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# The effects of dietary supplementation with mushroom or selenium enriched mushroom powders on the growth performance and intestinal health of post-weaned pigs

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## Abstract

# Background

There is an urgent need to identify natural bioactive compounds that can enhance gastrointestinal health and promote pig growth performance in the absence of in-feed medications. The objectives of this study were to: 1) compare the effects of mushroom powder supplemented with inorganic selenium (inSeMP) to mushroom powder enriched with organic selenium (orgSeMP) to pharmacological levels of zinc oxide (ZnO) on growth performance and faecal scores for the first 21 days post-weaning (Period 1); and 2) compare the molecular and microbial effects of inSeMP and orgSeMP in these pigs on day 39 post-weaning (Period 2).

# Methods

In Period 1, pigs (3 pigs/pen; 8 pens/treatment) were assigned to: (1) basal diet (control); (2) basal diet + zinc oxide (ZnO) (3100 mg/kg feed); (3) basal diet + mushroom powder supplemented with inorganic selenium (inSeMP) containing selenium (selenite) content of 0.3 mg/kg feed; (4) basal diet + mushroom powder enriched with organic selenium (orgSeMP) containing selenium (selenocysteine) content of 0.3 mg/kg feed. The inorganic selenium was in the form of sodium selenite and the organic selenium was in the form of selenocysteine. Mushroom powders were included at 6.5 g/kg of feed and contained a β-glucan content of 650 mg/kg.

# Results

In Period 1, there was no effect of diets on average daily gain (ADG) and gain:feed (G:F) ratio. The orgSeMP supplemented pigs had a lower average daily feed intake (ADFI) compared to all other groups. The ZnO supplemented pigs had reduced ($P < 0.05$) faecal scores compared to the basal and mushroom group, while the orgSeMP supplemented pigs had lower faecal scores compared to the basal group during the 21-day experimental period. In Period 2, there was no effect of diets on ADFI, ADG and G:F ratio. The orgSeMP supplementation increased the caecal abundance of bacterial members of the Firmicutes and Bacteroidetes phylum, including *Lactobacillus, Agathobacter, Roseburia* and *Prevotella* and decreased the abundance of *Sporobacter* compared to the basal group, while inSeMP increased the caecal abundance of *Prevotella* and decreased the caecal abundance of *Sporobacter*. Dietary supplementation with inSeMP increased expression of *TLR4* and anti-inflammatory cytokine gene *IL10* ($P < 0.05$) and decreased nutrient transporter gene *FABP2* ($P < 0.05$) compared to the orgSeMP group.

# Conclusion

OrgSeMP has potential, as a dietary supplement, to improve gastrointestinal health in pigs through improved faecal scores and modulation of the gastrointestinal microbiota.

# Background

In commercial pig production systems, weaning involves complex dietary, social, and environmental stressors which cause a transient reduction in feed intake [1]. This contributes to adverse gut morphological and functional changes which leads to epithelial permeability and upregulation of proinflammatory cytokines [2, 3]. As a result, the digestive and absorptive capacity of the small intestine is impaired, with consequent lower nutrient absorption and reduced energy availability [4]. Intestinal inflammation, mediated by the upregulation of proinflammatory cytokines, promotes the intestinal proliferation of pathogenic bacteria like *Escherichia Coli* leading to gut dysbiosis and post-weaning diarrhoea (PWD)[5].

Dietary supplementation with zinc oxide (ZnO) at pharmacological levels (2,000 to 3,000 mg/kg) during the immediate post-weaning period is an industry-wide practice to alleviate the negative impact of weaning on pig performance and gastrointestinal functionality and health [2, 5]. However, from June 2022, pharmacological doses of ZnO will no longer be authorized in the European Union (Commission Implementing Decision of 26.6.2017, C(2017) 4, 529 Final). Thus, novel nutritional strategies are urgently required to support growth, intestinal function and prevent diarrhoea in the weaned pig. β-glucans and selenium are interesting bioactives for use in post-weaning pig diets due to their immunomodulatory, prebiotic and antioxidant properties.

β-glucans with β-(1 → 3, 1 → 6) linked side chains represent major component of fungal and yeast cell walls [6] and have well recognized anti-inflammatory, antioxidant and immunomodulatory properties [7–9]. Beta-glucans are non-digestible polysaccharides and therefore have the potential to modulate the gastrointestinal microbiota of pigs. Yeast β-glucans in the diet of weaned pigs can increase faecal *Lactobacillus* populations [10] and decrease faecal *E. coli* numbers [11].

Selenium is an essential trace nutrient and has an integral role in promoting immune function, growth performance and meat quality [12, 13]. Selenium has also demonstrated bacterial-modulating activities, including increased *Lactobacilli* spp. and decreased *E. coli* spp. counts in the caecum of broilers [14] and faeces of pigs [15]. Dietary supplementation with selenium enriched yeast for 21 days post-weaning improved pig growth performance and reduced the production of cytokines associated with inflammation, including TNF-α and IL-6, in the liver and thymus of pigs exposed to oxidative stress [16].

Selenium occurs in both inorganic and organic forms [17]. Inorganic selenium is mainly used in the form of sodium selenite and is the most widely used selenium supplement in animal diets. However, replacing inorganic selenium in animal diets with an organic form has received considerable interest in recent years as organic sources of selenium exhibit lower toxicity and higher bioavailability in animals compared to inorganic sources [18]. While the National Research Council recommends 0.15–0.30 mg/kg of added selenium in weaned pig diets, the total maximum level of dietary selenium in swine diets is 0.5 mg/kg

[19]. Clinical selenium deficiency is a rare occurrence in commercial pig production, however sub-clinical selenium deficiency in young animals is more common and may be responsible for decreased pig health and performance [20]. As the inclusion levels of selenium in pig diets are bound by legal standards, incorporating selenium sources with high bioavailability, such as organic selenium, into pig diets may be an effective method of increasing selenium uptake in pigs.

The utilization of mushrooms as novel feed additives in animal diets is gaining considerable interest in recent years. Mushrooms are a rich natural source of bioactive compounds, such as phenolics, lectins, terpenoids, ergosterols and β-glucans [21, 22] and may offer a unique opportunity to incorporate organic selenium into the diet of pigs. Mushrooms are irrigated with sodium selenite solution as a method of selenium enrichment [23]. Sodium selenite, the inorganic form of selenium, is taken up by mushrooms through phosphate transporters and reduced to selenide, before being converted to selenocysteine, the organic form of selenium [24]. Thus, the first objective of this study was to compare the effects of mushroom powder supplemented with inorganic selenium (inSeMP), mushroom powder enriched with organic selenium (orgSeMP) and pharmacological levels of ZnO on growth performance of pigs during the first 21 days post-weaning (Period 1). The second objective of this study was to compare the molecular and microbial effects of inSeMP and orgSeMP supplementation in these pigs on day 39 post-weaning (Period 2).

