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Investigation of antioxidant vitamins (A, E and C) and selenium levels in chickens receiving estrogen or testosterone

Ihsan Halifeoglu*[1], Fikret Karatas[2], Halit Canatan[1,3], Ramiz Colak[4] and Erkan Karadas[5]

[1]*Department of Biochemistry and Clinical Biochemistry, College of Medicine, Firat (Euphrates) University, Elazig, Turkey*
[2]*Department of Chemistry, College of Science, Firat (Euphrates) University, Elazig, Turkey*
[3]*Department of Medical Biology, College of Medicine, Firat (Euphrates) University, Elazig, Turkey*
[4]*Department of Endocrinology, College of Medicine, Firat (Euphrates) University, Elazig, Turkey*
[5]*Department of Pathology, College of Veterinary Medicine, Firat (Euphrates) University, Elazig, Turkey*

In the present study estrogen or testosterone was administered to broiler chickens (6 weeks old) for 5 weeks and levels of antioxidant vitamins (A, E and C) and selenium (Se) were determined. In animals who received estrogen, vitamins A, E, C and Se levels were 0.70 ± 0.19, 11.0 ± 2.45, 20.0 ± 5.17 and $130.0 \pm 25.0\,\mu g\,l^{-1}$, respectively. Vitamins A, E, C and Se levels in the testosterone-administered group were found to be 0.54 ± 0.16, 9.9 ± 1.96, 18.0 ± 5.18 and $100.0 \pm 18.0\,\mu g\,l^{-1}$, respectively. Vitamins A, E, C and Se levels were found to be significantly increased in the estrogen-administered group compared to the controls ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$, respectively). Although all parameters were increased in testosterone-treated animals, only increases in vitamins A and E were found to be statistically significant ($p < 0.01$, $p < 0.01$, respectively). Based on the present findings, estrogen and testosterone show direct antioxidant effects by increasing the activities of some enzymes and they also cause an increase in antioxidant vitamin levels and hence indirectly also contribute to antioxidant capacity. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS — estrogen; testosterone; vitamin A; vitamin E; vitamin C; Se; HPLC

INTRODUCTION

Frequent development of infections in chickens results in reduction of meat and egg production and/or death of the animals. Therefore, huge efforts are made by both producers and health professionals to protect poultry from infections which may result in severe losses for both producers at the local level and in the country's general economy.[1]

There are several defence systems, named antioxidant defence systems or briefly, antioxidants. Antioxidants show their protective effect by scavenging reactive oxygen species and/or blocking peroxidation chain reactions and eventually inhibiting lipid peroxidation. Antioxidants can be divided into two major groups based on their sources: natural (endogenous) and exogenous. Mitochondrial cytochrome oxidase, superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione-S-transferase and hydroperoxidase are among the enzymic antioxidants. Non-enzymic antioxidants can be classified as lipid soluble (i.e. α-tocopherol, β-carotene) or water soluble (i.e. ascorbic acid, melatonin, cysteine, ceruloplasmin, hemoglobin, bilirubin).[2,3] Superoxide radicals are cleared by enzymic dismutation. Non-enzymic antioxidant systems also contribute to the clearing of oxygen radicals in organisms. Vitamins A, E, C and Se are among the important non-enzymic antioxidant defence systems.

In addition to reproductive, sexual and strong anabolic functions and effects, estrogen and testosterone are also known to have antioxidant potential. The exact mechanism of the antioxidative roles of estrogen and testosterone are not clearly understood yet.[4–6] In the present study we examined the effect of estrogen and testosterone administration on the levels of antioxidant vitamins A, E, C and Se in broiler chickens.

