Maternal cholecalciferol supplementation during gestation improves antioxidant capacities in gilts and piglets

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ABSTRACT

Various studies have evaluated the relationship between cholecalciferol (vitamin D3) and reproductive performance. This study aimed at investigating the effects of maternal D3 supplementation during gestation on reproductive performance and antioxidant capacities of gilts and their offsprings. Twenty-three Landrace × Yorkshire gilts were randomly allocated into two groups and fed on one of the following two diets during gestation: control diet or D3 supplemented diet. It was found that D3 supplementation had a tendency to increase the number of total born and born alive piglets. Moreover, it elevated serum 25-hydroxycholecalciferol concentrations in gilts and newborn piglets. Besides, D3 supplementation improved the activities of antioxidant enzymes (GSH-Px, T-AOC and T-SOD) in the blood of gilts, umbilical cord and newborn piglets' liver, while serum malondialdehyde concentrations of gilts and umbilical cord were reduced in the D3 group. In addition, D3 supplementation upregulated the expression of antioxidant related genes in the placenta and piglets' liver. Therefore, D3 supplementation has the potential to enhance the reproductive performance of gilts.

HIGHLIGHTS

- D3 supplementation has a significant role in the nutrition of gilts.
- Maternal D3 supplementation during gestation increased the number of born and born alive piglets.
- Maternal D3 supplementation during gestation improved the antioxidant capacities of gilts, placenta and newborn piglets.

Introduction

Various studies have evaluated the relationship between cholecalciferol (vitamin D3) deficiency and autoimmune diseases, intestinal microbiota disorders and reproductive disturbance (Simmons et al. 2000; Holick 2004; Coffey et al. 2012; Ooi et al. 2013). Sows, which are often raised in confinement facilities, exhibit inhibited capacities for endogenous D3 production (Thayer et al. 2019). Therefore, D3 supplementation might be beneficial for sow's health.

It has been reported that D3 has various effects on reproductive performance (Weber et al. 2014). Sows fed on higher doses (1400 or 2000 IU/kg) of D3 were found to have less stillborn piglets compared to those fed on lower doses (200 and 800 IU/kg) (Lauridsen et al. 2010). Zhou et al. reported that numbers of total born and born alive piglets were significantly increased by 50 µg/kg 25-hydroxycholecalciferol (25-OHD3) supplementation during gestation (Zhou et al. 2017). However, findings on the effects of D3 on reproductive performance of sows have not been conclusive. For instance, Thayer et al. reported that the performance of sows and suckling piglets were not affected by maternal 25-OHD3 (50 µg/kg)
supplementation, compared to the control group (Thayer et al. 2019). Flohr et al. documented that there were no significant differences in the number of total born and stillborn piglets between sows fed on high D₃ dose (2720IU/kg) and sows fed on low doses (680 and 1360IU/kg) (Flohr et al. 2014). Moreover, the specific mechanism through which D₃ improves sow performance has not been established.

Classically, D₃ has been implicated in calcium and phosphate homeostasis, as well as bone health. Active vitamin D analog was shown to upregulate the expression of nuclear factor E2-related factor 2 (NRF2), which activated the expressions of various reactive oxygen species (ROS) detoxifying and antioxidant genes, while suppressing nuclear factor-kappa B (NF-κB) and NADPH oxidase activity to improve antioxidant capacities in diabetic rat (Nakai et al. 2014). In Caenorhabditis elegans, D₃ promoted protein homeostasis and slow ageing by upregulating IRE-1, XBP-1, and SKN-1, which regulate a wide range of stress responses, detoxification factors while suppressing irreversible protein oxidation, nitration, and carbonylation (Mark et al. 2016). Vitamin D suppresses ROS levels by improving mitochondrial functions and integrity, and by suppressing the NF-κB inflammatory pathway (Ryan et al. 2016; Kim et al. 2020). A recent study indicated that D₃ may prevent human umbilical vein endothelial cell death by inhibiting superoxide anion generation, maintaining mitochondrial functions and cell viability, activating survival kinases, and inducing NO production (Uberti et al. 2014). Meanwhile, 1,25(OH)₂D₃ alleviated oxidative stress through the NF-κB/SOD signalling pathway to slow down male reproductive senescence in ageing mice (He et al. 2021). However, the effects of maternal D₃ supplementation on antioxidant capacities in gilts and piglets have not been clearly established.

