Theriogenology 102 (2017) 10–15

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Supplementation with dietary linseed oil during peri-puberty stimulates steroidogenesis and testis development in rams

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ARTICLE INFO

Article history:
Received 16 November 2016
Received in revised form
7 July 2017
Accepted 9 July 2017
Available online 10 July 2017

Keywords:
Linseed oil
Omega-3 polyunsaturated fatty acids
Rams
Steroidogenesis
Testis

ABSTRACT

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), such as α-linolenic acid (ALA), eicosapentaenoic acid, and docosahexaenoic acid, are involved in male reproductive function. In this study, we investigated the effects of linseed oil (LO) as a source of ALA on the steroidogenesis and changes of testicular histology in rams. Sixteen 3-month old rams during peri-puberty were randomly assigned into two groups. Eight rams were assigned as the control group, and the other received LO (4% dry matter of total diet) as the LO treatment group. After an 81-day feeding trial, the rams were slaughtered and investigated. Results revealed that compared with control group, diet containing LO did not affect body weight (36.87 ± 0.53 kg vs. 37.65 ± 0.64 kg, respectively; $P = 0.361$), average daily gain (227.47 ± 5.82 g vs. 237.95 ± 9.22 g, respectively; $P = 0.353$) and epididymis weight (40.77 ± 4.41 g vs. 45.53 ± 4.01 g, respectively; $P = 0.398$), however, it up-regulated PUFAs metabolism and steroidogenesis-related genes mRNA expression ($P < 0.05$), and increased plasma estradiol concentration (14.88 ± 0.67 pg/mL vs. 19.50 ± 1.27 pg/mL, respectively; $P < 0.05$). Therefore, LO stimulated seminiferous tubule development and increased the number of Sertoli cells (19.17 ± 2.14 vs. 27.2 ± 2.39, respectively; $P < 0.01$), germ-cell layers, as well as testis weight (148.65 ± 22.66 g vs. 249.96 ± 30.63 g, respectively; $P < 0.05$). All these results suggested that LO can improve testis development during peri-puberty by regulating steroidogenesis in rams' testes.

1. Introduction

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), such as α-linolenic acid (ALA) from linseed oil (LO), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) from fish oil, play important roles in reproductive physiological processes, including the regulation of prostaglandin synthesis and membrane properties [1]. The fatty acid composition and metabolism of spermatozoa, Sertoli and Leydig cells in adult male were studied, and found that the lipid composition of the sperm plasma membrane is a major determinant of the mobility characteristics and viability. PUFAs account for nearly 60% of the phospholipids that bind in the total fatty acid of cells and in sperm [2–4]. In several mammalian species including rodents, docosapentenoic acid is present in large concentrations, whereas in others including human, ram, and bull, the DHA content is relative [2–6].

Omega-3 PUFAs are semi-essential and should be sufficiently included in the diet. Omega-3 PUFAs greatly affect the fluidity of the plasma membrane and thus contribute to sperm structure formation, acrosome reaction, and sperm-oocyte fusion [2]. Testis development and spermatogenesis depend on many gonadal hormones. The ω-3 PUFAs, such as DHA, EPA, and their metabolites, in testis greatly affect the prostaglandin synthesis and steroidogenesis via the direct influence on steroid acute regulator (*StAR*) and cytochrome *P450*, which play critical roles in regulating steroid synthesis [7,8]. Omega-3 PUFAs also activate and stimulate the expression of peroxisome proliferator-activated receptor (*PPARγ*) [9], which regulates the gene transcription involved in lipid and glucose metabolism via binding to the peroxisome proliferator response element [10]. Regulated by the degree of saturation of the

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http://dx.doi.org/10.1016/j.theriogenology.2017.07.002
0093-691X/

lipid, sterol regulatory element binding protein 1 (*SREBP1*) is a strong regulator of the activity of fatty acid desaturases (*FADS1* and *FADS2*), PUFAs biosynthesis, and steroidogenesis [11].

LO also contributes to testis development and steroidogenesis. LO contains up to 50% ω-3 PUFAs, especially the ALA that can be converted into EPA and DHA through the alternating steps of elongation and desaturation involving ELOVL and FADS enzymes in mammals [12]. Most published data focused on the dietary fish oil as source of ω-3 PUFAs in improving the fresh or post-thaw semen quality in livestock [3,13–15]. However, the ω-3 PUFAs in the testis have dual effects on spermatogenesis: on the one hand, mammalian spermatozoa are sensitive to lipid peroxidation due to the phospholipid content of sperm membranes with high ω-3 PUFAs sidechains; on the other hand, the ω-3 PUFAs in testis provide some protection against peroxidation via its constituent antioxidants [16,17]. The function of LO on testis development during rams peripuberty is poorly reported. The number of Sertoli cells in testes determines the reproductive capacity of the adult male. Sertoli cells proliferation occurred during the early fetal and neonatal period and particularly the greatest during peri-pubertal stage [18,19]. The early stage is the most important period for testis development in rams. The present study aimed to investigate the effect of dietary LO on steroidogenesis and changes of testis development during peri-pubertal in rams.

