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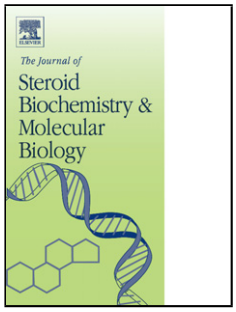

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Effect of UVB light on vitamin D status in piglets and sows

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Highlights

- Piglets was born with very low levels of vitamin D <2 ng/ml
- UVB light significantly improved vitamin D status in piglets and sows
- Improving vitamin D status did not increase the weaning weight of piglets
- No adverse effects were observed when exposing piglets to UVB light up to 1 SED

Abstract

Piglets are born with very low levels of vitamin D. Feed is the only source of vitamin D for pigs kept indoors, and the levels in feed are restricted by European legislation. We aimed to study the effect of lamps releasing ultraviolet type B (UVB) light on the vitamin D status (serum 25-hydroxyvitamin D) in sows and piglets in a Danish indoor herd.

A randomized trial with a parallel group design was initiated with two groups receiving a daily UVB-dose of maximum 0.7 standard erythema dose (SED) or 1 SED, in addition to a control group. The three groups included in the study consisted of 15 sows and their 195 offspring. Blood samples were taken from the piglets and sows on day 1, 12, and 24. Results showed no difference between the groups in serum levels of 25(OH)D_3 or vitamin D_3 on day 1, with the mean (±SD) for piglets being 0.96±0.26 ng/mL and 0.06±0.04 ng/mL, respectively. For sows, the values were 16±3 ng/mL 25(OH)D_3 and 3±0.8 ng/mL vitamin D_3 on day 1. A

significant difference ($p<0.001$) in serum 25(OH)D_3 between the groups receiving UVB light and the control group was observed on both day 12 and day 24. On day 24, the piglet control group had 5.5±2 ng/mL 25(OH)D_3 and 0.4±0.2 ng/mL vitamin D_3. For the UVB groups, the values were 21.6±10. ng/mL 25(OH)D_3 and 8.3±2.5 ng/mL vitamin D_3 for the 0.7 SED group and 19.5±6.0 ng/mL 25(OH)D_3 and 7.6±3.4 ng/mL vitamin D_3 for the 1 SED group. For the sows, the values were 25.6±5.5 ng/mL 25(OH)D_3 and 6.6±1.2 ng/mL vitamin D_3 for the control group, 66.7±13.5 ng/mL 25(OH)D_3 and 21.3±2.9 ng/mL vitamin D_3 for 0.7 SED group and 67±15 ng/mL 25(OH)D_3 and 25±5 ng/mL vitamin D_3 for the 1 SED. No significant difference was found between the two UVB groups for either piglets or sows. The use of lamps releasing UVB light is therefore suggested to be an efficient way to improve the vitamin D status of both sows and piglets.

Keywords: Vitamin D, UVB, pigs, piglets, 25-hydroxyvitamin D_3

1 Introduction

In humans, vitamin D deficiency is recognized worldwide as a problem[1,2]. The natural inducer of vitamin D production is the UVB wavelengths from sunlight (290-315 nm) that transform 7-dehydrocholesterol to pre-vitamin D_3, which, following a temperature-dependent process, converts to vitamin D_3 in the skin. If UVB exposure is not sufficient to produce adequate vitamin D, humans must rely on vitamin D in the diet. This is also the case for most indoor housed terrestrial vertebrates. Danish pig production uses mainly indoor facilities, where the pigs have no access to outdoor facilities and thereby no access to sunlight. The Danish recommendations for vitamin D for pigs relates to the amount in feed, while the levels in the serum are unknown. This raises the question of whether Danish pigs have a sufficient vitamin D status, which is measured as 25-hydroxyvitamin D in serum (S-25OHD).

European legislation allows the addition of up to 2,000 I.U./kg (50 µg/kg) of vitamin D i.e. vitamin D3, vitamin D2 or 25-hydroxyvitamin D3 to pig feed[3, 4]. The recommendations in Denmark are between 400 and 800 IU vitamin D per kg dry matter of diet to be added to the feed depending on the age of the pig[5]. To date, no recommendations relating to S-25OHD concentrations in pigs have been established. It is therefore difficult to

determine whether and when the vitamin D status of a pig is sufficient, insufficient or deficient. If the human definitions are applied, a level of 20 ng/mL would be considered adequate, a level of 12-20 ng/mL would be considered insufficient, and a level below 12 ng/mL would be considered deficient[6]. Since we do not know whether or not the vitamin D status of production pigs is optimal, it could be speculated that increasing vitamin D levels could improve the general health and welfare of pigs kept indoors.

