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Effect of Replacing Inorganic by Organic Selenium Sources in Diet of Male Broilers on Selenium and Vitamin E Contents and Oxidative Stability of Meat

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Abstract: An experiment was conducted to assess the effects of replacing Sodium Selenite (SS) by Se-Yeast (SY) in diet on selenium and α-tocopherol contents in male broilers tissue and it's oxidative stability. One day old 240 male birds were randomly assigned to 4 treatments with 4 replicates of 15 birds each. The experimental grower diets that were supplemented with SS or SY at 0.3 mg se kg^{-1} of feed, as follows: T1 = 0.3 SS, T2 = 0.2 SS + 0.1 SY, T3 = 0.1 SS + 0.2 SY and T4 = 0.3 SY were given *ad libitum* to the birds during a 21 days old grower period. The basal diet was also supplemented with 75 mg of vitamin E. SY enrichment of grower diets increased ($p \leq 0.05$) both contents of Se and vitamin E in breast and thigh tissues and their subsequent oxidative defence in birds compared to those fed diets supplemented with SS alone. Also, the replacing SY in the diet reduced Malondialdehyde (MDA) values in breast samples after 0, 3 and 5 days of chilled storage (4-6°C). Therefore, the replacing SS by SY in broilers diets protected with a strong natural antioxidant were more effective in Se-enrichment of tissues plus the oxidative stability of stored meat.

Key words: Sodium selenite, selenium-enriched yeast, vitamin E, male broiler, lipid oxidation, Iran

INTRODUCTION

Selenium, a component of glutathione peroxidase in animal's body can protect cells and cell membrane from damaging caused by oxidation (Rotruck *et al*., 1973). Selenium is a dietary essential nutrient for poultry and Se content of feed grains varies widely from region to region (National Research Council, 1994). Thus, it is a common practice in the poultry industry to supplement Se in broilers diets. Historically, the Se source that has been used is the inorganic sodium selenite (Na_2SeO_3). Organic Se has a couple of advantages compared to inorganic Se sources. First, the organic Se sources have a greater bio-availability and secondly, organic Se will not under go pro-oxidation because it is already in the organic form (Mahan, 1995). Therefore in June, 2000 an organic source of Se such as Se-enriched yeast was approved for use as a feed supplement in poultry diets (FDA, 2000). The amount of Se available for assimilation by the tissues is dependent on the form and concentration of the element while organic selenium is deposited in the body tissues more efficiently than inorganic selenium (Surai and Sparks, 2000). Selenium supplementation improves the immune function in Se deficient animals. When their bodies have a low concentration of Se, different diseases such as white muscle disease, blood capillary disease, cardiac muscle metamorphism, muscle atrophy, cancer, anemia, liver bleeding or putrescence and immunologic dysfunction were occur easily (Surai, 2002, 2006).

Recently, studies related to the effects of selenium sources in poultry were conducted in order to assess the growth performance, carcass characteristics, lipid peroxidation, selenium or vitamin E retention on meat/egg and it's quality especially in broiler chickens (Payne and Southern, 2005; Ryu *et al*., 2005; Sevcikova *et al*., 2006). These researchers reported that not significant differences of the growth performance were similar in all trials.

The supplementation of selenium especially organic selenium might improve meat quality and shelf life of poultry meat (Sevcikova *et al*., 2006). Increasing dietary selenium improved the Se status or retention of the muscle and oxidative stability of chicken meat during refrigerated storage (Yoon *et al*., 2007; Smet *et al*., 2008). Moreover, it is recognized that vitamin E as a strong natural antioxidant help to protect the polyunsaturated fatty acids in cell membranes from peroxidative damage. Dlouha *et al*. (2008a) and Skrivan *et al*. (2008a, b) reported on rats and broilers, respectively that a higher oxidative stability of lipids could be achieved if selenium is

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supplemented together with vitamin E in the diets. But according to Upton *et al.* (2008) the effects of two type sources of inorganic and organic Se (sodium selenite and Se-enriched yeast combined or alone) compared to control diet were assessed and the organic source of Se (Se-yeast, SY) did affect the performance of broilers. Body weights of broilers fed SY diets were increased compared to Control or SS treatment groups and the combination of SS and SY was no longer effective than SY alone. Also, Feed Conversion Ratio (FCR) was improved with Se supplementation as SY and SY + SS being superior to SS treatment.

