Effect of Dietary Energy Level on Ovarian Development and Related Gene Expression in Replacement Gilts (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary energy levels on ovarian development and the mRNA expression of ovarian follicle-stimulating hormone receptor (FSHR) and luteinizing hormone/chorionic gonadotropin receptor (LH/CGR) in replacement gilts. Twenty-seven Landrace × Large White crossbred gilts with a body weight of (61.0±3.1) kg were selected and randomly allocated into three groups (three replicates per group, three pigs per replicate), and fed diets with low, medium, and high energy levels [90%, 100%, and 110% of the digestible energy requirement recommended by NRC (1998), respectively]. During the experimental period, the average daily feed intake was identical across groups, but the levels of digestible energy intake differed. Experimental gilts were slaughtered on day 19 of the second estrous cycle. The results demonstrated that ovarian weight and the number of large follicles in the high-energy group were significantly higher than those in the low-energy group ($P<0.05$); no significant difference was observed in the number of small follicles among groups ($P>0.05$). The high-energy group exhibited the highest ovarian FSHR and LH/CGR mRNA expression levels, which were significantly higher than those in the low-energy group ($P<0.05$). It was concluded that high-energy-level diets could promote ovarian development in replacement gilts and facilitate the expression of ovarian FSHR and LH/CGR mRNA.

Full Text

Abstract

This study aimed to investigate the effects of dietary energy level on ovary development and mRNA expression of follicle stimulating hormone receptor (FSHR) and luteinizing hormone/chorionic gonadotropin receptor (LH/CGR) in prepubertal gilts. Twenty-seven crossbred (Landrace×Yorkshire) gilts with a body

weight of (61.0±3.1) kg were selected and randomly allocated to 3 groups (3 replicates per group, 3 pigs per replicate), and fed diets with low, medium, and high energy levels [90%, 100%, and 110% of the digestible energy (DE) requirements recommended by NRC (1998), respectively]. During the experimental period, gilts in all groups had the same average daily feed intake but consumed different DE levels. Gilts were slaughtered on day 19 of the second estrus cycle. The results showed that ovary weight and the number of large follicles in the high energy group were significantly higher than those in the low energy group ($P<0.05$). The number of small follicles did not differ significantly among groups ($P>0.05$). The high energy group exhibited the highest ovarian FSHR and LH/CGR mRNA expression levels, which were significantly higher than those in the low energy group ($P<0.05$). It was concluded that high dietary energy level promotes ovary development and facilitates the expression of ovarian FSHR and LH/CGR mRNA in prepubertal gilts.

Key words: energy level; prepubertal gilts; ovary development; gene expression

Introduction

As the gonads of mammals, ovaries are closely associated with reproductive performance in sows. Ovary development, oocyte maturation, and quality in prepubertal gilts directly affect ovulation rate and gonadotropin secretion, thereby influencing embryo number and litter size [1]. Numerous studies have reported on the effects of dietary energy on sow reproductive performance, but the conclusions are not entirely consistent. Most researchers believe that restricted feeding and refeeding strategies applied to sows at different growth or reproductive stages can affect follicular development (total follicle number, large follicle number, etc.), ovulation rate, and embryonic development [1-6]. However, conflicting results have also been reported. Almeida et al. [7] found that three feeding patterns during the estrous cycle (restricted feeding on days 1-7, restricted feeding on days 8-15, and ad libitum feeding on days 1-15) did not significantly affect ovulation rate. Moreover, few studies have investigated the effects of nutritional factors on the expression of ovarian gonadotropin receptors. Therefore, the mechanisms underlying nutritional regulation in prepubertal gilts remain to be further elucidated. In view of this, the present study used prepubertal gilts as experimental animals to investigate the effects of different dietary energy levels on ovary development and mRNA expression of follicle stimulating hormone receptor (FSHR) and luteinizing hormone/chorionic gonadotropin receptor (LH/CGR), aiming to provide evidence for improving the understanding of mechanisms through which dietary energy affects animal reproductive performance.

Materials and Methods

1.1 Experimental Design

Twenty-seven healthy crossbred (Landrace×Yorkshire) prepubertal gilts at (150±3) days of age with a body weight of (61.0±3.1) kg were selected and randomly divided into 3 groups: a medium energy group (14.28 MJ/kg DE) fed a diet formulated according to NRC (1998) (energy intake at 100% of DE requirements), a low energy group (12.86 MJ/kg DE) fed a diet with 10% reduced energy (energy intake at 90% of DE requirements), and a high energy group (15.71 MJ/kg DE) fed a diet with 10% increased energy (energy intake at 110% of DE requirements). The diet composition and nutrient levels are presented in Table 1 . Prior to the experiment, pig houses were thoroughly cleaned and disinfected. According to NRC (1998) recommendations for gilt nutrient requirements, during the experimental period, each pig was fed 2.1 kg/d when body weight was below 75 kg, and 2.4 kg/d when body weight reached 75 kg. All groups received the same intake of nutrients other than energy. Pigs were fed twice daily at 08:00 and 15:00 with equal amounts at each feeding. Experimental gilts were housed individually with ad libitum access to water. After reaching 80 kg body weight at approximately 26 weeks of age, estrus was checked twice daily, with the first day of standing reflex recorded as day 1 of estrus.

