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Effects of maternal vitamin D_3 concentration during pregnancy on adipogenic genes expression and serum biochemical index in offspring piglets

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ABSTRACT. The aim of the present study was to investigate the effects of different vitamin D_3 concentrations in sows during pregnancy on adipogenic genes expression in *musculus longissimus dorsi* tissue and serum biochemical parameters in their piglets. In total 27 pregnant sows (41st day of pregnancy) were randomly divided into low vitamin D_3 (LD), normal vitamin D_3 (ND) and high vitamin D_3 (HD) groups with 9 sows in each, and maintained on these diets throughout pregnancy till giving birth. Animals in LD, ND and HD groups were fed 200, 800 and 3200 IU of vitamin D_3/kg basal diet, respectively. From parturition to weaning (28 days of piglet life), all lactating sows were fed ND diet. At 28-day of life, 6 offspring pigs with similar body weight in each group (2 offspring pigs per replicate, sex balance) were weighed and slaughtered to investigate adipogenic genes expression and serum biochemical index. It was shown that piglets born in LD group had higher *PPARG* expression, serum IGF-I, FT3 and FT4 concentrations, while lower *VDR* and *ZFP423* expressions, serum leptin, 25(OH)D and insulin levels than those born in ND and HD groups ($P < 0.05$). Meanwhile, *FAS* expression and *FAS/HSL* ratio in piglets born in HD group were higher than in those born in LD and ND groups ($P < 0.05$). So, it is suggested that maternal vitamin D_3 concentration influenced adipocyte commitment and differentiation of *musculus longissimus dorsi* in piglets by altering adipogenic genes expression and serum biochemical parameters concentration.

Introduction

Adipocytes derive from multipotent mesodermal stem cells (MSCs) which could differentiate into preadipocytes, and then preadipocytes further develop into mature adipocytes (Dix et al., 2018). Intramuscular adipocytes are mainly generated at the foetal and neonatal stages (Tong et al., 2008) and can provide the sites for intramuscular fat (IMF) accumulation that generates marbling at fattening stage in offspring (Du et al., 2010). Therefore, foetal developmental stage has a crucial effect on the number of adipocytes in offspring. Enhancing adipogenesis in foetal muscle tissue could increase the amount of intramuscular adipocytes and improve meat quality (Du et al., 2015). Growing evidences suggested that maternal nutrients fluctuations during gestation impacted foetal development, growth performance and meat quality in offspring (Chango and Pogribny, 2015; Du et al., 2015). Meanwhile, adipogenesis is separated into commitment and differentiation of adipocyte, which is regulated by genetic, nutrition and

environmental factors (Harper and Pethick, 2004). Zinc finger protein 423 (ZFP423) plays an important regulatory function during the commitment stage, while peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) have crucial regulatory roles during the differentiation period of adipocytes (Wang et al., 2016). It was previously shown that vitamin D_3 is recognized as a potential regulator of adipogenesis (Wang et al., 2016), and vitamin D_3 could affect adipogenesis of 3T3-L1 cells by altering lipoprotein lipase (*LPL)* and fatty acid synthase (*FAS*) mRNA levels (Kong and Li, 2006). In addition, vitamin D_3 could inhibit differentiation and adipogenesis of 3T3-L1 preadipocytes by decreasing *PPARG*, *CEBPA*, fatty acid binding protein 4 (*FABP4*) and stearoyl-CoA desaturase-1 (*SCD1*) expressions (Ji et al., 2015). It also inhibited porcine preadipocyte differentiation by decreasing the expression of *PPARG* and retinoid X receptor α (*RXRA*) mRNA (Zhuang et al., 2007). These data indicated that vitamin D_3 modulated adipogenesis and lipid metabolism in animals by altering adipogenic genes expression. Meanwhile, hormone and growth factors could affect lipid metabolism and adipogeneis process in animals (Miao et al., 2008). Circulating serum leptin levels correlate strongly with fat deposition (Hynes and Jones, 2001), serum insulin and leptin levels increased LPL activity and glucose incorporation into fatty acids in adipose tissue, while insulin-like growth factor I (IGF-I), free triiodothyronine (FT3) and free thyroxine (FT4) could inhibit fat synthesis through reducing lipogenic enzyme activities in pigs (Miao et al., 2008).

