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Chrysin-induced sperm parameters and fatty acid profile changes improve reproductive performance of roosters

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ABSTRACT

Having antioxidant and androgenic effects, Chrysin was orally administrated to roosters and reproductive performance including sperm quality and fatty acid composition, testis index, fertility and hatchability rates as well as blood testosterone concentration were assessed. Twenty eight 40-week-old Ross 308 roosters were individually housed, equally divided into four groups and received different levels of capsulated Chrysin including 0 (Ch0), 25 (Ch25), 50 (Ch50) or 75 (Ch75) mg/bird/day for 12 consecutive weeks. Body weighting as well as semen and blood sampling were weekly done from 1st-10th weeks. A total of three artificial inseminations were carried out on the last two weeks of trial and collected eggs following second insemination were allotted to evaluate fertility and hatchability rates. Sperm fatty acid composition was determined using samples from 12th week. At the end of experiment (12th week) all roosters sacrificed, testis were carefully removed and testis index was calculated. Except for body weight, testis index, sperm abnormality percentage and ejaculated volume, other traits were significantly affected by Chrysin treatment. Sperm total and forward motility, plasma membrane integrity and functionality, semen concentration as well as fertility and hatchability rates were significantly higher in both Ch50 and Ch75 groups compared to control group. In spite of an increasing trend in most of n-3 and n-6 fatty acids, the n-6/n-3 ratio was significantly decreased in both Ch50 and Ch75 compared to other groups. Malondialdehyde concentration was also significantly decreased in Chrysin treated groups compared to control group. Blood testosterone level was only significantly higher in Ch75 group than that other groups. In conclusion, Chrysin administration particularly at higher levels alleviates post-peak fertility reduction in roosters; however, further research are needed to disclose involved mechanism(s).

1. Introduction

Broiler breeders industry has been dramatically changed during the last half century. Although genetic selection together with management has improved feed conversion ratio and meat production; however, excessive body weight [1], delayed sexual maturity [2] and fertility reduction [1,3,4] are current main concerns faced by commercial broiler breeder flocks [5]. Fertility of roosters peaks at about 37 week of age then progressively declines [3,4]. This phenomena is different from seasonal breeders in which spermatogenesis dysfunction and regression or atrophy of germinal epithelium in nonbreeding season occurs [3]. It seems that in low-fertile roosters not only less testicular testosterone is synthesized but also more metabolized to estradiol and lower plasma testosterone to estradiol ratio resulted [1,4]. Therefore, higher estradiol level reduces plasma levels of both LH and testosterone through negative feedback on Hypothalamus-Pituitary-Gonad (HPG) axis [4]. Recently, orally administered D-aspartate [6], rosemary leaves powder [7] and testosterone injection [8] have successfully alleviated reproductive aging of roosters.

Besides sexual steroids ratio, an optimal ratio of poly-unsaturated fatty acids (PUFA) in roosters' sperm plasma membrane are necessary to attain maximum fertility. The functional role proposed for PUFA may be related to the fertility, acrosome reaction or more specifically membrane fusion, leukotriene production and signal transduction [9]. A complex natural antioxidant system occurring in avian semen involving vitamin E, vitamin C and GSH together with enzymatic defenses (e.g. SOD and CAT) prevent or restrict formation and propagation of peroxidase. Higher PUFA concentration of spermatozoa [10], longer storage period in female reproductive tract [11,12] and higher dilution rate before artificial insemination [13] compromised aforementioned defensive system,

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thus antioxidant therapy is recommended.

In this regard, feeding several additives predominantly with antioxidant effect including dried tomato pomace [14], apple pomace [15], sage extract [16] and dried ginger rhizome [17] have improved sperm quality, fatty acid profile and reproductive performance of roosters. However, having some anti-nutrient compounds (e.g. tannins and pectins) restrict their inclusion in the diet [15]. In addition, semen antioxidant status and subsequently sperm quality of roosters have been improved by dietary antioxidants prior [18,19] or after dexamethasone, as a pro-oxidant challenge, injecting [20,21]. In the lights of mentioned studies, using an agent with both steroidogenic and antioxidant properties to improve post-peak reproductive performance of broiler breeders is worthwhile to test.

