Investigation of Ultraviolet B Radiation on Circulating 25(OH) Vitamin D Concentrations in Weaned Piglets

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Abstract

Pigs produced in standard operational units are known to have poor circulating concentrations of vitamin D (Alexander et al., 2017). Pigs rely solely on dietary vitamin D because they are generally raised in indoor confinement systems. Pigs have the ability to use ultraviolet radiation B (UVB) as an additional source of vitamin D (Alexander et al., 2017, Larson-Meyer et al., 2017, Kolp et al., 2017). This study compared a control group (n = 3 pens, 3 male and 3 female) that received no UVB exposure to a UVB treatment group (UV; n =3 pens, 3 male and 3 female). The UVB treatment group was given daily 10 minute exposure of 302nm UVB light at an intensity 250 $\mu W/cm^2$ for 28 days. An analysis of circulating 25(OH) vitamin D was used to determine vitamin D content of the pigs. There was no effect of treatment for bodyweight (P = 0.9151) or plasma vitamin D concentrations (P = 0.9008). The additional exposure to UVB light did not have an effect on circulating 25(OH) vitamin D.

Introduction

Vitamin D has an important role in the growth and maintenance of pigs. Vitamin D is essential for calcium-phosphorus homeostasis, cardiovascular health, and immune health. Swine, like many mammals, have the ability to generate vitamin D from UVB exposure on the skin. When exposed to UVB, 7-dehydrocholesterol (7-DHC) in the skin is converted to pre-vitamin D_3. Pre-vitamin D_3 is further transformed in the skin to vitamin D_3, also known as cholecalciferol, from heat produced by the animal. Following conversion to cholecalciferol, vitamin D_3 leaves the skin and is transformed to 25(OH) D (calcidiol) in the liver. The circulating form of 25(OH) D is then converted to its biologically active form, 1, 25-dihydroxyvitamin D in the kidneys. The most abundant circulating form of vitamin D is 25(OH) D, and it is widely distributed throughout the body and taken up by muscle and fat tissue (Heaney et al., 2009). It has been proposed that 25(OH) D is the form of vitamin D that should be considered when analyzing vitamin D concentrations in tissues because of its abundance and distribution throughout the body (Larson-Meyer et al., 2017, Heaney et al., 2009).

Pigs are generally raised in an indoor, confinement setting, limiting their exposure to the natural ultraviolet B radiation (UVB) gained from sunlight. According to Kolp et al. many pigs do not reach full potential or optimal levels of vitamin D because they rely solely on dietary intake. Exposure to sunlight or artificial UVB light has been shown to increase 25(OH) D concentrations in circulation (Alexander et al., 2017, Larson-Meyer et al., 2017, Kolp et al., 2017). Alexander et al. (2017) found a 200% increase in circulating 25(OH) D in pigs after two weeks of sunlight exposure for one hour per day in the summer. Larson-Meyer et. al (2017) also demonstrated an increase in serum and muscle tissue 25(OH) D concentrations, in pigs exposed to 1 hour of natural sunlight daily. The irradiance of the natural sunlight according to Alexander et al. (2017) and Larson-Meyer et al. (2017) was between 50 and 250 $\mu W/cm^2$. Kolp et al. (2017)

suggested that, with adequate exposure to UVB, there is no need for supplemental vitamin D in the feed. Kolp et al. found no differences in 25(OH) vitamin D status were found between pigs exposed and supplemented compared with pigs that were exposed and not supplemented with vitamin D. Kolp et al. (2017) claimed to reach adequate exposure of artificial UVB light with 23 $\mu W/cm^2$ for 15 minutes daily, this a much lower irradiance then the 50 - 250 μW/cm used by Alexander et al. (2017) and Laron-Meyer et al. (2017). Barnkob et al. (2016) found the optimal wave-length for vitamin D production in pigs to be 296 nm with the use of pig skin from cadavers. Barnkob et al. (2016) was also able to show a relationship between intensity and exposure length. According to Barnkob et al., (2106), the same dose of UVB can be received with a smaller irradiance and longer exposure time compared to a high irradiance and short exposure time (Barnkob et al., 2016). These studies confirm the effect of UVB exposure and its relation to 25(OH) D concentrations in the pig, but the optimal irradiation and exposure length has yet to be determined.

