Animal Feed Science and Technology 288 (2022) 115315

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

The influence of duration of feeding dietary vitamin D_2 enriched mushroom powder to finisher pigs on growth performance and meat quality parameters

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ARTICLE INFO

Keywords:
Pigs
Pork colour
Pork quality
Lipid peroxidation
Antioxidant capacity
Vitamin D_2 enriched mushrooms

ABSTRACT

Novel feed additives that replace the use of synthetic additives while enhancing pig performance and pork quality are continuously being explored. A complete randomised block design experiment was conducted to examine the effects of the duration of feeding of vitamin D_2 enriched mushrooms to finisher pigs and its effect on pig performance and pork quality. Sixty pigs were assigned to one of three dietary treatments for a 69-day feeding period. The dietary treatments were as follows: (T1) basal diet; (T2) basal diet + 1 g/kg of feed vitamin D_2 enriched mushroom powder for 48 days prior to slaughter (MPVD-48); and (T3) basal diet + 1 g/kg of feed vitamin D_2 enriched mushroom powder for 26 days prior to slaughter (MPVD-26). The mushroom vitamin D content was naturally enriched using synthetic ultraviolet B exposure to obtain a vitamin D_2 level of 50 μg/kg of feed. Dietary supplementation of vitamin D_2 enriched mushroom powder, irrespective of feeding duration, caused a reduction ($P < 0.05$) in feed intake, whilst improving ($P < 0.05$) gain-to-feed ratio. Supplementation of vitamin D_2 enriched mushroom powder for 26 days prior to slaughter caused pork lightness (L*) values to remain stable over the 21-day storage time-period compared with all other dietary groups. Supplementation for 26 days also caused a reduction in lipid peroxidation on day 4 of storage compared with supplementation for 48 days. Irrespective of feeding duration, vitamin D_2 enriched mushroom powder supplementation improved FRAP (ferric reducing antioxidant power) values of pork compared with the basal. In conclusion, vitamin D_2 enriched mushroom supplementation, irrespective of feeding duration, caused a reduction in feed intake but improved feed efficiency. The supplementation of vitamin D_2 enriched mushrooms for the shorter duration of 26 days prior to slaughter improved pork quality in relation to colour and antioxidant status.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; DM, dry matter; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; G:F, gain-to-feed ratio; LT, *Longissimus thoracis*; LPO, lipid peroxidation; MDA, malonaldehyde; MPVD-26, basal diet until 26 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder; MPVD-48, basal diet until 48 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder.

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https://doi.org/10.1016/j.anifeedsci.2022.115315
Received 4 September 2021; Received in revised form 25 April 2022; Accepted 26 April 2022
Available online 30 April 2022

1. Introduction

The use of antioxidants in the meat industry is an effective way to minimise the development of oxidative rancidity, extend the shelf life and improve the nutritional quality of the meat (Gupta and Abu-Ghannam, 2011; Rajauria et al., 2013). Synthetic antioxidants are commonly added to fresh and processed meat and have been successfully used in the meat industry (Gülçin, 2012; Kumar et al., 2015). However, due to synthetic antioxidants having toxicological and carcinogenic effects, extensive work is being carried out to find novel, and safe sources of antioxidants (Velasco and Williams, 2011; Kumar et al., 2015). Antioxidants can be applied directly to the meat products or through dietary supplementation (Falowo et al., 2014). Feeding antioxidants to pigs prior to slaughter may provide the meat industry with a novel and sustainable source of antioxidants and potentially enhancing the health benefits of the meat (Jiang and Xiong, 2016).

Mushrooms are a rich natural source of bioactive metabolites such as phenolic compounds and polysaccharides including β-glucans (Reis et al., 2012). Beta-glucans are natural biomolecules which exhibit antioxidant, prebiotic, anti-inflammatory and immunomodulatory activity (Sweeney et al., 2012; Du et al., 2015). Mushrooms possess antioxidant potential partly due to their phenolic compounds and their ability to scavenge free radicals (Cheung, 2010). The supplementation of mushroom powder in pig diets may have the potential to increase pork antioxidant status and in turn increase shelf-life of the pork products while simultaneously increasing animal performance (Duffy et al., 2018; Dowley et al., 2022). Dietary supplementation of *Agaricus bisporus* mushrooms (white button mushrooms) had positive effects on antioxidant capacity while also improving growth performance in chickens (Giannenas et al., 2010) and turkey poults (Giannenas et al., 2011). However, dietary supplementation with *Agaricus bisporus* mushrooms has been showen to reduce feed intake in weaner and finisher pigs (Conway et al., 2021; Dowley et al., 2022), suggesting a potential link between mushrooms and appetite control. The appropriate feeding duration of these natural feed additives prior to slaughter to enhance pork quality, feeding behaviour and pig performance has yet to be determined.

Vitamin D participates in antioxidant activity (Wiseman, 1993), meat quality and growth performance (Han et al., 2012). Mushrooms contain a high ergosterol content, which offers the potential to form vitamin D_2 when exposed to ultraviolet B radiation (Kalaras et al., 2012). When *Agaricus bisporus* mushrooms are exposed to ultraviolet light under certain conditions, they can produce at least 10 μg/100 g fresh weight of vitamin D_2 (Cardwell et al., 2018). Dietary supplementation of vitamin D_2 enriched mushrooms to

Table 1
Ingredient and chemical composition of the basal diet and vitamin D_2 enriched mushroom diet.

