



## Review Article

Use of 25-hydroxyvitamin D<sub>3</sub> in diets for sows: A review

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## ABSTRACT

Dietary supplementation with 25-hydroxyvitamin D<sub>3</sub> (25OHD<sub>3</sub>), as an alternative source of vitamin D, is becoming increasingly popular due to its commercialization and more efficient absorbability. The addition of 25OHD<sub>3</sub> rather than its precursor vitamin D<sub>3</sub> can circumvent the 25-hydroxylation reaction in the liver, indicating that supplementation of 25OHD<sub>3</sub> can rapidly improve the circulating vitamin D status of animals. Emerging experiments have reported that maternal 25OHD<sub>3</sub> supplementation could increase sow performances and birth outcomes and promote circulating vitamin D status of sows and their offspring. Increased milk fat content was observed in many experiments; however, others demonstrated that adding 25OHD<sub>3</sub> to lactating sow diets increased the contents of milk protein and lactose. Although an inconsistency between the results of different experiments exists, these studies suggested that maternal 25OHD<sub>3</sub> supplementation could alter milk composition via its effects on the mammary gland. Previous studies have demonstrated that adding 25OHD<sub>3</sub> to sow diets could improve the mRNA expressions of insulin-induced gene 1 (*INSIG1*) and sterol regulatory element-binding protein 1 (*SREBP1*) in the mammary gland cells from milk and increase the mRNA expressions of acetyl-CoA carboxylase  $\alpha$  (*ACC $\alpha$* ) and fatty acid synthase (*FAS*) in the mammary gland tissue. Maternal 25OHD<sub>3</sub> supplementation promotes skeletal muscle development of piglets before and after parturition, and improves bone properties including bone density and bone breaking force in lactating sows and their piglets. Interestingly, 25OHD<sub>3</sub> supplementation in sow diets could improve neonatal bone development via regulation of milk fatty acid composition related to bone metabolism and mineralization. In this review, we also discuss the effects of adding 25OHD<sub>3</sub> to sow diets on the gut bacterial metabolites of suckling piglets, and propose that butyrate production may be associated with bone health. Therefore, to better understand the nutritional functions of maternal 25OHD<sub>3</sub> supplementation, this paper reviews advances in the studies of 25OHD<sub>3</sub> for sow nutrition and provides references for practical application.

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## 1. Introduction

During reproductive periods such as pregnancy and lactation, the physiological requirement of calcium (Ca) is significantly higher as a result of milk production, growth, and development of the fetus (Halloran et al., 1979) as well as a substantial amount of Ca

flux existing from maternal blood to milk (Ardeshirpour et al., 2015). Recent studies in sows revealed that this physiological requirement of special stages could induce mobilization and loss of Ca from bone and finally result in locomotion-related injuries including bone weakness, lameness or even fractures (Kirk et al., 2005; Weber et al., 2014). A survey of Danish sows demonstrated that the primary reason for culling was associated with the locomotive system (72%), and the fracture rate was up to 16% (Kirk et al., 2005). The content of 25-hydroxyvitamin D<sub>3</sub> (25OHD<sub>3</sub>) in the blood of newborn pigs is the lowest (Horst and Littledike, 1982), because only a small amount of vitamin D or its metabolites passes into breast milk in sows. In humans, research has suggested that transplacental transfer and milk content of vitamin D are low in vitamin D deficient females during reproductive periods including pregnancy and lactation (Wagner and Greer, 2008; Jain et al., 2011; Chandu et al., 2016). Therefore, vitamin D deficient females are

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more likely to have vitamin D deficient infants, which could influence immune function and bone development in infants and further contribute to increasing fracture risk in both childhood and adult life. Further, maternal vitamin D supplementation has a positive effect on the improvements of birth outcomes and vitamin D status in suckling infants (Hollis and Wagner, 2004b, 2017; Oberhelman et al., 2013). In sows, maternal vitamin D<sub>3</sub> supplementation before farrowing has been proven to be a better way of supplementing cholecalciferol to young piglets via placental transport and breast milk (Goff et al., 1984).

Dietary 25OHD<sub>3</sub>, with its higher biopotency and more efficient absorbability, has been used for many years as a vitamin D supplement in sow diets. 25OHD<sub>3</sub> has lower daily excretion of metabolites than vitamin D<sub>3</sub>, and differences between vitamin D<sub>3</sub> and 25OHD<sub>3</sub> for net absorption and retention may account for the higher biopotency of 25OHD<sub>3</sub> (Chou et al., 2009). There are many experiments about the application of 25OHD<sub>3</sub> in the diets of sows because of many questions worthy of discussion including feeding value and in-service phase. The effects of 25OHD<sub>3</sub> supplementation in the diets of lactating animals are not consistent. Weber et al. (2014) demonstrated that the average improvement in newborn weight per piglet and total litter weight induced by 50 µg/kg 25OHD<sub>3</sub> were 7.6% and 17.3%, respectively. Zhang et al. (2019b) showed that feed 50 µg/kg 25OHD<sub>3</sub> to sows could significantly increase litter weight at weaning (+10.0%) and total litter weight gain (+13.3%). However, Lauridsen et al. (2010) and Flohr et al. (2016a) did not observe improvements in reproductive performance for sows fed diets supplemented with 25OHD<sub>3</sub>. Many reports demonstrated that increased milk fat content was found in lactating sows fed diets with 25OHD<sub>3</sub> (Zhang et al., 2019b; Wang et al., 2020), whereas increased contents of milk protein and lactose were observed in sows fed diets with 25OHD<sub>3</sub> (Zhou et al., 2017). Different responses of reproductive performance and milk composition were observed with supplementation of 25OHD<sub>3</sub> in the diets of lactating sows. The inconsistency between experiments indicates that more studies should be recommended to validate or refute the importance of supplementing 25OHD<sub>3</sub> as a feed supplement in the diets of lactating sows. Therefore, this review summarizes existing findings about the utilization of 25OHD<sub>3</sub> in the diets of sows, and provides references for its practical use in sow production.

