Animal Nutrition 1 (2015) 152–159

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: www.www.keaipublishing.com/en/journals/aninu

Original research article

Dietary energy intake affects fetal survival and development during early and middle pregnancy in Large White and Meishan gilts

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ARTICLE INFO

Article history:
Received 16 May 2015
Accepted 6 August 2015
Available online 1 September 2015

Keywords:
Energy level
Fetal survival
Corpus luteum
Endometrium
pig

ABSTRACT

This experiment was designed to determine the effects of variations in dietary energy intake on reproductive performance and gene expression of luteal and endometrium tissues in Large White (LW) and Meishan (MS) gilts during early and middle pregnancy. After insemination, 32 LW gilts were assigned to high and low (HE_L and LE_L, 14.23 and 12.56 MJ DE/kg, respectively) diet treatment groups, while 32 MS gilts were allocated to HE_M and LE_M (12.56 and 10.88 MJ DE/kg) groups. Gilts were slaughtered on days 35, 55 and 90 of gestation. The fetal survival and luteal progesterone (P_4) concentration in the HE_L group were higher on day 35 but lower on day 90 of gestation compared with the LE_L group ($P < 0.05$) for LW gilts. However, fetal survival and luteal P4 concentration on day 35 of gestation were greater ($P < 0.05$) in the LE_M group than in the HE_M group for MS gilts, but no significant difference in mid-gestation was showed. The fetal weights of both breeds were higher for the high energy diets compared with the respective control group on day 90 of gestation ($P < 0.05$). In addition, the mRNA levels of P_4 synthesis-related proteins had correlated with luteal P_4 concentration in both breeds. Further, endometrial levels of uteroferrin (ACP5), retinol-binding protein 4 (RBP4) and secreted phosphoprotein 1 (SPP1) mRNA were upregulated in the HE_L group on day 35 of gestation but ACP5 and SPP1 were downregulated on day 55 of gestation compared with the LE_L group ($P < 0.05$) for LW gilts. In MS gilts, diet only affected the expression of SPP1 ($P < 0.05$). Our results revealed the differential sensitivity of LW and MS breeds to variations in dietary energy intake. For LW gilts, the HE_L group improved fetal survival on day 35 but a sustained high energy diet decreased fetal survival on day 90 of gestation. The differences in dietary energy intake did not influence fetal survival on day 90 of gestation but the higher energy diet did increase fetal weight in the MS breed compared with the lower energy intake diet. These results may be due to differential luteal secretion activity and endometrium gene expression in these two breeds.

1. Introduction

Dietary energy intake levels play a major role in the regulation of swine reproductive performance (Quesnel et al., 2010; Hoving et al., 2011). An increased level of dietary energy intake after mating has been shown to reduce systemic progesterone concentrations (Jindal et al., 1997), and thus affects endometrial secretory functions (Lonergan et al., 2013) eventually leading to increased embryo mortality in early pregnancy (Xu et al., 2010). Interestingly, energy intake seems to display a specific action within the Chinese Meishan (MS) pig to modulate reproductive performance.

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2015.08.009

Studies have suggested that deficiencies of progesterone (P_4) secretion in some fatty pigs such as Iberian sows will lead to low reproductive efficiency (Astiz et al., 2013). In contrast, Ashworth et al. (1999) have demonstrated that dietary consumption after mating had no effect on P_4 release and embryo survival in MS pigs which are also fat pigs. Together, this evidence demonstrated that commercial breeds and MS pigs may exhibit different sensitivities to dietary alteration.

In general, P_4 synthesis-related proteins sense ovarian nutrient status, with the secretion of P_4 in the corpora lutea (CL) increasing when nutrients are abundant (Athorn et al., 2011, 2013). The synthesis of luteal P_4 depends on cholesterol which is absorbed by the scavenger receptor-BI (SR-BI) and the low-density lipoprotein receptor (LDLR). Intracellular cholesterol is transported by the steroidogenic acute regulatory protein (STAR) to the inner mitochondrial membrane and used to synthesize pregnenolone by cytochrome P450. Pregnenolone is then transported to the smooth endoplasmic reticulum, this is dependent on 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD), which converts pregnenolone to P_4 (Payne et al., 2004). Interestingly, numerous studies have explored the effect of dietary protein level on physiological parameters and reproductive performance in MS pigs (Liu et al., 2011; Pan et al., 2013), however, there is no report regarding the complex mechanisms controlling P_4 synthesis in the CL. Importantly, whether P_4 levels in the CL could be altered by changing dietary energy levels, as well as the subsequent effects on secretory activity of the uterus are largely unknown in MS pigs.