Vitamin D is used for many purposes in bodies functioning under normal conditions. The best-described vitamin D deficiency condition in pigs is rickets, which causes malformation of bone tissue, but, like in humans, vitamin D status also has an effect on immune functions[7], growth[8,9] and reproductive performance[10] among others.

When optimizing the vitamin D status of pigs via feed, there is a risk of both insufficient supply as well as toxicity. Vitamin D poisoning in pigs has been documented in experimental settings as well as being described in case studies. Vitamin D poisoning can lead to anorexia, vomiting, calcification of soft tissue such as the lungs, heart and kidneys, weight loss or reduced growth, lethargy, polyuria polydipsia and eventually death[11–14].

Investigation of health effect of feeding pregnant sows maximal level i.e. 2000 IU vitamin D/kg, as vitamin D_3 or $25OHD_3$ showed a significant increased birthweight in the $25OHD_3$ group, but no difference of weight at weaning [15]. Oral administration of vitamin D_3 for piglets at 2 days of age as a single dose of 40,000 IU vitamin D_3 resulted in an increased weight at weaning [8]. This could indicate that both sows and piglets may suffer from vitamin D insufficiency.

UVB light covers the wavelengths between 280 nm and 320 nm. Vitamin D production for both humans and animals occurs at wavelengths below 315 nm, with its peak at around 295-300 nm nm[16]. Vitamin D production in response to UVB light cannot reach toxic levels due to the negative feedback system where previtamin D_3 continuously exposed to UVB light will convert in to lumisterol and tachysterol[17,18]. If UVB-derived vitamin D_3 reaches a steady state in pigs, this level could be used as an optimal reference level. However, no steady state was achieved in the pigs with daily UVB exposure over 28 days[19].

The unit standard erythema dose (SED) takes into account the irradiant dose and the level of erythema on human skin, and 1 SED is equivalent to 100 J/m^2. It is a fixed measure that is not dependent on skin type, and

is easily comparable. In Denmark at 55° North 1 SED is equivalent to 10 minutes of sun exposure at zenith in summertime [20].

The aim of the present study was to investigate the effect of a daily UVB-dose on vitamin D levels in sows and their piglets until weaning.

2 Materials and Methods

The Danish Animal Experimentation Council approved the study. A veterinarian inspected the pigs daily.

2.1 Experimental design

The study was set up as a randomized parallel study with two treatment groups and a control group. Sample size was based on results from a similar study conducted in slaughterpigs by Barnkob et al.[19]. A required group size of 5 was determined based on an expected standard deviation of 25, a difference between treatment and control groups of 43 ng/ml, a significance level of 0.05 and a power of 80%.

2.2 Animals and facilities

Each group consisted of 5 sows with 13 piglets. The study was conducted over the period 1st to 29th June 2018 in a Danish herd with 1,270 sows and a health declaration of SPF+Myc+Ap2+Ap12. Danavl sows (Danish Landrace X Yorkshire (L/Y)) were mated with Danavl Duroc boars. Healty sows multiparious with expected farrowing dates between 1st and 5th June 2018 were selected for the study . Sows with expected farrowing dates between 1st and 5th June 2018 which belonged to parity 2-5 and showed no clinical signs of illnesses the week prior to farrowing were included. The sows were allocated to one of three groups (by random, using randomized numbers in Microsoft Excel) in the week prior to farrowing. The study began on the day of farrowing and ended on day 24 after farrowing. The sows and piglets were housed in two farrowing rooms each containing 20 identical pens. Each group included sows in both of the rooms to avoid issues with potential differences between the rooms. Calcium hydroxide was used to stain the windows in order to prevent UVB rays entering. Sows and piglets in the control group were placed in pens located at least 2 m from pens with

UVB light. Pigs with a birthweight of 900 g or more which had no health issues at birth were included in order to minimize the loss of piglets during the trial.

