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Effects of dietary soybean isoflavones (SI) on reproduction in the young breeder rooster

Dai Heng[a,1], Tao Zhang[b,1], Ye Tian[a,1], Shangyu Yu[a], Wenbo Liu[a], Kaili Xu[a], Juan Liu[a], Yu Ding[a], Baochang Zhu[a], Yanzhou Yang[c,*], Cheng Zhang[a,*]

[a] College of Life Science, Capital Normal University, Beijing 100048, People's Republic of China
[b] Animal Husbandry and Veterinary Medicine, Ningxia Agricultural School, Yinchuan, Ningxia 750021, People's Republic of China
[c] Key Laboratory of Fertility Preservation and Maintenance, Ministry of Education, Key Laboratory of Reproduction and Genetics in Ningxia, Department of Histology and Embryology, Ningxia Medical University, Ningxia 750004, People's Republic of China

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ABSTRACT

Soybean isoflavones (SIs) are phytoestrogens that competitive with estrogens in body. Although SIs play an important role in reproduction, their role in testicular development in roosters is unknown. This study was conducted to investigate the effect of SIs on testicular development and serum reproductive hormone profiles in young breeder roosters (70–133 days old). Gene expression of steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (P450scc), and 3β-hydroxysteroid dehydrogenase (3β-HSD), which are related to testosterone synthesis, in rooster testis were also evaluated after treatment with different SI doses. Although SIs had no significant effect on body weight, 5 mg/kg SIs significantly increased the testis index and serum levels of reproductive hormones (gonadotropin releasing hormone, follicle- stimulating hormone, luteinizing hormone, and testosterone).To further investigate whether SIs regulate hormone synthesis via StAR, p450scc, 3β-HSD, real time-PCR was performed to measure the mRNA levels of the corresponding genes. The results showed that 5 mg/kg of SIs significantly increased StAR mRNA levels. However, there were no significant effects on p450scc or 3β-HSD mRNA levels. Moreover, the spermatogonial development and the number of germ cell layers were increased by treatment with 5 mg/kg of SIs. These results suggest that SIs promote testicular growth by increasing reproductive hormone secretion, which is closely related to StAR expression, to positively regulate reproduction in young roosters.

1. Introduction

The reproductive performance of roosters is a critical aspect of poultry production, which is tightly regulated by endocrine, autocrine, and paracrine factors. As the male gonads, the testes secrete androgens and generate sperm and are important for maintaining normal reproductive function in males (McBride and Coward, 2016). Although there is no significant increase in testicular weight from 14 to 105 days of age during the chicken testis development, the number of spermatogonia reaches more than one mil-

Abbreviations: SI, soybean isoflavone; StAR, steroidogenic acute regulatory; P450scc, cholesterol side chain cleavage enzyme; 3β-HSD, 3β-hydroxysteroid dehydrogenase; GnRH, gonadotropin-releasing hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; T, testosterone.

* Corresponding authors.
E-mail addresses: alnord820119@163.com (Y. Yang), zhch8012@163.com (C. Zhang).

[1] These authors contributed equally to this work.

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lion (Anastasiadou et al., 2011; Sarabia Fragoso et al., 2013). Moreover, the growth and maturation of Sertoli and Leydig cells in the testes of roosters are critical steps during early testicular development from 14 to 105 days. The most important period for testicular development is from 70 to 105 days (Mucksova et al., 2009).

Testosterone (T) production depends on the binding of luteinizing hormone (LH), secreted from the pituitary gland, to receptors on Leydig cells (Kim et al., 2016). Meanwhile, steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (P450scc), and 3β-hydroxysteroid dehydrogenase (3β-HSD) play several important roles in the regulation of steroid synthesis (Stocco et al., 2005; Wathes et al., 2007). Furthermore, serum concentrations of sex hormones depend not only on steroidogenic capacity but also on the number of Leydig cells (Benton et al., 1995). It has been reported that testicular Leydig cells, which express estrogen receptors, and estrogen regulate T synthesis and secretion (Sherrill et al., 2010).