# Materials And Methods

### Period 1: The effects of inSeMP and orgSeMP supplementation on pig growth performance and faecal scores post-weaning (day 0-21)

### Experimental design and diets

At weaning (28 days), 96 pigs (progeny of Meatline boars × (Large White × Landrace sows)) with an average weight of 6.8 kg (SD 0.86) were selected from a commercial pig farm. The pigs were blocked by weaning weight, sex and litter of origin and within each block assigned to one of four dietary groups. The diets were as follows: (1) basal diet (control); (2) basal diet + zinc oxide (ZnO) (3100 mg/kg feed); (3) basal diet + mushroom powder supplemented with inorganic selenium (inSeMP) containing selenium content of 0.3 mg/kg feed; (4) basal diet + mushroom powder enriched with organic selenium (orgSeMP) containing selenium content of 0.3 mg/kg feed. The inorganic selenium was in the form of sodium selenite and the organic selenium was in the form of selenocysteine. Mushroom powders were included at 6.5 g/kg of feed and contained a β-glucan content of 650 mg/kg. The selenium mushroom powder contained an organic selenium concentration of 45.8 mg/kg mushroom powder and was included at 6.5 g/kg to achieve the selenium content of 0.3 mg/kg feed. Mushrooms were irrigated with sodium selenite solution at intermittent cycles as a method of selenium enrichment (23). All other diets contained a selenium (selenite) content of 0.3 mg/kg feed. The ZnO was sourced from Cargill (Naas, Kildare, Ireland). The ZnO was included at 3100 mg ZnO/kg feed and contained 80% zinc, resulting in an inclusion level of 2500 mg Zn/kg feed. The dried mushroom powder (*Agaricus bisporus*) was sourced from Monaghan

Mushrooms, Ireland. The mushroom powder contained 305 g/kg of crude protein, 34 g/kg of total fat, 2.34 mg/kg selenium, and 100 mg/g of β-glucan. The diets were formulated to have equivalent nett energy (10.6 MJ/kg), crude protein (208 g/kg) and standardised ileal digestible lysine (13.0 g/kg). All amino acid requirements were calculated relative to lysine [25] . The composition of diets are presented in Table 1.

### Housing and animal management

The pigs were penned in groups of three according to weight and sex and housed on fully slatted floors (1.68 m × 1.2 m). There were 8 replicate pens used per treatment with 3 pigs in each replicate pen. For the first 7 days, the temperature within the weaner house was controlled at 30 ºC and then reduced by 2 ºC per week until the temperature reached 26 ºC. The humidity was maintained at 65%. The experimental diets were given in meal form and pigs had ad libitum access to these diets immediately after weaning up to the final weighing. Clean drinking water was available ad libitum from a drinking nipple. Pigs received no medication throughout the experiment. Body weight (BW) was measured using a portable electronic scale (Prattley, Temuka, New Zealand) on days 1, 7, 14 and 21 and average daily gain (ADG) and average daily feed intake (ADFI) were calculated. Faecal scores were assessed twice daily for each individual pen to indicate the presence and severity of diarrhoea. The following scoring system was used to assign faecal scores: 1 = hard, 2 = slightly soft, 3 = soft, partially formed, 4 = loose, semi-liquid, 5 = watery, mucous like [27] .

### Period 2: The molecular and microbial effects of inSeMP and orgSeMPsupplementation in pig diets on day 39 post-weaning

### Experimental design and diets

At 21 days post-weaning, the ZnO group was removed from the experiment. Seventy-two pigs (average weight 12.6 kg (2.49 SD)) from (T1), (T3) and (T4) in experiment 1 proceeded to experiment 2. The pigs were kept on their original diets as described in experiment 1. The dietary treatments were as follows: (1) basal diet (control); (2) basal diet + mushroom powder supplemented with inorganic selenium (inSeMP) containing selenium content of 0.3 mg/kg feed; (3) basal diet + mushroom powder enriched with organic selenium (orgSeMP) containing selenium content of 0.3 mg/kg feed. The inorganic selenium was in the form of sodium selenite and the organic selenium was in the form of selenocysteine. Mushroom powders were included at 6.5 g/kg of feed and contained a β-glucan content of 650 mg/kg.

### Housing and animal management

The pigs were weighed at the beginning (day 21) and end (day 39) of Period 2. The housing and animal management were as described in Period 1.

### Feed analysis

All the feed samples were milled through a 1 mm screen (Christy and Norris Hammer Mill, Chelmsford, England) and kept for chemical analysis. The gross energy (GE) content was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA) as previously described [28] . The feed was dried for 72 hours at 55°C to determine the dry matter (DM) content of the feed. Feed samples were analysed for crude ash, nitrogen, crude fibre, acid detergent fiber (ADF), and ether extract according to the Association of Official Agricultural Chemists standard procedures [29], and neutral detergent fiber (NDF) was determined according to the method of Van Soest et al. [30] . The total glucans of the MP were determined using the kit K-YBGL, purchased from Megazyme (Bray, Co Wicklow, Ireland), following the manufacturer's recommendations, and as previously described [31]. The selenium content was measured by Eurofins Food Testing UK Ltd (Wolverhampton, United Kingdom) using the selenium in food method. All samples were measured in duplicate.

### Sample collection

On day 39 of the experiment, 8 pigs per treatment (one pig/pen) received a lethal injection with pentobarbitone sodium (Euthatal Solution, 200 mg/ml; Merial Animal Health, Essex, UK) at a rate of 0.71 ml/kg body weight to the cranial vena cava to humanely euthanise the animals. Sections from the duodenum, jejunum and ileum were processed for gut morphological analysis as previously described [32] . Digesta from the caecum was collected and stored in sterile containers (Sarstedt, Wexford, Ireland). This was then snap frozen on dry ice and stored at −80°C for subsequent 16s rRNA sequencing and volatile fatty acid (VFA) analysis. In addition, tissue samples were taken from the duodenum, jejunum, and ileum to measure the expression of cytokines, nutrient transporters, mucins, tight junctions, and appetite regulators using quantitative real-time PCR (QPCR). Tissue sections (1cm) from the duodenum, jejunum, and ileum were cut out, dissected along the mesentery, emptied, and rinsed using sterile phosphate buffered saline (Oxoid, Hampshire, UK). The tissue sections were stripped of the overlying smooth muscle before storage in RNAlater® (5ml) solution (Applied Biosystems, Foster City, CA, USA) overnight at 4°C. The RNAlater® was removed before storing the samples at -80°C.

### Gut morphological analysis

Standard paraffin embedding techniques were used to prepare the small intestinal tissue for gut morphological analysis, as previously described [32] . A light microscope with an image analyzer (Image-Pro Plus; Media Cybernetics, Oxon, UK) was used to measure the villus height and crypt depth. Fifteen measurements of villi and crypt were taken for each section.

### Gene expression in the small intestine

*RNA extraction and cDNA synthesis*

Total RNA was extracted from duodenal and ileal tissues using TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA) in accordance with the manufacturer's guidelines as previously described [33] . The total RNA (2 μg) was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied

Biosystems, Foster City, CA, USA) and oligo (dT) primers in a final reaction volume of 40 µL, in accordance with manufacturer's guidelines. The cDNA was then made up to a volume of 360 µL with nuclease-free water.

*Quantitative real-time polymerase chain reaction (QPCR)*

The qPCR reaction mixture (20 µl) consisted of GoTaq qPCR Master Mix (10 µl) (Promega, Madison, WI, USA), forward and reverse primers (5 µM), (1.2 µl), nuclease-free water (3.8 µl) and cDNA (5 µl). All the QPCR reactions were carried out in duplicate on the 7500 ABI Prism Sequence detection System (Applied Biosystems, Foster City, CA, USA). The cycling conditions consisted of a denaturation step of 95°C for 10 minutes which was followed by 40 cycles of 95°C for 15 seconds and then 60°C for 1 minute. All the primers were designed using the Primer Express Software (Applied Biosystems, Foster City, CA, USA) and made by MWG Biotech UK Ltd (Milton Keynes, UK) and are all described in Table 2. Dissociation curves were created to verify the specificity of the subsequent PCR products. The QPCR assay efficiencies were determined by plotting the cycling threshold (CT) values resulting from 4-fold serial dilutions of cDNA against their arbitrary quantities and only assays demonstrating 90 –110% efficiency and single products were accepted in this analysis. Normalised relative quantities were obtained using the software, qbase PLUS (Biogazelle, Ghent, Belgium) from stable reference genes; *H3F3A* and *YWHAZ* (duodenum and jejunum), *ACTB* and *H3F3A* (ileum). These genes were selected as reference genes based on their M value (<1.5) generated by the GeNorm algorithm within GeNorm. The genes analyzed in the current study are as follows: protein transporter *SLC15A1* (previously known as *PEPT1*); fatty acid transporter *FABP2*; glucose transporters *SLC2A2* (previously known as *GLUT2*) and *SLC2A5* (previously known as *GLUT5*); appetite regulator *CCK*, *NPY*, *GLP1* and *PYY*; cytokines *TNF, IL6, IL10, IFNG, TGFB1* and *IL17*; chemokine *CXCL8* (previously known as IL8); mucins *MUC2* and *MUC1*; tight junctions *CLDN3* and *CLDN1*; Toll like receptor *TLR4*; selenoproteins *DIO1*, *SELENOP* and *TXNRD1*.