Sows were fed a home-mixed liquid diet. Two batches of the liquid feed were analyzed, which showed a content of 4.5 vitamin D_3 per kg and 5.1 µg vitamin D_3 per kg. The piglets' primary diet consisted of sows milk, which from day 2 were supplemented with up to 0.5 liter of milk per litter per day (Danmilk supreme 1.0 from Agrokorn A/S). From day 7 ad libitum pelleted feed (Danish new wean from Danish Agro a.m.b.a.) was fed to the piglets on the floor. Analyses of the levels of vitamin D_3 showed that the aqueous milk supplement contained 12 µg vitamin D_3 per kg, and the pelleted feed contained 11 µg vitamin D_3 per kg.

2.3 UVB exposure

Piglets and sows in the treatment groups received UVB light in two different doses: 0.7 SED and 1.0 SED, while the control group received no UVB light. Two UVB light tubes were placed over each of the 10 pens containing the sows and piglets receiving a daily dose of UVB light. One tube was hung over the piglets nest (47 cm to the floor) and one tube was placed above the sow (180-190 cm to the floor). Pigs were gradually exposed to the UVB light, starting with one hour/day on day 1 (due to technical problems, the UVB exposure for sows started on day 2) up to 6 hours/day from day 8 to day 24. The 6 hours of UVB light was split between two hours in the morning (4 am-6 am) and 4 hours in the evening (6 pm-10 pm). These times were chosen to prevent personnel being present during UVB exposure, and to avoid disturbing the nocturnal rhythm of the pigs. The duration of exposure was chosen to be as long as possible in order to increase the chance of all piglets in the UVB groups receiving a daily dose of UVB light.

2.3.1 UVB light source

The UVB lights (Lucky Reptile UV sun T5, 24 W tubes, luckyreptile.com, Germany) in the piglet nests were covered by Plexiglas without UVB filter. A dimmer was attached to the lamp to enable adjustment and ensure that the correct UVB-dose was given to each treatment group. The UVB lamps (Lucky Reptile UV sun T5, 54 W tubes, luckyreptile.com, Germany) placed above the sows had no cover. For the sows, the UVB light were measured and the height adjusted to establish the correct dose for each treatment group.

Fig 1. UVB light placed above the sow and above the piglet nest in the corner of the pen.

2.3.2 UVB dose measurement

A full spectrum of the irradiance was recorded for one tube, scanning from 200 to 700 nm, with 1 nm increments (EOP146 detector probe, Instrument Systems-CAS140CT) at DTU Fotonik, Denmark. These data combined with the erythema reference action spectrum[16] enabled us to estimate the duration of UVB light required to achieve daily dose of 0.7 SED and 1.0 SED for the two treatment groups. For these estimations, it was assumed that a piglet remaining directly under the lamp for 6 hours would receive a maximum of either 0.7 SED or 1.0 SED. This was done to minimize the risk of pigs developing erythema in response to overexposure of UVB light. The calculation of UVB exposure for sows was based on the assumption that they would be standing for 25% of the time and lying for 75% of the time.

A handheld UVB meter (ILT 1400-BL photometer equipped with a SEL005/TLS312/TD detector, International Light Technologies, Peabody, MA, USA) was used to test the difference in UVB exposure between each of the 10 lamps used for the sows in order to allocate the correct height, and to adjust the dimmer for the piglets. This was also used to check the UVB exposure once during the trial.

2.4 Sampling of blood

Blood was sampled from sows and piglets on day 1, 12 and 24. For the piglets, blood sampling was performed by puncturing the jugular vein using a needle of 22G (BD ref 360210, Becton Dickinson, Franklin Lakes, New Jersey, United States) or 21G (BD ref 360212) and 4 mL dry tubes (BD ref 368975) with a vacutainer holder. The procedure for the sows was identical, but the needle used was 18Gx1.5 (BD ref 360748). Piglets were manually restrained during blood sampling and sows were restrained using a snout snare. Immediately after blood collection, the tube was placed at room temperature for 30 min to coagulate. Stored at 5 ℃, and within 48 hours centrifuged for 15 minutes at 2500 *g* (Sigma 4K15 Centrifuge, Sigma Laborzentrifuge GmbH, Osterrode am Harz, Germany. Serum stored at -80 °C until analyses within 1 month.

2.5 Sampling of feed

Feed was sampled twice during the trial. Sampling was done in one of the pens where the included sows were kept. Samples were obtained from the pipe that supplied the sow with liquid feed. A plastic bag was placed under the pipe at the time of feeding and was immediately sealed after filling and placed in a freezer at -18°C. When it had frozen solid, the sample was transported in a cooler bag to the laboratory where it was stored at -18°C until analysis.