Although, it is clear that the vitamin D_3 is involved in the regulation of differentiation and adipogenesis of adipocytes, studies on the role of maternal vitamin D_3 concentration during pregnancy on adipogenesis and serum biochemical index in piglets are missing. Hence, the aim of the present study was to explore the effects of maternal vitamin D_3 during pregnancy on adipogenic gene expression in *musculus longissimus dorsi* and serum biochemical index in piglets. These data would provide the theory basis for the regulation of adipose accumulation and pork quality in offspring pigs by vitamin D_3 nutrition during foetal period.

Material and methods

Animals and diets

All pig handling protocols in this study were approved by the Animal Care and Use Committee of Henan Institute Science and Technology (Xinxiang, China).

In total 27 pregnant sows (41st day of pregnancy) with the same parities and similar body weights (144.6 ± 2.3 kg) were randomly divided into low vitamin D_3 (LD), normal vitamin D_3 (ND) and high vitamin D_3 (HD) groups fed 200, 800 and 3200 IU of vitamin D_3/kg basal diet, respectively. In each group were 3 replicates with 3 sows per replicate. Animals were maintained on these diets throughout pregnancy until giving birth. During days 41–110 of gestation, sows were housed in gestation stalls (2.10 × 0.55 m), and fed 2.2 kg/day of the gestation diet per sow. On day 110, sows were transported to the farrowing house and were housed in farrowing crates (2.20 × 0.60 m for sow, and 2.20 × 1.2 m for piglets). Each farrowing crate was equipped with a single feeder and a nipple water. From parturition to weaning (28 days of piglet life), all lactating sows were switched to the ND diet. During the first 7 days after farrowing, the amount of feed was gradually increased according to sow appetite. After day 7, all sows were allowed *ad libitum* access to the lactation diet and water *via* nipple drinkers. After farrowing, all piglets obtained only breast milk.

Gestation and lactation diets were formulated to meet or exceed the National Research Council (NRC, 2012) recommendations, and are shown in Table 1 and Table 2, respectively.

Table 1. Composition of gestation diet fed to sows[1]

Ingredients, %		Nutrients[3]	
Maize	61.91	Digestible energy, MJ/kg	13.03
Wheat bran	16	Crude protein, %	16.45
Soybean meal	19	Ca, %	0.68
Fish meal	0	Available P, %	0.36
Limestone	1.5	Lysine, %	1.04
$CaHPO_4$	0.29	Methionine, %	0.24
Salt	0.3	Methionine + cysteine, %	0.52
Premix[2]	1		
Total	100		

[1] all animals received the same gestation diet from 41 days of age until giving birth, the difference was only in the vitamin D_3 concentrations: low vitamin D_3 (LD) group received 200 IU of vitamin D_3/kg basal diet, normal vitamin D_3 (ND) group – 800 IU of vitamin D_3/kg basal diet and high vitamin D_3 (HD) group – 3200 IU of vitamin D_3/kg basal diet; [2] per kg: mg: Cu 10, Fe 80, Mn 25, Zn 100, I 0.2, Se 0.2; IU: vit. A 4000, vit. D_3 200 (LD group), vit. D_3 800 (ND group), vit. D_3 3200 (HD group), vit. E 44; mg: vit. K_3 1, vit. B_1 1, riboflavin 3.75, vit. B_6 1, vit. B_{12} 15, pantothenic acid 12, niacin 10, choline 1.25; [3] all data were analysed values except digestible energy, which was calculated using swine National Research Council (NRC, 2012) values

Table 2. Composition of lactation diet fed to sows[1]

Ingredients, %		Nutrients[3]	
Maize	68	Digestible energy, MJ/kg	13.42
Wheat bran	8.02	Crude protein, %	16.77
Soybean meal	20	Ca, %	0.70
Fish meal	1	Available P, %	0.36
Limestone	1.5	Lysine, %	1.09
$CaHPO_4$	0.18	Methionine, %	0.27
Salt	0.3	Methionine + cysteine, %	0.54
Premix[2]	1		
Total	100		

[1] lactation diet was fed to all groups of animals; [2] per kg: mg: Cu 20, Fe 80, Mn 25, Zn 100, I 0.2, Se 0.2; IU: vit. A 2000, vit. D_3 800, vit. E 44; mg: vit. K_3 1, vit. B_1 1, riboflavin 3.75, vit. B_6 1, vit. B_{12} 15, pantothenic acid 12, niacin 10, choline 1.25; [3] all data were analysed values except digestible energy, which was calculated using swine National Research Council (NRC, 2012) values

