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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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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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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.

2.4. Metabolite and hormone assays

The concentrations of high-density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC) were measured using the colorimetric high- and low-density lipoprotein cholesterol assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The P_4 concentration in CL was determined by an equilibrium competitive RIA using a commercial iodine [^{125}I] kit (Diagnostic Product, Beijing North Institute of Biological Technology, Beijing, China) after extraction as described previously (Grzesiak et al., 2014). The concentration of P_4 in serum was determined using the same method without the extraction step.

2.5. RNA extraction and reverse transcription

Total tissue RNA were isolated from luteal and endometrial tissues using trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA purity and concentration were determined using a nucleic acid/protein analyzer (Beckman DU-800, CA, USA) by measuring the absorbance at 260 and 280 nm. Agarose gel electrophoresis was used to evaluate the quality of RNA. Reverse transcription was performed with a high-capacity cDNA reverse transcription kit (TaKaRa Biotechnology, Dalian, China). All RNA samples were treated with DNase I to avoid genomic DNA contamination before reverse transcription (RT). RT reaction mixtures were prepared in a total volume of 10 μL and performed according to manufacturer's guidelines. The cDNA was stored at −20°C for further use.

2.6. Real-time PCR

Real-time PCR was performed using the iQ5 Real Time PCR Detection System (ABI 7900HT, Applied Biosystems). The total volume of 10 μL reaction mixture consisted of 5 μL 2× SYBR Green Supermix (TaKaRa, Biotechnology, Dalian, China), 1 μL each of forward and reverse primers, 2 μL dHE$_M$O and 1 μL cDNA. The primers sequences used are shown in Table 2. PCR conditions were as follows: initial DNA denaturation at 95°C for 1 min, followed by 40 cycles of denaturation at 95°C for 5 s, the primer-specific annealing for 30 s and extension at 72°C for 30 s. Melt curve conditions at 95°C for 0 s, 50°C for 30 s and 95°C for 0 s (temperature change velocity: 0.5°C/s). All real-time PCR experiments were performed in triplicate. Agarose gel electrophoresis was used to confirm product sizeduct. β-actin was set as the housekeeping control.

2.7. Statistical analysis

All experimental data were shown as means ± standard error of the mean (SEM). Data were analyzed using SPSS statistical software program (v. 19.0 for windows, SPSS; IBM SPSS Company, Chicago, IL, USA). Firstly, normal distribution of data was tested with the Shapiro–Wilk test. Then, Student's *t*-test was used to detect differences between the two diet groups at each slaughtering stage along gestation and relative differences in the target gene of CL and endometrium were determined by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Results with $P < 0.05$ were considered significant.

3. Results

3.1. The effect of dietary energy intake on reproductive performance

Large White gilts in the HE$_L$ group had higher slaughter weight than the LE$_L$ group on days 55 and 90 of gestation ($P < 0.05$). There was no difference in uterine weight, uterine length and ovary weight between the LW treatment groups ($P > 0.05$) (Table 3). The HE$_L$ group had higher fetus survival on day 35 and lower fetus number and survival on day 90 of gestation compared with the LE$_L$ group ($P < 0.05$) (Table 4). In MS gilts, a higher slaughter weight was observed in the HE$_M$ than in the LE$_M$ group on day 90 of gestation ($P < 0.01$) (Table 3). The HE$_M$ group showed lower fetal survival and shorter left side uterine length ($P < 0.05$) than the LE$_M$ group on day 35 of gestation. There were no significant differences in uterine and ovary weights between the two treatment groups ($P > 0.05$) (Table 3). In addition, a greater fetal weight was observed in gilts of HE$_L$ and HE$_M$ groups on day 90 of gestation than the respective control groups ($P < 0.05$) (Table 4).