	Treatments	
Ingredient (g/kg)	Basal	Vitamin D_2 enriched mushrooms
Wheat	383	382
Barley	250	250
Soya bean meal	170	170
Maize	150	150
Soya oil	18	18
Salt	5	5
Monocalcuim phosphate	6.4	6.4
Limestone	12.5	12.5
Lysine HCl	2.2	2.2
L-threonine	0.5	0.5
Vitamin D_2 Mushroom Powder	0	1
Vitamins and mineral premix[a]	2.4	2.4
Analysed chemical analysis		
Gross energy (MJ/kg)	15.9	16.0
Dry matter	886.0	886.0
Crude protein (N × 6.25)	147.0	147.5
Lysine[b]	10.5	10.5
Methionine and cysteine[b]	6.3	6.3
Threonine[b]	7.2	7.2
Tryptophan[b]	1.9	1.9
Neutral detergent fibre	129.0	133.8
Acid detergent fibre	42.0	45.9
Ether extract	24.5	24.2
Ash	42.7	42.6
Calcium	5.9	5.9
Phosphorus	4.9	4.9
Additional vitamin D_2 (μg/kg)	0	50
Vitamin D_3 (μg/kg)	50	50

[a] The premix provided vitamins and minerals (per kg diet) as follows: 0.01 g/kg of retinol acetate, 0.16 g/kg of alpha tocopherol acetate, 0.007 g/kg of menadione, 0.00125 g/kg of thiamine mononitrate, 0.005 g/kg of riboflavin, 0.0025 g/kg of pyridoxine HCl, 0.003 g/kg of cyanocobalamin,0.0229 g/kg of nicotinamide, 0.0138 g/kg of calcium-D-pantohenate, 0.06 g/kg of copper as copper sulphate, 0.4167 g/kg of iron as iron sulphate, 0.0806 g/kg of manganese as manganese oxide, 0.0032 g/kg of iodine as calcium iodate, 0.1389 g/kg of zinc as zinc oxide, 0.0056 g/kg selenium, 0.05 g/kg of phytase, 1.24 g/kg of calcium.
[b] Calculated for the tabulated nutritional composition (Sauvant et al., 2004).

finisher pigs for 55 days prior to slaughter led to a delay in lipid peroxidation in pork while improving pig performance, carcass weight, antioxidant status and colour of pork (Duffy et al., 2018). The supplementation of marine polysaccharides to finisher pigs for a shorter duration of 21 days resulted in enhanced pork antioxidant potential compared with supplementation for 42 days (Moroney et al., 2015).

The objective of this study was to examine the feeding duration (48 and 26 days) prior to slaughter of vitamin D_2 enriched mushroom powder supplementation on pig performance, feeding behaviour and subsequent pork quality. It was hypothesised that vitamin D_2 enriched mushroom powder supplementation would improve pig performance and pork quality but would be influenced by the duration of feeding.

2. Materials and methods

All experimental procedures described in this work were approved under University College Dublin Animal Research Ethics Committee, Ireland (AREC-18–27-O'Doherty) and were conducted according to Directive 2010/63/EU (EC, 2010) for animal experimentation.

2.1. Experimental design and animal management

The experiment was designed as a completely randomised block comprising of three dietary treatments. Sixty 95-day old (30 males [entire], 30 females) pigs (Meat line boars × [Large White × Landrace] sows) (Hermitage, Co. Kilkenny, Ireland) with initial mean live weight of 47.2 kg (SD 3.8 kg) were blocked according to live weight and sex and assigned to one of three dietary treatments. The dietary treatments were as follows: (T1) basal diet, (T2) basal diet + vitamin D_2 enriched mushroom powder for 48 days prior to slaughter (MPVD-48), and (T3) basal diet + vitamin D_2 enriched mushroom powder for 26 days prior to slaughter (MPVD-26). The vitamin D_2 enriched mushroom powders were sourced from Monaghan Mushrooms (Tyholland, Co. Monaghan, Ireland) and contained a β-glucan content of 100 g/kg of mushroom powder. The mushroom vitamin D content was enriched using synthetic ultraviolet B exposure as described by Stepien et al. (2013) to obtain a vitamin D_2 level of 50 μg/kg of feed. The vitamin D_2 mushroom powder was included at 1 g/kg of feed achieving a β-glucan content of 100 mg/kg (Duffy et al., 2018). The vitamin D_2 enriched mushroom powder was analysed for vitamin D concentration by high performance liquid chromatography, as described by Mattila et al. (1994). The basal diets contained a vitamin D_3 level of 50 μg/kg of feed thus giving a total vitamin D content of 100 μg/kg when the mushroom powder was added to the diets. The experiment was divided into three time periods; period 1 (day 1–21), period 2 (day 22–43) and period 3 (day 44–69) which coincided with the feeding of vitamin D_2 enriched mushrooms to T2 on day 22 and T3 on day 44. All diets were milled on site and were provided ad libitum in a meal form and water was available ad libitum from nipple drinkers. The diets were formulated to have similar digestible energy (14MJ/kg) and standardised ileal digestible lysine (8.5 g/kg). All amino acid requirements were met relative to lysine (NRC, 2012). Detailed ingredient composition and chemical analysis of the diets are presented in Table 1.