Slaughter and samples collection

From birth, 72 piglets (sex balance) from all 348 offspring were allotted to 3 groups in accordance with their mother's gestational group. Each group consisted of 3 replicates with 8 offspring (sex balance) per replicate. Briefly, at birth, all piglets in each group (from 9 litters) were weighed individually and ear-tagged for identification. From each group 24 piglets were randomly selected (sex balance) according to the similar average birth weight, and the selected piglets (possibly from different litters) still stayed in their original litter until weaning. At their predesignated slaughter age (28 days of life), 6 offspring with similar average body weight in each group (2 offspring per replicate, sex balance) were randomly selected to weigh and slaughter for tissue collection according to the method described by Miao et al. (2009). The piglets were electrically stunned, exsanguinated, dehaired and eviscerated after fasting for 12 h. The head was removed and the carcass was split longitudinally, the *musculus longissimus dorsi* was 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 (6 pigs from each group according to average body weight), and allowed to cool off overnight at 4 °C. Serum samples were harvested following centrifugation (3 000 *g* for 10 min, at 4 °C) and stored at −80 °C until analysis.

Real-Time PCR

Total RNA of *musculus longissimus dorsi* was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and the DNA was removed *via* DNase treatment (NEB, Ipswich, MA, USA). Approximately 1 μg of the total RNA from each sample was used to synthesize cDNA by PrimeScript™ RT Reagent Kit (Takara Bio Inc., Tokyo, Japan). RT-PCR was performed with 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 3). Relative expressions of *ZFP423*, *VDR*, *PPARG*, *RXRA*,

Table 3. Primer sequences used for Real-Time PCR

Gene	Accession no.	Primer sequence	Size, bp
VDR	NM_001097414.1	F: 5'-CGGCAGCCAGCACTTCCTTAC-3'	211
		R: 5'-CGGCGGTTGTCCTTGGTGATG-3'	
PPARG	NM_214379.1	F: 5'-GACTCAGCTGTACAACAAACCTC-3'	185
		R: 5'-GACAGTTAAGATCGCACCTATC-3'	
FABP4	NM_001002817.1	F: 5'-GAAAGAAGTGGGAGTGGGCTTT-3'	212
		R: 5'-GGGCGCCTCCATCTAAGGTTAT-3'	
ZFP423	XM_0021094223.1	F: 5'-CACCTGACCGTGCACTACAT-3'	128
		R: 5'-CAGTGGTACAGCACGAAGGT-3'	
HSL	NM_214315.3	F: 5'-CTGGCGGAGGACAACATGGC-3'	268
		R: 5'-AGAAGATGCTGCGGCGGTTG-3'	
FAS	NM_001099930.1	F: 5'-CTACGAGGCCATTGTGGACG-3'	146
		R: 5'-AGCCTATCATGCTGTAGCCC-3'	
RXRA	XM_021071446.1	F: 5'-ACGAGGACATGCCGGTGGAG-3'	273
		R: 5'-TGGAGCGGTGCGAGAAGGAG-3'	
ACTB	XM_021086047.1	F: 5'-ACCTTCTACAACGAGCTGCGTG-3'	207
		R: 5'-GTCTCCGGAGTCCATCACGATG-3'	

VDR − vitamin D receptor, *PPARG* − peroxisome proliferator-activated receptor γ, *FABP4* − fatty acid binding protein 4, *ZFP423* − zinc finger protein 423, *HSL* − hormone-sensitive lipase, *FAS* − fatty acid synthase, *RXRA* − retinoid X receptor α, *ACTB* − β-actin

HSL, *FAS* and *FABP4* mRNA were determined after normalization to *β*-actin (*ACTB*) reference using $2^{-\Delta\Delta Ct}$ method.

Serum biochemistry analysis

Serum leptin levels were measured with a commercially available kit (Multispecies Radioimmunoassay Kit; Linco Research, St. Charles, MO, USA). Insulin, insulin-like growth factor I (IGF-I), free triiodothyronine (FT3) and free thyroxine (FT4) 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, IL, USA). Serum 25(OH)D concentration was determined using an EIA kit (IDS Immunodiagnostic Systems Ltd., Tyne and Wear, UK) according to pervious method described by Wallace et al. (2010).

Statistical analysis

Statistical analyses of variance (ANOVA) were performed using the one-way ANOVA procedure of SPSS software ver. 17.0 (2008; SPSS Inc., Chicago, IL, USA). Significant differences among all treatment means were measured at $P < 0.05$ by Duncan's multiple range tests. All data were presented as means ± SEM (standard errors of the mean).

