Vitamin D enhanced pork from pigs exposed to artificial UVB light in indoor facilities

Barnkob, Line Lundbæk; Petersen, Paul Michael; Nielsen, Jens Peter; Jakobsen, Jette

Published in:
European Food Research and Technology

Link to article, DOI:
[10.1007/s00217-018-3173-6](https://doi.org/10.1007/s00217-018-3173-6)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Barnkob, L. L., Petersen, P. M., Nielsen, J. P., & Jakobsen, J. (2018). Vitamin D enhanced pork from pigs exposed to artificial UVB light in indoor facilities. *European Food Research and Technology*, 245(2), 411-418. <https://doi.org/10.1007/s00217-018-3173-6>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Vitamin D enhanced pork from pigs exposed to artificial UVB light in indoor facilities**

2 Line Lundbæk Barnkob¹, Paul Michael Petersen², Jens Peter Nielsen³ and Jette Jakobsen¹

3

4 ¹ Technical University of Denmark, National Food Institute, Research Group for Bioactives – Analysis and
5 Application, Kemitorvet, 2800 Kgs. Lyngby, Denmark

6 ² Technical University of Denmark, Department of Photonics Engineering, Frederiksborgvej 399, 4000
7 Roskilde, Denmark

8 ³ University of Copenhagen, Division for Production, Nutrition and Health, Grønnegårdsvej 2, 1870
9 Frederiksberg C, Denmark

10

11

12 Jette Jakobsen

13 jeja@food.dtu.dk

14

15

16

17 **Funding**

18 A grant from the European Commission under its Seventh Framework Programme (ODIN; grant agreement no.
19 613977) and the Technical University of Denmark funded the project.

20 The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the
21 manuscript.

22

23

24 **Abstract**

25 Vitamin D deficiency is a recognized problem in Europe; this can be minimized by fortifying a broader range of foods.
26 Our aim was to investigate the potential for enhancing the vitamin D content of pork from pigs raised in indoor
27 facilities, by exposing the pigs to UVB for a period just before slaughter. Three groups of six pigs were exposed to 0,
28 0.7 or 1 SED/day for 28 days. A fourth group was exposed to 2 SED; this treatment was not completed due to mild
29 erythema. The highest increase of vitamin D₃ was achieved with 1 SED; the vitamin D₃ content in loin was 3.7 ng/g;
30 more than a factor of 2 higher compared to previously reported results from studies using 2000 IU/kg feed, the
31 maximum allowed level in Europe. This is the first time an increase in the vitamin D content of pork has been reported
32 as a result of using artificial UVB-exposure of slaughter pigs in indoor facilities. However, the maximum production of
33 vitamin D was probably not reached as a linear relationship between UVB dose and vitamin D content was found;
34 therefore, the UVB-lighting method described still calls for further investigation to realise its full potential to enhance
35 vitamin D in pork.

36

37 **Keywords**

38 Vitamin D; UVB; Pork; Pigs

39

40

41 **Introduction**

42

43 On annual basis around 13 % of the European population is experiencing vitamin D deficiency (vitamin D status < 30
44 nmol/L) and 40 % vitamin D insufficiency (vitamin D status < 50 nmol/L) [1]. Although the recommended intake of
45 vitamin D is between 10-20 µg/day [2, 3], the estimated intake is only around 3-7 µg/day[4]. It has been shown that
46 vitamin D intake may be increased by effective food-based strategies [5, 6]. A feasible strategy is to fortify a broader
47 range of foods and/or increase the natural content of vitamin D in foods, e.g. fatty fish, eggs and meat [4, 7, 8].

48 Adding vitamin D to the final food product e.g. milk dairy products, and breakfast cereals is the traditional way of
49 fortifying [9]. An alternative is using the natural mechanisms to enhance the vitamin D content in foodstuff;. Like
50 humans, livestock are able to produce vitamin D₃ in the skin when exposed to UVB light (290-320 nm) either
51 artificially or from the sun [10–15]. Also when adding more vitamin D to the livestock feed the vitamin D content in the
52 final food product will increase [16, 17].

53 Vitamin D exists in two forms, vitamin D₂ and vitamin D₃. Vitamin D and 25-hydroxyvitamin D (25(OH)D), the
54 major metabolite of vitamin D, is naturally found in products of animal origin; although there are other metabolites
55 only 25(OH)D is so far included when determining the total vitamin D content in food [18, 19].

56 EU has maximum limits for adding vitamin D to feed. For pigs this limit is 2000 IU/kg feed (Commission
57 Directive 91/248/EEC), that is reported to produce lean pork loin with a content of 1.2 ng vitamin D₃/g and liver with a
58 content of 8.4 ng vitamin D₃/g [17]. In Denmark, the pigs are not fed with the maximum allowed level of vitamin D as
59 the recommended level for pigs (30-100 kg) is 400 IU/kg feed [20], that result in lean pork with a vitamin D₃ content of
60 0.24 ng/g [21]. This is in contrast to earlier findings from our research group where a vitamin D₃ content of 5-11 ng/g
61 and 42 ng/g was measured in muscle tissue and in liver, respectively, from mini-pigs after they had been exposed to
62 UVB light; the daily exposure was similar to 10-20 min summer sun at 55 °N, for 120 days [10].