During gestation, the placenta and foetus secrete elevated levels of reactive oxygen species (ROS) (Al-Gubory et al. 2010). The imbalance between ROS levels (oxidative stress) and antioxidants is a feature of normal pregnancy. The oxidative stress induced by elevated ROS levels has been attributed to impaired rates of successful pregnancy (Agarwal et al. 2006; Zenclussen and Hämmerling 2015; Mullen et al. 2020). Oxidative stress affects embryonic and foetal development by modifying key transcription factors, including hypoxia-inducible factor (HIF-1), nuclear factor-kB (NF-kB), activator protein-1 (AP-1) and p53 (Dennery 2004). Moreover, oxidative stress is involved in the development of different placental lesions, including maternal or foetal vascular malperfusion or chronic villitis, leading to a decrease in the exchange of nutrients and oxygen between the mother and foetus (Schouts et al. 2018). Oxidative stress is associated with the pathogenesis of various reproductive diseases, such as endometriosis, infertility, polycystic ovarian syndrome (PCOA), premature delivery and abortion (Agarwal et al. 2012). Elevated maternal oxidative stress levels can affect their offsprings, which increases the risk of metabolic diseases in later life (Yin et al. 2013). Some nutrients are beneficial for embryonic and foetal survival as well as development by improving maternal, placental and foetal antioxidant capacities (Lin et al. 2012; Mou et al. 2020). Furthermore, it has not been determined whether maternal D₃ supplementation during gestation can improve antioxidant capacities of gilts and piglets, and whether it has a beneficial effect on reproductive performance of gilts. Therefore, the study aimed at evaluating the effects of D₃ supplementation during gestation on reproductive performance and antioxidant capacities of gilt and their offsprings.

**Materials and methods**

**Experimental animals and study design**

A total of 23 gilts (Landrace × Yorkshire) with similar body weights (BW) (initial body weight 163.73 ± 1.62 kg) and backfat thickness (initial backfat thickness 18.12 ± 0.47 mm) were used in this study. Gilts were artificially inseminated with pooled semen obtained from 2 purebred Duroc boars with the same genetic background (housed in the Research Farm) on the day of oestrus, and then, 24 h and 36 h later. Then, gilts were randomly assigned into two groups and provided with any one of the following two diets during gestation: control diet (CON, basal diet, 800 IU/Kg D₃, n = 12), and the D₃ supplemented diet (D₃, basal diet + D₃, 2000 IU/Kg D₃, n = 11). D₃ was premixed and added to both diets. D₃ (500 000 IU/Kg) was obtained from Chengdu Kefei Feed Technology Co. Ltd. Nutrition contents in the basal diet are showed in Table 1, which met or exceeded the daily recommendations of the National Research Council.

During gestation, the gilts were provided with 2.34 kg diet daily (9:00 and 15:00) from day 0 to day 90 of gestation and 2.92 kg diet daily (9:00 and 15:00) from day 91 of gestation to parturition. Gilts were moved to the farrowing pen on day 107 of gestation. At farrowing, the numbers of born alive, stillborn and mummy of newborn piglets were recorded. The newborn piglets were kept in piglet incubator away from the sows before suckling till the born of the last piglet.
of the litter. Then, 6 piglets were randomly selected from each group (male, one piglet per litter with body weight closest to the average body weight of the litter; CON: 1.56 ± 0.06 kg and D3: 1.55 ± 0.04 kg) for blood collection before suckling. After blood collection, the 6 piglets were euthanized by intravenous injection of sodium pentobarbital (60 mg/kg BW) for liver sample collection.

In the gestation room, gilts had a free access to drinking water while the ambient temperature was maintained at 22°C. The study began with 23 gilts, and the final number of gilts used for the analysis was 22. This was because 1 gilt from the D3 group had only 4 newborn piglets at farrowing and was, therefore, excluded.