The aim of this study was to evaluate the effect of different substitution of sodium selenite (SS, an inorganic sources of Se) by Se-yeast (SY, an organic enriched source of Se) and different levels of Se in grower broilers diets on performance, lipid oxidation, selenium and vitamin E content in meat of broiler chicks.

MATERIALS AND METHODS

Six hundred, one day old Ross 308 chicks obtained from a commercial hatchery were reared with commercial feed starter from 1-20 days. At 21 days of age, 240 male chickens were separated, individually weighed and randomly placed in 16 floor pens of 1.5 × 1.5 m (15 birds per pen). Up to 3 weeks of age, chicks were fed the same starter diet. The grower (experimental) diets were supplemented with organic Se-yeast (SY or Sel-Plex [SP], Alltech, Inc.) or sodium selenite (SS or Na_2SeO_3) at 0.3 mg se kg^{-1} of feed and were formulated in accordance with the National Research Council (1994) to contain 200.7 g of CP and 12.91 MJ of ME. The experimental treatments consisted of 3% SS (T1), 2% SS + 1% SY (T2), 1% SS + 2% SY (T3) and 3% SY (T4) and were fed to birds from 2-42 days of age. Vitamin E, sodium selenite and Se-yeast supplements were included in the premix. The chicks were maintained on a 24 h constant lighting schedule and both diets and fresh water were offered *ad libitum* until slaughter at 42 days of age. Ingredient composition and nutrient calculation for diets are shown in Table 1.

The analytical results of the experimental diets for Se are shown in Table 2. The levels of Se were found 0.365, 0.362, 0.371 and 0.375 mg kg^{-1} for treatments T1, T2, T3 and T4 and there were no major discrepancies between diets with different Se sources.

At 5 weeks of age samples of excreta were collected for the analysis of selenium content. By the end of the trial at 42 days of age, 8 birds from each treatment were slaugthered (two males per pen) after 12 h food deprivation. After evisceration, the breasts and thighs with skin were separated packed in plastics bags and chilled during transport to the laboratory. The breast (pectorals major) and thigh (gastrocnemius interna)

Table 1: Ingredients and analysed chemical composition of the starter and grower diets

Factors	Starter[1]	Grower[2]
Ingredients (%)		
Maize	55.70	730.00
Wheat	--	33.00
Soybean meal	37.00	30.00
Soybean oil	3.00	4.00
Fish meal	2.00	-
Limestone	1.00	-
Oyster shell	-	1.20
Dicalcium phosphate	0.50	1.00
Vitamin-mineral mix[3]	0.50	0.50
dl-methionine	0.10	0.10
Sodium chloride	0.20	0.20
Vitamin E (mg kg^{-1})	-	75.00
Se (sodium selenite/Se-yeast) (mg kg^{-1})[4]	-	0.30
Analysed chemical composition (%)		
Dry matter	89.22	89.35
Crude protein	22.23	20.07
Fat	6.24	6.29
Fibre	3.61	3.56
Ash	6.17	5.70
Calcium	0.82	0.81
Available P	0.55	0.55
Selenium (mg kg^{-1})	0.08	(0.365, 0.362, 0.371, 0.375)[4]
ME by calculation (MJ kg^{-1})	12.78	12.91

