USA
[2] - Sigma-Aldrich Chemical Co., St. Louis, MO, USA
[3] - Sel-Plex, Alltech, Inc., Nicholasville, KY, USA
[4] - Shanghai Stone Nano Technology Port Co., Ltd, China

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The plasma thyroid hormones, thyroxin (T4) and triidothyronine (T3), concentrations were measured by Radioimmunoassay as described previously (Valcic´ et al., 2011). The intra- and inter-assay coefficients of variation were less than 10%.

Statistical analysis

The data from this experiment were analyzed as a completely randomized design using the General Linear Model procedure of SAS (SAS Institute, 1996). Replicate was considered as the experimental unit for all data. Duncan's multiple range tests was performed to assess any significant differences at the probability level of $P \leq 0.05$ among the experimental treatments.

RESULTS AND DISCUSSION

Growth Performance

In terms of body weight (BW) and feed conversion efficiency (FCR), birds fed with SY diets performed significantly better ($P<0.01$) than those fed with other diets (Table 2). Whereas no differences ($P>0.05$) were found in BW and FCR among other Se sources and the unsupplemented control group during the experimental period. However, FCR in broilers fed with the Se supplemented diets were also lower than those of the control, during growing phase (14 to 21 days of age) post-hatch as well as for the cumulative period (1 to 28 days of age), but not starting phase (1 to 14 days of age). Data from the current study showed that average feed intake (FI) of the chickens were not affected ($P>0.05$) by dietary Se source in any period of growth or in the overall period (Table 2). Higher body weight and a similar feed consumption resulted in a significantly lower ($P<0.05$) feed: gain ratio for birds fed with the diets containing SY in growing and cumulative periods.The overall growth performance results were in agreement with the results of (Miller et al. 1972, Spears et al. 2003, Ryu et al., 2005; Kim et al., 2010) who reported that dietary supplementation with inorganic Se or SM did not affect the daily weight gain and feed efficiency of chickens. While Mahmoud and Edens (2005) reported a significant improvement in the body weight and FCR in broilers fed with SY ($P<0.05$) at 0.2 mg/kg feed. In our experiment, the NS-fed birds had no significant differences in growth performance in comparison to the control birds. Contrary to our finding, Zhou and Wang (2011) demonstrated a significantly ($P<0.05$) higher final live weight attributable to the supplemental nano-Se source. Improvement in the performance parameters of SY-fed birds is probably related to the effect of Se on the metabolism of thyroid hormones, which results in an increasing the efficiency of nutrient utilisation (Arthur et al., 1990). Also, the positive effect of dietary Se supplementation on FCR may be the result of better feathering, due possibly to the role of selenocysteine in feather formation (Edens, 1996). This is because correct feathering is important to maintain a protective covering for the skin of the birds and to regulate body temperature. Therefore, a combination of better absorption efficiency and thyroid hormone activation by organic selenium may explain the improved feed efficiency.

On the other hand, the sodium selenite supplement at 0.3 mg/kg caused a slight decrease in BW, which suggested early signs of toxicity in this group. Generally, the performance parameters data imply that an additional Se requirement may be necessary for the modern broiler chicken and organic Se form appeared to be more useful than inorganic form of Se.

Enzyme Activity

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The results of this study demonstrated that there was a significant difference (P<0.01) with respect to TR activity between all Se supplemented groups and the control in plasma, liver and breast muscle of broiler chickens (Figures. 1 and 2). Thioredoxin reductase activity in 28-day-old birds was lowest in the control-fed group, while at equi-selenium doses, NS elicited statistically the highest TR activity in serum and in the studied organs. As, NS supplementation resulted in a substantial (268% and 297%, respectively) increase in liver and breast TR activity comparison to the control. Dietary selenium supplementation significantly increased the plasma GPX activity in the broiler chicken at 2rd and 4th weeks of age (P<0.01). At these stages, the NS-fed chickens maintained the highest blood activity of GPX, while the control group showed the lowest value of GPX (Figure 1). Furthermore, the effect of selenium source was also very distinct on GPX activity in all above-mentioned tissues of broiler chicken at 4th week of age. Similar to TR activity, NS supplementations resulted in significant (P<0.01) higher induction of GPX activity compared with organic Se supplementation. At the end of the test, the GPX activity in the liver and breast muscle of chickens fed NS-supplemented diets was nearly 2.5 and 2.3 times more than the birds of the control diet, respectively (Figure 2). Thioredoxin reductase is a selenocysteine-containing enzyme that catalyzes the reduction of the redox protein thioredoxin by NADPH (Holmgren, 1989). Experimental studies have shown that supranutritional levels of dietary Se increases the activity of TR via increasing the incorporation of selenocysteine, leading to an increase in the activity of this antioxidative enzyme, and, to a lesser extent, via increasing thioredoxin reductase protein levels (Berggren et al., 1997; Burgos et al., 2006).

