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REVIEW

Pigs

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Dietary supplementation of 25-hydroxycholecalciferol as an alternative to cholecalciferol in swine diets: A review

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Abstract

25-hydroxycholecalciferol (25-OHD$_3$) formed via hepatic hydroxylation from vitamin D, cholecalciferol, represents the precursor of the biologically active vitamin D hormone, 1,25-dihydroxyvitamin D. Due to a higher absorption rate and the omission of one hydroxylation, dietary supplementation of 25-OHD$_3$ instead of vitamin D$_3$ is considered to be more efficient as plasma concentrations of 25-OHD$_3$ are increased more pronounced. The present review summarises studies investigating potential beneficial effects on mineral homeostasis, bone metabolism, health status and performance in sows, piglets and fattening pigs. Results are inconsistent. While most studies could not demonstrate any or only a slight impact of partial or total replacement of vitamin D$_3$ by 25-OHD$_3$, some experiments indicated that 25-OHD$_3$ might alter physiological processes when animals are challenged, for example, by a restricted mineral supply.

KEYWORDS

25-hydroxycholecalciferol, bone development, mineral supply, pig nutrition, vitamin D

1 | INTRODUCTION

Vitamin D, including cholecalciferol (D$_3$) and ergocalciferol (D$_2$), can be either synthesised in the skin by UVB light of the sun (D$_3$) or be absorbed from the diet (both, D$_2$ and D$_3$) (Holick, 2007). In conventional animal husbandry systems that restrict the access of livestock to sunlight, vitamin D supply mainly depends on feed (Alexander et al., 2017). Vitamin D, in most cases vitamin D$_3$, is added as a supplement to meet the demands despite the lack of UV exposure in animal houses, especially when mineral homeostasis is challenged, for example, during pregnancy, lactation and growth (Halloran et al., 1979; Hollis & Wagner, 2017; Kovacs, 2008).

As 25-hydroxycholecalciferol (25-OHD$_3$) is approved as a feed additive for poultry and pigs in the European Union, vitamin D can partially or completely be replaced by 25-OHD$_3$ (EFSA, 2009; European Union, 2009; von Rosenberg et al., 2016). The maximum allowed levels of vitamin D and 25-OHD$_3$ in feed for pigs are 50 μg/kg feed, respectively (conversion factor: 40 IU cholecalciferol = 0.001 mg = 1 μg cholecalciferol) (European Union, 2019).

Dietary supplementation of 25-OHD$_3$ is thought to have two advantages: First, 25-OHD$_3$ is absorbed more efficiently (Bar et al., 1980). Second, the first hydroxylation step in the liver is bypassed (Christakos et al., 2016). Therefore, the vitamin D status of animals, as determined by measuring serum levels of 25-OHD$_3$, can be increased about five times more effectively by application of 25-OHD$_3$ than by application of

vitamin D_3 (Cashman et al., 2012). Since vitamin D status is related not only to mineral homeostasis but also to immune function (Morán-Auth et al., 2013), cell differentiation (Holick, 2007), muscle strength and other physiological processes (Tieland et al., 2013), dietary supplementation of 25-OHD_3 instead of conventional vitamin D_3 could have beneficial effects on pig health and fitness.

The present review aims to give an overview about the impact of dietary supplementation of 25-OHD_3 compared to conventional vitamin D_3 on pigs' health and performance. Although potential benefits have been described the results are inconsistent.

2 | UPTAKE, SYNTHESIS AND METABOLISM OF VITAMIN D METABOLITES

In sun-exposed skin, previtamin D_3 is synthesised from 7-dehydrocholesterol under the influence of UVB radiation (280–320 nm). Previtamin D_3 isomerises to vitamin D_3 depending on temperature and time (Lehmann & Meurer, 2010). Excess previtamin D_3 is either converted to lumisterol, tachysterol, and toxisterol, or retransformed to 7-dehydrocholesterol (Holick, 2004; Lehmann & Meurer, 2010). UV exposure at doses above 1 MED (minimum erythema dose) is equivalent to approximately 20,000 IU vitamin D_3 in humans (Holick, 2009b) and does not result in a further increase in vitamin D_3 synthesis. Reversible isomerization and irreversible inactivation of previtamin D_3 and vitamin D_3 follows. Maximum 25-OHD_3 concentrations inducible by UVB radiation are thus reached in humans at UV doses below 1 MED (Gilchrest, 2008).

Vitamin D_2/D_3 can also be provided via the diet. While vitamin D_2 produced from the ergosterol of the cell membrane of fungi is found in UV-exposed mushrooms and grass, especially hay dried in the sunlight, significant amounts of vitamin D_3 are only present in products of animal origin, such as egg yolk, liver and fish (Benedik, 2022; Jaepelt et al., 2011; Schmid & Walther, 2013). Intestinal absorption of vitamin D_3 and 25-OHD_3 from exogenous sources occurs almost entirely in the duodenum (Gómez-Verduzco et al., 2013) and the upper jejunum (Bar et al., 1980) via micelle formation. In contrast, the absorption of 25-OHD_3 does not require the presence of bile acids (Nechama et al., 1978). For details, see Section 5.1.

Vitamin D_3, 25-OHD_3 and the other vitamin D metabolites are transported bound to plasma proteins in both, portal and systemic circulation. Vitamin D-binding proteins (DBP), including the specific vitamin DBP, circulate in plasma at concentrations 20 times higher than vitamin D metabolites and bind them almost completely (99%) (Kochupillai, 2008). Before it can exert effects, vitamin D_3 must be activated via two hydroxylation steps in the liver and kidney (Figure 1) (Lehmann & Meurer, 2010). For this purpose, vitamin D_3 synthesised in the skin or ingested with food is transported to the liver via DBP and hydroxylated to 25-OHD_3 at the 25-C atom in a largely unregulated manner via various vitamin D 25-hydroxylases (Christakos et al., 2019; Jones et al., 2014; Jovičić et al., 2012). Bound to DBP, 25-OHD_3 is transported to the kidneys (proximal tubule cells) where it is taken up with the help of the receptor protein megalin (Nykjaer et al., 1999). A

