Animal Feed Science and Technology 316 (2024) 116078

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Organic acid preservation of cereal grains improves grain quality, growth performance, and intestinal health of post-weaned pigs

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ARTICLE INFO

Keywords:
Cereal preservation
Piglets
Nutrient digestibility
Microbiota
Organic acids
Zinc oxide

ABSTRACT

This study investigated the effect of preserving wheat and barley grains with an organic acid liquid surfactant mould inhibitor compared to conventional artificial drying and assessed its effects on the health and performance of pigs post-weaning (PW). A 2 × 2 factorial arrangement was conducted to investigate the interaction between grain preservation method (dried vs. preserved) and zinc oxide (ZnO) inclusion (yes vs. no) on growth performance, diarrhoea scores and incidence, and total tract nutrient digestibility. One hundred and ninety-two pigs (3 pigs/pen; 16 replicates/treatment) were assigned to one of four experimental diets for 35 days: (1) dried grain diet, (2) preserved grain diet, (3) dried grain diet with ZnO and (4) preserved grain diet with ZnO. Diets were formulated to contain similar levels of net energy and standardised ileal digestible lysine. On day 35 PW, 24 pigs (12 replicates/treatment) from the non-ZnO supplemented groups were euthanised and digesta was collected for coefficient of apparent ileal digestibility (CAID), gut microbial population and volatile fatty acid analysis. Stomach tissue was also collected for gene expression analysis, as well as small intestine samples for gut morphology. Notable improvements in grain quality were observed in the preserved grain, including a reduction in grain pH, mould presence, and contamination by mycotoxins, specifically deoxynivalenol in wheat and barley, and ochratoxin A and HT-2 toxin in barley. The preserved grain diet improved average daily gain (ADG; $P < 0.01$) and body weight (BW; $P < 0.01$) compared to the dried grain diet. The incorporation of ZnO increased average daily feed intake (ADFI; $P < 0.01$) and BW ($P < 0.05$) in the dried grain diet, however, ZnO did not affect ADFI ($P > 0.05$), and reduced ADG and BW in the preserved grain diet ($P < 0.05$). Pigs offered the preserved grain diet had reduced faecal scores and diarrhoea incidence compared to those offered the dried grain diet ($P < 0.05$). Supplementation of ZnO reduced faecal scores and diarrhoea incidence compared to non-supplemented pigs ($P < 0.001$). On day 35 PW, the preserved grain diet had improved CAID of nitrogen and gross energy ($P < 0.05$), lower levels of colonic branched-chain volatile fatty acids

Abbreviations: ADF, acid detergent fibre; ADFI, average daily feed intake; ADG, average daily gain; AIA, acid-insoluble ash; BCFA, branched-chain fatty acids; BW, body weight; CAID, coefficient of apparent ileal digestibility; CATTD, coefficient of apparent total tract digestibility; CD, crypt depth; DE, digestible energy; DM, dry matter; DON, deoxynivalenol; G:F, gain-to-feed ratio; GE, gross energy; MC, moisture content; N, nitrogen; aNDF, neutral detergent fibre assayed with a heat-stable amylase and expressed inclusive of residual ash; OA, organic acid; OM, organic matter; OTA, ochratoxin A; OTU, operational taxonomic unit; PW, post-weaning; PWD, post-weaning diarrhoea; TGW, thousand-grain weight; TMC, total mould count; VH, villus height; VFA, volatile fatty acid; ZEN, zearalenone; ZnO, zinc oxide.

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https://doi.org/10.1016/j.anifeedsci.2024.116078
Received 14 February 2024; Received in revised form 1 August 2024; Accepted 2 August 2024
Available online 3 August 2024

($P < 0.05$), and beneficial shifts in gut microbial populations. Specifically, there was a reduction in ileal *Streptococcus* ($P < 0.001$) and an increased abundance of colonic *Faecalibacterium* ($P < 0.05$). In conclusion, organic acid preservation improved grain quality, benefiting post-weaned pigs through improved nutrient digestibility and gut health, thus enhancing overall growth performance PW.

1. Introduction

Cereal grains, such as wheat and barley, are fundamental components of pig diets and play a crucial role in determining the success of feed formulations (Aumiller et al., 2015; Gruber-Dorninger et al., 2019). The quality of these grains is of paramount importance as it directly impacts the nutritional value of the feed. However, preserving the quality of cereal grains during storage presents a formidable challenge due to a multitude of intrinsic and extrinsic factors that can lead to variations in their physical, chemical, and microbiological attributes (Ziegler et al., 2021). This challenge is further compounded by the natural presence of bacterial and fungal contaminants, particularly mycotoxins (Edwards, 2004; Clarke et al., 2018a). Among these mycotoxins, aflatoxins, fumonisins, trichothecenes [mainly T-2-toxin, HT-2 toxin and deoxynivalenol (DON)], zearalenone (ZEN), and ochratoxin A (OTA) are of particular concern (Kolawole et al., 2024). Their known toxicity threatens animal health and production efficiencies, resulting in substantial economic loss (Bryden, 2012; Pierron et al., 2016). Consequently, several countries have established maximum permitted and guidance levels of mycotoxins for both cereal grains and finished feeds to minimise exposure (van Egmond et al., 2007; Guerre, 2016).

Environmental conditions, including temperature, humidity, and moisture strongly influence the development of moulds and mycotoxins in stored grain. Controlling these parameters is crucial for mitigating contamination during storage (Neme and Mohammed, 2017). In Ireland, for example, the average moisture content of wheat and barley at harvest has exceeded 180 g/kg over the past 5 years (Teagasc, 2023), highlighting the requirement for effective preservation. While industrial drying is a conventional method of grain preservation (Kjær et al., 2018), it raises significant economic and environmental concerns due to its high energy consumption (Chojnacka et al., 2021). Moreover, variations in the drying process can lead to inconsistencies in moisture content within grain batches, potentially creating an environment conducive to mould and mycotoxin production (Raghavan et al., 1993; Magan and Aldred, 2006). Conversely, over-drying can result in the denaturation of proteins, thereby diminishing the nutritional value of the grains (Jokiniemi and Ahokas, 2014).

