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REVIEW

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The effects of mushroom powder and vitamin D_2-enriched mushroom powder supplementation on the growth performance and health of newly weaned pigs

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Abstract

A complete randomised block design experiment was conducted to examine the effects of mushroom powder (MP) and vitamin D_2-enriched mushroom powder (MPD_2) on growth performance, faecal scores, coefficient of apparent total tract digestibility (CATTD) of nutrients and selected microflora in weaned pigs up to day 35 post-weaning. One hundred and ninety-two weaned pigs (7.8kg [SD 1.08kg]) were blocked according to live weight, sex and litter of origin and randomly assigned to the following: (T1) control diet; (T2) control diet +MP; (T3) control diet + MPD_2; and (T4) control diet +zinc oxide (ZnO) (n = 12 replicates/treatment). Mushroom powders were included at 2 g/kg of feed achieving a β-glucan content of 200ppm. ZnO was included at 3100 mg/kg feed and halved to 1550 mg/kg after 21 days. Vitamin D content was enhanced in MPD_2 using synthetic UVB exposure to obtain a vitamin D_2 level of 100 μg/kg of feed. Faecal samples were collected on day 14 for microbial and nutrient digestibility analysis. There was no difference ($p > 0.05$) in ADG, G:F, faecal scores, microbial populations and CATTD of nutrients in pigs supplemented with MP or MPD_2 compared with the control diet. The supplementation of MP and MPD_2 caused a reduction ($p < 0.05$) in feed intake compared with the control and ZnO diet throughout the 35-day experimental period. ZnO supplementation increased ADG and ADFI ($p < 0.05$) during the first period (D0-21) compared with pigs offered MP and MPD_2. In conclusion, MP and MPD_2 supplementation resulted in similar ADG, G:F, faecal scores compared with the control but were not comparable to ZnO, mainly due to a reduction in feed intake.

KEYWORDS
Agaricus bisporus, pigs, post-weaning, vitamin D, zinc oxide, β-glucan

1 | INTRODUCTION

In conventional pig production systems, weaning is a source of significant stress resulting from exposure to environmental, psychological and nutritional stressors (Lallès et al., 2004; Pluske et al., 1997). The weaning period is marked by disruption of the mucosal integrity of the gastrointestinal tract leading to increased permeability allowing toxins, bacteria or feed-associated antigens to cross the epithelium (Campbell et al., 2013). This results in an increase in inflammatory cytokines and malabsorption, consequently increasing susceptibility to infection and post-weaning diarrhoea (PWD) (Campbell et al., 2013; Heo et al., 2013). Pharmacological levels of zinc oxide (ZnO) have been used as a supplement in post-weaned pig diets to enhance growth and control the proliferation of pathogenic bacteria (Sales, 2013). However, due to zinc accumulation in soils and the relationship between ZnO usage and the rise in antibiotic resistance (Long et al., 2017), the EU has begun phasing out the use of pharmacological doses of ZnO, with its complete ban by 2022 (Commission Implementing Decision of 26 June 2017, C(2017) 4529 Final). Therefore, identifying natural alternative feed additives to help alleviate the post-weaning growth check and intestinal dysfunction is of critical importance.

One feed supplement that exhibits beneficial properties is β-glucans. Beta-glucans are a non-digestible polysaccharide of D-glucose monomers linked through β-glycosidic bonds, which form the main constituent of the cell walls of cereals, mushrooms, yeast and macroalgae (Rahar et al., 2011; Zhu et al., 2015), and exhibit different biological activities depending on their source (Ryan et al., 2012). Beta-glucans contain glucans with β-(1–3), β-(1–4) and β-(1–6) glycosidic linkages (Manzi & Pizzoferrato, 2000; Volman et al., 2007) and have been identified as natural biomolecules which exhibit immunomodulatory, prebiotic and anti-inflammatory activity (Du et al., 2015; Sweeney et al., 2012). Yeast β-glucans in the diet of weaned pigs can decrease faecal *E. coli* numbers and has the potential to improve growth performance and immune response in weaned pigs (Li et al., 2006; Zhou et al., 2013) while β-glucans from macroalgae reduced Enterobacteriaceae counts and pro-inflammatory markers in the colon of the pig (Rattigan et al., 2019; Sweeney et al., 2012). Mushrooms are a rich natural source of bioactive metabolites such as phenolic compounds and polysaccharides, in particular β-glucans (Reis et al., 2012). The β-glucan content of mushrooms varies among species with *Agaricus bisporus* containing between 8.6 and 12.3% β-glucans on a dry matter basis (Sari et al., 2017). *Agaricus bisporus* supplementation at an inclusion rate of 10 and 20 g/kg of feed improved growth performance in both turkey poults and broiler chickens (Giannenas, Pappas, et al., 2010; Giannenas et al., 2011).