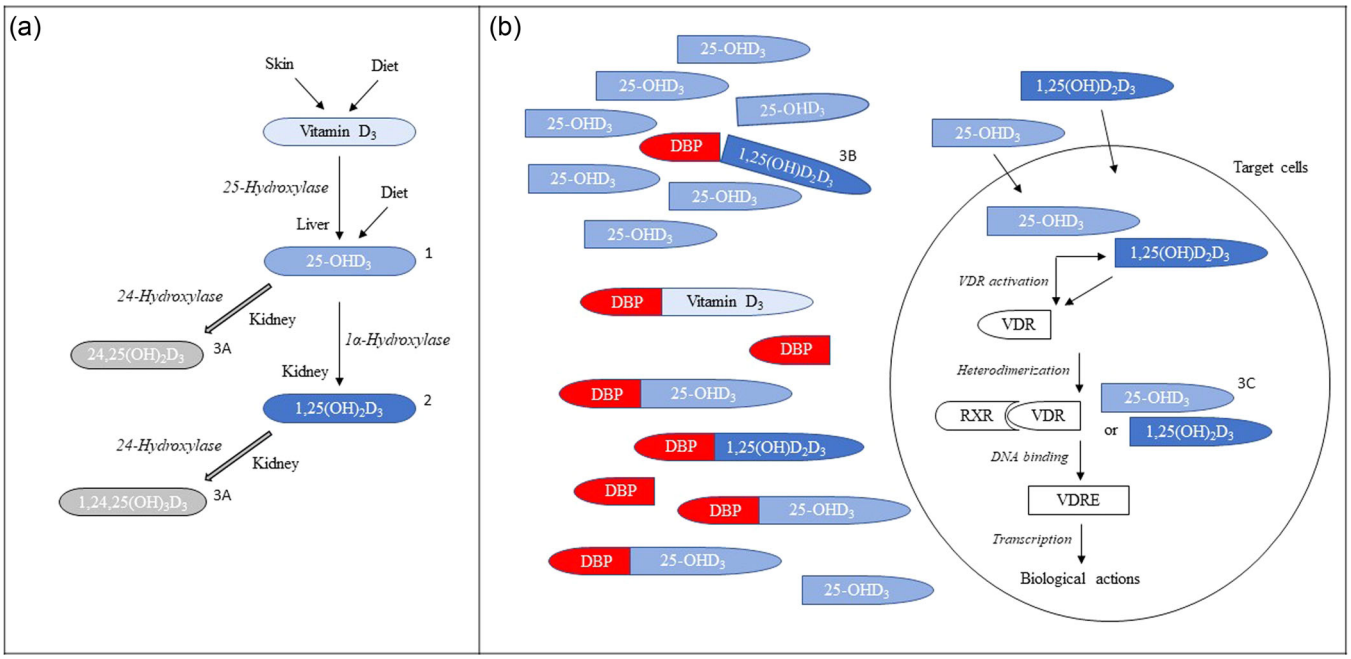

FIGURE 1 Vitamin D metabolism (a) and VDR activation (b) as impacted by high plasma concentrations of 25-OHD_3. [1]First hydroxylation and largely unregulated formation of 25-OHD_3; [2]Second hydroxylation and formation of 1,25$(OH)_2D_3$ (biologically most active metabolite) regulated by PTH, Ca, P_i, FGF23 and 1,25$(OH)_2D_3$; High concentrations of 25-OHD_3 could lead to [3A]inactivation of 25-OHD_3 to 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ to 1,24,25$(OH)_3D_3$, [3B]displacement of 1,25$(OH)_2D_3$ from the DBP and thus more free 1,25$(OH)_2D_3$ and [3C]binding of 25-OHD_3 to the VDR. DBP, D-binding proteins; VDR, vitamin D receptor.

decrease in plasma ionised calcium (Ca) concentration detected by the calcium-sensing receptor results in the release of parathyroid hormone (PTH) from the parathyroid glands (Kantham et al., 2009). In the kidney, PTH activates the 1-α-hydroxylase (CYP27B1) (Murayama et al., 1999). This enzyme mediates the second hydroxylation at the 1-C atom, the crucial step in the formation of the biologically active form of vitamin D, 1,25-dihydroxycholecalciferol (1,25$(OH)_2D_3$), a steroid hormone (Christakos et al., 2016; Jones et al., 2012; Jones et al., 2014). The 1α-hydroxylation by CYP27B1 is subject to a tight regulation not only by PTH, but also by Ca^{2+}, phosphate ($_{Pi}$), fibroblast growth factor 23 (FGF23), and 1,25$(OH)_2D_3$ itself as a negative feedback (Christakos et al., 2019; van Etten et al., 2008). High Ca^{2+} and $_{Pi}$ levels have a negative effect, while low levels have a positive effect on 1α-hydroxylase expression (van Etten et al., 2008). FGF23 is a phosphatonin produced by osteocytes in response to high plasma concentrations of $_{Pi}$ and 1,25$(OH)_2D_3$ (Schiavi & Kumar, 2004). FGF23 has been shown to increase urinary excretion of $_{Pi}$ and decrease the expression of CYP27B1 (Antoniucci et al., 2006; Perwad et al., 2005).

Incorporating another OH group at position 24, CYP24A1 (24-hydroxylase) initiates the inactivation of 25-OHD_3 to 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ to 1,24,25$(OH)_3D_3$ (Christakos et al., 2016; 2019; Jones et al., 2012). 24-Hydroxylation is activated by 1,25$(OH)_2D_3$, FGF23, Ca and P_i and inhibited by PTH (Bikle & Christakos, 2020). As the production of CYP24A1 is induced by 1,25$(OH)_2D_3$, CYP24A1 serves as a brake on the 1,25$(OH)_2D_3$ level (Bikle & Christakos, 2020). The biological activity of the formed 24,25$(OH)_2D_3$ is about 10,000 times lower than that of 1,25$(OH)_2D_3$ (Kanis et al., 1982).

3 | BIOLOGICAL ACTIONS OF VITAMIN D

The biological action of 1,25$(OH)_2D_3$ is mediated after binding to the vitamin D receptor (VDR) (Figure 1) (Haussler et al., 2013). Once VDR is occupied by 1,25$(OH)_2D_3$, it forms a heterodimer together with the retinoid X receptor and subsequently binds to so called vitamin D responsive elements in the promoter region of target genes (Haussler et al., 2013) which leads to the activation or repression of the transcription of vitamin D dependent genes (Christakos et al., 2016). It has been estimated that in humans and mice approximately <3% of the genome is regulated to a varying extent directly or indirectly by 1,25$(OH)_2D_3$ (Bouillon et al., 2022). The widespread abundance of the VDR in many tissues and cells highlights the potential multitude of physiological effects of 25-OHD_3 and 1,25$(OH)_2D_3$ (Rosen et al., 2012). The major biological networks of differentiation, metabolism, immune function, and so forth involve genes whose expression is directly modulated by 1,25$(OH)_2D_3$ (Haussler et al., 2013).

3.1 | Mineral homeostasis

The role of vitamin D in mineral homeostasis and bone metabolism has been known for a long time. The expression of RANKL, osteopontin, and osteocalcin (all bone mineral remodelling), TRPV6, CaBP9k, and claudin 2 (intestinal Ca absorption), and TRPV5, Klotho, and Npt2c (renal Ca and $_{Pi}$ reabsorption), and other relevant genes is regulated by 1,25$(OH)_2D_3$ (Haussler et al., 2013). In VDR-null mice, intestinal Ca absorption is severely impaired (Van Cromphaut et al., 2001). Although vitamin D is only the precursor of 1, 25-$(OH)_2D_3$ and intestinal Ca absorption correlates better with 1,25$(OH)_2D_3$ than with serum concentrations of 25-OHD_3 (Bouillon & Rosen, 2018), an appropriate vitamin D status is the prerequisite for maintaining mineral homeostasis. Heaney (2007) states that in humans, there is a significant, inverse relationship between vitamin D status determined by plasma concentrations of 25-OHD_3 and intestinal Ca absorption, bone mineral density and the risk of falls and cancer, especially for plasma concentrations of 25-OHD_3 below 32 ng/ml.

3.2 | Fertility

Pal et al. (2016) could show that in women diagnosed with polycystic ovary syndrome seeking pregnancy, the likelihood of achieving live birth was reduced by 44% for women presenting with 25-OHD_3 serum concentrations below 30 ng/ml. In addition, studies in humans suggest a lower birth weight in infants born to mothers with low 25-OHD_3 levels (Aghajafari et al., 2013; Theodoratou et al., 2014; Wei et al., 2013). Vitamin D might also influence fertility due to the direct effect of 1,25$(OH)_2D_3$ on target genes affecting implantation (Du et al., 2005). Furthermore, deficiency of 25-OHD_3 might be combined with lower ovarian reserve in humans (Dennis et al., 2012; Jukic et al., 2015; Merhi et al., 2012). Even when hypocalcemia was corrected, uterine hypoplasia and impaired folliculogenesis occurred in female VDR-null mice, while decreased sperm production and sperm motility were seen in male VDR-null mice (Kinuta et al., 2000). This indicates the importance of the VDR in reproduction.

