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Effects of maternal vitamin D_3 during pregnancy on *FASN* and *LIPE* mRNA expression in offspring pigs

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Abstract

In this study, sows were fed 200 (LD), 800 (ND) and 3200 (HD) IU of vitamin D_3/kg basal diet during pregnancy (from 41 d to birth), respectively. All their offspring pigs were fed the same vitamin D_3 replete die. At 150 days of age, a total of 18 offspring pigs (six offspring pigs per maternal diet group, sex balance) were weighed and slaughtered to investigate effects of maternal vitamin D_3 during pregnancy on fatty acids synthase (*FASN*) and hormone-sensitive lipase (*LIPE*) expression in offspring pigs. The results showed that LD offspring pigs had higher *FASN* mRNA expression and the ratio of *FASN/LIPE* mRNA expression in subcutaneous adipose tissue, as well as higher *LIPE* mRNA expression of *longissimus* dorsal muscle, whereas, had lower the ratio of *FASN/LIPE* mRNA expression in *longissimus* dorsal muscle compared with ND or HD offspring pigs, respectively. Meanwhile, LD offspring pigs had higher carcass fat, average backfat thickness (ABFT), serum insulin and leptin levels, lower intramuscular fat (IMF), serum free fatty acid and triglycerol levels compared with ND or HD offspring pigs. In addition, the ratio of *FASN/LIPE* mRNA expression was negatively correlated with IMF content, and positively correlated to carcass fat content and ABFT in offspring pigs. Meanwhile, *FASN* mRNA expression was positively correlated with carcass fat content, while negatively correlated with ABFT in offspring pigs. These results suggested that maternal vitamin D_3 affected fat accumulation and meat quality by regulating *FASN* and *LIPE* mRNA expression in offspring pigs.

Introduction

Intramuscular adipocytes were mainly generated at the foetal and neonatal stages (Tong *et al.*, 2008), they would provide the sites for intramuscular fat (IMF) accumulation that generate marbling at the fattening stage in offspring (Du *et al.*, 2010). Zhu *et al.* (2006) reported that adipose tissue occurred before mid-gestation in many mammals, and maternal malnutrition or over-nutrition affected the overall fat accumulation of offspring. These results suggested that lipid synthesis and degradation in offspring were impacted by maternal nutrition. Previous reports demonstrated that adipose tissue deposition depends on the balance between lipid synthesis and degradation (Qiao *et al.*, 2007; Miao *et al.*, 2010). This process is mainly regulated by fatty acids synthase (*FASN*) and hormone-sensitive lipase (*LIPE*). The *FASN* exerts a vital role in *de novo* lipogenesis of mammals (Smith *et al.*, 2003). Whereas, the *LIPE* plays an important role in hydrolysing triglycerol (TG) to free fatty acids (FFA) in adipose tissue, and regulates the lipolysis of animals (Haemmerle *et al.*, 2003). The ratio of *FASN/LIPE* mRNA expression and *FASN* mRNA expression was positively related to carcass fat content in pigs (Miao *et al.*, 2010). Growing evidence showed that vitamin D_3, the active metabolite of vitamin D, is recognized as a potential regulator of adipogenesis (Wang *et al.*, 2016). There is a negative relationship between obesity (excessive fat accumulation) and vitamin D deficiency (Caron-Jobin *et al.*, 2011; Marcotorchino *et al.*, 2013). In addition, serum vitamin D levels are negatively correlated with body fat content (Boon *et al.*, 2006; Fish *et al.*, 2010), and obese individual had lower serum vitamin D levels (Beckman *et al.*, 2013; Carrelli *et al.*, 2017). These results indicated that adipogenesis was affected by vitamin D status. Previous studies observed that vitamin D_3 inhibited differentiation and adipogenesis of 3T3-L1 preadipocytes by inhibiting peroxisome proliferator-activated receptor-γ (*PPARγ*), CCAAT/enhancer binding protein alpha, fatty acid binding protein 4 and stearoyl-CoA desaturase-1 expression (Ishida *et al.*, 1988; Ji *et al.*, 2015). Dix *et al.* (2018) found that TG accumulation in 3T3-L1 preadipocytes was increased by lower vitamin D_3 dosage. Zhuang *et al.* (2007) also reported that vitamin D_3 inhibited porcine preadipocyte differentiation via reducing the expression of *PPARγ* and retinoid X receptor alpha mRNA. Whereas, vitamin D_3 inhibited proliferation in bone marrow stromal cells from pigs and increased lipid accumulation (Zhuang *et al.*, 2007). Mahajan and Stahl, (2009) also observed that vitamin D_3 enhanced