To address these challenges, organic acids (OA) have been recognised as effective agents for grain and feed preservation (Wang et al., 2022). The application of OA in grain preservation has shown effectiveness in enhancing the hygienic quality of grain, inhibiting fungi and bacteria, and reducing mycotoxin contamination (Tung and Pettigrew, 2006). Of these OA, propionic acid is particularly effective; however, its use has raised concerns regarding feed palatability, equipment corrosion, volatility and potential microbial resistance (Rutenberg et al., 2018). In response to these issues, propionic acid is now blended with other OA and salts, enhancing its mould-inhibiting properties and reducing its corrosiveness and volatility. In contrast to the use of individual acids, blends of OA have been shown to be superior in terms of improving both the health and growth performance of pigs (Tugnoli, Piva 2020; Wang et al., 2022).

With recent EU regulatory changes aimed at reducing antimicrobial resistance and environmental pollution, the use of antimicrobials and zinc oxide (ZnO) in post-weaning (PW) pig diets have been restricted (Bonetti et al., 2021). This has prompted a closer examination of OA as an alternative strategy. Many studies have indicated that supplementing diets with OA in the form of a feed additive can improve nutrient digestibility (Mosenthin et al., 1992; Hanczakowska et al., 2011; Devi et al., 2016), modulate gut microbial populations (Papatsiros et al., 2011; Luise et al., 2017), reduce the incidence of post-weaning diarrhoea (PWD; Tsiloyiannis et al., 2001; Lei et al., 2017) and enhance pig growth performance (Partanen et al., 2007; Halas et al., 2008; Luise et al., 2017). However, studies investigating the impact of incorporating grain preserved with OA blends in contrast to ZnO supplementation on the health and growth of pigs during the PW period are limited.

Against this backdrop, the primary objective of this study was to compare the nutritional quality of wheat and barley grains preserved with an OA mould inhibitor against artificially dried grains. Furthermore, this study explored the effects of feeding these grains on various aspects of post-weaned pig performance, including growth performance, diarrhoea incidence and severity, nutrient digestibility, gene expression in the stomach, small intestinal morphology, and ileal and colonic volatile fatty acids (VFA) and microbial populations. Particular attention was paid to the potential benefits of integrating OA-preserved grains into pig diets with and without ZnO supplementation. In light of these objectives, this study hypothesised that preserving cereal grains with an OA mould inhibitor would enhance grain quality, ultimately improving the performance and health of post-weaned pigs.

2. Materials and methods

All experimental procedures in this study were approved under the University College Dublin Animal Research Ethics Committee (AREC-20-22-O'Doherty) and were conducted under 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 during the experiment.

2.1. Grain management and quality assessment

The winter wheat (cv. *JB Diego*) and spring barley (cv. *SY Errigal*) grains used in this study were sourced from McAuley Feeds (Burtonstown, Meath, Ireland). Both crops were established and harvested in the 2020 growing season. To ensure a high-quality wheat crop, sowing was carried out in early October 2019 and all recommended husbandry practises for winter wheat were followed (3 spray fungicide programme and a 3-split nitrogen (N) application rate of 180 kg N/ha). The wheat was harvested under ideal conditions in August 2020 at a moisture content (MC) of 180.0 g/kg. The barley was sown in March 2020, following the recommended husbandry practises for spring barley (2 spray fungicide programme and 2 split N application rate of 140 kg/ha) and was also harvested in August 2020 at an MC of 182.0 g/kg. Before storage, both crops were split into two batches and were either dried or preserved with an OA mould inhibitor. The dried grain was dried using a continuous flow-type grain dryer (Cimbria, Thisted, Denmark) for 3 h (h) at 65 °C followed by a 2-h cooling period. At the end of the drying process, the MC of both the wheat and barley dropped to 140 g/kg and 140.7 g/kg, respectively. The OA blend used was a liquid surfactant mould inhibitor [MycoCURB ES Liquid; propionic acid (650 g/kg), ammonium propionate (70 g/kg), glycerol polyethyleneglycol ricinoleate (17.5 g/kg) and a carrier] sourced from Adesco Nutricines (Dungarvan, Waterford, Ireland). It was applied before grain storage by spray action at an inclusion rate of 4 g/kg and a mixing auger ensured uniform distribution (Fig. 1). All grains were ventilated and stored for 240 days before diet manufacture.

The physical attributes of the grain at harvest were assessed by measuring MC, density (hectolitre weight) and thousand-grain weight (TGW). Grain moisture was determined using a DICKEY-john GAC 2500-UGMA electronic moisture metre (Auburn, IL, USA). Grain density was determined using a Pfeuffer Chondrometer and bulk density calibration chart and the TGW was determined by recording the weight of 1000 grains using a Pfeuffer Contador seed counter (Kitzingen, Germany). Before feed formulation, 20 representative samples of both wheat and barley were collected using the grab sample technique and analysed for dry matter (DM), ash, gross energy (GE), crude protein, crude fibre, starch, fat, pH, total mould count (TMC), and mycotoxins. The TMC of the grain was determined by the colony count technique (ISO21527-2:2008) as described by Laca et al. (2006). After storage, the pH of the grain was measured using a pH probe (Mettler-Toledo FiveEasy Plus; Greifensee, Switzerland), which was calibrated with certified pH 4 and pH 7 buffer solutions. The mycotoxin presence of aflatoxin B1, B2, G1 and G2, fumonisin B1 and B2, DON, T-2 Toxin, HT-2 Toxin, ZEN and OTA were determined by liquid chromatography-mass spectrometry as previously described by Soleimany et al. (2012). The chemical and mycotoxin analyses of the wheat and barley post-storage are presented in Table 1.

