VITAMIN E SUPPLEMENTATION REDUCES DEXAMETHASONE–INDUCED OXIDATIVE STRESS IN LAYING HENS

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ABSTRACT: The objective of the present study is to research the effect of dietary vitamin E (Vit E) on the antioxidative status and lipid peroxidation in laying hens as well as in egg yolks under experimentally induced oxidative stress conditions via Dexamethasone (DEX) administrations. A total of 60 Egyptian local strain (Gimmizah) laying hens at 36 weeks of age were housed in individual cages in an open-sided building under a 16 hr light: 8 hr dark lighting schedule. Birds were randomly divided into four experimentally treatments [DEX (4 mg/hen/day), Vit. E (200 mg/kg diet), DEX+Vit. E (4 mg/hen/day+ 200 mg/kg diet; respectively) and control, n= 15]. Birds were provided with commercial feed and water ad libitum. All treatments lasted for 7 successive days. Oxidative stress induced by DEX injections significantly reduced egg production and egg weight. Vit. E recovered this reduction when supplemented to stress-induced hens, compared to DEX treatment. Highly yolk lipid oxidation was associated with oxidative stress treatment and it was reduced by Vit E supplementation. The same trend was observed in lipid peroxidation in plasma and liver. It could be concluded that super-nutritional levels of Vit E (200 mg/kg diet) may contribute in enhancing laying performance and antioxidative properties of laying hens under stress conditions.
INTRODUCTION

Stress susceptibility of chickens is a major problem in the modern poultry industry. As a result of stress, feed consumption, growth rate, feed efficiency, egg quality, fertility and chick quality decline (Gross and Siegel, 1993; El-Lethey et al., 2000). In chickens, adrenal corticosteroids are secreted shortly after exposure to stress and elevated levels of plasma glucocorticoids have been used as an index of the response to stress in poultry (Siegel, 1995).

High levels of circulatory glucocorticoids accelerate the metabolic rates, and elevate free radicals levels (in particular reactive oxygen species, ROS). ROS can have beneficial roles, as in phagocytes where they protect against bacteria and parasites. However, if natural antioxidant mechanisms are not adequate to quench excess oxygen radicals then they can react with cell structures and attack proteins, lipids, carbohydrates and nucleotides within the cell, a state referred to as oxidative stress (Gutteridge, 1993). Various biomarkers like malondialdehyde (MDA) and specific enzymes that limit free-radical formation, such as glutathione peroxidase (GPX) play an important role in the protection of cell membranes against oxidative damage and may be used as indicators of antioxidative status. Previous studies indicated that glucocorticoid administration induced oxidative stress status in chicken (Eid et al., 2003, Eid et al., 2006 b). Similarly, synthetic glucocorticoid dexamethasone (DEX) administration mimics the adverse effects of increased corticosterone. Dexamethasone (doses ranging from 0.2 to 4.0 mg/kg) was used as an immune suppressive agent (Fowles et al., 1993), as a mediator of prenatal stress (Maccari et al., 2003) and to induce oxidative stress in laying hens (El-Habbak et al., 2005) and in cockerels (Eid et al., 2006 b).

Oxidative stress could be one of the factors negatively affecting egg production and quality. The oxidative stability of eggs has not been an area of major concern since their built-in antioxidant characteristics maintain the flavor during extended storage (Cuppert, 2001). However, this may be changed under stress condition due to the accelerating consumption of antioxidants to protect the birds’ cells. This may lead to low oxidative stability of the eggs and reduce its quality and shelf life.

Under stressful conditions, the requirement of antioxidants such as Vit E (α-tocopherol) is thought to increase to protect tissues from lipid peroxidation (Eid et al., 2006 b). A high dietary intake of Vit E (200 mg/kg diet) increases its concentration in both blood and seminal plasma and in
addition produces beneficial changes in the antioxidant capacity and lipid profile of chicken semen (El-Sebai, 2005) under normal conditions. Furthermore, use of α-tocopherol in hen feeding seems to be efficient way to improve the oxidative stability of eggs (Qi and Sim, 1998).