## 2. The vitamin D<sub>3</sub> metabolism in comparison to 25OHD<sub>3</sub> metabolism

Vitamin D<sub>3</sub> has 2 metabolites including 25OHD<sub>3</sub> and 1 $\alpha$ ,25-dihydroxycholecalciferol (1,25(OH)<sub>2</sub>D<sub>3</sub>). The product of 25OHD<sub>3</sub> has been authorized as a nutritional feed additive in animal nutrition, which could improve bone development and provide a variety of performance benefits and intestinal health when added to diets.

The metabolic pathways of vitamin D<sub>3</sub> and 25OHD<sub>3</sub> are presented in Fig. 1. Normally, vitamin D<sub>3</sub> can be obtained from 2 sources including diets and 7-dehydrocholesterol in skin. Vitamin D<sub>3</sub> has no biological activity and could be transported in the circulating blood through vitamin D binding protein into the liver (Christakos et al., 2016). Vitamin D is then converted to 25OHD<sub>3</sub> via the first activation reaction catalyzed by different 25-hydroxylase in the liver (Haussler et al., 2013). The cytochrome P450 (CYP)-containing enzymes, CYP2R1, is regarded as the best candidate for the enzyme 25-hydroxylase that are involved in the first step of activation because only CYP2R1 could hydroxylate vitamin D<sub>2</sub> or vitamin D<sub>3</sub> equally at the C-25 position of the side chain (Jones, 2012; Quesada-Gomez and Bouillon, 2018; Roizen et al., 2018). The previous review showed that other 25-hydroxylases, including CYP27A1 and CYP3A4, may act on the vitamin D<sub>3</sub> substrates if their concentrations are increased to a

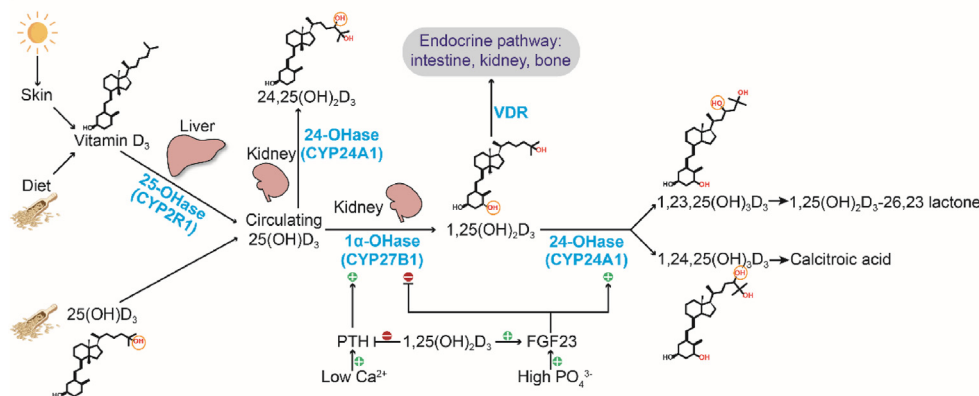
high nanomolar or micromolar range (Prosser and Jones, 2004). In the kidney, 25OHD<sub>3</sub> is hydroxylated at the C-1 position of the A ring, leading to the production of 1,25(OH)<sub>2</sub>D<sub>3</sub> (Christakos et al., 2016). Renal 1 $\alpha$ -hydroxylase (CYP27B1), existing mainly in the kidney, consists of a mitochondrial cytochrome P450, and a ferredoxin reductase and a ferredoxin (Haussler et al., 2013). The CYP27B1 enzyme is also present in extrarenal tissues including skin, prostate, immune organs, and mammary gland and could catalyze the production of 1,25(OH)<sub>2</sub>D<sub>3</sub> (Jones, 2012). The degradation and inactivation of 25OHD<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> are catalyzed by CYP24A1. CYP24A1 belongs to a cytochrome P450 component of 24-hydroxylase enzyme which catalyzes the conversion of 25OHD<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> into 24-hydroxylated products (Jones et al., 2012). CYP24A1 catalyzes the conversion of 1,25(OH)<sub>2</sub>D<sub>3</sub> into calcitric acid (Yu and Arnold, 2016) and produces 24,25(OH)<sub>2</sub>D<sub>3</sub> from 25OHD<sub>3</sub> by the C-24 oxidation pathway (Ketha et al., 2018). Calcitric acid is finally excreted in the bile and urine. CYP24A1 can also catalyze the formation of 25OHD<sub>3</sub>-26,23 lactone and 1,25(OH)<sub>2</sub>D<sub>3</sub>-26,23 lactone from 25OHD<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub>, respectively, by the C-23 oxidation pathway (Jones et al., 2012). The actions of CYP27B1 and CYP24A1 are under strict endocrine/mineral feedback control. In comparison to vitamin D<sub>3</sub>, the acquisition of 25OHD<sub>3</sub> in feed could avoid 25-hydroxylation reaction as a result of the presence of a hydroxyl group, indicating that the exogenous addition of 25OHD<sub>3</sub> can be directly utilized by animals in a ready-to-use active form. Therefore, adding 25OHD<sub>3</sub> as a feed additive to sow diets can ensure that there is a sufficient level of 25OHD<sub>3</sub> to produce 1,25(OH)<sub>2</sub>D<sub>3</sub>, which compensates for the limited natural ability of animals to transform 25OHD<sub>3</sub> into 1,25(OH)<sub>2</sub>D<sub>3</sub>.

