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Improving vitamin D content in pork meat by UVB biofortification

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ABSTRACT

Vitamin D deficiency is prevalent worldwide and identification of alternative food-based strategies are urgently warranted. In two studies, 12-week old crossbred pigs (Duroc x (Large White x Landrace)) were exposed daily to narrowband UVB radiation for ~10 weeks or control (no UVB exposure) until slaughter. In Study 1 ($n = 48$), pigs were exposed to UVB for 2 min and in Study 2 ($n = 20$), this duration was tripled to 6 min. All pigs were fed the maximum permitted 2000 IU vitamin D_3/kg feed. Loin meat was cooked prior to vitamin D LC-MS/MS analysis. In Study 1, pork loin vitamin D_3 did not differ between groups. Study 2 provided longer UVB exposure time and resulted in significantly higher loin vitamin D_3 (11.97 vs. 6.03 μg/kg), 25(OH)D_3 (2.09 vs. 1.65 μg/kg) and total vitamin D activity (22.88 vs. 14.50 μg/kg) concentrations, compared to control ($P < 0.05$). Pigs remained healthy during both studies and developed no signs of erythema. Biofortification by UVB radiation provides an effective strategy to further safely increase the naturally occurring vitamin D content of pork loin, alongside feed supplementation.

1. Introduction

Vitamin D deficiency is a serious public health concern, which manifests as various acute and chronic diseases, most prominently nutritional rickets or osteomalacia (Sizar, Khare, Goyal, Bansal, & Givler, 2021). Dietary requirements set to promote optimal musculoskeletal and immune health vary by country and life stage (approx 5–20 μg/day), yet whole-population insufficiency/deficiency prevalence (USA, Canada and Europe) remains at 23–40% and 6–13% respectively (Cashman et al., 2016; Herrick et al., 2019; Sarafin et al., 2015). Vitamin D exists in two main forms, vitamin D_2 and vitamin D_3; the latter of which is generally considered more effective in elevating vitamin D status (Wilson, Tripkovic, Hart, & Lanham-New, 2017). Endogenous synthesis, triggered by ultraviolet type B (UVB) exposure (290–315 nm), is the main and preferred source of vitamin D_3, providing approximately 80–90% of requirements in humans (Holick, 2007) but this is highly variable between individuals. Thus, dietary sources remain an important component to achieve optimal status and opportunity exists to fortify common foods to help increase vitamin D intakes and reduce risk of disease (Dunlop et al., 2021). Meat is a popular component of UK diets and pork, which naturally contains vitamin D_3 (0.1–1.4 μg/100 g) and 25-hydroxyvitamin D_3 (25(OH)D_3; 0.07–0.19 μg/100 g) as well as high biological value proteins, essential fatty acids, B-vitamins, iron and zinc, is the most widely consumed meat globally (Public Health England, 2020, 2021). As such, enriching the vitamin D content in pork meat may offer consumer protection against deficiency and improve current intake levels.

By feed supplementation or adjusting husbandry practices to include UVB exposure, it is possible to naturally enhance the vitamin D content in animal-based products, including pork, beef, chicken, eggs and fish (Neill, Gill, McDonald, McRoberts, & Pourshahidi, 2021). Pigs are traditionally raised indoors devoid of UVB exposure and European Member States are restricted to supplementing pig feed with 2000 IU vitamin D/kg (European Food Safety Authority (EFSA), 2017). Due to confined facilities, pigs are susceptible to vitamin D-dependent rickets (Dittmer & Thompson, 2011). Surveys and case studies from the Midwest United States (~37°N, USA) suggest vitamin D insufficiency is common and associated with mortality and morbidity in growing pigs

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(Madson et al., 2012). Coupled with restricted levels of vitamin D within animal feed, alternative strategies are warranted to further enhance vitamin D status in pigs and their meat. Similar to humans, pigs are capable of synthesising vitamin D_3 following UVB exposure as 7-dehydrocholesterol present in skin is converted to pre-vitamin D_3 and then vitamin D_3 (cholecalciferol) by thermal isomerization which is then sequentially hydroxylated by the liver and kidneys to 25-hydroxyvitamin D_3 (25(OH)D_3; calcidiol) and the final active metabolite 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$; calcitriol) (Holick, 1981; Kolp, Wilkens, Pendl, Eichenberger, & Liesegang, 2017). The production of vitamin D from UVB radiation is tightly regulated and controlled by a feedback system whereby pre-vitamin D_3 is converted to biologically inert tachysterol and lumisterol (Holick, 1981). Unlike dietary sources, this physiological regulation prevents vitamin D toxicity which can manifest as poor weight gain, haemorrhagic gastritis, mineralisation of various organs, lethargy, polyuria, polydipsia and hypercalcemia in pigs (Wimsatt, Marks, Campbell, Johnson, & Nachreiner, 1998). Therefore, UVB lighting in pig housing may provide a simple, feasible and cost-effective way in which to increase vitamin D content of pork meat and improve animal health.

To-date, on-farm vitamin D biofortification (also referred to as 'bio-enrichment' or 'bio-addition' research in pigs has been varied in approach, with some focusing on feed alone (Burild, Lauridsen, Faqir, Sommer, & Jakobsen, 2016; Clausen, Jakobsen, Leth, & Ovesen, 2003; Duffy et al., 2018; Jakobsen, Maribo, Bysted, Sommer, & Hels, 2007; Wilborn, Kerth, Owsley, Jones, & Frobish, 2004) and others investigating the impact of UVB exposure (Alexander et al., 2017; Barnkob, Petersen, Nielsen, & Jakobsen, 2019; Burild, Frandsen, Poulsen, & Jakobsen, 2015; Jakobsen, Nielsen, & Jakobsen, 2020; Kolp et al., 2017; Larson-Meyer et al., 2017). Of those reporting data following UVB exposure, both natural sunlight (Alexander et al., 2017; Larson-Meyer et al., 2017) and artificial lamps (Barnkob et al., 2019; Burild et al., 2015; Jakobsen et al., 2020; Kolp et al., 2017) have been used. Vitamin D status in pigs, classified by total circulating 25(OH)D (D_3 and, when above limit of detection and quantification, D_2) concentrations, consistently improves following supplementation or irradiation (Neill et al., 2021). With the exception of one of the earlier studies (Wilborn et al., 2004) which provided supranutritional concentrations (40,000 or 80,000 IU vitamin D_3/kg), more recently quantities in diet-focused biofortification studies have ranged from 200 to 2200 IU (5–55 μg) vitamin D/kg feed, with vitamin D_3, 25(OH)D_3, vitamin D_2 or vitamin D_2-enriched mushrooms being offered. The type of vitamin D ingested will determine the increases observed in tissue concentrations, for example, plasma 25(OH)D_3 or vitamin D_3 is highest depending on which metabolite was present within the feed. While some older research has explored the impact of natural UVB exposure from outdoor sunlight (Alexander et al., 2017; Larson-Meyer et al., 2017), inherently the opportunity to avail of such UVB exposure is limited, particularly during winter when no cutaneous vitamin D will be synthesised at latitudes higher than 35° North or South. Artificial UVB pig studies have been conducted in Denmark where recommendations vary compared to European legislation; advising between 400 and 800 IU vitamin D/kg feed depending on the age of the pig (Tybirk, Sloth, Kjeldsen, & Shooter, 2018). Studies providing 0.7–1.8 SED/day have observed increased plasma 25(OH)D_3 and vitamin D_3 concentrations (Barnkob et al., 2019; Burild et al., 2015; Jakobsen et al., 2020).