The objective of the present study is to research the effects of super-nutritional level of Vit E (200 mg/kg diet) supplementation on the antioxidative status and lipid peroxidation in laying hens as well as in egg yolks under oxidative stress conditions experimentally induced by DEX injections.

**MATERIALS AND METHODS**

*Birds, diets and experimental design:* A Total number of 60 layer hens (Egyptian local strain (Gimmizah)) at their 36 weeks of age were selected from about three fold larger population to obtain uniform body weight (2.21 ± 0.10 kg) and egg production (82.13 ± 1.22 %). Birds were housed in individual cages batteries, and provided with water and commercial corn-soybean diet ad libitum (16 % crude protein and metabolizable energy 2830 ME/Kg, 3.5% calcium, and 0.75% phosphorus). Diet composition was formulated to meet the recommended nutrient requirements (NRC, 1994). Ambient temperature averaged 25 ± 2 °C with relative humidity 60–70 % and a 16 hr light: 8 hr dark-cycle was applied. Hens were divided into 4 experimental treatments (n=15). The first one (DEX) was daily intramuscularly femur injected with 1 ml of the synthetic glucocorticoid Dexamethasone, DEX (as sodium phosphate 4 mg /ml). The second treatment (Vit E) received a diet supplemented with a super-nutritional level of Vit E in the form of α–tocopherol acetate (200 mg/kg diet) and equally daily intramuscularly femur injected with 1 ml sterile physiological saline solution (0.9% NaCl). The third treatment (DEX + Vit E) received both DEX injection and Vit E supplementation (4 mg/hen/day and 200 mg/kg diet; respectively). The fourth treatment served as control and was only intramuscularly femur injected with the same dose of sterile physiological saline solution. All treatments lasted for 7 successive days. DEX was purchased from Amriya Pharmaceutical Industries Co., Alexandria, Egypt; meanwhile Vit E (α–tocopherol acetate) was purchased from Cairo Pharmaceutical Industries Co., Egypt.

*Performance parameters and sampling:* Egg production, egg weight and feed consumption were recorded daily. On the seventh day of the treatments and after 4 hours from the last injection, ten females from each treatment were randomly chosen and slaughtered. Liver samples were taken
and kept at -20 °C for subsequent analysis. Blood samples were collected in heparinised tubes for blood count and other laboratory analysis. Blood smears were made and stained for differential leukocyte counts (100 cells/smear; Cook, 1959) and means were calculated for heterophils and lymphocytes (H/L) ratios. Plasma was separated by centrifugation at 10,000 g for 10 minutes and stored at -20 °C until analyzed.

Laboratory analysis: Glucose was estimated in plasma according to Lott and Turner (1975), by using “Glucose GOD-PAP kits” produced by Spinreact, S. A., Spain. Triglycerides was determined according to Fassati and Prencipe, (1983) by using "TRIGLYCERIDES kits" which produced by Bio-Diagnostic Egypt. Lipid peroxidation in the blood plasma, hepatic tissues and egg yolk was measured in the form of thiobarbituric acid reactive substance (malondialdehyde, MDA) according to Richard et al., (1992). MDA is a product of the oxidative degradation of polyunsaturated fatty acids, and thus used as index of oxidative stress. Activity of the antioxidative enzyme glutathione peroxidase (GPX) was determined by the method of Levander et al., (1983). One unite of GPX activity oxidizes 1.0 nmol of NADPH/ (mg protein/ min). Proteins were determined in the plasma by the method of Biuret as described by Armstrong and Carr (1964).

Statistical analysis: Differences among the experimental groups were tested by one-way ANOVA using SPSS® statistical software package for windows version 11.0. Duncan’s multiple-range test was applied. P≤0.05 was set as limit of significance.

RESULTS AND DISCUSSION

Data describing the effect of induced oxidative stress and supplemental dietary Vitamin E on body weight, feed intake, laying performance, egg weight, plasma triglyceride, plasma glucose and H/L ratio are presented in Table (1).

No significant differences were observed in body weight and feed intake. However, there were significant differences (P≤0.05) in egg production, and egg weight among the treatments Table (1).