Sample collection

Body weights (BW) and backfat thickness of gilts were measured at mating and day 110 of gestation. Backfat thickness was measured at 65 mm to the midline of the dorsal rib (P2) using an ultrasound scanner (Renco Lean-Meater; Renco Corporation, Minnesota, United States).

On the mating day, 6 gilts whose weights were closest to the average weights for each group were randomly selected for blood sample collection. Fasting blood samples (10 mL) of the gilts (n = 6) were obtained from marginal ear veins on day 30, 60, and 90 of gestation and on the farrowing day. Immediately after farrowing, umbilical cord blood was collected from as many umbilical cords as possible. Blood samples were centrifuged at 3500 × g for 20 min at 4°C. The obtained serum was stored at −80°C for further analysis.

Placental samples were collected from the placenta of newborn piglets whose BWs were closest to litter average BW immediately after parturition. Placental samples were collected as previously described (Mou et al. 2018). Immediately after sacrifice, liver samples were also obtained from newborn piglets whose BWs were closest to litter average BW. Then, placenta and liver samples were rinsed in ice-cold 0.9% NaCl, after which they were snap frozen followed by storage at −80°C for further analysis. Approximately 0.1 g of frozen liver samples were weighed and used to prepare a 10% homogenate. Liver samples were prepared as previously described (Mou et al. 2020).

Biochemical analysis

Determination of 25-OHD3 concentrations

Serum concentrations of 25-OHD3 were determined using a 25-Hydroxy Vitamin D ELISA kit for gilts and piglets (IDS Immunodiagnostic Systems Ltd, Tyne and Wear, UK) as previously described (Zhou et al. 2017).

Analysis of oxidant and antioxidant levels

Activities of glutathione peroxidase (GPH-Px), total superoxide dismutase (T-SOD) and total antioxidant capacity (T-AOC) as well as concentrations of malondialdehyde (MDA) in serum and liver lysates were determined using their respective commercial assay kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China), according to the manufacturers’ instructions.

RNA extraction and quantitative real-time PCR

Gene expression levels were detected by quantitative real-time PCR (qRT-PCR) as previously described (Bin et al. 2017). Briefly, total RNA was extracted from frozen placenta and liver samples using the TRIzol reagent (Invitrogen). Total RNA (1 µg) was used to synthesise cDNA using the Prime-Script™ RT reagent kit (Takara), according to the manufacturer’s instructions. qRT-PCR analyses were performed on an ABI Q5 Prism Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with SYBR Green RT-PCR reagents (Biorad). The PCR protocol was: 1 cycle of 95°C for 30 s and 40 cycles of 95°C for 15 s followed by 60°C

Table 1. Ingredients and chemical compositions of basal diet (as-fed basis, %).

| Ingredients                  | Contents   |
|------------------------------|------------|
| Corn                         | 70.87      |
| Soybean meal                 | 11.01      |
| Wheat bran (44% crude protein) | 4.59      |
| Fish meal (67% crude protein) | 1.10      |
| Soybean oil                  | 1.28       |
| Choline chloride (50%)       | 0.14       |
| Calcium carbonate            | 0.76       |
| Dicalcium phosphate          | 1.13       |
| Salt                         | 0.37       |
| Inulin                       | 1.83       |
| Pectin                       | 0.18       |
| Cellulose                    | 6.24       |
| L-Yls (98.5%)                | 0.03       |
| Vitamin and trace mineral premix† | 0.37     |

Calculated analysis

| Digestible energy, Mcal/kg | 3.03   |
| Crude protein, %           | 11.76  |
| Crude fibre, %             | 10.09  |
| Calcium, %                 | 0.73   |
| Total phosphorus, %        | 0.59   |
| Available phosphorus, %    | 0.34   |
| Total Lys                   | 0.56   |
| SID-Lys                     | 0.13   |
| SID- (Met + Cys)            | 0.19   |

†Supplied per kg of diet: 4000 IU vitamin A, 800 IU vitamin D3, 441 IU vitamin E, 0.5 mg vitamin K3, 1.0 mg vitamin B1, 1.75 mg vitamin B2, 1.0 mg vitamin B6, 15 µg vitamin B12, 10 mg niacin, 12 mg D-pantothenic, 1.3 mg folic acid, 200 µg biotin; 16 mg Cu, 165 mg Fe, 165 mg Zn, 30 mg Mn, 0.3 mg I, 0.30 mg Se.
for 1 min. The relative mRNA abundance of genes was calculated using the 2-delta CT method with β-actin as the reference gene. The mRNA expression level for each gene in the CON group was set as 1. The primers used in this study are shown in Table 2.