## Microbial analyses

*Microbial DNA extraction*

A QIAamp PowerFecal Pro DNA Kit (Qiagen, West Sussex, United Kingdom) was used to extract microbial genomic DNA in accordance with the manufacturer's instructions. A Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to measure the quantity and quality of the DNA.

*Illumina sequencing*

Bacterial DNA was extracted from the caecal digesta samples and high-throughput sequencing of the V3 - V5 hypervariable region of the bacterial 16S rRNA gene was performed on an Illumina MiSeq platform according to their standard protocols (Eurofins Genomics, Ebersberg, Germany).

*Bioinformatic*

The bioinformatic assessment of the sequences were conducted by Eurofins Genomics (Ebersberg, Germany) using the package (version 1.9.1) Quantitative Insights into Microbial Ecology [34]. All the raw reads passing the standard Illumina chastity filter were demultiplexed in accordance with their index sequences (read quality score >30). The primer sequences were clipped from the beginning of the raw forward/reverse reads. If primer sequences did not match perfectly, read pairs were eliminated to retain only high-quality reads. Paired-end reads were then merged, to get a single, longer read that covers the complete target region using the software FLASH 2.2.00 [35]. The pairs were merged with the lowest overlap size of 10 bp to decrease false-positive merges. The forward read was only kept for the subsequent assessment steps when merging was not viable. Merged reads were quality filtered in accordance with the expected and known length variations of the V3 - V5 region (ca. 445 bp). The ends of retained forward reads were clipped to a complete read length of 285 bp to eliminate low quality bases. Merged and retained reads comprising of ambiguous bases were removed. The filtered reads were then used for profiling of the microbiome. Chimeric reads were detected and deleted based on the de-novo algorithm of UCHIME [36] as implemented in the VSEARCH package [37] . The remaining set of high-quality reads were then processed using minimum entropy decomposition (MED) to partition reads to operational taxonomic units (OTU) [38, 39]. DC-MEGABLAST alignments of cluster representative sequences to the NCBI nucleotide sequence database were carried out for the taxonomic assignment of every OTU. A sequence identity of 70% across a minimum of 80% of the representative sequence was the minimal prerequisite for considering reference sequences. Abundances of bacterial taxonomic units were normalized using lineage-specific copy numbers of the appropriate marker genes to enhance estimates [40].

The data matrix was made up of the normalized OTU table in combination with the phenotype metadata and phylogenetic tree. The data matrix was then loaded into the phyloseq package in R (http://www.r-project.org; version 3.5.0). Differential abundance analysis was carried out on tables extracted from the phyloseq object at phylum, family, genus and species level. The model assessed the effect of 'group', with the individual pig being the experimental unit. Eight pigs per group were used for the statistical analysis of the relative bacterial abundances.

### Volatile fatty acid analysis

Gas liquid chromatography was used to determine the VFA concentrations in the caecal digesta as described previously [41].  1 g of digesta was diluted with water (2.5× sample weight) and centrifuged (1400× g for 10 min) using a Sorvall GLC-2B centrifuge (DuPont, Wilmington, DE, USA). 1 mL of 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 and then centrifuged for 10 minutes at (500 × g). The supernatant was then filtered through a syringe filter (0.45polytetrafluoroethylene (TFE)) into a chromatographic sample vial. Approximately 1 µL of this mixture was injected into a Varian 3800 GC (Ontario, Canada) with an ECTM 1000 Grace column (15 m × 0.53 mm I.D) with a film thickness of 1.20 µm. The temperature program was set to the range 75 °C – 95 °C which increased by 3 °C /min and 95 °C - 200 °C

which increased by 20 °C/min, and this was held for 0.50 min. The detector temperature was 280 °C and the injector temperature was 240 °C. The total analysis time was 12.42 minutes.

### Statistical analysis

All data on growth performance, gastrointestinal morphology, gene expression, and VFA were checked for normality using the univariate procedure of Statistical Analysis Software (SAS) 9.4. The PROC MIXED procedure of SAS was used to analyse growth performance in Period 1 and 2. In Period 1, faecal scores were averaged every 3 days for the first 21 days and analysed using repeated measures analysis in the PROC MIXED procedure of SAS. The general linearized model (GLM) procedure within SAS was used to analyse the data on gastrointestinal morphology, gene expression (Bonferroni adjusted $P < 0.05$) and VFA. The model included the effect of treatment and weight at weaning as a covariate. For the growth performance data, the pen was the experimental unit, whilst for the gastrointestinal morphology, gene expression, and VFA data, the pig was the experimental unit. The microbiome data was analysed using PROC GLIMMIX. Results are presented as least-square using Benjamini–Hochberg (BH) adjusted P-values. The probability level that denoted significance was $P < 0.05$. Data are presented as least-square means and standard error of the mean.

## Results

### Period 1 (day 0-21)

### Growth performance and faecal scores

The effects of dietary supplementation on ADG, ADFI and G:F ratio to day 21 post-weaning are presented in Table 3. While the orgSeMP supplemented pigs had a lower ADFI ($P < 0.05$) compared to all other groups, there was no difference ($p > 0.05$) in ADG and G:F ratio between groups over the 21-day period.

The effects of dietary supplementation on FS from day 0 - 21 post-weaning are presented in Figure 1. There was no treatment x time interaction on FS ($P > 0.05$). Overall the ZnO supplemented pigs had reduced ($P < 0.05$) FS compared to the basal and inSeMP group during the 21-day experimental period. The orgSeMP supplemented pigs had lower faecal scores compared to the basal group.

**Table 2.** Panel of porcine oligonucleotide primers used for real-time PCR.

**Table 3.** Effect of dietary treatment on pig growth performance (day 0-21; least square means with their standard errors).

| Target gene | Accession No. | Forward primer (5'-3') | Amplicon length (bp) |
|---|---|---|---|
| | | Reverse primer (5'-3') | |
| IL6 | NM_214399.1 | F: GACAAAGCCACCACCCCTAA | 69 |
| | | R: CTCGTTCTGTGACTGCAGCTTATC | |
| CXCL8 | NM_213867.1 | F: TGCACTTACTCTTGCCAGAACTG | 82 |
| | | R: CAAACTGGCTGTTGCCTTCTT | |
| IL10 | NM_214041.1 | F: GCCTTCGGCCCAGTGAA | 71 |
| | | R: AGAGACCCGGTCAGCAACAA | |
| IL17A | NM_001005729.1 | F: CCCTGTCACTGCTGCTTCTG | 57 |
| | | R: TCATGATTCCCGCCTTCAC | |
| IFNG | NM_213948.1 | F: TCTAACCTAAGAAAGCGGAAGAGAA | 81 |
| | | R: TTGCAGGCAGGATGACAATTA | |
| TNF | NM_214022.1 | F: TGGCCCCTTGAGCATCA | 68 |
| | | R: CGGGCTTATCTGAGGTTTGAGA | |
| TGFB1 | NM_214015.1 | F: AGGGCTACCATGCCAATTTCT | 101 |
| | | R: CGGGTTGTGCTGGTTGTACA | |
| TLR4 | NM_001293317.1 | F: TGCATGGAGCTGAATTTCTACAA | 140 |
| | | R: GATAAATCCAGCACCTGCAGTTC | |
| MUC1 | XM_001926883.1 | F: ACACCCATGGGCGCTATGT | 68 |
| | | R: GCCTGCAGAAACCTGCTCAT | |
| MUC2 | XM_021082584.1 | F: CAACGGCCTCTCCTTCTCTGT | 70 |
| | | R: GCCACACTGGCCCTTTGT | |
| CLND1 | NM_001244539.1 | F: CTGGGAGGTGCCCTACTTTG | 72 |
| | | R: TGGATAGGGCCTTGGTGTTG | |
| CLND3 | NM_001160075.1 | F: GAGGGCCTGTGGATGAACTG | 65 |
| | | R: GAGTCGTACACTTTGCACTGCAT | |
| CCK | NM_214237.2 | F: GGACCCCAGCCACAGAATAA | 61 |
| | | R: GCGCCGGCCAAAATC | |
| FABP2 | NM_001031780.1 | F: CAGCCTCGCAGACGGAACTGAA | 102 |