PPARγ, lipoprotein lipase and adipocyte fatty acid binding protein 2 expression in mature adipocytes from porcine subcutaneous adipose tissue. These results suggested that vitamin D had a stimulatory effect on adipogenesis in mature adipocytes. Although, it is clear that vitamin D_3 is involved in the regulation of fat accumulation and lipid metabolism by regulating adipogenic gene expression, reports about the role of maternal vitamin D_3 during pregnancy in adipogenic genes expression of muscle and adipose tissue from offspring pigs are missing. Because maternal nutrition could affect foetal epigenome by improving the intrauterine environment (Sinclair *et al.*, 2007; Chango and Pogribny, 2015). Therefore, the aim of this present study was to explore the effects of maternal vitamin D_3 during pregnancy on *FASN* and *LIPE* mRNA expression in *longissimus* dorsal muscle and subcutaneous adipose tissue from offspring pigs, and relation with carcass fat, IMF content and average backfat thickness (ABFT).

Material and methods

Experimental design and diets

All animals handing protocols in this study were approved by the Animal Care and Use Committee of Henan Institute of Science and Technology (Xinxiang, P.R. China). A total of nine pregnant sows (41 days of gestation) with the same parities and similar body weights (143.47 ± 2.1 kg) were randomly divided into low vitamin D_3 (LD), normal vitamin D_3 (ND) and high vitamin D_3 (HD) groups, which were fed 200, 800 and 3200 IU of vitamin D_3/kg basal diet, respectively. Each group includes three replicates with 1 sow per replicate. The feeding trials were separated into two stages, including pregnant sows and their offspring pigs in this present study. All diets for pregnant sows and their offspring pigs were formulated to meet or exceed the national research council (NRC 2012) recommendations, and shown in Tables 1–3, respectively. The feeding trial of pregnant sows from 41 days of gestation until birth. From birth, A total of 72 piglets (sex balance) from all their 119 offspring were allotted into three groups again according to their mother fed different vitamin D_3 concentrations. Each group has three replicates with eight offspring piglets (sex balance) per replicate, and all groups fed the same vitamin D_3 replete diet. The feeding trial of their offspring pigs from birth to 150 days of age. A total of 18 offspring pigs (six offspring pigs per maternal diet group, sex balance) were weighed and slaughtered for tissue collection at 150 days of age. During this period all piglets were reared in the same condition, and had *ad libitum* access to an experimental diet and water via nipple drinkers.

Table 1. Gestation diet composition of sow[a]

Ingredient %		Nutrients[b]	
Maize	61.91	DE, MJ/kg	13.03
Wheat bran	16	CP, %	16.45
Soybean	19	Ca, %	0.68
Fish meal	0	Available P, %	0.36
Limestone	1.5	Lys, %	1.04
$CaHPO_4$	0.29	Met, %	0.24
Salt	0.3	Met + Cys, %	0.52
Premix[c]	1		
Total	100		

DE, digestible energy; CP, crude protein; Lys, lysine; Met, methionine; Cys, cystine.
[a]Gestation diet for low vitamin D_3 (LD), normal vitamin D_3 (ND) and high vitamin D_3 (HD) groups from 41 days of age until birth. Their compositions were similar except vitamin D_3 levels.
[b]All data were analysed values except digestible energy, which was calculated using swine National Research Council (NRC) (2012) values.
[c]Provided the following (unit/kg): 10 mg of Cu, 80 mg of Fe, 25 mg of Mn, 100 mg of Zn, 0.2 mg of I and 0.2 mg of Se. A total of 4000 IU of vitamin A, 200 IU of vitamin D_3 (LD group), 800 IU of vitamin D_3 (ND group), 3200 IU of vitamin D_3 (HD group), 44 IU of vitamin E, 1.0 mg of vitamin K_3, 1 mg of vitamin B_1, 3.75 mg of riboflavin, 1 mg of vitamin B_6, 15 mg of vitamin B_{12}, 12 mg of pantothenic acid, 10 mg of niacin and 1.25 mg of choline.