No studies to-date have provided UVB exposure to pigs alongside the maximum vitamin D feed limit permitted by European legislation. Therefore, the objective of the study was to determine the impact of UVB irradiation of finishing pigs in commercial conditions on their total vitamin D concentration (including its vitamers and 25-hydroxymetabolites) in plasma and post-slaughter in pork cuts (loin and leg), hypothesising that UVB exposure would increase vitamin D concentrations compared to control.

2. Materials and methods

Two randomised control parallel intervention studies were conducted at Agri-Food and Biosciences Institute (AFBI, Hillsborough, UK) from September to November 2018 and 2019, referred to hereafter as Study 1 and Study 2, respectively. Methodology is adapted from a pilot study by the same research group. All experimental procedures described in this work were approved by the Agri-Food and Biosciences Institute (AFBI) Research Ethics Committee (AFBI-H-19063) and conducted under the Cruelty to Animal Act 1876 and the Animals (Scientific Procedures) Act 1986.

2.1. Animal housing and management

Both studies used crossbred pigs (Glenmarshal sires, Newry, UK) from pure Landrace X Large white sire F1 sows bred at AFBI (Hillsborough, UK) using PIC lines from Hermitage sires Kilkey. (Stapeley, Nantwich, UK). The F1 sow was crossed with a terminal Danish Duroc. Study 1 used 48 pigs (24 males, 24 females; baseline weight 44.0 ± 3.7 kg) and Study 2 used 20 pigs (10 males, 10 females; baseline weight 41.8 ± 3.1 kg). Sample size numbers were selected based upon maximum individual housing capacity at AFBI facilities and sufficient meat quantities required for a subsequent human intervention study, respectively.

Pigs in the study were fed and managed following commercial routine practice, including the time and age at when diets are changed from grower to a finisher diet. All feeds offered to pigs were commercially available diets. For both studies, at 10 weeks of age, all pigs were moved to the experimental facilities and were offered a cereal based grower diet (DE 15.0 MJ, CP 17.5%, Lys 1.2%) until 12 weeks of age (acclimatization period). At 12 weeks of age (start of the trial i.e. week 0), all pigs received a cereal based finisher diet (DE 14.0 MJ, CP 15.5%, Lys 1.1%). Both diets were sourced from John Thompson & Sons Limited (Belfast, UK) and contained the maximum permitted vitamin D_3 concentrations as outlined by European Union regulations (2000 IU/kg of complete feedstuff) (European Food Safety Authority (EFSA), 2017). Therefore, pigs received the same level of dietary vitamin D prior to the commencement of the study. Premixes were provided by Devenish Nutrition Limited (Belfast, UK). Pigs had ad libitum access to fresh water.

In Study 1, pigs were individually kept in solid floor pens (2.8 × 1 m) from 10 weeks of age until the end of the trial. Pigs were fed to appetite with constant access to the trough. Weekly weigh backs were performed for consumption data. In Study 2, pigs were individually identified with electronic ear tags and housed in plastic slatted pens (3.9 × 3.3 m) in groups of 10. Pens had an electronic feeder (0.65 × 2.2 m, Schauer Agrotronic GmbH, Prambachkirche, Austria) that allowed daily weight and feed intake of each pig. In both studies, pigs remained in indoor confinement. Temperature was set at 21 °C and Farm-x Dicam equipment (Finrone Systems Ltd., Londonderry, UK) used to automatically adjust and control ventilation settings to maintain temperature. Standard glass windows above the pens did not allow UVB permeation. From 00:00 to 02:00, there was total darkness in the housing facilities. Outside of these hours, two small passageway lights (1 ft. sq) were lit and fluorescent lights (5 ft) lit when staff were working in housing unit. No UVB radiation was emitted from these lights.

2.2. Experimental design

At 12 weeks of age, pigs were blocked using Microsoft Excel on the basis of weight, sex, age and mother, and randomly assigned by the AFBI Higher Scientific Officer in the pig unit to one of two treatment groups; either daily UVB exposure (Study 1 $n = 24$; Study 2 $n = 10$) or control treatment with no UVB exposure (Study 1 $n = 24$; Study 2 $n = 10$). Both studies followed the same protocol and were informed by earlier (unpublished) studies. Fig. 1 present flow diagrams of the study design and