2.2. Experimental design and diets

The pig experiment was arranged in a 2 × 2 factorial in a randomised complete block design consisting of four dietary treatments. The dietary treatments were as follows: (1) dried grain diet (control), (2) preserved grain diet, (3) dried grain diet with ZnO (positive control), and (4) preserved grain diet with ZnO. Each diet consisted of 600 g/kg of cereal grain, of which 450 g/kg was either dried or preserved wheat and 150 g/kg was dried or preserved barley. The remaining composition (400 g/kg) comprised a concentrate sourced from Cargill (Naas, Kildare, Ireland). The ZnO (Cargill) was included at 3.1 g ZnO/kg of feed and contained 80 % zinc, resulting in an inclusion level of 2.5 g Zn/kg of feed. After three weeks the inclusion level of ZnO was halved to 1.55 g ZnO/kg feed. Celite (5 g/kg) was added to the feed during the milling process to measure the coefficient of apparent total tract digestibility (CATTD) and the coefficient of apparent ileal digestibility (CAID) of nutrients using the acid-insoluble ash (AIA) method (McCarthy et al., 1977). The diets were formulated to contain similar levels of digestible energy (DE; 14.95 MJ/kg), net energy (10.6 MJ/kg), crude protein (190 g/kg) and standardised ileal digestible lysine (12.0 g/kg). The levels of amino acids were formulated to meet or exceed the

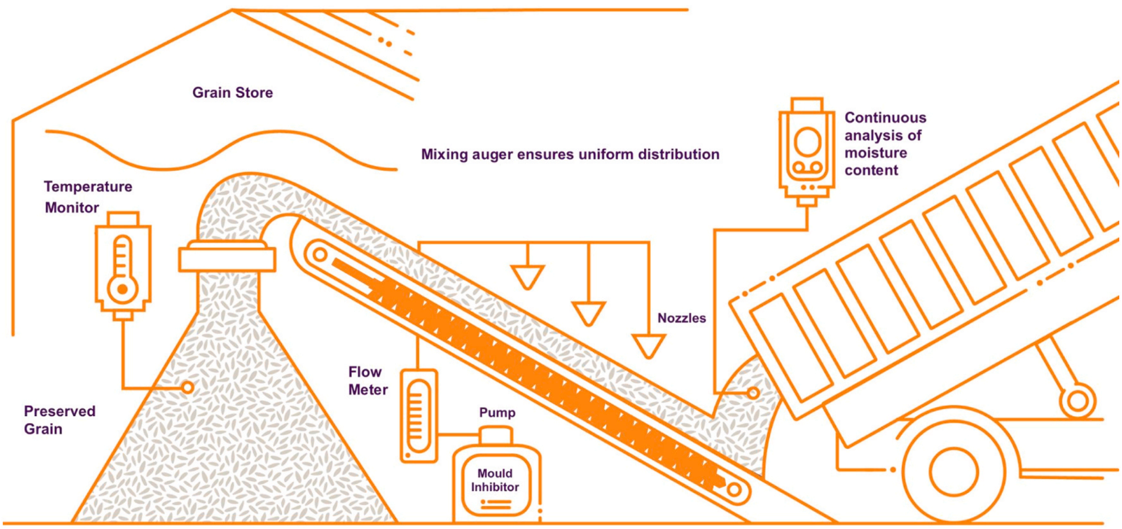

Fig. 1. The organic acid liquid surfactant mould inhibitor preservation process from grain intake to storage.

Table 1
The chemical analysis of experimental grain after storage on an as-fed basis (g/kg unless otherwise stated).

Cereal crop type	Wheat		Barley	
Preservation method	Dried	Preserved	Dried	Preserved
Analysis post storage (g/kg as fed)				
DM	860.0	820.8	859.3	818.8
Ash	11.5	12.3	12.1	13.1
GE (MJ/kg)	15.6	15.6	15.2	15.0
Crude protein	101.7	103.4	96.3	91.4
Crude fibre	23.5	21.5	50.5	45.5
Starch	618.5	593.0	521.3	510.7
Fat	13.7	13.0	15.3	14.7
pH	6.2	5.8	5.8	5.5
TMC (cfu/g)[a]	4.1	1.2	5.5	2.5
Mycotoxin levels (μg/kg)[b]				
Deoxynivalenol	87.0	<75.0	240.0	<75.0
T-2 Toxin	<4.00	<4.00	<4.00	<4.00
HT-2 Toxin	<4.00	<4.00	5.77	<4.00
Zearalenone	<10.0	<10.0	<10.0	<10.0
Ochratoxin A	<1.00	<1.00	15.76	<1.00

DM, dry matter; GE, gross energy; TMC, total mould count.
[a] These values were log transformed.
[b] The following mycotoxins were below detectable levels: Aflatoxin B1, B2, G1 and G2 (< 1 μg/kg); Fumonisin B1 (< 125 μg/kg) and Fumonisin B2 (< 50 μg/kg).

requirements of the NRC (2012). The diet compositions are presented in Table 2.

2.3. Housing and animal management

One hundred and ninety-two pigs [progeny of Meatline boar (Hermitage, Sion Road, Kilkenny, Ireland) × (Large White × Landrace sows)] were sourced from a commercial farm at weaning (28 days of age) with an average weight of 7.0 kg (SD ± 1.3 kg). The pigs were blocked based on weaning weight, sex, and litter of origin and within each block, assigned to one of the four dietary treatments for 35 days. The pigs were penned on fully slatted floors (1.68 × 1.22 m) in groups of three (16 replicates/treatment). The ambient environmental temperature within the house was thermostatically controlled at 30 °C for the first week and reduced by 2 °C per week until the temperature reached 22 °C. The relative humidity was maintained at 65 %. Feed and water were available ad libitum throughout the experiment. Feed consumed and pig body weight (BW) were measured using a portable electronic scale (Prattley, Temuka, New Zealand) on days 7, 14, 21, and 35 and average daily feed intake (ADFI), average daily gain (ADG), and gain-to-feed ratio (G:F) were subsequently calculated. During the first 21 days PW, faecal consistency scores were recorded for each pen twice daily by the same operator to assess the presence and severity of diarrhoea. The following faecal scoring system was used: 1 = hard firm faeces;

Table 2
The ingredient composition of experimental diets (g/kg).