Flavonoids with the general structure of a 15-carbon skeleton, consisting two phenyl rings (A and B) and a heterocyclic ring (C), are polyphenolic compounds ubiquitously found in many fruits, vegetables and beverage. Chrysin, chemically called 5,7-dihydroxyflavone, is a naturally occurring polyphenolic compound highly present in *Passiflora caerulea*, honey and several other plants. Protecting effects against oxidative and inflammatory injuries [22] along with steroidogenic [23] and anti-aromatase activities [24,25] make Chrysin an interesting candidate to evaluate its effect on reproduction. Chrysin treated male albino rats had significantly higher sperm count, fertility rate and litter size when they were permitted to mate with proven pro-estrous female rats [26]. These promoting effects of Chrysin was confirmed by Ciftci et al. [27] report in which testis antioxidant enzyme levels such as SOD, CAT and GSH-Px along with GSH were significantly improved following Chrysin administration. Higher sperm count and motility along with lower abnormality percentage were also recorded in their study. Beside antioxidant effect, both in vivo and in vitro studies have confirmed Chrysin potential for enhancing testosterone level and subsequently male sex drive [23,27].

According to the broad range of pharmacological activities of Chrysin and considering the fact that information on feeding this flavonoid to birds is lacking in literature. In this trail, we aimed at investigating the effectiveness of Chrysin administration on sperm quality and fatty acid composition, fertility and hatchability rates as well as blood testosterone level of broiler breeder roosters.

2. Materials and methods

2.1. Chemicals

All materials including Chrysin were purchased from Sigma (St. Louis, Mo, USA) and Merck (Darmstadt, Germany) unless indicated.

2.2. Animal ethics

This trial was conducted following approval given by Animal Care Committee and Animal Research Ethics Board from Department of Animal Science, University of Tehran, Iran.

2.3. Birds treatment and sampling

Twenty eight sexually mature Ross 308 roosters, 40-week-old, were individually housed in a pen ($1.2 \times 1.2 \times 1$ m^3). Each bird was offered 140 g standard diet (Table 1) supplemented with 0 (Ch0), 25 (Ch25), 50(Ch50) or 75 (Ch75) mg of capsulated Chrysin once daily at 8:00 am for 12 consecutive weeks. Body weighting as well as blood and semen sampling were done weekly from 1st-10th weeks. At the end of study, roosters were sacrificed, both testicles were carefully removed, weighted and testis index (testis weight/body weight) calculated [28].

A total of sixty 34-week-old female broiler breeders which had no previous contact with male birds, were individually kept in cage ($30 \times 40 \times 50$ cm^3) and daily fed a standard diet (170 g) including 15% Crude protein, 2800 kcal/kg diet Metabolizable energy, 3% Calcium and 0.35% available phosphorus. Other conditions including a 14L:10D lighting program, and 21 °C ambient temperature were similar for all birds and fresh water provided ad libitum.

2.4. Sperm characteristics

2.4.1. Gross assessment

The males were conditioned for semen collection using Burrows and Quinn [29] method. Semen was sampled individually into 1.5 ml plastic micro-tube and ejaculate volume estimated with a micropipette in microliters. Semen concentration was measured by placing a diluted sample (1:500 with distilled water) on both chambers of Neubauer hemocytometer [30]. According to the mean number of sperm counted in each chamber and dilution rate,

Table 1
Ingredient and chemical composition of standard diet fed Ross 308 roosters.

Item	Value (%)	Digestible amino acids	Value (%)
Corn	69.5	Lysine	0.46
Soybean meal	9	Methionine	0.39
Wheat bran	19.5	Methionine & Cysteine	0.49
Dicalcium phosphate	0.18	Tryptophan	0.12
Calcium carbonate	0.85	Arginine	0.67
Sodium chloride	0.35	Valine	0.5
DL-Met	0.12	Leucine	0.53
Vitamin premix[a]	0.25	Isoleucine	0.4
Trace mineral premix[b]	0.25	Threonine	0.37
Composition			
ME (kcal/kg)	2754.5	CP (%)	12
Ca (%)	0.7	Available P (%)	0.35
Na (%)	0.15	Cl (%)	0.15
K (%)	0.6		

[a] Supplied per kilogram of diet: vitamin A, 15,000 IU; vitamin E, 100 IU; vitamin K3, 4 mg; vitamin B12, 25 μg; vitamin D, 3000 IU; riboflavin, 7.5 mg; niacin, 50 μg; pantothenic acid, 18 mg; pyridoxine, 5.5 mg; biotin, 50 mg and folic acid, 1.5 mg.
[b] Supplied per kilogram of diet: Fe, 90 mg; Mn, 120 mg; Zn, 110 mg; I, 2 mg and Se, 0.3 mg.