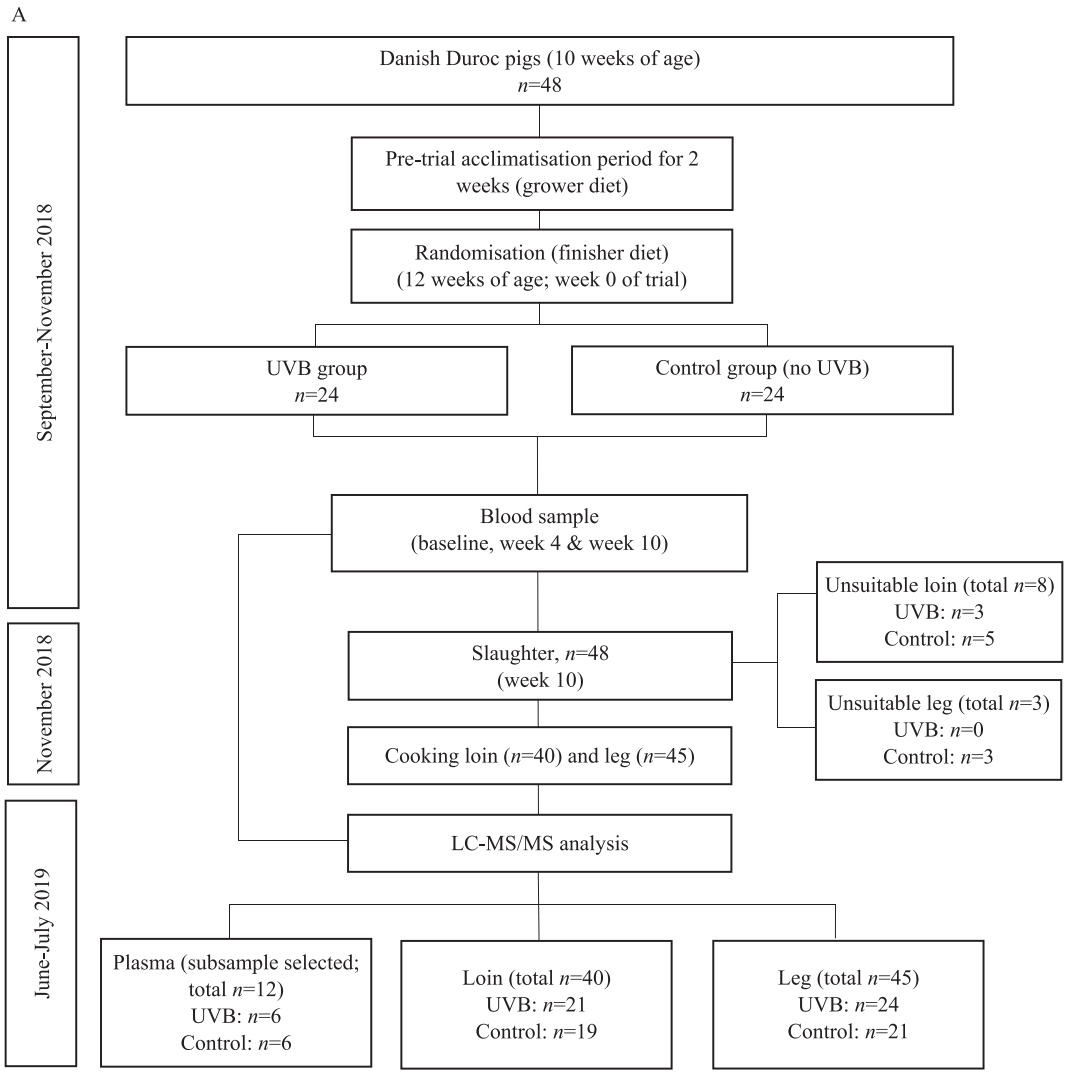

Fig. 1. A-B. Flowchart describing the design of Study 1 (Fig. 1A) Study 2 (Fig. 1B.). *n*, number of samples; UVB, ultraviolet-B; LC-MS/MS, liquid chromatography tandem mass spectrometry.

sampling; however, daily UVB radiation exposure time increased from Study 1 (2 min) to 2 (6 min). Baseline skin condition was recorded for all animals and health monitored. All pigs remained healthy for the duration of both studies and presented no signs of erythema (redness on the skin). No morbidity or mortality was observed.

Four UVB narrowband lamps (Koninklijke Philips N.V., Amsterdam, The Netherlands) providing a safe wavelength range effective for vitamin D synthesis (290–315 nm) were placed above a confined structure which fit one pig to ensure direct light exposure. This very narrow waveband emitted 305–315 nm, peaking at 311 nm on the UV spectrum, reduces erythema risk and allows extended exposure duration. The lamps were set at a fixed height (1.2 m from the ground) for the duration of the study. As such, the height of the light above the pigs, and therefore the UVB dose, constantly changed as the pigs grew in size and depending on the posture during UVB exposure (e.g. sit or stand). Radiation was measured from a set distance at three points along each bulb prior to the intervention commencing to confirm vitamin D effective irradiance, and then weekly at the height of the pigs back, using a handheld UVB meter (Solarmeter®, Solar Light Company, Pennsylvania, USA). The position of the lights remained fixed as the pig grew during the intervention. Pigs in the treatment group were moved individually to the confined structure and exposed to UVB radiation daily for 69 days in Study 1 and 63 days in Study 2. Pigs in the control group were also moved to a similar setting for the same duration, but without UVB exposure. UVB exposure was designed to administer at least 1 SED without the risk of erythema, and the duration of exposure was tripled from Study 1 (2 min) to Study 2 (6 min).

2.3. Weighing and posture measurement

In Study 1, pigs were weighed weekly and feed consumption recorded by weekly weigh backs. In Study 2, weight was individually recorded via the automatic electronic feeders. Additionally, in Study 2, pig posture was monitored and scored daily during UVB exposure. As pigs increased in size, it was noticed that their position whilst under the UVB light varied. Thus, from 18 weeks of age (week 6 of intervention) until endpoint, pig position was scored daily during irradiation exposure with 'standing' being assigned 3 points, 'sitting' 2 points and 'lying down' 1 point. Combination of the postures were awarded half of the posture points (e.g. stood/sat = 2.5 points). This was then calculated as a total posture score (range 35 to 105). Standing was the ideal posture as it was the closest distance to the UVB light and therefore the higher the posture score, the higher potential UVB dose received. Control pigs were scored during the same timeframe whilst also in individual confinement.