## 3. Efficacy and safety of 25OHD<sub>3</sub> as a feed additive

The use of 25OHD<sub>3</sub> can avoid 25-hydroxylation reaction as a result of the presence of a hydroxyl group (Susanna et al., 2010). Dietary 25OHD<sub>3</sub> not only has greater biological activity than vitamin D<sub>3</sub> (Chou et al., 2009), but also has fewer adverse effects and is more stable than 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> (Soares et al., 1995). As shown in Table 1, 36 sows were allocated to 4 treatments including supplementations of 5 µg vitamin D<sub>3</sub>/kg feed, 50 µg vitamin D<sub>3</sub>/kg feed, 50 µg 25OHD<sub>3</sub>/kg feed, and 50 µg of both vitamin D<sub>3</sub> and 25OHD<sub>3</sub>/kg feed (25 µg vitamin D<sub>3</sub> as well as 25 µg 25OHD<sub>3</sub>/kg). This study suggested that the plasma 25OHD<sub>3</sub> level was higher in sows fed diets supplemented with 25OHD<sub>3</sub> (EFSA, 2009), which supported the efficacy of 25OHD<sub>3</sub> production as a source of vitamin D supplementation. Information from tolerance trials on piglets was available, and piglets are taken as the most sensitive category of pigs to 25OHD<sub>3</sub>. A total of 48 weaned piglets were allocated to 4 treatments containing 50 µg vitamin D<sub>3</sub>/kg feed, 50, 250, and 500 µg 25OHD<sub>3</sub>/kg feed, respectively (EFSA, 2009). The results revealed that there were no adverse effects of 25OHD<sub>3</sub> supplementation at the recommended level and its 5- and 10-fold overdose, and renal calcification, which is a sensitive indicator of excessive vitamin D, was not detected. The results suggested that 25OHD<sub>3</sub> could be considered as a safe feed additive used in pig diets.

## 4. Maternal 25OHD<sub>3</sub> supplementation use as a means of improving vitamin D status of sow-offspring pairs

As fetal and neonatal circulating 25OHD<sub>3</sub> levels are dependent on maternal 25OHD<sub>3</sub> status, it is important to ensure maternal vitamin D sufficiency during reproductive periods including pregnancy and lactation. Maternal vitamin D supplementation has been proven to ensure proper vitamin D status of the mothers, thereby preventing vitamin D deficiency of their offspring. Given this, females who are pregnant or breastfeeding should take vitamin D



**Fig. 1.** Acquisition of vitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>3</sub> (25OHD<sub>3</sub>), modulation of anabolism/catabolism, and receptor-mediated endocrine actions of 1,25(OH)<sub>2</sub>D<sub>3</sub>. CYP2R1 = 25-hydroxylase; CYP27B1 = 1 $\alpha$ -hydroxylase; CYP24A1 = 24-hydroxylase; VDR = vitamin D receptor; PTH = parathyroid hormone; FGF23 = fibroblast growth factor 23.

**Table 1**  
Efficacy and safety of 25-hydroxyvitamin D<sub>3</sub> (25OHD<sub>3</sub>) as a feed additive.

Species	Treatments	Dose, $\mu\text{g}/\text{kg}$ feed	Treatment effects			
			Body weight, kg		Plasma 25OHD <sub>3</sub> , ng/mL	
			Start <sup>1</sup>	End <sup>1</sup>	Start <sup>1</sup>	End <sup>1</sup>
Sows	Vitamin D <sub>3</sub>	5	167	193	29.1	15.5 <sup>b</sup>
		50	162	234	32.1	26.5 <sup>b</sup>
	25OHD <sub>3</sub>	50	163	201	39.8	70.0 <sup>a</sup>
		25 + 25	185	212	42.1	63.8 <sup>a</sup>
Weaned piglets	Vitamin D <sub>3</sub> 25OHD <sub>3</sub>	50	Growth performance: ND			
		50	Haematology and serology: ND			
		250	Urinary parameters: ND			
		500	Renal tubular calcification: ND			

<sup>a, b</sup> Means in the same column with different letter superscripts differ at  $P < 0.05$ . “ND” represents that significant effect was not detected. From EFSA (2009).

<sup>1</sup> Start means mating during the first reproductive period, and end means weaning during the second reproductive period.

daily to meet their recommended requirements, which contribute to ensuring vitamin D sufficiency and prevention of low pregnancy and birth outcomes (Mulligan et al., 2010; Souberbielle et al., 2010). It has been reported that maternal 25OHD<sub>3</sub> could cross the placenta and transport to the fetus via the umbilical cord (Hosseini-nezhad and Holick, 2013), and maternal serum 25OHD<sub>3</sub> level is directly associated with the concentration of 25OHD<sub>3</sub> in the umbilical cord at birth (Lucas et al., 2008). Coffey et al. (2012) demonstrated that circulating 25OHD<sub>3</sub> level was increased in gilts fed with 25OHD<sub>3</sub> on days -13 (224.3 vs 141.8 nmol/L), 46 (239.5 vs 139.3 nmol/L), and 89 (232.0 vs 145.5 nmol/L) of gestation compared with that of gilts fed with the control diet, and fetuses from gilts fed with 25OHD<sub>3</sub> had higher plasma 25OHD<sub>3</sub> concentration than the control group. As reported by a previous study in our lab, a correlation analysis of 25OHD<sub>3</sub> concentrations in sows, newborns (umbilical cord blood) and suckling piglets showed that adding 25OHD<sub>3</sub> to lactating sows increased serum 25OHD<sub>3</sub> concentration in the umbilical cord and suckling pigs (Zhang et al., 2019a). Zhou et al. (2017) reported that 25OHD<sub>3</sub> supplementation increased serum concentration of 25OHD<sub>3</sub> in sows at farrowing and weaning, and serum concentration of 25OHD<sub>3</sub> was higher in the umbilical cord and neonatal piglets from sows fed with 25OHD<sub>3</sub> in comparison with the control. In addition, another finding in our lab showed that maternal 25OHD<sub>3</sub> supplementation improved levels of milk 25OHD<sub>3</sub> during lactation (Zhang et al., 2019b). Although limited vitamin D metabolites can be transferred into milk (Hollis and Wagner, 2004a), the concentration of 25OHD<sub>3</sub> in milk is positively correlated with the concentration of 25OHD<sub>3</sub> in maternal serum (Specker et al., 1985). The results of correlation analysis from our lab showed