Table 2. Lactation diet composition of sow[a]

Ingredient %		Nutrients[b]	
Maize	68	DE, MJ/kg	13.42
Wheat bran	8.02	CP, %	16.77
Soybean	20	Ca, %	0.70
Fish meal	1	Available P, %	0.36
Limestone	1.5	Lys, %	1.09
$CaHPO_4$	0.18	Met, %	0.27
Salt	0.3	Met + Cys, %	0.54
Premix[c]	1		
Total	100		

DE, digestible energy; CP, crude protein; Lys, lysine; Met, methionine; Cys, cystine.
[a]Lactation diets with the same vitamin D_3 levels were fed lactating sows in low vitamin D_3 (LD), normal vitamin D_3 (ND) and high vitamin D_3 (HD) groups, and their offspring piglets were weaned 28 days of age.
[b]All data were analysed values except digestible energy, which was calculated using swine National Research Council (NRC) (2012) values.
[c]Provided the following (unit/kg): 20 mg of Cu, 80 mg of Fe, 25 mg of Mn, 100 mg of Zn, 0.2 mg of I and 0.2 mg of Se. A total of 2000 IU of vitamin A, 800 IU of vitamin D_3, 44 IU of vitamin E, 1.0 mg of vitamin K_3, 1 mg of vitamin B_1, 3.75 mg of riboflavin, 1 mg of vitamin B_6, 15 mg of vitamin B_{12}, 12 mg of pantothenic acid, 10 mg of niacin and 1 mg of choline.

Slaughter and samples collection

At 150 days of age, 18 offspring pigs (six pigs per group) were selected to weight and slaughter according to the method described by Miao *et al.* (2009). Briefly, the pigs were electrically stunned, exsanguinated, dehaired and eviscerated after fasting 12 h. The head was removed and the carcass was split longitudinally, the subcutaneous adipose tissue and *longissimus* dorsal muscle were quickly dissected and frozen in liquid nitrogen, and then stored at −80°C until extraction for total RNA. Samples of blood were collected from 18 offspring pigs (six pigs each group), and allowed to clot overnight at 4°C. Serum was harvested following centrifugation (3000 *g* for 10 min, at 4°C) and stored at −80°C until analysis (Miao *et al.*, 2008). Left half-carcasses without head, legs and guts (except kidney) were weighed. Adipose and muscle tissue in the left half-carcass was dissected and weighed, the carcass fat content and carcass dressing percentage was calculated. The ABFT was taken in the midline with a sliding caliper, and the average of three backfat thickness, measured on the first rib, last rib and last lumbar vertebrae. The analysis of IMF in the *longissimus* dorsal muscle was measured according to the AOAC (1990) procedures.

Real-time PCR

Total RNA of subcutaneous adipose tissue and *longissimus* dorsal muscle was extracted using TRIzol reagent (Invitrogen, Carlsbad,

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Table 3. Ingredients and nutrients of basal experiment diets of offspring pigs

Item	28–90 days	91–150 days
Ingredient %		
Maize	71.95	76.5
Soybean	24	20
Limestone	0.7	0.9
$CaHPO_4$	1.7	1.2
Lysine	0.25	0.21
Salt	0.4	0.4
Premix[a]	1	1
Total	100	100
Nutrients[b]		
DE, MJ/kg	13.75	13.79
CP, %	17.78	15.65
Ca, %	0.71	0.67
Available P, %	0.42	0.35
Lys, %	0.96	1.11
Met, %	0.27	0.26
Met + Cys, %	0.55	0.52

DE, digestible energy; CP, crude protein; Lys, lysine; Met, methionine; Cys, cystine.
[a]Provided the following (unit/kg): 10 mg of Cu, 80 mg of Fe, 30 mg of Mn, 80 mg of Zn, 0.5 mg of I and 0.3 mg of Se. A total of 5850 IU of vitamin A, 1251 IU of vitamin D_3, 20 IU of vitamin E, 1.86 mg of vitamin K_3, 3 mg of vitamin B_1, 3.6 mg of riboflavin, 1.5 mg of vitamin B_6, 20 mg of vitamin B_{12}, 18 mg of pantothenic acid, 26 mg of niacin and 56 mg of choline.
[b]All data were analysed values except digestible energy, which was calculated using swine National Research Council (NRC) (2012) values.