| | | | |
|---|---|---|---|
| | | R: GTGTTCTGGGCTGTGCTCCAAGA | |
| SLC2A2/GLUT2 | NM_001097417.1 | F: CCAGGCCCCATCCCCTGGTT | 96 |
| | | R: GCGGGTCCAGTTGCTGAATGC | |
| SLC2A5/GLUT5 | XM_021095282.1 | F: CCCAGGAGCCGGTCAAG | 60 |
| | | R: TCAGCGTCGCCAAAGCA | |
| SLC5A1/SGLT1 | NM_001164021 | F: GGCTGGACGAAGTATGGTG | 153 |
| | | R: ACAACCACCCAAATCAGAGC | |
| SLC15A1/PEPT1 | NM_214347.1 | F: GGATAGCCTGTACCCCAAGCT | 73 |
| | | R: CATCCTCCACGTGCTTCTTGA | |
| NPY | NM_001256367.1 | F: CAGGCAGAGATACGGAAAACG | 71 |
| | | R: TCCGTGCCTCTCTCATCAAG | |
| GLP1 | NM_001256594.1 | F: CAGTGCAGAAATGGCGAGAA | 61 |
| | | R: GGTGGAGCCTCAGTCAGGAA | |
| PYY | XM_005668763.1 | F: CTCCTGATTCGGTTTGCAGAA | 61 |
| | | R: GGACAGGAGCAGCAGGAAGA | |
| SELENOP | NM_001134823.1 | F:CAGGCCAGCTGATACCTGTGT | 21 |
| | | R:TTAGAATATCCTTCTTTCTCCAGTTTTACTC | |
| DIO1 | NM_001001627.1 | F:GGCTCTGGGTGCTCTTTCAG | 20 |
| | | R:CAGGAAACAATGTCATGAGCACTT | |
| TXNRD1 | NM_214154.3 | F:CACCGTGACGGACTCAAAACT | 21 |
| | | R:GCTTGAGGCTGGTGACTTCAT | |
| H3F3A | NM_001014389.2 | F:CATGGCTCGTACAAAGCAGA | 136 |
| | | R:ACCAGGCCTGTAACGATGAG | |
| ACTB | AY550069.1 | F:CAAATGCTTCTAGGCGGACTGT | 75 |
| | | R:TCTCATTTTCTGCGCAAGTT | |
| YWZHAZ | XM_001927228.1 | F:GGACATCGGATACCCAAGGA | 71 |
| | | R:AAGTTGGAAGGCCGGTTAATTT | |

| | Dietary treatments[1] | | | | SEM | P Values |
|---|---|---|---|---|---|---|
| | Basal[2] | ZnO[2] | inSeMP[2] | orgSeMP[2] | | Treatment |
| Final body weight (kg) | 12.58 | 12.55 | 12.91 | 12.22 | 0.326 | 0.688 |
| ADG (kg/day) | 0.28 | 0.26 | 0.27 | 0.27 | 0.014 | 0.630 |
| ADFI (kg/day) | 0.39[b] | 0.38[b] | 0.39[b] | 0.35[a] | 0.010 | 0.002 |
| Gain:feed (kg/kg) | 0.68 | 0.62 | 0.65 | 0.72 | 0.048 | 0.488 |

ADG, average daily gain; ADFI, average daily feed intake.

[1]Treatments: (1) Basal diet; (2) basal diet + ZnO (3100 mg/kg d 1-14, 1550 mg/kg d 15-21); (3) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (4) basal diet + selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

[2]A total of 8 replicates were used per treatment group.

**Period 2 (day 39)**

**Growth performance**

The effect of dietary supplementation on ADG, ADFI, G:F ratio and final BW is presented in Table 4. Overall, there was no difference ($p > 0.05$) in ADG, ADFI, G:F ratio and final BW between groups.

**Table 4.** Effect of dietary treatment on pig growth performance (day 21-39; least square means with their standard errors).

| | Dietary treatments[1] | | | SEM | P Values |
|---|---|---|---|---|---|
| | Basal[2] | inSeMP[2] | orgSeMP[2] | | Treatment |
| Final body weight (kg) | 23.7 | 24.7 | 23.4 | 0.759 | 0.455 |
| ADG (kg/day) | 0.60 | 0.65 | 0.63 | 0.029 | 0.171 |
| ADFI (kg/day) | 0.92 | 0.87 | 0.93 | 0.027 | 0.287 |
| Gain:feed (kg/kg) | 0.67 | 0.75 | 0.69 | 0.026 | 0.058 |

ADG, average daily gain; ADFI, average daily feed intake.

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet +

selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

[2]A total of 8 replicates were used per treatment group.

### Small intestinal morphology

The effect of dietary supplementation on small intestinal morphology is presented in Table 5.

In the duodenum, there was no difference in villus height (VH), crypt depth (CD) and villus height to crypt depth ratio (VH:CD) between groups. In the jejunum, pigs supplemented with inSeMP had decreased VH and VH:CD ($P < 0.05$) compared to the orgSeMP and basal group. In the ileum, pigs supplemented with inSeMP had decreased VH ($P < 0.05$) compared to the orgSeMP and basal group.

**Table 5.** Effect of dietary supplementation on villus height and crypt depth in the small intestine (least square means with their standard errors).

| Dietary treatments[1] | | | | SEM | P-values |
|---|---|---|---|---|---|
| | Basal[2] | inSeMP[2] | orgSeMP[2] | | |
| **Duodenum** | | | | | |
| VH µm | 306.55 | 283.11 | 304.50 | 12.648 | 0.366 |
| CD µm | 136.16 | 133.48 | 140.18 | 7.611 | 0.823 |
| VH:CD | 2.31 | 2.17 | 2.19 | 0.142 | 0.778 |
| **Jejunum** | | | | | |
| VH µm | 292.49$^{b}$ | 227.79$^{a}$ | 297.99$^{b}$ | 15.567 | 0.007 |
| CD µm | 138.30 | 131.89 | 143.19 | 8.408 | 0.641 |
| VH:CD | 2.15$^{b}$ | 1.74$^{a}$ | 2.10$^{b}$ | 0.107 | 0.029 |
| **Ileum** | | | | | |
| VH µm | 267.90$^{b}$ | 216.84$^{a}$ | 265.27$^{b}$ | 15.600 | 0.053 |
| CD µm | 134.41 | 118.23 | 127.05 | 8.832 | 0.445 |
| VH:CD | 2.03 | 1.89 | 2.12 | 0.129 | 0.457 |

VH, villus height; CD, crypt depth; VH:CD, villus height to crypt depth ratio.

