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Effects of the different levels of dietary vitamin D on boar performance and semen quality

Yan Lin[a,*], Gang Lv[b], Hong-Jun Dong[a], De Wu[a], Zhi-Yong Tao[c], Sheng-Yu Xu[a], Lian-Qiang Che[a], Zheng-Feng Fang[a], Shi-Ping Bai[a], Bin Feng[a], Jian Li[a], Xue-Yu Xu[a]

[a] Key Laboratory for Animal Disease Resistance Nutrition of the Ministry of Education of China, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
[b] Tongwei Group Co., Ltd, Hi-tech Development Zone, Chengdu, Sichuan 610041, China
[c] Veterinary Station of Ming Shan, Ya'an, Sichuan 625100, China

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ABSTRACT

Vitamin D plays a major role primarily in bone mineral homeostasis; however, the action of vitamin D on male reproduction of boars remains unknown. This study was conducted to evaluate the effects of dietary vitamin D_3 supplementation on reproductive performance of boars. Twenty-four Yorkshire boars with eighteen months were randomly allocated to one of three vitamin D_3 levels diets: 200 IU/kg (C), 2000 IU/kg (T1) and 4000 IU/kg (T2) of vitamin D_3 for a 16-week period. Results showed that sperm motility, effective sperm number per ejaculate, acrosome intactness ratio, and membrane intactness ratio in T1 group were significantly higher than those in C group. The sperm membrane intactness ratio was higher in T1 group than in T2 group, whereas the deformity ratio was significantly lower in T1 than in C and T2 groups. Dietary supplementation with vitamin D_3 significantly increased 25-OHD_3 concentration in the blood and seminal plasma, but there was no significant difference in its concentration in the seminal plasma between T1 and T2 groups. The concentrations of blood testosterone and aromatase activity increased following vitamin D supplementation. Seminal plasma Ca^{2+}, fructose concentration, and acid phosphatase activity were higher in T1 group than in C group, but not significantly different from the corresponding values in T2 group. Furthermore, expression of CYP19, CYP24A1, and VDR mRNAs in sperm was up-regulated in T1 group compared to that in C group. In conclusion, supplementation of boar diets with 2000 IU/kg vitamin D_3 increased the sperm motility and effective sperm number, which was in line with the elevated concentrations of vitamin D, hormone secretion and gene expression.

1. Introduction

Vitamin D is primarily known for its critical role in calcium homeostasis; however, recent studies have shown that this nutrient has many effects on female (Kinuta et al., 2000) and male reproduction (Wehr et al., 2010). The nuclear receptor (vitamin D receptor, VDR) for 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) as well as the vitamin D-activating enzyme, 1α-hydroxylase, are expressed in the human testis, male reproductive tract (Mahmoudi et al., 2013), and mature spermatozoa (Corbett et al., 2006). This suggests that vitamin D has important roles in spermatogenesis and sperm function (Aquila et al., 2009; Jensen et al., 2010) and highlights its potential involvement in male reproduction responses in addition to the traditional mineral homeostasis.

Research has shown that deficiency or excess of vitamin D is harmful to male reproduction. The insemination of rats with sperm from vitamin D-deficient male rats resulted in 71% fewer pregnancies than did the insemination with sperm from vitamin D-sufficient rats (Kwiecinski et al., 1989). Vitamin D deficiency results in reduced sperm counts in male rats and lower fertility rates in female rats inseminated with semen from vitamin D-deficient male rats (Hirai et al., 2009). In rats, fertility can be restored by correcting the hypocalcaemia associated with vitamin D-deficiency and is, therefore, not directly affected by vitamin D (Uhland et al., 1992). Male mice deficient in $1,25(OH)_2D_3$, the active form of vitamin D, are infertile (Sun et al., 2015). However, other studies found that pregnancy rates following insemination with sperm from normocalcemic vitamin D-deficient rats remained 43% lower than that from insemination with sperm from normocalcemic vitamin D-replete rats (Kwiecinski et al., 1989; Uhland et al., 1992). Furthermore, cross-sectional studies have suggested that vitamin D deficiency or insufficiency in men results in significantly lower sperm motility than is observed under conditions of vitamin D sufficiency (Yang et al., 2012; Jensen et al., 2011).

* Correspondence to: Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu 611130, Sichuan, China.
E-mail address: linyan936@163.com (Y. Lin).

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Artificial insemination (AI) is commercially applied worldwide in swine industry, yielding fertility outcomes similar to those of natural mating. However, it is not fully efficient because of semen quality and quantity. Although, vitamin D is necessary for reproductive functions in rats and human, research on the effects of vitamin D supplementation in boars' diet has been lacking. Audet et al. (2004) reported that dietary supplementation with water- and fat-soluble vitamins may increase semen production during intensive semen collection. But it is difficult to distinguish the effects of each vitamin, and whether vitamin D has effect on semen production is unclear. Therefore, the objective of the present study was to determine the effects of different levels of vitamin D_3 supplementation in boar diets on the semen quality and to decipher the possible mechanism of vitamin D action.

2. Materials and methods

All the procedures involving animals were approved by the Biosafety and Animal Care and Use Committees of Sichuan Agricultural University, Sichuan, China. The study was conducted at the Institute of Animal Nutrition, Sichuan Agricultural University.

2.1. Experimental design

Twenty-four 18-month-old Yorkshire boars were randomly allocated to one of the three dietary treatments with vitamin D supplementation at 200 (C, recommended by NRC, 2012), 2000 (T1), and 4000 IU/kg (T2), administered for 16 weeks. Each group included eight boars, which were housed individually in 2.3 × 2.3-m pens on partial-slatted floors. The compositions of the diets are shown in Table 1. Diet was formulated weekly and stored at 4 °C until it was fed to the boars. Boars were fed 2.5–2.6 kg/day of feed twice daily such that at least the minimum NRC, 2012 requirements were met. The boars were provided water ad libitum. The temperature of the pen was maintained between 22 °C and 25 °C.