	Treatments			
Preservation method	Dried	Preserved	Dried	Preserved
Zinc oxide inclusion	No	No	Yes	Yes
Ingredients (g/kg)				
Wheat	452	452	448.9	448.9
Barley	150	150	150	150
Full-fat soya	150	150	150	150
Soya bean meal	101.5	101.5	101.5	101.5
Whey powder	60	60	60	60
Soya oil	65	65	65	65
Vitamins and mineral premix[a]	2.5	2.5	2.5	2.5
Salt	2	2	2	2
Mono calcium phosphate	4	4	4	4
Calcium carbonate (limestone)	6	6	6	6
L-Lysine HCl, 78.8 %	4	4	4	4
DL-Methionine	1.5	1.5	1.5	1.5
L-Threonine	1.5	1.5	1.5	1.5
Zinc oxide	0	0	3.1	3.1

[a] Vitamin and mineral premix (per kg diet): 250 mg of choline chloride; 140 mg of Fe as $FeSO_4$; 47 mg of Mn as MnO; 120 mg of Zn as ZnO; 25 mg of Cu as $CuSO_4$; 0.6 mg of I as calcium iodate on a calcium sulphate/calcium carbonate carrier; 0.3 mg Se as sodium selenite; 12 mg of nicotinic acid; 10 mg of pantothenic acid; 67 mg of α-tocopherol; 4 mg of phytylmenaquinone; 2 mg of riboflavin; 2 mg of thiamine; 1.8 mg of retinol; 0.025 mg of cholecalciferol; 0.015 mg of pyridoxine; 0.01 mg of cyanocobalamin.

2 = slightly soft faeces; 3 = soft, partially formed faeces; 4 = loose, semi-liquid faeces and 5 = watery mucous-like faeces as described by Walsh et al. (2013). Diarrhoea incidence was characterised by a faecal score greater than 3.

2.4. Sample collection

On day 35 PW, twelve pigs each from the dried grain diet and the preserved grain diet, which were selected at weaning, were humanely euthanised following a lethal injection of pentobarbitone sodium (Euthatal Solution, 200 mg/ml; Merial Animal Health, Essex, UK) at a rate of 0.71 ml/kg BW to the cranial vena cava. Euthanasia was completed by a competent person in a separate room away from the sight and sound of other animals. Pigs were not fasted before sacrifice. Following confirmation of death, the entire digestive tract was immediately removed. The stomach was dissected at the oesophagus and the duodenum. After a 2-point calibration using standard solutions of pH 4 and 7, gastric digesta pH was measured by inserting a pH probe (Mettler Toledo FiveEasy Plus) into a small incision at the top of the stomach. The stomach was further incised along the greater curvature and its contents were removed. Following this, the stomach lining underwent a gentle rinse with sterile phosphate-buffered saline. Tissue Section (1 cm each) were collected from both the fundic gland and the pyloric gland regions, based on the description of the regions by Kiernan et al. (2023). These tissue samples were then washed with phosphate-buffered saline, stripped of the overlying smooth muscle, and immersed in RNAlater® solution (5 ml) overnight at 4 °C. After 24 h, the RNAlater® was discarded, and the samples were stored at − 80 °C. Sections from the duodenum (10 cm from the stomach) and the jejunum (60 cm from the stomach) were excised and fixed in 10 % neutral-buffered formalin for gut morphological analysis as previously described (Rattigan et al., 2020). Digesta from the ileum and colon were aseptically collected and placed in sterile containers (Sarstedt, Wexford, Ireland) before storage at − 80 °C for quantification of selected microbial populations using 16S rRNA sequencing. In addition, digesta from the ileum and colon were also collected for the determination of the CAID of nutrients and VFA analysis respectively and stored at − 20 °C. The CAID was calculated using AIA as an internal marker (McCarthy et al., 1977). The equation used was:

CAID of nutrient = (1 – [nutrient in digesta/nutrient in diet] × [AIA-diet/AIA-digesta]), where nutrient in digesta and nutrient in diet were the nutrient concentration (g/kg) in the digesta and diet DM, respectively and AIA-diet and AIA-digesta represent the marker concentrations (g/kg) in the diet and digesta DM, respectively (Clarke et al., 2018b).

Table 3
The chemical analysis of experimental diets on an as-fed basis (g/kg unless otherwise stated).

	Treatments			
Preservation method ZnO inclusion	Dried No	Preserved No	Dried Yes	Preserved Yes
Chemical analysis (g/kg)				
DM	885.2	864.7	888.3	861.9
Ash	48.2	47.2	52.5	50.5
GE (MJ/kg)	16.6	16.3	16.8	16.4
Crude fat	53.8	53.0	54.0	53.3
Crude protein	190.4	189.8	190.0	189.4
Crude fibre	33.0	32.0	33.7	32.0
aNDF	135.0	120.7	129.0	114.0
ADF	43.0	40.0	39.7	37.3
Starch	341.3	327.5	334.8	322.4
pH	6.1	6.0	6.2	6.2
TMC (cfu/g)	<100	<100	<100	<100
Mycotoxin levels (μg/kg)[a]				
Deoxynivalenol	<75.0	<75.0	<75.0	<75.0
T-2 toxin	<4.00	<4.00	<4.00	<4.00
HT-2 toxin	<4.00	<4.00	<4.00	<4.00
Zearalenone	<10.0	<10.0	<10.0	<10.0
Ochratoxin	2.13	<1.00	2.60	<1.00
Essential amino acids (g/kg)				
Arginine	9.3	9.9	9.7	10.2
Histidine	4.1	4.3	4.3	4.2
Isoleucine	6.9	7.0	7.0	6.7
Leucine	12.3	12.1	12.3	12.3
Lysine	13.3	13.2	13.7	13.7
Methionine	5.0	4.9	5.1	4.7
Phenylalanine	8.1	7.8	8.3	8.0
Threonine	8.8	8.8	8.9	8.9
Tryptophan	2.7	2.6	2.7	2.6
Valine	9.0	9.2	9.5	9.1

ZnO, zinc oxide; DM, dry matter; GE, gross energy; aNDF, neutral detergent fibre assayed with a heat-stable amylase and expressed inclusive of residual ash; ADF, acid detergent fibre; TMC, total mould count.

[a] The following mycotoxins were below detectable limits: Aflatoxin B1, B2, G1 and G2 (< 1 μg/kg); Fumonisin B1 (< 125 μg/kg) and Fumonisin B2 (< 50 μg/kg).