63 Already in 1924 it was shown that pigs produce vitamin D when exposed to sunlight [22]; and in 1925 it was
64 reported that indoor livestock production reduces the vitamin D content in animal products for human consumption
65 [23]. A recent study shows that the vitamin D content will increase in the meat when pigs are exposed to sunlight
66 during the summer; specifically 1 hour of summer sunlight per day for 14 days before slaughter resulted in lean pork loin
67 with a content of 7.2 ng vitamin D₃/g [13]. However during winter if above or below 35 °N and 35 °S, respectively, no
68 cutaneous vitamin D will be produced [24]. Giving pigs' access to an outdoor area will therefore not alleviate the
69 problem with human vitamin D deficiency, which is a problem, especially in winter.

70 The aim of this study was to investigate the potential to enhance the vitamin D content of pork from pigs raised in
71 indoor facilities, by exposing the pigs to UVB-light for a period just before slaughter.

72

73 **Materials and methods**

74

75 **Animals and test environment**

76

77 The study included 24 slaughter pigs, all females and fair-skinned, of the Danish breed LYD (Landrace, Yorkshire and
78 Duroc); purchased from Askelygård, Roskilde, Denmark. At delivery the pigs had a mean weight of 50 kg (range 44-79
79 kg). All pigs were clinically healthy at arrival.

80 The pigs were allocated at random into 4 groups of 6 and placed in pens of 3x4.5 m, which were separated by UV-
81 impenetrable walls.

82 The pigs were fed standard feed with a vitamin D₃ content of 420 IU/kg (Danish Top S1 611+3+5, Danish Agro,
83 Karise, DK). The feed was administered twice a day. In the last 28 days of the trial the amount was up to 1 kg per pen
84 per feeding. Water was offered ad libitum, and fresh straw was provided every day. The stable was lit by fluorescent
85 tubes from 8:00 to 14:30 during the entire study. The fluorescent tubes did not emit any UVB light.

86 The trial ran from November 2016 to February 2017. The pigs were kept at the research facility for large animals
87 at University of Copenhagen (Rørrendegård, Taastrup, Denmark).

88

89 **Study design and sample size determination**

90

91 The UVB-treatment was a parallel study with three treatment groups and one control group; it lasted 28 days. Based on
92 sample size calculation with $\alpha=5\%$, $1-\beta=0.8$, $SD=24\%$ [16] and an expected increase of 50 %, a group size of four was
93 needed; however six were chosen to be on the safe side.

94

95 **UVB-exposure**

96

97 In each of the pens housing a treatment group, four UVB tubes (Lucky Reptile UV sun T5, 54 W tubes,
98 luckyreptile.com, Germany) were placed at a height of approximately 180 cm above the floor. Normal stable armatures
99 were used, but without plastic covers, as they absorbed the UVB-light. The pigs in the 3 treatment groups were
100 gradually adapted to UVB light over the first 8 days of exposure. The doses are stated in units of standard erythema
101 dose (SED) where 1 SED is equivalent to 100 J/m^2 erythema dose [25]. During the first 7 days the 3 treatment groups
102 were given the same doses: day 1-2, 0.3 SED; day 3-5, 0.6 SED; day 6-7, 0.7 SED. From day 9 the remaining 2 groups
103 were exposed to the planned doses of 1.0 SED and 2.0 SED, respectively, while the third group continued with 0.7
104 SED. The control group received no UVB exposure. The pigs were slaughtered on day 29, after 28 days of UVB-
105 exposure.

106

107 **UVB measurements**

108

109 At DTU Fotonik, Department of Photonics Engineering, Technical University of Denmark, a full spectrum of the
110 irradiance was recorded for one tube, scanning from 200 nm to 700 nm, with 1 nm increment (EOP146 detector probe,
111 Instrument Systems-CAS140CT). The tube emitted UVB from 286 nm to 320 nm, and had a maximum at 312 nm. The
112 full spectrum is shown in Online Resource 1.

113 The erythema effective irradiance was calculated using the measured spectrum and the erythema reference action
114 spectrum [25] which couples irradiance to the development of erythema. A figure of the measured spectrum with the
115 erythema reference action spectrum superimposed is shown in Online Resource 2. For measurements in the stable a
116 handheld ILT 1400-BL photometer equipped with a SEL005/TLS312/TD detector (International Light Technologies,
117 Peabody, MA) was used to measure the irradiance at 16 positions at two distances from the floor; 30 cm and 60 cm, to
118 illustrate pigs lying and standing. The average of the 32 measurements combined with the calculated erythema
119 effective irradiance was used to assess the exposure times of 150, 214, and 427 minutes, in order to achieve the target
120 doses of 0.7, 1 and 2 SED, respectively.

121

122 **Weighing**

123

124 The pigs were weighed 7 days before the UVB treatment started and just before slaughter.

125

126 Slaughtering process

127

128 The 24 pigs were electrically stunned, then stunned with a captive-bolt pistol, pithed and then hoisted by one leg and
129 stuck. After bleeding, the pigs were scalded and dehaired in a scalding tub, followed by singeing using a hand-held gas
130 torch. After singeing, black deposits and singed hairs were scraped off with a knife and washed off before evisceration;
131 the liver was collected and kept at -20 °C until further treatment. The carcasses were cut in half and hung in cold storage
132 overnight.