Table 2
Real-time PCRprimer sequences

Gene	Primer	Sequence (5′ to 3′)	Product size, bp	Accession No.
SR-BI	Forward	TCGCCACACCTCCACAAC	130	AF467889
	Reverse	CCCAAGACCAGAAGCCCG		
LDLR	Forward	GGATAAGCACAGATGCGAAGATA	177	NM001206354
	Reverse	CGGTTGGTGAAGAAGAGGTAGG		
STAR	Forward	CTGCCGATTTCTCTGCTTCAA	77	NM213755
	Reverse	TTACCCCCAACTATCCCTTCC		
CYP11A1	Forward	GGCTCCAGAGGCCATAAAGA	142	X13768.1
	Reverse	ACTCAAAGGCGAAGCGAAAC		
3β-HSD	Forward	CGTGGATGTGGGTGTGAGG	85	AF232699
	Reverse	TGTATGAAGCCAGTGGCGG		
PGR	Forward	GGCGGGCTGCTGCATGAGA	180	NM213911
	Reverse	ACGCAGCTCGGCAGGGGTGA		
RBP4	Forward	ATGGCAAATCGGAAAGAAACAT	128	NM214057
	Reverse	GGGGAAGGAGAGAGGGACAAAC		
FGFR2	Forward	TACACCCACCAGAGTGATGTCT	120	NM001099924
	Reverse	TCCTTCTTTGAGCAGCTTAAAA		
SPP1	Forward	GGTGGGAGAAAATATGAAAGGC	196	X16575
	Reverse	ATTACAAATCAGTGACGGCTTG		
ACP5	Forward	CAGGAGACCTTTGAGGATGTGT	112	NM214209
	Reverse	AATAGGCTATCTGTGCCGAGAC		
β-actin	Forward	GTGCTGAGTATGTCGTGGAGTC	183	AY550069.1
	Reverse	CAGTTGGTGGTACAGGAGGC		

SR-BI = scavenger receptor-BI; LDLR = low-density lipoprotein receptor; STAR = steroidogenic acute regulatory protein; CYP11A1 = cytochrome P450scc; 3β-HSD = 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase; PGR = progesterone receptor; RBP4 = retinol-binding protein 4; FGFR2 = fibroblast growth factor receptor 2; SPP1 = secreted phospoprotein 1; ACP5 = uteroferrin.

Table 3
Influence of energy level on slaughter weight, uterine length and ovary weight in Large White and Meishan gilts.[1]

Parameter	Days of gestation, d	LW gilts			MS gilts		
		HE_L	LE_L	*P*-value	HE_M	LE_M	*P*-value
Body weight, kg	0	135.48 ± 1.07	135.60 ± 0.80	0.927	72.70 ± 0.94	72.98 ± 0.95	0.840
	35	156.12 ± 1.34	153.04 ± 0.76	0.054	93.46 ± 1.80	89.91 ± 1.32	0.123
	55	178.08 ± 2.62[a]	171.93 ± 1.20[b]	0.049	107.26 ± 1.99	101.09 ± 2.15	0.057
	90	201.65 ± 3.60[a]	188.30 ± 1.82[b]	0.005	127.93 ± 0.91[a]	117.03 ± 2.49[b]	0.002
Uterine weight, kg[2]	35	5.32 ± 0.52	5.23 ± 0.08	0.877	3.42 ± 0.19	4.24 ± 0.75	0.332
	55	11.36 ± 1.59	13.64 ± 1.33	0.315	10.30 ± 0.73	12.68 ± 1.28	0.145
	90	23.70 ± 1.12	25.44 ± 2.26	0.517	13.86 ± 2.09	15.97 ± 0.33	0.357
Uterine length (left), cm	35	72.88 ± 11.75	85.50 ± 9.75	0.470	92.00 ± 10.95[b]	115.33 ± 4.91[a]	0.030
	55	154.67 ± 17.07	158.00 ± 10.04	0.865	128.25 ± 6.28	136.00 ± 16.58	0.677
	90	167.75 ± 4.75	160.67 ± 6.17	0.396	134.25 ± 8.44	127.75 ± 11.74	0.669
Uterine length (right), cm	35	89.17 ± 29.55	99.00 ± 21.71	0.75	90.50 ± 4.84	102.00 ± 15.54	0.510
	55	162.00 ± 12.66	165.75 ± 20.26	0.892	128.25 ± 6.28	136.00 ± 23.44	0.727
	90	152.33 ± 11.10	162.25 ± 9.20	0.519	137.00 ± 10.07	133.25 ± 4.66	0.725
Ovary weight, g	35	22.68 ± 2.03	21.43 ± 1.74	0.677	19.36 ± 1.38	16.63 ± 1.14	0.177
	55	18.58 ± 2.30	24.13 ± 0.62	0.059	17.82 ± 0.94	17.56 ± 1.40	0.879
	90	25.20 ± 2.89	26.60 ± 2.52	0.728	19.00 ± 2.64	20.19 ± 1.36	0.701

LW = Large White; MS = Meishan.