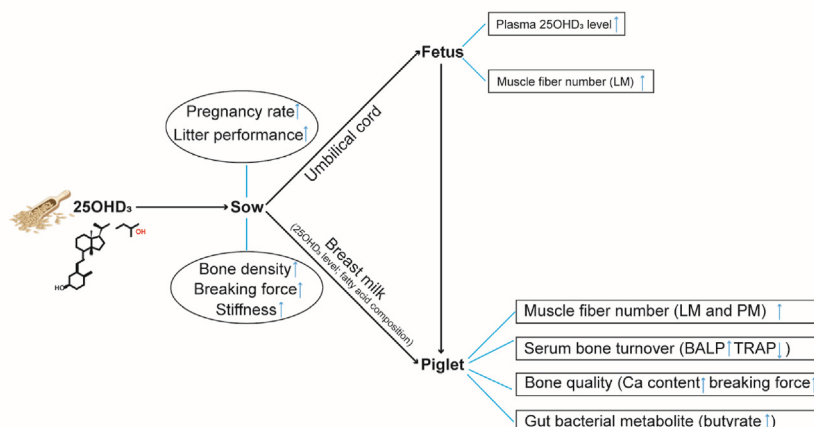
that change of 25OHD<sub>3</sub> concentration in maternal or neonatal serum was correlated to change of milk 25OHD<sub>3</sub> concentration during lactation ( $R^2 = 0.82$  to  $0.86$ ,  $P < 0.01$ ) (Zhang et al., 2019a). Collectively, adding 25OHD<sub>3</sub> to sow diets could contribute to improvements of 25OHD<sub>3</sub> concentration in maternal and neonatal serum during lactation compared with the control group, thereby increasing vitamin D status of sows and their offspring.

## 5. Function and application of 25OHD<sub>3</sub> in diets for the sow industry

Generally, available literature has confirmed the feasibility of maternal 25OHD<sub>3</sub> supplementation in sow diets for improving performance and health (Fig. 2). Maternal 25OHD<sub>3</sub> supplementation improved piglet performance and vitamin D status via its regulation of milk composition including improvements of fat content, fatty acid composition, and 25OHD<sub>3</sub> level. Changes of vitamin D status and fatty acid composition have beneficial effects on bone mineralization and skeletal muscle development of piglets, and modulate gut bacterial metabolites including butyrate of piglets. This section discusses this in detail from the following aspects.

### 5.1. Enhancement of reproductive performance and nutrient digestibility of sows

Many studies have addressed the benefits of 25OHD<sub>3</sub> for sow performance during reproductive periods including pregnancy and lactation (Table 2), but corresponding results are limited and inconsistent. Adding 50  $\mu\text{g}$  25OHD<sub>3</sub>/kg feed to gilts increased the



**Fig. 2.** An overview of the roles of 25-hydroxyvitamin D<sub>3</sub> (25OHD<sub>3</sub>) in sow diets. LM = longissimus dorsi; PM = psoas major; BALP = bone-specific alkaline phosphatase; TRAP = tartrate-resistant acid phosphatase; Ca = calcium.

**Table 2**  
Effect of 25-hydroxyvitamin D<sub>3</sub> (25OHD<sub>3</sub>) supplementation to diets on reproductive performance of sows.

Species	Diet types	Treatments	Treatment effects (% difference to control) <sup>1</sup>	References
Gilts	Corn-soybean meal-based diet	62.5 µg/kg VD <sub>3</sub> (control), 12.5 µg/kg VD <sub>3</sub> + 50 µg/kg 25OHD <sub>3</sub>	Pregnancy rate (+23.0%), litter size (+24.5%)	Coffey et al. (2012)
Gilts	Corn-soybean meal-based diet	62.5 µg/kg VD <sub>3</sub> (control), 12.5 µg/kg VD <sub>3</sub> + 50 µg/kg 25OHD <sub>3</sub>	Fetal muscle fiber number (+9.3%)	Hines et al. (2013)
Primiparous sows	Corn-soybean meal-based diet	0 (control), 50 µg/kg 25OHD <sub>3</sub>	Live born (+10.0%), litter size at weaning (+14.9%), weaning litter weight (+19.7%)	Zhou et al. (2017)
Primi- and multiparous sows	Corn-soybean meal-based diet	50 µg/kg VD <sub>3</sub> (control), 50 µg/kg 25OHD <sub>3</sub>	Litter weight at weaning (+10.0%), total litter weight gain (+13.3%)	Zhang et al. (2019b)
Primi- and multiparous sows	Barley-soybean meal-based diet	50 µg/kg VD <sub>3</sub> (control), 50 µg/kg 25OHD <sub>3</sub>	Birth weight per piglet (+7.6%), total litter weight (+17.3%)	Weber et al. (2014)
Primi- and multiparous sows	Barley-soybean meal-based diet	VD <sub>3</sub> : 5, 20, 35, 50 µg/kg; 25OHD <sub>3</sub> : 5, 20, 35, 50 µg/kg	Vitamin D form: ND	Lauridsen et al. (2010)
Multiparous sows	Corn-soybean meal-based diet	VD <sub>3</sub> : 20, 50, 240 µg/kg; 25OHD <sub>3</sub> : 50 µg/kg	50 µg/kg 25OHD <sub>3</sub> vs. 20, 50, 240 µg/kg VD <sub>3</sub> : ND	Flohr et al. (2016a)
Multiparous sows	Corn-soybean meal-based diet	150 µg VD <sub>3</sub> /d (control); 200 µg 25OHD <sub>3</sub> /d	Total piglet weight gain (+11.2%), Piglet average daily gain (+14.2%)	Wang et al. (2020)

VD<sub>3</sub> = vitamin D<sub>3</sub>.