2.5. Feed and faecal analysis

Representative feed samples were collected at regular intervals during the experiment and retained for chemical analysis. Faecal samples were collected from all pens on day 14 PW (16 samples/treatment) and immediately frozen at − 20 °C for the determination of CATTD of nutrients. Before analysis, faecal and digesta samples were dried at 55 °C for 72 h. The feed, dried faeces and digesta were milled through a 1-mm screen (Christy and Norris Hammer Mill; Chelmsford, UK). The crude ash content was determined after ignition of a weighted sample in a muffle furnace (Nabertherm, Bremen, Germany) at 550 °C for 6 h. The GE content was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL, USA). The nitrogen (N) content was determined using the LECO FP 528 instrument (Leco Instruments Ltd., Stockport, Cheshire, UK). The dietary concentration of essential amino acids was determined by HPLC (Iwaki et al., 1987). The crude fibre was determined according to the AOAC (2005; method 962.09) and the neutral detergent fibre (assayed with a heat-stable amylase and expressed inclusive of residual ash; aNDF) and acid detergent fibre (ADF) were determined according to Mertens (2002; AOAC method 2002.04) using the Ankom 220 Fibre Analyser (Ankom Technology, Macedon, NY, USA). Starch concentration was determined using a Megazyme assay kit (Megazyme Int. Wicklow, Ireland). The CATTD of nutrients were calculated using AIA as an internal marker, as previously described. The chemical analysis of the diets is presented in Table 3.

2.6. Gut morphological analysis

Standard paraffin embedding techniques were used to prepare preserved duodenal and jejunal tissue samples for gut morphological analysis as previously described by Rattigan et al. (2020). The samples were sectioned at a thickness of 5 μm and stained with haematoxylin-eosin. A light microscope with an image analyser (Image-Pro Plus; Media Cybernetics, Oxon, UK) was used to measure the villus height (VH) and crypt depth (CD). Fifteen measurements of well-orientated and intact villi and crypts were taken for each section. The VH was measured from the tip to the crypt-villus junction and the CD was measured from the crypt-villus junction to the base of the crypt.

2.7. Gene expression

2.7.1. RNA extraction and cDNA synthesis

Total RNA was isolated from 100 mg of tissue using TriReagent (Sigma-Aldrich, St. Louis, Missouri, USA) following the manufacturer's instructions. Subsequent purification was conducted with the GenElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich), including a DNase step utilizing an on-column DNase 1 Digestion set (Thermo Scientific, Waltham, MA, USA). The purity and quantity of RNA were determined by measuring the absorbance ratio at 260 nm and 280 nm with a Nanodrop-ND1000 spectrophotometer (Thermo Scientific). 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. The resulting cDNA was then diluted to a final volume of 400 μL using nuclease-free water.

2.7.2. Quantitative PCR

The quantitative PCR (qPCR) reaction mixture (20 μL) consisted of GoTaq qPCR Syber Green Master Mix (10 μL) from Promega (Madison, WI, USA), forward and reverse primers (1.2 μL of a 5 μM mix) for a final concentration of 300 nM/RXN, nuclease-free water (3.8 μL), and cDNA (5 μL) equivalent to 25 ng total RNA. All qPCR reactions were carried out in duplicate on the 7500 ABI Prism Sequence Detection System (Applied Biosystems). The cycling conditions comprised a denaturation step at 95 °C for 10 min (min), followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Primers were designed using Primer Express™ software (Applied Biosystems) and synthesised by Eurofins (Milton Keynes, Buckinghamshire, UK). Optimal reference genes were selected using the GeNorm algorithm in the qBase PLUS software (BioGazelle, Ghent, Belgium). The geometric mean of the optimal reference genes *ACTB* and *RPL27* was utilised for normalising target expression. Normalised relative quantities were determined using qbase PLUS software (BioGazelle). The genes analysed in the current study were involved in a range of stomach functions and were selected on the findings from Kiernan et al. (2023) which included *ATP4A; CLIC6; HRH2; KCNQ1; KCNE1; CBLIF; CHIA; PGA5; GAST; CCKBR; GHRL; HDC; MUC1; MUC2; MUC5AC; MUC6; OLFM4; PIGR; AQP4; SST; ACTB; B2M; GAPDH; PPIA; OAZ1; RPS29; RPL27; RPL29*. The accession numbers, primer sequences, and amplicon lengths are provided in Supplementary Table 1.

2.8. Microbiological analysis

2.8.1. Microbial DNA extraction

Microbial DNA was extracted from the ileal and colonic digesta using a QIAamp PowerFecal Pro DNA Kit (Qiagen, West Sussex, UK) according to the manufacturer's instructions. Using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific), the quality and quantity of DNA was assessed.

2.8.2. Illumina sequencing

High throughput sequencing of the V3-V5 hypervariable region of the bacterial 16S rRNA gene was performed on an Illumina MiSeq platform according to standard protocols (Eurofins Genomics, Ebersberg, Germany). The V3-V5 region was PCR-amplified using universal primers containing adapter overhang nucleotide sequences for forward and reverse index primers. Amplicons were purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) and set up for the index PCR with Nextera XT index primers

(Illumina, San Diego, CA, USA). The indexed samples were purified using AMPure XP beads, quantified using a fragment analyser (Agilent, Santa Clara, CA, USA) and equal quantities from each sample were pooled. The resulting pooled library was quantified using the Bioanalyser 7500 DNA kit (Agilent) and sequenced using the v3 chemistry (2 × 300 bp paired end reads).