It is widely known that vitamin D enhances calcium and phosphorus absorption but is also involved in immune system regulation and has both anti-inflammatory and immunomodulatory properties (Prietl et al., 2013). Cultivated mushrooms contain high levels of the steroid ergosterol, which has the potential to form a bioavailable source of vitamin D_2 by post-harvest ultraviolet irradiation (Cardwell et al., 2018; Kalaras et al., 2012; Teichmann et al., 2007). When *Agaricus bisporus* mushrooms are exposed to UV light under certain conditions, they produce vitamin D_2 in amounts that exceed the required adequate daily intake in humans (Koyyalamudi et al., 2009; Roberts et al., 2008). Supplementation of vitamin D_2-enriched mushroom powder to finisher pigs improved animal performance (Duffy et al., 2018). Therefore, the supplementation of mushroom powder, in particular vitamin D_2-enriched mushroom powder, to newly weaned pigs may enhance the growth performance during the post-weaning period.

The objective of this experiment was to investigate the effects of supplementing mushroom powder and vitamin D_2-enriched mushroom powder on growth performance, faecal scores, nutrient digestibility and selected faecal microflora in weaned pigs. It was hypothesised that mushroom powder and particularly vitamin D_2-enriched mushroom powder supplementation in the post-weaned pig diet would improve pig performance by enhancing beneficial microbial populations and reducing diarrhoea compared with a control diet and would give performance results similar to those observed following supplementation with ZnO.

2 | MATERIALS AND METHODS

All experimental procedures described in this work were approved under University College Dublin Animal Research Ethics Committee (AREC-18–27-O'Doherty) and conducted in accordance with Irish legislation (SI no. 543/2012) and the EU directive 2010/63/EU for animal experimentation. All efforts were taken to minimise pain and discomfort to the animal while conducting these experiments.

2.1 | Experimental design and animal management

This study was designed as a complete randomised block design comprising of 4 dietary treatments. One hundred and ninety-two pigs (progeny of Meatline boars × (Large White × Landrace sows)) were sourced from a commercial pig farm at weaning (28 days of age) with an average weaning weight of 7.8 kg (SD 1.08 kg). The pigs were blocked by weaning weight, sex and litter of origin and within each block assigned to one of four dietary treatments for the duration of the experiment (day 0–day 35 post-weaning). The dietary treatments were as follows: control diet; control diet +mushroom powder (MP); control diet +ultraviolet treated vitamin D_2 mushroom powder (MPD_2) and control diet +ZnO. Both mushroom powders were sourced from Monaghan Mushrooms (Tyholland, Co. Monaghan) and contained 100 g/kg β-glucans. The mushroom powder contained 0.4 μg/100 mg of vitamin D prior to UVB exposure. The mushroom vitamin D content was naturally enhanced using synthetic UVB exposure as described by Stepien et al. (2013) to obtain a vitamin D_2 level of 100 μg/kg of feed. Both mushroom powders were included at 2 g/kg of feed achieving a β-glucan content of 200 ppm (Rattigan et al., 2019; Sweeney et al., 2012) and a vitamin D_2 content of 100 μg/kg in the vitamin D_2-enriched mushroom diet. All diets contained a vitamin D_3 content of 50 μg/kg of feed.

The ZnO (Cargill, Naas) was included at 3100 mg ZnO/kg feed and contained 80% zinc, resulting in an inclusion level of 2500 mg Zn/kg of feed. After three weeks the inclusion level of ZnO was halved to 1550 mg ZnO/kg. The diets were formulated to contain similar levels of net energy (10.6 MJ/kg) and standardised ileal digestible lysine (13.0 g/kg). The levels of amino acids were formulated to meet or exceed the requirements of the NRC (2012). All diets were milled on-site and fed in meal form. The ingredient composition and analysis of the diets are shown in Table 1. Celite was added to all diets at a rate of 1 g/kg of feed to calculate acid-insoluble ash. Faecal samples were collected on day 14, for microbial and nutrient digestibility analysis, as this coincides with the end of the critical post-weaning challenge.