133

134 Sampling and homogenization

135

136 The day after slaughter, half of each carcass was divided into primal cuts and kept at -20 °C until further treatment
137 within four days. The middle third of the pork loin with rind (*Longissimus dorsi* with subcutaneous fat and skin;
138 approximately 1.2 kg) was carefully divided into lean meat, subcutaneous fat and rind. Before analysis the liver, lean
139 meat and subcutaneous fat was homogenized (Tecator 1094 Homogenizer (Foss Tecator, Höganäs, Sweden) and
140 Bamix® SliceSy®, respectively). The rind was divided into 20 strata, and from each stratum a random small biopsy
141 (diameter: 4 mm) was taken and combined into one sample.

142

143 Vitamin D analysis

144

145 The analytical method used on tissue samples, which included vitamin D₃, 25(OH)D₃, vitamin D₂ and 25(OH)D₂, was
146 developed by combining and optimizing different parts of two published methods for the quantification of vitamin D₃
147 and 25(OH)D₃ in serum and tissue [26, 27]. A full description of the method is available in Online Resource 3. In short,
148 the food samples were saponified over night at room temperature, clean-up by liquid-liquid extraction and solid phase
149 extraction. For quantification on LC-MS/MS vitamin D metabolites were derivatized by 4-phenyl-1,2,4-triazoline-3,5-
150 dione (PTAD). In serum samples the protein was precipitated and clean-up by solid phase extraction.

151 The precision of the method was < 10 % in food and < 5 % in serum. Furthermore the correctness of the method
152 was checked by analyses of certified reference materials (Milk powder, CRM421, IRMM, Geel, Belgium) and recovery
153 of added vitamin D. The result was 151 ng vitamin D₃/g ± 2.4 % (n=8) which was within the certified values of 0.143 ±

154 0.008 mg/kg, while the recovery for vitamin D₃, vitamin D₂, 25(OH)D₃ and 25(OH)D₂ was 90-127 % (n=9). All of the
155 analyses were conducted in laboratory environment accredited to perform the analyses according to ISO17025 [28].

156

157 **Content of fat**

158

159 The fat content was determined gravimetrically using a Schmid-Bondynski-Ratslaff (SBR) method [29]. The sample
160 was boiled in hydrochloric acid, ethanol was then added and the lipids were extracted using diethyl ether:petroleum
161 ether (1:1) that was subsequently evaporated and the fat was weighed.

162

163 **Statistical analysis**

164

165 One-way analysis of variance (ANOVA) with Tukey tests was used to determine significant difference in the vitamin D
166 content between the individual groups using 95 % family-wise confidence interval. The means were log transformed to
167 fulfill the assumption of equal variance and normal distribution; except for vitamin D₂ in liver. The 2 SED group was
168 excluded from the analyses as the treatment was not completed. One-way ANOVA was used to test for difference in fat
169 content between the groups. The statistical analyses were performed in R [30] using RStudio [31]. Association between
170 vitamin D status and content of vitamin D in other parts of the pigs were assessed with Pearson's correlation coefficient
171 using the Data Analysis function in Excel (version 2010, Microsoft, Redmond, WA). Data is reported as mean ±
172 standard deviation (SD).

173

174 **Results**

175

176 **Clinical findings**

177

178 No effect on health or behavior was observed in three of the groups: control, 0.7 SED and 1 SED. The pigs in the 2 SED
179 group developed mild erythema on the ears and backs after 10 days with the dose of 2 SED, i.e. day 19, therefore, the
180 UVB treatment was immediately ceased for this group. The veterinarian did not find the redness severe and the general
181 well-being and behavior of the pigs were normal; the pigs were therefore kept alive until the end of the trial and

slaughtered together with the other pigs at day 29. Thus this group did not complete the treatment and was left out from further analyses.

Content of vitamin D and fat

The measured content of vitamin D₃, 25(OH)D₃, vitamin D₂, 25(OH)D₂ is displayed in Table 1 from the three groups that completed the treatment. The main finding was that the treatment with 1 SED resulted in the highest content of vitamin D₃; specifically the content in rind, subcutaneous fat, lean meat, and liver was 316 ng/g, 120 ng/g, 3.7 ng/g, and 29 ng/g, respectively.

Table 1. Content of the four vitamers in rind, subcutaneous fat and lean meat from pork loin, in liver and in serum, and fat percentage for the samples from loin from pigs treated with different doses of UVB for 28 days.