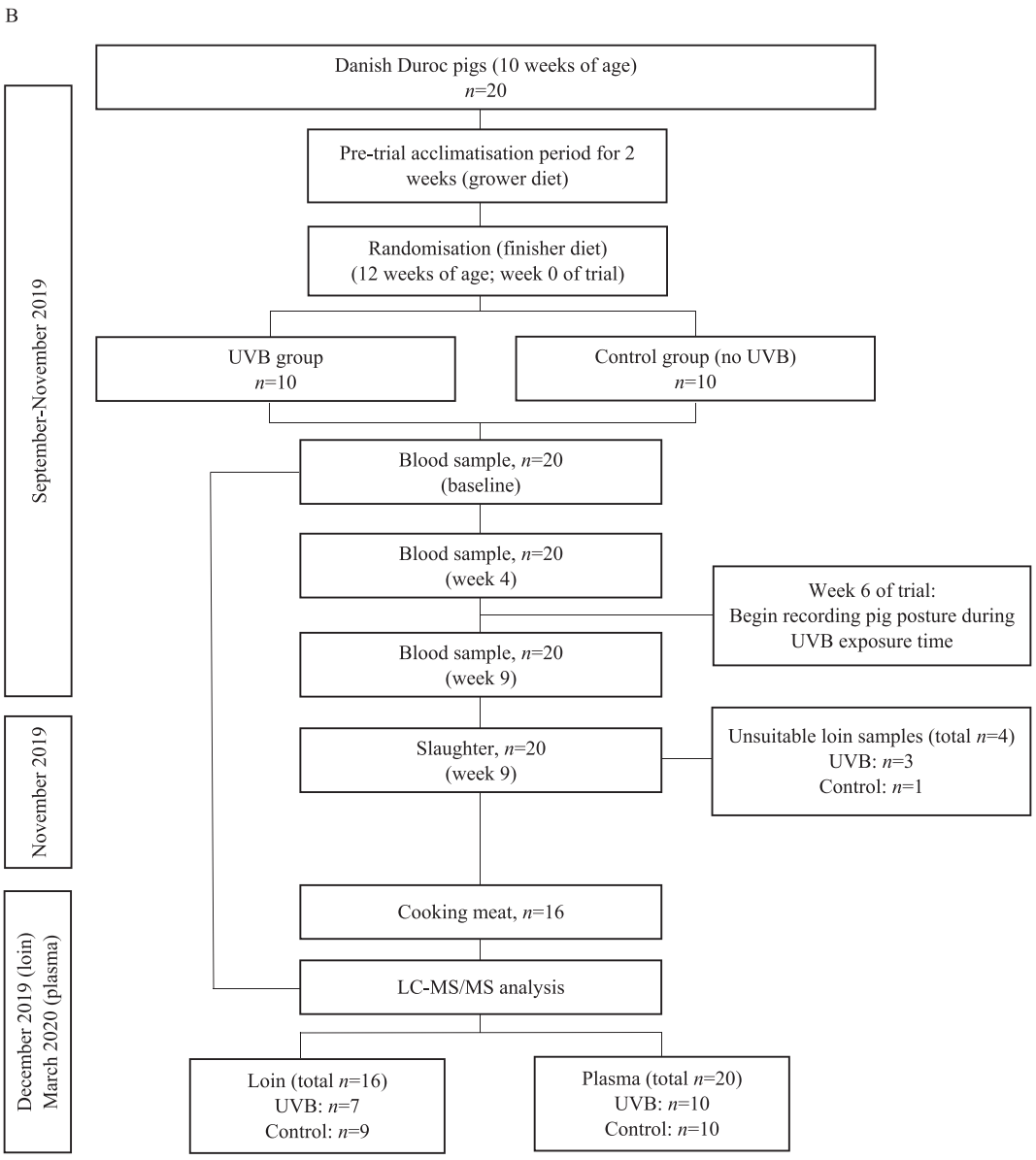

Fig. 1. (*continued*).

2.4. Sampling of blood

For both studies, blood samples by vena cava puncture (10 ml; 21-gauge needles) were obtained at baseline, midpoint (week 4 of trial) and endpoint prior to slaughter (week 10 for Study 1 and week 9 of trial for Study 2) by a trained handler. Samples were centrifuged (2200 x*g* for 15 min at 4 °C) before decanting plasma supernatant and storing within −80 °C freezers until required for analysis.

2.5. Slaughter procedure

Following the last day of UVB exposure, pigs were slaughtered at the Karro Food Group (Cookstown, UK) abattoir. The pigs were gas stunned followed by exsanguination as per standard slaughter procedures. The carcasses were fast chilled for the first 2 h after slaughter, bisected longitudinally and hung in cold storage overnight. Full loin muscle from both sides of the back muscle (*Longissimus thoracis et lumborum* (LTL)) was collected and kept at −20 °C until further treatment. In study 1, leg (topside and silverside) was also collected and backfat (mm) was measured as the average at the 10th and last rib. Eight loin and three leg samples from Study 1 and four loin samples from Study 2 were deemed unsuitable for analysis due to spoilage and thus were disposed and excluded from the remainder of the study. Food safety and hygiene protocols were adhered to throughout as samples in Study 2 were being retained for human consumption.

2.6. Cooking of meat

Following slaughter, all meat samples from Study 1 and 2 were subsequently prepared at the College of Agriculture Food & Rural Enterprise (CAFRE, Loughry, UK) and Ulster University (Nutrition Innovation Centre for Food and Health, Coleraine, UK), respectively. Pork loin (Study 1 and 2) and pork leg (Study 1 only) were roasted in a conventional oven (Falcon Pro-lite, Stirling, Scotland) at 180 °C for approximately 1 h 20 min to an internal temperature of at least 75 °C which was checked using a digital probe food thermometer (Electronic Temperature Instruments Ltd., Sussex, UK). Rind was trimmed (but subcutaneous fat was not removed) and the cooked samples were minced (Kenwood Meat Mincer MG510, Hampshire, UK) prior to vitamin D analysis. Raw and cooked weights were recorded to at least 2

decimal places (Mettler Toledo, Leicester, UK). Post-cooking, samples were individually labelled in storage bags and kept at −20 °C until subsequent analysis.

2.7. Vitamin D analysis

A full description of the liquid chromatography tandem mass spectrometry (LC-MS/MS) method is available in the Supplemental Material. Analysis was adapted from previously described methods (Ding et al., 2010; Strobel, Buddhadasa, Adorno, Stockham, & Greenfield, 2013; Trenerry, Plozza, Caridi, & Murphy, 2011). All samples were prepared and measured in duplicate.

Briefly, in plasma samples, the protein was precipitated and extracted under liquid-liquid extraction (LLE) using hexane (epi-25(OH)D_3 and 25(OH)D_3). The solvent was then removed by evaporation under nitrogen and the residue dissolved in 50% methanol. Plasma (μg/l) samples were quantified using the AB Sciex 6500/Shimadzu Nexera X2 UHPLC/HPLC system equipped with a Phenomenex Kinetex F5 100 (150 mm × 2.1 × 2.6) column (Phenomenex, Macclesfield, UK) and run in positive atmospheric pressure chemical ionization (APCI) mode.

The determination of vitamin D and 25(OH)D (both D_3 and D_2) in pork meat involved four main steps: saponification, solid-liquid extraction (SLE), solid-phase extraction (SPE) and derivatisation. Meat samples were saponified overnight at room temperature and four main vitamin D forms (vitamin D_3, vitamin D_2, and their respective hydroxylated forms, 25(OH)D_3 and 25(OH)D_2) were extracted by SLE (Chem-Elut, Agilent Chem-Elut Agilent Chem-Elut) with petroleum ether and purified by SPE (Bond-Elut, Agilent Chem-Elut Agilent Chem-Elut). The solvent was then removed by evaporation and the residue dissolved in methanol before derivatisation with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) solution. Meat (μg/kg) extracts were separated on a Nexera X2 UHPLC/HPLC system consisting of two LC-30AD pumps, a SIL-30 AC autosampler, a CTO-20 AC column oven, a CBM-20A control module and a DGU-20A5R degasser unit (Shimadzu, Kyoto, Japan) with a Synergy Hydro-RP (150 × 2.0 mm × 4 μm) polar endcapped reverse-phase C18 column (Phenomenex, Macclesfield, UK) incorporated and run in positive electrospray ionization (ESI) mode.