1.2 Sample Collection

On day 19 of the second estrus, gilts were slaughtered and ovaries were collected. After blotting off surface moisture with absorbent paper, ovaries were weighed and the numbers of large follicles (diameter >3 mm) and small follicles (diameter <3 mm) were counted. Ovaries were then snap-frozen in liquid nitrogen and stored at -80°C for subsequent molecular biological analysis.

1.3 Experimental Materials

The following reagents and equipment were used: diethylpyrocarbonate (DEPC) (Sigma, USA), Trizol reagent (Invitrogen, USA), reverse transcriptase (M-MLV) (Promega, USA), dNTP (TaKaRa, China), RNase inhibitor (TaKaRa, China), Oligo(dT)18 (TaKaRa, China), ABI System-7000 PCR instrument (ABI, USA), GeneQuant-100 nucleic acid concentration analyzer (Pharmacia Biotech, UK), and Sigma-3k15 high-speed refrigerated centrifuge (Sigma, Germany).

1.4 Molecular Analysis

1.4.1 Total RNA Extraction Total RNA was extracted from 50-100 mg samples using the one-step Trizol method. RNA integrity was assessed by electrophoresis, and concentration and purity were measured using the GeneQuant-100 nucleic acid concentration analyzer.

1.4.2 Primer Design and Synthesis Primers were designed using Primer 5.0 software and synthesized by Shanghai Bioengineering Technology Co., Ltd., as shown in Table 2 .

1.4.3 Target Fragment Amplification Conventional PCR was used to amplify target fragments and internal controls. Using FSHR and LH/CGR primers, target fragments were amplified from cDNA templates derived from ovaries of high, medium, and low energy groups, with expected fragment lengths of 103 and 145 bp, respectively. The reaction conditions were: pre-denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 30 s; final extension at 72°C for 6 min; and hold at 4°C.

1.4.4 Purification, Ligation, Transformation and Sequencing of PCR Products Target gene products were recovered using the DNA gel recovery kit from V-gene Biotechnology Limited. After ligation to the pMD 18-T vector, positive clones were initially screened using blue/white colony selection, and plasmids were extracted using a routine plasmid DNA rapid preparation kit. Recombinant plasmids identified as correct by PCR were sent to TaKaRa for sequencing. Sequencing results were compared with known nucleic acid sequences online and analyzed for homology with GenBank sequences using DNAMAN software.

1.4.5 Determination of Ovarian FSHR and LH/CGR mRNA Expression Levels Ovarian FSHR and LH/CGR mRNA expression levels were detected using a real-time fluorescence quantitative PCR instrument with the TaqMan probe method. Serially diluted recombinant plasmids were used as positive control standards to generate standard curves. The PCR reaction system (25.0 µL) contained: 1.00 µL cDNA, 2.50 µL 10×Ex Taq Buffer, 2.00 µL 2.5 mmol/L dNTP Mixture, 0.50 µL each of 10 pmol/L forward and reverse primers, 0.25 µL 10 pmol/L FAM-TAMRA probe, 0.15 µL Ex Taq HS, and 18.10 µL ddH2O. The reaction conditions were: pre-denaturation at 95°C for 6 min; 48 cycles of denaturation at 94°C for 30 s and annealing at 59°C for 30 s. The copy number in each sample was determined by comparing the Ct value with the corresponding standard curve.

1.5 Data Processing

Experimental data are expressed as mean ± standard deviation. SPSS 15.0 software was used for analysis of variance and LSD multiple comparisons, with $P<0.05$ considered statistically significant.

Results

2.1 Effects of Dietary Energy Level on Ovary Development in Prepubertal Gilts

As shown in Table 3 , dietary energy level significantly affected the number of large follicles and ovary weight in prepubertal gilts ($P<0.05$), but had no significant effect on the number of small follicles ($P>0.05$). The number of large follicles and ovary weight increased with dietary energy level. Specifically, the high energy group had significantly higher numbers of large follicles and ovary weight compared with the low energy group ($P<0.05$), but did not differ significantly from the medium energy group ($P>0.05$). No significant differences were observed between the low and medium energy groups for either large follicle number or ovary weight ($P>0.05$).