CA, USA), and then removed DNA via DNase treatment (NEB, Ipswich, MA, USA). Approximately 1 μg of the total RNA in each sample was used to synthesize cDNA by the PrimeScript™ RT Reagent Kit (Takara Bio Inc., Tokyo, Japan). The reverse transcription polymerase chain reaction (RT-PCR) was performed with the ViiA™ 7 real-time PCR System (Applied BioSystems, Foster City, CA, USA) using a SYBR green RT-PCR kit from Bio-Rad (Hercules, CA, USA). Primer sequences were designed according to the basis of known sequences deposited in GenBank (Table 4). Relative expression of mRNA was determined after normalization to β-actin reference using the $2^{-\triangle\triangle Ct}$ method.

Serum biochemistry analysis

Serum 25OHD concentration was determined using an EIA kit (IDS Immunodiagnostic Systems Ltd., Tyne and Wear, UK) according to the previous method described by Wallace *et al.* (2010). Insulin concentrations were measured with the RIA kits (Beijing North Institute of Biotechnology, Beijing, China) in a Gamma-counter (Packard 8500, Packard Instrument Co., Downers Grove, Illinois, USA). Leptin levels were measured with a commercially available kit (Multispecies Radioimmunoassay Kit; Linco Research, St. Charles, MO). Serum FFA and TG concentrations were determined with an enzymatic colorimetric procedure (Nanjing Jiancheng Bioengineering Institute, China) in a UV-visible spectrophotometer (Ultrospec 2000, Sweeden).

Statistical analysis

Statistical analysis of variance (Uhlirova *et al.*) was performed using the one-way ANOVA procedure of SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The *post-hoc* analysis for comparing group means (offspring pigs) was measured by Duncan's multiple range tests, and significance was declared at $P < 0.05$. Adipogenic genes expression analysis (*FASN* and *LIPE*) was performed using REST 2009 software (https://www.gene-quantification.de/rest-2009.html). Linear and quadratic polynomials were performed to study the effect of vitamin D_3 levels. Replicate was used as experimental materials unit for the study of carcass traits, meat quality, serum biochemical indicators and gene expression. Bivariate correlations were used to evaluate the correlation between meat quality (carcass fat, IMF content and ABFT) and adipogenic gene (*FASN* and *LIPE*) expression.

Results

Carcass characteristics and meat quality

The effects of maternal vitamin D_3 during pregnancy on carcass characteristics and meat quality in offspring pigs are shown in Table 5. There were no significant differences in carcass weight ($P = 0.440$) and dressing percentage ($P = 0.910$) among all groups. The offspring pigs born to the LD group had higher carcass fat content (($P = 0.001$) and ABFT ($P = 0.001$)) compared with the ND and HD groups, respectively. Whereas, IMF content of *longissimus* dorsal muscle in offspring pigs born to the LD group was lower than those born to the ND and HD groups, respectively ($P = 0.001$). In addition, no significant differences in carcass fat, ABFT and IMF content were measured between the ND and HD groups.

Serum biochemical index

As shown in Table 6, no significant differences in serum 25OHD concentration were observed among all groups ($P = 0.376$). The offspring pigs born to the LD group had higher concentrations of serum insulin ($P = 0.001$) and leptin ($P = 0.010$) compared with the ND and HD groups, respectively. Whereas, serum FFA ($P = 0.020$) and TG ($P = 0.026$) concentrations of offspring pigs born to the LD group were lower than those born to the ND and HD groups, respectively. Meanwhile, the offspring pigs born to the HD group had lower serum insulin, leptin and higher FFA, TG levels compared with the ND group, respectively.