In TR protein the insertion of selenocysteine has been shown to be critical for catalytic function (Allan et al., 1999). During selenocysteine metabolism, the incorporation of Se instead of sulfur occurs in a concentration dependent manner, so there is a correlation between dietary levels of Se and tissue concentration of this antioxidant protein (Allan et al., 1999; Toufektsian et al., 2000). This is consistent with our present study where an increase in TR activity was observed with increasing selenium content in the Se-supplemented diet compared with the control. Also, the enhancement of the primary indicator of antioxidant status, GPX activity, in blood and the studied organs by Se supplementation is in accordance with the earlier reports of (Zhou and Wang, 2001; Mahmoud and Edens, 2003) who observed that GPX activity significantly (P<0.05) improved with dietary Se supplementation in chickens. Plasma and tissues GPX activities is closely related to dietary Se concentration and bioavailability of various Se supplements. As would be expected, the activity of the selenoprotein enzyme GPX is greatly improved by dietary Se supplementation. Higher pronounced increases in GPX activity by supplementation of inorganic Se forms compared to organic Se form (SY and SM) are probably due to the fact that Se, regardless of its form, has to be converted into selenocysteine before it can be incorporated into the GPX enzyme (Forstrom et al., 1978). Henry and Ammerman, (1995) indicated that inorganic Se from was metabolized into selenocysteine more efficiently than selenomethionine (Se-Met), the primary fraction of SY and SM. Another possibility is that Se-Met might be incorporated into body proteins in place of methionine (Met; Wang et al., 2011). White and Hoekstra, (1979) reported that Se-Met be initially incorporated into a wide spectrum of non-Se-requiring proteins and afterwards be incorporated into GPX, whereas Se from inorganic source is quickly incorporated into GPX. So, the competition with Met for incorporation in non-Se-requiring proteins possibly influences the availability of Se from Se-Met for the synthesis of GPX and other specific Se enzymes.

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Broilers given the SY-supplemented diet had higher breast GPX activity than those broilers given SS-supplemented diet. Apart from different chemical forms of dietary Se, the quantity of Se in the muscles is also an important regulator of GPX activity. Broilers fed SY-supplemented diet had greater breast Se concentrations on d 28 than those fed SS, thus this organ provided adequate Se to promote GPX activity. So, greater GPX activity in broilers fed the Se-supplemented diet was accompanied with greater plasma, breast, and liver Se concentrations in comparison to the control.
In general, NS showed a higher potential to improve the antioxidant status than other Se sources, which is a result of its high catalytic efficiency and strong adsorbing ability, and consequently its excellent bioavailability (Zhang et al., 2008). An age-related increase in plasma antioxidant enzyme levels by Se supplementation in broilers diets was observed. This phenomenon is consistent with the concept that within one week after the birth, the antioxidant status of chicks mostly depends on the Se accumulated in the liver during embryogenesis, and does not related to the feed Se content (Sunde, 1997). The improved activity of plasma GPX in 4-week-old broilers in comparison with 2-week-old chicks is in accordance with the results reported by Valcic´ et al., 2011. Similarly, Kuricova et al. (2003) observed a steady GPX activity increase in chickens from week 2 to week 4 of age in birds supplemented with 0.20 mg/kg various Se sources.