### Statistical analysis

SPSS Statistics 20.0 was used for data analysis. Each gilt or piglet was considered to be an experimental unit. The Shapiro-Wilk test was performed to assess the normality of continuous variables. Differences in reproductive performance as well as antioxidant related indicators of the umbilical cord, placenta and newborn piglets between the two groups were determined by Independent-sample T-test. Two-way repeated-measures ANOVA were used to investigate serum antioxidant indicators and serum 25-OHD3 concentrations of gilts at each stage during gestation. The model is as follows:

\[
Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}
\]

where \(Y_{ijk}\) is the response variable, \(\mu\) is the overall mean, \(\alpha_i\) the fixed effects of dietary D3 supplementation, \(\beta_j\) the fixed effects of gestation stage, \(\alpha\beta)_{ij}\) is the interaction between the dietary D3 supplementation and gestation stage, \(\epsilon_{ijk}\) is the residual error. When the main effects were significant, a Tukey post-hoc was conducted to determine where the differences occurred. Data are expressed as mean ± standard error. p < .05 was set as the threshold for statistical significance, while tendency was defined as .05 ≤ p < .10.

### Results

#### Effects of maternal D3 supplementation on the reproductive performance of gilts

The number of total born piglets \((p = .074)\) and born alive piglets \((p = .073)\) tended to be increased by maternal D3 supplementation during gestation, when compared to the CON group (Table 3). Besides, placenta weights were significantly increased by maternal D3 supplementation \((p < .05)\) compared to the CON group. However, body weights (BWs) and backfat of gilts were not affected by D3 supplementation \((p > .05)\). Stillborn weights were significantly increased by maternal D3 supplementation \((p < .05)\) compared to the CON group. Placental weight, kg 5.45 ± 0.46. Farrowing duration, min 297.25 ± 67.19. Piglet birth interval, min 17.19 ± 0.39.

#### Maternal D3 supplementation increased serum 25-OHD3 concentrations in both gilts and newborn piglets

Serum 25-OHD3 concentrations of gilts were significantly affected by gestation stage \(p < .01\). Gilts of the D3 supplemented group exhibited elevated \((p < .01)\) concentrations of serum 25-OHD3 on days 60 and 90 of gestation and at farrowing, when compared to gilts of CON group (Figure 1A). Besides, serum concentrations of 25-OHD3 \((p < .01)\) in newborn piglets were significantly elevated by maternal D3 supplementation, compared to the CON group (Figure 1B).

#### Effects of maternal D3 supplementation on serum antioxidant indicators in gilts

Serum GSH-Px, T-SOD and MDA of gilts were significantly affected by the stage of gestation \((p < .01)\). The serum GSH-Px activities of gilts...
on G90 and farrowing were higher than those on G30 and G60. The serum T-SOD activities of gilts at farrowing were higher than those on G30, G60 and G90. Besides, the serum activities of GSH-Px and T-AOC of sows were significantly elevated by D3 supplementation ($p < .05$). Furthermore, serum MDA concentrations in gilts were affected by interactions between treatments and gestation stage ($p < .01$, Table 4). Serum MDA concentrations in gilts were significantly reduced by D3 supplementation on day 60 of gestation, compared to the CON group ($p < .05$).

**Effects of maternal D3 supplementation on antioxidant indicators in umbilical cord blood**

Activities of T-SOD and T-AOC ($p < .05$) in blood obtained from the umbilical cord were higher in the D3 supplemented group than those in the CON group (Table 5). Besides, compared to the CON group, MDA concentrations in umbilical cord blood were reduced by maternal D3 supplementation ($p < .05$).