2.2 | Housing and animal management

The pigs were **housed** in groups of four (twelve replicates per treatment) on fully slatted pens (1.68 × 1.22 m). The ambient environmental temperature within the house was thermostatically controlled at 30°C for the first 7 days and then reduced by 2°C each week, and the humidity was maintained at 65%. Feed-in meal form and water were available ad libitum from four-space feeders and nipple drinkers. Pig performance was measured from weaning (day 0) to 35 days post-weaning. Pigs and feed were weighed weekly to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F).

2.3 | Faecal scoring

The piglets were observed for clinical signs of diarrhoea, and a scoring system was applied to indicate the presence and severity of the diarrhoea as described by Walsh et al. (2013). Faecal scoring was recorded twice daily for individual pens from day 0 until day 35 by the same operator on a scale ranging from 1 to 5. The following scoring system was used: 1 = hard, firm faeces; 2 = slightly soft faeces; 3 = soft, partially formed faeces; 4 = loose, semi-liquid faeces and 5 = watery, mucous-like faeces.

2.4 | Feed and faecal analysis

Faecal samples were collected on day 14 and dried at 55°C for 72 h. The feed and dried faecal samples were milled through a 1 mm screen (Christy and Norris hammer mill, Ipswich, UK). The dry matter of the feed was determined after drying overnight at 104°C. The crude ash content of the faeces was determined after ignition of a known weight of a concentrate in a muffle furnace (Nabertherm) at 550°C for 6 h. Faecal gross energy content was determined using an adiabatic bomb calorimeter (Parr Instructions). The faecal nitrogen content was determined using the LECO FP 528 instrument (Leco Instruments). The neutral detergent fibre content of the feed samples was determined according to Van Soest et al. (1991) using the Ankom 220 Fibre Analyser (Ankom^{tm} Technology). The ether extract concentrations of the diet were determined using light petroleum ether and Soxtec instrumentation (Tecator). The concentration of acid-insoluble ash was determined according to the method of McCarthy et al. (1977). The β-glucan content of the mushroom powder and feed was determined in triplicate using the Megazyme assay kit (Megazyme©, Bray, Co) according to the manufacturer's instructions. The vitamin D concentration in the MPD_2 and feed was analysed by high-performance liquid chromatography, as described by Mattila et al. (1994).

2.5 | Extraction of microbial DNA from faecal samples

On day 14 of the experiment, fresh faecal samples were collected from each pen (12 pens/treatment) and stored in sterile 50 ml tubes (Sarstaedt) at −20°C and transported to the laboratory within 2 h. Bacterial genomic DNA was extracted from the faecal samples using the QIAamp DNA stool kit (Qiagen) following manufacturer's instructions. The quantity and quality of DNA were assessed using the Nanodrop spectrophotometer (Nanodrop, ND1000; Thermo Scientific) and the purified DNA stored at −20°C.

Estimation of selected bacterial groups in faecal samples: Primers specifically designed for qPCR for; total bacteria, *Lactobacillus* spp., *Bifidobacterium* spp. and *Enterobacteriaceae* are presented in the (Table S1). The numbers of the selected bacterial groups were estimated based on gene copy number (GCN) in the digesta using QPCR on the 7500 Fast Real-Time PCR System (Applied Biosystems, Warrington, UK). A final reaction volume of 20 μl contained 3 μl template DNA, 1 μl of forward and reverse primers (10 pM/ul), 10 μl GoTaq® qPCR Master Mix (Promega) and 5 μl nuclease-free water. The thermal cycling conditions included an initial denaturation step at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Dissociation curves confirmed the specificity of the final PCR products. All samples were prepared in duplicate, and the mean threshold cycle (Ct) value was used for calculations. Bacterial counts were determined using a standard curve derived from the log Ct and the log gene copy number per gram of faeces (LogGCN/g faeces).