FASN and LIPE gene expression

As shown in Table 7, *FASN* mRNA expression ($P = 0.009$) and the ratio of *FASN/LIPE* mRNA expression ($P = 0.002$) in subcutaneous adipose tissue of offspring pigs born to the LD group was higher than those born to the ND and HD groups, respectively. Meanwhile, offspring pigs born to the HD group had lower expression of *FASN* and the ratio of *FASN/LIPE* mRNA expression compared with the ND group. Whereas, no differences in *LIPE* expression were observed among all groups ($P = 0.268$).

As shown in Table 8, offspring pigs born to the LD group had lower the ratio of *FASN/LIPE* mRNA expression in *longissimus* dorsal muscle compared with those born to the ND and HD groups, respectively ($P = 0.011$), and the ratio of *FASN/LIPE* mRNA expression in offspring pigs born to the ND group was lower than that born to the HD group ($P = 0.011$). Compared

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Table 4. Primer sequences used for quantitative RT-PCR analyses

Gene name	Accession no.	Primer sequence	Product size, bp
FASN	AY952929	F:5′-CTACGAGGCCATTGTGGACG-3′ R:5′-AGCCTATCATGCTGTAGCCC-3′	148
LIPE	AF141958.1	F:5′-CTGGCGGAGGACAACATGGC-3′ R:5′-AGAAGATGCTGCGGCGGTTG-3′	268
β-Actin	XM_021086047	F:5′-ACCTTCTACAACGAGCTGCGTG-3′ R:5′-GTCTCCGGAGTCCATCACGATG-3′	207

FASN, fatty acid synthase; *LIPE*, hormone-sensitive lipase.

Table 5. Carcass characteristics and meat quality in offspring pigs

	Treatment				Contrast		
Item	LD	ND	HD	S.E.M.	Treatment	Linear	Quadratic
Carcass weight (kg)	66.44	67.05	68.45	0.568	0.440	0.110	0.597
Dressing percentage (%)	70.02	72.67	72.61	0.589	0.910	0.010	0.283
Carcass fat (g/100 g)	26.45	23.21	22.18	0.2227	0.001	0.002	0.281
ABFT (cm)	1.96	1.41	1.34	0.052	0.001	0.001	0.157
IMF (g/100 g)	1.62	1.83	1.87	0.021	0.001	0.001	0.028

LD, low vitamin D_3 group; ND, normal vitamin D_3 group; HD, high vitamin D_3 group; ABFT, average backfat thickness; IMF, intramuscular fat; SEM, standard error of the mean.

Table 6. Serum biochemical index in offspring pigs

	Treatment				Contrast		
Item	LD	ND	HD	S.E.M.	Treatment	Linear	Quadratic
25OHD (ng/ml)	9.68	9.85	9.97	0.343	0.376	0.325	0.773
Insulin (μmol/ml)	16.61	12.48	10.15	0.810	0.001	0.001	0.323
Leptin (ng/ml)	1.62	1.34	1.13	0.072	0.010	0.001	0.711
FFA (μmol/l)	352.60	420.44	443.39	6.018	0.020	0.001	0.231
TG (mmol/l)	1.09	1.34	1.53	0.066	0.026	0.001	0.618

LD, low vitamin D_3 group; ND, normal vitamin D_3 group; HD, high vitamin D_3 group; FFA, free fatty acids; TG, triacylglycerol; SEM, standard error of the mean.

with the LD group, the ND and HD groups had lower expression of *LIPE* mRNA in *longissimus* dorsal muscle ($P = 0.001$). The *FASN* mRNA expression in the ND group was lower than that in the LD and HD groups, respectively ($P = 0.006$). Whereas, no differences in *FASN* mRNA expression between the LD and HD groups, as well as *LIPE* expression between the ND and HD groups, respectively.