Deuterated internal standards (vitamin D_2 and vitamin D_3 diluted in methanol) were used for quantification alongside a series of calibration standards (0, 0.2, 1, 5, 10, 50, 100 and 200 ng/ml for meat; 0, 1, 2, 5, 10, 20 and 50 ng/ml for plasma) which were introduced at the beginning and end of each analysis. Calibrations curves were constructed by plotting the peak response ratio for each analyte against the corresponding stable labelled internal standard verses the corresponding concentration, and fitting the data using linear regression with a weighting factor of 1/x. For meat samples, the correctness of the method was checked by analyses of one 'in house produced' reference material and duplicate spiked recovery samples with the results of the reference material plotted on a Shewart chart to determine the method under control. For plasma samples, ClinChek® serum control level 135,080 was used as a CRM (RECIPE Chemicals + Instruments GmbH, 2019). Correlation coefficient was >0.98 for all analytes. The precision of the method was <5% in plasma and < 10% in meat. In Study 1, the recovery for plasma 25(OH)D_3 was 89.3–95.2%. The recovery for vitamin D_3, vitamin D_2, 25(OH)D_3 and 25(OH)D_2 in meat was 86.8–91.5%, 88.8–90.9%, 86.0–92.6% and 93.6–97.2%. For Study 2, the recovery for plasma 25(OH)D_3 was 78.3–109.8%. The recovery for vitamin D_3, vitamin D_2, 25(OH)D_3 and 25(OH)D_2 in meat was 92.3–98.2%, 84.7–88.9%, 82.6–89.8% and 93.2–95.1%.

The limit of quantification (LOQ) and limit of detection (LOD) was 0.02 μg/kg and 0.01 μg/kg for meat, and 1.0 ng/ml and 0.5 ng/ml for plasma, respectively.

2.8. Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (IBM SPSS Statistics 25, Chicago, IL, USA). Significance level was set at $P < 0.05$ throughout. Normality of the data was assessed using Shapiro-Wilk test. Nonparametric data were log-transformed to achieve normal (or approaching normal) distributions. Results are presented as medians with interquartile range (IQR), unless otherwise specified. Independent *t*-tests, one-way repeated measures ANOVA and Mann-Whitney *U* tests were used to compare the mean of parametric and nonparametric data, respectively. Friedman test was conducted on nonparametric data and post hoc analysis with Wilcoxon signed-rank tests was then conducted with a Bonferroni correction applied (0.05/ 3 = 0.017 *P* value).

3. Results

No significant difference was observed between number of pigs, distribution of sex, baseline weight or endpoint weight of both groups in either Study 1 or Study 2 (Table 1). Median (IQR) backfat was reported in Study 1 as 13.4 (11.5–14.6) mm and 13.6 (12.2–14.6) mm in UVB and control group, respectively ($P < 0.05$).

3.1. Plasma 25(OH)D_3 concentration

Mean (95% CI) 25(OH)D_3 plasma concentrations (μg/l) for baseline, midpoint and endpoint are presented in Fig. 2A-B. In Study 1, one-way repeated measures ANOVA showed there was a significant difference in plasma 25(OH)D_3 between the three timepoints in UVB group ($P < 0.05$). At baseline in Study 1 and 2, there was no significant difference in plasma 25(OH)D_3 concentrations between control and UVB treatment. However, at midpoint and endpoint, UVB exposure had a significantly positive effect (see Fig. 2A-B). A significant increase in plasma 25(OH)D_3 concentration was observed between the three timepoints in both groups in Study 2 (UVB $P < 0.001$; Control $P < 0.01$). Absolute change in plasma 25(OH)D_3 from baseline to midpoint (11.08 ± 2.51 μg/l) was greater than midpoint to endpoint (6.11 ± 2.33 μg/l) for the UVB group. The opposite effect (i.e. greater increase midpoint to endpoint) was observed in the control group.

3.2. Vitamin D concentration in loin and leg

In Study 1, UVB exposure (narrowband bulbs, 305–315 nm wavelength, ~10 weeks) had no effect on loin vitamin D_3, 25(OH)D_3 or total vitamin D activity concentrations when compared to control (Fig. 2C). A larger range in loin vitamin D_3 concentration was observed in the UVB group compared to control (median (IQR): 6.45 (4.90–9.65) μg/kg vs 5.17 (4.35–5.73) μg/kg, see Supplemental Material). In the UVB group, vitamin D_3 concentration in loin meat was significantly greater than leg meat (6.45 (4.90–9.65) vs 3.37 (2.29–4.98) μg/kg, $P < 0.001$). There was no difference between leg meat in UVB and control (3.06

Table 1
Baseline characteristics of pigs in Study 1 and 2.

	Study 1			Study 2		
	UVB	Control	All	UVB	Control	All
n	24	24	48	10	10	20
Sex, M:F (M%)	12:12 (50)	12:12 (50)	24:24 (50)	5:5 (50)	5:5 (50)	10:10 (50)
Baseline weight (kg)	43.18 ± 3.43	44.73 ± 3.78	43.95 ± 3.65	42.29 ± 3.14	41.34 ± 3.15	41.82 ± 3.10
Endpoint weight (kg)	127.97 ± 8.61	130.08 ± 8.90	129.02 ± 8.73	118.50 ± 6.92	124.10 ± 8.53	121.30 ± 8.09

Data presented as mean ± SD, unless otherwise specified. No significant difference between UVB and control groups; independent samples *t*-test ($P > 0.05$). Control group received no UVB exposure. UVB, ultraviolet-B; *n*, number of pigs; M, males; F, females; SD, standard deviation.