[a,b] Within a same row, means with different superscripts are significantly different at $P < 0.05$.

[1] Energy levels were varied by supplementing soybean oil and they were 14.23 and 12.56 MJ DE/kg (HE_L and LE_L) for LW gilts and 12.56 and 10.88 MJ DE/kg (HE_M and LE_M) for MS gilts, respectively. $n = 4$ per group of days 35, 55, and 90 of gestation.

[2] Uterine weight is the weight of uterus and its contents.

3.2. The effects of dietary energy intake on serum metabolites and hormones

In LW gilts, the concentration of serum P_4 was not affected by dietary energy treatment ($P > 0.05$), however, the luteal P_4 concentration was higher in the HE_L group on day 35 of gestation ($P < 0.05$) than in the LE_L group. This was subsequently reversed on days 55 and 90 of gestation ($P = 0.092$ and $P = 0.071$) (Table 5). Compared with the HE_L group, serum HDLC was higher on day 55 of gestation ($P < 0.01$). No significant difference was observed in serum LDLC between the treatment groups in LW gilts ($P > 0.05$) (Table 5). In contrast, interestingly, the level of luteal P_4 was higher ($P < 0.05$) in the LE_M group than in the HE_M group on day 35 of gestation. However, it was not markedly affected by different diet treatments on day 55 or 90 of gestation (Table 5).

3.3. The effect of different dietary energy intake on gene expression in CL

On day 35 of gestation, the level of 3β-HSD mRNA expression in luteal tissue was higher ($P < 0.05$) in the HE_L group than that in the LE_L group of LW gilts. Interestingly, SRBI, STAR and 3β-HSD were significantly lower ($P < 0.05$) in the HE_L group as compared with the LE_L group on day 55 of pregnancy (Fig. 1). In MS gilts, the expression of SRBI, LDLR and STAR mRNAs were significantly higher ($P < 0.05$) in the LE_M group compared with the HE_M group on day 35 of gestation. However, the luteal genes involved in steroid hormone synthesis were not affected by dietary energy intake on days 55 and 90 of pregnancy with the exception of LDLR ($P < 0.05$) (Fig. 2). The mRNA expression of P_4 synthesis-related proteins had a similar variation as luteal P_4 concentration in both of the breeds.

Table 4
Influence of energy level on corpora lutea number, fetal number and survival, fetal weight and length in Large White and Meishan gilts.[1]

Parameter	Days of pregnancy, d	LW gilts			MS gilts		
		HE_L	LE_L	*P*-value	HE_M	LE_M	*P*-value
Corpora lutea number	35	17.75 ± 1.11	18.75 ± 1.89	0.664	16.33 ± 2.33	15.67 ± 0.88	0.802
	55	17.67 ± 0.33	19.00 ± 0.71	0.191	16.50 ± 0.50	15.50 ± 0.64	0.267
	90	18.00 ± 0.93	19.33 ± 1.20	0.408	16.75 ± 1.11	15.75 ± 0.75	0.483
Number of living fetuses	35	15.00 ± 1.08	13.25 ± 1.31	0.343	13.00 ± 2.00	14.66 ± 1.33	0.526
	55	13.67 ± 0.33	14.25 ± 1.18	0.699	12.25 ± 0.85	12.75 ± 0.95	0.708
	90	12.25 ± 0.48[b]	14.33 ± 0.67[a]	0.047	12.00 ± 0.58	11.75 ± 1.38	0.873
Fetal survival, %[2]	35	84.49 ± 2.42[a]	71.04 ± 3.88[b]	0.026	79.37 ± 0.80[b]	93.28 ± 4.14[a]	0.030
	55	77.34 ± 0.44	74.97 ± 5.69	0.739	74.80 ± 7.18	82.66 ± 7.18	0.469
	90	68.19 ± 1.16[b]	74.30 ± 1.50[a]	0.022	72.47 ± 8.38	75.13 ± 4.78	0.791
Fetal weight, g	35	5.28 ± 0.17	6.00 ± 0.39	0.146	5.09 ± 0.52	4.36 ± 0.24	0.247
	55	92.59 ± 2.21	86.07 ± 3.56	0.195	74.64 ± 2.10[a]	61.58 ± 1.41[b]	0.007
	90	780.03 ± 11.81[a]	647.18 ± 31.12[b]	0.016	574.40 ± 7.98[a]	507.71 ± 24.01[b]	0.039
Fetal length, cm	35	3.72 ± 0.10	4.00 ± 0.10	0.097	3.90 ± 0.13	3.58 ± 0.12	0.113
	55	12.15 ± 0.18	11.85 ± 0.28	0.421	11.15 ± 0.14	10.98 ± 0.28	0.594
	90	24.53 ± 0.18[a]	23.46 ± 0.33[b]	0.030	22.08 ± 0.28	21.82 ± 0.28	0.530