Results

Gene expression

As shwon in Figure 1A, *PPARG* expression in *musculus longissimus dorsi* of piglets born in the LD group was significantly higher than of those born in ND and HD groups ($P < 0.05$).Whereas, *VDR* expression in piglets born in the LD group was lower than in those born in ND and HD groups ($P < 0.05$). No differences in *PPARG* and *VDR* expressions between ND and HD groups ($P > 0.05$) were observed. In additioin, *ZFP423* experssion in piglets born in the ND group was higher than in those born in the LD group, whereas lower than in those born in the HD group ($P < 0.05$). There were no differences in *RXRA* expression among all groups (LD, ND and HD groups, $P > 0.05$).

There were noted no differences in *HSL* and *FABP4* expressions in offspring piglets (Figure 1B) among all groups ($P > 0.05$). Whereas, *FAS* expression and *FAS/HSL* ratio in piglets born in the HD group were higher than in those born in LD and ND groups ($P < 0.05$); no differences were observed between LD and ND groups ($P > 0.05$).

Blood biochemical index

As shown in Table 4, serum leptin and 25(OH)D concentrations in piglets born in the ND group were higher than in those born in LD group, and lower than in those born in HD group ($P < 0.05$). Serum IGF-I concentration in the ND group was lower than that in the LD group, and higher than that in the HD group ($P < 0.05$). In addition, serum FT3 and FT4 levels in the LD group were higher than those in ND and HD groups, while insulin concentration was lower than in ND and HD groups ($P < 0.05$).

Table 4. Effects of maternal vitamin D_3 on serum hormonal and biochemical indices in offspring piglets

Indices	Groups			SEM	*P*-value
	LD	ND	HD		
Leptin, ng/ml	0.56^{c}	0.74^{b}	0.95^{a}	0.080	0.001
IGF-I, ng/ml	41.79^{a}	34.51^{b}	24.89^{c}	0.975	0.001
FT3, fmol/ml	0.74^{a}	0.56^{b}	0.45^{b}	0.070	0.004
FT4, fmol/ml	5.03^{a}	4.26^{b}	3.60^{b}	0.427	0.015
25(OH)D, nmol/ml	13.44^{c}	15.52^{b}	17.13^{a}	0.569	0.015
Insulin, µmol/ml	6.63^{b}	7.88^{a}	8.19^{a}	0.574	0.007

^{a,b} – means with different superscripts in the same row are significantly different at $P < 0.05$; IGF-I – insulin-like growth factor I, FT3 – free triiodothyronine, FT4 – free thyroxine

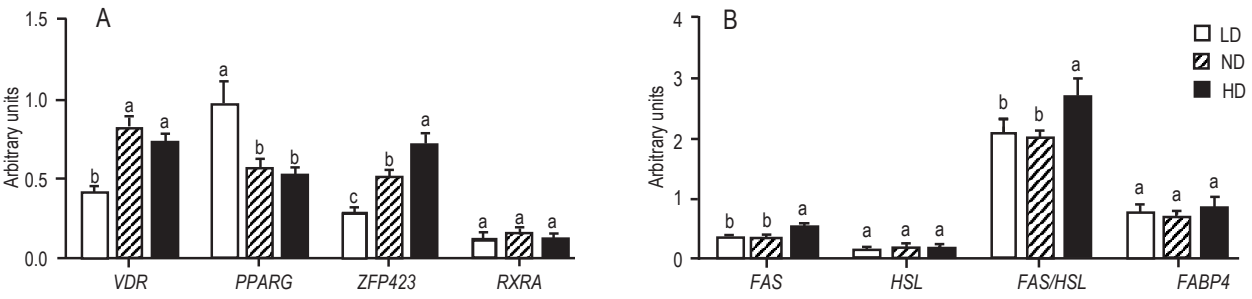

Figure 1. A) *VDR*, *PPARG*, *ZFP423* and *RXRA* mRNA expressions in *musculus longissimus dorsi* of piglets born in LD, ND and HD groups; a,b,c – bars with different superscripts for each gene seperatly are significantly different at $P < 0.05$; *VDR* – vitamin D – receptor, *PPARG* – peroxisome proliferator-activated receptor γ, *ZFP423* – zinc finger protein 423, *RXRA* – retinoid X receptor α, LD – low vitamin D_3 group, ND – normal vitamin D_3 group, HD – high vitamin D_3 group; B) *FAS*, *HSL* and *FABP4* mRNA expressions and *FAS/HSL* ratio in *musculus longissimus dorsi* of offspring born in LD, ND and HD groups; a,b,c – bars with different superscripts for each gene seperatly are significantly different at $P < 0.05$; FAS – fatty acid synthase, HSL – hormone-sensitive lipase, *FABP4* – fatty acid binding protein 4, LD – low vitamin D_3 group, ND – normal vitamin D_3 group, HD – high vitamin D_3 group

No differences were observed in FT3, FT4 and insulin concentrations between ND and HD groups ($P > 0.05$).