Previous studies have shown that administration of glucocorticoids develops oxidative stress which mimics the effect of stressors in domestic fowls (Eid et al., 2003; 2006 b). In the present experiment this model was used to study the effect of induced stress by DEX and dietary Vit E supplementation on antioxidative status and lipid peroxidation of laying hens and the lipid oxidation in its eggs.
Induced stress by daily injection of DEX for 7 successive days caused a significant (P ≤ 0.05) decline in egg production and egg weight (Table 1). It was observed that egg production was reduced sharply on the 6th and 7th day of DEX administration. These responses are in agreement with the results obtained by El-Lethey et al. (2001; 2003) and El-Habbak et al. (2005). They noted that increases of circulating levels of glucocorticoids caused a reduction in egg production, delayed the onset of egg laying and also inhibited the reproduction. A possible explanation for this could be found in Etches et al. (1984) who established that DEX administration or infusions of corticosterone blocked ovulation. These results were confirmed by Huang and Shirley (2001) who observed that treatment of the follicles with increasing concentrations of DEX suppressed LH and progesterone production. Similarly, Deitemeyer et al. (1985) indicated that DEX markedly inhibited the synthesis of prostaglandin E2. Dexamethasone markedly decreased inhibin production by granulosa cells in vitro, and consequently, decreased the plasma inhibin concentration (Vanmontfort et al., 1997).

Dietary Vit E under stress conditions significantly enhanced egg production and egg weight compared to stress group (Table 1), this may refer to the antioxidant properties of Vit E (Trader and Atkinson, 2007). Another explanation is that dietary Vit E may reduce the circulatory levels of glucocorticoids (Eid et al., 2003) which in turn will reduce the formation of free radicals. However, we have no data in this paper to emphasize this point of view. Vit E alone did not show any effect on egg production or egg weight comparing to control group. These results are in correspondence with the observations of Hossain et al. (1998) and Panagiota et al., (2006).

Glucocorticoids treatment develops insulin resistance, hyperglycaemia, hypertriglyceridemia, resulting in increasing abdominal fat content and fatty liver, which are accompanied by increases in lipid peroxidation (Hidalgo et al., 1988). Laying hens treated for 7 successive days with DEX (4 mg/bird/day) had significantly (P ≤ 0.05) higher plasma glucose (Table 1). Plasma glucose levels were increased in DEX- treated birds relative to control birds. These results are in agreement with Kobayashi et al. (1989) and El-Habbak et al., (2005) who found that DEX increased the level of plasma glucose. Corticosterone-induced hyperglycemia in chickens is, in part, supported by enhancing of tissue glycogenolysis and hepatic glucose-6-phosphate (Joseph and Ramachandran, 1992). These effects of exogenous corticosterone on intermediary metabolism may be mediated directly by specific receptors in the relevant target tissue. In addition, the effects may be indirect in that exogenous corticosterone produces changes in other hormones of
intermediary metabolism such as thyroid hormones, growth hormone, prolactin, somatomedin C and norepinephrine (John et al., 1987; Saadoun et al., 1987). Our results show that plasma triglycerides concentrations in DEX treatment were increased significantly by 144.6% of the control treatment (Table 1). Furthermore, it is shown in the present study that Vit E significantly decreased plasma triglyceride concentration in both DEX treated or untreated birds as compared to control. These results suggesting that the antioxidants like Vit E play an important role in lipid metabolism of the whole body (Taniguchi et al., 1999; Eid et al., 2003, 2006 a,b).

The H/L ratio was increased by DEX administration. The data in Table1 show that DEX infusion induced higher H/L ratio than infusion of vehicle only (control). These observations were repeatedly reported by Huff et al. (2001) who noted that treatment with DEX resulted in increases in H/L ratio. Similarly, El-Lethey et al. (2001; 2003) proved that birds fed on corticosterone had higher H/L ratios. Moreover, the upward shift in the H/L ratio occurs during stress (Gross and Siegel, 1993). Also H/L ratio could be a tool for resistant to stress selection in poultry (AL-Murrani et al.,2006). It is noteworthy to indicate that dietary Vit E could ameliorate these increases under stress condition. However Vit E alone does not have any significant effect when compared with control.