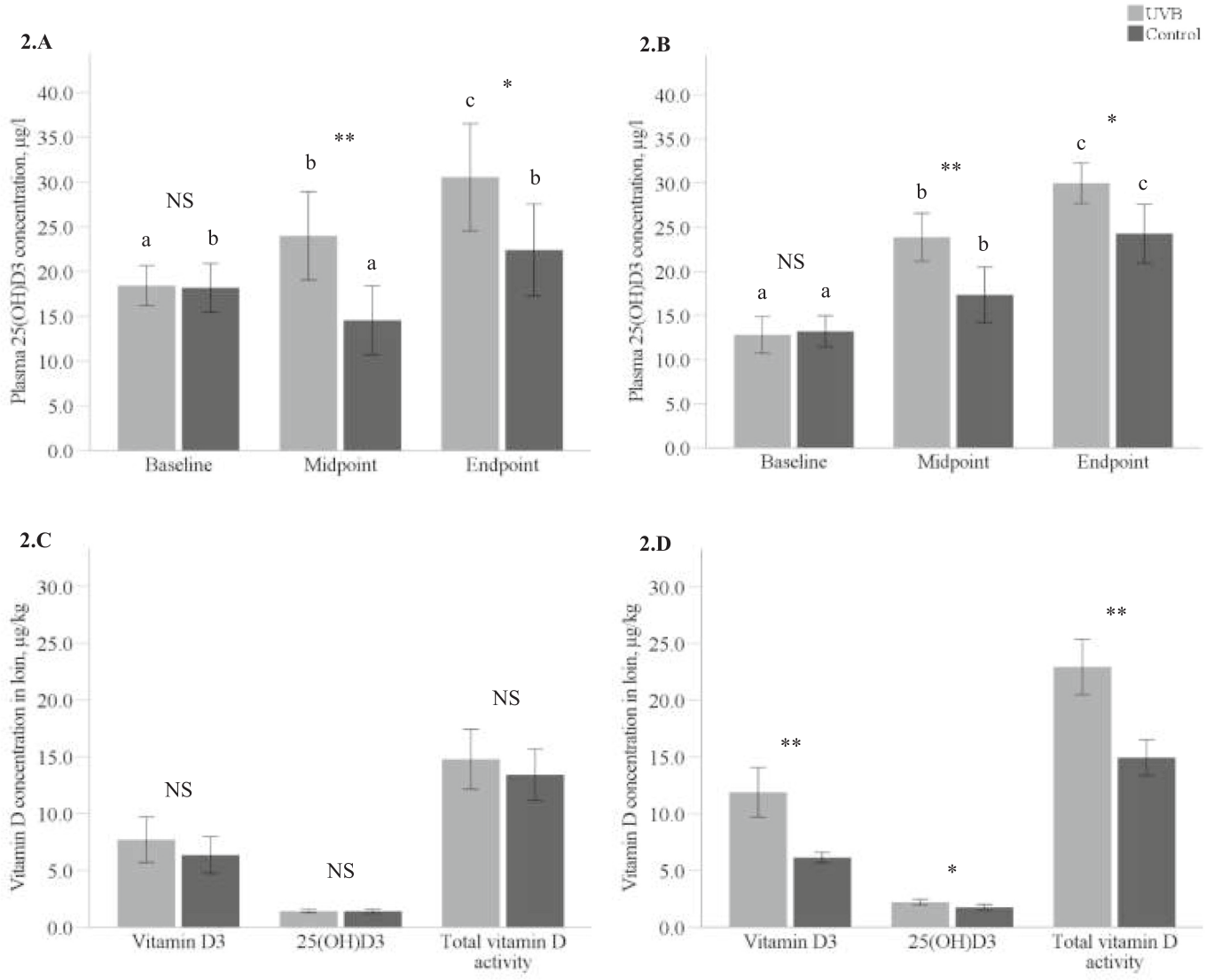

Fig. 2. A-D. Effect of UVB exposure on plasma in Study 1 (2.A; UVB $n = 6$, Control $n = 6$) and Study 2 (2.B; UVB $n = 10$, Control $n = 10$) and loin in Study 1 (2.C; UVB $n = 21$, Control $n = 19$) and Study 2 (2.D; UVB $n = 7$, Control $n = 9$). Data presented as mean (95% CI). *P*-value from Mann-Whitney *U* test showing difference between UVB and control groups at each timepoint and in metabolite concentrations (*$P < 0.05$; **$P < 0.01$; NS, non-significant). Values not sharing a common superscript letter in Fig. 2A and B are significantly different (Study 1 $P < 0.05$; Study 2 $P < 0.01$) between blood sample timepoints within UVB and control groups; one-way repeated measures ANOVA. UVB, ultraviolet-B; $25(OH)D_3$, 25-hydroxyvitamin D_3; *n*, number of samples; CI, confidence interval. Total vitamin D activity = vitamin D_3 + [$25(OH)D_3$ x 5].

(3.57–5.03), $P < 0.05$) groups.

UVB exposure in Study 2 (narrowband bulbs, 305–315 nm wavelength, ~9 weeks) resulted in significantly higher vitamin D_3, $25(OH)D_3$ and total vitamin D activity concentrations in loin meat, compared to control group (Fig. 2D). Similar to Study 1, the UVB group had a larger loin vitamin D_3 concentration range compared to control (median (IQR): 11.97 (9.53–14.60) µg/kg vs 6.03 (5.58–6.65) µg/kg, see Supplemental Material). There was a significant difference between Study 1 and 2 UVB groups in loin vitamin D_3 ($P < 0.01$), $25(OH)D_3$ ($P < 0.001$) and total vitamin D activity ($P < 0.01$) concentration.

Table 2
Weekly pig posture scores whilst in individual daily treatment pens in Study 2.

	Week 16	Week 17	Week 18	Week 19	Week 20	Week 21
All ($n = 20$)	2.5 (1.0–3.0)	1.7 (1.3–2.5)	1.4 (1.1–2.1)	1.8 (1.1–2.3)	1.5 (1.1–2.4)	1.5 (1.0–2.0)
UVB ($n = 10$)	1.8 (1.0–3.0)	1.6 (1.0–2.5)	1.4 (1.2–2.4)	1.5 (1.0–2.4)	1.6 (1.3–2.6)	1.7 (1.3–2.3)
Control ($n = 10$)	2.5 (1.8–3.0)*	1.9 (1.5–6.7)	1.4 (1.0–2.1)*	1.9 (1.2–2.4)	1.3 (1.1–2.0)	1.3 (1.0–2.1)

Data presented as median (IQR). No significant difference between UVB and control groups at any week; Mann-Whitney U test ($P > 0.05$). Scores were appointed as follows: standing = 3 points, sitting = 2 points, lying down = 1 point (minimum-maximum range: 35–105 points). Combination of the postures were awarded half of the posture scores (e.g. stood/sat = 2.5 points). *Denotes significant difference ($P = 0.011$) between weeks; Friedman test and post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $P < 0.017$ (i.e. 0.05/3). All other timepoints were non-significant. UVB, ultraviolet-B; *n*, number of pigs; IQR, interquartile range.

3.3. Pig posture

Table 2 presents the average weekly pig posture score in Study 2 (not recorded in Study 1). The total pig posture score was 56.00 (44.25–79.00) (UVB 49.50 (43.00–86.00) and control 62.00 (47.50–77.75), $P < 0.05$) out of a maximum possible 105 points. There was no significant difference in average weekly pig posture within the six weeks, $\chi^2(2) = 7.772$, $P = 0.169$. However, when treatment groups were split, a significant difference was observed within the control group (UVB $\chi^2(2) = 4.513$, $P = 0.478$; Control $\chi^2(2) = 22.815$, $P < 0.001$). Within the control group, there was a significant difference in average pig posture between week 16 and 18 ($Z = -2.552$, $P = 0.011$). No significant difference in average weekly pig posture between the two groups was identified ($P > 0.05$).

4. Discussion

To the best of our knowledge, we are the first to demonstrate a positive impact of UVB light exposure on vitamin D status in slaughter pigs, while also offering the maximum vitamin D feed limit permitted by European legislation. UVB exposure is an effective and safe way to elevate the vitamin D content in plasma and pork loin (as seen in Study 2, but not Study 1). Further diet-induced enhancements are not possible due to set limits, therefore UVB radiation offers an alternative strategy and confirms our hypothesis. Additionally, for the first time, we observed that pigs which stood more frequently during UVB exposure were shown to have greater total vitamin D activity and vitamin D_3 concentrations in loin.