The pigs were grouped in mixed sex (50:50) groups of ten with a space allowance of 1.0 m^2 per pig (two pens per treatment). Each pen had a solid floor lying space with access to slats at the rear of pen. The pens were equipped with single space computerised feeders (Mastleistungsprufung MLP-RAP; Schauer Agrotronic AG, Sursee, Switzerland), as described by Varley et al. (2011), which allowed individual ad libitum feeding and daily recording of dietary intake and feeding behaviour. The recorded data was used to calculate the number of visits per day, total time spent at feeder per day, time per visit, consumption per visit, eating rate and total consumption per day. Feeding behaviour was measured during period 2 (day 22–43) and period 3 (day 44–69). The house was mechanically ventilated, temperature was maintained at 18 °C and there was no sunlight exposure inside the pig barn. Pigs were weighed individually on day 1, day 21 (end of period 1), day 43 (end of period 2) and day 69 (end of period 3), which coincided with the addition of the vitamin D_2 enriched mushroom powder to the diet. Feed samples were collected at diet manufacture and throughout the experimental period and stored at − 20 °C for chemical analysis.

2.2. Slaughter procedure

The pigs were slaughtered at a nearby commercial abattoir (Rosderra Irish Meats, Edenderry, Co. Offaly, Ireland [distance:50 km]) at the end of the feeding period with an average live weight of 119 kg. The pigs were stunned using carbon dioxide and killed by exsanguination. The backfat thickness was measured as described by Egan et al. (2015) using the Hennessy grading probe (Hennessy and Chong, Auckland, New Zealand). The lean meat content was estimated as described by O'Meara et al. (2020) using the following equation:

$$\text{Estimate lean meat content (g/kg)} = (60{\cdot}3 - 0{\cdot}847x + 0.147\,y) \times 10$$

where x is fat depth (mm) and y is muscle depth (mm).

Further carcass data were determined using the following equations:

$$\text{Carcass weight (kg)} = \text{hot carcass weight} \times 0.98$$

$$\text{Kill out } \textit{proportion } (\text{g}/\text{kg}) = \left(\frac{\textit{carcass weight}}{\textit{live weight}} \times 100\right) \times 10$$

Following overnight chilling of the carcasses at 4 °C, the *Longissimus thoracis* (LT) muscle was excised from 9 pigs/treatment (5 males and 4 females) and subdivided into three pork steaks/pig. Three steak pieces were placed in vacuum pack pouches (200 × 300 mm, Mc Donnells Ltd., Dublin, Ireland) and flushed with 80% O_2: 20% CO_2 (modified atmosphere packs technology), as described by Rajauria et al. (2016) using a single chamber vacuum sealing unit (Webomatic vacuum packaging system, C10 H with a Km100–3 M gas mixer, Bochum, Germany) for colour and antioxidant analysis. The LT steaks in modified atmosphere packs were stored for up to 21 days at 4 °C for antioxidant and colour analysis. The gas atmosphere (oxygen and carbon dioxide) in the modified atmosphere packs was checked using a handheld gas analyser (Checkpoint handheld gas analyser, PBI Dansensor, Ringsted, Denmark). The remaining LT samples were vacuum packed using a single chamber vacuum-sealing unit and were stored at − 20 °C until required for water holding capacity and cook loss analysis.

2.3. Chemical analysis

Feed samples collected during the experiment were milled through a 1 mm screen (Christy and Norris Hammer Mill, Chelmsford, England) and retained for chemical analysis. The proximate analysis of the feed for dry matter was determined after drying overnight at 105 °C (16 h minimum). Ash was determined after ignition of a known weight of concentrate in a muffle furnace (Nabertherm, Bremen, Germany) at 550 °C for 6 h. The nitrogen content was determined as N × 6.25 using the LECO FP 528 instrument (LECO instruments UK Ltd., Cheshire, UK). Gross energy content was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, Illinois, USA). The ether extract concentrations of the diet were determined using light petroleum ether and Soxtec instrumentation (Tecator, Sweden). The neutral detergent fibre content was determined by the method of Van Soest et al. (1991) whereas the acid detergent fibre content was determined by procedure 973.18 of AOAC International (2000).

2.4. Cook loss

Longissimus thoracis samples were de-frosted overnight at 4 °C, trimmed of external fat, weighed and cooked in open vacuum bags in a circulating water bath (Grant Instruments Ltd., Barrington, Cambridge, UK) set at 72 °C, until an internal temperature of 70 °C was achieved. Internal temperature was monitored by placing a thermocouple in the geometric centre of each steak, four steaks were cooked per water bath to ensure water circulation was consistent around all samples. All juices were poured out of bag immediately after removing from water bath. The LT steaks were cooled to room temperature, dabbed gently with tissue paper to absorb excess moisture and reweighed. The percentage cook loss was then determined using the equation:

$$Cook\ loss\ (\%) = \frac{(W1 - W2)}{(W1)} \times 100$$

where W1 = raw weight of LT and W2 = cooked weight of LT.