Carcass characteristics and meat quality

Data concerning carcass characteristics and meat quality is part of another article that is prepared for publication. In brief, LD offspring pigs had higher carcass fat, average backfat thickness and lower intramuscular fat in comparison with ND or HD offspring.

Discussion

The aim of this research was to explore the impact of maternal vitamin D_3 concentration (excess or deficiency) during pregnancy on adipogenesis genes expression and serum biochemical index in offspring piglets. It was found that there were differences in expressions of *PPARG*, *VDR*, *ZFP423* and *FAS, FAS/HSL* ratio, and serum IGF-I, FT3, FT4, leptin, 25(OH)D and insulin concentrations in piglets. These results suggested that maternal vitamin D_3 during pregnancy had lasting effects on factors regulating adipogenesis in their piglets. The process of adipogenesis is mediated by many transcription factors, such as ZFP234, PPARγ, C/EBPα, FABP4, SCD-1 and so on (Hausman et al., 2009). In a previous research it was shown that vitamin D_3 is recognized as a potential regulator of adipogenesis (Wang et al., 2016), and inhibits differentiation and adipogenesis of 3T3-L1 preadipocytes by decreasing *PPARG, CEBPA*, *FABP4* and *SCD1* expressions (Ji et al., 2015). In *in vitro* studies it was also observed that vitamin D_3 inhibited porcine preadipocyte differentiation by decreasing *PPARG* and *RXRA* expressions (Zhuang et al., 2007). PPARγ is the critical regulator of adipogenesis, which can stimulate it (Belenchia et al., 2018). In the present study, in the group of animals with maternal vitamin D_3 deficiency (LD group) increased *PPARG* expression of *musculus longissimus dorsi* in offspring was noted. So, it can be indicated that maternal vitamin D_3 status can influence adipogenesis in *musculus longissimus dorsi* of piglets by alternating *PPARG* mRNA expression. Similar results were observed by Belenchia et al. (2018), who found that vitamin D deficient-exposed offspring mice had greater *PPARG* expression in perigonadal white adipose tissue.

VDR has an inhibitory effect on adipogenesis. As it was previously shown, *VDR* expression in 3T3-L1 cells inhibited *PPARG* mRNA levels and decreased adipogenesis, which suggested that VDR inhibited adipogenesis *via* decreasing *PPARG* expression (Kong and Li, 2006; Ji et al., 2015). In the present study, maternal vitamin D_3 deficiency decreased *VDR* mRNA expression in piglets. Therefore it can be indicated that maternal vitamin D_3 deficiency increased *PPARG* by decreasing *VDR* expression, and further enhanced adipogenesis (Kong and Li, 2006). The molecular mechanism of inhibitory effect of *VDR* on adipogenesis may be due to RXR, which is a heterodimeric partner for both PPARγ and VDR, and the fact that VDR competes RXR with PPARγ to decrease adipogenesis (Ji et al., 2015; Wang et al., 2016). Inconsistent results were reported by Belenchia et al. (2018) who observed that vitamin D_3 deficient-exposed offspring mice had higher *VDR* expression. These dissimmilarities may be due to different species, tissue, as well as duration of feeding vitamin D_3.

ZFP423 is identified as a key transcriptional initiator of adipogenic differentiation in adipose tissue which promotes adipogenic commitment and adipogenesis (Gupta et al., 2010). It was previously found that vitamin A administration at birth could increase *ZFP423* expression, and promote intramuscular fat development in Angus beef cattle (Harris et al., 2018). In addition, maternal vitamin A administration expanded $PDGFa^+$ adipose progenitor population in progeny, and the $PDGFa^+$ progenitors can further differentiate into white adipocytes, and promote adipogenesis (Wang et al., 2017). In the current study, maternal vitamin D_3 supplementation increased the *ZFP423* expression in longissimus dorsal muscle of piglets. These results indicated that maternal nutrition could affect foetal epigenome by improving the intrauterine environment (Chango and Pogribny, 2015), and maternal vitamin D_3 could promote adipogenic commitment of progenitor cells in *musculus longissimus dorsi* by increasing *ZFP432* expression in offspring piglets. The molecular mechanism might be due to maternal vitamin D_3 increased *ZFP423* expression which increases $PDGFa^+$ progenitor population in *musculus longissimus dorsi* of piglets, and further promotes the numbers of intramuscular adipocytes.