<sup>1</sup> Note: “+” represents a significant effect, “ND” represents significant effect was not detected.

pregnancy rate and litter size by 23.0% and 24.5%, respectively (Coffey et al., 2012). Dietary supplementation with 50 µg/kg 25OHD<sub>3</sub> in gilt diets could improve total muscle fiber number in the fetus (Hines et al., 2013). Zhou et al. (2017) reported that the average improvement in live born, litter size at weaning and weaning litter weight induced by 25OHD<sub>3</sub> were 10.0%, 14.9% and 19.7%, respectively. In this study, dietary 25OHD<sub>3</sub> supplementation increased the contents of protein and lactose in milk, which caused an improvement in the growth performance of suckling piglets. Weber et al. (2014) suggested that the average improvement in newborn weight per piglet and total litter weight induced by 50 µg/kg 25OHD<sub>3</sub> were 7.6% and 17.3%, respectively. The previous study in our lab showed that adding 50 µg/kg 25OHD<sub>3</sub> to sows can significantly increase litter weight at weaning and total litter weight gain by 10.0% and 13.3%, respectively (Zhang et al., 2019b). However, Lauridsen et al. (2010) and Flohr et al. (2016a) did not observe improvements in reproductive performance for sows fed diets supplemented with 25OHD<sub>3</sub>. The differences in experimental results are possibly caused by the origin of the 25OHD<sub>3</sub>, the quantity added to the diets, the feeding period and the environmental conditions used in the trials. Regassa et al. (2015) demonstrated that vitamin D metabolites could enhance Ca utilization and decrease faecal excretion in growing pigs. Our lab investigated the benefits of dietary 25OHD<sub>3</sub> on nutrient

digestibility in lactating sows. In this experiment, 2 dietary treatments (2,000 IU vitamin D<sub>3</sub>/kg feed, 50 µg 25OHD<sub>3</sub>/kg feed) were fed to sows, and the results showed that feeding 25OHD<sub>3</sub> to lactating sows significantly increased the apparent digestibility of Ca compared with ND sows (Zhang et al., 2019b). These studies indicated that maternal 25OHD<sub>3</sub> supplementation could improve sow performance including pregnancy rate and litter performance and increase the utilization rate of Ca in diets.

### 5.2. Improvement of milk composition of sows

Breastfeeding is considered as an important source of nutrients for neonates and provides essential components for neonatal growth and development. Bhattacharjee et al. (1987) demonstrated that vitamin D<sub>3</sub> plays an important role in modulating milk quality (Bhattacharjee et al., 1987). Improvement of milk quality could contribute to an increase in growth performance in piglets from sows fed with 25OHD<sub>3</sub> supplementation. Zhou et al. (2017) reported that maternal 25OHD<sub>3</sub> supplementation significantly increased milk contents of protein and lactose during lactation. However, Wang et al. (2020) showed that dietary 25OHD<sub>3</sub> supplementation enhanced the content of fat in sow colostrum in comparison to that of the control group. Our study reported that the content of fat in milk was

increased in sows fed with 25OHD<sub>3</sub> compared with the control on day 21 of lactation (Zhang et al., 2019b). Although there have been different views about the changes in milk composition induced by 25OHD<sub>3</sub>, these studies unanimously believe that maternal 25OHD<sub>3</sub> supplementation can improve the ability of the sows for lactating. In mammary glands, the vitamin D receptor (VDR) may participate in modulating the metabolic process of epithelial cells in the mammary gland during reproductive periods (Zinser and Welsh, 2004). Cant et al. (2018) reported that the synthesis of milk fat may be affected by changes in mRNA translation efficiency, and the number and synthetic capacity of mammary gland secretory cells. Rudolph et al. (2010) reported that sterol regulatory element-binding protein 1 (SREBP1) could promote gene expressions of fatty acid synthesis in the mammary epithelium and increase fat content in milk, and Wang et al. (2011) indicated that SREBP1 appears to be involved in the activation of VDR. In addition, Li et al. (2019) suggested that insulin-induced gene 1 (INSIG1) is considered as an essential transcriptional factor modulating fat synthesis in breast tissue, which can inhibit translocation of the SCAP/SREBP1 complex in cells, thereby preferentially activating SREBP1 and promoting the synthesis of milk fat (Rincon et al., 2012). An increase in milk fat content when 25OHD<sub>3</sub> was added to sow diets may be partly explained by the higher gene expressions of SREBP1 and INSIG1 in the mammary gland cells from milk (Wang et al., 2020). Although this study did not directly analyze the effects of 25OHD<sub>3</sub> on breast tissue, Brenaut et al. (2012) and Toral et al. (2016) reported that the status of breast cells in milk is closer to the expression state in the breast tissue of animals compared with the breast cells in vitro culture. Our previous study directly analyzed the influence of 25OHD<sub>3</sub> on the breast tissue in lactating sows, and the results indicated dietary 25OHD<sub>3</sub> supplementation improved the higher gene expressions of acetyl-CoA carboxylase  $\alpha$  (ACC $\alpha$ ) and fatty acid synthase (FAS) in the breast tissue of lactating sows (Zhang et al., 2019b). These experiments provided some reasonable evidence for effects of 25OHD<sub>3</sub> on the changes in milk composition, especially an increase in milk fat content of lactating sows. Passive immunity could be transferred from sows to piglets via the ingestion of breast milk and changes in the levels of immunoglobulins in milk reflect the maternal ability to pass immunity to neonates (Theil et al., 2014). The results from our lab reported that 25OHD<sub>3</sub> supplementation in sow diets during the last week of gestation and lactation enhanced milk immunoglobulin G (IgG) content (Zhang et al., 2019b). These studies indicated that supplementation of 25OHD<sub>3</sub> to sows could contribute to increasing milk quality through affecting the gene expression of fatty acid synthesis in the mammary gland.