4.1. Impact of UVB exposure on 25(OH)D_3 plasma

Both studies observed a significant effect of UVB exposure at midpoint and endpoint plasma 25(OH)D_3 concentrations. Unlike humans, no limits exist to determine deficiency or insufficiency within pigs or domestic farm animals. However, if compared to human cut-off levels set by the Endocrine Society Task Force, pig baseline plasma 25(OH)D_3 in both present studies would be deemed insufficient (< 20 μg/l) (Holick et al., 2011). At midpoint, only UVB groups were considered sufficient. Interestingly, at endpoint, both UVB and control in Study 1 and 2 were above this threshold. This may suggest feed alone is sufficient to increase the plasma 25(OH)D_3 concentrations, albeit the addition of UVB seems to influence the rate and ultimately results in greater vitamin D concentrations in meat which is of benefit to human consumption. This is in agreement with previous studies however, lower levels of vitamin D were provided in feed compared to the present work (~1000–1239 IU vitamin D_3/kg) (Alexander et al., 2017; Kolp et al., 2017). Improving vitamin D status in animals has been alluded to health benefits such as immune functions, mortality, growth, bone development, end weight and reproductive performance as shown in hens, chickens, dairy cattle and pigs (Yang & Ma, 2021). Conversely, mild erythema has previously been reported in slaughter pigs exposed to 2 SED/day, stressing the need to optimise UVB exposure without compromising animal welfare (Barnkob et al., 2019). If pig vitamin D status was further elevated via UVB exposure, and provided additional health benefits to the animal, this may be advantageous to both animals and producers as welfare parameters are becoming an increasing priority for consumers (Alonso, González-Montaña, & Lomillos, 2020).

In Study 2, from midpoint to endpoint plasma 25(OH)D_3 the absolute change is almost half of what was observed in the first ~4 weeks of the study (baseline to midpoint). Initially, it may be postulated that 25(OH)D_3 plasma saturation is being reached, resulting in a plateau effect and providing an argument for shorter intervention duration, such as 50 days suggested for minipigs (Burild et al., 2015). Similar previous studies have shown elevated plasma 25(OH)D_3 concentrations (39.5–85 μg/l) in pigs exposed to 0.7–1.8 SED with varying levels of vitamin D_3 provided in feed (180–420 vitamin D_3 IU/kg and 600–2400 vitamin D_3 IU/day) (Barnkob et al., 2019; Burild et al., 2015; Jakobsen et al., 2020). It could also be argued that by providing supplemental vitamin D feed alongside irradiation in our work, the full potential of UVB endogenous synthesis is limited due to the innate feedback loop to prevent toxicity. This may offer another rationale for the plasma 25(OH)D_3 change from baseline to midpoint being much greater than midpoint to endpoint in the UVB group of Study 2.

Conducting future research with a greater number of pigs per treatment may complement previous similar pig studies (Barnkob et al., 2019; Larson-Meyer et al., 2017) in further strengthening evident correlations between vitamin D metabolites in plasma and loin, and therefore enable the vitamin D content in pork products to be predicted by analysis of plasma alone. This may offer a simple screening process to highlight pigs responding optimally to UVB exposure and potentially inform the future short-term direction for producers, with underperforming pigs moved or redirected to another production outside of anticipated vitamin D biofortified food ranges. Thus, resulting in reduced time and cost. Moreover, a greater number of blood sampling timepoints could be considered in future studies to clearly plot the plasma 25(OH)D_3 change over time and better identify where potential plateaus exist. This may highlight where additional cost and time does not equate to additional vitamin D benefits and thus, help to inform optimal cost-effective interventions of potentially shorter durations.

4.2. Impact of UVB exposure on loin

Our results demonstrate that increasing the UVB duration resulted in higher total vitamin D activity, vitamin D_3 and 25(OH)D_3 concentrations in pork loin in Study 2. Whilst modest in comparison (94% increase), findings are in accordance with previous pig research which observed 1750% (Barnkob et al., 2019) and 228% (Jakobsen et al., 2020) change in vitamin D_3 loin concentration between UVB and control groups. However, direct comparisons prove challenging due to varying UV exposure period owed to the selected height of light above the pigs, intervention duration, source of UV (i.e. natural or artificial), wavelength and diet, in addition to whether fat was removed prior to analysis and quantification of raw or cooked samples (Barnkob et al., 2019; Larson-Meyer et al., 2017). The use of health claims is based on the vitamin D concentration in food as consumed (EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA), 2010) and hence, in the present work, cooked meat was analysed rather than raw due to commercial relevance.

4.3. Pig posture

A wider variance in loin vitamin D_3 concentrations was observed in the UVB group compared to control in both Study 1 and 2 which may be attributed, in part, to posture variation (Barnkob et al., 2019). The maximum and minimum posture score possible in our study were 105 and 35 points, respectively. On average, the UVB and control group respectively achieved 20.7% and 38.6% of the maximum posture score, implying pigs spent a greater time sitting or lying down, rather than standing. This suggests it may be of benefit to position the UVB light on an adjustable height to accommodate the pig's movement accordingly. However, this is impractical and certainly not realistic in a larger scale intervention or in commercial application. It has previously been suggested that a fixed height and irradiance with variable duration is the optimal set-up and should be investigated further (Barnkob et al., 2019; Jakobsen et al., 2020). Owing to the structure of the individual confined pens during radiation, pigs were unable to move away from under the lights as they grew and thus could not avoid UVB exposure which is a strength of the present study. Evading radiation may be an issue if the UVB light was over a larger pen where pigs may prefer to remain in parts outside the UVB exposure area (Jakobsen et al., 2020) and possibly result in heterogenous vitamin D concentrations post-intervention. It has previously been estimated that pigs in vitamin D-enriched UVB trials

spend 75% of time standing and 25% lying down (Jakobsen et al., 2020), however the present work only assessed posture for the duration of UV exposure.

4.4. Commercial application

Literature suggests pork meat contains 0.5–4 μg/kg and < 0.5–4.44 μg/kg of vitamin D_3 and 25(OH)D_3, respectively (Bilodeau et al., 2011; Clausen et al., 2003). The vitamin D content of pork meat recorded in food composition databases from Europe and North America ranges between 1.0 and 23.0 μg/kg (Schmid & Walther, 2013). Specifically considering the UK McCance and Widdowson's Composition of Foods Integrated Dataset (CoFID; Public Health England, 2021), vitamin D pork data is reported as total vitamin D activity, whereby a factor of five is applied for 25(OH)D potency. Key cuts of pork were recently re-analysed for CoFID by liquid chromatography-mass spectrometry (LC-MS), replacing the 1992 analytical method high-performance liquid chromatography (HPLC) owing to the greater specificity and sensitivity of LC-MS (Pinchen et al., 2020). In the UK composition of foods integrated dataset (CoFID), roasted pork loin is reported as 9 μg/kg vitamin D activity (vitamin D_3 + 25(OH)D_3 x 5; Public Health England, 2021), which is modestly lower than the range of 10.6 to 17.6 μg/kg we reported in control pigs and greater than the vitamin D_3 range of 4.4–6.7 μg/kg. Inter and intra-variability is to be expected owing to the cut of meat, cooking methods, differing laboratory analysis, single analysis, accepted potency of 25(OH)D and diet supplementation. However, considering cooking methods, Neill et al. (2022) showed favourable retention of vitamin D_3 (102–135%) and 25(OH)D_3 (88–108%) in pork loin across various thermal treatments. If the use of UV exposure became widespread common practice amongst producers, nationally representative food databases should be updated to reflect the anticipated change to vitamin D metabolite concentrations in pork.