	0 SED		0.7 SED		1 SED	
	Mean	SD	Mean	SD	Mean	SD
Rind						
Fat (%)	11.7 ^a	2.3	11.0 ^a	2.4	12.2 ^a	2.8
Vitamin D ₃ (ng/g)	1.7 ^a	0.2	146 ^b	76	316 ^c	159
25(OH)D ₃ (ng/g)	2.0 ^a	1.0	12 ^b	4	21 ^c	8
Vitamin D ₂ (ng/g)	0.5 ^a	0.1	0.8 ^b	0.2	1.1 ^b	0.3
25(OH)D ₂ (ng/g)	0.9	0.3	< 0.5		< 0.5	
Subcutaneous fat						
Fat (%)	77.9 ^a	5.5	74.5 ^a	5.1	78.4 ^a	4.7
Vitamin D ₃ (ng/g)	4.1 ^a	1.1	66 ^b	21	120 ^c	56
25(OH)D ₃ (ng/g)	0.9 ^a	0.2	4.1 ^b	0.8	6.9 ^c	1.8
Vitamin D ₂ (ng/g)	1.7 ^a	0.9	2.6 ^b	1.0	2.8 ^b	0.7
25(OH)D ₂ (ng/g)	< 0.5		< 0.5		< 0.5	
Lean meat						
Fat (%)	1.9 ^a	0.6	1.8 ^a	0.3	1.5 ^a	0.2
Vitamin D ₃ (ng/g)	0.2 ^a	0.03	2.3 ^b	0.4	3.7 ^c	1.0
25(OH)D ₃ (ng/g)	0.3 ^a	0.08	1.6 ^b	0.2	2.4 ^c	0.8
Vitamin D ₂ (ng/g)	0.3 ^a	0.08	0.4 ^b	0.1	0.5 ^b	0.1
25(OH)D ₂ (ng/g)	< 0.5		< 0.5		< 0.5	
Liver						
Vitamin D ₃ (ng/g)	0.8 ^a	0.3	14.9 ^b	3.8	29 ^c	9
25(OH)D ₃ (ng/g)	0.8 ^a	0.2	4.9 ^b	0.8	8.3 ^c	2.4
Vitamin D ₂ (ng/g)	0.7 ^a	0.6	2.3 ^b	0.3	2.7 ^b	0.4
25(OH)D ₂ (ng/g)	< 1.0		< 1.0		< 1.0	
Serum						
Vitamin D ₃ (ng/ml)	0.8 ^a	0.3	17 ^b	4	31 ^c	9

25(OH)D ₃ (ng/ml)	11 ^a	3	52 ^b	8	95 ^c	25
Vitamin D ₂ (ng/ml)	0.4 ^a	0.2	0.8 ^b	0.1	1.0 ^b	0.3
25(OH)D ₂ (ng/ml)	5.1 ^a	1.3	2.3 ^b	0.2	2.2 ^b	0.4

^{a,b,c} Mean values within a row with unlike superscript were significantly different (One-way ANOVA with Tukey test, $p < 0.05$).

There was a significant association between the serum 25(OH)D₃ (vitamin D status) and the vitamin D₃ content in the other tissues of the pig (Pearson's R 0.92-0.98). A table with the exact values is available in Online Resource 4.

There was also a significant association between the UVB dose and the content of vitamin D₃ and 25(OH)D₃ in all parts of the pig (Pearson's R 0.96-1.00). The vitamin content as a function of UVB dose is shown in Online Resource 5.

Weight

The average live weight at slaughter was 112±6 kg; equal to the average live weight of slaughter pigs in Denmark. The average weight gain during the UVB treatment was 22±3 kg; there were no significant difference between groups.

Discussion

The main goal of this study was to investigate the potential to enhance the vitamin D content of pork from pigs raised in indoor facilities, by exposing pigs to artificial UVB for a period just before slaughter.

This is the first study that reports data on the effect of different UVB-exposure levels in slaughter pigs. The doses of 0.7 SED, 1 SED and 2 SED were chosen to simulate approximately 7 to 20 minutes of midday sun exposure during summertime in Denmark (56 °N) [32]. We had planned to investigate the effect of increasing the dose to a level of 2 SED, but unfortunately the treatment with 2 SED had to be stopped, due to mild erythema (see Clinical findings in the Results section). However, the results for 0, 0.7 and 1 SED showed that the vitamin D₃ and 25-hydroxyvitamin D₃ content in the analysed parts of the pig was linearly associated with the daily exposure time with a Pearson coefficient > 0.96. This indicates that the maximum production of vitamin D has not been reached using 1 SED as the vitamin D production after exposure to UVB has been shown to be non-linear in humans and hens [33, 34].

In an earlier study from our research group, mini-pigs did not develop erythema when exposed to a UVB dose of 0.9 SED for 84 days followed by 1.8 SED for 35 days [10]. The adaption period in the present study was 5, 7 and 9

221 days for the groups 0.7 SED, 1 SED and 2 SED, respectively. We therefore assume that the mild erythema in the 2 SED
222 group was due to the shorter adaption, which was used in the present study.

223 The high content of vitamin D₃ in skin is expected to decline if the period between the last UVB exposure and
224 slaughter is extended beyond the 1 day in the present study; in rats it has been shown that vitamin D₃ is released from
225 the skin into the circulation for up to three days after UVB exposure and around 70 % of the possible vitamin D₃ is
226 released within the first day [35].

227 Kolp et al. [14] investigated a single level of UVB-exposure on pigs (1.25 SED/week¹) for 14 weeks before slaughter
228 with the aim of investigating the effect on calcium and bone metabolism for the purpose of studying the health effects
229 for the pigs. However, besides 25(OH)D₃ in serum, they also included analyses of vitamin D₃ in skin. They reported no
230 effect of the UVB-exposure on the content of vitamin D₃ in skin, but they did show an increase in serum 25(OH)D₃.
231 Compared to our study, they used less than 0.2 SED daily, which could be the reason for the different results.