### 5.3. Improvement of skeletal muscle development of piglets and carcass characteristics of growing pigs

Vitamin D can influence body Ca homeostasis, which is an important factor for interaction between cytosol and mitochondria involved in the energy metabolism of skeletal muscles (Glancy and Balaban, 2012). Supplementation of 1,25(OH)<sub>2</sub>D<sub>3</sub> to C2C12 myoblasts caused changes in gene expressions of transcription factors related to muscle development, and promoted the size and diameter of muscle fiber (García et al., 2011). García et al. (2011) and Girgis et al. (2013) reported that vitamin D<sub>3</sub> has beneficial effects on regulating skeletal muscle development. In the research conducted by Zhou et al. (2016), a total of 20 gilts were assigned randomly to regular vitamin D treatment supplemented with 50  $\mu$ g vitamin D<sub>3</sub>/kg feed or high vitamin D treatment supplemented with an additional 50  $\mu$ g of 25OHD<sub>3</sub>/kg feed. Maternal 25OHD<sub>3</sub> supplementation significantly increased the amount of muscle fiber in the longissimus dorsi (LM) of neonatal piglets and weaned piglets by 23.53% and 27.47%, respectively. In addition, maternal 25OHD<sub>3</sub> supplementation also enhanced cross-sectional areas of muscle

fiber in the psoas major (PM) and LM of weaned piglets by 12.82% and 22.62%, respectively. Real-time PCR results showed that LM and PM of piglets from sows fed with 25OHD<sub>3</sub> had higher mRNA expressions of insulin-like growth factor 2 (IGF2), insulin-like growth factor 2 receptor (IGF2R), myogenic differentiation factor 1 (MyoD1), and myogenin, but had lower mRNA expressions of myosin heavy chain I (MyHC I) and myostatin. These findings implied that maternal supplementation with 25OHD<sub>3</sub> could improve skeletal muscle development of newborn and weaning piglets via modulating the expressions of muscle transcription factors. Clements and Fraser (1988) suggested that serum 25OHD<sub>3</sub> level in the fetus is directly associated with 25OHD<sub>3</sub> level in maternal serum, and 25OHD<sub>3</sub> in maternal serum could be passed into skeletal muscle in the fetus. Therefore, changes in maternal 25OHD<sub>3</sub> level may influence development of skeletal muscle in the fetus. A study in gilts showed that the addition of 25OHD<sub>3</sub> in diets to replace 80% of vitamin D<sub>3</sub> supplementation improved the 25OHD<sub>3</sub> level in maternal serum, and significantly increased the numbers of the LM muscle fibers in the fetus on day 90 of gestation (Hines et al., 2013). Consequently, these studies have highlighted that maternal 25OHD<sub>3</sub> supplementation could promote the development of skeletal muscle in piglets before and after parturition. The previous study suggested that growing pig from sows fed with 50  $\mu$ g/kg 25OHD<sub>3</sub> had better carcass traits than growing pigs from sows fed with 9,600 IU/kg vitamin D<sub>3</sub>, but there was no difference compared with growing pigs from sows fed with 2,000 IU/kg vitamin D<sub>3</sub> (Flohr et al., 2016b). In fact, it is not clear whether this phenomenon was due to the different vitamin D treatments of sows or the different weaning weight of piglets from sows supplemented with the medium dose of vitamin D<sub>3</sub>. These results indicated that adding 25OHD<sub>3</sub> to sow diets could improve prenatal and postnatal skeletal muscle development of piglets.

### 5.4. Improvement of bone turnover markers in serum

Bone formation markers are derived from osteoblastic cells or produced by procollagen metabolism (Harada et al., 2013). Bone resorption markers are produced by the degradation products of osteoclasts or collagen degradation (Ram et al., 2015). Among enzyme activity markers, alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) belong to one of bone formation and resorption markers, respectively (Harada et al., 2013; Ram et al., 2015). In the results presented by Zhou et al. (2017), maternal 25OHD<sub>3</sub> supplementation elevated ALP activity in sow serum on day 110 of lactation and at weaning. The higher activity of serum ALP in sows supplemented with 25OHD<sub>3</sub> during gestation and lactation indicated an increased bone formation. Accurately, serum bone-specific alkaline phosphatase (BALP) is considered as a superior parameter for bone formation compared with total ALP (Tripathi et al., 2018). Seibel (2005) and Golub and Boesze-Battaglia (2007) reported that BALP accounts for more than 90% of the total ALP and is an important index associated with the calcification process of bone matrix. Recently, the results from our lab showed that maternal 25OHD<sub>3</sub> supplementation could increase BALP activity in the serum of sows and suckling piglets (Zhang et al., 2019a). The effects of maternal 25OHD<sub>3</sub> supplementation on osteocalcin (OC) and cross laps (CL) in sow serum during gestation and lactation were conducted by Weber et al. (2014). In this study, a total of 227 sows were randomly assigned to one of two dietary treatments: 114 sows fed diets with 2,000 IU/kg vitamin D<sub>3</sub>, and 113 sows fed diets with 50  $\mu$ g/kg 25OHD<sub>3</sub>. No difference was observed for the level of OC in plasma between the two groups, and 25OHD<sub>3</sub> supplementation markedly reduced the level of CL in plasma of sows at insemination and tended to reduce the level of CL in plasma of sows during early

lactation. However, 25OHD<sub>3</sub> supplementation markedly improved CL concentration in plasma of sows during late pregnancy compared with the control group. An increase of CL concentration in plasma during late pregnancy indicated that Ca reserves in maternal bones were mobilized during the rapid accumulation of skeletal Ca in the fetus (Kovacs and Kronenberg, 1997). These studies indicate that maternal supplementation with 25OHD<sub>3</sub> improves the bone biochemical indexes in the serum of sows and piglets, which could contribute to maternal and neonatal bone formation and ossification.