**Effects of maternal D3 supplementation on antioxidant indicators in newborn piglets**

Serum activities of T-SOD ($p < .05$) in newborn piglets were significantly elevated by maternal D3 supplementation, compared to the CON group (Table 5). Besides, compared to the CON group, serum activities of GSH-Px ($p = .067$) tended to be elevated while serum MDA ($p = .063$) levels tended to be reduced by maternal D3 supplementation (Table 5). However, serum activities of T-AOC in newborn piglets were not affected by maternal D3 supplementation (Table 5).

Compared to the CON group, the hepatic MDA concentrations ($p = .084$) tended to be reduced by maternal D3 supplementation (Table 5). Besides, piglets in the D3 group exhibited elevated hepatic T-AOC ($p < .05$) activities when compared to piglets in the CON group.

**Maternal D3 supplementation regulated the expression of redox related genes in the placenta and newborn piglet liver**

Maternal D3 supplementation significantly upregulated mRNA expression levels of GPx3 ($p < .05$) and GPx4 ($p < .01$) in the placenta, while expression levels of VDR ($p = .059$) tended to be elevated by maternal D3 supplementation, compared to the CON group (Figure 2).

Moreover, maternal D3 supplementation significantly upregulated mRNA expression levels of GPx1 ($p < .01$), GPx3 ($p < .01$), GPx4 ($p < .01$), SOD2 ($p < .01$) and NRF2 ($p < .05$) in the liver of newborn piglets (Figure 3).

**Discussion**

Oxidative stress impairs female health and the reproductive process (Agarwal et al. 2005; Lu et al. 2018). Maternal oxidative stress can be passed on to offsprings, thereby damaging the health of offsprings in later life (Yin et al. 2013). Therefore, this study aimed at evaluating the effects of maternal D3 supplementation during gestation on reproductive performance and on the antioxidant abilities of gilts and their newborn offsprings.
In this study, maternal dietary 2000 IU/kg D3 supplementation during gestation had a tendency to increase the number of total born and live born piglets without changing the average born alive weight, compared to the CON group. Sows supplemented with 200 ng/day 25-OHD3 from day 90 of gestation to

Table 5. Effects of maternal vitamin D₃ supplementation during gestation on oxidant status of umbilical cord blood and newborn piglets.<sup>a,b</sup>

| Treatment | CON | VD₃ | p-value |
|-----------|-----|-----|---------|
| Umbilical cord blood | | | |
| GSH-Px, U/mL | 92.07 ± 16.09 | 128.73 ± 21.45 | .201 |
| MDA, nmol/mL | 7.89 ± 1.42 | 4.14 ± 0.56 | .047 |
| T-AOC, U/mL | 1.18 ± 0.15 | 1.86 ± 0.25 | .043 |
| T-SOD, U/mL | 36.29 ± 6.33 | 53.35 ± 2.22 | .092 |
| Newborn piglets’ sera | | | |
| GSH-Px, U/mL | 154.04 ± 12.33 | 183.02 ± 6.79 | .067 |
| MDA, nmol/mL | 1.41 ± 0.15 | 1.06 ± 0.09 | .063 |
| T-AOC, U/mL | 1.71 ± 0.16 | 1.84 ± 0.10 | .512 |
| Newborn piglets’ livers | | | |
| GSH-Px, U/mg protein | 125.26 ± 17.54 | 114.16 ± 16.48 | .654 |
| MDA, nmol/mg protein | 1.74 ± 0.17 | 1.33 ± 0.50 | .084 |
| T-AOC, U/mg protein | 0.72 ± 0.19 | 2.06 ± 0.41 | .015 |

aData are expressed as mean ± standard error.

<sup>b</sup>n = 6 for each group.

GSH-Px: glutathione peroxidase; MDA: malondialdehyde; T-AOC: total antioxidant capacity; T-SOD: total superoxide dismutase; CON: basal diet, 800 IU/Kg D₃; D₃: basal diet + D₃, 2000 IU/Kg D₃.