Correlation between genes expression and meat quality parameters

As shown in Table 9, the ratio of *FASN/LIPE* mRNA expression in *longissimus* dorsal muscle was negatively correlated with IMF content of offspring pigs ($r = -0.868$, $P = 0.002$). Whereas, there is no relationship between *FASN*, *LIPE* mRNA expression and IMF content. In addition, the *FASN* mRNA expression ($r = 0.843$, $P = 0.021$) and the ratio of *FASN/LIPE* mRNA expression ($r = 0.890$, $P = 0.001$) in subcutaneous adipose tissue were both positively correlated with carcass fat content. Meanwhile, the *FASN* mRNA expression was negatively correlated with the ABFT ($r = -0.746$, $P = 0.021$). Whereas, the ratio of *FASN/LIPE* mRNA expression was positively correlated with ABFT in offspring pigs ($r = 0.795$, $P = 0.010$).

Discussion

Carcass characteristics and meat quality

Previous research showed that production efficiency and meat quality in mammals (including cattle, sheep and pigs) were affected by nutrient fluctuations during the foetal stage (Du *et al.*, 2015). Yang *et al.* (2013) also found that offspring growth performance was impacted by nutrition concentrations during gestation. Our research observed that there were differences in carcass fat content, ABFT and IMF content in offspring pigs born to the LD group compared with the ND group. These results suggested that maternal vitamin D_3 status could improve growth performance, carcass characteristics and meat quality of offspring pigs. These results are in accordance with previous research studies. Belenchia *et al.* (2018) observed that maternal vitamin D concentrations during

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Table 7. *FASN*, *LEPE* and the ratio of *FASN/LIPE* mRNA expression in subcutaneous adipose tissue of offspring pigs at 150 days of age

	Treatment				Contrast		
Gene	LD	ND	HD	S.E.M.	Treatment	Linear	Quadratic
FASN	1.242	0.955	0.638	0.128	0.009	0.003	0.899
LIPE	1.194	1.277	1.352	0.087	0.268	0.119	0.965
FASN/LIPE	1.044	0.745	0.475	0.088	0.002	0.001	0.853

LD, low vitamin D_3 group; ND, normal vitamin D_3 group; HD, high vitamin D_3 group; SEM, Standard error of the mean.

Table 8. *FASN*, *LEPE* and the ratio of *FASN/LIPE* mRNA expression in *longissimus* dorsal muscle tissue of offspring pigs at 150 days of age

	Treatment				Contrast		
Gene	LD	ND	HD	S.E.M.	Treatment	Linear	Quadratic
FASN	1.908	1.348	1.863	0.119	0.006	0.719	0.002
LIPE	2.163	1.187	1.290	0.068	0.001	0.001	0.001
FASN/LIPE	0.884	1.132	1.447	0.088	0.011	0.001	0.673

LD, low vitamin D_3 group; ND, normal vitamin D_3 group; HD, high vitamin D_3 group; SEM, standard error of the mean.

Table 9. Correlations between *FASN* and *LIPE* mRNA expression levels and carcass fat content, ABFT and IMF in offspring pigs

Item	*FASN* mRNA levels	*LEPE* mRNA levels	*FASN/LIPE*
Longissimus dorsal muscle tissue			
IMF	$r = -0.409$, $P = 0.275$	$r = -0.561$, $P = 0.116$	$r = -0.868^*$, $P = 0.002$
Subcutaneous adipose tissue			
Carcass fat content	$r = 0.843^*$, $P = 0.004$	$r = -0.555$, $P = 0.121$	$r = 0.890^{**}$, $P = 0.001$
ABFT	$r = -0.746^*$, $P = 0.021$	$r = -0.475$, $P = 0.196$	$r = 0.795^*$, $P = 0.010$

ABFT, average backfat thickness; IMF = intramuscular fat tissue.