Se Content

Figure 3 shows the Se content of plasma, liver and breast muscle of chicks after 28 d of treatment with different sources of Se. Compared with the control group, the addition of various sources of Se increased Se concentration in the studied organs ($P<0.05$). Broilers fed the SY diet maintained the highest hepatic and muscle Se contents on d 28, as liver and breast Se concentration in SY-fed chicks were 0.64 and 0.33 mg/kg, respectively in comparison to 0.27 and 0.12 mg/kg achieved in the control.Day 28 plasma Se concentration in all Se supplemented groups except SS was significantly higher compared to the control ($P<0.01$), although group receiving Se supplemented in the form of SS had numerically higher concentration of plasma Se in comparison to the control group ($P>0.05$). Regarding plasma and tissues breast Se content, organic forms of Se (SY and SM) proved to be significantly more efficient Se source compared to the inorganic Se, because the predominant form of Se in organic chemical forms is selenomethionine (Beilstein and Whanger, 1985), which is readily incorporated into body proteins (Sunde, 1997). This result was consistent with previous findings (Choct et al., 2004; Radmila et al., 2008) who demonstrated a considerably higher tissue Se concentration in broilers fed Se-enriched yeast supplemented diets in compassion to inorganic Se form. Furthermore, Wang (2009) reported that nano elemental Se has a higher bioavailability than inorganic Se for the Se deposition in the tissues. Higher bioavailability of organic Se than inorganic Se for the Se deposition in the tissues is probably due to their different absorption and metabolism mechanisms. As, inorganic Se is passively absorbed from the intestinal wall by a simple diffusion process, whereas organic Se (SY and SM) is actively absorbed and utilized in the intestine through the amino acid transport mechanisms (Wolfram et al., 1989; Rayman, 2004; Schrauzer, 2003). Furthermore, some reports indicated that nanoparticles are also absorbed in duodenum by active transportation, so usually high levels of this Se form is deposited in various tissues (Zhang et al., 2001; Shi et al., 2011). The other likely possibility is that the chemical similarity between selenomethionine and Met allows the body to use them interchangeably during synthesis of protein because the tRNAMet cannot discriminate between

selenomethionine and Met (Schrauzer, 2000). This makes it possible to build a reversible Se storage in organs and tissues (Schrauzer, 2003). The plasma Se results on d 28 are in general agreement with (Wang et al., 2011) who reported that plasma Se level is increased in broilers fed a diet containing organic Se relative to those fed a diet containing SS (Also see Appendix). Furthermore, higher Se content was observed in liver compared with that in muscle. Animal studies have illustrated that the liver is the major target organ of selenium accumulation (Diskin et al., 1979). The observed pattern of overall tissue Se contents in this study is similar to that reported by Surai, 2002, who demonstrated that Se concentration of liver is higher than muscle and Se content of muscle is also greater than plasma.

Thyroid Hormone

At the age of 14 d, plasma T4 concentrations did not differ significantly among treatments but tended to be lower ($P>0.05$) in all Se supplemented chicks, while T3 concentration was significantly higher in Se-treated birds than in the unsupplemented control group (Table 3). At the age of 28 d, the Se supplemented groups except SS ($P<0.01$) had significantly higher plasma T3 concentration and T3/T4 ratio compared to the control. Moreover, the serum T4 levels were higher in birds within the no supplemental Se treatment in comparison to those supplemented with Se ($P<0.01$). The highest plasma T3 level and T3/T4 ratio were recorded in group SY at day 14 (2.75 ng/ml-28.66%, respectively) and at day 28 (2.57 ng/ml- 31.36%, respectively). Also, a decline in hormone concentration was observed with age (Table 3).

Selenium constitutes an essential part of type 1 deiodinase, which is responsible for deiodinating thyroxine (the inactive prohormone) to make the more metabolically active form T3 thyroid hormone (Sunde, 1997). Thus, selenium deficiency may results in a decreased conversion of T4 to the T3 and consequently an increased T4 concentration in serum. Also, the ratios between serum T4 and T3 indicate that SY and NS treatments facilitated the conversion of T4 to T3 more efficiently, possibly extrathyroidally in the liver (Edens, 2001).

These results are consistent with previous study by (Kohrle et al., 1992) who demonstrated that there is a strong correlation between the amount of Se in the diet and thyroid hormone synthesis.

The increased activation ratio in the SY and NS supplemented groups was a consequence of a remarkably lower plasma T4 concentration compared to inorganic Se (SS) supplemented groups. This is probably the result of the negative feedback control mechanism through the thyroid axis, which under normal conditions prevents an excessive increase of plasma T3. The implications of this mechanism could be relevant in slightly hypothyroid individuals in which a diet enriched with easily available Se, such as Se-enriched yeast, could increase the conversion of T4 to T3 without a simultaneous increase of T4 release from the thyroid gland (Valcic´ et al., 2011).