Figure 2. Effects of maternal vitamin D₃ supplementation during gestation on the expression of antioxidant activity related genes in the placenta. n = 6 for each group. CAT, catalase; GPx1, glutathione peroxidase 1; GPx2, glutathione peroxidase 2; GPx3, glutathione peroxidase 3; GPx4, glutathione peroxidase 4; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; NRF2, nuclear erythroid 2-related factor 2; VDR, vitamin D receptor. CON, basal diet, 800 IU/Kg D₃; D₃, basal diet + D₃, 2000 IU/Kg D₃ Data are normalised against β-actin, with results expressed relative to the control using the 2-delta CT method (Ct is cycle threshold); #, 0.05 < p < .10; *, p < .05; **, p < .01.

In this study, maternal dietary 2000 IU/kg D₃ supplementation during gestation had a tendency to increase the number of total born and live born piglets without changing the average born alive weight, compared to the CON group. Sows supplemented with 200 μg/day 25-OHD₃ from day 90 of gestation to
Farrowing had increased numbers of total born and live born piglets (Wang et al. 2020). A recent study reported that numbers of total born and live born piglets were significantly increased by dietary supplementation of 50 \( \mu \)g/kg 25-OHD3 during gestation (Zhou et al. 2017). The above findings imply that D3 can effectively improve the reproductive performance of sows. However, the effects of D3 on reproductive performance of sows are not conclusive. For instance, Weber et al. reported that total numbers of born and live born piglets were not affected by dietary D3 supplementation (2000 IU/kg) and different parities (1–4) of sows (Weber et al. 2014). In a previous study, variations in D3 supplementation concentrations (800–9600 IU/kg) had no effect on the reproductive performance of sows (Flohr et al. 2016). Differences in outcomes among studies were attributed to different doses and forms of D3, treatment times, or to the different parities of sows (Flohr et al. 2014, 2016). Besides, there were no significant changes in BW and backfat of gilts in the two treatments. It is worth noting that gilts were used in this experiment, therefore, their growth needs could also be attributed to this outcome. A previous study showed that 25-OHD3 supplementation during gestation had no significant effects on the growth performance of gilts (Zhou et al. 2017).

Serum 25-OHD3 is the main circulatory form of D3 and is used to determine the body’s vitamin D status (Holick 2007). A previous study showed that 2720 IU/lb D3 supplementation during gestation increased serum levels of 25-OHD3 in sows and in piglets (Flohr et al. 2014). Zhang et al. reported that maternal 25-OHD3 supplementation elevated serum levels of 25-OHD3 in sows and in piglets (Zhang et al. 2020). In agreement with these studies, we found that gilts supplemented with D3 during gestation exhibited elevated concentrations of serum 25-OHD3, compared to the control group, as well as to newborn piglets. Genomic actions of VDR are important in VD3 functions, and it is regulated by 1,25(OH)2D3 (Christakos et al. 1996). VDR, the receptor for the active form (1,25(OH)2D3) of D3, is widely distributed in tissues. 1,25(OH)2D3/VDR/RXR regulates the expression of target genes to execute the traditional genomic functions of D3 (Haussler et al. 2011). We found that maternal D3 supplementation during gestation had a tendency to improve the expression of the VDR gene in the placenta.

Severe oxidative stress occurs during gestation, and is one of the reasons that impair the success of female gestation (Hempstock et al. 2003; Agarwal et al. 2005). Maternal oxidative stress levels, which are correlated with MDA levels, can be transferred to the foetus through the placenta (Takahashi and Oishi 2000; Yi et al. 2011). Studies have reported on the antioxidant effects of D3, however, the antioxidant properties of vitamin D in pigs have not been reported before. We found that maternal dietary supplementation of 2000 IU/kg D3 significantly elevated serum 25(OH)D3 concentrations in gilts and in newborn piglets, had a tendency to improve the expression levels of VDR in the placenta, significantly improved serum activities of GSH-Px, T-SOD and T-AOC in gilts, umbilical cord blood and in newborn piglets, as well as elevated T-AOC activities in the liver of newborn piglets. Serum MDA concentrations were significantly reduced by D3 supplementation in gilts and umbilical cord blood. As previously mentioned, D3 can exert its antioxidant functions by modifying the activities of antioxidant enzymes. A recent study showed that dietary 50 \( \mu \)g/kg 25(OH)D3 supplementation enhanced serum GSH-Px activities in sows and serum SOD activities in suckling piglets, compared to the control group (Zhang et al. 2020). In a previous study, D3 pre-treatment (25 \( \mu \)g/kg) for 1, 24 and 48 h before LPS injection alleviated LPS-induced mice renal oxidative stress, by elevating the...
expression of inducible nitric oxide synthase, SOD1 and SOD2 genes in kidneys (Xu et al. 2015).

