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Effects of maternal dietary selenium (Se-enriched yeast) on testis development, testosterone level and testicular steroidogenesis-related gene expression of their male kids in Taihang Black Goats

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ABSTRACT

To investigate the effects of maternal dietary selenium (Se-enriched yeast) on testis development, testosterone level and steroidogenesis-related gene expression in testis of their male kids, selected pregnant Taihang Black Goats were randomly allotted to four treatment groups. They were fed the basal gestation and lactation diets supplemented with 0 (control), 0.5, 2.0 and 4.0 mg of Se/kg DM. Thirty days after weaning, testes were collected from the kids. After the morphological development status of testis was examined, tissue samples were collected for analyzing testosterone concentration and histological parameters. Testosterone synthesis-related genes were detected using real-time PCR. Localization and quantification of androgen receptor (AR) in testis of goats were determined by immunohistochemical and western blot analysis. The results show that Se supplementation in the diet of dams led to higher ($p < 0.05$) testicular weight, volume, length, width, transverse and vertical grith of their male kids. Excessive Se (4.0 mg/kg) can inhibit the development of testis by decreasing testicular weight and volume. The density of spermatogenic cells and Leydig cells in the Se treatment groups was significantly ($p < 0.05$) higher than that in the control. Maternal dietary Se did not affect the thickness of testes, thickness of germinal epithelium and diameter of seminiferous tubule. Se supplemented in the diet of dams improved the testosterone level in testis tissue and serum, and promote the expression of testosterone-related genes. The mRNA expression of StAR, 3β-HSD and CYP11A1 was decreased with the increasing dietary Se levels of dams. Maternal dietary Se can improve the AR protein abundance in testis of their offspring. AR immunopositive product was detected in Leydig cells, peritubular myoid cells, perivascular smooth muscle cells, primary spermatocytes and spermatids. The expression of AR in spermatogenetic cells is stage specific. This study suggests that maternal dietary Se can influence the testis development and spermatogenesis of their male kids by modulating testosterone synthesis in goats. More attention should be given to the potential role of maternal nutrition in improving reproductive performance of their offspring.

1. Introduction

Selenium (Se) is an essential trace element for man and animals, especially required for the maintenance of male fertility. Se deficiency or low Se status is related to numerous reproductive disorders including abnormal testicular morphology, lower semen quality, impaired sperm structure and fertilization ability [1–3]. Se source is supplemented to animal diet mainly in two forms, inorganic Se and organic Se. Compared to the sodium selenite, Se-enriched yeast is an ideal additive for domestic animals because it can be easily absorbed and retained [4–6].

Spermatogenesis takes place in the seminiferous epithelium of mammalian testis in which male primordial germ cells give rise to mature spermatozoa by way of mitosis and meiosis. As an indicator

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of male fertility, the morphological and histological characteristics of testis are closely related to the spermatozoa productivity and male reproductive performance. The continuous and asynchronous production of spermatozoa depends on the involvement of complex cell signaling, including hormonal, prarcrine and autocrine signals [7,8]. Androgens play key roles in the male sexual differentiation, maintenance of spermatogenesis and sexual behavior [9–11]. In mammals, testosterone is one of the principal androgens in regulating the progression of spermatogenesis. Its biosynthesis is mainly mediated by steroidogenic acute regulatory (StAR), hydroxysteroid dehydrogenase (3β-HSD, 17β-HSD) and cytochrome P450 family (P450c17, P450scc) in response to the gonadotropins luteinizing hormone (LH) in Leydig cells [12,13]. The biological activity of testosterone in target cells can be regulated by their own receptors, such as androgen receptor (AR) and sex hormone binding globulin (SHBG), also known as androgen binding protein (ABP) [14,15]. Testosterone can stimulate the AR-dependent progression of spermatogenesis by binding to the AR of target cells and interacting with membrane receptor/second messenger cascades [16–19].

Since its discovery in 1817, Se has been extensively used to improve the reproductive performance of male animals. Studies have demonstrated that dietary Se can influence the spermatogenesis and semen quality in a number of species [20–23]. However, the foundation of reproductive gland in male animals has been basically established in prepuberty. To improve semen quality by directly supplementing Se in diet after sexual maturity is relatively limited. Based on the promoting role of Se in male reproductive function, it should be considered to further improve the testicular development of animals through the transmission of Se from dams to their fetus and/or newborn. Accumulating evidence indicates that maternal Se can improve the body weight and growth performance of their offspring in rat [24], pig [25], sheep [26,27] and goat [28]. However, research work on the influence of maternal dietary Se on the testicular development of their offspring is rather limited, especially in goat from Se deficient areas. The present study was performed to determine whether Se supplementation to gestation and lactation diets could affect the testicular development, testosterone level and the expression of steroidogenesis-related genes in testis of their male kids in Taihang Black Goats.