Meanwhile, adipose tissue deposition depends on the balance between lipid synthesis and degradation (Qiao et al., 2007; Miao et al., 2010). This process is regulated by *FAS* and *HSL* expressions. In this study, *FAS* expression and *FAS/HSL* ratio in piglets born in the HD group were higher than in those born in ND and LD groups. Similar results were reported by Vu et al. (1996) who observed that vitamin D_3 promoted adipocyte differentiation in

3T3-L1 cells and induced *FAS* expression. In other research it was noted that vitamin D_3 stimulated *FAS* expression in primary human adipocytes and adipose organ cultures (Menendez et al., 2001). These data suggested that maternal vitamin D_3 could increase lipid accumulation in *musculus longissimus dorsi* of piglets by enhancing *FAS* expression and *FAS/HSL* ratio. The one of possible reason may be that maternal vitamin D_3 increased adipogenic gene expression by enhancing $PDGFRa^+$ adipose progenitor population (Wang et al., 2017). Certainly, the underlying molecular regulatory mechanism still needs to be proved by further investigation.

Leptin is an important regulator of energy homeostasis in mammals, and adipocytes can produce and secrete leptin (Salmeron et al., 2015). Circulating leptin levels are strongly correlated with adipose tissue stores (Hynes and Jones, 2001). In this study, serum leptin concentration in piglets born in the HD group was higher than in those born in ND and LD groups. The data suggested that high levels of maternal vitamin D_3 supplementation probably increase the adipose accumulation in *musculus longissimus dorsi,* and then further enhance serum leptin levels of offspring. In mice, maternal vitamin D_3 deficiency did not affect serum leptin in offspring mice (Belenchia et al., 2018). Inconsistent results concerning serum leptin concentrations may be due to differential species and dosage of vitamin D_3.

IGF-I, T3 and T4 can increase basal energy expenditure through altering lipid metabolism pathway (Miao et al., 2008). In this study, it was observed that serum IGF-I, FT3 and FT4 levels in offspring piglets born in the LD group were higher than in those born in ND and HD groups, which indicated that maternal vitamin D_3 deficiency increased basal energy expenditure and lipolysis in *musculus longissimus dorsi* of piglets. Insulin is able to increase glycogen synthesis and fat deposition, while decrease gluconeogenesis. In the present study, serum insulin levels in offspring piglets born in the LD group were lower than in those born in ND and HD groups, which indicated that maternal vitamin D_3 deficiency decreased lipid synthesis in *musculus longissimus dorsi* of piglets *via* decreasing serum insulin levels. No differences in serum insulin levels between offspring mice born in maternal vitamin D_3 deficiency group and those born in the control groups (Belenchia et al., 2018). Inconsistent research results concerning serum insulin might result from differential species, tissue and dosage of vitamin D_3, but the reasons and its mechanism have not been unclear.

Serum 25(OH)D concentration usually reflects the vitamin D_3 status of pigs (Jakobsen et al., 2007). In this present study, serum 25(OH)D concentration in piglets born in the LD group was lower than in those born in ND and HD groups, which indicated that maternal vitamin D_3 deficiency decreased the serum 25(OH)D concentration in early piglets (at 28 day of life). Similar result was reported by Flohr et al. (2016) who observed that maternal dietary vitamin D_3 influenced serum 25(OH)D levels in piglets (early after weaning). In other reports it was shown that enhancing maternal vitamin D_3 supplementation increased serum 25(OH)D in offspring (Flohr et al., 2014). Maternal vitamin D_3 deficiency groups decreased serum 25(OH)D concentration in offspring mice (Wen et al., 2018). Whereas, there were no differences in serum 25(OH)D levels in offspring mice born in vitamin D_3 deficient and normal groups. Different species of animals used in the experiments may influence the final results. Taken together, maternal vitamin D_3 could also affect adipogenesis in *musculus longissimus dorsi* of piglets by altering serum biochemical parameters. Certainly, its underlying mechanism still needs to be identified by further investigation.

Conclusions

It was demonstrated that vitamin D_3 status in sows influenced adipocyte commitment and differentiation of *musculus longissimus dorsi* in their piglets by altering mRNA expressions of *PPARG*, *VDR*, *ZFP423* and *FAS*, *FAS/HSL* ratio, and serum IGF-I, FT3, FT4, leptin, 25(OH)D and insulin concentrations.

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