CONCLUSION

From the results of this study it could be concluded that providing of Se concentrations in the broiler diet above the level of Se requirement could improve the antioxidant status and deposition of Se into muscle tissue. In general, SY showed a higher potential to Se deposition in plasma and some tissues, as well as more proficient conversion of T4 to T3. Furthermore, NS supplementation

of chicken diets was superior form of Se in the improvement of the serum and tissue GPX and TR activities of chicken.

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Table 1: Ingredients and composition of experimental diets

Ingredients	Starter (1 to 14 days)	Grower (15 to 28 days)
Maize	57.56	61.51
Soybean meal	32.80	27.50
Corn gluten meal	3.30	3.20
Oil	2.20	3.90
Dicalcium phosphate	2.00	1.80
Oyster shell	1.15	1.10
Salt	0.32	0.32
L-Lysine	0.09	0.09
DL-Methionine	0.08	0.08
Vit. and Min. permix[1,2]	0.50	0.05
Calculated analysis		
ME (kcal/kg)	2980	3140
CP %	21.54	19.50
Se	0.18	0.17

[1]Vitamin premix provided per kg of diet: vitamin A, 7,040 IU; vitamin D3, 2,000 IU; vitamin E, 8.8 IU; vitamin K3, 1.76 mg; biotin, 0.12 mg; thiamine, 1.2 mg; riboflavin, 3.2 mg; pentothenic acid, 6.4 mg; pyridoxine, 1.97 mg; niacin, 28 mg; vitamin B12, 0.008 mg; choline, 320 mg; folic acid, 0.38 mg.

[2]Mineral premix provided per kg of diet: Mn, 60 mg; Fe, 60 mg; Zn, 51.74 mg; Cu, 4.8 mg; I, 0.69 mg; Se, 0.16 mg.

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Table 2: Broiler chicken performance in response to experimental diets

Parameter	Days of age	Treatment[1]					SEM	P Value
		C	SS	SM	SY	NS		
Body weight	1-14 d	352.26±4.1[b]	350.09±10.7[b]	352.09±4.9[b]	379.02±4.5[a]	358.32±3.7[b]	3.45	**
	15-28 d	893.55±26.0[b]	888.65±13.3[b]	924.99±19.4[b]	1010.6±17.7[a]	939.18±20.5[b]	12.77	**
	1-28 d	1243.9±22.3[b]	1240.9±12.9[b]	1277.1±21.8[b]	1389.6±19.8[a]	1297.5±21.7[b]	14.75	**
Feed consumption	1-14 d	410.87±3.9	410.93±5.3	409.79±4.8	411.57±4.4	407.17±8.6	2.28	NS
	15-28 d	1588.13±16.3	1593.83±13.2	1592.38±12.9	1598.09±15.1	1605.15±16.0	6.01	NS
	1-28 d	1999.07±13.8	2004.71±9.5	2002.17±16.8	2009.66±17.1	2012.32±23.6	6.76	NS
Feed conversion efficiency	1-14 d	1.17±0.01	1.19±0.04	1.16±0.02	1.09±0.01	1.12±0.02	0.01	NS
	15-28 d	1.78±0.05[b]	1.79±0.03[b]	1.72±0.03[b]	1.59±0.02[a]	1.71±0.03[b]	0.02	*
	1-28 d	1.61±0.03[b]	1.62±0.02[b]	1.57±0.03[b]	1.45±0.02[b]	1.55±0.02[a]	0.02	**

[a-e] Values in the same column with no common superscript differ significantly.
[1]C: Basal diet; SS: Diet with 3 mg/kg sodium selenite; SM: Diet with 3 mg/kg selenomethionine; SY: Diet with 3 mg/kg selenized yeast; NS: Diet with 3 mg/kg nano elemental selenium.
Ns: P> 0.05; * P ≤ 0.05, ** P ≤ 0.01.