232 Another strategy which has been investigated is the exposure of pigs to sunlight during summer in order to increase the
233 vitamin D content in pork [13]; this study includes a control and one treatment group of 1 hour of summer sunlight per
234 day for 14 days before slaughter. An effect of sunlight was shown for content of vitamin D₃ and 25(OH)D₃ in lean pork
235 loin, and for 25(OH)D₃ in subcutaneous fat, but no increase in the vitamin D₃ content in the subcutaneous fat was
236 observed. Larson-Meyer et al. [13] suggest that they reached the limit of how much vitamin D₃ can be stored in the
237 adipose tissue, however, in light of our findings this seems not to be the explanation.

238 During winter if above or below 35 °N and 35 °S, respectively, no cutaneous vitamin D will be produced [24];
239 giving pigs' access to an outdoor area will therefore not alleviate the problem with human vitamin D deficiency, which
240 is a problem, especially in winter.

241

242 **Effect of UVB exposure compared to effect of feeding trials**

243

244 Maximum increase in vitamin D₃ was obtained with a daily exposure of 1 SED; compared to the control group the
245 content in lean meat was increased 19 times. The dose of 1 SED was used in the last 19 of the 28 days the UVB trial
246 lasted and resulted in a content of 3.7 ng/g vitamin D₃ (range: 2.3-5.3 ng/g) in lean meat; this is more than two-fold
247 higher compared to the vitamin D₃ content of 1.2 ng/g loin from pigs fed 2000 IU/kg, which is the maximum allowed
248 vitamin D₃ in feed in Europe [17]. The vitamin D₃ content in subcutaneous fat was found to be 120 ng/g (range 40-240

¹ 0.96 MED (minimal erythema dose) where 1 MED is 125 J/m² according to their reference and thereby equal to 1.25 SED.

249 ng/g) that is 5-20 times higher than formerly reported values of 7.5-7.6 ng/g in subcutaneous fat from pigs fed 2000
250 IU/kg [16, 17]. In rind, 1 SED resulted in a vitamin D₃ content of 259 ng/g (range 170-530 ng/g) that is 55-170 times
251 higher than the 3 ng/g reported in a feeding study at maximum feeding level [16]. The liver from pigs exposed to 1 SED
252 had a content of 29 ng/g (range 20-45 ng/g) that is 2-5 times higher than the 8.4 ng/g in liver from pigs fed 2000 IU/kg
253 [17]. This content was lower than in liver from mini-pigs exposed to UVB [10], but the duration of the UVB treatment
254 differed, being 120 days for mini-pigs and 28 days in this study.

255 Similar to vitamin D₃ the content of 25(OH)D₃ was highest in the 1 SED group, and higher in the UVB-exposed
256 pigs compared to the results from feeding trials using 2000 IU/kg feed [16, 17].

257

258 **Unexpected increase in vitamin D₂**

259

260 Unexpectedly there was found an increase in the vitamin D₂ content in the UVB treatment groups compared to the
261 control. We suggest that it originates from either feed or straw contaminated with fungi. In another study it is shown
262 that when grass contaminated with fungi is exposed to UVB the ergosterol in the fungi is converted to vitamin D₂ [36].
263 This would explain why only the groups with UVB-exposure show an increase in vitamin D₂ as the straw and the feed
264 were exposed to the UVB when administered to the pigs and thereby functioned as a source of vitamin D₂ in the UVB
265 exposed groups. We cannot confirm if this is the explanation, as neither the straw nor the feed were analysed for
266 vitamin D metabolites.

267

268 **Interaction between vitamin D₂ and vitamin D₃**

269

270 In contrast to vitamin D₂, the control group had the highest content of 25(OH)D₂. The 25(OH)D₂ content in serum from
271 the UVB treated groups were significantly lower than in serum from the control group. Furthermore 25(OH)D₂ was
272 present in rind from the control group but not found in the in rind from the UVB treated groups. To our knowledge this
273 is the first time an increase in vitamin D₃ has been reported to result in a decrease in 25(OH)D₂. Previously, only a
274 decreasing effect of vitamin D₂ intake on 25(OH)D₃ serum concentration has been reported as summarised by Wilson et
275 al. [37]. Lehmann et al. [38] administered vitamin D₂, D₃ or placebo to healthy humans for a period of 8 weeks. They
276 suggested that vitamin D₂ impairs the hydroxylation of vitamin D₃ as it explained that they observed a higher decrease
277 in serum 25(OH)D₃ in the group administered with vitamin D₂ than in the placebo group [38]. However, based on our

278 results we suggest that the hydroxylation of vitamin D₂ and vitamin D₃ is a classic case of competitive inhibition
279 where the substrate present in the highest concentration inhibits the hydroxylation of the substrate with the lower
280 concentration [39].

281

282 **Effect of UVB exposure on variation in vitamin D content**

283

284 The within group variation, in vitamin D content, is generally low in feeding trials [16, 17]. The variation in the control
285 group in this study was also low, while the variation in the UVB exposure groups was higher. The relative precision
286 (RSD) for the vitamin D₃ content measured in rind, subcutaneous fat and lean meat from the 1 SED group was 4.3, 1.7
287 and 1.8 times higher, respectively, compared to RSD from the control group. Similarly, sun exposure causes higher
288 variation [13].