* Correspondence to: Dr I. Halifeoglu, Department of Biochemistry and Clinical Biochemistry, College of Medicine and Firat Medical Center, Firat (Euphrates) University, Elaziğ 23119, Turkey.
Fax: +90-424-238-7688. E-mail: halifeoglu@hotmail.com

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MATERIALS AND METHODS

A total of 60 broiler chickens (ROSS PM-3) was used in the study. When the animals were 6 weeks old, they were divided into three group. The first group ($n = 20$) was given testosterone (25 mg kg^{-1}, Sustason 250, Budapest, Hungary) whereas the second group of animals ($n = 20$) was given estrogen (2 mg kg^{-1}, Akrofolline Chinoin, BioFarma, Budapest, Hungary) by intramuscullar injection. Control animals ($n = 20$) were injected with saline. Blood samples were collected at the end of the fifth week of the experiment and sera were kept at $-20°C$ until analysed.

Levels of vitamins A and E were determined as described by Catignani[7] and Miller *et al*.[8] For determination of vitamin C, samples were initially precipitated by the method of Cerhata *et al*.[9] and assayed according to Tavazzi *et al*.[10] as we recently reported.[11]

Serum samples (1.0 ml) obtained for determination of Se levels were treated in a teflon bomb according to Breyer and Gilbert[12] and were determined according to Shofstal and Hardy.[13] CECIL 1100 (Cambridge, England) High Performance Liquid Chromatography (HPLC) was used for determination of Se and vitamin levels. All the chemicals used in the study were of analytical grade and purchased from Merck (Darmstad, Germany).

Statistical analysis was carried out using an SPSS program for Windows, Ver.10 (SPSS Inc. Chicago, IL, USA). The data obtained are expressed as mean values $\pm$ SD. Student's *t*-test was used to determine whether differences between the means were significant, with $p < 0.05$ taken as the significance level.

RESULTS AND DISCUSSION

Levels of vitamins A, E, C and Se are summarized in Table 1. Recovery rates using the assay systems were 98.2, 99.4, 96.5 and 95.7% for vitamins A, E, C and Se, respectively.

Estrogen and testosterone which are important steroid hormones have strong anabolic effects. In addition to their roles in metabolism and development of tissues such as skin, bone and muscle, it is known that estrogen and testosterone have antioxidant capacity although their mechanism of action has yet to be clarified.[4–6] Huh *et al.*[4] claimed that sex hormones supress the systems producing free radicals and hence they reduce formation of lipid peroxidation in mice. On the other hand, Arteaga *et al*.[5] reported that estradiol, but not progesterone or testosterone, possess antioxidant capacity and have an antioxidant effect on LDL. Ahlbom *et al*.[6] reported that catalase and SOD activity levels in the cerebral granule cells of mice administered testosterone were increased two-fold. In addition, testosterone has been reported to be protective against hydrogen peroxide and nitric oxide toxicity in mice. Biswas *et al*.[14] examined the effect of ascorbic acid on testicular steroid dehydrogenase and testosterone concentration and reported that ascorbic acid stimulates enzyme activity and increases testosterone content.

Tidus proposed that estrogen can act as an antioxidant against oxidative stress arising from exercise.[15] It was also stated that administration of estrogen affects levels of tissue glutathione, SOD and GSH-Px activity and vitamin A and E levels after exercise. Interestingly, female mice were better protected against tissue damage due to exercise and lipid peroxidation than males. Natural estrogen was reported to have 2.5-fold more activity than vitamins C and E, so that it is capable of scavenging more free radicals demostrating its antioxidant capacity.[16]

Vitamin A is reported to have a dose-dependent effect on the development of antioxidant systems in neonatal chicks.[17] Trash *et al.* reported that serum SOD activity levels were increased when vitamin E and Se were administered.[18] SOD, catalase and GSH-Px activity levels in chicken tissues were reported to be increased whereas lipid peroxidation levels were reduced upon supplementation with vitamins E and C.[19]

In the present study serum vitamins A, E, C and Se levels were increased in chickens that received testosterone or estrogen compared to the control group (Figures 1–4). These increases were higher in estrogen-administered animals than those given testosterone (Figures 1–4). This increase is possibly related to increased estrogen binding proteins in plasma but