2.8.3. Bioinformatics

The bioinformatic analysis of the sequences was conducted by Eurofins Genomics (Germany) using the open-source package Quantitative Insights into Microbial Ecology (Version 1.9.1; Caporaso et al., 2010). All 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 and reverse reads. If primer sequences were not perfectly matched, read pairs were eliminated to retain only high-quality reads. Paired-end reads were then merged, to obtain a single, longer read that covered the entire target region using the software FLASH 2.200 (Magoč and Salzberg, 2011). The pairs were then merged with a minimum overlap size of 10 bp to reduce false-positive merges. The forward read was only retained for the subsequent assessment steps when merging was not possible. Quality filtration of merged reads was then carried out according to the expected and known length variations in the V3–V5 region (ca. 445 bp). The ends of retained forward reads were clipped to a total read length of 285 bp to eliminate low-quality bases. Merged and retained reads comprising ambiguous bases were removed. The filtered reads were then used for the microbiome profile. Chimeric reads were detected and discarded based on the de-novo algorithm of UCHIME (Edgar et al., 2011) as implemented in the VSEARCH package (Rognes et al., 2016). The remaining set of high-quality reads were processed using minimum entropy decomposition to partition reads into operational taxonomic units (OTU; Eren et al., 2013, 2015). The DC-MEGABLAST alignments of cluster representative sequences to the NCBI nucleotide sequence database were conducted for the taxonomic assignment of every OTU. A sequence identity of 70 % across at least 80 % of the representative sequence was the minimal requirement for considering reference sequences. Abundances of bacterial taxonomic units were normalised using linear-specific copy numbers of the appropriate marker genes to enhance estimates (Angly et al., 2014). The normalised OTU table combined with the phenotype metadata and phylogenetic tree comprised the data matrix. The data matrix was loaded into the phyloseq package in R (Version 3.5.0, accessed on 14/03/2022). The dynamics of richness and diversity were computed with the Observed, Chao1, ACE, Shannon, Simpson, InvSimpson and Fisher indices. Shannon and Simpson accounted for richness and evenness parameters. In addition, beta diversity was estimated by normalising the data so the taxonomic feature counts were comparable across all samples. Several distance metrics were considered to calculate the distance matrix of the various multidimensional reduction processes including weighted and unweighted UniFrac distance/non-phylogenetic distance metrics (Bray-Curtis, Jensen-Shannon divergence and Euclidian) using phyloseq in R as previously described by Dowley et al. (2021). Differential abundance testing was carried out on tables extracted from the phyloseq object at phylum, family and genus levels.

2.9. Volatile fatty acids

The VFA concentrations of collected digesta samples were determined using gas-liquid chromatography according to the method described by Clarke et al. (2018b). A 1 g digesta sample was diluted with distilled water (2.5 × weight of sample) and centrifuged (1400 × *g* for 10 min) using a Sorvall GLC-2B laboratory centrifuge (DuPont, Wilmington, DE, USA). One 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. The reaction mixture was centrifuged (500 × *g*) for 10 min and the supernatant was filtered through a 0.45 polytetrafluoroethylene syringe filter into a chromatographic sample vial. An injection volume of 1 μl was injected into a Varian 3800 GC (Markham, Ontario, Canada) equipped with a EC™ 1000 Grace column (15 m × 0.53 mm I.D) with 1.20 μm film thickness. The temperature programme set was to the range 75–95 °C increasing by 3 °C/min and 95–200 °C increasing by 20 °C/min and 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.

2.10. Statistical analysis

Model suitability was investigated by checking normality of scaled residuals using the UNIVARIATE procedure of SAS® software 9.4 (SAS Institute). The growth performance (ADFI, ADG, G:F and BW) and faecal scores were analysed as a 2 × 2 factorial by repeated measures analysis using the PROC MIXED procedure of SAS. The growth parameters were divided into two time periods; days 0–21 and days 21–35. The model included grain preservation method, ZnO inclusion, time of weighing and their associated two and three-way interactions. Diarrhoea incidence in the first 21 days was analysed using the PROC GENMOD procedure of SAS. The pen was the experimental unit for the performance, faecal score and diarrhoea incidence data analysis. The CATTD and CAID of nutrients, gastric pH, small intestinal morphology, stomach gene expression and digesta VFA concentrations were analysed using PROC GLM (general linear model) procedure of SAS. The model included grain preservation method. The data is presented as least-square means with their standard errors of the mean (SEM). Microbial populations of the ileal and colonic digesta were analysed using PROC GLIMMIX for nonparametric data. The model examined the effect of grain preservation method and the microbiome results are presented using Benjamini-Hochberg adjusted P-values. The probability level that denoted significance was $P < 0.05$, while P values between 0.05 and 0.10 were considered tendencies.

3. Results

3.1. Grain quality

Before grain preservation, the MC, hectolitre weight, and TGW of the wheat samples at harvest were determined to be 180 g/kg, 73 kg/hL and 49.8 g, respectively. The MC, hectolitre weight, and TGW of the barley at harvest were determined to be 182 g/kg, 61 kg/hL and 48.2 g, respectively.

Preserving grain with the OA mould inhibitor lowered the pH, DM content, and starch composition of the preserved wheat and barley in contrast to the dried wheat and barley on an as fed basis. The levels of Aflatoxin B1, B2, G1 and G2 (< 10 μg/kg), Fumonisins B1 and B2 (< 1000 μg/kg), DON (< 900 μg/kg), T-2 Toxin and HT-2 Toxin (< 50 μg/kg), ZEN (< 250 μg/kg), and OTA (< 50 μg/kg) were below the detectable levels for both dried and preserved grains. However, the dried wheat had numerically greater levels of DON and mould in comparison to the preserved wheat and the dried barley had numerically greater levels of DON and mould compared to the preserved barley. Additionally, the dried barley had numerically greater levels of HT-2 toxin and OTA compared to preserved barley.

3.2. Performance, faecal scores, and diarrhoea incidence

The effect of grain preservation method and ZnO inclusion on the growth performance, faecal scores, and the incidence of diarrhoea during the PW period (days 0–21 and days 21–35) are presented in Table 4. During days 0–21 PW, supplementing the dried grain diet with ZnO increased ADG ($P < 0.05$), however ZnO inclusion had no effect on ADG in the preserved grain diet. During days 21–35 PW, ZnO had no effect on ADG in the dried grain diet, and reduced ADG in the preserved grain diet ($P < 0.05$).

During the overall experimental period (days 0–35), the inclusion of ZnO increased ADFI ($P < 0.01$) and BW ($P < 0.05$) in the dried grain diet, however ZnO had no effect on ADFI and reduced final BW ($P < 0.05$) in the preserved grain diet. There was no effect of dietary treatment on G:F ratio.

