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ORIGINAL ARTICLE

The Effects of Organic, Inorganic and Nano-Selenium on Blood Thyroid Hormones Concentration in Broiler Chickens Exposed to Oxidative Stress

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ABSTRACT
Two essential trace elements, iodine and selenium are involved in thyroid hormone metabolism. Supplementation of avian diets with selenium in particular, has been shown to have an important role in maintaining bird health and production. Selenium is necessary for the increasing conversion of serum tetraiodothyronine (T_4) to tri-iodothyronine (T_3). The present study aimed to compare different sources of selenium (Se) on blood thyroid hormones concentration of male broilers subjected to oxidative stress. Corn grain and soybean meal-base diets were formulated to meet the nutritional requirements of Cobb 500 broiler chickens. The birds (n=320) were randomly assigned to receive either selenium-free diet, control with (CON_O) or without (CON_N) oxidative stress or diets supplemented with 0.3 mg/kg Nano-Se with (NAN_O) or without (NAN_N) oxidative stress, organic Se with (ORG_O) or without (ORG_N) oxidative stress and inorganic Se with (INO_O) or without (INO_N) oxidative stress. The study was conducted as a completely randomized designed with a 2×4 factorial arrangement comprising 4 replicates of 10 birds per each. Tert-butyl hydro peroxide (tert-BHP) was used to induce oxidative stress. Groups fed Se supplementation had higher T_3 and lower T_4 concentration as compared to control group ($p<0.05$). Experimental groups exposed to oxidative stress had lower T_3 concentration compared to non-oxidative stress groups ($p<0.05$). The thyroid hormone in this study showed that conversion of T_4 to T_3 was more preference when ORG_N, INO_N and NAN_N were the supplemented selenium source as compared to CON_N. Interaction between Se source, oxidative and non-oxidative condition was significant different ($p<0.05$).
Key words: *Thyroid hormones, selenium, oxidative stress, broiler*

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INTRODUCTION

Oxidative stress is a condition where there is an imbalance between antioxidant defense system and the production of free radical, so that the defense is overcome by radical formation causing oxidative stress [**3**]. When birds exposed to stress, selenium is used in antioxidant enzymes to decrease the harmful effects of free radicals [8,10,11,12]. Selenium deficiency causes decrease in plasma 3,5,3'-triiodothyronine (T_3) concentration and this can cause reduced growth in birds and selenium supplementation increased it [**5**]. Selenium has important role in thyroid hormone metabolism, because it is necessary to convert the thyroxine (T_4) to active form (T_3) of this hormone. For this conversion selenoenzyme is necessary, mainly in the liver and kidneys [1].

We hypothesized that in the oxidative stress, selenium pool of body maybe depleted for antioxidant activity and deficiency in selenium occur for conversion of T_4 to T_3. Another issue is the source of selenium, which they have different bioavailability to fulfill the requirement of bird in this condition. In the literature, there was no information regarding the effect of different sources of selenium on blood thyroid hormone concentration in animals exposed in oxidative stress.

The aim of the present work was to study the effect of nano-selenium, organic Se, and inorganic Se on thyroid hormones in broiler subjected to oxidative stress.

MATERIALS AND METHODS

A total of 320 one-day old male broiler chicken (Cobb 500) were used in a completely randomized design with a 2×4 factorial arrangement. The study consisted of 8 treatment groups with 4 replicates of 10 birds each. The chicks were weighed and were randomly assigned to experimental units (Floor pens). The rearing temperature was 32˚C in the first week rearing to 21˚C at the end of the trial (week 6). The birds were kept under a 23L:1D lighting program and had a free access to fresh water. Experimental diets were fed from the first day of the trial the corn-soybean-meal-based diets were formulated to meet the requirements for Cobb 500 broilers in mash form (Table 1). The Se sources used were organic Se (Sel-Plex; Alltech Inc. Chemical Co.), Inorganic Se (sodium selenite) and nono-selenium (American Elements Co.). The particle size of nono-selenium ranged from 10 to 45 nm with a 99.9% purity.

Experimented treatment were as follows: control with (CON_O) or without (CON_N) oxidative stress, nano-selenium with (NAN_O) or without (NAN_N) oxidative stress, organic selenium with (ORG_O) or without (ORG_N) oxidative stress and inorganic selenium with (INO_O) or without (INO_N) oxidative stress. All Se sources were included at 0.3 mg/kg of diet. Tert-butyl hydroperoxide (tert- BHP) purchased from Sigma-Aldrich Chemical Co. was used to induce the oxidative stress. The birds belong to oxidative stressed received 0.2 mmol/kg body weight tert-BHP intraperitoneally. A sham operation was conducted in birds belonging to non- stressed groups through injection of normal saline.

In day 42 of age, blood samples (2 ml) were taken from the brachial vein of two chickens per each replicate into EDTA-containing tubes. Then tubes were centrifuged (3000 × g, 12 min, 17°C) and the plasma was stored at −20°C, pending T_3 and T_4 assays. Plasma T_3 and T_4 concentration were measured with commercial kites using ELISA as method described by Sauer et al. [**7**].