2.6 Analytical methods

2.6.1 Weighing

Piglet weight was recorded on day 1 and 24. Weights on day 1 were recorded using a handheld Ryom digital scale (Center-Gros A/S, Denmark) ranging from 100 g to 40 kg with 10 g intervals. Weights on day 24 were recorded using a Diesella table scale (Diesella A/S, Denmark) ranging from 100 g to 30 kg and with 5 g intervals. Piglets were placed in a bucket when weighed.

2.6.2 Vitamin D

Blood samples were analyzed using a previously described method [19]. In short, the protein in 100 µL of serum was precipitated by acetonitrile and cleaned up by solid-phase extraction, followed by derivatization by 4-phenyl-1,2,4-triazoline-3,5-dione, and quantification of 25(OH)D_3 and vitamin D_3 by liquid chromatography coupled with tandem mass spectrometry. The precision of the method was <6% for both metabolites estimated from an in-house serum reference (n=35).

3 Statistical analysis

The statistical software Rstudio (Rstudio, Inc., Boston) was used to perform all statistical analysis. A linear mixed model for 25(OH)D_3 and vitamin D_3 with group, parity and birthweight as fixed effects and sow as a random effect was fit using analysis of variance (ANOVA) with Tukey's test. Analysis of other parameters including sex, mortality and birthweight was performed using ANOVA and Tukey's test. Birthweight was grouped in to one of three groups: "Small" (n=10) referring to piglets <900 grams, "Medium" (n=131) was 1,400 grams> piglets ≥900 grams and "Large" (n=54) described piglets ≥1,400 grams. Results are given as mean ± standard deviation (SD).

3.1 Results

None of the pigs developed erythema during the trial.

3.2 Sows

The parity of the sows included in the trial ranged from 2-5 and the sows had no health issues prior to farrowing. After farrowing, 11 of the 15 sows received antibiotic treatment for MMA (Mastitis, Metritis, Agalactia). The four sows that did not receive treatment belong to one in the control, two in the 0.7 SED, and one in the 1.0 SED group.

Serum values for 25(OH)D_3 and vitamin D_3 for sows on all sampling days can be found in Table 1. Vitamin D levels in serum of the sows showed no significant difference in 25(OH)D_3 or vitamin D_3 on day 1. However,

on day 12 and day 24, a significant increase in 25(OH)D_3 and vitamin D_3 for the UVB-treated groups compared to the control.

Table 1. Results for 25(OH)D_3 and vitamin D_3 in serum (ng/mL) from sows

		Control		0.7 SED		1.0 SED	
		mean	SD	Mean	SD	Mean	SD
Day 1							
	25(OH)D_3	15.1	3.7	16.3	3.4	16.4	2.8
	Vitamin D_3	2.92	0.72	3.29	0.50	2.95	1.23
Day 12							
	25(OH)D_3	20.6[a]	3.5	35.7[b]	6.5	33.3[b]	6.5
	Vitamin D_3	5.23[a]	1.24	22.4[b]	3.41	23.12[b]	5.00
Day 24							
	25(OH)D_3	25.6[a]	5.5	66.7[b]	13.5	66.9[b]	15.0
	Vitamin D_3	6.59[a]	1.17	21.33[b]	2.91	24.48[b]	5.07

[a,b] Mean values with different superscript letters within a row were significantly different ($p<0.001$)

3.3 Piglets

3.3.1 Sex, mortality and weight gain

Each of the 15 sows kept 13 piglets. Information about the 195 piglets are given in Table 2. Piglets under 900 g were included in three of the litters in order to include 13 piglets from each litter. Of the piglets under 900 g, six were from a sow in the control group (range: 720-895 g), one piglet weighing 855 g was from a sow in the 0.7 SED group, and three from a sow in the 1.0 SED group (range: 780-890). One piglet was excluded prior to random selection due to a birth defect; the remaining excluded piglets were excluded due to their size or through random selection.