The inclusion of ZnO reduced faecal scores and diarrhoea incidence during the first 21 days PW compared to the non-ZnO supplemented pigs ($P < 0.001$). Pigs offered the preserved grain diet had reduced faecal scores and diarrhoea incidence compared to the dried grain diet ($P < 0.05$).

3.3. Nutrient digestibility

3.3.1. Coefficient of apparent total tract digestibility

The effects of the preservation method and ZnO supplementation on the CATTD of nutrients at 14 days PW are presented in Table 5. The inclusion of ZnO increased the CATTD of DM, OM, N, aNDF, and GE and DE content compared to the dried grain diet, however ZnO inclusion reduced the CATTD of DM, OM, ash, and GE and DE content compared to the preserved grain diet ($P < 0.05$). Pigs offered the preserved grain diet had increased CATTD of DM, OM, ash, N, aNDF, and GE and DE content compared to pigs offered the dried grain diet ($P < 0.001$).

3.3.2. Coefficient of apparent ileal digestibility

The effect of grain preservation method on the CAID of nutrients at day 35 PW are presented in Table 6. Pigs offered the preserved grain diet had improved CAID of DM, OM, N, and GE compared to the dried grain diet ($P < 0.01$).

3.4. Stomach gene expression and gastric digesta pH

There was no effect of diet on gene expression in the fundic gland region at day 35 PW. In the pyloric gland region, *CCKBR* was the only gene that was differentially expressed between the treatment groups, with higher expression in the preserved grain group compared to the dried grain group (0.101 vs. 0.038; $P = 0.002$). The gastric digesta pH did not differ between pigs offered the dried grain diet and the preserved grain diet (3.59 vs. 3.57; $P = 0.975$).

3.5. Small intestinal morphology

There was no effect of diet on the VH in the duodenum (319 vs. 324 μm; $P = 0.753$) or in the jejunum (296 vs. 286 μm; $P = 0.702$), nor was there any significant difference of the CD in the duodenum (127 vs. 127 μm; $P = 0.991$) or the jejunum (129 vs. 140 μm; $P = 0.408$). The VH:CD ratio in the duodenum and the jejunum was also not affected between treatments.

3.6. Ileal and colonic microbiota

3.6.1. Bacterial richness and diversity

The alpha and beta microbial diversity of ileal and colonic digesta samples were measured from pigs fed the non-ZnO supplemented diets at day 35 PW, however, no statistical difference or clustering according to dietary treatment was observed. The Observed, Chao1, ACE, Shannon, Simpson, InvSimpson and Fisher index measures of alpha diversity are presented in Table 7.

Table 4
The effect of grain preservation method and zinc oxide supplementation on pig growth performance, faecal scores and diarrhoea incidence (least square mean with their SEM).

Preservation method	Dried		Preserved		Dried		Preserved			P-value*				
ZnO inclusion	No		No		Yes		Yes							
Time	D 0–21	D 21–35	D 0–21	D 21–35	D 0–21	D 21–35	D 0–21	D 21–35	SEM	Grain	ZnO	Grain × ZnO	Time	Grain × ZnO × Time
No of replicates	16		16		16		16							
ADFI (kg/d)	0.39	0.94	0.43	1.06	0.44	1.03	0.45	1.04	0.013	**<0.001**	**<0.001**	**0.002**	**<0.001**	**0.012**
ADG (kg/d)	0.27	0.59	0.32	0.65	0.32	0.58	0.33	0.57	0.014	**0.010**	0.533	**0.012**	**<0.001**	0.439
G:F (kg/kg)	0.67	0.63	0.74	0.61	0.73	0.56	0.72	0.55	0.021	0.690	0.210	0.201	**<0.001**	0.088
Body weight (kg)	12.5	20.9	13.7	23.0	13.7	21.9	13.9	22.0	0.337	**0.005**	0.205	**0.018**	**<0.001**	**0.036**
Faecal Score[a]	2.70	-	2.57	-	2.14	-	2.05	-	0.054	**0.049**	**<0.001**	0.745	-	-
Diarrhoea incidence (%)[b]	30.2	-	19.1	-	1.0	-	1.5	-	2.268	**0.036**	**<0.001**	0.303	-	-

SEM, standard error of the mean; ZnO, zinc oxide; ADFI, average daily feed intake; ADG, average daily gain; G:F, gain-to-feed ratio.

* Grain = the effect of grain preservation method; ZnO = the effect of ZnO inclusion; Grain × ZnO = the two-way interaction between grain preservation method and ZnO inclusion; Time = the effect of time period (days 0–21 and days 21–35); Grain × ZnO × Time = the three-way interaction between grain preservation method, ZnO inclusion and time period.

[a] Faecal consistency score range: 1 = hard, firm faeces; 2 = slightly soft faeces; 3 = soft partially formed faeces; 4 = loose, semi-liquid faeces; and 5 = watery, mucous like faeces.

[b] A faecal score of > 3 was characterised as diarrhoea.

Table 5
The effect of grain preservation method and zinc oxide supplementation on the coefficient of apparent total tract digestibility of dry matter (DM), organic matter (OM), ash, N (nitrogen), neutral detergent fibre assayed with a heat-stable amylase and expressed inclusive of residual ash (aNDF), gross energy (GE) and digestible energy (DE) on day 14 post-weaning (least square mean with their SEM).