LW = Large White; MS = Meishan.

[a,b] Within a same row, means with different superscripts are significantly different at $P < 0.05$.

[1] Energy levels were varied by supplementing soybean oil and they were 14.23 and 12.56 MJ DE/kg (HE_L and LE_L) for LW gilts and 12.56 and 10.88 MJ DE/kg (HE_M and LE_M) for MS gilts, respectively. $n = 4$ per group of days 35, 55, and 90 of gestation.

[2] Fetal survival is the percentage of the number of corpora lutea represented by all living fetuses.

Table 5
Influence of energy level on serum metabolites and hormone concentrations in Large White and Meishan gilts.[1]

Parameter	Days of pregnancy, d	LW gilts			MS gilts		
		HE_L	LE_L	*P*-value	HE_M	LE_M	*P*-value
Serum P_4, ng/mL	35	14.11 ± 1.79	10.65 ± 0.89	0.113	14.20 ± 1.29	23.77 ± 5.21	0.093
	55	7.03 ± 1.09	9.89 ± 1.06	0.098	12.42 ± 0.60	14.32 ± 1.68	0.499
	90	6.63 ± 0.12	7.91 ± 0.47	0.058	11.30 ± 1.47	13.24 ± 3.52	0.597
Luteal P_4, ng/mL	35	9,829.6 ± 710.50[a]	6,671.3 ± 701.79[b]	0.027	6,704.1 ± 1,045.08[b]	9,628.7 ± 817.44[a]	0.044
	55	3,823.8 ± 254.21	6,263.6 ± 1,192.91	0.092	5,532.3 ± 432.64	7,170.9 ± 1,099.62	0.149
	90	3,654.1 ± 770.02	5,625.7 ± 580.36	0.071	4,354.6 ± 677.46	5,524.1 ± 1,084.76	0.382
HDLC, mmol/mL	35	0.85 ± 0.06	0.86 ± 0.03	0.935	0.76 ± 0.05	0.72 ± 0.02	0.553
	55	0.75 ± 0.04[b]	1.00 ± 0.05[a]	0.002	0.72 ± 0.05[a]	0.56 ± 0.04[b]	0.040
	90	0.87 ± 0.03	0.86 ± 0.03	0.828	0.66 ± 0.03	0.58 ± 0.06	0.237
LDLC, mmol/mL	35	3.91 ± 0.32	3.64 ± 0.24	0.560	4.59 ± 0.22[a]	3.57 ± 0.33[b]	0.043
	55	3.59 ± 0.21	3.30 ± 0.27	0.411	3.32 ± 0.58	3.23 ± 0.38	0.897
	90	2.99 ± 0.13	2.95 ± 0.10	0.836	3.33 ± 0.30	3.57 ± 0.31	0.602

LW = Large White; MS = Meishan; P4 = progesterone; HDLC = high-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol.

[a,b] Within a same row, means with different superscripts are significantly different at $P < 0.05$.

[1] Energy levels were varied by supplementing soybean oil and they were 14.23 and 12.56 MJ DE/kg (HE_L and LE_L) for LW gilts and 12.56 and 10.88 MJ DE/kg (HE_M and LE_M) for MS gilts, respectively. $n = 4$ per group of days 35, 55 and 90 of gestation for luteal P4, $n = 8$ per group of three points of gestation for serum P4, HDLC and LDLR.