#### 5.5. Improvement of milk and neonatal bone fatty acid composition

Watkins et al. (2000) and Korotkova et al. (2004) reported that the fatty acid profile of bone could be influenced by polyunsaturated fatty acid (PUFA) levels in diets such as lower n-6 PUFA level or higher n-3 PUFA content, which may result in higher bone mineral content, bone mineral density and breaking force. Interestingly, the new data from our lab reported that 25OHD<sub>3</sub> supplementation in sow diets significantly reduced the ratio of n-6 PUFA to n-3 PUFA and tended to decrease C20:4n-6 content in milk (Zhang et al., 2019a). Breast milk is an important source of nutrients including energy and protein for suckling piglets. The changes in milk n-6 PUFA and n-3 PUFA contents could influence neonatal bone formation and development. The systematic explanation for PUFA and bone health was not available, but several possible theories can clarify the influences of PUFA on bone development. It has been demonstrated that n-3 PUFA promotes intestinal Ca absorption, which in turn affects the development of bone marrow cells (Lau et al., 2013). Adipocytes or osteoblasts can be differentiated from mesenchymal stem cells present in the bone marrow (Casado-Díaz et al., 2013). Several important PUFA including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) has been proven to influence bone development. The addition of DHA and EPA could not only improve bone status markers of mineralization, but also stimulate the formation of osteoblasts differentiated from mesenchymal stem cells, whereas AA could promote the activity of adipogenesis (Casado-Díaz et al., 2013). Therefore, a lower ratio of n-6 PUFA to n-3 PUFA present in the bone may be positively correlated with osteoblast activity, leading to better bone development. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is an important regulator of normal bone development, and stimulates normal bone growth at the physiological level (Korotkova et al., 2004; Gao et al., 2009). However, osteoblasts could express receptor-activated nuclear factor-κB ligand (RANKL) under the higher level of PGE<sub>2</sub>, and RANKL binds to its receptor on osteoclast precursors to promote the formation of mature osteoclasts (Lau et al., 2013). Another finding in our lab showed that the ratio of n-6 PUFA to n-3 PUFA and the content of C20:4n-6 in milk declined, which led to a decrease in the ratio of n-6 PUFA to n-3 PUFA in tibias and the contents of C20:4n-6 and PGE<sub>2</sub> in femurs of suckling piglets (Zhang et al., 2019a). It was indicated that dietary 25OHD<sub>3</sub> supplementation in sow diets during the whole lactation could improve bone development and mineralization of neonatal piglets through regulating fatty acid composition such as the lower ratio of n-6 PUFA to n-3 PUFA and the content of C20:4n-6 in milk. However, the specific mechanisms involved and the impacts of adding 25OHD<sub>3</sub> to sow diets on the fatty acid profile of breast milk remain unclear. Further studies should be undertaken to understand the influences of 25OHD<sub>3</sub> on the fatty acid profile in milk, and to examine the relationship between 25OHD<sub>3</sub> and its function on key genes related to fatty acid synthesis in the breast tissue.

#### 5.6. Increase in bone mineralization and bone biomechanical properties

Reproductive periods including pregnancy and lactation, reflect a challenge for the mothers, because they need to supply the added nutritional demands for Ca requirement of developing fetuses and neonates (Kovacs, 2016). Halloran et al. (1979) reported that the total requirement of Ca is dramatically elevated due to the growth and development of fetus and milk production (Halloran et al., 1979). The intestinal absorption rate of Ca doubles during pregnancy (Kovacs, 2016), and breastfeeding has a relationship with higher bone turnover rate, which induces the release of maternal Ca reserves during lactation (Costa et al., 2012). However, interventions limited to dietary Ca supplementation have mild effects on preventing loss of bone mass during the whole lactation (Kalkwarf et al., 1997). Several studies showed that 25OHD<sub>3</sub> supplementation in sow diets has beneficial impacts on bone quality of sows and their piglets in comparison with the use of regular vitamin D<sub>3</sub> in sow diets (Zhou et al., 2017; Zhang et al., 2019a). As presented by Zhou et al. (2017), improved femoral bone strength and density were observed in newborn piglets from sows fed with 25OHD<sub>3</sub> and dietary supplementation with 25OHD<sub>3</sub> in sow diets also tended to increase tibial ash content of newborn piglets. The data from our lab showed that bone Ca content, bone density, breaking force and stiffness in tibias and femurs were higher in lactating sows supplemented with 25OHD<sub>3</sub> (Zhang et al., 2019a). Our lab also reported that 25OHD<sub>3</sub> supplementation markedly increased tibial Ca content and tended to increase femoral Ca content of suckling piglets (Zhang et al., 2019a). The mRNA expressions of duodenal VDR, transient receptor potential vanilloid 6 (TRPV6), and calcium-binding protein D9k (CaBP-D9k) were detected in our study, and the data showed that higher mRNA expressions of these genes were observed in sows after 25OHD<sub>3</sub> supplementation, which promoted a higher rate of intestinal Ca absorption and improved bone mineralization and development of sows (Zhang et al., 2019a). Collectively, the results received from an analysis of gene expression could account for higher bone Ca content of tibias and femurs and improvement of bone biomechanical properties in sows as a result of adding 25OHD<sub>3</sub> to diets. In addition, our lab reported that piglets suckling sows fed diets with 25OHD<sub>3</sub> had higher gene expressions of VDR and claudin-2 in the ileum as well as VDR and CaBP-D9k in the colon (Zhang et al., 2019a). For neonatal piglets, the levels of milk Ca and P is relatively large and constant, and the composition of breast milk does not seem to depend on these minerals being present in diets (Mahan and Vallet, 1997). It has been reported that adequate Ca intake results in reduced transcellular transport, and when Ca intake is high or sufficient, passive absorption of Ca in the jejunum and ileum is the main route of absorption (Bronner and Pansu, 1999). The tight junctions such as claudin 2 and claudin 12 can modulate paracellular absorption of Ca (Alexander et al., 2014). Pointillart et al. (2000) reported that only a small amount of Ca that is not absorbed by the small intestine could enter the large intestine as a result of higher utilization of Ca in milk, which could explain the higher mRNA expressions of ileal claudin 2 and colonic CaBP-D9k in suckling piglets from sows fed with 25OHD<sub>3</sub> supplementation (Zhang et al., 2019a). These results suggested that maternal 25OHD<sub>3</sub> supplementation increased higher mRNA expressions of key genes related to intestinal Ca absorption, which could contribute to maternal and neonatal bone health.