Isoflavones are a subgroup of flavonoids found in soybeans and clover (Reinli and Block, 1996) at concentrations ranging from 100 to 5000 ppm (Xu et al., 1994); they are comprised mainly of soy isoflavones (SIs), formononetin, and biochanin A. Since isoflavones are similar to natural estrogens in terms of structure and function (Kurzer and Xu, 1997), they can bind weakly to estrogen receptors and act as weak agonists/antagonists, competing with natural estrogens; thus, they are an important class of phytoestrogens in animals and humans (Kelly et al., 1993). Soybean has been reported to be the most important dietary source of isoflavones, and SIs are useful ingredient as a nutrient supplement. Although the consumption of soy-based foods or phytoestrogen supplements has been associated with beneficial health effects, their potentially adverse effects on the reproductive and endocrine systems are likely underappreciated. Several lines of evidences have shown that isoflavones exert different effects on male reproduction (Cederroth et al., 2012), depending on the species and dose, and that soy consumption and isoflavone exposure decrease testicular weight or size, spermatogenesis, and follicle-stimulating hormone (FSH) (Atanassova et al., 2000; Cederroth et al., 2012) and T levels (Wisniewski et al., 2003) in rats. However, dietary isoflavones increase serum T levels during the perinatal period in rat (Akingbemi et al., 2007). It has been reported that the neonatal period is sensitive to SIs (Napier et al., 2014), which increases the proliferative activity of developing Leydig cells and suppresses the T concentration in male rat (Napier et al., 2014). Meanwhile, SIs affect the development of steroidogenic capacity by upregulating StAR and steroidogenic enzyme expression in adult Leydig cells (Akingbemi et al., 2007). Sherrill et al. (2010) demonstrated that exposure of male rats to high isoflavone concentrations affected testicular development by regulating the proliferative activity of Leydig cells during the perinatal period. Increased Leydig cell numbers during the prepubertal period were found to alleviate deficits in androgen biosynthesis and/or augmented serum and testicular T concentrations during adulthood (Sherrill et al., 2010). At low doses of isoflavones, puberty onset and the increase in serum T levels were delayed in rats. However, at high doses of isoflavones, rats showed no significant increase in T levels and did not reach puberty (Caceres et al., 2015). Moreover, decreased plasma T and androstenedione levels have been observed in young rats exposed to a phytoestrogen-rich diet (Weber et al., 2001). In contrast, diets with higher levels of phytoestrogens had less of an effect on plasma steroid contents and most morphometric parameters of testis in male bilgoraj geese (Opalka et al., 2008). This is consistent with previous results in adult males demonstrating that 40 mg of isoflavones as a dietary supplement for 2 months had no significant effect on serum T concentrations, testicular volume, or sperm parameters (Mitchell et al., 2001).

Although isoflavones are critical to male reproduction capacity, the function and appropriate dose of SIs for testicular development and reproductive hormone production in young roosters are not known. The present study investigated the effects of SIs on testicular development, the plasma levels of reproductive hormones, and the expression of protein/enzymes related to T synthesis in young breeder roosters. The aims were to provide scientific data regarding the appropriate dose of SIs to administer in animal feed and to explore the possible mechanisms and biological functions of isoflavones.

2. Materials and methods

2.1. Materials

SIs were purchased from Beijing Zhizheng biotechnology limited company, and the SI concentration is ninety-eight percent. All reagents were from Sigma Chemical Co. (St.Louis, MO, USA) unless otherwise stated. The ELISA kit was purchased from Nanjing Jian Cheng Bioengineering Institute (Nanjing, Jiangsu, China). Trizol reagent was obtained from Takara Bio, Takara Holdings Inc. (Otsu, Shiga,Japan). The M-MLV Reverse Transcriptase kit was obtained from Omega Bio-Tek, Inc. (Norcross, GA, USA).

2.2. Animals and treatment

The same batch of 70-day-old Jing Hong I breeder roosters were purchased from Beijing Yukou poultry limited company (Beijing, China). Roosters had a similar weight and healthy bodies and were fed under controlled cage individually conditions at 22 °C with a 13-h light and 11-h dark phases. The basal meal is corn-fish meat (Table 1) and SI with different doses were added according to the experiment design respectively. All the procedures used in the present study were in accordance with the Guidelines for the Care and Use of Laboratory Animals and the China Council on Animal Care and was approved by the Institutional Animal Care and Use Committee of Capital Normal University. The breeder roosters were randomly divided into six groups (six roosters per group at 0 days). All breeder roosters were fed basic diet for 7 days and then received different dose of SI [0 mg/kg (Treatment 1), 5 mg/kg (Treatment 2), 10 mg/kg (Treatment 3), 15 mg/kg (Treatment 4), 20 mg/kg (Treatment 5) and 25 mg/kg (Treatment 6)].