2.5. Water holding capacity

Water holding capacity was determined according to Honikel (1998). Briefly samples were weighed and suspended using thread and placed in a polythene bag, which was tied to prevent dehydration. Samples were hung freely inside the bag to ensure exudate did not remain in contact with the meat. The bag was hung freely for 48 h at 4 °C. *Longissimus thoracis* steaks were removed from the bag, gently blotted dry using tissue paper and reweighed. Water holding capacity was expressed as a percentage of the final weight. Water holding capacity was calculated using the equation:

$$Water\ holding\ capacity\ (\%) = \frac{(W1 - W2)}{(W1)} \times 100$$

Where W1 = initial weight and W2 = weight after 48 h.

2.6. Colour measurement

The surface colour of the LT steaks was measured using a colorimeter (CR-400handheld Chroma metre, Konica Minolta, Inc., Osaka, Japan) as described by Rajauria et al. (2016). The Chroma meter was calibrated on the CIE LAB colour system using a CR-A43 calibration plate (D_c: L^* = 97.79, a^* = −0.11, b^* = 2.69). The "L^*" value represents lightness while "a^*" and "b^*" values represent redness and yellowness, respectively. Colour measurements were recorded on steaks following storage in modified atmosphere packs at 4 °C for 0, 4, 7, 11, 14 and 21 days. Values were recorded as the mean of three measurements made on non-overlapping regions of the LT steak in triplicate (averaged from three locations) from each side of the cut of LT.

2.7. Antioxidant activity analysis

Five gram of raw meat sample was homogenised (Stomacher 400 circulator, Steward Ltd., UK) with 50 ml phosphate buffer (0.05 M, pH 7) for 3 min. The resulting homogenate was centrifuged at 4731 gx at 4 °C for 15 min (Rotanta 460 R, Zentrifugen, Hettich) and the supernatant was collected for further analysis. The collected meat supernatant was tested for the determination of

Table 2
Effect of dietary treatment on animal performance during the experimental period. (Least-square means ± S.E.M.).

	Treatments* 1							P-value		
	Basal (Male)	Basal (Female)	MPVD-48 (Male)	MPVD-48 (Female)	MPVD-26 (Male)	MPVD-26 (Female)	SEM[2]	Treatment	Sex	Treatment × Sex
Weight (kg)										
Day 1	46.60	47.25	46.65	48.25	46.90	47.45	0.504	0.949	0.543	0.935
Day 21	68.02	68.67	68.07	69.67	68.32	68.87	1.276	0.919	0.375	0.902
Day 43	92.59	90.09	93.27	92.35	92.89	90.29	1.286	0.473	0.059	0.763
Day 69	124.99	117.36	121.62	115.84	121.51	112.16	1.278	0.005	< 0.001	0.383
ADG (kg)										
Day 1–21	1.06	0.99	1.05	1.00	1.04	1.01	0.032	0.899	0.119	0.917
Day 22–43	1.17	1.02	1.18	1.09	1.16	1.01	0.055	0.504	0.037	0.620
Day 44–69	1.20	1.00	1.05	0.94	1.07	0.91	0.059	0.109	0.002	0.705
Day 1–69	1.15	1.03	1.10	0.99	1.10	0.95	0.025	0.178	0.020	0.594
ADFI (kg)										
Day 1–21	2.23	2.14	2.19	2.15	2.20	2.15	0.059	0.919	0.288	0.928
Day 22–43	2.79	2.53	2.52	2.44	2.80	2.54	0.121	0.149	0.183	0.467
Day 44–69	3.27	3.16	2.77	2.46	2.74	2.59	0.122	< 0.001	0.065	0.578
Day 1–69	2.94	2.77	2.57	2.31	2.46	2.29	0.097	< 0.001	0.016	0.869
G:F										
Day 1–21	0.51	0.46	0.52	0.46	0.50	0.47	0.024	0.929	0.190	0.907
Day 22–43	0.42	0.40	0.48	0.46	0.41	0.42	0.016	0.002	0.203	0.959
Day 44–69	0.37	0.31	0.38	0.39	0.39	0.36	0.016	0.031	0.060	0.149
Day 1–69	0.39	0.37	0.43	0.45	0.45	0.42	0.018	0.005	0.439	0.377

*Treatments: (1) basal diet; (2) basal diet until 48 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-48); (3) basal diet until 26 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-26).
1A total of 2 pens (5 males and female pigs/pen) were used per treatment (experimental unit = individual pig).
[2]SEM = Standard error of the mean.
Abbreviations; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain-to-feed ratio.

total antioxidant status by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity and FRAP (ferric reducing antioxidant power) assays.