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Table 3: Plasma T3 and T4 concentrations in broiler chickens at 2 to 4 wk of age (ng/ml)

Treatment [1]	2rd week			4th week		
	Plasma T3 (ng/ml)	Plasma T4 (ng/ml)	Activation ratio (T3/T4 × 100)	Plasma T3 (ng/ml)	Plasma T4 (ng/ml)	Activation ratio (T3/T4 × 100)
C	1.40±0.02^{e}	9.75±0.08	14.36±0.34 ^{e}	1.31±0.01 ^{d}	9.06±0.03^{a}	14.46±0.10^{d}
SS	1.64±0.02^{d}	9.67±0.07	16.93±0.24 ^{d}	1.28±0.02 ^{d}	8.83±0.03^{b}	14.56±0.27^{d}
SM	1.82±0.04^{c}	9.61±0.09	18.95±0.49 ^{c}	1.85±0.03^{c}	8.43±0.05^{c}	21.94±0.37^{c}
SY	2.75±0.05^{a}	9.59±0.30	28.66±0.61 ^{a}	2.57±0.05^{a}	8.19±0.03^{d}	31.36±0.66^{a}
NS	2.38±0.03^{b}	9.63±0.40	24.74±0.41 ^{b}	2.25±0.04 ^{b}	8.23±0.07^{d}	27.31±0.44^{b}
SEM	0.11	0.03	1.21	0.11	0.08	1.56
P Value	**	NS	**	**	**	**

[a–e]Within rows, values with different superscripts differ significantly ($P \leq 0.05$).
[1]C: Basal diet; SS: Diet with 3 mg/kg sodium selenite; SM: Diet with 3 mg/kg selenomethionine; SY: Diet with 3 mg/kg selenized yeast; NS: Diet with 3 mg/kg nano elemental selenium.
Ns: $P > 0.05$; * $P \leq 0.05$, ** $P \leq 0.01$.

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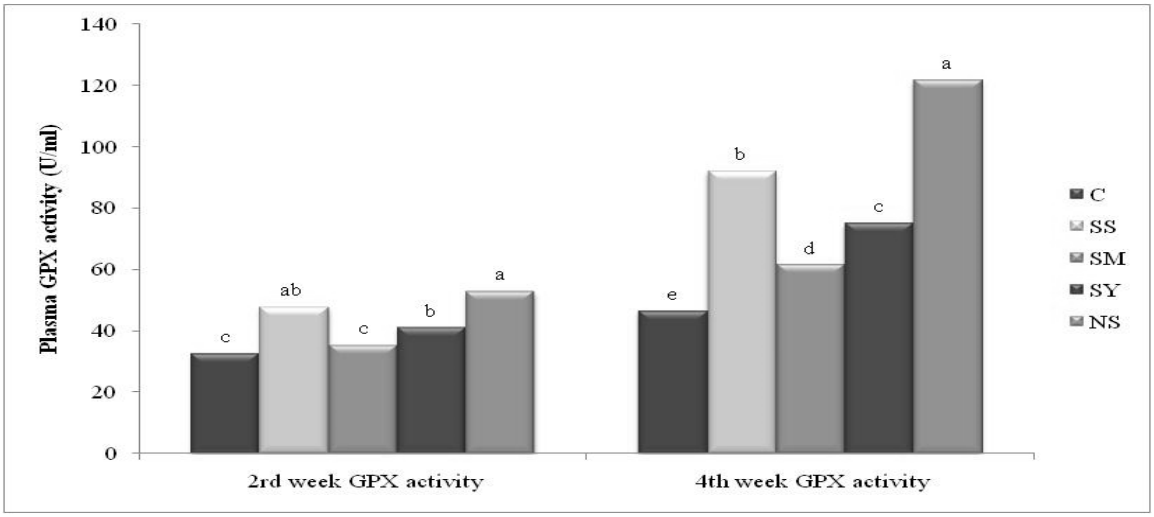
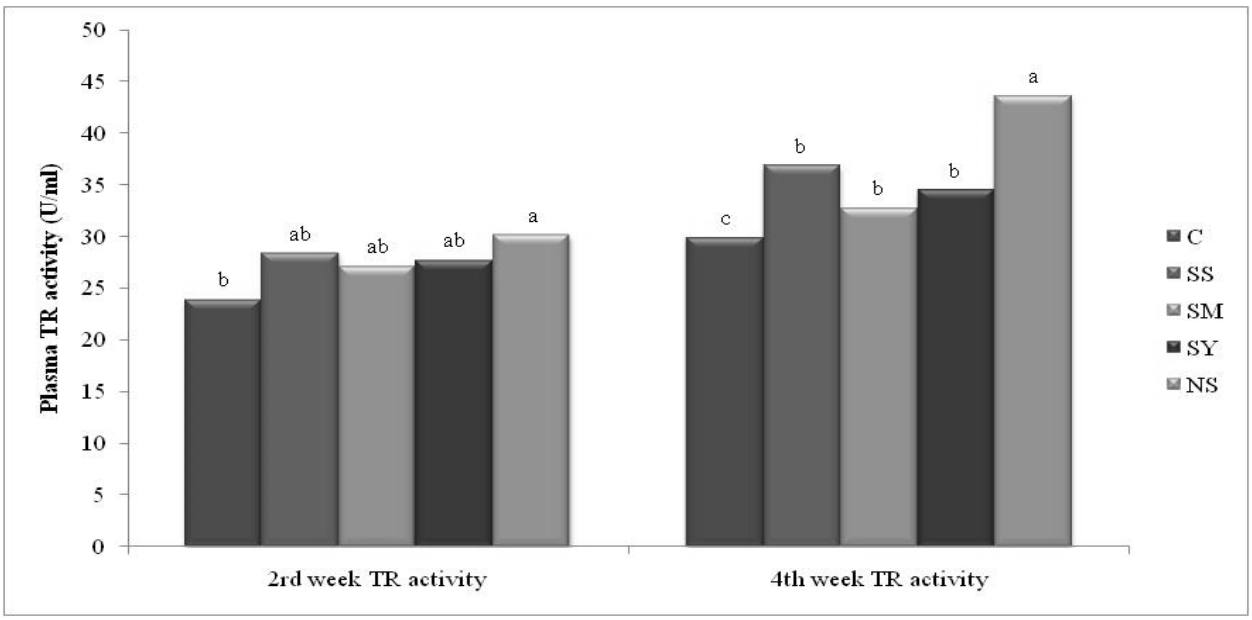
Figure 1: Serum glutathione peroxidase (GPX) and thioredoxin reductase (TR) activities in 14 and 28-day broiler chicks