In addition to increased circulatory 25(OH) concentrations, exposure of pigs to UVB increases vitamin D concentrations in muscle and adipose tissue (Larson-Meyer et al., 2017). Vitamin D insufficiency has become a global problem (J. Guo et al., 2019). Guo et al. (2019) stresses the importance of fortifying foods with vitamin D where applicable and furthers this point by suggesting that 25(OH) concentrations in meat may be a more effective and preferable form of vitamin D fortification, because of its high digestibility and bioavailability. Larson-Meyer et al. (2017) were able to increase 25(OH) vitamin D concentrations in the loin tissue from 0.4ug/100g to 1ug/100g from 1 hour of daily exposure to natural UVB light. Exposure to UVB light has the ability to enhance pork, the most consumed animal meat worldwide, as source

of vitamin D for human consumption. UVB exposure has the potential to be a simple method of natural bio-fortification of vitamin D concentrations in pork products.

This study investigated the effect of UVB light on vitamin D status of pigs by mimicking the natural sunlight irradiance during the summer using artificial UVB light. The aim was to clarify the effect of the time pigs are exposed to UVB light and the total amount of radiation at the surface of the skin on circulating concentrations of vitamin D. I was hypothesized that exposure to daily UV light for ten minutes will increase circulating concentrations of 25(OH) vitamin D in weaned piglets.

Methods

Animals and measurement

This study was reviewed and approved by The Ohio State University's Institutional Animal Care and Use Committee (IACUC). Twelve crossbred (Yorkshire x Landrace x Exotic) piglets (6 male, body weight 15.4 kg ± 4 kg, 24-26 days-of-age and 6 female body weight 14.8 kg ± 4 kg, 24-26 days-of-age) were weaned and grouped into 6 pens by bodyweight (1 male and 1 female in each pen). The pens were randomly allocated to one of two treatments: 1) No UV exposure (CONTROL; n = 3 pens, 3 male and 3 female total); and, 2) Exposed to UV light daily for 28 days (UV; n =3 pens, 3 male and 3 female total). The piglet nursery was blocked from any natural UV entrance. Piglets were given three days as an adjustment period after weaning. On day 0, 14 and 28, bodyweights and blood samples were collected from all piglets. All piglets were raised using standard practice, and feed and water were given *ad libitum*. Pigs were fed one of three commercial diets (Provimi North America, Inc.) formulated to meet the requirements: a medicated starter pig diet for pigs weighing 12 to 16 lb., a prestarter diet for pigs weighing 13 to 22 lb., and a starter pig diet for pigs weighing 18 to 30 lb.

Piglets in the UV pens were placed by pen into a box (61 cm high, 43 cm wide, and 79 cm long) and exposed daily to 10 minutes of 302 nm UVB light. The UV intensity ($\mu W/cm^2$) was measured with a Sper Scientific UVA/B light meter, after a 10 minute warming period. The 10 minute warming period was determined based on the amount of time that the UVA/B light meter would take to measure a constant irradiance emitting from the artificial UVB light. The distance between the light and back of the piglets was adjusted daily to measure an average intensity of 250 $\mu W/cm^2$ in the center and 40 $\mu W/cm^2$ at the sides of the box. One piglet was removed on day 3 due to UV skin damage, and was replaced by another piglet. The loss of day 0 data was accounted and adjusted for in the statistical analysis.

Blood was collected on day 0, 14, and 28. Within twenty-four hours blood was centrifuged and serum was removed. An ELISA kit for25 (OH) Vitamin D concentrations was used to measure all samples in one assay (Abcam, ab213966 25(OH) Vitamin D)

Statistical analysis

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc.). Pen (1-6) was included in the model as a random effect, while treatment (CONTROL or UV), day (0, 14, or 28), and their interaction were included in the model as fixed effects. Repeated measures were assessed using the first-order autoregressive covariance structure for bodyweight data and the heterogeneous autoregressive covariance structure for vitamin D data. Selection of appropriate covariance structure was based on lowest Bayesian information criterion between first-order autoregressive, heterogeneous autoregressive, compound symmetry, and heterogeneous compound symmetry structures. Significance was determined at $P < 0.05$ and trends are reported at $0.05 < P \leq 0.10$.

Results

Day tended to effect plasma vitamin D concentrations (P = 0.0786) such that day 0 had greater vitamin D concentrations (42.333ng/mL) compared with day 14 (28.910 ng/mL). Day 0 and day 14 did not differ from day 28 (27.294 ng/mL) due to the increase in variation detected in day 28 samples (SEM = 1.2238). There was an effect of day on bodyweight (P < 0.0001), as expected for growing starter pigs.

No effect of treatment was detected for bodyweight (P = 0.9151) or plasma vitamin D concentrations (P = 0.9008). There was no treatment by day interaction for bodyweight (P = 0.4447) or plasma vitamin D concentrations (P = 0.4445).