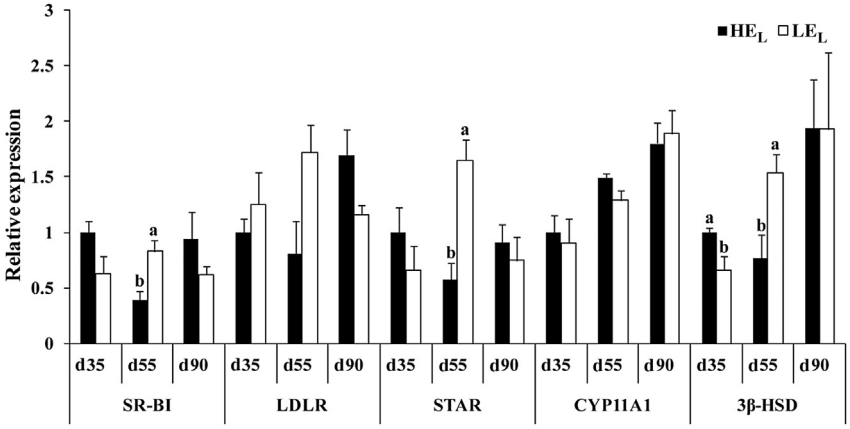

Fig. 1. Relative mRNA expressions of SR-BI, LDLR, STAR, CYP11A1 and 3β-HSD in luteal from different energy intake on days 35, 55 and 90 of gestation in Large White gilts. Energy levels were varied by supplementing soybean oil and they were 14.23 and 12.56 MJ DE/kg (HE_L and LE_L) for LW gilts.[a,b]bars with different letters are significantly different at $P < 0.05$. SR-BI = scavenger receptor-BI; LDLR = low-density lipoprotein receptor; STAR = steroidogenic acute regulatory protein; CYP11A1 = cytochrome P450scc; 3β-HSD = 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase.

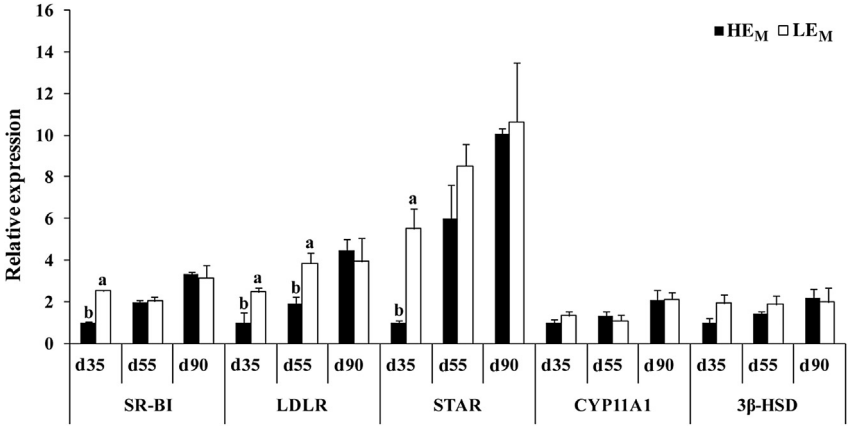

Fig. 2. Relative mRNA expressions of SR-BI, LDLR, STAR, CYP11A1 and 3β-HSD in luteal from different energy intake on days 35, 55 and 90 of gestation in Meishan gilts. Energy levels were varied by supplementing soybean oil and they were 12.56 and 10.88 MJ DE/kg (HE_M and LE_M) for MS gilts.[a,b]Bars with different letters are significantly different at $P < 0.05$. SR-BI = scavenger receptor-BI; LDLR = low-density lipoprotein receptor; STAR = steroidogenic acute regulatory protein; CYP11A1 = cytochrome P450scc; 3β-HSD = 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase.