3.3 | Immune function

Other studies show that vitamin D is an important regulator of immune function irrespective of Ca homeostasis (Tamblyn et al., 2015). Vitamin D deficiency assessed by 25-OHD_3 serum concentrations as well as low 1,25$(OH)_2D_3$ levels seem to compromise the transcription of genes involved in immunological functions (Morán-Auth et al., 2013). According to Wimalawansa (2018) and Wimalawansa (2019), the incidence and severity as well as complications of various diseases can be reduced by higher serum concentrations of 25-OHD_3 (30–60 ng/ml). The VDR is expressed in B cells and different types of T cells. Monocytes and macrophages express extrarenal CYP27B1 that is needed for local production of 1,25$(OH)_2D_3$ that exerts its effects via autocrine and paracrine pathways (Bouillon & Rosen, 2018; Hewison, 2010; Stoffels et al., 2006; van Etten et al., 2008). Therefore, a link between immune dysfunction and vitamin D status is likely. This assumption is corroborated by the association of a poor vitamin D deficiency with infections like tuberculosis, HIV, and pulmonary diseases (Bouillon & Rosen, 2018; Holick, 2010; Janssens et al., 2009; Liu et al., 2006). It has also been reported that the severity of various intestinal diseases worsens in VDR

null mice, accompanied by an increased expression of inflammatory cytokines (Froicu & Cantorna, 2007). Yu et al. (2008) confirms that the absence of the VDR can lead to an inflammatory response to non-pathogenic bacteria.

In chicks from hens supplemented with 25-OHD$_3$, phagocytic activity of leucocytes was stimulated in comparison to the control group treated with vitamin D$_3$ (Saunders-Blades & Korver, 2015). Furthermore, in ovo treatment with 25-OHD$_3$ increased the plasma concentrations of immunglobulin G (IgG) of the chicks in comparison to a treatment with vitamin D$_3$ (Fatemi et al., 2021).

3.4 | Muscle function

As the VDR has also been identified in smooth and skeletal muscle, it can be assumed that muscle is a target for vitamin D and its metabolites (Bischoff et al., 2001; Bischoff-Ferrari, Borchers, et al., 2004; Boland et al., 1985; Ceglia et al., 2010; Srikuea et al., 2012). VDR knockout mice showed altered gait, shorter strides and decreased muscle coordination and balance with shorter holding times in rotarod and tilting box tests, among others (Burne et al., 2005; Minasyan et al., 2009). Various studies showed that not only contractility but also gene expression and differentiation of smooth muscle is influenced by vitamin D metabolites in different organs (Bossé et al., 2007; Giraldi et al., 2015; Morelli et al., 2008). Wimalawansa (2018) confirms that vitamin D has a significant role in supporting the cardiovascular system and cardiac, endothelial, and smooth muscle cell functions. Adequate vitamin D status is also required for skeletal muscle maintenance, growth and strength (Ceglia, 2008; Tieland et al., 2013). In a study by Endo et al. (2003), the disruption of the VDR gene in mice interfered with the regulation of transcription factors and decreased skeletal muscle fibre diameter independently of Ca homeostasis. Clinical studies in humans demonstrated an association between serum concentrations of 25-OHD$_3$ and muscle strength (Mowé et al., 1999; Stein et al., 1999) and suboptimal vitamin D status can increase the risk for muscle pain and compromised muscle performance (Bischoff-Ferrari, Dietrich, et al., 2004; Larson-Meyer, 2015). In addition, the stimulated expression of VDR and CYP27B1 in injured muscle suggests a role of vitamin D in muscle regeneration (Makanae et al., 2015).

3.5 | Biological activity of the different vitamin D metabolites

In vivo and in vitro studies have shown that pharmacological plasma concentrations of 25-OHD$_3$ result in activation of the VDR, producing effects similar to those of 1,25(OH)$_2$D$_3$ (Jones, 2008). Studies using CYP27B1 knockout mice confirm that 25-OHD$_3$ can bind to the VDR and induce transcription (DeLuca et al., 2011). But due to the lower affinity of 25-OHD$_3$ to the VDR and the higher affinity to the DBP compared to 1,25(OH)$_2$D$_3$, it can be assumed to be a poor agonist. Probably, 25-OHD$_3$ itself is only able to activate the VDR when serum concentrations are above 150 ng/ml (Quesada-Gomez & Bouillon, 2018).

In addition, competition for binding to DBP could increase the concentration of the free and thus more biologically active 1,25(OH)$_2$D$_3$ (Jones, 2008). Lower concentrations of free 1,25(OH)$_2$D$_3$ in pseudo-vitamin D-deficiency rickets type I piglets versus in normal piglets were recognised as exclusive triggers of rachitic symptoms (Kaune et al., 1990).

The compounds formed during inactivation, 24,25(OH)$_2$D$_3$ and 1,24,25(OH)$_3$D$_3$ (Christakos et al., 2019), are also thought to have effects. Studies suggest that 24,25(OH)$_2$D$_3$ regulates chondrocytes and promotes their maturation in the endochondral lineage and thus may be important in endochondral ossification (Boyan et al., 2001). In addition, 24,25(OH)$_2$D$_3$ has been associated with fracture healing (Demay, 2018; Martineau et al., 2018).

Overall, it can be assumed that vitamin D does have additional effects beyond mineral status and bone health. For humans, there is a constant debate about the definitions of vitamin D insufficiency that has been changed from levels below 20 ng/ml to levels below 30 ng/ml. Therefore, a re-evaluation of the optimal supply might be worthwhile not only in human, but also in animal nutrition.

4 | VITAMIN D STATUS

4.1 | Humans

Due to the good correlation with vitamin D supply or endogenous production and a long half-life (10–21 days, Flohr et al., 2014), serum concentrations of 25-OHD$_3$ are generally accepted as a measure for vitamin D status (DeLuca, 2008; Heaney et al., 2003; Holick, 2009a). In humans, blood levels of 25-OHD$_3$ ≤20 ng/ml are considered as a vitamin D deficiency. A level of 21–29 ng/ml is considered insufficient, while >30 ng/ml represents an optimal state (Holick, 2009b). To avoid the potential health risks associated with vitamin D deficiency, serum 25-OHD$_3$ levels should be maintained in the range of 30–50 ng/ml (Sosa Henríquez & Gómez de Tejada Romero, 2020). von Domarus et al. (2011) confirmed that 25-OHD$_3$ concentrations of at least 30 ng/ml are necessary to ensure adequate bone mineralisation. A study by Luxwolda et al. (2012) confirmed that people with traditional lifestyles who have lifelong, year-round exposure to tropical sunlight have a mean circulating 25-OHD$_3$ concentration of 46 ng/ml. The highest plasma concentrations of 25-OHD$_3$ probably ever measured in a healthy human exposed to sunlight was 90 ng/ml (Haddock et al., 1982). The normative range for 25-OHD$_3$ can therefore be defined up to 100 ng/ml (Holick, 2009a). With oral supplementation, 25-OHD$_3$ blood levels increase by 1 ng/ml for every 100 IU of vitamin D ingested (Holick, 2009b). Vitamin D intoxication occurs when blood 25-OHD$_3$ levels exceed 150–200 ng/ml (Holick, 2009b). In contrast, Jones (2008) state that the concentration of 25-OHD$_3$ must increase to 300 ng/ml to cause vitamin D toxicity.

Adequate maternal vitamin D$_3$ supply during the reproductive period, including pregnancy and lactation, is important because circulating foetal and neonatal 25-OHD$_3$ levels depend on maternal 25-OHD$_3$ status (Brannon & Picciano, 2011). Maternal 25-OHD$_3$ can cross the placenta and reach the foetus via the umbilical cord, whereas maternal 1,25(OH)$_2$D$_3$ cannot readily do so (Hossein-nezhad

& Holick, 2013; Noff & Edelstein, 1978; Shin et al., 2010). Brown et al. (1980) confirm that the concentration of 25-OHD_3 in human umbilical cord blood is about 87% of that in maternal blood. This suggests a relatively free transfer of 25-OHD_3 between mother and foetus. O'Callaghan et al. (2018) confirmed that the concentrations of 25-OHD_3 in human, maternal and cord blood were highly correlated. However, the 25-OHD_3 concentration in the umbilical cord was on average 52% of maternal levels in this study.