Free radicals trigger the metabolic disorder, cell death and growth retardation (Okada, 1996). However, little attention has been paid to the point that free radicals formation is significantly increased when animals are exposed to stress. By using malondiaidehyde (MDA) as a marker of the oxidative stress, we studied the effect of induced oxidative stress and supplemental dietary Vit E on lipid oxidation in egg yolk and lipid peroxidation in plasma and hepatic tissues. Over the course of the trial DEX injections elevated (P ≤ 0.05) egg yolk MDA and Vit E supplementation appeared to antagonize this effect of DEX. In case of normal (unstressed) birds dietary Vit E alone significantly reduced yolk MDA comparing to the control (Figure 1A). As for plasma MDA (Figure 1B), induced stress via DEX administration significantly elevated plasma MDA to more than three times its value in control samples, where the third group (DEX+Vit E) was significantly lower than that for the treatment receiving DEX alone, reaching about 54% of its value. However, it was still significantly higher than the control value. The same trend was observed in hepatic MDA (Figure 1C), unless dietary Vit E alone significantly reduced hepatic MDA lower than the other treatments. The effect of induced stress on plasma and hepatic MDA are in agreement with our previous work (Eid et al., 2003; 2006 a, b). There is a high level of MDA in yolk of the stress birds and this
was correlated with high level of MDA in both plasma and liver. This may refer to the fact that liver is the major metabolic center in the body where, yolk precursors are synthesized then transferred to the ovary. So, it could be possible that elevated MDA was transferred to the yolk from the liver. This elevated yolk MDA may affect the shelf life of the eggs and reduced its quality. The oxidative stability of eggs has not been an area of major concern since their built-in antioxidant characteristics maintain the flavor during extended storage (Cuppert, 2001). However, this may be changed under stress conditions due to the accelerating consumption of antioxidants to protect the birds’ cells. This may lead to low oxidative stability of the eggs and reduce its quality and shelf life. Because of the free radical scavenging ability of Vit E either under stress or alone, levels of MDA were found to be decreased. It could be speculated that feeding laying hens with high levels of Vit E under stress seems to be an efficient way to improve the oxidative stability of eggs; this is in agreement with (Qi and Sim, 1998). Free radicals are neutralized by α-tocopherol before lipid oxidation propagates among highly unsaturated fatty acids in cellular and subcellular membranes (Burton and Traber, 1990). Oxidative damage induces a cascade of downstream reactive oxygen species, some of which are relatively transient, such as hydroxynonenal, while others appear later and accumulate, such as malondialdehyde

The liver and kidney are major metabolic centers inside the body, according to their multifunction and the significant role of liver in lipid metabolism that they are a good targets for free radicals attack. GPX is one of the components of the enzymatic defense system against free radicals. GPX catalyze the reduction of a variety of hydroperoxides (ROOH and H$_2$O$_2$) using glutathione, thereby protecting cells against oxidative damage. Nutrition and environmental factors of the animal play crucial role in normal enzyme activity (Mézes, et al., 2003) thus measuring such parameters may be useful in evaluating the whole body defense system. Data of the present work showed that induced stress by DEX significantly (P ≤ 0.05) decreased the activity of GPX in the hepatic tissue homogenate (Figure 1 D). Vit E enhanced the activity of GPX under stress significantly (P ≤ 0.05) compared the stress treatment however; Vit E alone had no significant effect comparing to control (Figure 1 D). There are a number of studies showing the relation between glucocorticoids and antioxidative defense systems. Dexamethasone was reported to reduce the activity of GPX (Eid, 2006 b). This could lead to the speculation that Vit E may improve the antioxidative system and this assumption agrees with Ohtsuka et al. (1998).
In conclusion, based on the previous results, it could be mentioned that the antioxidants such as Vit E play an important role under the oxidative stress condition by decreasing lipid peroxidation level and enhancing the antioxidative defense. This could be translated to a normal and better performance and high quality products, according to the low effect of free radicals in the presence of the antioxidants. However, dietary guidelines for using supplemental antioxidants under stressful conditions should be studied. This study may contribute in guiding the egg production under stressful conditions since, recent consumers concern more on the quality and nutritive value of foods. The interest in high quality eggs now extends beyond production considerations to designing a high-quality food for consumption by health-conscious humans.