2.6 | Volatile fatty acids

The VFA concentrations of the collected faecal samples (collected on day 14) were determined using gas-liquid chromatography according to the method described by Clarke et al. (2018). A 1 g sample was diluted with distilled water (2·5 × weight of sample) and centrifuged at 1400 x g for 10 min (Sorvall GLC–2 B laboratory centrifuge; DuPont). One ml of the supernatant and 1 ml of internal standard (0.05% 3-methyl-n-valeric acid in 0.15 M oxalic acid dihydrate) were mixed with 3 ml of distilled water. The reaction mixture was centrifuged at 500 g for 10 min, and the supernatant was filtered through 0.45 polytetrafluoroethylene syringe filter into a chromatographic sample vial. An injection volume of 1 μl was injected into a Varian 3800 GC equipped with an EC™ 1000 Grace column (15 m × 0.53 mm I.D) with 1.20 μm film thickness. The

TABLE 1 Ingredient and chemical composition of all diets

Ingredients (g/kg unless otherwise stated)	Treatments			
	Control	MP	MPD_2	ZnO
Wheat	355.4	353.4	353.4	352.3
Full-fat soya bean	170.0	170.0	170.0	170.0
Soya bean meal	105.0	105.0	105.0	105.0
Whey powder (90%)	50.0	50.0	50.0	50.0
Mushroom Powder	0.00	2.0	2.0	0.0
Zinc oxide	0.00	0.0	0.0	3.1
Soya oil	30.0	30.0	30.0	30.0
Soya concentrate	65.0	65.0	65.0	65.0
Flaked wheat	130.0	130.0	130.0	130.0
Flaked maize	70.0	70.0	70.0	70.0
Lysine-HCl	4.0	4.0	4.0	4.0
DI-Methionine	2.0	2.0	2.0	2.0
L-Threonine	1.8	1.8	1.8	1.8
Tryptophan	0.3	0.3	0.3	0.3
Sodium bicarbonate	2.0	2.0	2.0	2.0
Monocalcium phosphate	4.0	4.0	4.0	4.0
Vitamins and minerals[c]	2.5	2.5	2.5	2.5
Calcium carbonate (limestone)	6.0	6.0	6.0	6.0
Salt	2.0	2.0	2.0	2.0
Analysed chemical analysis				
DM	899.0	897.5	898.1	899.5
NDF	99.0	99.5	99.3	98.7
GE (MJ/kg)	16.9	16.8	16.9	16.9
Ash	46.2	46.0	46.2	46.1
Crude fat	79.9	80.1	80.0	80.3
β-glucan[a] (mg/kg)	3.0	195	205	4.0
Crude fibre	28.0	28.2	28.3	28.0
Vitamin D_3 (μg/kg)	50.0	50.0	50.0	50.0
Vitamin D_2 (μg/kg)	0.00	0.0	100.0	0.0
Crude protein	208.0	208.5	208.5	208.3
Lysine (%)[b]	1.43	1.43	1.43	1.43
Methionine (%)[b]	0.50	0.50	0.50	0.50
Threonine (%)[b]	0.93	0.93	0.93	0.93
Methionine and cysteine (%)[b]	0.84	0.84	0.84	0.84
Tryptophan (%)[b]	0.30	0.30	0.30	0.30

Abbreviations: CP, crude protein; DM, dry matter; GE, gross energy; MP, mushroom powder; MPD_2, vitamin D_2 mushroom powder; NDF, neutral detergent fibre; ZnO, zinc oxide.

[a]Analysed for β(1–3)-(1–6) β-glucan.

[b]Calculated for the tabulated nutritional composition (Sauvant et al., 2004).

[c]Provided (per kg diet): 25 mg Cu; 140 mg Fe; 47 mg Mn; 120 mg Zn; 0.6 mg I; 0.3 mg S; 1.8 mg retinol; 0.025 mg cholecalciferol; 67 mg tocopherol; 4 mg menaquinone; 0.01 mg cyanocobalamin; 2 mg riboflavin; 12 mg nicotinic acid; 10 mg pantothenic acid; 250 mg choline chloride; 2 mg thiamine; and 0.015 mg pyridoxine.

temperature programme set was as follows: 75–95°C increasing by 3°C/min and 95–200°C increasing by 20°C/min, which was held for 0.50 min. The detector and injector temperature were 280°C and 240°C, respectively, while the total analysis time was 12.42 min.

2.7 | Statistical analysis

Growth parameters (ADG, ADFI and G:F) and faecal scores were analysed using the PROC MIXED procedure of SAS® software 9.4

(SAS Institue). The growth parameters were divided up into two time periods; day 0–21 and day 21–35. The model for growth parameters included fixed effects of dietary treatment, and time while the model for faecal scores included fixed effects of dietary treatment and time and their associated interactions. 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 microbiology data and VFA concentrations were analysed using PROC GLM procedure of SAS® software 9.4 (SAS Institue). The results are presented as least-square means with their standard errors. The probability level that denotes significance is $p < 0.05$; a numerical trend is $p > 0.05$ and $p < 0.10$.