2. Materials and methods

This study was approved by the Shanxi Agricultural University Animal Care and Ethics Committee. All experimental procedures involving animals and their care were conducted in conformity with the guidelines for the care and use of laboratory animals, formulated by the Ministry of Science and Technology of China (the Ministry of Science and Technology of the People's Republic of China, Beijing, China, 2006).

2.1. Study site

The feeding experiment was conducted at the Lichen Breeding Goat Center in Shanxi province of China, located at longitude 36.56°E and latitude 113.4°N (Se deficiency region in China) and at an altitude of 840 m. This region has a typical north temperate continental monsoon climate with an average annual precipitation of 540 mm and an average temperature 10.4 °C.

2.2. Animals, management, and treatments

Before the trial, animals were kept in the same shed and grazed as one flock on the mountain pasture (containing 0.03–0.06 mg Se/kg DM). One hundred and sixty 3-year-old Taihang Black Goat does (with an average body weight of 38.6 ± 0.8 kg) were selected to synchronize estrus with progesterone-based protocols. They were bred using artificial insemination with diluted fresh semen after being observed in estrus. The does that did not exhibit estrus (n = 119) were randomly allocated to four treatment groups They were individually housed in 1.0 m × 1.2 m wooded pens with concrete floors and offered the basal diet for early gestation. After a 20-days adaption period, the basal diet was gradually switched to the experimental diet (the basal diet supplemented with 0, 0.5, 2.0 and 4.0 mg Se/kg DM) for early gestation. The does received the late gestation diet from the 90th day of gestation to kidding. After kidding, the lactation diet was continuously supplied to the does which produce male kids until weaning at four months of age. Daily feed allocations to each pen were adjusted according to the minimal feed refusals (<5%) in the feed bunk. The basal diets (Table 1) were formulated to meet or exceed the nutrient requirements of goats except for Se (NRC, 2007). The male kids were fed green hay (0.039 mg Se/kg DM) from 10 days of age and offered a common basal diet (without Se supplementation) [29] from 20 days of age until the end of the experiment. Feed was offered daily at 07:00 and 18:00 in equal allotments. Drinking water was freely available all the time.

2.3. Sample collection

Sample collection was carried out at the end of the study (30 d after weaning). Briefly, 10-mL blood samples were taken via jugular venipuncture using an 18-gauge needle into a vacutainer tube without anticoagulants. Blood samples were allowed to stand for 20 min at room temperature (RT) and centrifuged at 700 ×g for 15 min. Serum was separated into 2-mL Eppendorf tubes and frozen at −20 °C until analysis. Testicular tissue sampling was performed immediately after the morphological measurements.

Table 1
Ingredients and chemical composition of the basal gestation, lactation and common diets.

Item	Early gestation	Late gestation	Lactation
Ingredient, g/kg DM			
Alfalfa	150.3	300.4	300.1
Corn stalk	352.0	221.1	252.7
Soybean straw	194.4	145.7	145.5
Cracked corn	190.8	200.7	180.4
Wheat bran	10.0	22.0	22.0
Soybean meal	40.9	64.5	45.9
Sunflower meal	50.6	30.3	38.4
Salt	5.0	6.0	6.0
Calcium phosphate	3.0	5.0	5.0
Trace mineral mix[1]	3.0	4.3	4.0
Chemical composition[2] g/kg DM			
ME[3] (MJ/kg)	9.55	9.94	9.64
Crude protein	91.2	114.9	100.8
Acid detergent fiber	339.8	307.8	320.9
Neutral detergent fiber	500.0	445.7	465.3
Calcium	5.9	7.5	7.5
Phosphorus	2.5	3.0	3.0
Selenium (mg/kg)	0.046	0.045	0.045

[1] Provided per kilogram of the gestation diet: 35 mg of Zn as $ZnSO_4 \cdot 7H_2O$; 26 mg of Mn as $MnSO_4 \cdot H_2O$; 0.6 mg of I as KI; 0.2 mg of Co as $CoCl_2 \cdot 6H_2O$; 40 mg of Fe as $FeSO_4 \cdot 7H_2O$; 20 mg of Cu as $CuSO_4 \cdot 5H_2O$; 1800 IU of Vitamin A; 500 IU of Vitamin D and 260 IU of Vitamin E.
Provided per kilogram of the lactation diet: 45 mg of Zn as $ZnSO_4 \cdot 7H_2O$; 18 mg of Mn as $MnSO_4 \cdot H_2O$; 1.2 mg of I as KI; 0.2 mg of Co as $CoCl_2 \cdot 6H_2O$; 20 mg of Fe as $FeSO_4 \cdot 7H_2O$; 25 mg of Cu as $CuSO_4 \cdot 5H_2O$; 2000 IU of Vitamin A; 500 IU of Vitamin D and 225 IU of Vitamin E.
[2] Analyzed values except ME.
[3] ME, metabolizable energy.