Table 2: The distribution of sex, mortality and excluded piglets, percentage in brackets. Mean and SD for birthweight and weaning weight

		Control	0.7 SED	1.0 SED	All
Sex					
	M (%)	30 (46)	27 (42)	32 (49)	89 (46)
	F (%)	35 (54)	38 (58)	33 (51)	106 (54)
Mortality					
	n (%)	3 (5)	1 (2)	6 (9)	10 (5)

Excluded piglets[1]					
	n (%)	3 (5)	0 (0)	5 (8)	8 (4)
Birthweight					
	Mean, g	1,390	1,156	1,198	1,248
	SD, g	392	216	264	315
Weaning weight					
	Mean, g	6,833	6,022	5,870	6,246
	SD, g	1,618	1,398	1,722	1,622

[1]Due to unthriftiness

More females than males were included in the study (54% *vs*. 46%), but no significant difference for serum 25(OH)D_3, serum vitamin D_3 or weight between gender were observed. Sows and their offspring being allocated a group before farrowing did not allow adjusting the groups according to birthweight. The control group had an average birthweight that was significantly higher ($p<0.001$) than both of the groups receiving UVB light. A mixed linear model including group, parity and sex as fixed effects and sow as a random effect found no significant difference in birthweight between the groups. When including birthweight in the model, no significant difference in weight on day 24 was found between groups. Categorizing the piglet in to three groups based on birthweight, "Small", "Medium" and "High", show a significant difference in weight on day 24 between the group "Large" and each of the two other groups ($p<0.01$). No significant difference in weight on day 24 was seen between the groups "Small" and "Medium".

3.3.2 Vitamin D in serum

Table 3 shows the results relating to 25(OH)D_3 in serum i.e. vitamin D status and for vitamin D_3 in serum. On day 1, neither for vitamin D status nor vitamin D_3 in serum a significant difference was found between the groups, showing an overall mean for vitamin D status of 0.96±0.19 ng/mL. On day 12 and 24, a significant difference was seen between the control group and each of the two UVB-treatments i.e. 0.7 SED and 1.0 SED ($p<0.001$) for vitamin D status as well as for vitamin D3 in serum. The UVB light significantly increased the vitamin D levels in piglets compared to the unexposed control group, but no significant difference was observed between the two treatment groups.

Table 3. Results for 25(OH)D_3 and vitamin D_3 in serum (ng/mL) from piglets

	Control	0.7 SED	1.0 SED

		Mean	SD	Mean	SD	Mean	SD
Day 1							
	$25(OH)D_3$	0.93	0.28	1.00	0.27	0.94	0.22
	Vitamin D_3	0.06	0.03	0.07	0.04	0.04	0.03
Day 12							
	$25(OH)D_3$	4.10^{a}	1.23	12.95^{b}	2.41	12.9^{b}	3.65
	Vitamin D_3	0.47^{a}	0.46	8.21^{b}	2.32	8.44^{b}	3.51
Day 24							
	$25(OH)D_3$	5.54	1.97	21.6^{b}	3.97	19.5^{b}	6.14
	Vitamin D_3	0.36^{a}	0.23	8.25^{b}	2.50	7.61^{b}	3.40

[a,b] Mean values with different superscript letters within a row were significantly different ($p<0.001$)

4 Discussion

This is the first study to investigate the effect of UVB light on vitamin D status in sows and piglets.

The UVB light tubes worked well and no adverse effect (erythema) was observed in the skin of exposed animals. During the experiment, setting up the lights presented some challenges. The lights above the piglet nests were slightly movable, and when the piglets had grown, they were able to manipulate and move the lights around. Also, the piglets were able to move freely around the entire pen, and on some days, the floor in some of the piglet nests became soiled. As a result, the piglets preferred to remain in other parts of the pen outside the range of the UVB light tubes. This meant that the assumption that piglets would stay 6 hours under the tubes and thereby receive of a daily dose of 0.7 SED or 1.0 SED was not met for these piglets. The sows were housed in farrowing crates and therefore remained under the UVB tubes for the entire exposure time. However, we could not ascertain whether the estimation of 75% standing and 25% lying time was met.

Even though the UVB-dose did not reach the intended dose for piglets, the results showed a significant difference in vitamin D status on day 12 and 24 between the groups that received UVB light and the control group, for both sows and piglets. We did not, however, find any significant difference between the 0.7 SED group and the 1.0 SED group, as previously reported for slaughter pigs by Barnkob et al.[19]. In our study, the dose was controlled by adjusting the irradiance and the height of UVB tubes, but the duration of exposure was fixed. In the study by Barnkob et al. [19], the dose was controlled by adjusting the duration of exposure, with a fixed irradiance and height. This suggests that having a fixed height and irradiance but variable duration is a

better way to adjust the dose in an experimental setup. These results are encouraging for further studies on enhancing vitamin D levels using UVB light.