3 | RESULTS

3.1 | Performance

The effects of dietary treatment on average daily feed intake (ADFI), average daily gain (ADG), gain to feed ratio (G:F) and body weight during the post-weaning period (day 0–21, day 21–35, day 0–35) are presented in Table 2.

During the first phase (D0-21), pigs supplemented with MP and MPD_2 had a lower ($p < 0.05$) ADFI compared to pigs supplemented with ZnO but were not different to the controls. During the second phase (D21-35), pigs supplemented with MP and MPD_2 had a lower ADFI ($p < 0.05$) compared to pigs supplemented with both the ZnO and control diet. Overall, pigs supplemented with MP and MPD_2 had a lower ($p < 0.05$) ADFI compared with pigs offered both the ZnO and control diets over the 35-day period.

During the first phase, ADG was increased ($p < 0.05$) in pigs offered the ZnO diet compared with all other groups. However, during the second phase, ADG was decreased ($p < 0.05$) in pigs offered the ZnO diet compared with all other groups. Overall, there was no difference ($p > 0.05$) in ADG between groups over the 35-day period.

There was no difference in G:F between groups during the first phase or over the 35-day period. However, during the second phase, pigs offered the ZnO diet had a lower ($p < 0.05$) G:F compared with all other groups. Pigs offered the ZnO diet had a higher final body weight ($p < 0.05$) at the end of phase 1 and phase 2 compared with all other groups.

3.2 | Faecal scores

The effects of dietary treatment on faecal scores is presented in Figure 1. There was an interaction between treatment and time ($p < 0.05$) on faecal scores. Pigs offered the ZnO diet had reduced ($p < 0.05$) faecal scores on days 0–7, 7–14 and 14–21 compared with all other groups. However, there was no difference ($p > 0.05$) in faecal scores between groups on days 21–28 and 28–35.

TABLE 2 Effect of mushroom powder, vitamin D_2-enriched mushroom powder and zinc oxide inclusion on pig growth performance

	Treatments[1]					
	Control	MP	MPD2	Zinc	SEM	P
ADFI (kg)						
Day 0–21	0.53[a]	0.54[a]	0.52[a]	0.62[b]	0.012	<0.001
Day 21–35	1.15[b]	1.08[a]	1.11[a]	1.18[c]	0.012	<0.001
Day 0–35	0.84[b]	0.81[a]	0.81[a]	0.90[c]	0.008	<0.001
ADG (kg)						
Day 0–21	0.38[a]	0.38[a]	0.37[a]	0.46[b]	0.015	<0.001
Day 21–35	0.71[b]	0.71[b]	0.69[b]	0.65[a]	0.015	<0.001
Day 0–35	0.54	0.55	0.53	0.56	0.010	0.309
G:F						
Day 0–21	0.70	0.70	0.70	0.74	0.020	0.098
Day 21–35	0.62[b]	0.66[b]	0.62[b]	0.56[a]	0.020	<0.001
Day 0–35	0.66	0.68	0.66	0.65	0.014	0.432
Weight (kg)						
Day 21	15.97[a]	15.68[a]	15.29[a]	17.45[b]	0.360	<0.001
Day 35	25.97[a]	25.64[a]	24.96[a]	26.55[b]	0.360	<0.001

Note: (Least-square mean values with their standard error).

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$).

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; G:F, gain to feed ratio; MP, mushroom powder; MPD_2, vitamin D_2 mushroom powder; ZnO, zinc oxide.

[1]A total of twelve replicates were used per treatment group (replicate = pen, 4 pigs/pen).

3.3 | Microbiology and volatile fatty acids

The effects of dietary treatment on the abundance of a selected microbial populations in the faecal samples are presented in Table 3. There was no effect of dietary treatment on the numbers of total bacteria, *Lactobacillus* spp. or *Bifidobacterium* spp. ($p > 0.05$). *Enterobacteriaceae* was reduced ($p < 0.05$) in the ZnO group compared with all other groups.

The effects of dietary treatment on the concentrations and molar proportions of faecal VFA are presented in Table 4. Pigs offered MP had increased concentrations of total faecal VFA ($p < 0.05$) compared with the MPD_2 and ZnO groups. The acetate concentration was increased ($p < 0.05$) in the faeces from pigs offered MP compared with faeces from pigs offered MPD_2. The molar proportions of valerate were reduced ($p < 0.05$) in the ZnO group compared with all other groups.