289 The effect of UVB-exposure on vitamin D content in pork possibly depends on three factors: the height of the
290 pigs; the individual behavior, especially standing or lying; and the effectiveness of the endogenous production of
291 vitamin D₃ in the skin. There was noticeable height difference between the pigs; the height of the individual pigs was
292 however not measured but was estimated to be around 60 cm at arrival and 70 cm at slaughter. The higher the pig, the
293 closer the pig was to the UVB tube and thereby the dose received would be higher giving raise to higher vitamin D
294 production; the same reasoning applies for some pigs standing more than others. Some of the pigs had pigmentation on
295 part of their bodies, this could in theory decrease the effectiveness of the endogenous production of vitamin D [40],
296 though for cows no effect is observed [11].

297

298 **25(OH)D₃ as biomarker for vitamin D content**

299

300 One study has shown that there is a linear relationship between the serum 25(OH)D₃ and the vitamin D₃ and 25(OH)D₃
301 content in meat when vitamin D₃ is added to the feed [17]. In the present study serum 25(OH)D₃ was associated to the
302 content of vitamin D₃ and 25(OH)D₃ in rind, fat, lean meat, liver and serum. This indicates that a model may be set up
303 between serum 25(OH)D₃ and the content of vitamin D₃ and 25(OH)D₃ in pork products. Such a model could be useful
304 in quality control of vitamin D content in pork products, as the analysis of serum 25(OH)D₃ is easier, faster and
305 cheaper compared to analysis of other tissues. Larson-Meyer et al. [13] also found a positive association between serum
306 25(OH)D₃ concentrations and vitamin D₃ in loin (R 0.63) although not as high as ours (R 0.96). They also found a

307 positive association with 25(OH)D₃ in loin (R 0.83). The reason for the stronger association shown in our study might
308 be due to the difference in analytical assays for serum 25(OH)D₃, namely RIA in Larson-Meyer et al. [13] and a
309 chemical assay in this study. A concentration dependent difference has been shown between the two analytical
310 principles of quantification ; the linear correlation between the two is segmented with a shift around 50 nmol/L[41, 42].
311

312 **Effect of UVB exposure on weight gain**

313

314 UVB exposure did not have an effect on the weight gain of the pigs. The same was found in studies where vitamin D
315 status was followed in pigs exposed to 1 hour of sunlight for a total of 28 days, and in pigs exposed for 14 weeks [14,
316 15].
317

318 **Strengths and limitations**

319

320 The strength of our study is the characterisation of the UVB-source as well as the documentation of the UVB-exposure
321 of the pigs during the study. Furthermore, a sensitive and specific analytical method capable of quantifying all four
322 vitamin D metabolites was used for food and serum samples.

323 However, if the length of the exposure prior to slaughter had been extended beyond the 28 days it might lead to an
324 additional increase in vitamin D as 50 days was needed to reach maximum serum vitamin D₃ concentration in mini-pigs
325 exposed to UVB-light [10]. The UVB-tubes used had a maximum at 312 nm, however, the optimal wavelength for the
326 production of vitamin D₃ in skin is 296 nm; at this wavelength approximately 10 times more vitamin D is produced
327 compared to 312 nm [7]. If the spectrum of the used UVB-tubes was shifted so it had a maximum at 296 nm it would,
328 however, only increase the erythema effective irradiance with a factor of 3; therefore, in theory, the exposure time
329 needed to obtain 1 SED would be 1/3, compared to at 312 nm, but the production of vitamin D₃ would increase
330 approximately 3 times.

331 The variation caused by individual behavior could possibly be lowered by extending the exposure time, which
332 could be implemented by increasing the height from lamp to pigs or using a UVB source with lower intensity.
333

334 **Perspectives**

335

336 It was demonstrated that it is possible to enhance the vitamin D content in pork by use of artificial UVB-exposure in
337 indoor facilities for pigs, providing pork products with higher vitamin D content than has been shown in feeding trials.
338 The obtained content by use of UVB-exposure is similar to the levels found in fish [43, 44]. Using UVB is a natural
339 process for fortification with vitamin D that may be useful for marketing this pork given the public aversion to
340 genetically modified organisms (GMO) products in Europe. Additionally, the vitamin D content is expected to be
341 unchanged during cooking as there is no significant difference between the vitamin D content in raw and cooked pork
342 loin with rind [45].

343 The potential of our results (with only 28 days of exposure before slaughter) may be expressed based on the pig
344 with the highest content of vitamin D; the total vitamin D activity in 100 g of minced meat with 10 % fat is calculated to
345 be 3.0 µg and in a traditional Danish liver paté (35 % liver and 15 % lard) the activity is calculated to be 4.9 µg/100g;
346 this is enough to be labelled 'high content of vitamin D' under current European legislation (Regulation (EC) No
347 1924/2006). However, the maximum production of vitamin D was probably not reached as we found a linear
348 relationship between UVB dose and vitamin D content. Therefore, the UVB-lighting method described still calls for
349 further investigation to realise its full potential to enhance vitamin D in pork.