In this study, expression levels of antioxidant activity related genes in the placenta (GPx3 and GPx4) and livers of newborn piglets (GPx1, GPx3, GPx4 and SOD2) were found to be significantly upregulated by maternal D3 supplementation. GPxs are usually involved in defences against oxidative stress (Santi et al. 2013). Among them, GPx1 is a major intracellular antioxidant enzyme that is involved in protection against oxidative stress (Spanier et al. 2009), GPx3 plays an important role in protecting tissues from oxidative stress by inactivating ROS (Chen et al. 2011). GPx4 inhibits lipid peroxidation damage by decreasing the levels of phospholipid hydroperoxides in membranes and lipoproteins (Yant et al. 2003). SOD2 polymorphisms regulate mitochondrial ROS levels through SOD (Flekac et al. 2008). NRF2 is an important node against oxidative stress (Yamamoto et al. 2018). NRF2 is negatively regulated by Keap1, and it activates the expression of various antioxidant activity related genes. Chen et al. documented that 1,25(OH)2D3 improves the transcription of NRF2 to exert antioxidant functions (Chen et al. 2019). In this study, although the expression levels of VDR in the livers of newborn piglets were not significantly altered after maternal D3 supplementation, expression levels of genes such as NRF2, SOD and GPx were significantly upregulated. A previous study reported that D3 elevated VDR protein expression in cultured keratinocytes, without affecting VDR mRNA expression levels. Moreover, VDR was ubiquitinated in cells, but this ubiquitination was inhibited by D3 (Li et al. 1999). Besides, we found that serum 25-OHD3 concentrations in newborn piglets were significantly elevated by maternal D3 supplementation. Therefore, elevated 25-OHD3 concentrations may increase the expression levels of the VDR protein, thereby upregulating the NRF2 level, and further improving the expression of genes associated with antioxidant and related enzyme activities. Maternal D3 supplementation improved antioxidant capacities in gilts, placenta and in newborn piglets by elevating serum 25(OH)D3 concentrations and expression levels of the VDR gene. Improvement in maternal and placental antioxidant capacity ensures a smooth progression of pregnancy, and improves the reproductive performance of gilts. Besides, transmission of placental and umbilical cord antioxidant capacities between gilts and foetus and improvement in VD status might further enhance antioxidant capacities in newborn piglets.

Conclusion
Maternal D3 supplementation during gestation elevated serum 25(OH)D3 levels and might improve antioxidant capacities in gilts, placenta and newborn piglets, induces the expression of antioxidant related genes in the placenta and in newborn piglets’ liver, and has the potential to improve the reproductive performance of gilts. Therefore, D3 supplementation is important in improving reproductive health for both animals and humans.

Ethical approval
All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Sichuan Agricultural University. Ethical approval was obtained from the Animal Care and Use Committee of Sichuan Agricultural University (Approval number: DKYB20131704).

Author contributions
The authors’ responsibilities were as follows: BF, LPZ and DW designed the study; LPZ, ZYM, DLM, LH, MY and DJD conducted the experiments; HY, XMJ, ZFF, LQC, YZ, SYX, YL, JL, CH, YFZ and LXL analysed the data; LPZ wrote the manuscript; BF and DW revised the manuscript; all authors read and approved the manuscript for publication.

Disclosure statement
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Data availability statement
For privacy reasons, the data analysed in this manuscript is not publicly available, however, it is available from the corresponding author upon reasonable request.

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