Effects of dietary antioxidant supplementation on the adrenal glands in quails (Coturnix coturnix japonica) reared under heat stress

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SUMMARY

The objective of this study was to assess the effects of the dietary antioxidant supplementation on adrenal glands in Japanese quails (Coturnix coturnix japonica) reared under heat stress using morphometric techniques. A total of 250 quails, 21 days old, were reared under heat stress (night temperature: 24°C and day temperature: 34°C) and divided into 5 equal groups composed of 30 females and 20 males each according to the dietary regimen: the control group was not supplemented whereas lipoic acid, vitamin C, vitamin E and vitamin E + vitamin C were daily mixed to the basal starter and grower diets at the dose of 250 mg/kg in the 4 other groups for 21 days. After slaughtering, adrenal glands were examined for morphometric histology. Although the proportions of the different zones of the adrenal tissues have varied according to the sex (males generally exhibited larger zones) and to the dietary treatment, it was observed that the hyperplasia of the cortical tissue induced by heat stress, particularly of the fasciculate and reticular zones, was markedly reduced when birds were supplemented with antioxidants. Treatments with the vitamin E or vitamin C (especially in males) used alone appeared to be the most efficient, whereas the proportions of the medulla zone were significantly increased when quails were supplemented with vitamin C alone or coupled to the vitamin E. These results suggest that a dietary antioxidant supplementation may alleviate the deleterious effects of heat stress by limiting steroid synthesis in the fasciculate and reticular zones in quails.

Keywords: Heat stress, antioxidant, quail, adrenal gland, fasciculate zone, reticular zone, corticosterone.

Introduction

Stress causes considerable increase in the activity of both pituitary adrenocortical and sympathetic adrenomedullary systems [1]. The adrenal gland, as a stress marker organ, is known to react to stressful stimuli, including noise, by an increase in corticosterone release [6, 34, 36], as well as in free cholesterol content [30]. Hormonal products of the adrenal medulla are known to assume key roles in cell and tissue injury brought on by stress [33].

One of the hormonal responses to heat stress is an increase in blood concentrations of corticosterone, the primary glucocorticoid hormone produced by the avian adrenal gland. Ascorbic acid supplementation improved performance of heat challenged broiler chickens and has been associated with lower plasma corticosterone concentrations [13, 17, 22]. The inhibitory action of ascorbic acid on adrenal steroidogenesis is mediated via modulation of steroid hydroxylating enzymes in the adrenal gland [11]. Therefore, high dietary intake of ascorbic acid regulates the production of corticosterone and plasma hormone concentrations during heat stress. The extent of the deleterious effects of heat stress is determined not only by its magnitude but also by the status of cellular defence systems, particularly the cellular antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, and water or lipid soluble antioxidants, such as ascorbic acid and vitamin E [23]. A deficiency of these antioxidants or decrease in defensive enzyme activities may cause irreparable and severe oxidant damage to cellular com-

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Ascorbic acid supplementation strengthens body energy stores that may be used for energy metabolism during heat stress [18]. Consequently, antioxidant nutrient supplementation, especially ascorbic acid can be used to attenuate the negative effects of environmental stress [27].

The adrenal gland exhibits structural changes in accordance to environmental states of the bird. Hence this study aims to record the effects of antioxidants added to rations, on the adrenal glands of Japanese quail submitted to heat stress.

**Materials and Methods**

**ANIMAL AND PROTOCOL DESIGN**

The Research Animal Ethic Committee of Ataturk University approved the protocol of this experiment. Two hundred and fifty (150 females and 100 males) 21-day old Japanese quails (Coturnix coturnix japonica), were randomly divided into five equal groups according to the antioxidant supplementation in the diet. Each group was constituted by 50 quails reared in 5 cages of 10 birds (6 females and 4 males) each. Lots were drawn to determine the placement of groups in the cages. The quail cages were placed in temperature-controlled rooms during the study, receiving 17 hours of daylight. A room temperature of 34°C was maintained between 8h00 and 16h00 and for the rest of the time the animals were housed at a temperature of 24°C. The study lasted 21 days.