4.2 | Pigs

Information on reference values for swine is inconsistent. A study from New Zealand reported plasma concentrations of 25-OHD_3 between 11.0 and 33.7 ng/ml for juvenile pigs and between 49.4 and 102.0 ng/ml for adult pigs kept indoors (Fairweather et al., 2013). Madson et al. (2012) summarised data from four studies and also describe differences depending on age: according to this study from the USA, the reported intervals for newborn piglets are 5–15 ng/ml, for 10-day-old piglets 8–23 ng/ml, for growing piglets (post-weaning) 18–30 ng/ml and for mature pigs 35–70 ng/ml. Around parturition, however, 25-OHD_3 concentrations of sows can range from 35 to 100 ng/ml (Madson et al., 2012). The rather low concentrations reported for piglets are probably related to the transfer to milk. The concentration in porcine and human colostrum amounts to approximately 20% of the maternal serum concentration (Madson et al., 2012; Pilz et al., 2018). In interpreting the reference data, it must be taken into account that both solar radiation (Kolp et al., 2017) and the supplied dietary vitamin D levels (Lauridsen et al., 2010) have an influence on the plasma concentrations of 25-OHD_3. Unfortunately, the studies by Fairweather et al. (2013) and Madson et al. (2012) allow only limited informations in this regard. In addition, precise animal numbers are not provided.

Arnold et al. (2015) investigated serum 25-OHD_3 concentrations of pigs of different age groups. For this purpose, 1200 serum samples sent to a diagnostic laboratory located in Iowa for various reasons other than vitamin D status surveillance were analysed for serum 25-OHD_3 concentration. A seasonal effect was observed in nursery and fattening herds (indoor confinement) between the months of January and June, with significantly higher concentrations in June (Nursery, +58%; Finisher, +15%; Boar, +44%). In contrast, in the grower age category, 25-OHD_3 concentrations were under indoor conditions significantly higher in January (+16%). No difference was detected in sows. In samples taken from animals kept in indoor housing lower 25-OHD_3 concentrations were detected compared to samples from animals in outdoor housing (Nursery, −77%; Grower, −70%; Finisher, −67%; Sow, −36%). The most interesting observation was the large variations detected in all age groups: In pigs aged 10–14 weeks, serum concentrations ranged between 3.4 and 54.1 ng/ml, at the age of 6–8 months between 3.7 and 77.9 ng/ml, and in mature sows and boars, the authors reported serum concentrations of 25-OHD_3 between 4.7 and 94.5 ng/ml and between 8.9 and 93.8 ng/ml, respectively. Minimum and optimal plasma concentrations of 25-OHD_3 in pigs at different age and reproductive stages and different housing systems are currently poorly available. However, the large variations shown in the study by Arnold et al. (2015) suggest that individual animals probably have a suboptimal vitamin D status. In a very recent study conducted by Jakobsen et al. (2022), 97 sows from five different Danish outdoor housing systems were sampled. The mean serum concentration was 67 ng/ml, with a range from 32 to 134 ng/ml. Interestingly, these concentrations are approximately twice as high in comparison to indoor housing systems with a dietary supply of 20 µg (800 IU) per kg feed according to the Danish recommendations (Jakobsen et al., 2022). So far, optimal dietary supply of vitamin D has been estimated by dose-response studies, not by measuring 25-OHD_3 in plasma. To date it is not known whether the differences between vitamin D status in sun-exposed pigs and animals from indoor housing systems are of any relevance. Furthermore, even in human samples the analysis of 25-OHD_3 is not trivial and the different methods applied complicate the interpretation and the comparison of results from different studies (Enko et al., 2014). Reliable reference values should be urgently developed in future studies to better assess vitamin D status of pigs.

5 | DIETARY SUPPLEMENTATION WITH 25-OHD_3 VERSUS VITAMIN D_3

5.1 | Vitamin D-status

5.1.1 | Absorption

In terms of absorption, vitamin D_3 and 25-OHD_3 show differences that can be attributed to their polarity (polar hydroxylated form and non-polar non-hydroxylated form) and solubility (Maislos et al., 1981). The absorption mechanism of the non-hydroxylated vitamin D_3 depends on micelle formation and thus on fat digestion and secretion of bile (Gómez-Verduzco et al., 2013; Maurya & Aggarwal, 2017; Raimundo et al., 2015). Maislos et al. (1981) administered vitamin D_3, 25-OHD_3 and 1,25$(OH)_2D_3$ directly into the duodenum of rats. They could show that the hydroxylated metabolites were absorbed faster and to a larger extent. From the appearance in blood and mesenteric lymph they concluded that the polar vitamin D metabolite 25-OHD_3 is absorbed directly into the portal blood and not only via chylomicrons into the lymph. This results in a significantly faster and more efficient absorption of 25-OHD_3 compared to that of vitamin D_3 reported not only in rats but also in chicken (Bar et al., 1980; Maislos et al., 1981; Sitrin et al., 1982). While the absorption efficiency of supplied vitamin D_3 is about 70% in healthy humans, it is close to 100% for 25-OHD_3 (Quesada-Gomez & Bouillon, 2018). Especially when the intestinal absorption capacity is reduced because of other health issues the higher intestinal absorption rate of orally administered 25-OHD_3 may be of critical importance (Quesada-Gomez & Bouillon, 2018).

5.1.2 | Fattening pigs

In a study by Duffy, Kelly, Rajauria, Jakobsen, et al. (2018), fattening pigs supplemented with 50 µg/kg 25-OHD_3 instead of 50 µg/kg

vitamin D_3 showed significantly higher serum concentrations of 25-OHD_3 (+112%). In an older work by Sugiyama et al. (2013), an additional supplementation of 50 µg/kg 25-OHD_3 also resulted in significantly increased serum concentrations of 25-OHD_3 in fattening pigs (+326%). However, as the exchange of vitamin D_3 and 25-OHD_3 was not equivalent, a quantitative interpretation of these results is difficult. Table 1 summarises the results of several comparative studies on the influence of vitamin D form (vitamin D_3 vs. 25-OHD_3, equivalent exchange) on vitamin D status (25-OHD_3 serum concentrations, ng/ml) of finishing pigs, sows and their offspring.

5.1.3 | Sows and their offspring

Dietary replacement of vitamin D_3 (62.5 µg/kg feed) with 25-OHD_3 (12.5 µg vitamin D_3 and 50 µg 25-OHD_3 per kg feed) in the diet significantly increased plasma concentrations of 25-OHD_3 in both sow (mean, +63%) and foetus (+27%) (Coffey et al., 2012). In the studies by Weber et al. (2014) and by Flohr et al. (2016), plasma 25-OHD_3 concentrations were also elevated more pronounced with dietary supplementation of 25-OHD_3 compared to vitamin D_3 in sows at each sampling time point (mean, +124% and 101%). Lauridsen et al. (2010) used different dosages (5, 20, 35 and 50 µg) of either vitamin D_3 or 25-OHD_3. The enhancement of plasma 25-OHD_3 with increasing dosage was 2–3 times higher in animals fed 25-OHD_3 instead of vitamin D_3.

The concentrations of 25-OHD_3 in human cord blood amounted to 52% to 87% of the concentrations detected in maternal blood (see Section 4.1). In a study in sows, the cord blood concentrations of 25-OHD3 amounted to 38% in comparison to maternal blood when animals were fed 50 µg/kg vitamin D_3 and to 58% when vitamin D_3 was supplemented with an additional 50 µg/kg 25-OHD_3 administration (Zhou et al., 2017). Unfortunately, the authors did not give any explanation for this difference in transfer.