pregnancy have lasting effects on adipose tissue development in offspring mice. Zhou *et al.* (2016) also found that improving maternal vitamin D status promoted postnatal skeletal muscle development of pig offspring. Flohr *et al.* (2016) observed that pigs from sows fed 25(OH)D_3 had higher ADG compared with pigs from sows fed 800 of vitamin D_3, and higher final body weight and hot carcass weight compared with pigs from sows fed 9600 IU of vitamin D_3. These data indicated that maternal vitamin D supplementation affected subsequent growth performance and carcass characteristics in offspring pigs. In addition, offspring pigs born to the LD group had higher carcass fat and ABFT compared with the ND and HD groups, which suggested that maternal vitamin D_3 deficiency could increase adipogenesis in adipose tissue of offspring pigs. The season may be supported that maternal vitamin D_3 inhibited the differentiation of preadipocyte, decreased the number of preadipocytes in foetal, which reduced the fat deposition in offspring pigs. Similar results are reported by Kong and Li (2006), who demonstrated that vitamin D_3 decreased 3T3-L1 preadipocyte differentiation by inhibiting adipogenic genes expression. Wang *et al.* (2017) observed that maternal vitamin A administration expanded PDGFRa$^+$ adipose progenitor population in offspring mice. PDGFRa$^+$ adipose progenitor is differentiated into both beige and white adipocytes (Lee *et al.*, 2012). In this study, we observed that maternal vitamin D_3 increased IMF content in offspring pigs. The reason may be that maternal vitamin D_3 increased PDGFRa$^+$ adipose progenitor population which differentiated into white adipocytes in *longissimus* dorsal muscle, thereby enhanced IMF content in offspring pigs. Certainly, the mechanism underlying still needs to be proved by further investigation.

Serum biochemical index

Serum 25OHD is the liver metabolite and primary circulating form of vitamin D, and used to determine the vitamin D status of pigs (Jakobsen *et al.*, 2007). In this study, no differences in serum vitamin D status were observed among all groups, which indicated that maternal vitamin D_3 didn't change the serum vitamin D concentration in later offspring pigs (at 150 days of age). A similar result was reported by Flohr *et al.* (2016), who observed that maternal vitamin D influenced serum concentration in growing offspring pigs until 35 days post weaning. Flohr *et al.* (2014) demonstrated that serum vitamin D_3 of weaned pigs (21 days of age) was not affected by maternal vitamin D status. These results suggested that maternal vitamin D_3 during pregnancy mainly affected the serum vitamin D levels in early offspring pigs, but had no significant effect on the later offspring pigs. The previous study has shown that insulin suppressed lipolysis of rats by increasing *FASN* and acetyl-COA carboxylase (*ACC*) expression,

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which indicated that lipogenesis is regulated by insulin (Scherer *et al*., 2011). Whereas, leptin could induce lipolysis and inhibit lipogenesis (Buettner and Camacho, 2008). Serum 25OHD decreased leptin concentrations, which was negatively associated with leptin levels (Gangloff *et al*., 2020). Meanwhile, vitamin D deficiency is correlated with elevated insulin resistance, which regulated lipid metabolism process (Leung, 2016). In addition, offspring pigs born to the LD group had higher serum insulin, leptin levels and lower FFA and TG concentrations compared with the ND and HD groups, which indicated that maternal vitamin D_3 status affected adipogenesis in offspring pigs by regulating the levels of serum biochemical parameters related to lipid metabolism. A similar result was reported by Wen *et al*. (2018), who found that maternal vitamin D deficiency increased the adiposity in offspring mice through regulating serum biochemical index concentrations. However, no significant differences in serum insulin and leptin levels in offspring mice were observed between maternal vitamin D deficiency and the control groups (Belenchia *et al*., 2018). Inconsistent research results in serum biochemical parameters might be due to differential species of animals, dosage of vitamin D, duration of feeding, and feeding methods, but the reasons and its mechanism have not been unclear.

FASN and LIPE gene expression

The *FASN* promotes the conversion of acetyl-CoA and malonyl-CoA to TG, which controlling *de novo* lipogenesis of mammals (Semenkovich, 1997). Whereas, *LIPE* mainly catalyses hydrolysis of stored TG in adipose tissue into FFA and glycerol to regulate lipolysis in animals (Haemmerle *et al*., 2003). Fat accumulation is determined by the balance between *FASN* and *LIPE* (the ratio of *FASN/LIPE*) expression, *FASN* mRNA expression and the ratio of *FASN/LIPE* mRNA expression is positively correlated with carcass fat content in pigs (Miao *et al*., 2010). In this study, we observed that maternal vitamin D_3 deficiency increased carcass fat content and ABFT by increasing *FASN* mRNA expression and the ratio of *FASN/LIPE* mRNA expression. These results are in accordance with previous reports, Yao *et al*. (2015) demonstrated that obesity is associated with vitamin D deficiency, and vitamin D suppresses adipogenesis. Whereas, Bhat *et al*. (2014) observed that vitamin D-deficient rats decreased visceral fat content and *FASN* and *PPARγ* expression. Inconsistent research results may be due to differential adipose tissue and species of animals. Meanwhile, our research also found that maternal vitamin D_3 deficiency had lower the ratio of *FASN/LIPE* expression in *longissimus* dorsal muscle, and these results are in accordance with lower IMF content. The reason may be that maternal vitamin D_3 deficiency suppressed $PDGFRa^+$ adipose progenitor population which differentiation into white adipocyte in *longissimus* dorsal muscle, further decreased IMF of offspring pigs by decreasing the ratio of *FASN/LIPE* mRNA expression. So, the ratio of *FASN/LIPE* mRNA expression was opposite between the *longissimus* dorsal muscle tissue and subcutaneous adipose tissue with the same VD_3 diet in this study. Certainly, the mechanism underlying still needs to be proved by further investigation.