[a–e]Within rows, values with different superscripts differ significantly ($P \leq 0.05$).



[a–c]Within rows, values with different superscripts differ significantly ($P \leq 0.05$).

Figure 2: Liver and breast glutathione peroxidase (GPX) and thioredoxin reductase (TR) activities of 28 d-old broiler chicks

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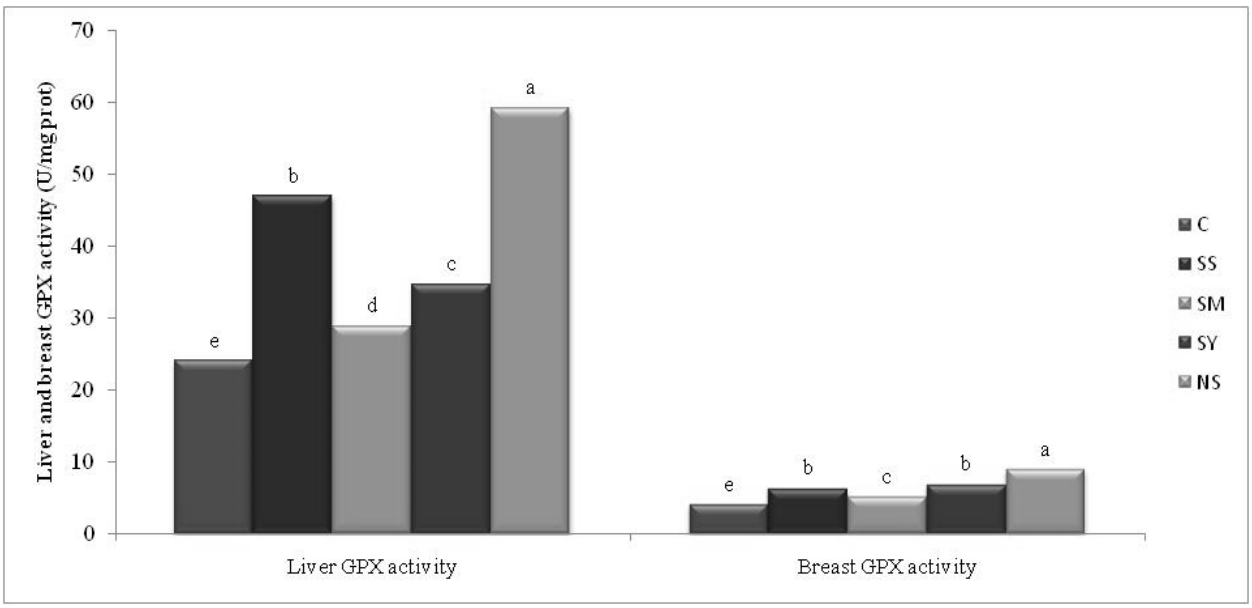