### 5.7. Improvement of gut bacterial metabolites of suckling piglets and the possible action on immune function and bone health

Vitamin D<sub>3</sub> can impact microbial colonization and composition of the intestinal tract as a result of its anti-inflammatory properties. Li et al. (2015) indicated that the underlying mechanism of vitamin D<sub>3</sub> regulating gut microbiota is associated with the roles of VDR in preventing damage to the intestinal barrier and controlling the inflammatory response in the mucosa. Results of the previous study in our lab reported that maternal 25OHD<sub>3</sub> supplementation in sow diets increased butyrate content in the caecal digesta of suckling piglets (Zhang et al., 2019b). Butyrate is considered as one of the bacterial metabolites that can promote proliferation and differentiation of epithelial cells (Guilloteau et al., 2010; Morrison and Preston, 2016), increase intestinal barrier function (Kim et al., 2012), and encourage an appropriate immune response (Zeng and Chi, 2015; Schilderink et al., 2016). Butyrate derived from gut bacteria can be oxidized by host tissues, which could limit the available amount of oxygen to the mucosa (Rivera-Chávez et al., 2016). Therefore, this established environment in the mucosa is conducive to the process of microbial fermentation and contributes to synthesize short-chain fatty acids (SCFA). Emerging differences between different treatments with or without maternal 25OHD<sub>3</sub> supplementation may be due to feedback interactions between gut bacterial metabolites and host, thereby exerting beneficial effects on the intestinal barrier. Collins et al. (2016) and Villa et al. (2017) indicated that gut microbiota has positive effects on bone mineral density and breaking strength. Previous studies have suggested that the potential mechanisms included the production of SCFA or decreased intestinal inflammation (Campbell et al., 1997; Sjögren et al., 2012). Butyrate could promote bone formation and acquisition of bone mass by regulatory T cell pool-mediated up-regulation of osteogenic Wnt10b expression, because Wnt10b can improve bone formation via stimulating Wnt signaling in osteoblasts (Tyagi et al., 2018; McHugh, 2019). Limited information is available about the impacts of maternal supplementation with 25OHD<sub>3</sub> on the production of intestinal bacterial metabolites of piglets and the possible action on bone health, but it provides a theoretical link between gut bacterial metabolites influenced by 25OHD<sub>3</sub> and bone health. Interestingly, these results suggest that changes in intestinal microbiota could be a potential research direction for maternal 25OHD<sub>3</sub> supplementation to improve bone development of piglets.

### 6. Economic considerations for the application of 25OHD<sub>3</sub> in the animal industry

The conversion of substrates into intermediate or final products in animals is not complete. As a derivative of vitamin D<sub>3</sub>, 25OHD<sub>3</sub> has higher bioactivity than vitamin D<sub>3</sub> for promoting Ca absorption in the intestine. Therefore, the addition level of 25OHD<sub>3</sub> in diets could be relatively smaller than regular vitamin D<sub>3</sub>. However, the application of 25OHD<sub>3</sub> as a feed additive is limited by the costs of production and the availability of stabilizers allowed to be used in the diets. As a result of progress in production technology, it can be expected that 25OHD<sub>3</sub> would be used in animal husbandry at a relatively reasonable price (Keshavarz, 2003).

### 7. Adverse effects of 25OHD<sub>3</sub> in animals

Besides numerous beneficial aspects, there are some negative aspects attributed to 25OHD<sub>3</sub>. Vitamin D<sub>3</sub> has beneficial effects on maintaining Ca homeostasis and improving bone mineralization and formation. Paradoxically, Sato et al. (2007) and Kogawa et al. (2010) reported that vitamin D<sub>3</sub> and its metabolites could promote bone resorption in both in vitro and in vivo experiments. The

impacts of vitamin D<sub>3</sub> and its metabolites on bone development may be dependent on the supplemented dosage, physiological status, and experimental subjects. Low physiologic levels of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> could elicit osteoblast proliferation and improve bone formation and mineralization via VDR signaling pathway (Hausler et al., 2013). However, the addition of high-dose vitamin D<sub>3</sub> may cause bone catabolism and significantly inhibit bone mineralization (Yamaguchi and Weitzmann, 2012). The beneficial impacts of adding 25OHD<sub>3</sub> and canthaxanthin to maternal diets on growth performance and serum concentration of phosphorus (P) in ducklings were found in only in the lower but not higher vitamin regimen (Ren et al., 2017). Different responses of 25OHD<sub>3</sub> supplementation to distinct vitamin regimen may be caused by different doses of vitamin D or interaction among vitamins. Therefore, these findings suggest that animal physiology, dietary composition, and dosage should be taken into account when adding 25OHD<sub>3</sub> to the diet for best results.

### 8. Conclusion

This paper reviews the application of 25OHD<sub>3</sub> in diets for sow nutrition. Dietary 25OHD<sub>3</sub> can bypass the 25-hydroxylation response that exists in the liver, indicating that its biological activity is higher than that of vitamin D<sub>3</sub>, and has fewer adverse effects compared with 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub>. According to this review, maternal 25OHD<sub>3</sub> supplementation has several more beneficial effects, including increased sow performance, improved milk composition and enhanced bone characteristics in sows and piglets, promoting skeletal muscle development and influencing gut bacterial metabolites of piglets compared with vitamin D<sub>3</sub>. Collectively, 25OHD<sub>3</sub> may be a more valid and favorable alternative of vitamin D supplement in diets for the sow industry.

### Author contributions

**Lianhua Zhang:** Investigation, Data curation, Formal analysis, Writing-Original draft preparation, Conceptualization, Methodology, Software, and Editing.

**Xiangshu Piao:** Conceptualization, Methodology, Writing-Reviewing and Editing, Supervision.

### Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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