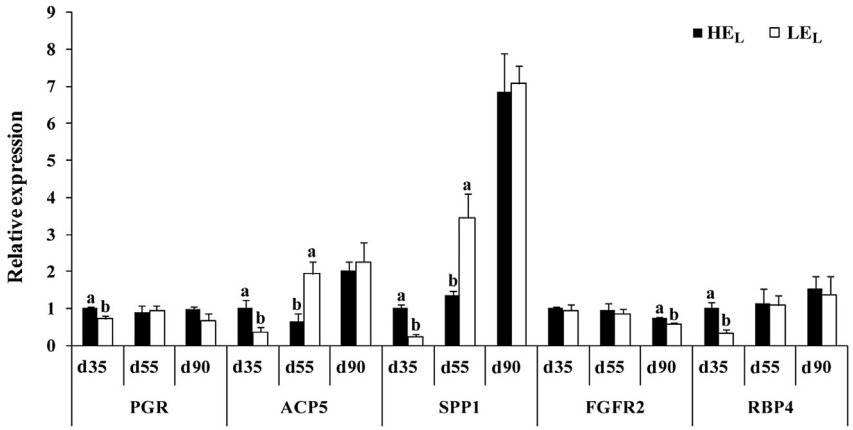

Fig. 3. Relative mRNA expressions of PGR, ACP5, SPP1, FGFR2 and RBP4 in endometrium from different energy intake on days 35, 55 and 90 of gestation in Large White gilts. Energy levels were varied by supplementing soybean oil and they were 14.23 and 12.56 MJ DE/kg (HE$_L$ and LE$_L$) for LW gilts. [a,b]Bars with different letters are significantly different at $P < 0.05$. PGR = progesterone receptor; ACP5 = uteroferrin; SPP1 = secreted phospoprotein 1; FGFR2 = fibroblast growth factor receptor 2; RBP4 = retinol-binding protein 4.

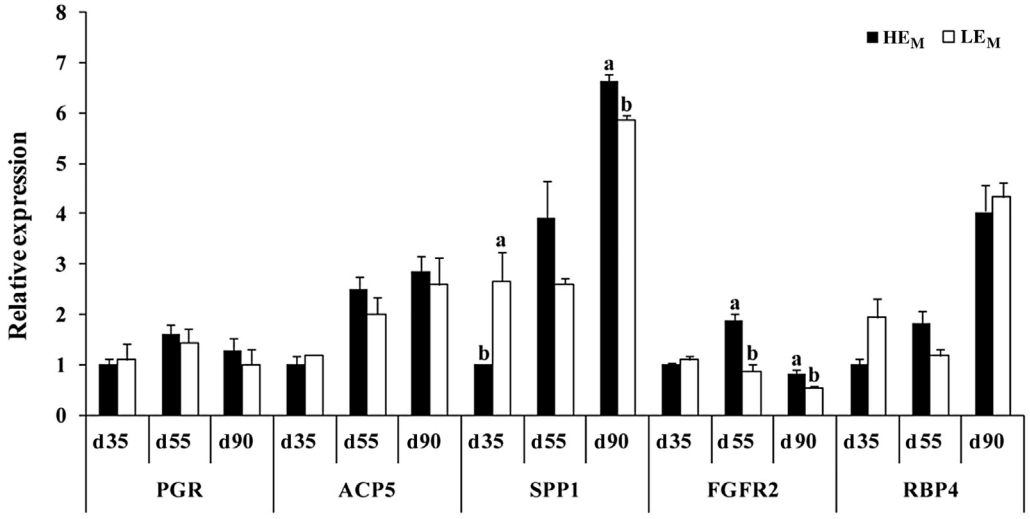

Fig. 4. Relative mRNA expressions of PGR, ACP5, SPP1, FGFR2 and RBP4 in endometrium from different energy intake on days 35, 55 and 90 of gestation in Meishan gilts. Energy levels were varied by supplementing soybean oil and they were 14.23 and 12.56 MJ DE/kg (HE$_L$ and LE$_L$) for LW gilts. [a,b]Bars with different letters are significantly different at $P < 0.05$. PGR = progesterone receptor; ACP5 = uteroferrin; SPP1 = secreted phospoprotein 1; FGFR2 = fibroblast growth factor receptor 2; RBP4 = retinol-binding protein 4.

3.4. The effect of dietary energy intake on gene expression in endometrium

The effect of dietary energy intake on expressions of ACP5, SPP1, FGFR2 and RBP4 mRNA in endometrial tissue was determined on days 35, 55 and 90 of gestation. The transcript expression levels of PGR, ACP5, SPP1 and RBP4 in endometrium were significantly higher ($P < 0.05$) in the HE$_L$ than in the LE$_L$ group on day 35 of gestation in LW gilts. However, on day 55 of gestation, ACP5 and SPP1 expressions were lower ($P < 0.05$) in the HE$_L$ group than in the LE$_L$ group (Fig. 3). For MS gilts, the HE$_M$ group had lower expression of SPP1 mRNA than the LE$_M$ group on day 35 but it was higher on day 90 of gestation ($P < 0.05$). An non-significant increase of RBP4 expression level was observed on day 35 of gestation in the LE$_M$ group of MS gilts ($P = 0.075$) (Fig. 4). The expression of FGFR2 was greater in the HE$_L$ group on day 90 ($P < 0.05$) and in the HE$_M$ group on days 55 and 90 ($P < 0.01$ and $P < 0.05$) of gestation than that in the control groups, respectively.