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet +

selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

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

[2]A total of 8 replicates were used per treatment group

**Table 6.** The effects of dietary supplementation on the expression of nutrient transporters, immune markers and tight junctions in pigs duodenum, jejunum and ileum (least square means with their standard errors)

| | Treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| | Gene | Basal[2] | inSeMP[2] | orgSeMP[2] | SEM | P-values |
| **Duodenum** | | | | | | |
| Appetite regulators | *CCK* | 1.13 | 1.15 | 1.05 | 0.221 | 0.946 |
| | *NPY* | 1.37 | 1.13 | 0.90 | 0.279 | 0.502 |
| | *PYY* | 0.97 | 0.89 | 1.27 | 0.189 | 0.331 |
| | *GLP1* | 0.67 | 0.93 | 0.65 | 0.176 | 0.483 |
| Tight junctions and immune markers | *CLDN1* | 1.37 | 1.13 | 0.90 | 0.279 | 0.502 |
| | *CLND3* | 0.81 | 1.19 | 1.50 | 0.321 | 0.352 |
| | *IL10* | 1.04[a] | 1.61[b] | 1.03[a] | 0.179 | 0.047 |
| | *IL17a* | 1.09 | 1.73 | 0.94 | 0.223 | 0.069 |
| | *IL6* | 1.33 | 1.28 | 0.88 | 0.146 | 0.154 |
| | *CXCL8* | 1.22 | 1.34 | 1.12 | 0.170 | 0.825 |
| | *INFG* | 0.93 | 1.30 | 1.13 | 0.226 | 0.682 |
| | *TLR4* | 1.15[ab] | 1.90[b] | 0.87[a] | 0.234 | 0.026 |
| | *TNFa* | 1.04 | 1.12 | 1.07 | 0.083 | 0.439 |
| | *MUC1* | 0.95 | 1.15 | 1.46 | 0.338 | 0.562 |
| | *MUC2* | 1.13 | 1.15 | 1.05 | 0.221 | 0.946 |
| | *SLC15A1* | 0.81 | 1.19 | 1.50 | 0.321 | 0.352 |
| Nutrient transporters | *FABP2* | 0.78 | 1.19 | 1.20 | 0.151 | 0.120 |
| | *SLC2A2* | 0.93 | 1.19 | 0.97 | 0.156 | 0.472 |
| | *SLC2A5* | 1.00 | 1.18 | 1.31 | 0.900 | 0.777 |
| Selenoproteins | *DIO1* | 0.97 | 0.89 | 1.27 | 0.189 | 0.331 |
| | *SelenoP* | 1.22 | 1.41 | 0.87 | 0.240 | 0.229 |
| | *TXNRD1* | 0.92 | 1.1 | 1.25 | 0.176 | 0.600 |
| **Jejunum** | | | | | | |
| Appetite regulators | *NPY* | 0.84 | 1.54 | 1.41 | 0.348 | 0.362 |
| | *PYY* | 1.09 | 1.42 | 0.72 | 0.205 | 0.123 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *GLP1* | 0.95 | 1.23 | 0.88 | 0.162 | 0.281 |
| Tight junctions and immune markers | | | | | | |
| | *CNDN1* | 0.89 | 1.25 | 1.30 | 0.232 | 0.442 |
| | *CLND3* | 1.11 | 1.24 | 0.90 | 0.159 | 0.391 |
| | *IL10* | 1.10 | 1.38 | 1.10 | 0.174 | 0.227 |
| | *IL17a* | 0.84 | 1.10 | 0.79 | 0.136 | 0.160 |
| | *IL6* | 0.89 | 1.59 | 1.54 | 0.392 | 0.480 |
| | *CXCL8* | 1.01 | 1.08 | 0.90 | 0.147 | 0.843 |
| | *INFG* | 0.78 | 1.03 | 1.15 | 0.183 | 0.564 |
| | *TLR4* | 1.31 | 0.76 | 0.96 | 0.173 | 0.107 |
| | *TNFa* | 0.93 | 0.96 | 0.99 | 0.030 | 0.404 |
| | *MUC1* | 1.25 | 1.69 | 0.56 | 0.336 | 0.126 |
| | *MUC2* | $1.34^{ab}$ | $1.49^{b}$ | $0.70^{a}$ | 0.211 | 0.038 |
| Nutrient transporters | *SLC15A1* | 1.13 | 0.73 | 1.13 | 0.153 | 0.099 |
| | *FABP2* | $1.22^{b}$ | $0.67^{a}$ | $1.13^{b}$ | 0.149 | 0.037 |
| | *SLC2A2* | 1.20 | 0.72 | 1.07 | 0.150 | 0.095 |
| | *SLC2A5* | 1.31 | 0.76 | 0.96 | 0.173 | 0.107 |
| Selenoproteins | *DIO1* | 2.03 | 0.92 | 0.95 | 0.464 | 0.190 |
| | *SelenoP* | 0.77 | 1.08 | 1.87 | 0.187 | 0.329 |
| | *TXNRD1* | 0.78 | 0.82 | 1.15 | 0.150 | 0.174 |
| **Ileum** | | | | | | |
| Appetite regulators | *NPY* | 1.21 | 1.13 | 0.86 | 0.410 | 0.196 |
| | *PYY* | 0.93 | 1.61 | 0.87 | 0.09 | 0.221 |
| | *GLP1* | 1.21 | 1.91 | 1.32 | 0.247 | 0.154 |
| Tight junctions and immune markers | *CLDN1* | 1.21 | 1.13 | 0.86 | 0.410 | 0.196 |
| | *CLDN3* | $0.84^{ab}$ | $1.38^{b}$ | $0.64^{a}$ | 0.347 | 0.008 |
| | *IL10* | 1.13 | 1.42 | 1.28 | 0.545 | 0.232 |
| | *IL17a* | 1.00 | 1.55 | 0.81 | 0.365 | 0.500 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *IL6* | 0.77 | 1.34 | 1.36 | 0.290 | 0.265 |
| | *CXCL8* | 1.30 | 1.13 | 1.07 | 0.867 | 0.248 |
| | *INFG* | 1.03 | 1.16 | 1.06 | 0.908 | 0.130 |
| | *MUC1* | 0.99 | 0.94 | 0.99 | 0.040 | 0.672 |
| | *MUC2* | 0.72 | 0.73 | 0.91 | 0.189 | 0.089 |
| | *TLR4* | 1.30 | 1.53 | 1.46 | 0.954 | 0.297 |
| | *TNFa* | 0.88 | 1.14 | 1.16 | 0.618 | 0.204 |
| Nutrient transporters | *SLC15A1* | 1.83 | 1.18 | 1.00 | 0.299 | 0.269 |
| | *FABP2* | 2.21 | 1.37 | 1.14 | 0.145 | 0.362 |
| | *SLC2A2* | 1.72 | 1.41 | 0.96 | 0.306 | 0.232 |
| | *SLC2A5* | 1.63 | 1.17 | 0.78 | 0.296 | 0.164 |
| Selenoproteins | *DIO1* | $0.35^{a}$ | $1.24^{b}$ | $0.79^{ab}$ | 0.169 | 0.011 |
| | *SelenoP* | 1.19 | 1.61 | 1.12 | 0.213 | 0.350 |

*SLC15A1/PEPT1*, peptide transporter 1; *FABP2*, fatty acid binding protein 2; *SLC2A1/GLUT1*, glucose transporter 1; *SLC5A1/SGLT1*, sodium glucose linked transporter 1; *SLC2A2/GLUT2*, glucose transporter 2; *SLC2A5/GLUT5*, glucose transporter 5; *CCK*, cholecystokinin; *TNF*, tumor necrosis factor alpha*; CXCL8*, interleukin 8; *IL6*, interleukin 6; *IL10*, interleukin 10; *IFNG*, interferon gamma; *ZO1*, zonulin; *MUC2*, mucin 2; TGF-β, transforming growth factor beta*; IL17*, interleukin 17; *TLR4*, toll like receptor 4; *CLDN3*, claudin 3; *CLDN1*, claudin 1; *MUC1*, mucin 1; DIO1, Deiodinase Type 1; SelenoP, Selenoprotein P; TXNRD1, thioredoxin reductase 1.