Thayer et al. (2019) compared the combination of 25 µg/kg 25-OHD_3 and 12.5 µg/kg vitamin D_3 with 37.5 µg/kg conventional vitamin D_3 in the diets fed to the sows during gestation and lactation as well as in the nursery, rearing and fattening diet used later on in the offspring. Although vitamin D status was improved in sows (+56%) and 25-OHD_3 concentrations were higher in colostrum (+61%) and mature milk (+49%, 21 day post-partum [pp]) in the treatment group, serum concentrations of 25-OHD_3 in piglets at birth and at weaning were below the reference range reported by Madson et al. (2012) in both groups. During rearing and fattening, serum concentrations of 25-OHD_3 were significantly improved with maternal dietary supplementation of 25-OHD_3. This is in line with a study by Witschi et al. (2011). In addition to the treatment of the sows, the piglets were offered a corresponding nursery diet starting on Day 21. While there was no difference between the groups when the complementary feeding was started, an increase in serum 25-OHD_3 could be seen on Day 33. The difference became even more pronounced on Day 77, indicating that the transfer of 25-OHD_3 from the sow to the piglet via milk is limited. This assumption is further supported by the above-mentioned study by Flohr et al. (2016) in which the piglets did not present with higher 25-OHD_3 serum concentrations at weaning although a treatment effect was demonstrated at birth (+59%).

However, in the studies by Zhang Li, et al., (2019) Zhang, Hu, et al., (2019), supplementation of 25-OHD_3 to sows instead of vitamin D_3 resulted in higher concentrations of 25-OHD_3 in the umbilical cord and in milk on 7, 14 and 21 day pp (between +80% and +100%), but not in colostrum—probably because the treatment was started only 1 week before farrowing. Nevertheless, the vitamin D status in the piglets was significantly improved from birth until weaning although there was no additional supply of vitamin D metabolites via a nursery diet. Furthermore, the authors reported increased serum concentrations of 1,25$(OH)_2D_3$ in sows (farrowing, +27%; weaning, +108%) and piglets (Day 14, +14%; Day 21, +18%) as well as increased renal messenger RNA (mRNA) expression of CYP27B1. In sows, increased CYP24A1 expression was also detected, which is a counter regulation to protect against excessive accumulation of toxic hormone levels. A strong induction of renal CYP24A1 after supplementation with 25-OHD_3 was also found by Wilkens et al. (2016) in sheep and Thayer et al. (2019) reported increased 24,25$(OH)_2D_3$ concentrations in the serum of offspring of 25-OHD_3-fed sows (in equivalent comparison to vitamin D_3) at birth (+47%), rearing (+119%), and fattening (+69%).

Further studies examining the counter regulation and its physiological effects in pigs could provide important new insights. Although serum 25-OHD_3 is generally accepted as a measure of vitamin D status and supply, the rise in serum 25-OHD_3 after oral supplementation of vitamin D_3 is less pronounced when baseline levels of 25-OHD_3 are high. With very high daily intakes the increase of serum 25-OHD_3 becomes non-linear and seems to reach a plateau in humans (Brouwer-Brolsma et al., 2016; Gallagher et al., 2012; Ross et al., 2011). In contrast, oral administration of 25-OHD_3 linearly increases serum concentrations of 25-OHD_3 irrespective of basal values, probably due to the difference in respect to the mechanism of absorption and the skipping of the hydroxylation step in the liver.

Taken together, serum concentrations of 25-OHD_3 in fattening pigs and sows were increased more efficiently with 25-OHD_3 treatment in comparison to an equivalent amount of vitamin D_3 at any time. However, this did not result in a clear beneficial effect on vitamin D status of the offspring. While the association between the dietary vitamin D metabolite administered to the sows and the vitamin D status of the offspring at birth supports the assumption that maternal 25-OHD_3 reaches the foetus via the umbilical cord, an influence on the vitamin D status of the offspring at weaning via milk seems very unlikely. However, oral supplementation of 25-OHD_3 via the prestarter does seem to have an effect on the vitamin D status of the suckling piglets.

5.2 | Reproduction and offspring development

5.2.1 | Litter size

Coffey et al. (2012) compared two diets containing either 62.5 µg/kg vitamin D_3 or a combination of 12.5 µg/kg vitamin D_3 and 50 µg

TABLE 1 Influence of dietary vitamin D form (vitamin D_3 vs. 25-OHD_3) on vitamin D status (25-OHD_3 serum or plasma concentrations) of pigs

Study	Dose	Ca (g/kg)[a]	P (g/kg)[a]	Type	Time of sampling	RI (ng/ml)[b]	25-OHD_3 (ng/ml) in the control group	25-OHD_3 (ng/ml) in the 25-OHD_3 group	*p* Value
Duffy, Kelly, Rajauria, Jakobsen, et al. (2018)	50 μg/kg vitamin D_3 versus 50 μg/kg 25-OHD_3	-	-	Fattening pig, 58 kg (start)	Trial day 55 (108 kg)	18–30	27.7	58.8	<0.05
Coffey et al. (2012)	62.5 μg/kg vitamin D_3 versus 12.5 μg/kg vitamin D_3 + 50 μg/kg 25-OHD_3	7.4	7.3	Sow (Gestation)	43 day ai (start)	35–70	53.8	57.4	N.s.
					13 day ai		56.7	89.7	<0.05
					46 day pi		55.7	95.8	<0.05
					89 day pi		58.2	92.8	<0.05
				Foetus (M)	90 day pi	–	6.5	8.2	<0.05
Weber et al. (2014)	50 μg/kg vitamin D_3 versus 50 μg/kg 25-OHD_3	7.6/8.1	6.6/6.9	Sow (Gestation/ Lactation)	1 day pi	35–70	14.0	23.0	<0.05
					28 day pi		22.6	48.0	<0.05
					80 day pi		17.1	41.3	<0.05
					5 day pp		11.6	26.3	<0.05
					28 day pp		15.5	43.0	<0.05
Flohr et al. (2016)	50 μg/kg vitamin D_3 versus 50 μg/kg 25-OHD_3	8.2/8.3	6.4/7.0	Sow (Gestation/ Lactation)	1 day pi (start)	35–70	43.9	45.9	N.s.
					100 day pi		29.2	59.5	<0.05
					1 day pp	35–100	26.1	55.4	<0.05
					21 day pp	35–70	50.9	94.6	<0.05
				Piglet (M)	1 day pn	5–15	2.2	3.5	<0.05
					21 day pn	8–23	7.0	6.1	N.s.
Thayer et al. (2019)	37.5 μg/kg vitamin D_3 versus 12.5 μg/kg vitamin D_3 + 25.0 μg/kg 25-OHD_3	7.6/7.7	4.8/5.2[c]	Sow (Gestation/ Lactation)	100 day pi	35–70	21.2	31.4	<0.05
					1 day pp	35–100	17.8	25.3	<0.05
					21 day pp	35–70	27.6	48.8	<0.05
				Piglet (M)	1 day pn	5–15	2.1	2.0	N.s.
					21 day pn	8–23	4.7	3.6	<0.05
		7.9/6.9	5.5/5.2[c]	Pig (M + D)	59 day pw	18–30	16.6	36.4	<0.05
		5.5/4.5	3.3/2.6[c]		156 day pw		17.8	30.0	<0.05
				Colostrum	1 day pp	–	0.3	0.5	<0.05

TABLE 1 (Continued)

Study	Dose	Ca (g/kg)[a]	P (g/kg)[a]	Type	Time of sampling	RI (ng/ml)[b]	25-OHD$_3$ (ng/ml) in the control group	25-OHD$_3$ (ng/ml) in the 25-OHD$_3$ group	*p* Value
				Milk	21 day pp	–	0.5	0.7	<0.05

Abbreviations: ai, ante insemination; D, Direct; M, Maternal; N.s., Not significant; -, no indication; pi, post-insemination; pn, post-natum; pp, post-partum; pw, post-weaning; RI, Reported intervals.