4. Discussion

Numerous studies have indicated a role for nutrition in the central regulation of fetal survival. Here we demonstrated that in LW gilts, there was a trend towards greater fetal survival on day 35 in the group with higher dietary energy intake but not on day 90 of gestation. However, in MS gilts lower energy intake led to greater fetal survival than higher energy intake at 90 days with no significant difference in mid-gestation for the different diet treatment groups. These results may be dependent on the breed of pig. The LW and MS gilts have different sensitivities to changes in dietary energy intake during gestation, and this may reflect the different strategies employed by the two breeds to achieve optimal breeding.

During the early stages of gestation, independent lines of investigation have demonstrated that progesterone is an important driver of embryonic implantation, survival and development. Some studies of commercial breeds indicated that high feeding levels are linked to decreased embryo survival in gilts (Wu et al., 2009; Jindal et al., 1997) due to increased P_4 metabolic clearance rate, which in turn results in low peripheral P_4 concentrations (Jindal et al., 1997). In contrast with the previous research, our study demonstrated that LW gilts in the HE$_L$ group had a significantly greater fetal survival rate compared with the LE$_L$ group. These results may relate to the supply of progesterone. Athorn et al. (2011) demonstrated that local progesterone supply from the ovaries to the uterus contributes to the probability of embryo

survival. The local supply of progesterone is direct and hence not modulated by hepatic metabolism. Our results showed that an appropriate increase of dietary energy intake had no effect on peripheral P_4 but significantly increased the P_4 concentration in luteal tissue of LW gilts in early pregnancy. Further, studies have shown the positive impact of higher energy intake on activity and development of luteal tissue as well as on steroid hormone secretion (Ying et al., 2013; Athorn et al., 2013). In contrast with the LW gilts, a lower dietary energy level led to a higher luteal P_4 concentration and fetal survival in MS gilts in the early stages of pregnancy. The change of luteal P_4 concentrations in both breeds may be associated with mRNA levels of P_4 synthesis-related proteins in CL. In the current study, SR-BI, LDLR and STAR genes were affected by nutritional treatment in the CL tissues of MS gilts in early gestation suggesting that lower dietary energy intake in MS gilts may promote luteal P_4 secretion, thereby enhancing fetal survival. However, diet only affected the expression of 3β-HSD in LW during early pregnancy. Thus, these results indicated that these pig breeds may utilize different strategies to regulate luteal P_4 secretion. Although it has been shown that MS gilts are different from LW gilts in progesterone section mode (Pickard, 1996), the physiological and molecular mechanisms associated with altered dietary energy intake that contribute to controlling progesterone concentration still need further research. In general, the effects of pig genotype on the efficiency of nutrient use have been widely reported (Noblet et al., 1999; Barea et al., 2011). Comparative studies varying dietary protein levels indicate that a low-protein diet during gestation will not affect reproductive performance of MS gilts (Liu et al., 2011). Furthermore, MS pigs are able to select appropriate pairs of foods to meets their nutritional requirements when given a diet choice with different crude protein content (Kyriazakis et al., 1993). Therefore, we presume that the genotype of MS pigs is geared toward a lower nutrient intake for meeting their genotypes in early of pregnancy. Thus, the modification of dietary energy levels lead to different fetal survival between the two breeds at day 35 of gestation.