350

351 **Acknowledgements**

352 We would like to acknowledge the staff at Rørrendegård (University of Copenhagen) for their commitment to take care
353 of the pigs, Heidi Jahn and Tamaris Phipps (DTU Food) for their skillful performance of the analysis for vitamin D and
354 fat, and Dennis Dan Corell (DTU Fotonik) for performing the UVB measurements.

355

356 **Compliance with Ethical Standards**

357 The Animal Experiments Inspectorate, part of the Danish Veterinary Food and Administration, judged that the trial did
358 not need a governmental approval. No ethics committee was involved as the pigs lived similar to pigs at a farm except
359 that they were exposed to UVB light similar to a daily exposure of approximately 5 to 20 minutes summer sunshine in
360 Denmark. The project plan was assessed and approved by a veterinarian at the Department of Experimental Medicine
361 at University of Copenhagen (Number P16-414). The animals were overseen by keepers and veterinarians during the
362 trial.

363

364 **Conflict of interest** None.

365

366 **References**

- 367 1. Cashman KD, Dowling KG, Gonzalez-Gross M, et al (2016) Vitamin D deficiency in Europe: pandemic? Am J
368 Clin Nutr 103:1033–1044
- 369 2. Institute of Medicine (2011) Dietary Reference Intakes for Vitamin D and Calcium. The National Academies
370 Press, Washington DC
- 371 3. Nordic Council of Ministers (2012) Nordic Nutrition Recommendations 2012 : Integrating nutrition and
372 physical activity
- 373 4. Cashman KD, Kiely M (2016) Tackling inadequate vitamin D intakes within the population: fortification of
374 dairy products with vitamin D may not be enough. Endocrine 51:38–46
- 375 5. O’Mahony L, Stepien M, Gibney MJ, et al (2011) The potential role of vitamin D enhanced foods in improving
376 vitamin D status. Nutrients 3:1023–41
- 377 6. Black LJ, Seamans KM, Cashman KD, Kiely M (2012) An Updated Systematic Review and Meta-Analysis of
378 the Efficacy of Vitamin D Food Fortification. J Nutr 142:1102–1108
- 379 7. Barnkob LL, Argyraki A, Petersen PM, Jakobsen J (2016) Investigation of the effect of UV-LED exposure
380 conditions on the production of vitamin D in pig skin. Food Chem 212:386–391
- 381 8. Kiely M, Black LJ (2012) Dietary strategies to maintain adequacy of circulating 25-Hydroxyvitamin D
382 concentrations. Scand J Clin Lab 72:14–23
- 383 9. Holick MF (2007) Vitamin D Deficiency. N Engl J Med 357:266–281
- 384 10. Burild A, Frandsen HL, Poulsen M, Jakobsen J (2015) Tissue content of vitamin D3 and 25-hydroxy vitamin
385 D3 in minipigs after cutaneous synthesis, supplementation and deprivation of vitamin D3. Steroids 98:72–9
- 386 11. Jakobsen J, Jensen SK, Hymøller L, et al (2015) Short communication: Artificial ultraviolet B light exposure
387 increases vitamin D levels in cow plasma and milk. J Dairy Sci 6492–6498
- 388 12. Schutkowski A, Krämer J, Kluge H, et al (2013) UVB exposure of farm animals: study on a food-based strategy

389 to bridge the gap between current vitamin D intakes and dietary targets. PLoS One 8:e69418

390 13. Larson-Meyer DE, Ingold BC, Fensterseifer SR, et al (2017) Sun exposure in pigs increases the Vitamin D
391 nutritional quality of pork. PLoS One 12:

392 14. Kolp E, Wilkens MR, Pendl W, et al (2017) Vitamin D metabolism in growing pigs: influence of UVB
393 irradiation and dietary vitamin D supply on calcium homeostasis, its regulation and bone metabolism. J Anim
394 Physiol Anim Nutr (Berl) 101:79–94

395 15. Alexander BM, Ingold BC, Young JL, et al (2017) Sunlight exposure increases vitamin D sufficiency in
396 growing pigs fed a diet formulated to exceed requirements. Domest Anim Endocrinol 59:37–43

397 16. Jakobsen J, Maribo H, Bysted A, et al (2007) 25-Hydroxyvitamin D3 affects vitamin D status similar to vitamin
398 D3 in pigs—but the meat produced has a lower content of vitamin D. Br J Nutr 98:908–913

399 17. Burild A, Lauridsen C, Faqir N, et al (2016) Vitamin D3 and 25-hydroxyvitamin D3 in pork and their
400 relationship to vitamin D status in pigs. J Nutr Sci 5:e3

401 18. Ovesen L, Brot C, Jakobsen J (2003) Food contents and biological activity of 25-hydroxyvitamin D: A vitamin
402 D metabolite to be reckoned with? Ann Nutr Metab 47:107–113

403 19. Robertson CE (2003) McCance and Widdowson's The Composition of Foods - Sixth Summary Edition. Nutr
404 Bull 28:81–83

405 20. Tybirk P, Sloth NM, Kjeldsen N, Shooter L (2016) Nutrient requirement standards. 24th edition

406 21. Jakobsen J, Mikkelsen AÆ, Bysted A, Knuthsen P (2017) Næringsindhold i kødudskæringer - Danske grise.
407 Søborg