2.7.1. DPPH free radical scavenging assay

The DPPH radical scavenging assay was carried out by adding 1 ml of DPPH to 1 ml of sample. This was incubated at room temperature for 20 min and then centrifuged at 2500 rpm for 15 min and read on the spectrometer at 515 nm. The amount of scavenging can be quantified by the change in colour from a deep violet to a yellow (Rajauria et al., 2016).

$$\text{Scavenging capacity } (\%) = \left(\frac{A\ control - A\ sample}{A\ control}\right) \times 100$$

Where 'A control' is the absorbance of the control (DPPH solution without sample) and A sample is the absorbance of the test sample (DPPH solution plus test sample).

2.7.2. FRAP assay

The FRAP assay was carried out to assess the reducing power of the sample according to the method reported by Benzie and Strain (1996), with some modifications by Rajauria et al. (2010). Trolox (6-hydroxy-2, 5, 7, 8-tetrametmethylchroman-2-carboxyl acid) was used as a standard, and the results were expressed as mg of Trolox equivalents/kg meat sample. Increased absorbance of the reaction mixture indicated higher reducing power of the supplements in the meat samples.

2.7.3. Lipid peroxidation assay

For the lipid peroxidation (LPO) assay, meat samples were cooked according to the cook loss procedure. Five gram of cooked meat sample was then homogenised (Stomacher 400 circulator, Steward Ltd., UK) with 50 ml phosphate buffer (0.05 M, pH 7) for 3 min. The resulting homogenate was centrifuged at 4731 gx at 4 °C for 15 min (Rotanta 460 R, Zentrifugen, Hettich) and the supernatant was collected for further LPO analysis. A deproteinisation step was added where 2 ml of trichloro acetic acid was added to 2 ml of the sample (supernatant), mixed vigorously and centrifuged at 2739 gx for 15 min. Samples were measured on day 0, 2 and 4 of storage.

The lipid peroxidation level is measured in meat samples using thiobarbituric acid-reactive substances, based on the method of Qwele et al. (2013) with slight modifications. The thiobarbituric acid reacts with the malonaldehyde (MDA) to form a pink chromogen, which can be detected spectrophotometrically at 535 nm. The amount of MDA formed in each of the samples was assessed by measuring absorbance of samples against a reagent blank. MDA was used as a standard and results are expressed as mg MDA equivalent/kg of cooked meat.

2.8. Statistical analysis

Growth parameters (average daily gain, average daily feed intake, gain-to-feed ratio and body weight), carcass characteristics and pork quality parameters were analysed using the PROC general linearized model procedure of SAS® software 9.4 (SAS Institute, Inc., 2006). The model included dietary treatment, sex, their associated interactions and pen being the fixed effect. The experimental unit was the individual pig. Feeding behaviour was analysed by repeated measures using the PROC MIXED procedure of SAS® (Littell et al., 1996) with dietary treatment, time, their associated interactions and pen being the fixed effect. Colour variation and antioxidant activity was analysed by repeated measured using the PROC MIXED procedure of SAS®. Model suitability was investigated by checking normality of scaled residuals using the Shapiro-Wilk test within the UNIVARIATE procedure of SAS, and the data were transformed when required. The probability level that denotes significance is $P < 0.05$, while P values between 0.05 and 0.1 are considered numerical trends.

3. Results

3.1. Growth performance and feeding behaviour

During the first period (day 1–21), there was no effect of treatment, sex or treatment × sex interaction on body weight, average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F) (Table 2).

During the second period (day 22–43), male pigs had a higher ADG compared with female pigs (1.17 vs. 1.04 kg/day, $P = 0.037$). Pigs offered the vitamin D_2 enriched mushroom powder (MPVD-48) had an improved G:F compared with pigs offered the basal diets (basal and MPVD-26) (0.47 vs. 0.41 and 0.42, $P = 0.002$). There was no effect of treatment, sex or treatment × sex interaction on body weight or ADFI during this period.

During the third period (day 44–69), male pigs had a higher ADG compared with female pigs (1.11 vs. 0.95 kg/day, $P = 0.002$). Vitamin D_2 enriched mushroom powder supplementation, irrespective of duration, reduced ADFI (2.62 and 2.67 vs. 3.22 kg/day, $P < 0.001$) and improved G:F (0.39 and 0.38 vs. 0.34, $P = 0.031$) compared with pigs offered the basal diet.

Overall (day 1–69), male pigs had a higher ADG (1.12 vs. 0.99 kg/day, $P = 0.020$) and higher ADFI (2.66 vs. 2.46 kg/day, $P = 0.016$) compared with female pigs. Vitamin D_2 enriched mushroom powder supplementation, irrespective of duration, reduced ADFI (2.44 and 2.38 vs. 2.86 kg/day, $P < 0.001$) and improved G:F (0.44 and 0.44 vs. 0.38, $P = 0.005$) compared to pigs offered the basal diet.

Pigs offered vitamin D_2 enriched mushroom powder in period 2 (MPVD-48) had a lower number of visits (12.84 vs. 17.64 and 16.14 visits/day, P = 0.032) per day to the feeder compared with pigs offered the basal diets (basal and MPVD-26) (Table 3a). During period 3, there was no effect of dietary treatment on feeding behaviour (Table 3b).