Taken together, results from the present work will prove valuable for commercialisation. Pork meat 25(OH)D_3 concentrations may be of particular relevance for consumers as this metabolite is considered more potent than parental vitamin D in raising circulating 25(OH)D_3 concentrations in humans (Quesada-Gomez & Bouillon, 2018). Despite this, only the content of vitamin D (cholecalciferol and ergocalciferol) is recognised when quantifying and marketing a nutrition or health claim on a food product (EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA), 2010). As such, individual companies require clear rationale regarding which form of vitamin D is their primary focus when producing biofortified pork. Considering industry application, our UV-enriched pork loin achieves 15% of the recommended daily intake (RDI) (≥ 7.5 μg/kg) and thus would be permitted to use the EFSA regulated "source of vitamin D" nutrition claim, and its respective health claims (EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA), 2010). The highest individual vitamin D_3 loin concentration in Study 2 was 14.78 μg/kg. Therefore, if the UVB exposure time was further extended, it may be possible to achieve a "high in vitamin D" nutrition claim set at 30% of the RDI (≥ 15.0 μg/kg) (Regulation (EC) No 1924/2006). Applying these findings to real-life consumption patterns, a typical average serving of vitamin D_3-enriched pork (177 g loin chop) could provide consumers with 21% of estimated average requirement (EAR) 10 μg/day and 11–14% of recommended daily allowance (RDA) 15–20 μg/day (Institute of Medicine, 2011). Consumer evaluation testing will be crucial to better understand perception and market potential of novel biofortified foods to ensure their acceptability and integration to UK diets, in addition to the identification of barriers or facilitators to their adoption.

4.5. Strengths and limitations

No erythema was detected in either study, therefore confirming a safe, yet effective UVB intensity. The UVB light remained in a permanent position meaning radiation intensity increased as the pigs grew in size. This allowed a natural acclimatization phase to limit risk of redness. It has been postulated that extending the UVB adaption period may have safely permitted a higher dosage later. Despite providing the European Union (EU) maximum vitamin D level permitted in pig feed, a response was observed following UV exposure and, by adhering to these restrictions, results show commercial viability and a response effect even when restrictions are adopted. Recording pig posture provided greater depth of understanding regarding anticipated stance as pigs grew and its impact on vitamin D concentrations in loin. By providing UVB exposure via artificial lights, rather than sunlight which is susceptible to daily and seasonal variation as seen as in previous studies (Burild et al., 2015; Larson-Meyer et al., 2017), a more consistent and controlled radiation environment can be ensured. Animal welfare benefits may be attributed to increased vitamin D status in pigs, which provides clear advantages to the present work, beyond consideration for potential human health implications. Whilst the UVB set up and intensity in Study 2 provides proof of concept, further work is needed to upscale in a larger production setting to enable numerous pigs to receive a radiation dose at the same time and therefore, ensure commercialisation and marketability of vitamin D enriched meat. Pigs were individually exposed to the UVB light which is inefficient and unfeasible considering industry application. As the height of the UVB light was fixed in the current study, radiation intensity was determined by pig posture. Adjusting the height of the UVB light during each pig's individual exposure time could have ensured adequate radiation even when pigs lay down. However, as discussed, whilst this may offer insight within a research setting, it does not offer a realistic solution in a commercial setting whereby a vast number of pigs would receive UVB exposure simultaneously within the same pen. The interventions lasted ~9 weeks prior to slaughter, however this time could be reduced by increasing the wavelength of the light tubes and exposure duration. Further studies, including both a larger number of pigs and longer exposure duration, are planned in order to identify the maximum safe radiation whilst optimising vitamin D concentrations in pork meat. Due to public health messaging regarding saturated fat and red or processed meat consumption, analysis of vitamin D in lean meat may be of benefit to better reflect consumer habits and provide a more accurate representation of vitamin D intake from UVB-enriched pork meat. Of note, whilst a limitation within the present work, the fat content should be quantified independently, and this adopted as a standard to enable future comparisons between similar vitamin D pig studies. Importantly, future research warrants better understanding regarding any changes in sensory attributes, as well as randomised controlled trials (RCTs) to confirm the bioavailability and bioaccessibility of vitamin D present in pork meat to ensure its efficacy within the diet of human participants. In addition, future research should focus on exploring additional parts of the pig carcass to identify whether all pork cuts are biofortified to the same degree and investigate the optimal way to administer radiation (lighting set up) to ensure the body surface receiving radiation is maximised (Rosbotham et al., 2021). Thus, informing new product development industry practices and ensuring optimisation of biofortified meat. Lastly, the use of supervised machine learning techniques to identify factors influencing vitamin D biofortification of pork could be advantageous to inform future studies and enable more robust model development.

5. Conclusion

Results from this research confirm that exposing pigs to UVB radiation during the finishing period, in addition to offering the maximum EU amount of vitamin D-enriched feed, is more effective at increasing vitamin D in loin and plasma than feed alone. Pork biofortification may complement traditional fortification measures to offer an additional food-based strategy to help consumers achieve vitamin D intake recommendations without change to their habitual diet, and thus reduce rates of hypovitaminosis D and respective negative health outcomes.

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CRediT authorship contribution statement

H.R. Neill: Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **C.I.R. Gill:** Conceptualization, Methodology, Writing – review & editing. **E.J. McDonald:** Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **R. McMurray:** Resources. **W.C. McRoberts:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing. **R. Loy:** Methodology, Validation, Resources, Writing – review & editing. **A. White:** Methodology, Validation, Resources, Writing – review & editing. **R. Little:** Project administration. **R. Muns:** Writing – review & editing. **E.J. Rosbotham:** Data curation, Writing – review & editing. **U. O'Neill:** Resources, Writing – review & editing. **S. Smyth:** Resources. **L.K. Pourshahidi:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

EJM and RM were employed by the industrial partner Devenish Nutrition Ltd. The authors declare that this study received funding from Devenish Nutrition Ltd., in the form of a CAST PhD Studentship awarded to the lead author. The funder had the following involvement in the study: study design, review and approval of the final manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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