[1]Starter diet fed to birds from 0-20 days. [2]T1, control diet = 0.3 SS; T2 = 0.2 SS + 0.1 SY; T3 = 0.1 SS + 0.2 SY; T4 = 0.3 SY of mg kg^{-1}. [3]Provides per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 2,000, IU; vitamin E, 18 IU; vitamin B1, 1.8 mg; vitamin B2, 6.6 mg; vitamin B3, 10 mg; vitamin B5, 30 mg; vitamin B6, 3.0 mg; vitamin B9, 1 mg; vitamin B12, 1.5 mg; vitamin K3, 2 mg; vitamin H2, 0.01 mg; folic acid, 0.21 mg; nicotinic acid, 0.65 mg; choline chloride, 500 mg; Fe, 50 mg; Mn, 100 mg; Cu, 10 mg; Zn, 85 mg; I, 1 mg; Se, 0.2 mg. [4]1% basal premix was made with the selenium products for mixing of dietary treatments in grower phase. Selenium contained 1000 mg Se kg^{-1} and it was supplemented, individually or mixed (sodium selenite/se-yeast) to the diet. Se-enriched Yeast (SY) provided per kg of diets: selenium 0.3 mg, calcium 0.75 mg, phosphorus 2.33 mg, sulphur 1.21 mg, potassium 3 mg, magnesium 0.94 mg, iron 0.074 mg, manganese 0.034 mg, copper 0.015 mg, zinc 0.107 mg sodium selenite (Na2SeO3; SS) content were >98%

Table 2: Concentration of selenium and a-tocopherol in diets (mg kg^{-1}), breast and thigh muscles and excreta[1]

	Experimental diets[2]					
Time of storage	T1	T2	T3	T4	SE	P[3]
Diet						
Selenium	0.36	0.36	0.37	0.37	0.004	NS
α-tocopherol	71.60	71.50	72.20	72.70	0.380	NS
Breast muscle						
Selenium	0.65^{e}	0.70^{e}	1.00^{b}	1.49^{a}	0.380	**
α-tocopherol	45.70^{e}	46.10^{e}	52.30^{b}	56.70^{a}	1.120	**
Thigh muscle						
Selenium	0.64^{e}	0.78^{e}	1.05^{b}	1.21^{a}	0.040	**
α-tocophero	151.10^{b}	50.80^{b}	52.20^{ba}	54.20^{a}	0.690	*
Excreta selenium	1.17^{a}	0.93^{b}	0.65^{c}	0.45^{d}	0.050	**

[a,b,c,d]Averages with different superscripts differ at $p<0.05$. [1]Values are means of eight observations per treatment and their standard errors. [2]Treatments:T1 = 0.3 SS; T2 = 0.2 SS + 0.1 SY; T3 = 0.1 SS + 0.2 SY; T4 = 0.3 SY mg kg^{-1}. [3]NS = non significant; *= $p<0.05$; **= $p<0.01$

muscles were ground and divided into several samples for the determination of selenium, α-tocopherol and lipid oxidation at 0, 4 or 8 days during storage at 5°C.

Analyses: Experimental diets, meat and excreta samples were analysed for humidity determined by oven at 105°C and ash content. To determine Se in diets, frozen-dried meat and excreta samples were mineralized using a Closed-vessel Microwave Digestion System (MDS-2000, 630 W) equipped with pressure monitoring option (maximum operating pressure, 13.8 bar), sample carousel and advanced composite vessels (CEM Corp., Matthews, NC, USA) in the presence of HNO_3 and H_2O_2. Standard addition method was used for electrothermic atomization ET AAS measurements after mineralization. Total Se in plasma and tissues samples were measured by electrothermal atomic absorption spectrophotometry, using a Shimadzu A A-680 (Shimadzu Corporation, Tokyo, Japan) flame Atomic Absorption Spectrophotometer (AAS).

The α-tocopherol content of diets and meat was determined according to the EVS-EN 12822 European Standards (2000) by HPLC (Shimadzu, VP series) equipped with a diode-array detector. Lipid peroxidation in breast and thigh muscles was measured by the thiobarbituric acid method accordingly to Piette and Raymond (1999) and the results were expressed as Thiobarbituric Acid-Reactive Substances (TBARS) in mg of malondialdehyde kg^{-1}. All analyses were carried out in Research Station of Medical Sciences, Tabriz University, Iran.