408 22. Steenbock H, Hart EB, Jones JH (1924) Fat-soluble vitamins. XVIII. Sunlight in its relation to pork production
409 on certain restricted rations. J Biol Chem 61:775–794

410 23. Steenbock H, Hart E, Hoppert C, Black A (1925) Fat-soluble vitamin. XXVI. The antirachitic property of milk
411 and its increase by direct irradiation and by irradiation of the animal. J Biol Chem 66:441–449

412 24. Webb AR, Kline L, Holick MF (1988) Influence of season and latitude on the cutaneous synthesis of vitamin

413 D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin.
414 J Clin Endocrinol Metab 67:373–8

415 25. International Commission on Illumination (1999) Erythema Reference Action Spectrum and Standard Erythema
416 Dose. ISO/CIE Standard ISO 17166:1999

417 26. Burild A, Frandsen HL, Poulsen M, Jakobsen J (2014) Quantification of physiological levels of vitamin D3 and
418 25-hydroxyvitamin D3 in porcine fat and liver in subgram sample sizes. J Sep Sci 37:2659–63

419 27. Burild A, Frandsen HL, Jakobsen J (2014) Simultaneous quantification of vitamin D3, 25-hydroxyvitamin D3
420 and 24,25-dihydroxyvitamin D3 in human serum by LC-MS/MS. Scand J Clin Lab Invest 74:418–423

421 28. International Standard Organization (2005) ISO/IEC 17025 General requirements for the competence of testing
422 and calibration laboratories. Int Stand 2005:1–36

423 29. Nordic Committee on Food Analysis. (1989) Fat. Determination according to SBR (Schmid–Bodynzki–
424 Ratslaff) in meat and meat products. Method no. 131

425 30. R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical
426 Computing

427 31. RStudio Team (2016) RStudio: Integrated Development for R.

428 32. Bogh MKB, Schmedes A V., Philipsen PA, et al (2012) A small suberythematous ultraviolet B dose every second
429 week is sufficient to maintain summer vitamin D levels: A randomized controlled trial. Br J Dermatol 166:430–
430 433

431 33. Grigalavicius M, Moan J, Dahlback A, Juzeniene A (2015) Vitamin D and ultraviolet phototherapy in
432 Caucasians. J Photochem Photobiol B Biol 147:69–74

433 34. Kühn J, Schutkowski A, Hirche F, et al (2015) Non-linear increase of vitamin D content in eggs from chicks
434 treated with increasing exposure times of ultraviolet light. J Steroid Biochem Mol Biol 148:7–13 . doi:
435 10.1016/j.jsbmb.2014.10.015

436 35. Holick MF, MacLaughlin JA, Clark MB, et al (1980) Photosynthesis of Previtamin D3 in Human Skin And the

- 437 Physiologic Consequences. *Science* (80-) 210:203–205
- 438 36. Jäpelt RB, Didion T, Smedsgaard J, Jakobsen J (2011) Seasonal Variation of Provitamin D 2 and Vitamin D 2
439 in Perennial Ryegrass (*Lolium perenne* L.). *J Agric Food Chem* 59:10907–10912
- 440 37. Wilson LR, Tripkovic L, Hart KH, Lanham-New SA (2016) Conference on “New technology in nutrition
441 research and practice” Postgraduate Symposium Vitamin D deficiency as a public health issue: using vitamin D
442 2 or vitamin D 3 in future fortification strategies. In: *Proceedings of the Nutrition Society*. pp 11–14
- 443 38. Lehmann U, Hirche F, Stangl GI, et al (2013) Bioavailability of Vitamin D 2 and D 3 in Healthy Volunteers, a
444 Randomized Placebo-Controlled Trial. *J Clin Endocrinol Metab* 98:4339–4345
- 445 39. Berg JM, Tymoczko JL, Stryer L (2006) *Biochemistry*, sixth. W.H. Freeman and Company
- 446 40. Clemens TL, Henderson SL, Adams JS, Holick MF (1982) Increased skin pigment reduces the capacity of the
447 skin to synthesise vitamin D3. *Lancet* 319:74–76
- 448 41. Cashman KD, Kiely M, Kinsella M, et al (2013) Evaluation of Vitamin D Standardization Program protocols
449 for standardizing serum 25-hydroxyvitamin D data: a case study of the program’s potential for national nutrition
450 and health surveys. *Am J Clin Nutr* 97:1235–1242
- 451 42. Jakobsen J, Bysted A, Andersen R, et al (2009) Vitamin D status assessed by a validated HPLC method: within
452 and between variation in subjects supplemented with vitamin D 3. *Scand J Clin Lab Invest* 69:190–197
- 453 43. Padula D, Greenfield H, Cunningham J, et al (2016) Australian seafood compositional profiles: A pilot study.
454 Vitamin D and mercury content. *Food Chem* 193:106–111
- 455 44. Jakobsen J, Smith C (2017) Farmed Salmon and Farmed Rainbow Trout - Excellent Sources of Vitamin D?
456 *Fish Aquac J* 8:
- 457 45. Clausen I, Jakobsen J, Leth T, Ovesen L (2003) Vitamin D3 and 25-hydroxyvitamin D3 in raw and cooked pork
458 cuts. *J Food Compos Anal* 16:575–585

459