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Feeding rosemary leaves powder ameliorates rooster age-related subfertility

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ABSTRACT

Having a high proportion of polyunsaturated fatty acids avian spermatozoa predispose to lipoperoxidation which results in fertility reduction. In the current study, rosemary leaves powder (RLP) was fed to senescent breeder roosters to improve their reproductive performance. Twenty four 70-week-old roosters were randomly divided into four groups and received following treatments including 0 (RLP-0), 2.5 (RLP-2.5), 5 (RLP-5) or 7.5 (RLP-7.5) g of RLP/kg of diet for eight consecutive weeks. Semen characteristics were evaluated weekly. Sperm penetration rate was assessed once, however, fertility, hatchability, embryonic mortality and hatchling quality evaluated twice (using eggs collected during 1st and 2nd weeks following AI) at the end of experiment. Excluding body weight and sperm abnormality percentage, other traits including semen concentration (RLP-2.5 = 3.57, RLP-5 = 4.21 and RLP-7.5 = 3.79; SEM = 0.12; $p < 0.01$), total sperm production (RLP-2.5 = 1.33, RLP-5 = 1.8 and RLP-7.5 = 1.47; SEM = 0.07; $p < 0.01$), forward motility (RLP-2.5 = 72.96, RLP-5 = 83.65 and RLP-7.5 = 78.84; SEM = 0.47; $p < 0.01$) and viability (RLP-2.5 = 82.93, RLP-5 = 88.69 and RLP-7.5 = 86.85; SEM = 0.45; $p < 0.01$) were improved in RLP treated groups compared to control group (3.08 ± 0.12, 1.05 ± 0.07, 68.39 ± 0.47 and 76 ± 0.45 for semen concentration, total sperm production, sperm forward motility and viability, respectively). In addition, semen volume and sperm plasma membrane functionality were higher in both RLP-5 (0.43 ± 0.01 and 66.66 ± 0.55) and RLP-7.5 (0.39 ± 0.01 and 65.52 ± 0.55) than control group (0.34 ± 0.01; $p < 0.05$ and 62.89 ± 0.55; $p < 0.05$). Supplementation of RLP significantly decreased semen Malondialdehyde (MDA) concentration. Moderate level of RLP (RLP-5) had significantly higher numbers of sperm penetration holes compared to other groups. Fertility rate of collected eggs from both RLP-5 (first week: 91.09 ± 1.27 ($P < 0.01$); second week: 88.73 ± 1.27 ($p < 0.05$)) and RLP-7.5 (first week: 93.11 ± 1.27 ($P < 0.01$); second week: 90.87 ± 1.27 ($p < 0.05$)) groups was higher than other groups at 1st and 2nd weeks of egg collection. Hatchability of eggs set at 2nd week (83.64 ± 3.54; $p < 0.05$) was higher and embryonic mortality at 1st week (1–6 day mortality: 5.03 ± 1.25 ($p < 0.05$); 18–21 day and pipped mortality: 8.55 ± 1.31 ($p < 0.05$)) was in RLP-0.5 group than other groups, respectively. To conclude, RLP supplementation could successfully attenuate age-related sub-fertility in senescent roosters. Further studies are needed to divulge the causal mechanisms involved.

1. Introduction

Hatching eggs production is the main income source of broiler breeders industry. Thus, infertility could result in major economic loss. Both male and female birds contribute in chicken production. However, because of a lower male to female ratio, roosters play more important role in flock fertility [1,2].

Fertility of broiler breeders reaches to peak (above 90%) at the beginning of the reproductive period (30–40 week of age) and declines shortly afterward. Hindering fertility reduction by artificial insemination and spiking (replacing old bird with young) mainly links this subfertility to male birds [3]. The deterioration in fertilizing ability of aged roosters has been attributed to many factors like weight gain, reduced sperm and testosterone production and decline in semen antioxidant capacity [4]. The antioxidant capacity

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of sperm is low, but enzymatic and non-enzymatic antioxidants in the seminal plasma protect sperm by scavenging the reactive oxygen species (ROS) [5]. In human studies, it has been mentioned that total antioxidant capacity of seminal plasma in infertile men may be lower than in fertile men [6]. Furthermore, an increased production of ROS is associated with male infertility [7]. Plasma membrane lipids play a vital role in sperm fertilizing capacity [8,9]. Avian sperm cell membranes have a much greater concentration of polyunsaturated fatty acids (PUFAs) than mammalian sperm cells and therefore be more prone to lipid peroxidation (LPO) in the presence of ROS [10]. Duo to lacking or low activity of hexose phosphate shunt, NADPH production and subsequently antioxidants recycling is also limited in avian spermatozoa [7].

In light of the results of aforementioned studies, several approaches have been introduced to enhance the semen quality and the anti-oxidative capacity of seminal plasma, including the use of dietary dried apple pomace [11], lycopene [12], carnitine [13], sage extract [2], dried tomato pomace [14], dried ginger rhizome [15], Satureja khuzistanica powder [16], Vitamin E [1,17], Rosemary oil [18] Selenium [19,20]. Most of these studies have shown the beneficial effect of antioxidant therapy against oxidative stress in avian spermatozoa. However, further research is required to evaluate other dietary sources to improve reproductive performance of male broiler breeders, particularly those exhibit age-related subfertility [21].