3.2. Carcass characteristics, pork cook loss and water holding capacity

There was an effect of sex on carcass weight, with male pigs having a higher carcass weight compared with female pigs (91.21 vs. 85.54 kg, P < 0.001) (Table 4). There was no effect of treatment, sex or treatment × sex interaction on kill out (g/kg), lean meat (g/kg), backfat depth (mm) and muscle depth (mm). There was no effect of treatment, sex or treatment × sex interaction on cook loss and water holding capacity.

3.3. Colour stability and total antioxidant of LT muscle

Pork lightness (L^*) values remained lower (P = 0.007) over the storage time-period in pork from pigs supplemented with vitamin D_2 enriched mushroom powder for 26 days compared with all other dietary groups (Table 5). There was no effect of sex or a treatment × sex interaction on pork lightness.

Redness (a^*) values were not influenced by the supplementation of vitamin D_2 enriched mushroom powder regardless of duration.

There was an interaction between dietary group and time (P < 0.05) on yellowness (b^*) values. Pigs offered the basal diet had a lower (P < 0.05) pork yellowness on day 11 and day 14 of storage compared with pigs offered vitamin D_2 enriched mushroom powder, regardless of duration. However, there was no difference in yellowness values on days 4, 7 and 21 of storage between dietary groups (data not shown). Pigs offered the basal diet had a lower pork yellowness value (4.52 vs. 5.17, P = 0.001) compared with pigs supplemented with vitamin D_2 enriched mushroom powder for 48 days (MPVD-48).

Irrespective of feeding duration, vitamin D_2 enriched mushroom powder supplementation caused overall pork FRAP values to increase (26.04 and 26.85 vs. 25.41, P = 0.039) compared with the basal (Table 5).

There was an interaction between dietary group and time (P < 0.05) on pork FRAP values. Feeding duration of vitamin D_2 enriched mushroom powder had no effect on FRAP values on day 0, 11, 14 and 21 of storage. However, on day 4 of storage, pigs supplemented for 26 days (MPVD-26) had an increase in pork FRAP values compared with all other dietary groups. Also, on day 7 of storage, pigs supplemented with vitamin D_2 enriched mushroom powder, regardless of duration, had increased pork FRAP values compared with the basal (data not shown). DPPH was unaffected by vitamin D_2 enriched mushroom powder supplementation, irrespective of duration.

Lipid peroxidation increased in meat from all dietary groups as a function of storage time. However, on day 4 of storage, lipid peroxidation was reduced (P < 0.05) in pork from pigs supplemented with vitamin D_2 enriched mushroom powder for 26 days (MPVD-26) compared with pigs supplemented for 48 days (MPVD-48) (data not shown). Lipid peroxidation was increased (129.18 vs. 107.66 and 103.65, P = 0.006) in pigs supplemented with vitamin D_2 enriched mushroom powder for 48 days (MPVD-48) compared with basal pigs and pigs supplemented for 26 days (MPVD-26).

4. Discussion

In the present study vitamin D_2 enriched mushroom powder supplementation, irrespective of feeding duration, caused a reduction in feed intake whilst improving feed efficiency. The supplementation of vitamin D_2 enriched mushrooms for the shorter duration of 26 days prior to slaughter improved pork quality in relation to colour and antioxidant status.

The supplementation of vitamin D_2 enriched mushroom powder to weaned pigs caused a reduction in feed intake (Conway et al., 2021). While the aim of the current study was not to find the mechanism behind the reduced feed intake due to the addition of vitamin D_2 enriched mushroom powder, it may be due to (1) reduced palatability, (2) food components and/or (3) increased satiety. Mushrooms contain an array of bioactive compounds such as tannins, saponins and phenolic acids (Yıldız et al., 2017) which may negatively

Table 3a
Effect of dietary treatment on feeding behaviour in period 2 (day 22–43) (Least-square means ± S.E.M.).

	Treatments* 1				
	Basal	MPVD-48	MPVD-26	SEM[2]	P-value
Number visits/day	17.64[b]	12.84[a]	16.14[b]	1.322	0.032
Total time/day (minute)	96.84	85.06	85.57	5.755	0.264
Time/visit (minute)	6.04	7.18	5.86	0.483	0.112
Intake/visit (g)	178.94	200.06	153.03	14.299	0.066
Eating rate (g/sec)	0.45	0.44	0.46	0.020	0.795

*Treatments: (1) basal diet; (2) basal diet until 48 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-48); (3) basal diet until 26 days prior to slaughter serving as a second control during period 2 (MPVD-26).
1A total of 2 pens (5 males and 5 female pigs/pen) were used per treatment (experimental unit = individual pig).
[2]SEM = Standard error of the mean.
[a,b] Mean values within a row with unlike superscript letters were significantly different (P < 0.05).

Table 3b
Effect of dietary treatment on feeding behaviour in period 3 (day 44–69) (Least-square means ± S.E.M.).