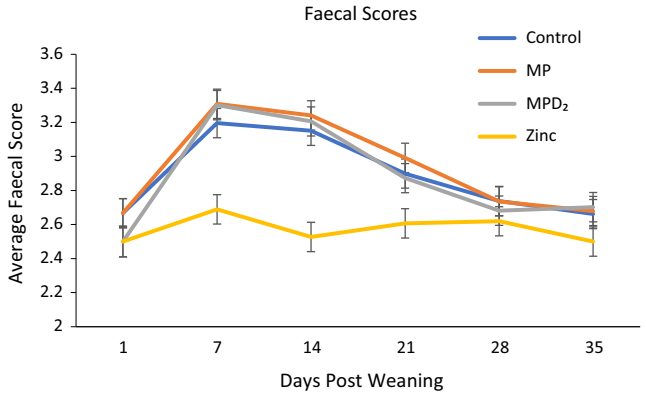

FIGURE 1 Effect of mushroom powder, vitamin D_2-enriched mushroom powder and zinc oxide inclusion on faecal scores from day 0–35 post-weaning. Values are means, with their standard errors represented by vertical bars. Scale from 1 to 5: 1 = hard, firm faeces; 2 = slightly soft faeces; 3 = soft, partially formed faeces; 4 = loose, semi-liquid faeces and 5 = watery, mucous-like faeces. Treatment ($p < 0.001$), Time ($p < 0.001$), Treatment x Time ($p < 0.05$)

3.4 | Coefficient of apparent total tract digestibility

The effects of dietary treatment on the coefficient of apparent total tract digestibility (CATTD) of nutrients are presented in Table 5. There was no difference between groups in CATTD of DM, OM, N, GE and DE content of the diets ($p > 0.05$). However, the ZnO group had a lower CATTD of ash ($p < 0.05$) compared with all other groups.

4 | DISCUSSION

The hypothesis of this study was that mushroom powder, particularly vitamin D_2-enriched mushroom powder supplementation to the diet of newly weaned pigs would improve pig performance during the first 35 days post-weaning by reducing faecal scores and enhancing beneficial microbial populations compared with a control diet and would give performance results similar to ZnO. Supplementation of MP and MPD_2 to the diet of weaned pigs for the first 35 days post-weaning resulted in similar ADG, G:F, faecal scores and microbial populations compared with the control diet. When compared to ZnO, MP and MPD_2 supplementation reduced feed intake and increased faecal scores.

Maintaining adequate feed intake in the immediate post-weaning period is of critical importance to reduce intestinal permeability, PWD and to improve growth performance (Dong & Pluske, 2007; O'Doherty et al., 2017). The reduction in feed intake that was observed in this experiment may be due to either a reduced appetite and/or reduced palatability of the diet. A reduced appetite may be attributed to the presence of chitin in the mushroom powder. *Agaricus bisporus* mushrooms are abundant in fungal chitinous biopolymers (chitin and chitosan). They contain approximately 7% chitin (Hassainia et al., 2018; Vetter, 2007) which is found in the cell walls (Wu et al., 2004). Chitosan supplementation, the deacetylated form of chitin, can cause a reduction in feed intake in both mice (Kumar et al., 2009) and pigs (Egan et al., 2016; Walsh et al., 2013). In contrast to our results, diet supplementation with the mushroom species *Pleurotus ostreatus* to post-weaned pigs increased feed intake (Adams et al., 2019). However, the fact that *Pleurotus ostreatus*

TABLE 3 Effect of mushroom powder, vitamin D_2-enriched mushroom powder and zinc oxide inclusion on selected faecal microbial populations

	Treatments[1]					
	Control	MP	MPD_2	ZnO	SEM	P
Log GCN/g faeces						
Lactobacillus spp.	9.21	9.44	9.21	9.53	0.145	0.12
Total bacteria	10.82	11.08	10.89	11.11	0.135	0.107
Enterobacteriaceae	7.27[b]	7.28[b]	7.25[b]	6.18[a]	0.331	0.039
Bifidobacterium spp.	6.43	6.5	6.37	6.33	0.425	0.951

Note: (Least-square mean values with their standard error).

Abbreviations: GCN, gene copy number; MP, mushroom powder; MPD_2, vitamin D_2 mushroom powder; ZnO, zinc oxide.

[a,b]Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$).

[1]A total of twelve replicates per treatment (replicate = pen).