There is an increasing interest of natural antioxidants because of the safety and toxicity problems of synthetic ones generally used in lipid containing foods [22,23]. In this regards, it is also noteworthy to mention that two-thirds of the world's plant species have medicinal properties with great antioxidant potential [24]. For instance, the plant species such as sage [2,25], oregano [26], and rosemary [27,28] have been tested to develop natural antioxidant formulations in the areas of medicine and nutrition.

Rosemary (*Rosmarinus officinalis L.*) contains biologically active substances such as diterpenes, triterpenes, flavonoids and polyphenols as well as monoterpenes and sesquiterpenes [29,30]. Its antioxidant capacity is mainly related to the presence of components like carnosol, rosmanol, isorosmanol, epirosmanol, carnosic and rosmarinic acids [31]. Carnosic and rosmarinic acids are two major phenolic constituents present in rosemary leaves which their antioxidant properties have been previously reported [32–35]. Further, the antioxidant potential of pure Carnosic acid is several times greater than butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) [35]. Supplementing boar diet with Rosemary oil extract containing 5.5% rosmarinic has been resulted in an increase of sperm concentration, viability and a significant reduction of return to estrus in sows inseminated with semen from treated males [36]. In addition, Rosemary oil alleviated heat stress induced testicular lipid peroxidation and reduction in spermatogenic cell numbers in growing Japanese quail [18]. In vitro inclusion of rosemary aqueous extract has also been successful in improving post-thawed sperm total and forward motility, viability, plasma membrane functionality and Malondialdehyde (MDA) concentration [37]. Despite the antioxidant properties of Rosemary has been well documented, its effect on roosters' reproductive performance received scant attention. Therefore, the present study was designed to evaluate the seminal attributes and reproductive performance in breeder roosters fed different levels of rosemary leaves powder.

2. Materials and methods

2.1. Animal ethics

All issues about the experimental setups and evaluation techniques have been approved by the local scientific ethical committee, Department of Animal Science, University of Tehran, Karaj, Iran.

2.2. Chemicals and rosemary

All chemicals were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Fresh rosemary leaves were first collected, cleaned, sliced and dried in the shade. Dried rosemary leaves were then milled (Moulinex/A320, Indonesia) and stored (22–25 °C) in airtight plastic bags away from sunlight until use.

2.3. Birds and treatments

Twenty-four 68-week-old Ross 308 broiler breeder roosters were randomly divided into four groups (n = 6 birds/group), individually caged (60 × 50 × 75 cm) and kept under similar management conditions (a 15 L: 9D light schedule and 21 °C ambient temperature). Treatment groups were including 0 (RLP-0), 2.5 (RLP-2.5), 5 (RLP-5) or 7.5 (RLP-7.5) g of RLP/kg of diet, capsulated and daily fed (149 g/day at 70 wk up to 153 g/day at 78 wk) at the beginning of photoperiod (06:00 a.m.) with a basal diet formulated to meet or slightly exceed Ross 308 catalog recommendations (Table 1). Water was provided ad libitum. A total of 140 female broiler breeders (55-wk-old) were also individually caged (60 × 60 × 75 cm) and fed a standard diet with 2700 kcal of Metabolizable energy/kg, 16.5% crude protein, 4% calcium and 0.4% phosphorus. At the end of experiment, hens were artificially inseminated and collected eggs assigned to sperm penetration assay, fertility and hatchability rates estimation.

2.4. Semen quality indices

2.4.1. Gross evaluations

Roosters were conditioned to semen collecting by abdominal

Table 1
Ingredients and the chemical composition of basal diet fed to broiler breeder roosters (DM basis).

Ingredient (%)	Value (%)[a]
Corn	69.18
Soybean meal (44%)	8.5
Wheat bran	19.19
Dicalcium phosphate	1.4
CaCO3	0.8
Sodium chloride	0.32
Vitamin premix[b]	0.25
Trace-mineral premix[c]	0.25
DL-Met	0.11
Total	100
Composition	
ME (kcal/kg)	2754
CP (%)	11.99
Ca (%)	0.70
P (%)	0.35
Lys (%)	0.46
Met (%)	0.30
Thr (%)	0.38

[a] The birds in the control group received diets without R. Other birds received, 2.5 ($R_{2.5}$), 5 (R_5), or 7.5 ($R_{7.5}$) g Rosemary leaves powder (R) in Kg of diet for 8 weeks (56–64 wk of age).

[b] Supplied per kg diet: vitamin A, 12,000 IU; vitamin D3, 3500 IU; niacin, 50 mg; vitamin E, 100 IU; vitamin K3, 5 mg; riboflavin, 12 mg; thiamin, 3.0 mg; D-pantothenic acid, 13 mg; folic acid, 2 mg; pyridoxine, 6 mg; vitamin B12, 0.03 mg, and biotin, 0.66 mg.

[c] Supplied per kg diet: Fe (FeSO4·H2O), 50 mg; Mn (MnSO4·H2O), 120 mg; Zn (ZnO), 110 mg; Cu (CuSO4·5H2O), 10 mg; iodine (KI), 2 mg; and Se (Na2SeO3), 0.3 mg.