Statistical analysis: All data were analysed by ANOVA using the GLM procedure of SAS software (SAS Institute, 1998) which were appropriate for a randomized complete block design. When significances were detected ($p<0.05$) values were compared post-hoc using the Duncan test. The results are expressed as averages and their Standard Error (SE).

RESULTS AND DISCUSSION

Dietary replacement of inorganic selenium with organic form increased ($p<0.05$) the concentration of Se and α-tocopherol in breast and thigh muscles (Table 2). By replacing selenium source from SS to SY in diet of male broilers, the concentration of Se in excreta was decreased. The influence of replacement of inorganic with organic Se on lipid oxidation measured as MDA formation in the breast and thigh muscles is shown in Table 3. The concentration of malondialdehyde increased in all treatments during chilled storage (4-6°C) for up to 8 days but the extent of lipid oxidation was lower in broilers fed diets containing SY compared to the control (SS diet). The dietary utilization of Se-enriched yeast reduced MDA values in breast and thigh meat samples after 0, 4 and 8 days under chilled storage. All differences were statistically different ($p<0.05$) with the exception of thigh samples after 8 days of storage in which the average of MDA concentration was not significantly decreased by the substitution of Se source.

Table 3: Effect of Se supplementation and chilled storage (4-6°C) on the concentration of malondialdehyde (mg kg^{-1}) in breast and thigh muscles

	Experimental diets[2]					
Time of storage	T1	T2	T3	T4	SE	P[3]
Breast muscle (day)						
0	0.37[a]	0.35[a]	0.30[ba]	0.26[b]	0.024	*
4	0.85[a]	0.83[a]	0.77[ba]	0.70[b]	0.025	**
8	1.20[a]	1.20[a]	1.07[b]	0.96[b]	0.038	**
Thigh muscle (day)						
0	0.36[a]	0.35[a]	0.32[ba]	0.30[b]	0.014	*
4	0.86[a]	0.86[a]	0.82[b]	0.78[c]	0.011	**
8	1.24	1.23	1.13	1.03	0.066	NS

[a,b,c,d]Averages with different superscripts differ at $p<0.05$. [1]Values are averages of eight observations per treatment and their standard errors. [2]Treatments: T1 = 0.3 SS; T2 = 0.2 SS + 0.1 SY; T3 = 0.1 SS + 0.2 SY; T4 = 0.3 SY of mg kg^{-1}. [3]NS = non significant; *= $p<0.05$; **= <0.01

Choct *et al.* (2004) found that an increasing supplementation rate of Se from 0.1-0.25 mg kg^{-1} increased the breast muscle selenium concentration from 0.232-0.278 mg kg^{-1} and both selenium source (organic and inorganic) and concentration significantly influenced ($p\leq0.05$) the selenium content of the excreta at day 28. They reported that the amount of Se available for assimilation by the tissues was dependent on the source and concentration of the element while organic Se is deposited in the body tissues more efficiently than inorganic selenium.

Inorganic selenium is passively absorbed from the intestine by a simple diffusion process, whereas organic selenium is actively absorbed through the amino acid transport mechanisms (Wolfram *et al.*, 1989). For this reason, inorganic Se (sodium selenite) was retained at a much lower concentration in muscle tissue was less efficiently absorbed and was excreted at a higher rate than organic Se due to their different metabolic pathways. Echevarria *et al.* (1988) and Downs *et al.* (2000) stated that the Se concentration in several tissues, particularly in kidneys and liver increased linearly with the increase of Se content of the diet.

Spears *et al.* (2003) reported that broiler chickens fed 0.15 ppm Se-Methionine showed increased breast Se concentrations compared to those fed sodium selenite. Payne and Southern (2005) observed that the increased Se concentration in breast muscle and blood plasma of broiler chickens fed diets supplemented with 0.3 ppm Se as Se-enriched yeast. Results found in the present study are in accordance with those stated by Sevcikova *et al.* (2006) who found that Se and α-tocopherol retention

increased in muscles of birds receiving organic Se in combination with 50 mg of vitamin E in their diets. It was reported that selenium as Se-enriched yeast has more efficient utilization than Se-alga as indicated by the high Se concentration in breast and thigh muscle and low Se concentration in the excreta. The content of selenium in muscle may be influenced by the method of determination used (ICP, hydride system, atomic absorption spectrophotometry) in both trials.