[a]as-fed basis;

[b]Madson et al. (2012);

[c]Standardized digestible P.

25-OHD$_3$/kg fed from Day 43 before mating. On Day 90 after insemination, gilts were sacrificed. Gilts fed the experimental diet that included 25-OHD$_3$ showed more fetuses per litter on 90th day of gestation (12.7 vs. 10.2). However, the studies by Flohr et al. (2016), Weber et al. (2014) and Thayer et al. (2019) did not demonstrate any beneficial effect 25-OHD$_3$ on the number of piglets born.

Lauridsen et al. (2010) found that an increased dosage of vitamin D$_3$ (35 and 50 μg/kg) compared to a lower dosage (5 and 20 μg/kg) reduced the number of stillborn piglets. However, the form of supplementation (vitamin D$_3$ vs. 25-OHD$_3$), had no influence. In respect to the number of weaned piglets and suckling piglet losses, Flohr et al. (2016) and Thayer et al. (2019) could not show effects of the vitamin D source and Weber et al. (2014) even found a lower ratio of weaned to live-born piglets with 25-OHD$_3$ treatment.

Overall, several studies suggest that replacing vitamin D$_3$ with 25-OHD$_3$ does not to have any positive effect on the number of piglets born and weaned. However, it should be noted that the experimental feeding was often started after insemination. In the study conducted by Coffey et al. (2012), who could show a positive effect on the number of fetuses, the experimental feeding was started 43 days prior to insemination. Future studies to analyse the potential effects of 25-OHD$_3$ on reproductive performance should therefore consider an early administration of the different vitamin D metabolites or span several parities.

5.2.2 | Body condition of sows and birth weight of the piglets

Replacement of vitamin D$_3$ with 25-OHD$_3$ in sows had no effect on feed intake, weight and backfat thickness gain during gestation as well as weight and backfat thickness loss during lactation in the studies by Flohr et al. (2016), Zhang Li, et al. (2019), Coffey et al. (2012), Lauridsen et al. (2010) and Thayer et al. (2019).

Coffey et al. (2012) reported more fetuses per litter detected on the 90th day of gestation with 25-OHD$_3$ treatment. As the mean foetal weight was not influenced, total litter weight was increased (+26%). This finding is of particular interest because in pigs, individual birth weight is negatively associated with the number of fetuses due to a phenomenon known as 'uterine crowding' that may affect the prenatal and post-natal development of the offspring (Bérard et al., 2010). Uterine crowding can compromise the hyperplasia of secondary and total muscle fibres necessary for a normal development, thus reducing the cross-sectional area and the weight of the muscle (Bérard et al., 2010; Town et al., 2004). Although the weight of a foetus on the 90th day of gestation amounts only about 50% of the birth weight (McPherson et al., 2004), it might be speculated from the results reported by Coffey et al. (2012) that 25-OHD$_3$ has the potential to ameliorate the effects of uterine crowing.

While dietary supplementation of 25-OHD$_3$ instead of vitamin D$_3$ resulted in an increase in birth weight per piglet (+8%) and per total litter weight (+17%) in the study by Weber et al. (2014),

Flohr et al. (2016) and Thayer et al. (2019) could not demonstrate any effects. It must be taken into account that in the study by Weber et al. (2014), sows were not inseminated at the same time (first week, sows with vitamin D_3 supplementation; second week, sows with 25-OHD_3 supplementation). Thus, possible influences on birth weight could also be related to environmental or management influences. In addition, litter size might be a confounder. In the studies by Coffey et al. (2012) and Weber et al. (2014), the numbers of fetuses or piglets (10.2–12.7) were rather low in comparison to actual litter sizes in high performing modern sows.

However, the inconsistency between studies could also be related to differences regarding the vitamin D status of the control groups. According to the reference values reported by Madson et al. (2012), the vitamin D status of the control sows in the study by Weber et al. (2014) as suboptimal, while animals supplemented with 25-OHD_3 presented with adequate plasma concentrations. In the experiment conducted by Flohr et al. (2016), 25-OHD_3 plasma concentrations were higher not only in the experimental, but also in the control group, while Thayer et al. (2019) reported lower concentrations in both groups on Day 100 pi. It could be assumed that the effect of the replacement of vitamin D_3 with 25-OHD_3 on birth weight depends on the maternal vitamin D status, that is, that the animals only benefit from the addition of 25-OHD_3 if a suboptimal vitamin D status is corrected by the treatment.

5.2.3 | Weighth development and feed intake

Sugiyama et al. (2013), Duffy, Kelly, Rajauria, Jakobsen, et al. (2018) and Duffy, Kelly, Rajauria, Clarke, et al. (2018) did not find any advantage of 25-OHD_3 supplementation over vitamin D_3 on average daily feed intake (ADFI), weight gain (ADG) and feed conversion rate in growing and finishing pigs. In contrast, O'Doherty et al. (2010) reported an increase in ADFI (growing and finishing phase, +3%) and ADG (finishing phase, +3%) when they replaced half of the vitamin D_3 in the diet (25 μg/kg) by an equivalent amount of 25-OHD_3. In contrast to the above-mentioned studies, O'Doherty et al. (2010) fed a P-restricted diet (4.0 g total P per kg). It might be speculated that beneficial effects of 25-OHD_3 only become apparent when mineral supply is comprised.

With respect to weaning weight, neither Weber et al. (2014), nor Flohr et al. (2016), or Thayer et al. (2019) showed differences between the 25-OHD_3 and vitamin D_3 groups. Interestingly, Zhang Li, et al. (2019) demonstrated significantly increased litter weight gain when sows were supplemented with 25-OHD_3 instead of vitamin D_3. The authors discussed an increased milk fat content to be the cause of the increased weight gain.

In the study conducted by Witschi et al. (2011), the suckling piglets were offered a creep diet corresponding to the treatment of the dam starting on Day 21. The animals were weaned on Day 35 and continuously supplemented up to an age of 70 days. Daily gain, feed intake and feed conversion were not affected by the equivalent use of vitamin D_3 versus 25-OHD_3. Similarly, maternal and direct dietary supplementation of 25-OHD_3 versus vitamin D_3 did not affect rearing or fattening performance in the study by Thayer et al. (2019).

In line with these findings, the equivalent replacement of vitamin D_3 with 25-OHD_3 in the diet of weaned piglets in the study by Konowalchuk et al. (2013) had no effect on the weight development of the animals. Also, carcass traits like kill out and lean meat percentage were not affected (Duffy, Kelly, Rajauria, Clarke, et al., 2018; Duffy, Kelly, Rajauria, Jakobsen, et al., 2018; O'Doherty et al., 2010).

Taken together, supplementation with 25-OHD_3 instead of vitamin D_3 does not seem to have any effect as long as the diet meets the requirements of the animals. However, the previously presented results of O'Doherty et al. (2010) suggest that advantages related to performance may occur when dietary supply of minerals is restricted.

5.3 | Immune function

Fundamental and applied studies on the supplementation of vitamin D_3 and its metabolites on porcine immune function and health are scarce. Konowalchuk et al. (2013) investigated the effect of an additional administration of 50 μg/kg vitamin D_3 versus 50 μg/kg 25-OHD_3 in weanling piglets. An increasing effect of 25-OHD_3 on the number of leucocytes was demonstrated. Moreover, functional traits like viability and phagocytic capacity were enhanced in both serum-derived and bronchoalveolar leucocytes from pigs treated with 25-OHD_3 in comparison to the vitamin D_3 group. However, the exact mechanism remains unclear.