Table 1. Main effects of UVB light on bodyweight and 25 (OH) Vitamin D in piglets.

	Day				*P*-values		
	0	14	28	SEM[1]	Trt[2]	Day	Trt x Day
Bodyweight	15.9[c]	26.4[b]	48.3[a]	1.2	0.92	< 0.0001	0.45
25 (OH) Vitamin D	40.2[x]	28.9[y]	27.3[y]	0.8	0.90	0.08	0.45

[1]Standard error of the mean
[2]Treatment
[a-c] Means within a row differ by day ($P \leq 0.50$)
[x-z] Means within a row tend to differ by day ($P \leq 0.10$)

Discussion

Pigs produced in standard operational units are known to have poor circulating concentrations of vitamin D primarily due to the lack of exposure to natural UVB light (Alexander et al., 2017). Exposure of weaned pigs to UVB light at 302nm at an intensity of 250 $\mu W/cm^2$ for 10 minutes in the present study however, did not have an effect on the circulating concentrations of vitamin D in the blood. The time that pigs are exposed to UVB light and the total amount of radiation at the surface of the skin appear to be differentiating factors in the

circulating concentrations of vitamin D. Exposing pig skin *in vitro* to LED-UVB light at 296 nm for seven seconds was sufficient to increase vitamin D concentrations in skin (Barnkob et al., 2016). The light source in the study of Barnkob et al. (2016) was placed at 2 cm above the skin surface and delivered a maximum applied dose of 2000 J/m^2 to the surface. Kolp et al. (2017) used two light sources of UVB to deliver 1200 J/m^2 onto the pig's back for 15 minutes a day, and was able to increase circulating 25(OH) vitamin D. The present study delivered a dose of 2500 J/m^2 as measured at the surface of the highest point on the pigs back. It is possible that an even distribution of UVB light to the total available skin surface of the pigs was not achieved in the present study and, therefore, the actual amount of total radiation was less than the expected dose. The artificial UVB light delivers more localized exposure compared to natural sunlight. The study of Kolp et al. (2017) used artificial UVB light given with two lamps to create a more even distribution of UVB. Pigs exposed to summer sunlight at midday for one hour demonstrated greater concentrations of 25 (OH) vitamin D in the serum after 14 days compared with pigs housed indoors without access to sunlight (Larson-Meyer et al., 2017). Pigs exposed to a lower irradiance (23 $\mu W/cm^2$) than this study (250 $\mu W/cm^2$) for 15 minutes daily showed significant increases in 25 (OH) vitamin D concentrations. It may be that a greater time period of exposure at a lower dose is required to create greater concentrations of circulating vitamin D alternatively a lesser time period at a greater UVB dose rate may also be sufficient (Barnkob et al., 2016).

Kolp et al. (2017) observed serum 25(OH) vitamin D concentrations begin to decrease after week 6 of UVB exposure. Kolp et al. (2017) speculated this was because the UVB treated groups had greater concentrations of the enzyme 24-hydroxylase, resulting in more degradation of 25(OH) vitamin D. Alexander et al. (2017) contrasts this reasoning by finding no difference in 25-hydroxylase concentrations in UVB exposed pigs. The activity of 25-hydroxylase was not

measured in this study, but it is known to affect the circulating concentrations of 25(OH) vitamin D. Although not measured in the current study, 25-hydroxylase may explain the trend decrease in serum 25 (OH) concentrations measured.

Alexander et al. (2017) and Larson-Meyer et al. (2017) both used grower pigs over 11 weeks of age, while in the present study weaned pigs of less than 4 weeks were used. Alexander et al. (2017) used 18-30ng/mL of serum 25(OH) vitamin D as a reference range for sufficiency. Larson-Meyer found serum 25(OH) vitamin D concentrations for sunlight exposed pigs to be 46.1± 1.7 ng/mL. The day 28 plasma concentration found in this study was 27 ng/mL. The differences in plasma vitamin D concentrations noted in the current study compared to others could be due to the age difference in the pigs between the studies.

Conclusion

In this study additional vitamin D through UVB exposure did not increase circulating 25(OH) D levels. This contradicts the results gathered by Alexander et al. (2017), Kolp et al. (2017), and Larson-Meyer (2017). The difference in results could be due to an uneven distribution of the UVB light, the intensity and exposure time of the UVB light, or the age of animals exposed to treatment. Further studies are needed to determine the capacity for pigs to synthesize vitamin D from UVB light and the role UVB exposure has on vitamin D homeostasis in the pig.

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