Table 1. Levels of vitamins A, E, C and Se in chicken

Groups	Control ($n = 20$)	Estrogen treated ($n = 20$)	Testosteron treated ($n = 20$)
Vitamin A (mg l^{-1})	0.40 ± 0.15	0.70 ± 0.19*	0.54 ± 0.16*
Vitamin E (mg l^{-1})	7.9 ± 3.10	11.0 ± 2.45*	9.9 ± 1.96**
Vitamin C (mg l^{-1})	16.0 ± 4.85	20.0 ± 5.17**	18.0 ± 5.18
Se (μg/l)	90.0 ± 20.0	130.0 ± 25.0**	100.0 ± 18.0

*$p < 0.01$, **$p < 0.05$ when compared to control.

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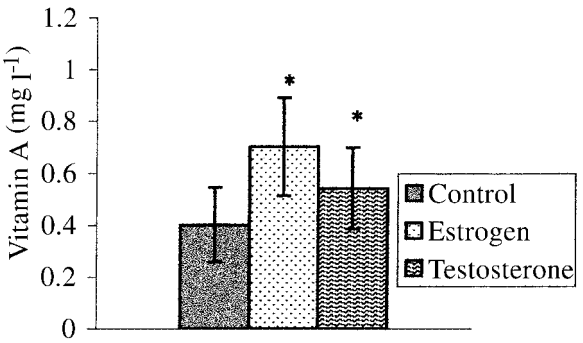


Figure 1. Vitamin A levels in control and testosterone- or estrogen-administered chickens. *$p < 0.01$

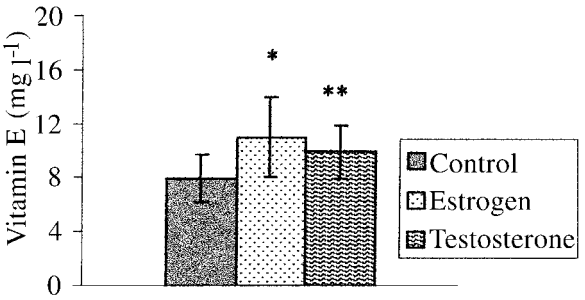


Figure 2. Vitamin E levels in control and testosterone- or estrogen-administered chickens. *$p < 0.01$, **$p < 0.05$

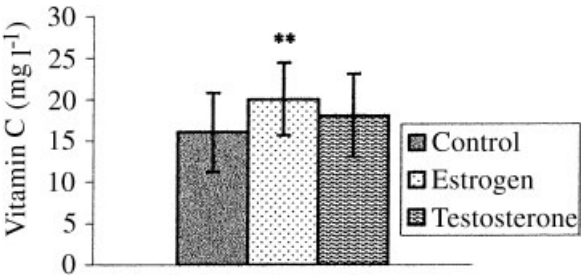


Figure 3. Vitamin C levels in control and testosterone- or estrogen-administered chickens. **$p < 0.05$

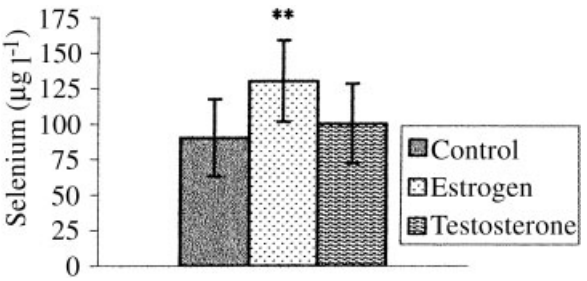


Figure 4. Se levels in control and testosterone- or estrogen-administered chickens. **$p < 0.05$

we did not measure binding proteins in our investigation. Overall, this increase contributes to the antioxidant capacity directly or indirectly.

In conclusion, in addition to enhancing the activity of antioxidant enzymes directly, estrogen and testosterone can also contribute indirectly to antioxidant activity by protecting levels of antioxidant vitamins and Se.

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