A basal standard ration with no antioxidant was distributed to the control group (BS group), whereas basal diets supplemented with lipoic acid alone (250 mg α-lipoic acid/kg of diet) or vitamin E alone (250 mg dl-α-tocopheryl acetate/kg of diet) or vitamin C alone (250 mg of L-ascorbic acid/kg of diet) or vitamins E and C at the same dosages as previously were distributed to the four other groups (named BSLA, BSE, BSC and BSEC respectively). The starter and grower basal diets (Table I) were formulated using National Research Council [20] guideline and contained 20% to 22.2% crude protein (CP) and 2 844 to 2 901 kcal/kg metabolizable energy (ME). Small amounts of the basal diet were first mixed with the respective amounts of vitamin E and C as small batch, and then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. Food and water were given ad libitum during the experiment.

**HISTOLOGICAL ANALYSIS**

At the end of experiment, animals were killed by decapitation and the whole adrenal glands was rapidly removed from the fresh cadaver, subsequently cut open and washed with running water and immediately immersed in 10% formalin. After complete inspection of the adrenal glands, each segment removed was then put back into 10% formalin, where it remained for 24 hours. The segments were then embedded in paraffin blocks. Following this, sections of 5 µm in thick-

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Starter diet (0 – 3 weeks)</th>
<th>Grower diet (4 – 6 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>27.90</td>
<td>30.40</td>
</tr>
<tr>
<td>Corn</td>
<td>28.20</td>
<td>31.70</td>
</tr>
<tr>
<td>Corn gluten (43% CP)</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>21.20</td>
<td>15.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Wheat bran1</td>
<td>3.80</td>
<td>3.80</td>
</tr>
<tr>
<td>Di calcium phosphate</td>
<td>1.80</td>
<td>2.00</td>
</tr>
<tr>
<td>DL Methionine</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>L Lysin hydrochloride</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin - Mineral premix2</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

**Composition**

| Metabolizable energy (kcal/kg) | 2 844 | 2 901 |
| Crude protein (%)             | 22.2  | 20.0  |
| Ca (%)                        | 0.81  | 0.84  |
| P (%)                         | 0.43  | 0.45  |

*CP:* Crude protein; 1the wheat bran was substituted with the various additives (Vitamin E: 50 mg/kg, Vitamin C: 50 mg/kg, Lipoic acid: 25 mg/kg) in the antioxidant enriched diets; 2The vitamin-mineral premix provides per kg: all-trans-retinyl acetate, 1.8 mg; all-rac-α-tocopherol acetate, 1.25 mg; menadione sodium bisulfate, 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B-6, 2.2 mg; niacin, 35 mg; Co-pantothenate, 10 mg; vitamin B-12, 0.02 mg; folic acid, 0.55 mg; D-biotin, 0.1 mg, manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

**TABLE I:** Ingredients and chemical analysis of the starter and grower standard diets distributed to the Japanese quails.
ness were cut and stained with haematoxylin and eosin. The stained sections were observed under light microscope [25]. Thicknesses of cortex, cortical zones, medulla and total dimensions of adrenal glands were measured with micrometric ocular with regard to direct optical measurements as a classical method for small particles [9]. All results were tabulated for subsequent statistical study.

STATISTICAL ANALYSIS

The effect of antioxidants on the adrenal glands was tested with One-Way ANOVA test (total). Finally, male and female animals were divided into two different groups and the effect of the antioxidants on the adrenal components was determined with One-Way ANOVA test. Differences between vitamin groups were determined with the Multiple Comparison Range Test of Duncan. All statistical analyses were made with Statistical Packages for the Social Sciences 10.00 package software [31]. Differences were considered as significant when P values were less than 0.05.

Results

The adrenal glands were surrounded by a capsule of dense irregular connective tissue. The glomerulous zone was formed of clusters cells. The fasciculate zone was consisted of radial cords of cells and the cytoplasm of the cells was foamiert in the upper part than in the lower part. The cells of the reticular zone were smaller and darker than the cells of the fasciculate zone (figure 1). Macroscopically, no obvious difference between the adrenal glands from quails treated with various antioxidants and from controls was recorded.

The mean micrometric proportions of the various components of the adrenal glands were reported in Table II. The dimensions of the various zones were usually higher in males than in females for all groups except for the BSLA group (quails treated with lipoic acid) in which the medulla, the reticular and the glomerulous zones appeared slightly but not significantly more developed in females and the capsule was significantly larger in females (P < 0.001). In the other cases, differences between males and females were significant (P < 0.001) for the capsule width in BS and BSEC groups, for the glomerulous zone in the BS, BSE and BSC groups, for the reticular zone in BSE group, and for the medulla in the BSC group.