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet + selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

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

[2]A total of 8 replicates were used per treatment.

### Gene expression in the small intestine

The effects of inSeMP and orgSeMP supplementation on the intestinal expression of genes related to nutrient digestion and absorption, mucosal barrier function, immunity and appetite regulation in the duodenum, jejunum and ileum are presented in Table 6.

In the duodenum, orgSeMP supplementation decreased the expression of *TLR4* (Toll like receptor 4, P < 0.05) compared to the inSeMP group. The inSeMP supplementation increased the expression of *IL10* (interleukin 10, P < 0.05) compared to all other groups.

In the jejunum, inSeMP supplementation decreased the expression of *CCK* (cholecystokinin, P < 0.05) and *FABP2* (fatty acid binding protein 2, P < 0.05) compared to all other groups. The orgSeMP supplementation decreased the expression of *MUC2 (*Mucin 2, P < 0.05) compared to the inSeMP group.

In the ileum, orgSeMP reduced the expression of *CLDN3* (claudin 3, P < 0.05) compared to the inSeMP group. The inSeMP supplementation increased the expression of *DIO1* (Thyroxine deiodinase type 1, P < 0.05) compared to the basal group.

## Effects of mushroom powder supplementation on the caecal microbiota

*Differential bacterial abundance analysis*

All data on bacterial abundances at phylum, family and genus level are presented in Table 7, 8 and 9. There were five bacterial phyla identified with Firmicutes being the dominant phyla (~79.6%) followed by Bacteriodetes (~14.64%), Proteobacteria (~1.34%) and Actinobacteria (~1.05%). The inSeMP and orgSeMP increased the relative abundance of Bacteriodetes compared to the basal group (P < 0.05).

At family level, dietary supplementation with orgSeMP increased the relative abundance of *Lactobacillaceae* and decreased the relative abundance of *Ruminococcaceae* within the Firmicutes phylum compared to the basal group (P < 0.05).  Dietary supplementation with orgSeMP and inSeMP increased the relative abundance of *Prevotellaceae* within the Bacteroidetes phylum.

At the genus level, dietary supplementation with inSeMP and orgSeMP increased the relative abundance of *Prevotella* within the family *Prevotellaceae* (P < 0.05) and decreased (P < 0.05) the relative abundance of *Sporobacter* and *Ruminococcus* within the family *Ruminococcaceae* compared to the basal group. Dietary supplementation with orgSeMP, increased the relative abundance of *Lactobacillus* within the family *Lactobacillaceae*, and *Agathobacter* within the family *Lachnospiraceae* compared to the basal group (P < 0.05). Supplementation with orgSeMP increased the relative abundance of *Roseburia* within the family *Lachnospiraceae* compared to the inSeMP group (P < 0.05).

**Table 7.** The effect of dietary treatment on the % bacterial abundance at phylum level (Least-square means with their standard errors).

| Phylum | Treatments[1] | | | | |
| --- | --- | --- | --- | --- | --- |
| | Basal[2] | inSeMP[2] | orgSeMP[2] | SEM | P-values |
| Bacteroidetes | 8.87[a] | 16.91[b] | 18.14[b] | 1.374 | 0.0002 |
| Firmicutes | 83.73 | 77.43 | 77.64 | 3.142 | 0.298 |
| Actinobacteria | 0.48 | 1.26 | 1.43 | 0.399 | 0.192 |
| Proteobacteria | 1.05 | 1.61 | 1.34 | 0.406 | 0.634 |

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet + selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

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

[2]A total of 8 replicates were used per treatment

**Table 8.** The effect of dietary treatment on the % bacterial abundance at family level (Least-square means with their standard errors).

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Family | Basal[2] | inSeMP[2] | orgSeMP[2] | SEM | P-values |
| Prevotellaceae | 8.89$^{a}$ | 17.83$^{b}$ | 18.71$^{b}$ | 1.3587 | <.0001 |
| Selenomonadaceae | 0.09 | 0.40 | 0.44 | 0.188 | 0.467 |
| Clostridiaceae | 10.71 | 9.23 | 8.46 | 1.092 | 0.352 |
| Lachnospiraceae | 18.32 | 18.89 | 22.90 | 1.584 | 0.108 |
| Hungateiclostridiaceae | 1.06 | 0.66 | 0.64 | 0.334 | 0.584 |
| Streptococcaceae | 0.24 | 1.06 | 1.54 | 0.315 | 0.079 |
| Lactobacillaceae | 1.23$^{a}$ | 1.74$^{a}$ | 3.29$^{b}$ | 0.502 | 0.031 |
| Veillonellaceae | 0.26 | 1.07 | 1.08 | 0.264 | 0.180 |
| Ruminococcaceae | 44.78$^{b}$ | 37.50$^{ab}$ | 34.81$^{a}$ | 2.165 | 0.013 |
| Acidaminococcaceae | 0.98 | 1.06 | 0.78 | 0.349 | 0.839 |
| Muribaculaceae | 0.48 | 0.25 | 0.54 | 0.232 | 0.668 |
| Erysipelotrichaceae | 1.20 | 0.86 | 0.62 | 0.325 | 0.490 |
| Oscillospiraceae | 0.73 | 0.53 | 0.27 | 0.259 | 0.473 |

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet + selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

$^{a\text{-}c}$Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

[2]A total of 8 replicates were used per treatment

**Table 9.** The effect of dietary treatment on the % bacterial abundance at genus level (Least-square means with their standard errors).

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Genus | Basal[2] | inSeMP[2] | orgSeMP[2] | SEM | P-values |
| Blautia | 2.30 | 1.69 | 2.33 | 0.516 | 0.622 |
| Prevotella | 4.38[a] | 10.77[b] | 13.49[b] | 1.111 | <.0001 |
| Clostridium | 10.28 | 8.62 | 7.85 | 1.060 | 0.275 |
| Dorea | 1.24 | 2.56 | 1.82 | 0.471 | 0.191 |
| Coprococcus | 2.47 | 2.31 | 1.38 | 0.538 | 0.288 |
| Oscillibacter | 0.59 | 0.51 | 0.21 | 0.203 | 0.514 |
| Anaerobacterium | 0.90 | 0.66 | 0.61 | 0.321 | 0.772 |
| Streptococcus | 0.25 | 1.07 | 1.56 | 0.959 | 0.075 |
| Lactobacillus | 1.25[a] | 1.75[ab] | 3.33[b] | 0.505 | 0.029 |
| Fournierella | 1.24 | 1.84 | 2.14 | 0.426 | 0.162 |
| Anaerobium | 0.96 | 0.61 | 0.57 | 0.266 | 0.399 |
| Mediterraneibacter | 0.91 | 0.66 | 0.63 | 0.285 | 0.734 |
| Eubacterium | 2.56 | 2.17 | 1.44 | 0.534 | 0.198 |
| Lachnobacterium | 0.48 | 0.31 | 0.41 | 0.248 | 0.534 |
| Dialister | 0.26 | 0.79 | 0.63 | 0.218 | 0.240 |
| Butyricicoccus | 0.38 | 0.58 | 0.65 | 0.265 | 0.865 |
| Gemmiger | 9.4 | 10.67 | 8.22 | 1.034 | 0.302 |
| Agathobacter | 4.82[a] | 6.93[ab] | 9.05[b] | 0.871 | 0.016 |
| Faecalibacterium | 15.77 | 16.03 | 17.75 | 1.416 | 0.580 |
| Phascolarctobacterium | 0.97 | 0.97 | 0.78 | 0.343 | 0.942 |
| Kineothrix | 1.09 | 0.28 | 0.41 | 0.323 | 0.129 |
| Roseburia | 2.81[ab] | 1.81[a] | 4.40[b] | 0.605 | 0.027 |
| Prevotellamassilia | 2.33 | 4.16 | 4.05 | 0.607 | 0.122 |
| Sporobacter | 10.31[b] | 4.96[a] | 3.87[a] | 0.949 | 0.0001 |
| Ruminococcus | 5.88[b] | 2.03[a] | 2.50[a] | 0.668 | 0.001 |
| Intestinimonas | 0.39 | 0.31 | 0.14 | 0.224 | 0.146 |

| Alloprevotella | 2.22 | 3.02 | 1.36 | 0.568 | 0.115 |
|---|---|---|---|---|---|