Table 1: Effect of DEX-induced oxidative stress and dietary Vit E supplementation on performance, plasma triglycerides and glucose and H/L ratio in laying hens. Values are expressed as means ± standard error.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DEX</th>
<th>Vit E</th>
<th>DEX + Vit E</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (Kg)</td>
<td>2.11 ± 0.7</td>
<td>2.32 ± 0.1</td>
<td>2.14 ± 0.2</td>
<td>2.06 ± 0.3</td>
</tr>
<tr>
<td>Feed Intake (g/hen/day)</td>
<td>104.3 ± 5.3</td>
<td>108.6 ± 3.8</td>
<td>106.3 ± 4.8</td>
<td>110.1 ± 7.8</td>
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<tr>
<td>Egg Production (%)</td>
<td>26.5 ± 3.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.1 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.7 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.9 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>44.2 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48.9 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.1 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.4 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>455.9 ± 10.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>273.4 ± 11.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>381.8 ± 10.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>315.2 ± 12.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>375.8 ± 18.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.4 ± 20.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>302.7 ± 16.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>210.2 ± 18.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>H/L ratio</td>
<td>0.62 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.39 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d</sup> Means within a row with no common superscript differ significantly (<i>P</i>≤0.05).
Oxidative stress; egg production; vitamin E.

Figure 1: Effect of DEX-induced oxidative stress and dietary Vit E supplementation on MDA in (A) egg yolk, (B) blood plasma and (C) liver and GPX (D) activity in the liver of laying hens. Values are expressed as means ± standard error; means with different script are significantly different from each other (P ≤ 0.05).

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الملخص العربي

الضافة فيتامين ه تقلل من الإجهاد التأكسدي المستحدث بواسطة الديكساميثازون في الدجاج البياض

يخيز عبد الوهاب عيسى و طارق عبيد و محمد معوض خليفة و محمد الحباك

قسم إنتاج الدواجن - كلية الزراعة - جامعة فرفر الشيخ *** محطة بحوث الدواجن بالصحيحة

معهد بحوث الإنتاج الحيواني

أجري هذا البحث لدراسة تأثير ضافة فيتامين ه في علاج الدجاج البياض على أدائها الإنتاجي والدهون المتراكمة والوظائف المضادة للتأكسدي تحت ظروف الإجهاد التأكسدي المستحدث بح lcm مساوكة الجلوكوكورتيكود الديكساميثازون. تم استخدام 60 دجاج عمر 36 أسبوع وموزعين على 4 معاملات (n=15)، و تم اختبارهم في أقصى فردية في عبير مفتوح تحت 16 ساعة أطلاع و 8 ساعات اطلاع، كما تم إعدادهم بعلاق تجارب و ماء لح الشهد. تم حقن المعاملة الأولى ب 1 ملج يعمل على الديكساميثازون 4 ملجم/ طائر و المعاملة الثانية تم ضافة فيتامين ه للعلاقة بين 200 ملجم/ كجم علف و تم حقنها ب 1 مل محلول فيسيولوجي و المعاملة الثالثة كانت كلا من الحق بالديكساميثازون و ضافة فيتامين ه للعلاقة، أما المعاملة الرابعة فكانت مجموعة مقارنة و تم حقنها ب 1 مل محلول فيسيولوجي وذلك لمدة أسبوع متواصل. أظهرت النتائج أن الإجهاد التأكسدي أدى إلى خفض إنتاج وزن البيض معا و كان لإضافة فيتامين ه أثر معنوي في تحسين هذه الصفات مقارنة بالطوري تحت الإجهاد. زادت نسبة الدهون المتراكمة في صف بيضه كتئبيه للإجهاد. و قلت هذه النسبة بالمعاملة فيتامين ه نفس الوضع تم ملاحظته بالنسبة لارتفاع في نسبة تأكسد الدهون و انخفاض في نشاط أنزيم الجلوكاتيون بروفوبرسيدي في بلازم الدم و الكبد. ويمكن القول بأن إضافة مستويات مرتفعة من فيتامين ه يمكن أن تحسن من إنتاجية و النظام المضاد للأكسد في الدجاج البياض تحت ظروف الإجهاد.

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