Among domestic farm animals tested, pigs are born with the lowest vitamin D status, which may predispose to neonatal rickets [21]. We report levels for vitamin D status in newborn piglets at 1 ng 25(OH)D_3/mL serum, which is even lower than previously reported at 2.5-5.5 ng/ml serum [22,23]. In the present study the sows were followed from farrowing until weaning and thus no analysis on the vitamin D status of feed during gestation were performed. However, the gestation feed was supplemented with vitamin D at a level of 800 IU/kg feed according to Danish recommendation standards [5]. No reference values for adequate vitamin D status for pigs are available. If adequate vitamin D status are considered similar for pigs and for humans, then the newborn piglets would be categorized as vitamin D deficient [6] a condition that may lead to retarded skeletal growth and reduced weight gain. This might indicate that sows supplementation with vitamin D during pregnancy had been insufficient.

At weaning, the vitamin D status of the piglets in the UVB-treated groups would in humans be considered sufficient (≥20 ng/mL), while the piglets in control group would be described as deficient (<12 ng/mL) [6]. The vitamin D source for piglets was apart from the milk and feed provided from day 2 and day 7, respectively, sows milk. The vitamin D intake from the sows milk is difficult to estimate partly because of the individual level of content of vitamin in sows milk and the variation of milk uptake from individual piglets [24]. Levels of vitamin D in sows milk has been reported to be approximately 2 ng vitamin D_3/g and 5 ng 25(OH)D_3/g in some studies [15,25]. The result for the control group indicate that this vitamin supply do not ensure an adequate vitamin D status for the piglets. The vitamin D status of the sows at parturition should be seen as the lowest levels occurring during their reproduction cycle due to both placental transfer to the fetuses as well as the increased demand in the calcium metabolism after parturition [15, 26]. This study showed that UVB exposure of sows for 22 days resulted in vitamin D levels comparable to those (53 ng/mL) that previously has been shown to increase birthweight of piglets [9, 15].

No significant difference in weight gain was found between treatment groups. However, the study was not designed to detect small improvements in daily weight gain and therefore does not exclude such effects. Other studies have shown an increase in growth rate for piglets following a single oral dose of vitamin D at farrowing

[8]. Also the present study was not designed to test if UVB exposure of the pregnant would improve farrowing results.

The advantage of UVB light as the source of vitamin D seems to be the strategy, which can ensure a sufficient vitamin D status at an earlier stage than through feeding for the piglets. Piglet will start to eat creep feed only at day 10-21. In our study the piglets in the control group could be categorized as deficient at weaning. The piglets in UVB-treated groups at day 12 were not deficient but had an inadequate vitamin D status, while at weaning the piglets could be categorized as having a sufficient vitamin D status. Further studies including a greater number of sows and piglets are currently planned, in order to determine the effects of the increased vitamin D status achieved by UVB-exposure, on sow reproduction and average daily weight gain in piglets.

5 Conclusion

Sows and their newborn piglets exposed for 24 days to a daily dose of UVB light to a maximum of 0.7 SED or 1.0 SED had a significantly higher vitamin D status and level of vitamin D_3 in serum compared to a control group. This study showed no significant difference in vitamin D levels between the groups receiving 0.7 SED and 1.0 SED. Vitamin D status in newborn piglets at 1 ng 25(OH)D_3/mL serum, would in humans be catergorized as deficient. At weaning, the vitamin D status of the piglets in the UVB-treated groups would be caterorized as sufficient, while the piglets in control group would be described as deficient. Further studies including a greater number of pigs are currently planned, since several other studies have shown an effect on average daily weight gain in piglets when either the sows or the piglets received extra vitamin D.

Author statement.

Sine Stricker Jakobsen: Methodology, Investigation, Formal Analysis, Writing – Original Draft. Jens Peter Nielsen: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Funding Acquisition.

Jette Jakobsen: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Project

administration, Funding Acquisition.

Author contributions

SSJ: Methodology, Investigation, Formal Analysis, Writing – Original Draft. JPN: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Funding Acquisition. JJ: Conceptualization, Methodology, Writing – Review & Editing, Supervision, Project administration, Funding Acquisition.

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Conflict of interest

Authors of this paper have no conflict of interest.

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