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet + selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

[a-c]Mean values within a row with unlike superscript letters were significantly different (P < 0.05)

[2]A total of 8 replicates were used per treatment

**Volatile fatty acids**

The effects of dietary supplementation on the total concentrations of caecal VFA are presented in Table 10. Supplementation with inSeMP and orgSeMP decreased (P<0.05) the concentration of branched-chain volatile fatty acids (BCVFA) and the concentration of isovalerate compared to the basal group.

**Table 10.** The effects of dietary supplementation on total concentrations of volatile fatty acids (VFA) in the caecum

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| | Basal[2] | inSeMP[2] | orgSeMP[2] | SEM | P -values |
| VFA (mmol/L digesta) | | | | | |
| Total | 176.79 | 180.01 | 175.87 | 17.439 | 0.985 |
| Acetate | 126.24 | 129.21 | 123.51 | 13.835 | 0.959 |
| Propionate | 32.23 | 33.91 | 34.00 | 2.732 | 0.876 |
| Isobutyrate | 0.69 | 0.51 | 0.50 | 0.098 | 0.333 |
| Butyate | 14.62 | 14.36 | 16.03 | 2.140 | 0.839 |
| Isovalerate | 1.27[b] | 0.79[a] | 0.64[a] | 0.124 | 0.005 |
| Valerate | 1.75 | 1.23 | 1.19 | 0.192 | 0.098 |
| Acetpropionate | 3.92 | 3.77 | 3.62 | 0.231 | 0.666 |
| Branched-chain VFA | 3.71[b] | 2.53[a] | 2.33[a] | 0.331 | 0.016 |

[1]Treatments: (1) Basal diet; (2) basal diet + unenriched mushroom powder containing an inorganic selenium (selenite) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed; (3) basal diet + selenium enriched mushroom powder containing an organic selenium (selenocysteine) content of 0.3 mg/kg feed and a β-glucan content of 650 mg/kg feed.

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

[2]A total of 8 replicates were used per treatment (experimental unit = pig).

## Discussion

In the present study, it was hypothesised that mushroom powder enriched with organic selenium (orgSeMP) would be more effective than mushroom powder supplemented with inorganic selenium (inSeMP) at enhancing gastrointestinal health parameters and modulating the caecal microbiota of post-weaned pigs. Supplementation with orgSeMP altered the pigs caecal microbiota by increasing the relative abundance of bacterial members of the Firmicutes and Bacteroidetes phylum,including *Lactobacillus, Agathobacter, Roseburia* and *Prevotella* and decreasing the abundance of *Sporobacter,* while inSeMP supplementation increased the caecal abundance of *Prevotella* and decreased the caecal abundance of *Sporobacter.* Although supplementation with both inSeMP and orgSeMP decreased BCVFA concentrations, only the orgSeMP supplemented pigs had improved faecal scores compared to the basal group. The findings from this study indicate that orgSeMP supplementation was superior to inSeMP in supporting pig health through improving faecal scores and promoting the development of a healthier microbiome composition in the caecum.

Maintaining adequate feed intake in the immediate post-weaning period is considered to be of critical importance to improve pig growth performance. However, growth performance was unaffected by the observed drop in feed intake in Period 1 and 2 of this study. The effects of mushroom powders on feed intake are consistent with a previous study investigating the effects of mushroom and vitamin D enriched mushrooms on the growth performance of pigs [42] . While the exact mechanisms regulating feed intake are highly complex, it is possible that the reduced feed intake in the current study is attributed to the wide array of bioactive compounds present in mushrooms that may influence feed intake through a satiety effect. Mushrooms contain non-digestible polysaccharides, including chitin [43], which have been associated with increased satiety. Dietary chitosan, the deacetylated form of chitin, influences feed intake and the expression of associated adipokines and genes in pigs [44]. The gastrointestinal tract plays a key role in the regulation of food intake through the release of food intake-regulatory peptides such as PYY and GLP1, which induces satiety and reduces food intake [45-47] . Supplementation with inSeMP had a numerical tendency to increase the gene expression of *PYY* and *GLP1*, indicating a potential satiety effect of mushroom powder. The gut hormone PYY is often co-expressed and secreted with GLP-1; these play a role in suppressing appetite [48]. Indeed, further research is warranted to investigate the satiety effects of mushroom powders.

Faecal scoring is an established method of evaluating the gut health of newly weaned pigs. Faecal scores remained within a healthy range throughout the duration of this study, which is likely attributed to the pristine condition of research farms compared to commercial farms. Nevertheless, orgSeMP enhanced faecal scores comparable to ZnO. These healthier faecal scores were concurrent with a decrease in the concentration of caecal BCVFA. The inSeMP supplementation also reduced caecal BCVFA

concentrations compared to the basal group but did not have the same effects as orgSeMP on faecal scores, suggesting the improvement in faecal scores may be attributed to the effects of organic selenium. Branched chain volatile fatty acids are toxic metabolites associated with PWD and poor growth performance in pigs [49] . The reduced BCVFA in both mushroom groups are likely attributed to the β-glucan content of the mushrooms as in previous studies, pigs fed high fibre diets had reduced caecal [50] and colonic [51] BCVFA concentrations. The improved faecal scores in association with lower concentrations of BCVFA in the caecum, suggest that these orgSeMP-supplemented pigs had a healthier digestive tract compared to the basal group.

The intestinal microbiota of pigs may be positively influenced via dietary interventions to improve animal productivity and health and to inhibit the activity of pathogenic bacteria. In the current study, supplementation with orgSeMP was superior to inSeMP in terms of modulating the caecal microbiota, which may be attributed to the source of selenium, as organic selenium is less toxic and more bioavailable for the animal [52]. The orgSeMP supplementation altered the gut microbiota of the pigs by increasing the population of potentially beneficial bacteria, within the phylum Firmicutes, including *Lactobacillus, Roseburia*, and *Agathobacter* compared to the basal group. Bacterial members of the genus *Lactobacillus* can enhance host gastrointestinal health through the competitive exclusion of pathogenic bacteria, producing antimicrobial peptides and enhancing immune function [53, 54]. In broilers, dietary supplementation with bacterial organic selenium was associated with increased caecal *Lactobacilli* spp. counts when compared to diets with inorganic selenium [15], further supporting the advantageous effects of organic selenium over inorganic selenium on the gut microbiota. *Roseburia* and *Agathobacter* are beneficial gut bacteria that produce SCFAs, particularly butyrate [55, 56]. Thus, it may be anticipated that butyrate levels would be increased in the orgSeMP group, however caecal butyrate levels were unaffected in this study and unfortunately colonic butyrate levels were not measured in this study.