2. Materials and methods

2.1. Reagents

Trizol reagent, transScript one-step gDNA removal and cDNA synthesis super-mix kit, and transStart tip green qPCR super-mix were purchased from Transgen Biotech (Beijing, China). Architect 2nd generation testosterone kit, Architect estradiol assay kit, Architect 2nd generation testosterone calibrators and estradiol calibrators were purchased from Abbott (Abbott Park, USA). Hematoxylin and eosin (H&E) staining kit was purchased from Boster Biotech (Wuhan, China). All the other reagents were purchased from Sangon Biotech (Shanghai, China).

2.2. Animals and feeding trial

Chinese Hu sheep is famous for its early sexual maturity and high fecundity with starting estrus and gestation in all seasons of the year. Hu sheep reached puberty at a mean age of 120 days. Yue [14] mentioned that spermatids and spermatozoa are first observed in seminiferous tubules after 80 days, and many mature spermatozoa are found in the epididymis after 120 days. In this study, sixteen Hu rams (average body weight of 22.84 ± 0.26 kg) aged 3 months were housed in individual pens (1 m × 1.5 m) and randomly assigned into two groups. Eight rams were used as the control group, whereas the remaining received LO (4% dry matter of total diet) as the LO treatment group. The diets were prepared to complete diet pellets with 6 mm in diameter. The levels of crude protein and calculated metabolized energy content were similar in the control and LO diet groups (Table 1).

The entire experimental period lasted for 81 days. The parasites were expelled, and disinfection and epidemic prevention were performed during the adaptation period from D0 to D20 to ensure that the rams adapt to the diets and surroundings. The normal commencing trial period was 60 days from D21 to D80, during which all rams were fed thrice a day at 8:00, 14:00, and 19:00 with ad libitum access to fresh water and multi-nutrient blocks. The daily feed offerings and refusals from D21 to D30 and D71 to D80 were recorded to calculate the dry matter intake (DMI). All rams were weighted at the beginning and the end of the 60-day commencing trial prior to feeding.

The trial was performed at the Minqin Zhongtian Sheep Industry Co., Ltd. (Minqin, China) from August to November 2015. This study was conducted in strict accordance with the recommendations from the Guide for the Animal Care and Use Committee of Lanzhou University. No ram was harmed during the feeding trial.

2.3. Sample collection

At the end of the feeding trial, blood samples were collected in heparinized vials through jugular venepuncture. The samples were centrifuged at × 1000 g at 4 °C for 30 min and the blood plasma was stored at −20 °C. Immediately after blood sampling, the rams were humanely slaughtered by a licensed slaughter man in a way of severing the carotid artery, ingular vein, trachea, esophagus, and vagus nerve according to the halal slaughter procedure as outlined in the Chinese Local Standards DB13/T 963–2008 [20] at the Minqin Zhongtian Sheep Industry Co., Ltd. Their body, testes, and epididymis weight without spermatic cord were measured. Testes tissues were rapidly collected from the left testis without tunica albuginea using liquid nitrogen for frozen tissues and 10% formalin for fixed tissues.

2.4. RNA isolation and cDNA synthesis

Total RNA was isolated using Trizol reagent according to the manufacturer's instructions. The RNA quantity and quality was detected using Nano Drop 2000 (Thermo Fisher, Waltham, USA). Total RNA (2.5 μg) was reverse-transcribed to cDNA using TransScript one-step gDNA removal and cDNA synthesis super-mix kit at 42 °C for 15 min following the manufacturer's instructions. The cDNA was diluted 1:10 with nuclease-free water and stored at −80 °C.

2.5. Quantitative real-time PCR analysis

Relative mRNA abundance of luteinizing hormone receptor (*LHR*), follicle stimulating hormone receptor (*FSHR*), androgen-binding protein (*ABP*), *StAR*, aromatase (*P450scc* and *P450arom*),

Table 1
Chemical characteristics of the experimental diets.

Item (% of DM)	Control	LO
Ingredient, % of DM		
Barley straw	26.00	26.00
Malt root	8.00	8.00
Corn	45.25	39.55
Concentrate feed[a]	20.75	22.45
LO	–	4.00
Chemical composition, % of DM		
Metabolizable energy (MJ/kg) (calculated)	12.60	12.60
Crude protein	16.30	16.30
Neutral detergent fibre	35.10	35.10
Calcium	0.65	0.65
Phosphorus	0.34	0.34
Fatty acid composition (% of FAs)		
Total saturates	39.02	25.31
Total MUFAs	25.85	40.77
Total PUFAs	35.12	33.91
ω-3 PUFAs	3.24	6.19
ω-6 PUFAs	31.88	27.02

LO = linseed oil, DM = dry matter, FAs = Fatty acids, MUFAs = Monounsaturated fatty acids, PUFAs = Polyunsaturated fatty acids.

[a] Concentrate feed composed with flax meal, soybean meal, cotton seed meal, salt, powder, vitamin and mineral premix. Concentration of vitamin and mineral per kilogram of DM: 2500 IU vitamin A; 23 IU vitamin E; 0.3 mg selenium; 70 mg ferrum; 41 mg zinc; 8 mg copper.