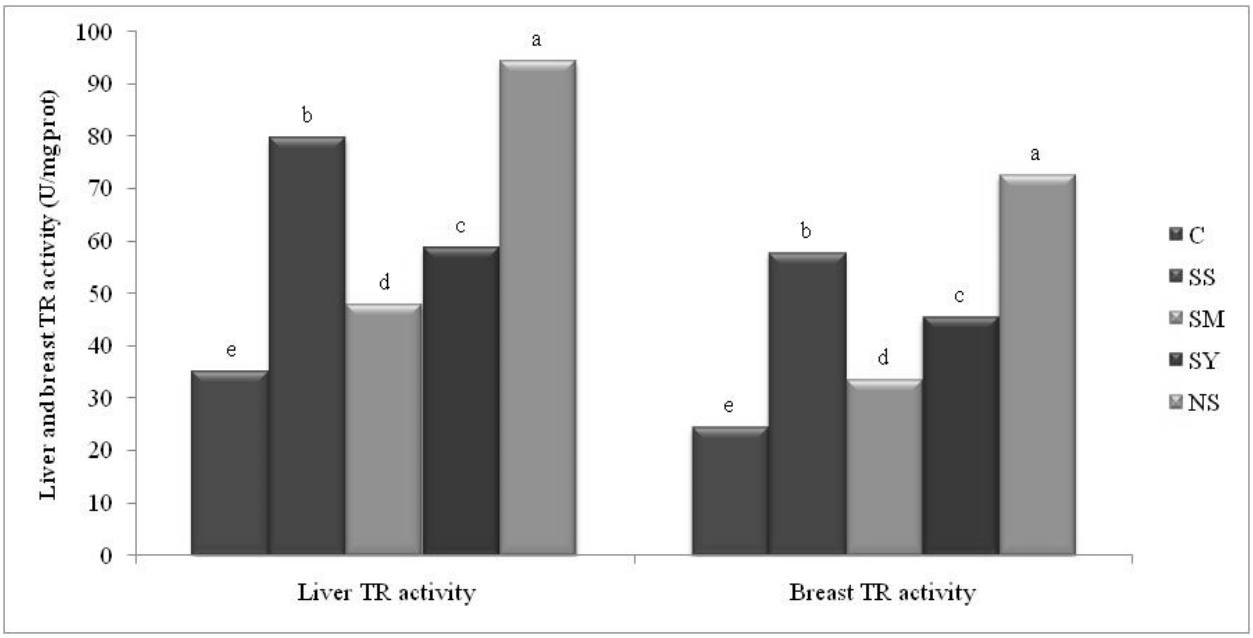


[a–e]Within rows, values with different superscripts differ significantly ($P \leq 0.05$).



[a–e]Within rows, values with different superscripts differ significantly ($P \leq 0.05$).

Figure 3: Selenium concentration in serum and tissues of 28-day-old broiler chicks (milligram per kilogram)

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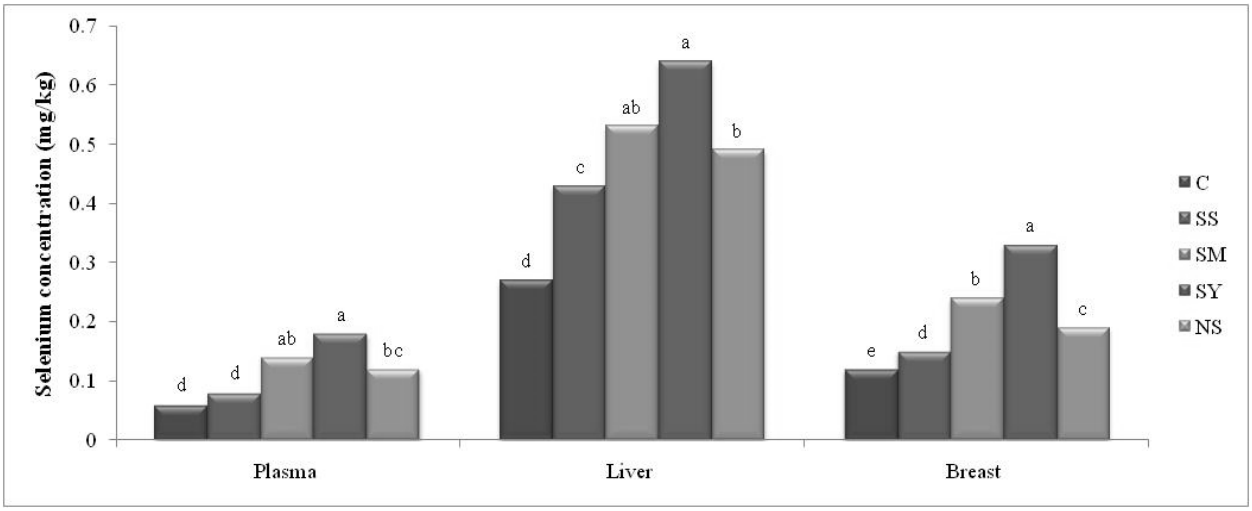