On the other hand, adding α-tocopherol to Se supplemented diets could be effective to improve selenium retention and oxidative stability of chicken meat. Ryu *et al.* (2005) studied the effects of supplemental dietary selenium on growth performance, lipid oxidation and color stability of male broiler chicks and reported that dietary selenium supplementation of 8 ppm in combination with 100 IU of α-tocopherol was more effective in reducing lipid oxidation compared to 100 IU of α-tocopherol kg^{-1} feed only. In agreement with previous findings (Sevcikova *et al.*, 2006; Skrivan *et al.*, 2008b), the present study shows evidence that dietary Se supplementation increased the α-tocopherol content of broiler meat. However, the mechanism of this synergism remains unclear (Dlouha *et al.*, 2008b). It can be speculated that Se as a component of glutathione peroxidase, actively participates on lipid peroxide removal from cells, sparing the use of vitamin E for this purpose (Surai, 2002). Thus, the substitution of inorganic selenium by organic Se in combination to α-tocopherol was more effective on Se and vitamin E deposition in muscle tissues when compared to other types of supplementation.

For adult humans, the recommended selenium intake for adult humans is 55 μg day^{-1} with tolerable upper intake level 300 μg day^{-1} (Rayman, 2004) and the recommended vitamin E intake is 10-15 mg day^{-1} (Food and Nutrition Board, Institute of Medicine, 2000). Considering this the replacement of 0.3 mg of organic Se kg^{-1} of the diet in combination to 50 mg of vitamin E of the diet in broilers diet would ensure the content od Se and vitamin E in meat of T4-fed chickens that achieve the human recommended intake (Se and vitamin E content in breast = 38 and 1.14 mg 100 g^{-1}, respectively and in t high = 27 and 1.05 mg 100 g^{-1}, respectively).

In this study, TBARS formation in breast meat decreased in SY treatment compared with SS or SS in mixed with SY treatment. However, for all treatments and tissues, concentration of MDA increased with storage time as expected but replacing SS with Se-enriched yeast in the diet decreased lipid peroxidation speed in breast and thigh samples of T4 treatment which showed the lowest values of MDA.

Sevcikova *et al.* (2006) found that the supplementation of selenium especially organic might improve poultry meat quality and shelf life by reducing the drip loss. Increasing dietary selenium improved the Se status or retention of the muscle and oxidative stability of chicken meat during chilled storage (Yoon *et al.*, 2007; Skrivan *et al.*, 2008a, b). Poultry meat is quite sensitive to oxidative deterioration due to its high content of polyunsaturated fatty acids. Moreover, it is recognized that vitamin E as a strong antioxidant helps to protect the polyunsaturated fatty acids in cell membranes from peroxidative damage. The researchers by studies on broiler chickens (Smet *et al.*, 2008; Villaverde *et al.*, 2008) or Turkeys (Mikulski *et al.*, 2009) reported that a higher oxidative stability of lipids could occur if selenium in combination with vitamin E were supplemented in diets. This finding suggested that dietary supplementation of Se especially in combination with vitamin E, more influenced oxidative stability of the meat during refrigerated storage.

CONCLUSION

In this study, modification of dietary Se source by replacing SS for SY increased the selenium and α-tocopherol content of broiler meat and can prevent the oxidation of breast and thigh meat during chilled storage. Incorporation of selenium and vitamin E in diet had several effects on antioxidant defense system that could help to improve body protection from peroxidative damage. Therefore, healthy broiler diets can be appropriate to ensure human daily requirements of selenium and α-tocopherol and improve meat quality and shelf life.

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