In females, the capsule width was not significantly affected by the dietary supplementation with antioxidants whereas this parameter was significantly decreased in males supplemented with lipoic acid or with tocopherol compared to the other groups (P < 0.001). Similarly, the extend of the glomerulous zone did not significantly differ between the various groups of females but, in males, this zone was minimal in birds simultaneously supplemented with the vitamins E and C and maximal in the controls (BS group); the difference in the dimensions of the glomerulous zone was significantly between males from these 2 groups (P < 0.001). The dimensions of the fasciculate zone were markedly and significantly reduced in both males and females supplemented with the lipoic acid or with the vitamins E and C used alone compared to birds co-treated with the 2 vitamins (group BSE vs. group BSEC; P < 0.001 for females; groups BSC, BSLA or BSE vs. group BSEC: P < 0.001 for males) and to the corresponding controls (P < 0.001). However, this parameter did not significantly differ between the groups BSEC and BS. The dimensions of the reticular zone were minimal in birds (females

<table>
<thead>
<tr>
<th></th>
<th>BS</th>
<th>BSLA</th>
<th>BSE</th>
<th>BSC</th>
<th>BSEC</th>
<th>SEM</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td><strong>Capsule</strong></td>
<td>15.67cd</td>
<td>18.98a</td>
<td>16.52bc</td>
<td>14.03d</td>
<td>14.70cd</td>
<td>14.92cd</td>
</tr>
<tr>
<td><strong>Glomerulous zone</strong></td>
<td>87.78cde</td>
<td>102.45a</td>
<td>96.85abc</td>
<td>93.77abcd</td>
<td>83.57de</td>
<td>98.45ab</td>
</tr>
<tr>
<td><strong>Fasciculate zone</strong></td>
<td>423.90a</td>
<td>438.42a</td>
<td>361.07cd</td>
<td>352.20cd</td>
<td>320.28ed</td>
<td>356.53cd</td>
</tr>
<tr>
<td><strong>Reticular zone</strong></td>
<td>205.10ab</td>
<td>212.72a</td>
<td>184.70cd</td>
<td>179.72cd</td>
<td>151.08e</td>
<td>173.98d</td>
</tr>
<tr>
<td><strong>Medulla</strong></td>
<td>300.25de</td>
<td>321.05cd</td>
<td>309.68de</td>
<td>271.67e</td>
<td>310.88de</td>
<td>330.53cd</td>
</tr>
</tbody>
</table>

M : male ; F : female.
Different superscripts a,b,c,d,e in the same row indicate significant differences between groups (P < 0.001).

TABLE II: Volumetric proportions (μm) of the various components of the adrenal glands in Japanese quails reared under heat stress according to the dietary supplementation with antioxidants (BS: no supplementation; BSLA: + lipoic acid (250 mg/kg of diet); BSE: + vitamin E (250 mg/kg of diet); BSC: + vitamin C (250 mg/kg of diet); BSEC: + vitamins E (250 mg/kg) and C (250 mg/kg). Results are expressed as means ± SEM (standard error of the mean).

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and males) supplemented with the vitamin E alone, intermediate in groups treated with the lipoic acid, the ascorbic acid and with both vitamin E and C, and maximal in the controls; these differences between groups were more evident in females (group BSE vs. groups BSEC, BSLA, BSC or BS: \( P < 0.001 \), groups BSEC, BSLA or BSC vs. group BS: \( P < 0.001 \)) than in males (groups BSE vs. groups BSEC or BS: \( P < 0.001 \) and groups BSLA or BSC vs. group BS: \( P < 0.001 \)). Finally, the various dietary treatments have differently affected the medulla size in females and in males. In females, the significantly largest medulla was achieved in the group co-treated with the 2 vitamins compared to all the other groups \( (P < 0.001) \) whereas this parameter reached maximal values in males treated with the vitamin C alone or coupled with the vitamin C (groups BSC or BSEC vs. groups BSLA, BS or BSE: \( P < 0.001 \)). It was also noted that for the both sexes, medulla reached highest dimensions in vitamin C-treated males (BSC males vs. BSEC females: \( P < 0.001 \)). Furthermore, the significantly smallest medulla in males was observed when they were supplemented with the lipoic acid compared to the other dietary treatments \( (P < 0.001) \).