TABLE 4 Effect of mushroom powder, vitamin D_2-enriched mushroom powder and zinc oxide inclusion on total and molar populations of volatile fatty acids (VFA) in faeces

	Treatments[1]					
	Control	MP	MPD_2	ZnO	SEM	P
VFA (mmol/L digesta)						
Total	195.5^{ab}	228.6^{b}	166.0^{a}	175.3^{a}	15.657	0.039
Acetate	144.1^{ab}	172.9^{b}	121.7^{a}	129.6^{a}	12.484	0.034
Propionate	30.7	36.4	28.1	27.6	2.643	0.098
Butyrate	10.4	11.7	9.6	10.9	0.878	0.387
Isobutyrate	2.4	2.0	2.2	2.1	0.210	0.613
Isovalerate	4.0	3.3	3.9	3.5	0.374	0.560
Valerate	2.6^{b}	2.3^{b}	2.2^{b}	1.6^{a}	0.174	0.002
Molar proportions						
Acetate	0.732	0.755	0.729	0.734	0.008	0.121
Propionate	0.157	0.160	0.162	0.158	0.006	0.922
Butyrate	0.056^{ab}	0.051^{a}	0.055^{a}	0.064^{b}	0.003	0.044
Isobutyrate	0.013	0.009	0.014	0.012	0.001	0.090
Isovalerate	0.021^{a}	0.015^{ab}	0.025^{ac}	0.021^{a}	0.003	0.007
Valerate	0.014^{b}	0.010^{a}	0.013^{b}	0.010^{a}	0.001	0.008

Note: (Least-square mean values with their standard error).
Abbreviations: MP, mushroom powder; MPD_2, vitamin D_2 mushroom powder; ZnO, zinc oxide.
^{a,b,c}Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$).
[1]A total of twelve replicates per treatment (replicate = pen).

TABLE 5 Effect of mushroom powder, vitamin D_2-enriched mushroom powder and zinc oxide inclusion on the coefficient of apparent total tract digestibility (CATTD) of dry matter (DM), organic matter (OM), nitrogen (N), gross energy (GE), ash and digestible energy content (DE)

	Treatments[1]					
	Control	MP	MPD_2	ZnO	SEM	P
DM	0.76	0.76	0.76	0.75	0.006	0.652
OM	0.84	0.84	0.84	0.84	0.380	0.903
N	0.79	0.78	0.79	0.79	0.660	0.844
GE	0.81	0.80	0.81	0.81	0.452	0.736
Ash	0.57^{b}	0.55^{b}	0.55^{b}	0.51^{a}	1.087	0.004
DE (MJ/kg)[2]	15.17	15.07	15.19	15.19	0.085	0.736

Note: (Least-square mean values with their standard error).
Abbreviations: MP, mushroom powder; MPD_2, vitamin D_2 mushroom powder; ZnO, zinc oxide.
^{a,b}Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$).
[1]A total of twelve replicates per treatment (replicate = pen).
[2]Calculated from DE = GE x GE digestibility.

contains as little as 3% chitin versus 7% in *Agaricus bisporus* (Vetter, 2007) may explain the difference in feed intake between the two studies. Feed palatability can also influence feed intake. Palatability can be affected by the composition of feed with some feedstuffs of plant origin containing anti-nutritional factors (Dong & Pluske, 2007). Mushrooms contain an array of compounds such as tannins and phenolic acids (Yıldız et al., 2017), which may negatively affect their palatability due to their bitter taste and thus reduce feed intake (Dong & Pluske, 2007). In the present study, there was no difference observed in the CATTD of DM, OM, N, GE and DE content suggesting mushroom powder supplementation or additional vitamin D_2 supplementation had no effect on nutrient utilisation or uptake. In contrast to our results, pigs supplemented with β-glucans from seaweed had increased CATTD of DM, ash, N and GE (Heim et al., 2014).

It is widely accepted that feeding ZnO at pharmacological dietary levels to post-weaned pigs increases ADG, ADFI and G:F (Sales, 2013). In the present study, ZnO supplementation to post-weaned pigs lead to an increase in ADG and ADFI during the first phase (D0-21). However during the second phase (D21–35), although the amount of ZnO in the diet was halved, the supplementation of ZnO caused a reduction in ADG and G:F, suggesting a negative impact of ZnO supplementation after 21 days. This supports findings from previous studies whereby ZnO lost its effectiveness after 21 days (Heim et al., 2014; O'Doherty et al., 2005). Interestingly, although ZnO supplementation caused a

reduction in ADG during the second phase, pigs offered ZnO had higher body weight at the end of the experimental period (D35) compared with pigs supplemented with MP and MPD_2. The long-term feeding of pharmacological ZnO can lead to zinc toxicity and thus impacting animal health and wellbeing (Brugger & Windisch, 2017). A reduction in weight gain and feed efficiency, which was evident in our study, as well as lameness are clinical signs of zinc toxicity in swine (Burrough et al., 2019).

