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Letrozole, an aromatase inhibitor, reduces post-peak age-related regression of rooster reproductive performance

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ABSTRACT

This study was designed to evaluate orally administrated Letrozole (Lz) on reproductive performance, plasma testosterone and estradiol concentrations and relative abundance of mRNA of GnRH, FSH and LH in roosters. Ross 308 roosters (n = 32) that were 40-weeks of age were individually housed and received a basal standard diet supplemented different amounts of capsulated Lz [0 (Lz-0), 0.5 (Lz-0.5), 1 (Lz-1) or 1.5 (Lz-1.5), mg Lz/bird/day] for 12 weeks. Sperm quality variables and plasma testosterone and estradiol concentrations were assessed from the first to the tenth week of the treatment period. Semen samples from the 11th to 12th week were used for artificial insemination and eggs were collected and allotted to assess fertility and hatchability rates. Relative abundance of hypothalamic and pituitary GnRH, LH and FSH mRNA was evaluated at the end of 12th week. The results indicated that total and forward sperm motility as well as egg hatchability rate were greater in the Lz-0.5 group. Greater sperm concentrations, ejaculate volume, sperm plasma membrane integrity, testis index and fertility rates were recorded for both Lz-0.5 and Lz-1 groups compared with the Lz-0 group ($P < 0.05$). Body weight, percentage of sperm abnormalities, and sperm plasma membrane functionality were not affected by treatment. Testosterone and estradiol concentrations were negatively related with greater testosterone concentrations in the Lz-1.5 group which had lesser estradiol concentrations. Relative mRNA transcript abundance for GnRH, LH and FSH was Lz dose responsive being greater in the treated groups; however, this trend plateaued for GnRH and for the relative abundance of both LH and FSH mRNA was less in the Lz-1.5 group than the other treatment groups. It is concluded that Lz may be an effective treatment to improve age related post-peak reproductive performance of roosters.

1. Introduction

Incubating eggs with greater fertility rates subsequently results in greater chick production which is the main goal of the commercial broiler breeder industry. Broiler breeders undergo a decrease in fertility within the first year of their life (Avital-Cohen et al., 2015). This reproductive defect is associated with a decrease in sperm quality and quantity as well as blood testosterone concentration (Rosenstrauch et al., 1998; Weil et al., 1999; Sarabia Fragoso et al., 2013). It has been proposed that the lesser fertility is associated with greater aromatase activity and subsequently greater plasma and intra-testicular estradiol concentrations in roosters (Weil et al., 1999) and humans (de Ronde and de Jong, 2011). Greater concentrations of estradiol in combination with lesser

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testosterone concentrations lead to impaired spermatogenesis as well as hypothalamic-hypophyseal-gonadal (HPG) axis function and subsequently less LH and FSH production (Dabaja and Schlegel, 2014). In addition, excessive estrogen up-regulates the amount of aromatase in the testes (Bharti et al., 2013). Exogenous testosterone therapy in roosters has improved reproductive success after 40-weeks of age (Ordas et al., 2015). Testosterone injection can, however, is costly and is an invasive method, thus, negating its commercial application. In addition, in humans using anabolic steroids has resulted in HPG-axis impairment, poor sperm motility and lesser sperm numbers as well as greater numbers of abnormal sperm (Kim et al., 2013). Selective estrogen receptor modulators competitively bind to estrogen receptors in the hypothalamus and pituitary gland, thus, inhibiting estradiol negative feedback which in turn leads to stimulation of the HPG axis. Serum testosterone concentrations are increased as a result of treatment with testosterone and spermatogenesis is subsequently stimulated, however, results with this treatment are variable, unpredictable and inconsistent (Moss et al., 2013; Rambhatla et al., 2016). Treatments to inhibit aromatase enzyme actions have had positive impacts on sperm variables and blood and/or testicular testosterone/estradiol ratios (Moss et al., 2013).

Aromatase is a member of the cytochrome P450 superfamily found in testis, brain, liver and adipose tissues that promotes the conversion of an androgen to an estrogen via removal of the 19-methyl group and aromatization of the A-ring of androgens. Aromatase inhibitors inhibit this reaction; consequently increasing serum concentrations of LH, FSH and testosterone/estradiol ratios. There has been some success in administering aromatase inhibitors to treat breast cancer (Smith and Dowsett 2003; Karnon, 2006) with the beneficial effect being a reduction in concentrations of estradiol at the desired tissue sites. Letrozole is a non-steroidal, late-generation aromatase inhibitor that is more specific in its action by binding to the heme iron of estrogen synthase (de Ronde and de Jong, 2011). Aromatase inhibitors also gained popularity as off-label options in men who need hormonal manipulation (Rambhatla et al., 2016). Using Lz has resulted in significant increases of testosterone, LH and FSH in healthy and non-obstructive azoospermia patients (T'Sjoen et al., 2005; Cavallini et al., 2013). In addition, sperm total motility; sperm concentrations in semen and ejaculate volume have improved in infertile men with less than optimal testosterone/estradiol ratios (Saylam et al., 2011; Stahl et al., 2012).

In avian species, studies on aromatase inhibition have been mostly focused on sexual differentiation and singing behavior (Balthazart et al., 1994; Alward et al., 2016). Because of the greater specificity and well-documented reports about the effect of Lz on spermatogenesis and normalization of testosterone concentration (T'Sjoen et al., 2005; Saylam et al., 2011; Moss et al., 2013; Dabaja and Schlegel, 2014; Ribeiro et al., 2016), it was selected in the current study to evaluate whether reduced aromatase activity may slow the rate of decrease in reproductive performance of broiler breeder roosters that are post-peak for age related reasons in reproductive function.

2. Materials and methods

2.1. Chemicals

Materials were purchased from Sigma-Aldrich Company (St. Louis, Mo, USA) and Merck (Darmstadt, Germany) otherwise indicated.

2.2. Birds housing and treatments

This study was approved by Department of Animal Science at University of Tehran Ethics Committee. Ross 308 roosters ($n = 32$) that were 40 weeks of age were housed separately in pens ($1.2 \times 1.2 \times 1$ m^3) with a controlled photoperiod regimen (14:10 h light:

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

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