	Treatments* 1				
	Basal	MPVD-48	MPVD-26	SEM[2]	P-value
Number visits/day	18.40	12.94	17.17	2.480	0.264
Total time/day (minute)	87.54	77.03	78.03	6.861	0.492
Time/visit (minute)	5.31	6.53	5.50	0.606	0.310
Intake/visit (g)	195.73	214.57	180.29	26.618	0.658
Eating rate (g/sec)	0.51	0.54	0.57	0.024	0.182

*Treatments: (1) basal diet; (2) basal diet until 48 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-48); (3) basal diet until 26 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-26).
1A total of 2 pens (5 males and 5 female pigs/pen) were used per treatment (experimental unit = individual pig).
[2]SEM = Standard error of the mean.

Table 4
Effect of dietary treatment on carcass characteristics, water holding capacity and cook loss (Least-square means ± S.E.M.).

	Treatments* 1							P-value		
	Basal (Male)	Basal (Female)	MPVD-48 (Male)	MPVD-48 (Female)	MPVD-26 (Male)	MPVD-26 (Female)	SEM[2]	Treatment	Sex	Treatment × Sex
Carcass weight (kg)	93.47	86.85	89.51	86.65	90.65	83.11	0.949	0.671	< 0.001	0.079
Kill out (g/kg)	747.88	740.51	736.42	748.49	746.96	741.11	8.974	0.978	0.959	0.501
Lean meat (g/kg)	586.67	589.50	588.00	595.40	582.60	607.60	7.408	0.633	0.058	0.284
Back-fat depth (mm)	13.02	13.22	13.46	11.78	13.18	9.96	1.046	0.312	0.074	0.255
Muscle depth (mm)	51.78	55.70	51.20	50.58	46.85	50.04	2.308	0.073	0.258	0.590
Cook loss (g/kg)	293.61	263.90	296.83	253.75	274.80	242.00	1.833	0.506	0.068	0.930
Water holding capacity(g/kg)	67.71	62.15	49.08	49.54	49.27	70.41	1.453	0.558	0.657	0.636

*Treatments: (1) basal diet; (2) basal diet until 48 days prior to slaughter and then supplemented with 100 mg/kg vitamin D2 enriched mushroom powder (MPVD-48); (3) basal diet until 26 days prior to slaughter and then supplemented with 100 mg/kg vitamin D2 enriched mushroom powder (MPVD-26).
1A total of 2 pens (5 males and 5 female pigs/pen) were used per treatment (experimental unit = individual pigs).
[2] SEM = Standard error of the mean.

Table 5
Effect of dietary treatment on surface colorimeter values L* , a* , b* and antioxidant activity including FRAP, DPPH and LPO (Least square means ± S.E.M).

		Treatments*[1]							P-value		
		Basal (Male)	Basal (Female)	MPVD-48 (Male)	MPVD-48 (Female)	MPVD-26 (Male)	MPVD-26 (Female)	SEM[2]	Treatment	Sex	Treatment × Sex
Colour	L	52.55	52.38	52.23	52.41	51.20	51.40	0.397	0.007	0.822	0.872
	a	4.52	4.39	4.66	4.61	4.68	3.88	0.211	0.256	0.058	0.155
	b	4.89	4.14	4.91	5.42	5.02	4.64	0.167	0.001	0.141	0.079
Antioxidant activity	FRAP	25.96	24.86	26.72	25.35	26.85	26.85	0.931	0.039	0.281	0.733
	DPPH	65.08	66.64	61.57	62.37	59.41	61.40	2.273	0.055	0.438	0.965
	LPO	109.72	105.60	122.96	135.39	100.38	106.92	8.190	0.006	0.462	0.595

*Treatments: (1) basal diet; (2) basal diet until 48 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-48); (3) basal diet until 26 days prior to slaughter and then supplemented with 100 mg/kg vitamin D_2 enriched mushroom powder (MPVD-26).
1A total of 2 pens (5 males and 4 female pigs/pen) were used per treatment (experimental unit = individual pig).
[2]SEM = Standard error of the mean.
Abbreviations; L, lightness value; a, redness value; b, yellowness value; FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl; LPO, lipid peroxidation

affect their palatability due to their astringent taste (Dong and Pluske, 2007). Food components, including the presence of non-digestible polysaccharides in mushroom powder such as chitin, may increase the satiety effect leading to a reduction in feed intake. *Agaricus bisporus* mushrooms contain approximately 7% chitin (Vetter, 2007; Hassainia et al., 2018). Chitosan supplementation, the deacetylated form of chitin, has been shown to reduce feed intake in both mice (Kumar et al., 2009) and pigs (Walsh et al., 2013; Egan et al., 2016). Satiety, defined as the feeling of fullness that persists after eating, is affected by numerous factors such as episodic signals, hormone fluctuations and food components (Benelam, 2009). Increased satiety was interpreted during period 2 when pigs supplemented with vitamin D_2 enriched mushroom powder visited the feeder less often compared with pigs on the basal diets, perhaps suggesting that the vitamin D_2 enriched mushroom powder caused the pig to remain full for longer periods of time. Previous work has also shown that mushroom powders had no effect on diet digestibility in weaner pigs (Conway et al., 2021). Even though the mushroom powder is added at a low level (1 g/kg of feed), Egan et al. (2016) demonstrated that chitosan supplementation at similar levels (1 g/kg) affected satiety signals in finisher pigs.

