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Dietary rosemary oil alleviates heat stress-induced structural and functional damage through lipid peroxidation in the testes of growing Japanese quail

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ABSTRACT

Supplementation of natural antioxidants to diets of male poultry has been reported to be effective in reducing or completely eliminating heat stress (HS)-induced reproductive failures. In this study, the aim is to investigate whether rosemary oil (RO) has a protective effect on HS-induced damage in spermatozoa production, testicular histologic structures, apoptosis, and androgenic receptor (AR) through lipid peroxidation mechanisms in growing Japanese quail. Male chicks ($n = 90$) at 15-days of age were assigned to two groups. The first group ($n = 45$) was kept in a thermo-neutral (TN) room at 22 °C for 24 h/d. The second group ($n = 45$) was kept in a room with a greater ambient temperature of 34 °C for 8 h/d (from 9:00 AM to 5:00 PM) and 22 °C for 16 h/d. Animals in each of these two groups were randomly assigned to three subgroups (RO groups: 0, 125, 250 ppm), consisting of 15 chicks (six treatment groups in 2×3 factorial design). Each of subgroups was replicated three times with each replicate including five chicks. The HS treatment significantly reduced the testicular spermatogenic cell counts, amount of testicular Bcl-2 (anti-apoptotic marker) and amount of AR. In addition, it significantly increased testicular lipid peroxidation, Bax (apoptotic marker) immunopositive staining, and the Bax/Bcl-2 ratio in conjunction with some histopathologic damage. Dietary supplementation of RO to diets of quail where the HS treatment was imposed alleviated HS-induced almost all negative changes such as increased testicular lipid peroxidation, decreased numbers of spermatogenic cells, and decreased amounts of Bcl-2 and AR, increased ratio of Bax/Bcl-2 and some testicular histopathologic lesion. In conclusion, dietary supplementation of RO for growing male Japanese quail reared in HS environmental conditions alleviates the HS-induced structural and functional damage by providing a decrease in lipid peroxidation.

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1. Introduction

Environmental factors often affect physiological functions of each organ in the body in almost all animal species. However, avian species seem to be particularly sensitive to environmental challenges, especially heat stress (HS) because of the increased production of body heat by modern poultry genotypes due to the greater metabolic activity associated with this increased productivity (Deeb and Cahaner, 2002). Exposure to HS has a deleterious effect on fertility and is considered to be one of the significant risk factors causing infertility in males. In most homoeothermic birds and mammals, including humans, testicular function is influenced by ambient temperature. Temperatures outside of the animal's thermo-neutral (TN) zone are often not optimal for testicular function and can potentially disrupt spermatogenesis (Durairajanayagam et al., 2014). Reductions have been reported for testis weight (McDaniel et al., 2004), semen volume, numbers of normal-shaped spermatozoa (Joshi et al., 1980), spermatozoa count, spermatozoa motility, and there is an increase in number of dead spermatozoa (McDaniel et al., 2004; Ebeid, 2012) as well as testicular degenerative disorders (Terim Kapakın et al., 2013; Türk et al., 2015) in different poultry species in response to HS.

The increased intra-testicular temperature resulting from increased body temperature (McDaniel et al., 2004), and in particular greater production of reactive oxygen species (ROS) leading to lipid peroxidation due to the increased metabolic activity under stress conditions (Ebeid, 2012), have been considered to be responsible mechanisms for the HS-induced reproductive failures in poultry. The plasma membrane of avian spermatozoon contains abundant polyunsaturated fatty acids (PUFAs). While PUFAs provide the fluidity that is necessary for flagellar movement and fusion-related events, it also makes the spermatozoa susceptible to lipid peroxidation. Spermatozoa need adequate antioxidant capacities because lipid peroxidation can lead male reproductive dysfunction. Therefore, avian testes need a precise oxidant/antioxidant balance for regular spermatozoa production and subsequently semen with high quality (Surai et al., 2001).

Supplementation of natural antioxidants to diets of male poultry is effective in reducing or completely eliminating HS-induced reproductive failures (Lara and Rostagno, 2013). Various antioxidants such as cinnamon bark oil (Türk et al., 2015), betaine, vitamin C, folic acid (Ezzat et al., 2011), selenium, and vitamin E (Ebeid, 2012) have been added to the diets to prevent HS-related reproductive disturbances in avian species. Rosemary (*Rosmarinus officinalis*) is a common household plant, which belongs to the family of Lamiaceae that is grown in many parts of the world. Rosemary contains active anti-oxidative substances such as phenolic diterpenes, flavonoids, phenolic acids (Ho et al., 2000), and volatile oils (Begum et al., 2013). The volatile oils consist of borneol, bornyl acetate, camphene, cineol, pinene, and camphor (Begum et al., 2013). Rosemary oil (RO) has various biological properties including great antioxidant and free radical scavenging activities (Adorjan and Buchbauer, 2010). However, there is inconsistent information regarding the effects of rosemary on the male reproductive system, because there are studies indicating that rosemary and its different extracts have been harmful (Nusier et al., 2007; El-Din et al., 2012; Heidari-Vala et al., 2013) while other studies have found there were beneficial (Luno et al., 2014; Motlagh et al., 2014; Uyeturk et al., 2014) effects on structure and functions of spermatozoa. One study (Superchi et al., 2005) was conducted that relates to the protection of rosemary extract against spermatozoa damage caused by HS in boars, but to the best of our knowledge there is no previous research regarding the effect of RO on HS-induced reproductive disturbance in male quail. The present study was, therefore, conducted to investigate whether RO has a preventive or aggravating effect on HS-induced disturbance impacting reproductive efficiency of male quail by examining the changes in spermatogenic cell counts, testicular oxidant-antioxidant markers, testicular histologic structures, and quantity of testicular apoptotic cells and androgenic receptors (AR).

2. Materials and methods

2.1. Rosemary oil and chemicals

Rosemary oil was purchased from a local store (Agromiks Food Additive Co., İzmir, Turkey). The compounds and percentages of volatile components within the RO have been reported to be 1,8 cineole (39.31%), camphor (14.69%), α-pinene (13.85%), β-pinene (9.87%), camphene (6.17%), limonene (3.17%), P-cymene (2.58%), borneol (2.33%), α-terpineol (2.28%), myrcene (2.02%), bornyl acetate (1.46%), and others (2.27%) by manufacturer. RO was kept at 4 °C until being used. The other chemicals were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA).

2.2. Experimental protocol and dietary regimen

The Animal Experimentations Local Ethics Committee of Fırat University (Elazığ, Turkey) approved the experimental protocol of the present study. A total of 90 male Japanese quail chicks (*Coturnix coturnix japonica*) at 5-days of age were purchased from a commercial company (Deva-Yum Co., Elazığ, Turkey). After a 10-day adaptation period to experimental conditions of the Poultry Unit of Fırat University, chicks were placed in wire cages in temperature-controlled rooms, and the study was initiated. The chicks were randomly divided into two groups. The first group ($n = 45$) were housed in a TN condition at 22 °C for 24 h/d. The second group ($n = 45$) were placed in a room with high ambient temperature (HS) at 34 °C for 8 h/d (from 9:00 AM to 5:00 PM) and at 22 °C for 16 h/d. The chicks in the two groups were then randomly assigned to three subgroups (RO groups: 0, 125, 250 ppm) consisting of 15 chicks (six treatment groups in 2 × 3 factorial design). The study with each of the subgroups was replicated three times with each replicate being with five chicks. The relative humidity of both TN and HS rooms was 60–65%. At both temperatures, chicks were fed either a basal diet (0 ppm) or the