**Discussion**

In most of previous studies, biochemical parameters were investigated for evaluating the effects of supplementation with vitamins and minerals on the adrenal glands [5, 7, 12, 13, 28-30, 37-39]. In this study, the effects of dietary antioxidant supplementation on the adrenal glands in Japanese quails reared under heat stress were determined by histology.

The adrenal medulla in mammalian species is surrounded by a cortex that contains three distinct layers, whereas the cortex and the medulla are intermingled in poultry species [2, 10]. In birds, the adrenal gland is characterised by the presence of cortical or inter-renal tissue which intermingles with the medullar or chromaffin tissue with a relatively uniform distribution but the ratio of one type of tissue to the other remains variable [35]. The relative volume proportions of the medullar tissue are usually considered to be higher than those of the cortical tissue in adult but this ratio varies with age, sex, health and environmental factors [35]. In the present study, the adrenal gland in Japanese quails was enclosed by a peri-capsular sheath of connective tissue with ganglion cells, bundles of myelinised nerve fibbers and blood vessels. Three components, cortical tissue, medullar tissue and sinusoids, mainly constituted the parenchyma of the gland, as previously described by BASHA and RAMESH [3]. The adrenal glands are known to respond to stress conditions, particularly environmental conditions, by inter-renal hyperplasia in birds [8]. In the current study, supplementation with antioxidants such as vitamin C, vitamin E, and lipoic acid, appeared to prevent hyperplasia, particularly in the fasciculate and reticular zones, in quails submitted to heat stress.

SAHIN et al. [27] evaluated the effects of vitamins C, E and folic acid supplementation on performance, carcass characteristics and biochemical parameters in Japanese quails exposed to high ambient temperature. They have observed that the dietary antioxidants prevent the decline in growth performances as well as the occurrence of an oxidative stress by sparing the endogenous antioxidant status. SAHIN and KUCUK [26] have also reported that vitamin C supplementation increased performance and carcass traits in broilers reared under conditions of heat stress (32°C). It has been reported that dietary ascorbic acid supplementation may alleviate the effects of heat stress on the performance of broiler chickens [12, 14, 15] and quails [29]. Besides, broilers seem to have a special appetite for ascorbic acid and tend to consume more diet supplemented with ascorbic acid at high temperature [14]. Although some investigators reported that dietary ascorbic acid supplementation did not positively affect performance [16, 32] or even decreased weight [21] in broilers, CAKIR et al. [5] has also stated that beneficial effects of dietary supplements could be more evident during stressful conditions. At high temperatures, it was previously demonstrated that corticosteroid secretion increased [4]. KUTLU and FORBES [13] reported that ascorbic acid induced a significant reduction of the glucocorticoid synthesis in birds. Similarly, SAHIN et al. [28] reported low plasma concentrations of ACTH in quail reared at 32°C and supplemented with vitamin C. On the contrary, STILBORN et al. [32] reported that neither acetylsalicylic acid nor ascorbic acid had beneficial effects on broiler growth or on food efficiency. Nevertheless, a significant and marked reduction in the volume of the fasciculate zone responsible for the corticosterone synthesis was evidenced in females and males quails supplemented with antioxidants compared to the not supplemented birds reared under heat stress in the present study. Moreover, the specific effect on the fasciculate zone was observed whatever the antioxidant added to the ration and was more pronounced with the vitamin E supplementation in both sexes.

Some authors [13, 22, 27] reported that supplementation with antioxidants were protected management practice in preventing heat-stress-related depression in the performance. We have observed that during supplementation with antioxidants, the quantitative importance of the reticular zone is significantly reduced in supplemented birds (males and females) compared to the not supplemented birds. This effect was considered as beneficial for birds submitted to heat stress. The medulla zone appeared on the contrary to be increased with co-supplementation with vitamins C and E in females and in males, and with specific vitamin C supplementation only in males. On the other hand, this zone was not affected by heat stress whereas epinephrine secretion would be exacerbated in stressful conditions.

In conclusion, antioxidant supplementation may offer protection against heat stress and inherent performance reduction in Japanese quails by preventing hyperplasia in the adrenal glands, particularly in the fasciculate and reticular zones, and limiting in this way overproduction of steroids.

**References**