The successful establishment and maintenance of pregnancy require the action of P_4 on the uterus to regulate endometrial function (Lonergan et al., 2013). The changes in target gene expression in endometrium further explain the mechanism underlying the different fetal survival rate during early gestation of LW and MS gilts. The RBP4 gene is a strong candidate to litter size in pigs by supplying vitamin A to the developing fetus (Terman et al., 2011). Retinol would improve the uniformity of fetal size and the synchronism of development (Bao et al., 2012) therefore improving the fetal survival rate. Uteroferrin is an iron-containing glycoprotein secreted by uterine GE, which plays a vital role in stimulating hematopoiesis and promoting iron utilization (Nuttleman and Roberts, 1990). A previous report demonstrated that increased erythrocyte numbers in the uterus enhance embryo survival rates (Pearson et al., 1998). Secreted phosphoprotein 1 has been identified to play an important physiological role in placentation, porcine embryo development and survival (Hao et al., 2008). In the current study on LW gilts, the increased expression levels of RBP4, ACP5 and SPP1 in the HE_L group on day 35 of gestation suggest that higher dietary energy intake may promote the secretion of uterine protein, the transportation of vitamin A, iron and calcium, thereby enhancing fetal survival. However, we also observed the differential expression of SPP1 in endometrium in MS gilts with altered energy intake. The increased abundance of SPP1 mRNA expression in the LE_M group in MS gilts was observed in the current study which suggests again that different strategies are employed between the two breeds in response to changes in dietary energy levels.

In the middle of pregnancy, however, a greater fetus number was observed in the LE_L group of LW gilts on day 90 of gestation. This change may be associated with crowding of fetuses in the uterus which ultimately limits fetal development (Sysyn, 2004). Town et al. (2005) suggested a negative relationship between the number of conceptuses and uterus volume. In our results, although the gene expression of FGFR2, a regulator of uterine cell growth, was increased in the HE_L and HE_M groups compared with their respective controls, the uterine length was not significantly different. In addition, fetal growth is strongly influenced by nutrient supply, which is dependent on placental transport functions (Jansson and Powell, 2006). A previous study showed that a high-fat diet during pregnancy causes marked upregulation of placental nutrition transport and fetal overgrowth in mice (Jones et al., 2009). Amdi et al. (2013) also demonstrated that the piglets weight was increased when gilts consumed higher quantities of feed during gestation. Therefore, we presume that higher fetal weight and length in the higher dietary energy intake groups led to the occurrence of uterus crowding in commercial breeds. On the other hand, the lower concentration of CL P_4 in the middle of pregnancy of the HE_L group may contribute to the effects on fetal survival and development. Furthermore, the lower expression of SR-BI, STAR and 3β-HSD mRNA in LW gilts treated with higher energy diet may contribute to the reduced concentration of P_4. However, the decreased P_4 concentration in CL tissue on day 55 of pregnancy may lead to a lower mRNA level of ACP5 and SPP1 in the HE_L group which suggests that high dietary energy levels are helpful for fetal growth but not survival in commercial breeds during middle pregnancy. In contrast, there was no difference in fetal numbers between the two groups of MS gilts during mid-gestation. During mid-gestation, comparison of commercial breeds and MS gilts demonstrated that MS gilts have a greater density of placental blood vessels (Biensen et al., 1998) and within-litter uniformity in fetal weight (Finch et al., 2003). It is noteworthy that the MS gilts uterus may exhibit some type of growth inhibition which limits on fetal size (Biensen et al., 1998). Thus, the MS gilts can ensure the higher effectiveness of placental nutrient transport to achieve prolificacy. Thus, although higher fetal weights were observed in the HE_M group during middle pregnancy, the special uterus and placental functions of MS gilts ensured fetal survival and development. The P_4 concentration did not differ between the two groups of MS gilts during mid-gestation, although a higher LDLR level was observed in the LE_L group on day 55 of pregnancy. This result was associated with the observation that HDLC may be the major source of cholesterol for the CL (Jiménez et al., 2010). Nevertheless, the gene expression levels within the uterus were similar to those observed in early pregnancy in MS gilts, except for SPP1 which was higher in the HE_M than in the LE_M group. This is believed to be because SPP1 plays a key role in the uterus of MS pigs in response to changes in energy levels. These data indicate that dietary intake during middle pregnancy is also important for fetal development and increasing dietary energy level appropriately is helpful to increase MS gilts' fetal weight. However, the mechanism by which breed differences contribute to endometrial protein changes are not known and warrants further study.

5. Conclusion

In conclusion, the LW and MS gilts have different sensitivities to variations in dietary energy intake levels. In LW gilts, the HE_L group could improve fetal survival on day 35 but a sustained supply of high energy diet decreased the fetal number on day 90 of gestation. The LE_M group could increase fetal survival of MS gilts on day 35 of gestation. The variational dietary energy intake levels did not influence the fetal survival on day 90 of gestation but higher energy diet can increase fetal weight of MS gilts. These

results were associated with different luteal secretion activity and endometrial gene expression in two breeds.

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