In sows, an equivalent replacement of the vitamin D with 25-OHD_3 resulted in a higher immunglobulin G (IgG) concentration in milk on the 21st day pp (Zhang Li, et al., 2019). However, the major antibody class for protection of mucosal epithelia is immunglobulin A (IgA) (Woof & Kerr, 2006). Studies by Van der Stede et al. (2001) and Van der Stede et al. (2004) have shown that the IgA response in pigs can be increased by an intramuscular injection of $1,25(OH)_2D_3$. Zhang et al. (2021) supplemented weaned piglets with five different dosages of 25-OHD_3 and demonstrated a positive, linear effect on the serum concentrations of IgA. Other studies could not show any impact on IgG and immunglobulin M (IgM) concentrations (Yang, Tian, Chen, Zheng, Yu, Mao, He, Luo, Luo, Huang, Wu et al., 2019; Yang, Tian, Chen, Zheng, Yu, Mao, He, Luo, Luo, Huang, Yu, 2019).

Again, this inconsistency might be related to the dietary background. When Ca and phosphorus (P) supply was restricted (5.6/4.5 g Ca per kg and 4.7/3.9 g total P per kg vs. 8.1/7.2 g Ca per kg and 6.0/5.3 g total P per kg on Days 0–14/15–28), Zhang et al. (2022) found in weaned piglets that additional supplementation of 25-OHD_3 resulted in enhanced levels of IgA (+44%), IgG (+28%) and IgM (+21%) at Day 28, when 25-OHD_3 levels were significantly increased, but not on Day 14, when vitamin D status was not affected by the treatment. Interestingly, in animals kept on adequate Ca and P supply, differences could only be found for IgM (+13%). From these results, it might be concluded that the effect of additional

administration of 25-OHD_3 on the serum immunoglobulin levels in piglets is probably related to both, increased serum concentrations of 25-OHD_3 and compromised supply of minerals.

5.4 | Bone and muscle development

Lameness is a common health and welfare problem in both, sows and fattening pigs (Huneau-Salaün et al., 2021; KilBride et al., 2009). Besides infectious causes, non-infectious conditions like claw-horn lesions and metabolic diseases of bone and cartilage have been reported. Of course, every form of 'leg weakness' also increases the risk of traumatic injuries and infections with opportunistic pathogens.

Clinical metabolic bone disease, which can develop due to compromised bone mineralisation caused by dietary imbalances or dysregulations of mineral homeostasis, is a possible cause of lameness and fractures (Madson et al., 2012; Wegner et al., 2020). Due to their rapid growth rate, especially in the late rearing to early finishing phase, and limited sunlight in conventional housing systems, pigs are particularly susceptible to developing rickets (Dittmer & Thompson, 2011; Madson et al., 2012). As endogenous vitamin D synthesis is insufficient in indoor housing, vitamin D_3 is added to the feed (Alexander et al., 2017). To minimise the risk of bone diseases, animals used to be provided with rather high amounts of dietary Ca and P and adequate crude protein. Today, many countries have implemented regulations limiting the amount of nitrogen and P applied to farmland with manure resulting in the necessity to reduce the content of crude protein and P in the diet. This feeding regime might increase the animals's risk for impaired bone health. In growing goats, it has been shown that protein restriction in the diet can lead to impaired skeletal mineralisation (Elfers et al., 2016). Furthermore, in a study by Schlegel and Gutzwiller (2020), it was shown that a reduction of digestible P or total P to a level below the current recommended level (2.5/1.7 g vs. 3.0/2.4 g dP/kg or 3.8/2.9 g vs. 4.4/4.1 g P/kg) in the growing/fattening diets of pigs can lead to reduced bone mineralisation despite unaffected growth performance. This was evident in lower ash (−3%), Ca (−5%), and P (−4%) content in bone, reduced bone mineral content (−8%), reduced bone mineral density (dual energy X-ray absorptiometry, DXA) (−6%), and lower bone density (−3%).

5.4.1 | Digestibility of Ca and P

In beef cattle with marginal P supply, additional supplementation with 25-OHD_3 had a beneficial effect on retention of Ca and P (McGrath et al., 2012). In finishing pigs, in the study by O'Doherty et al. (2010), exchanging half of the vitamin D_3 with 25-OHD_3 (25 µg/kg feed) in a P-restricted diet (4.0 g total P per kg) resulted in a proportional improvement of the digestibility of P and Ca. In contrast, serum and urinary P and Ca levels were not affected.

In the study by Duffy, Kelly, Rajauria, Clarke, et al. (2018), fattening pigs supplemented with 25-OHD_3 showed a higher apparent total tract digestibility of ash, crude protein and P. Regassa et al. (2015) demonstrated significantly reduced faecal Ca and P concentrations and an increase in the expression of intestinal RNA coding for the sodium-dependent phosphate transporter 1 in fattening pigs with additional supplementation of 25-OHD_3. Unfortunately, renal excretion of Ca and P was not determined in these studies, therefore the results only indicate an increase in digestibility, but no conclusions can be drawn in respect to retention.

As plasma concentrations of Ca and P are regulated and the surplus of minerals absorbed from the diet is either excreted via the kidney or accreted in the skeleton, it is not surprising that alterations of plasma concentration only become visible when mineral homeostasis is challenged by an increased demand. Weber et al. (2014) showed that plasma concentrations of P were not affected in sows (7.6/8.1 g Ca per kg and 6.6/6.9 g P per kg in gestation/lactation). However, serum concentrations of Ca were even lower in the group treated with 25-OHD_3 in comparison to control sows supplemented with vitamin D_3 on Day 5 pp. This observation indicates that the administration of 25-OHD_3 might interfere with vitamin D metabolism and the endocrine control of Ca homeostasis (see also Section 5.1). In line with this hypothesis, replacement of the vitamin D source in the maternal and creep diet of growing piglets had no effect on serum concentrations of P but decreased Ca concentrations on Day 77 post-natum (pn) (Witschi et al., 2011). Also, in the study by Zhang, Hu, et al. (2019), an exchange of vitamin D_3 with 25-OHD_3 neither influenced serum concentrations of Ca and P in sows nor in their piglets. Nevertheless, a significantly improved apparent digestibility of Ca but not of P was observed in the 25-OHD_3 group. The improved absorption of Ca was explained by an increase in mRNA expression of some genes related to vitamin D metabolism (renal CYP27B1) and gastrointestinal Ca transport (duodenal VDR, TRPV6 and CaBP-D9k) (Zhang, Hu, et al., 2019). As in the above-mentioned studies, renal excretion was not addressed.

Based on the studies presented, it is difficult to make a conclusive statement on whether the use of 25-OHD_3 instead of vitamin D_3 clearly improves Ca and P digestibility and retention. Future studies should be conducted with restricted diets and include the quantification of renal excretion and bone accretion.

5.4.2 | Bone markers

An approach to investigate bone metabolism, that is, bone formation and bone resorption, of living animals is to determine the dynamics of different biomarkers in plasma or serum (Leeming et al., 2006). An increase in the concentration of bone-specific alkaline phosphatase that is, associated with enhanced bone formation (Seibel, 2005) was found in sows at weaning (+64%) with dietary supplementation of 25-OHD_3 in the study by Zhang, Hu, et al. (2019). This is in line with a study conducted with vitamin D deficient humans that also reported a positive effect of 25-OHD_3 supplementation on serum alkaline phosphatase activity (Bordier et al., 1978). Again, the effect of these interventions could be related to the actual vitamin D status and the challenge of mineral homeostasis. In the above-mentioned

study by Zhang, Hu, et al. (2019), no differences were seen in sows at birth before Ca demand was enhanced by lactation. In the piglets, alterations were only found at weaning (21 day pn, +67%).