[a–e]Within rows, values with different superscripts differ significantly ($P \leq 0.05$).

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Appendix

Serum and tissues glutathione peroxidase (GPX) and thioredoxin reductase (TR) activities of 28 d-old broiler chicks

Treatment[1]	2rd week Plasma		4th week Plasma		Liver		Breast muscle	
	GPX activity	TR activity	GPX activity	TR activity	GPX activity	TR activity	GPX activity	TR activity
C	32.46±1.8^{c}	23.90±1.1^{b}	46.42±1.36^{e}	29.84±1.62^{c}	23.93±1.42^{e}	35.09±1.60^{e}	3.92±0.22^{d}	24.44±0.37^{e}
SS	47.61±3.7^{ab}	28.34±2.0^{ab}	92.18±1.95^{b}	36.82±0.86^{b}	46.91±1.07^{b}	79.84±1.44^{b}	6.24±0.29^{b}	57.83±0.93^{b}
SM	35.34±1.2^{c}	27.09±1.2^{ab}	61.46±1.19^{d}	32.72±0.52^{bc}	28.77±1.01^{d}	47.92±1.43^{d}	5.01±0.37^{c}	33.43±1.23^{d}
SY	40.99b±4.1^{b}	27.70±1.2^{ab}	75.05±1.68^{c}	34.43±1.86^{bc}	34.59±1.61^{c}	58.66±1.29^{c}	6.80±0.31^{b}	45.58±0.99^{c}
NS	52.77±3.8^{a}	30.12±1.4^{a}	121.59±3.87^{a}	43.57±1.01^{a}	59.15±1.69^{a}	94.39±1.65^{a}	8.88±0.53^{a}	72.62±3.33^{a}
SEM	2.13	0.72	6.02	1.17	2.98	4.94	0.41	3.96
P Value	*	NS	**	**	**	*	**	**

$^{a–e}$Within rows, values with different superscripts differ significantly ($P \leq 0.05$).
[1]C: Basal diet; SS: Diet with 3 mg/kg sodium selenite; SM: Diet with 3 mg/kg selenomethionine; SY: Diet with 3 mg/kg selenized yeast; NS: Diet with 3 mg/kg nano elemental selenium.
Ns: $P > 0.05$; * $P \leq 0.05$, ** $P \leq 0.01$.

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Selenium concentration in serum and tissues of 28-day-old broiler chicks (milligram per kilogram)

Treatment[1]	**Se concentration**		
	Plasma (mg/L)	Liver (mg/kg)	Breast (mg/kg)
C	0.06±0.004^{d}	0.27±0.01^{d}	0.12±0.005^{e}
SS	0.08±0.010^{d}	0.43±0.01^{c}	0.15±0.006^{d}
SM	0.14±0.007^{ab}	0.53±0.01^{ab}	0.24±0.006^{b}
SY	0.18±0.003^{a}	0.64±0.03^{a}	0.33±0.01^{a}
NS	0.12±0.004^{bc}	0.49±0.01^{b}	0.19±0.003^{c}
SEM	0.01	0.03	0.02
P Value	**	*	**

$^{a–d}$Within rows, values with different superscripts differ significantly ($P \leq 0.05$).

[1]C: Basal diet; SS: Diet with 3 mg/kg sodium selenite; SM: Diet with 3 mg/kg selenomethionine; SY: Diet with 3 mg/kg selenized yeast; NS: Diet with 3 mg/kg nano elemental selenium.

Ns: P>