Table 1
Composition and nutrient levels of diets (as-fed basis)[a].

Items	C	T1	T2
Ingredients,%			
Corn	62.73	62.05	61.47
Wheat bran	10.00	10.40	10.70
Soybean meal (CP 43%)	21.00	21.00	21.00
Soybean oil	2.50	2.50	2.50
Lysine-HCl (98.5%)	0.40	0.61	0.61
Ttryptophan (98%)	0.10	0.08	0.08
Vitamin D_3	0.04	0.32	0.60
Limestone	0.60	0.75	0.75
Dicalcium phosphate	2.00	2.20	2.20
Choline chloride (50%)	0.15	0.15	0.15
Sodium chloride	0.20	0.50	0.50
Vitamins premix[b]	0.10	0.10	0.10
Minerals premix[c]	0.13	0.13	0.13
Total	100.0	100.0	100.0
Nutrient levels			
DE, Mcal/kg[d]	3.30	3.29	3.29
CP, %[d]	16.19	16.20	16.20
Ca, %[d]	0.79	0.79	0.79
Available phosphorus, %[d]	0.51	0.51	0.51
Lysine, %[d]	1.08	1.08	1.08
Methionine + Cystine, %[d]	0.31	0.31	0.31
Vitamin D_3, IU/kg[d]	200.0	2000	4000

[a] C, 200IU/kg vitamin D_3, T1, 2000IU/kg vitamin D_3, T2, 4000IU /kg vitamin D_3.
[b] Provided per kg of diets: Vitamin A, 8000 IU; Vitamin K, 5 mg; Vitamin E, 200 IU; Vitamin B_1, 2 mg; Vitamin B_2, 16 mg; Vitamin B_6, 6 mg; Vitamin B_{12}, 0.03 mg; Folic acid, 1 mg; Biotin,0.3 mg; Pantothenic acid, 25 mg; Nicotinic acid, 35 mg.
[c] Provided per kg of diets: Copper, 10 mg; Zinc, 60 mg; Iron, 95 mg; Manganese, 30 mg; Selenium e, 0.3 mg; Iodine, 0.15 mg.
[d] Calculated.

2.2. Sperm motility and morphology analysis

Boar semen was collected by the "gloved-hand technique" twice weekly during the experiment. Boar libido, as well as sperm production and quality, were determined for each ejaculate during the experiment. Boar libido was assessed by the method described by Louis et al. (1994). The time to ejaculation was the time from when the boar entered the collection area until it mounted the semen-collection dummy and started ejaculating and the duration of ejaculation was the time of boar ejaculation. The semen volume was measured after straining through two layers of disposable filter membranes to remove the gelatinous fraction by weighing each ejaculate and converting the weight to volume, as described by Lovercamp et al. (2013). The semen samples were immediately diluted with a commercial extender (ROC®, JiNan-Roc Co. Ltd., China) to a semen:extender ratio of 1:5. A computer-assisted sperm analysis system (Mailang, Nanning Song Jing Tianlun Bio-technology Co. Ltd., China) was used by a trained technician to determine the sperm concentration and characteristics of sperm motility. The mobility measurements included the following variables associated with sperm motility: average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat-cross frequency (BCF), straightness (STR), linearity (LIN), and mean angular displacement (MAD). Morphological abnormalities of sperm cells (including abnormal head, abnormal tail, detached head, bent tail, proximal droplet, and distal droplet) were determined under a bright field microscope at a total magnification of 200× (mL-8000, Nanning Song Jing Tianlun Bio-technology Co. Ltd., China) using the Gentian violet alcohol method. A minimum of 200 spermatozoa were counted per boar. The sperm acrosome integrity was determined by the Giemsa method, and sperm plasma membrane integrity was determined using the hypo-osmotic swelling (HOS) test, as described previously (World Health Organization, 1999), under a bright field microscope at 200× total magnification (ML-8000, Nanning Song Jing Tianlun Bio-technology Co. Ltd., China). All the measurements were analyzed in triplicate, and recorded for each ejaculation during the entire experimentation period.

In addition, the sperm and seminal plasma samples from individual boars were taken on days 56 and 112. Semen was immediately centrifuged (Biofuge Heraeus Primo, Heraeus, Germany) at 1800×*g* for 20 min at 4 °C (Castellano et al., 2010) and the seminal plasma was aliquoted and frozen at −20 °C until further analysis. Sperm was gently washed twice with phosphate-buffered saline (PBS) and then stored in 200-μL EP tubes at –80 °C for future analysis.

2.3. Blood collection

Blood samples (6 mL) were collected via jugular venipuncture, as described previously (Castellano et al., 2010). The samples were collected on days 1, 56, and 112, and processed by centrifugation (Biofuge Heraeus Primo) at 3500 rpm for 10 min at 4 °C. The plasma was aliquoted and frozen at –20 °C for subsequent analyses.

2.4. Assessment of 25-OHD$_3$ concentrations

The concentrations of 25-OHD$_3$ in blood and seminal plasma were determined using an EIA kit (IDS Immunodiagnostic Systems Ltd., Tyne and Wear, UK), as previously described (Wallace et al., 2010). The minimum detectable level was 2 ng/mL.

2.5. Biochemical analysis

The concentrations of total testosterone (T) and estradiol (E2), and the aromatase activity in the blood plasma were analyzed using a commercial ELISA kit (Immunotech, R & D, Minneapolis, MN, USA) in accordance with the manufacturer's protocol; the absorbance was measured at a wavelength of 450 nm. The concentrations of calcium

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