2.2 Molecular Validation and Gene Expression Analysis

2.2.1 Identification of Total RNA from Ovarian Tissue Total RNA extracted from ovarian tissue was analyzed by 1% agarose gel electrophoresis, as shown in Figure 1 [Figure 1: see original paper]. Three distinct bands corresponding to 28S, 18S, and 5S were clearly visible, indicating intact RNA without degradation and meeting the requirements for reverse transcription. The OD260/OD280 ratios ranged from 1.8 to 2.0, confirming adequate purity.

2.2.2 Identification of Target Gene PCR Products Using recombinant plasmids as templates, conventional PCR identification was performed with FSHR and LH/CGR primers. As shown in Figure 2 [Figure 2: see original paper], specific bands were observed at 103 and 145 bp, consistent with the expected fragment sizes. Sequence analysis revealed 100% homology with the FSHR and LH/CGR gene sequences published in GenBank.

2.2.3 Effects of Dietary Energy Level on Ovarian FSHR and LH/CGR mRNA Expression As shown in Table 4 , dietary energy level significantly affected ovarian FSHR and LH/CGR mRNA expression levels ($P<0.05$). Both FSHR and LH/CGR mRNA expression increased significantly with dietary energy level ($P<0.05$). The high energy group exhibited significantly higher FSHR mRNA expression than both the low and medium energy groups ($P<0.05$), while the difference between medium and low energy groups was not significant ($P>0.05$). The high energy group also showed significantly higher LH/CGR mRNA expression than the low energy group ($P<0.05$), but did not differ significantly from the medium energy group ($P>0.05$). No significant difference in LH/CGR mRNA expression was observed between medium and low energy groups ($P>0.05$).

Discussion

3.1 Effects of Dietary Energy Level on Ovary Development in Prepubertal Gilts

Follicle maturation is one of the hallmarks of puberty onset in prepubertal gilts. Follicle development involves sequential processes of recruitment, selection, dominance, and ovulation, which are regulated by endocrine, paracrine, and autocrine factors. Multiple studies have confirmed that energy intake can affect follicle development in sows at different reproductive stages. In the present study, dietary energy level influenced the number of large follicles and ovary weight in prepubertal gilts, with high energy levels significantly increasing both parameters. These findings are consistent with the results of Wang [8] and Zhou [9], who studied the effects of dietary fat supplementation (29% of DE from mixed fats, lard:rapeseed oil = 1:1) in 59 kg prepubertal gilts. Booth et al. [1] reported that restricted feeding of prepubertal gilts from 75 to 85 kg significantly reduced follicle number compared with ad libitum feeding. Similar conclusions have been drawn in studies on primiparous lactating sows [5,6,10]. Ovary weight is closely correlated with reproductive performance in sows; increased ovary weight can promote gonadotropin secretion and facilitate the continued development of more small follicles. The number of large preovulatory follicles represents the potential ovulation rate [8]. The increased ovary weight and large follicle number observed in gilts fed high-energy diets may lead to corresponding improvements in ovulation rate, embryo number, and litter size after breeding. However, in contrast to the findings of Wang [8] and Zhou [9], the present study found no significant effect of dietary energy level on small follicle number, which may be attributed to differences in the types of dietary fat supplemented.

3.2 Effects of Dietary Energy Level on Ovarian FSHR and LH/CGR mRNA Expression

Few studies have investigated the effects of dietary energy level on ovarian FSHR and LH/CGR mRNA expression in prepubertal gilts. The present study demonstrated that dietary energy level caused significant changes in the expression of these receptors, with high energy levels promoting ovarian FSHR and LH/CGR mRNA expression. The high energy group exhibited the highest expression levels, which were significantly higher than those in the low energy group, consistent with the findings of Wang [8]. This significant difference was also observed in the ovarian FSHR and LH/CGR mRNA expression levels of 50-day-old prepubertal gilts [11], suggesting that the differential effects of dietary energy level on ovarian FSHR and LH/CGR mRNA expression have long-term effects that may persist throughout the entire growth period of prepubertal gilts.

As is well known, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are important regulators of female reproductive function. FSH initiates follicle recruitment and promotes follicle development and maturation to the

preovulatory stage, while LH synergizes with FSH to promote follicle growth and maturation and can trigger ovulation. The physiological actions of FSH and LH are mediated through specific binding to FSHR and LH/CGR, respectively. The molecular mechanisms through which energy status affects the expression of ovarian FSHR and LH/CGR are not yet fully understood. Energy may influence follicle development, ovulation rate, and litter size by modulating the expression of ovarian FSHR and LH/CGR, thereby altering follicular sensitivity to FSH and LH [11]. However, the specific mechanisms remain to be further elucidated.

Conclusions

Increasing dietary energy level can increase ovary weight and the number of large follicles in prepubertal gilts.

Increasing dietary energy level promotes ovarian FSHR and LH/CGR mRNA expression in prepubertal gilts.

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Note: Figure translations are in progress. See original paper for figures.