Taken together, these results indicated that maternal vitamin D_3 status could change adipose tissue metabolism, carcass characteristics and meat quality in offspring pigs by regulating gene expression involved in lipid accumulation. Whereas, the mechanism and signal pathway underlying still needs to be proved by further investigation.

Correlation between genes expression and meat quality parameters

Fat accumulation in adipose tissue is associated with *FASN* mRNA expression (Huang *et al*., 2008). Backfat thickness, a good indicator of fat deposition, is closely correlated with carcass fat and IMF of pigs (Suzuki *et al*., 2009). In this present experiment, we found that the ratio of *FASN/LIPE* mRNA expression in adipose tissue was positively correlated with carcass fat content and ABFT, which suggested that carcass content and ABFT in pigs were affected by the ratio of *FASN/LIPE* mRNA expression. Similar results were reported by Miao *et al*. (2010), who demonstrated that there was a positive relationship between the ratio of *FASN/LIPE* mRNA expression and carcass fat in pigs. Meanwhile, *FASN* mRNA expression was negatively correlated with AFBT, whereas, there was a positive correlation between the ratio of *FASN/LIPE* mRNA expression and AFBT. These results confirmed that fat accumulation in subcutaneous adipose tissue of pigs was a balance between *FASN* and *LIPE* expression levels, and fat deposition was increased when *FASN* expression was higher than *LIPE* expression. In addition, our study also observed that the ratio of *FASN/LIPE* mRNA expression in *longissimus* dorsal muscle was negatively correlated with IMF content in offspring pigs. Similar results were reported by Qiao *et al*. (2007), who found that there was a negative relationship between the ratio of *FASN/LIPE* expression and IMF content in Kazak sheep. However, other study reported that the ratio of *FASN/LIPE* expression was positively correlated with IMF content in Sutai pigs (Chen *et al*., 2004). Inconsistent research results may be due to the different patterns of IMF storage or breeds (Barber *et al*., 2000). Taken together, our results in the present study indicated that maternal vitamin D_3 status regulated adipogenic genes expression in IMF, whereas, didn't alter the relationship between the ratio of *FASN/LIPE* expression and carcass content, ABFT, as well as IMF content in offspring pigs.

Conclusion

LD offspring pigs had higher carcass fat and ABFT, serum levels of insulin and leptin, *FASN* and *FASN/LIPE* mRNA expression in subcutaneous adipose tissue, *LIPE* mRNA expression of *longissimus* dorsal muscle, whereas, had lower IMF, serum FFA and TG levels, *FASN/LIPE* mRNA expression in *longissimus* dorsal muscle compared with ND and HD offspring pigs. Meanwhile, the ratio of *FASN/LIPE* mRNA expression was negatively correlated with IMF content, and positively correlated with carcass fat content, as well as ABFT in offspring pigs. In addition, *FASN* mRNA expression was positively correlated with carcass fat content, and negatively correlated with ABFT in offspring pigs. These results suggested that maternal vitamin D_3 status during pregnancy has a long-lasting impact on lipid accumulation in offspring pigs.

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Conflict of interest. None.

Ethical standards. All experimental animal procedures were approved by the Animal Care and Use Committee of Henan Institute of Science and Technology.

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