Mushroom powder and orgSeMP supplementation increased the abundance of the phylum Bacteroidetes, including members *Prevotella* and decreased the abundance of the genera *Ruminococcus* and *Sporobacter,* within the phylum *Firmicutes. Sporobacter* is increased in pigs challenged with F4+ ETEC and may have a detrimental impact on gut health [57]. Both *Prevotella* and *Ruminococcus* are involved in the degradation of complex plant carbohydrates, thus their abundance usually increases in plant rich diets [58]. The reduced abundance of *Ruminococcus* in relation to *Prevotella* is an interesting finding. This may reflect differences between *Ruminococcus* and *Prevotella* in their utilization of carbohydrate substrates and that *Prevotella* in these pigs were primed towards mushroom β-glucan degradation. The increase in *Prevotella* in response to beta-glucan supplementation is in agreement with previous studies investigating the microbial effects of yeast cell wall [59] and cereal β-glucans [60, 61].

Gastrointestinal homeostasis is of utmost importance to the health of the weaned pig and disruption to this gives rise to intestinal inflammation. In the present study, inSeMP supplementation increased the gene expression of *TLR4* and had a tendency to increase the gene expression of *IL17* compared to the

orgSeMP group. TLR4 is a pathogen recognition receptor and activation of TLR4 can lead to the production of proinflammatory cytokines, including IL-17, which has implications in the pathogenesis of chronic disease [62]. It is well known that increased expression of inflammatory cytokines compromise epithelial barrier function [63]. In the current study, the reduced villus height in the jejunum and ileum of inSeMP supplemented pigs, alongside the increased expression of *CLDN3* compared to the orgSeMP group, may be indicative of disruption of epithelial barrier integrity. Furthermore, inSeMP supplemented pigs had increased gene expression of *IL10*. IL-10 is an anti-inflammatory cytokine which is elevated in patients at early stages of infection, preceding elevations in pro-inflammatory cytokines [64]. As β-glucans are immunostimulatory compounds and initiate the inflammation process, it is possible that feeding beta-glucans at high inclusion levels (650 ppm) may over activate the immune response, as observed in inSeMP supplemented pigs. In previous studies, the recommended inclusion level of β-glucans in nursery and post-weaned pig diets was reported to be between 250 ppm and 500 ppm, and feeding β-glucan levels above this may have a negative effect on the growth performance of pigs which may be attributed to enhanced immune activation [65, 66]. It is worthy to note that supplementation with orgSeMP had a tendency to decrease expression of proinflammatory cytokine gene *IL6*; indicating a potential immunomodulatory effect of orgSeMP. Supplementation with orgSeMP attenuated the inflammatory response, and this is likely due to the source of selenium. These findings suggest that orgSeMP supplementation may be more effective at maintaining immune homeostasis compared to inSeMP supplementation.

# Conclusion

Mushroom powder supplemented with inorganic selenium (inSeMP) had a positive effect on caecal BCVFA concentrations and the microbial population of pigs, represented by a higher abundance of *Prevotella* from the phylum Bacteroidetes and decreased abundance of *Sporobacter* from the phylum Firmicutes. However, mushroom powder enriched with organic selenium (orgSeMP) showed greater potential than inSeMP at enhancing intestinal health. Supplementation with orgSeMP improved faecal scores, caecal BCVFA concentrations and the microbial population of pigs, represented by a higher abundance of several bacterial members of the Firmicutes and Bacteroidetes phyla, including *Lactobacillus*, *Agathobacter*, *Roseburia* and *Prevotella* and decreasing the abundance of *Sporobacter* from the phylum Firmicutes. Furthermore, supplementation with orgSeMP was more effective at maintaining immune homeostasis compared to inSeMP supplementation. These beneficial effects suggest that orgSeMP may have an important role in supporting the intestinal health of post-weaning pigs.

# Abbreviations

ADFI, average daily feed intake; ADG, average daily gain; β-glucan, beta-glucan; BCVFA, branched-chain volatile fatty acids; BW, body weight; CCK, cholecystokinin; CD, crypt depth; CLDN1, claudin 1; CLDN3, claudin 3; CP, crude protein; CXCL8/IL8, interleukin 8; DM, dry matter; FABP2, fatty acid binding protein 2;

G:F, gain to feed ratio; GCN, gene copy number; Ct, threshold cycle; GE, gross energy; GLM, general linearized model; IFNG, interferon gamma; IL10, interleukin 10; IL17, interleukin 17; IL6, interleukin 6; inSeMP, mushroom powder supplemented with inorganic selenium; MUC1, mucin 1; MUC2, mucin 2; NDF, neutral detergent fibre; orgSeMP, mushroom powder enriched with organic selenium; PW, post-weaning; QPCR, quantitative real-time PCR; Se, selenium; SLC15A1/PEPT1, peptide transporter 1; SLC2A2/GLUT2, glucose transporter 2; SLC2A5/GLUT5, glucose transporter 5; SLC5A1/SGLT1, sodium glucose linked transporter 1; TGFB1, transforming growth factor beta 1; TLR4, toll like receptor 4; TNF, tumor necrosis factor; VFA, volatile fatty acid; VH, villous height; ZnO, zinc oxide.

# Declarations

### Ethics Statement

In this study, all procedures were approved under the University College Dublin Animal Research Ethics Committee, Ireland (AREC-20-22-O'Doherty) and were conducted in accordance with Irish legislation (SI no. 543/2012) and the EU directive 2010/63/EU for animal experimentation.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated and/or analysed during this study are available from the corresponding author upon reasonable request.

### Competing Interest

The co-authors (S.Y. and J.W.) are employees of a company that partially financed the project and participated in the design of the study, in the analysis of mushroom supplements, and in the final review of the manuscript. The other authors have no competing interests.

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### Author Contribution

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

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# Tables

Table 1 is not available with this version

# Figures

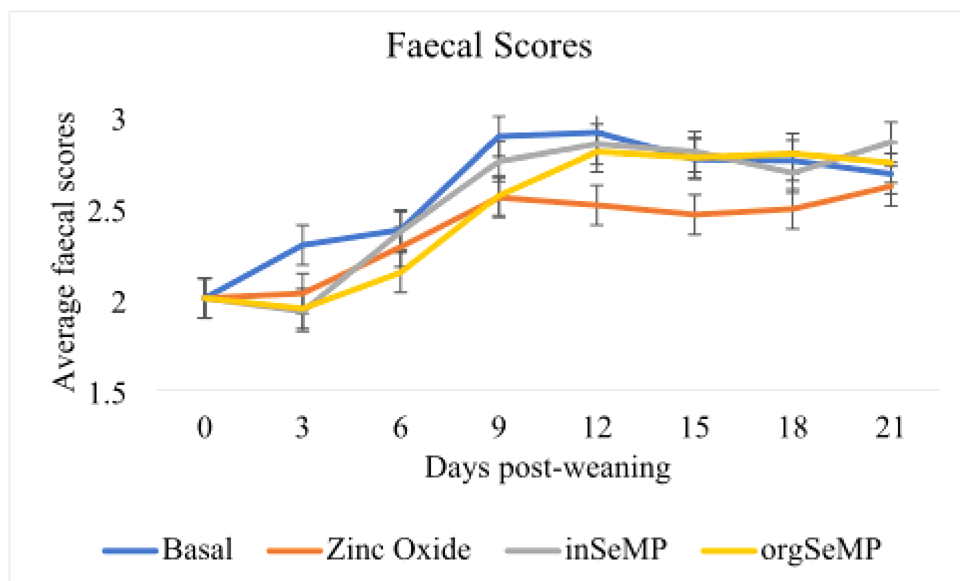


**Figure 1**

Effect of dietary treatment on faecal scores from day 0-21 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. A total of 8 replicates were used per treatment (replicate = pen, 3 pigs/pen). Treatment ($P < 0.001$), Time ($P < 0.001$), Treatment x Time ($P > 0.05$).