Research studies have demonstrated that P_4 regulates embryonic survival and gene expression in endometrium via the P_4 receptor (PGR) (Kastner et al., 1990). Genes altered by P_4 include retinol-binding protein 4 (RBP4) (Mullen et al., 2012), uteroferrin (ACP5) (Spencer et al., 2010), fibroblast growth factor receptor 2 (FGFR2) (Bailey et al., 2010) and secreted phosphoprotein 1 (SPP1) (Johnson et al., 1999). In commercial breeds, especially, there are limited reports regarding the effect of levels of energy intake on gene expression and the relationships between genes expressed in the endometrium and embryo survival. Furthermore, there is no evidence for meeting whether MS gilts will have similar changes in gene expression as commercial breeds in response to increased dietary energy levels. Thus, the aim of this study was to investigate the response to different dietary energy intake levels in the Large White (LW) and MS pig breeds via measurement of reproductive performance and gene expression of in the CL and endometrium.

2. Materials and methods

2.1. Animal management and experimental design

Animal studies were conducted in accordance with the actual law of animal protection approved by the Agricultural Animal Care and Use Committee of Sichuan Agricultural University. Thirty-two pure-bred LW gilts with an average weight of 135.54 ± 0.66 kg and the same number of MS gilts with an average weight of 72.84 ± 0.66 kg were used in this experiment. In the third estrus, all LW gilts were artificially inseminated twice with fresh diluted semen from the same LW boar by one well-trained person from 18 to 24 h after the first observation of standing heat. Estrus detection and mating dates of MS gilts were similar to those of LW gilts, and the semen was obtained from the same MS boar. After mating, the LW and MS gilts were randomly allocated to two feeding groups. The experimental diet included 13.9% crude protein, 0.69% Lys, 0.96% calcium and 0.79% phosphorus but energy levels were varied by supplementing soybean oil and they were 14.23 or 12.56 MJ DE/kg (HE_L or LE_L) for LW gilts and 12.56 or 10.88 MJ DE/kg (HE_M or LE_M) for MS gilts, respectively (Table 1). Feed intake of all gestating gilts was 2.0 kg/d from days 0 to 30 of pregnancy and 2.4 kg/d from days 31 to 90 regardless of treatments. All gilts were housed in individual feeding stalls and allowed to consume water ad libitum.

Table 1
The ingredients and nutrient contents of diets (as-fed basis).

Item	Dietary energy level, MJ of DE/kg		
	14.23	12.56	10.88
Ingredient, %			
Corn	45.00	45.00	45.00
Soybean meal	13.60	13.60	13.60
Wheat bran	27.80	27.80	27.80
Soy oil	9.10	4.50	0
Wheat fiber	0	2.54	5.02
Soybean fiber	0	1.10	2.17
Corn fiber	0	0.96	1.91
Salt	0.40	0.40	0.40
Choline chloride	0.14	0.14	0.14
Calcium carbonate	1.24	1.24	1.24
Dicalcium phosphate	1.99	1.99	1.99
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.50	0.50	0.50
Lysine	0.10	0.10	0.10
Threonine	0.10	0.10	0.10
Total	100.00	100.00	100.00
Chemical compositions, %			
DE, MJ/kg	14.23	12.56	10.88
CP	13.49	13.92	14.35
Ca	0.96	0.96	0.96
Total P	0.79	0.79	0.79
Lysine	0.69	0.69	0.69
Threonine	0.46	0.46	0.46

[1] Supplied the following per kilogram of complete diet: 15,500 IU of vitamin A; 3,250 IU of vitamin D_3; 16 IU of vitamin E; 5.2 mg of riboflavin; 20 mg of nicotinic acid; 11 mg of pantothenic acid; 0.12 mg of vitamin B_{12}; 0.13 mg of biotin.
[2] Supplied the following per kilogram of complete diet: 170 mg of Fe; 17 mg of Cu; 160 mg of Zn; 35 mg of Mn; 0.3 mg of Se; 0.28 mg of I.

2.2. Blood collection

Gilt body weights were measured before feeding, and peripheral blood was collected from eight gilts (including four from slaughtered gilts at the same time point and others randomly selected from each feeding group) on days 35, 55 and 90 by acute jugular venipuncture. All blood samples were centrifuged immediately after collection (3,000 × *g* for 15 min at 4°C). Serum samples were collected and stored at −20°C for future analysis.

2.3. Collection of reproductive tracts

Four gilts were selected randomly from each group to collect reproductive tracts ($n = 4$) after being slaughtered at a local abattoir on days 35, 55 and 90 of gestation following deep anaesthesia with Zoletil 50 (Zoletil 50 Vet, Virbac, France) at a dose of 0.1 mg/kg of body weight administered by intramuscular injection. After slaughter, the uterus was immediately removed from each gilt and total weight of the gravid uterus, length of each uterine horn, number of fetuses per horn, fetal weight and crown-rump length were measured. Both ovaries of each horn were examined and counted to determine the number of CL. The fetal survival rate was calculated by the percentage of the number of CL represented by all living fetuses (Jindal et al., 1997). Luteal tissues were separated from the ovaries, and a piece of endometrium from the middle of each uterine horn was separated from the myometrium. All luteal tissues and endometrial samples were frozen rapidly in liquid nitrogen after rinsing with cold sterile saline, and then stored at −80°C for further hormone concentration measurements and RNA isolation.