However, Zhang, Hu, et al. (2019) reported unaffected plasma concentrations of osteocalcin, another bone formation marker, in both, sows and piglets at any time. In the study by Weber et al. (2014) osteocalcin concentrations were also not affected and Witschi et al. (2011) showed even lower levels at Day 77 pn when 25-OHD_3 was used.

As bone turn-over is not only bone formation but also its ratio to bone resorption, respective markers should also be addressed (Leeming et al., 2006). In the study by Zhang, Hu, et al. (2019) bone resorption, estimated by measuring by tartrate-resistant acid phosphatase concentration, was not affected by 25-OHD_3 supplementation. Interestingly, Weber et al. (2014) found in sows that 25-OHD_3 supplementation resulted in a decrease of the bone resorption marker CrossLaps at insemination (−22%) but an increase on the 80th day of gestation (+19%) in comparison to vitamin D_3. In weaned piglets, the vitamin D source had no impact on the plasma concentrations of CrossLaps (Witschi et al., 2011). Taken together, these results suggest that dietary supplementation of 25-OHD_3 has no clear effect on bone resorption in pigs.

5.4.3 | Bone composition, density and breaking strength

Bone composition, that is, bone ash, Ca and P content, is considered to be a parameter reflecting long-term mineral balance (O'Doherty et al., 2010; Prentice, 2003). Despite improved Ca absorption or improved apparent total tract digestibility of crude ash, crude protein and P and reduced faecal content of Ca and P, respectively, O'Doherty et al. (2010), Duffy, Kelly, Rajauria, Clarke, et al. (2018) and Regassa et al. (2015) could not demonstrate any increase in bone ash, Ca and P levels in the bones of fattening pigs supplemented with 25-OHD_3. Both bone density, determined either by Archimedes' principle or by dual energy X-ray absorptiometry, and fracture strength were not affected in these studies.

A comparable outcome was reported in two of the experiments that addressed vitamin D status and mineral homeostasis of piglets after dietary treatment of the sows with 25-OHD_3 instead of vitamin D_3. Flohr et al. (2016) found no alterations of bone ash content, Ca and P content or the ratio of Ca to P in the bone ash of the second rib and the femur in the offspring euthanized after birth and at weaning. Witschi et al. (2011) also conducted measurements of bone mineral density and breaking strength. The vitamin D metabolite did not have any impact on these parameters. In contrast, significantly higher Ca contents in the tibia (+7%) and the femur (+5%) of the sows and in the tibia (+13%) of the piglets were demonstrated by Zhang, Hu, et al. (2019). In the sows, bone density was increased by 4% to 5% and breaking force was also improved by 10% in the tibia and by 42% in the femur. In the offspring, density was not affected, and breaking strength tended to be higher. The widely varying study results do not allow a conclusive, definitive statement on the effect of 25-OHD_3 and vitamin D_3 replacement on bone mineralisation.

5.4.4 | Osteochondrosis

Osteochondrosis is a degenerative, multifactorial disorder of the immature skeleton that results in lesions of the growth plate cartilage (Olstad et al., 2015, 2018, 2019) and lameness (Wegner et al., 2020). Sugiyama et al. (2013) found in growing pigs (6–10 kg) that dietary supplementation of 50 μg/kg 25-OHD_3 was able to reduce the incidence of osteochondrotic lesions analysed by macroscopic examination of articular cartilage of the distal humerus (32.4% vs. 59.3%) and distal femur (47.1% vs. 87.5%). Microscopic examination of humerus (20.6% vs. 43.8%) and femur (52.9% vs. 87.5%) confirmed the beneficial effect of 25-OHD_3. Based on histopathological findings, the authors speculated that 25-OHD_3 in swine diets might promote endochondral ossification, inhibit progression of osteochondrosis, and possibly regenerate destroyed cartilage tissue. Further studies are needed to understand the exact mechanisms. As reduced concentrations of Ca in the diet of pigs can lead to osteochondrosis and, together with osteodystrophia fibrosa, can cause the leg weakness syndrome (Kääntee, 1983), experiments addressing the influence of 25-OHD_3 when dietary Ca supply in restricted would be interesting.

5.4.5 | Muscle development

Muscle weakness is considered another possible cause of lameness symptoms, either directly or indirectly by increasing the risk of injuries (van Riet et al., 2013). In a study by Hines et al. (2013), it was observed that cultured myoblasts of fetuses from gilts fed vitamin D_3 (12.5 μg/kg feed) and 25-OHD_3 (50 μg/kg feed) (90th day of gestation) exhibited a prolonged proliferation phase (+13%) compared to those from fetuses of sows fed vitamin D_3 alone (62.5 μg/kg feed). In addition, an increase in total fibre number (+9%) was observed. The post-natal growth potential of skeletal muscle of fetuses from sows supplemented with 25-OHD_3 was enhanced by the formation of additional muscle fibres and a tendency to increase the number of Pax7+ myoblasts with prolonged proliferative capacity (Hines et al., 2013). Thus, for the development of foetal skeletal muscle, the vitamin D status of the sow seems to be of crucial importance. However, it remains to be seen whether muscle development is altered at the time of birth or during subsequent rearing and fattening. Pigs with a high number of muscle fibres tend to grow faster and more efficiently (Dwyer et al., 1993). Primary muscle fibre myogenesis is completed by approximately 50th–60th day of gestation. Secondary muscle fibre myogenesis then occurs by 85th–90th day of gestation (Wigmore & Stickland, 1983). After birth, the final number of muscle fibres does not change anymore. Post-natal growth is realised via hypertrophy, that is, an increase in size of the preformed fibres not the number (Rehfeldt et al., 2000).

In the studies by Flohr et al. (2016) and Thayer et al. (2019), supplementation of sows with 25-OHD$_3$ instead of vitamin D$_3$ had no effect on the total number of primary or secondary muscle fibres, their ratio or muscle cross-sectional area. This contrasts the results obtained by Hines et al. (2013). A possible explanation could be that the experimental feeding in this study was started 43 days before insemination, while Flohr et al. (2016) and Thayer et al. (2019) only supplemented the sows during gestation. Furthermore, Hines et al. (2013) only enroled gilts and the fetuses were sacrificed on the 90th day of gestation, so that potential alterations occurring during the last days of gestation could not have been revealed.

6 | CONCLUSION

Up to date, results on effects of 25-OHD$_3$ instead of vitamin D$_3$ in diets for pigs are inconsistent. In healthy animals and as long as dietary requirements are met, beneficial effects can probably not be expected. But dietary supplementation with 25-OHD$_3$ clearly provides the opportunity to increase serum concentrations of 25-OHD$_3$ more pronounced than using conventional vitamin D$_3$. This might be of special importance when the animals are vitamin D deficient, for example because of a malabsorption caused by diarrhoea, or challenged by suboptimal environmental conditions, high infection pressure or restricted nutrient supply. To mitigate emissions from livestock production, it is becoming common practice to feed diets restricted in protein and phosphorus content. Most of the studies that reported positive effects of 25-OHD$_3$ in comparison to vitamin D$_3$ were conducted under the above-mentioned challenging conditions or included a control group with a vitamin D status that could be considered low.

Reference values for humans had been adapted after several studies had demonstrated extra-skeletal effects of vitamin D, especially in respect to its impact on immune function (Ismailova & White, 2022). For pigs, accurate reference values to evaluate vitamin D status at different ages and reproductive status are still lacking. As higher plasma concentrations of 25-OHD$_3$ are found in outdoor housing systems that allow endogenous synthesis (Arnold et al., 2015; Jakobsen et al., 2022), future studies should clarify the efficiency of absorption of vitamin D metabolites from the diet as well as the optimal plasma levels in high performing modern pigs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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