Feed efficiency is critical in pig production as it can increase profitability while reducing the environmental impact of pig production (McCormack et al., 2017). The improved efficiency observed in pigs supplemented with vitamin D_2 enriched mushroom powder may be attributed to the β-glucans and/or the additional vitamin D present in the mushroom powder. Supplementation of β-glucans to weaned pigs improves the composition of the intestinal microbiota and gastrointestinal health (Sweeney and O'Doherty, 2016) while vitamin D has been shown to have anti-inflammatory and immunomodulatory properties (Prietl et al., 2013). Research has suggested that the improvement in intestinal microbiota is associated with increased feed efficiency in pigs through improved digestion and gut function (Vigors et al., 2016; McCormack et al., 2017). Immune system activation causes an increase in energy demands due to the host's response to infection (Romanyukha et al., 2006). The production of acute phase proteins due to immune system activation increases the demand for amino acids thus increasing the energy demand of the pig (Gruys et al., 2005). Therefore, reducing the demand placed on the pig's immune system is another successful way to improve feed efficiency (Patience et al., 2015). We hypothesised that the improvement in G:F in the current study could potentially be due to a reduced immune system activation, thus allowing more nutrients go towards growth.

One of the most important quality traits of fresh pork determined from a consumer perception is colour (Risvik, 1994), with expectations of fresh pork to have a homogenous reddish-pink colour (Van Oeckel et al., 1999). The rate of fresh meat colour deterioration is influenced by the rate of pigment oxidation and their effect on the oxygen content on the meat surface (Faustman et al., 1989). In the present study, vitamin D_2 enriched mushroom powder supplementation to finisher pig diets for 26 days prior to slaughter caused pork lightness (L^*) to remain stable over a 21-day storage time-period. Interestingly, this trend was not observed when pigs were supplemented for 48 days prior to slaughter. In contrast to our study, the supplementation of vitamin D_2 enriched mushroom for 55 days prior to slaughter caused pork to have an overall lower L^* value (Duffy et al., 2018). It is worth noting that the total vitamin D content in our study was twice the concentration (100 μg/kg) of that used by Duffy et al. (2018), perhaps suggesting the feeding of an increased level of vitamin D or other mushroom components for a longer period had a negative impact on pork colour compared with feeding for a shorter duration.

Dietary supplementation of marine carbohydrates (laminarin and fucoidan) for a shorter duration of 21 days resulted in enhanced pork antioxidant potential compared with supplementation for 42 days (Moroney et al., 2015). While the current experiment was not designed to explore the mode of action of the enhanced meat quality observed in the shorter feeding period, it is possible that we see improved antioxidant activity and colour stability in the shorter feeding duration due to (1) the increased utilisation of the polysaccharides by gut microbes, (2) the stage of development of the pig or (3) the amount of fat storage of the pig. Supplementing a higher amount of *Agaricus bisporus* mushroom powder (10 or 20 g) caused antioxidant activity to increase in both turkey poults (Giannenas et al., 2011) and broiler chickens (Giannenas et al., 2010). The decrease in lipid peroxidation and increase in colour stability due to the supplementation of vitamin D_2 enriched mushroom powder for 26 days could potentially be due to a higher antioxidant activity (FRAP). Higher antioxidant activity leads to the increased ability to scavenge free radicals, therefore protecting the phospholipid content against lipid oxidation resulting in improved colour stability (Kumar et al., 2015). However, there is very little research in the literature on the effects of mushroom powder supplementation to animals on the antioxidant status in subsequent meat products.

5. Conclusion

Dietary supplementation to finisher pigs with vitamin D_2 enriched mushroom powder, irrespective of duration, reduced feed intake whilst improving feed efficiency. Additionally, pork from pigs supplemented with vitamin D_2 enriched mushroom powder for the shorter duration of 26 days exhibited the highest antioxidant activity and improved the overall colour stability of fresh pork. Supplementation of vitamin D_2 enriched mushroom powder to finisher pig diets for the shorter duration of 26 days seems to be a promising alternative to synthetic antioxidants thus potentially providing a natural animal feed supplement for finisher pig diets.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed the EU standards for the protection of animals used for scientific purposes.

Financial support

This work was funded by the Science Foundation Ireland (Dublin, Ireland) and Monaghan Mushrooms Ireland Unlimited Company (Co. Monaghan, Ireland) [grant number: 16/RC/3889].

Authorship

The author's contributions were as follows: E.C performed the experiment, collected the samples, carried out the laboratory analyses and wrote the manuscript; J.V.O.D. and T.S. designed the experiment, supervised data collection and statistical analyses and corrected the manuscript; S.V. contributed to statistical analysis; A.D., and G.R. contributed to sample collection and laboratory analyses. S.Y., J.W., and W.G. developed and manufactured the mushroom powder. All authors approved the final version of the manuscript.

Declaration of Competing Interest

Eadaoin Conway, Torres Sweeney, Alison Dowley, Gaurav Rajauria, Stafford Vigors and John V. O'Doherty had no financial or personal conflict of interest in relation to the present study. Supriya Yadav, Jude Wilson and William Gabrielli work for MBio, Monaghan, Ireland, the mushroom producing company.

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