Data were subjected to the PROC GLM of SAS 9.2 [**6**] and mean comparison was done using the Duncan, s Multiple Range Test at $P<0.05$.

RESULTS AND DISCUSSION

Table 2 shows the effect of selenium sources and oxidative stress on thyroid hormones. Groups with Se supplementation had higher T_3 concentration compare to control group ($p<0/05$). Groups with oxidative stress had lower T_3 concentration compare to groups not exposed to oxidative stress ($p<0/05$). Groups with Se supplementation had lower T_4 concentration compare to control group ($p<0/05$) and groups with oxidative stress had higher T_4 concentration compare to groups without oxidative stress. There were no differences between CON_O and CON_N in T_3 and T_4concentration ($p>0/05$). But significant differences found in T_3 concentration between chicks exposed to oxidative and non-oxidative stress in NAN, ORG and INO groups ($p<0/05$). Higher amount of T_3 found in NAN_N group and higher amount of T_4 found in CON_O group.

In the present study, we found that oxidative stress created by tert-BHP could decrease plasma T_3 and increase T_4 concentration in birds exposed to oxidative stress fed different source of selenium as compare to birds with non-oxidative stress condition. Similar T_3 levels in selenium supplementation (nano, organic and inorganic) in oxidative and non oxidative groups broilers is most likely to reflect the fact that a sufficient amount of T_3 was produced in broilers in both oxidative and no-oxidative conditions by selenium supplementation.

Selenium is needed to synthesis type I iodothyronine deiodinase which catalyses conversion of T_4 to T_3 [5]. Plasma T_3 concentration is produced by 5'-deiodination of thyroxine in non thyroidal tissues, particularly the liver and kidney [2].

Nano, organic and inorganic selenium (0.3 mg/kg) increased plasma T_3 concentration and decreasing T_4 in oxidative and non oxidative condition compared to control group. Diets with different selenium source were significantly increasing plasma T_3 concentration. Jensen et al. [5] observed plasma thyroxine was increased when a diet was supplemented with either selenium or vitamin E. T_3 was also significantly increased by selenium supplementation. In their study, no significant increase in these hormones was observed in birds fed a corn-soybean-meal without selenium and vitamin E.

Jianhua et al. [**5**] and Jensen et al. [4] observed that selenium supplementation effected on thyroid hormones metabolism. A selenium deficiency in broilers resulted in decrease of plasma T_3 while plasma T_4 concentrations were increased. Also they reported that selenium deficiency on broilers chicken causes reducing thyroid hormones and direct correlation between plasma thyroid hormones and T_3 concentration. The control group (without Se supplementation) had lower T_3 concentration compare to other treatments.

In the present study, we found an increase in blood T_3 concentration by nano, organic and inorganic selenium in oxidative and non oxidative condition. This study showed for the first that negative effects of oxidative stress might be attenuated by dietary supplementation of Se. There was no study in the literature concerning this issue to compare our result with them.

Table 1. Feed ingredients (%, as fed) and composition of the basal diets			
Ingredients	Starter	Grower	Finisher
Yellow corn	49.53	53.04	53.64
Soybean meal (44%)	38.31	31.51	30.02
Wheat grain	5.02	8	10
Fish meal (66%)	2.5	2.5	0
Vegetable oil	1.03	1.6	2.8
Di-calcium phosphate	1.53	1.44	1.52
Oyster shell	0.95	0.84	1.02
Salt	0.15	0.14	0.18
Vitamin-mineral premix*	0.6	0.55	0.5
Coccidiostat	0.04	0.04	0.04
DL- Methionine	0.23	0.22	0.20
L-Lysine	0.11	0.12	0.08
Total	100	100	100
Calculated chemical composition			
ME (kcal/kg)	2950	3000	3050
Crude protein (%)	22.2	20.2	18.2
Calcium (%)	1	0.97	0.92
Available phosphorus (%)	0.5	0.48	0.45
*Vitamin and mineral provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; Choline chloride, 200000 mg, Manganese; 40000 mg; Iron, 20000 mg; Zinc, 40000 mg; Copper, 4000mg; Iodine, 400 mg.			

Table2: Effect of organic, inorganic and nano selenium on thyroid hormones concentration of broiler in oxidative and non-oxidative conditions

Item	Treatment *									P-Value		
	CON_O	CON_N	NAN_O	NAN_N	INO_O	INO_N	ORG_O	ORG_N	SEM	Se source	Oxidative condition	Interaction
T_3(ng/ml)	1.86^{cd}	1.50^{d}	2.41^{bc}	4.46^{a}	2.58^{b}	4.35^{a}	2.21^{bc}	4.16^{a}	0.219	<0.0001	<0.0001	<0.0001
T_4(ng/ml)	10.99^{a}	10.61^{a}	9.10^{b}	7.83^{c}	8.83^{b}	7.98^{c}	7.22^{cd}	6.68^{d}	0.255	<0.0001	0.0003	0.3370

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

*** CON**: Control, **NAN**: Nano selenium 0.3 mg/kg, **INO**: Inorganic selenium 0.3 mg/kg, **ORG**: Organic selenium 0.3 mg/kg, **O**: Oxidative condition, **N**: Non oxidative condition.

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