Faecal scoring has been considered as an indicator of gut health where a high faecal score correlates with diarrhoea, and hence, a lower faecal score is desirable (Pierce et al., 2005). One of the main causative factors of PWD in pigs is Enterotoxigenic *Escherichia coli* (ETEC) (García et al., 2020; Gresse et al., 2017). The results from the present study support findings from previous research that feeding pharmacological levels of ZnO alleviates PWD (Lei & Kim, 2018; Milani et al., 2017; Rattigan et al., 2020). No difference was observed in faecal scores or faecal *Enterobacteriaceae* populations in pigs supplemented with MP or MPD_2 compared with pigs offered the control diet, whereas faecal scores and faecal *Enterobacteriaceae* populations were decreased in pigs supplemented with ZnO compared with all other dietary groups. Contrary to the present study, *Pleurotus ostreatus* mushroom powder supplementation at a higher rate of 5–15 g/kg of feed to post-weaned pigs caused a reduction in the incidence of diarrhoea compared with pigs fed a basal diet (Adams et al., 2019). The relevance of measuring Enterobacteriaceae populations as an indicator of pathogenic bacteria is widely debated; however, an increase in Enterobacteriaceae has been reported in diarrhoeic pigs post-weaning (Dou et al., 2017).

It was anticipated that MP and MPD_2 supplementation would increase beneficial faecal microbial populations. However, the results indicate no effect was observed on the numbers of *Lactobacillus* spp and *Bifidobacterium* spp in pigs supplemented with MP and MPD_2 compared with control and ZnO pigs. In similar studies, *Agaricus bisporus* supplementation increased L*actobacilli* spp. and B*ifidobacteria* spp. counts in the ileum and caecum of broiler chickens (Giannenas, Tontis, et al., 2010) and turkey poults (Giannenas et al., 2011). Interestingly, as no difference was observed in faecal microbiota when pigs were supplemented with MP and MPD_2 compared with control pigs, total faecal VFA and acetate concentration were increased in pigs supplemented with MP. In a similar study, *Pleurotus ostreatus* supplementation at a rate of 5 g/kg and 10 g/kg to post-weaned pigs increased total VFA and acetate production (Adams et al., 2019). The supplementation of MPD_2 caused a reduction in total VFA and acetate production compared to MP supplementation suggesting that treating mushrooms with ultraviolet light had a negative effect on faecal VFA production.

In the present study, whole mushroom powder was supplemented to the diet of post-weaned pigs, resulting in a reduction in feed intake. As a result, the functional dosage of β-glucans may not have been achieved in the current study due to the negative effect on feed intake. Mushrooms contain many anti-nutrients such as tannins, alkaloids, phytates and saponins (Majesty et al., 2019) attributing to the reduction in feed intake. The removal of these anti-nutrients, through the extraction of the glucans, may potentially remove the negative effects on feed intake associated with the supplementation of whole mushroom powders. Glucans are commonly extracted from various sources (Garcia-Vaquero et al., 2019; Maheshwari et al., 2017) and supplemented to the diet of post-weaned pigs, resulting in enhanced pig performance and gastrointestinal health (Rattigan et al., 2019).

5 | CONCLUSION

In conclusion, the results presented in this study reveal that dietary supplementation with mushroom powder or vitamin D_2-enriched mushroom powder caused similar post-weaned pig performance compared with the control but did not give results similar to ZnO, mainly due to a reduction in feed intake. Treating mushrooms with ultraviolet light had no additional benefits. Further investigation is necessary to elucidate why whole mushroom powder supplementation negatively affects feed intake.

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CONFLICT OF INTEREST

None of the authors had a financial or personal conflict of interest in relation to the present study.

AUTHOR CONTRIBUTION

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; A.D., S.M. and G.R. contributed to sample collection and laboratory analyses. S.Y., J.W. and W.G. manufactured the mushroom powders. All authors approved the final version of the manuscript.

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.

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SUPPORTING INFORMATION

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