	Treatments				P-value*			
Preservation method ZnO inclusion	Dried No	Preserved No	Dried Yes	Preserved Yes	SEM	Grain	ZnO	Grain × ZnO
DM	0.855^{w}	0.904^{z}	0.876^{x}	0.889^{y}	0.002	<**0.001**	0.228	<**0.001**
OM	0.868^{w}	0.914^{z}	0.890^{x}	0.901^{y}	0.002	<**0.001**	0.076	**<0.001**
Ash	0.682^{w}	0.763^{x}	0.667^{w}	0.697^{w}	0.006	<**0.001**	**<0.001**	**<0.001**
N	0.820^{w}	0.879^{y}	0.856^{x}	0.874^{y}	0.005	<**0.001**	**0.019**	**<0.001**
aNDF	0.421^{w}	0.647^{y}	0.545^{x}	0.611^{y}	0.015	**<0.001**	**0.005**	<**0.001**
GE	0.834^{w}	0.890^{z}	0.860^{x}	0.874^{y}	0.003	<**0.001**	0.089	**<0.001**
DE content, (MJ/kg)[a]	13.83^{w}	14.53^{y}	14.42^{xy}	14.29^{x}	0.047	**<0.001**	**0.001**	<**0.001**

SEM, standard error of the mean; ZnO, zinc oxide.
[w,x,y,z] Means values within a row with different superscript letters were significantly different.
[*] Grain = the effect of grain preservation method; ZnO = the effect of ZnO inclusion; Grain × ZnO = the two-way interaction between grain preservation method and ZnO inclusion.
[a] Calculated DE (MJ/kg) = [analysed dietary GE value (MJ/kg) × GE digestibility coefficient].

Table 6
The effect of grain preservation method on the coefficient of apparent ileal digestibility of dry matter (DM), organic matter (OM), ash, nitrogen (N) and gross energy (GE) on day 35 post-weaning (least square mean with their SEM).

	Treatments		SEM	P-value
Preservation method	Dried	Preserved		
DM	0.691	0.762	0.016	**0.009**
OM	0.704	0.774	0.016	**0.008**
Ash	0.525	0.592	0.028	0.124
N	0.686	0.769	0.022	**0.012**
GE	0.649	0.737	0.019	**0.006**

SEM, standard error of the mean.

3.6.2. Differential bacterial abundance analysis

The effects of grain preservation method on bacterial phylum, family and genus of ileal and colonic digesta are presented in Tables 8–10. The only bacterial phyla identified in the ileal digesta were Firmicutes, which were also the most dominant phyla in the colonic digesta (~ 92.3 %), followed by Actinobacteria (~ 2.2 %), Bacteroidetes (~ 1.7 %), Tenericutes (~ 1.3 %) and Proteobacteria (~ 1.2 %). There was a grain preservation effect on the relative abundance of Actinobacteria in the colon with pigs offered the preserved grain diet having a higher abundance compared to pigs offered the dried grain diet ($P < 0.05$).

At family level, the preserved grain diet increased the relative abundance of *Clostridiaceae* and decreased the relative abundance of *Streptococcaceae* in the ileum compared to the dried grain ($P < 0.01$). Within the phylum Actinobacteria, an increase in the relative abundance of *Coriobacteriaceae* was observed in the colon of pigs offered the preserved grain diet ($P < 0.05$).

At genus level, the preserved grain diet increased the relative abundance of *Clostridium* ($P < 0.01$) and decreased the relative abundance of *Streptococcus* ($P < 0.001$) in the ileum. In the colon, the preserved grain diet increased the relative abundance of *Collinsella* and *Faecalibacterium* ($P < 0.05$) and decreased the relative abundance of *Dorea*, *Eubacterium* and *Roseburia* compared to the dried grain diet ($P < 0.05$).

3.7. Volatile fatty acids

The effect of grain preservation method on ileal and colonic digesta VFA concentrations at day 35 PW are presented in Table 11. Pigs offered the preserved grain diet had reduced propionate, iso-butyrate and valerate concentrations in the ileum compared to pigs offered the dried grain diet ($P < 0.05$). Pigs offered the preserved grain diet also had reduced iso-valerate and branched-chain fatty acids (BCFA) in the colon compared to pigs offered the dried grain diet ($P < 0.05$).

4. Discussion

Grain preservation is essential for successful cereal production, particularly in regions where grain moisture fluctuates due to weather conditions. Ensuring the safe storage of grain harvested below 850 g/kg DM is crucial (Yu and Pedroso, 2023). This study aimed to investigate whether an OA liquid surfactant mould inhibitor is a superior alternative to conventional drying for preserving wheat and barley below this critical DM threshold.

Despite the variations in DM after storage, both preservation methods resulted in grains with similar chemical compositions on a

Table 7
The effect of grain preservation on measures of alpha diversity at day 35 post-weaning (least square mean with their SEM).

Preservation method	Treatments: Dried	Treatments: Preserved	SEM	P-value
Ileum				
Observed	72.10	69.67	1.384	0.219
Chao1	78.30	73.02	2.501	0.144
ACE	75.64	73.26	1.714	0.329
Shannon	3.70	3.62	0.030	0.079
Simpson	0.96	0.96	0.001	0.080
InvSimpson	27.10	24.51	0.931	0.061
Fisher	18.55	17.70	0.472	0.210
Colon				
Observed	68.42	69.92	2.367	0.659
Chao1	70.50	72.53	2.416	0.559
ACE	70.26	72.70	2.472	0.494
Shannon	3.76	3.76	0.041	0.967
Simpson	0.97	0.97	0.002	0.972
InvSimpson	30.77	31.07	1.471	0.886
Fisher	17.30	17.89	0.811	0.616

SEM, standard error of the mean.

Table 8
The effect of grain preservation method on the % bacterial abundance at day 35 post-weaning at phylum level (least square mean with their SEM).

Phylum Preservation method	Treatments Dried	 Preserved	SEM	P-value
Ileum				
Firmicutes	99.71	99.72	3.084	0.999
Colon				
Actinobacteria	1.35	3.02	0.419	**0.013**
Bacteroidetes	2.00	1.30	0.369	0.202
Firmicutes	92.72	91.85	2.773	0.827
Proteobacteria	1.07	1.27	0.312	0.646
Tenericutes	1.49	1.07	0.326	0.371

SEM, standard error of the mean.

fresh weight basis. This corroborates with previous research suggesting that OA can serve as a practical and effective alternative to conventional drying methods for cereal grains (Young et al., 1970; Higgins and Brinkhaus, 1999; Rutenberg et al., 2018). Traditionally, a lower DM content is associated with an increased risk of fungal growth and mycotoxin contamination. However, the results of the current study suggest that the OA mould inhibitor effectively mitigated mould and mycotoxins in both the wheat and barley during storage. Dried wheat and barley exhibited higher concentrations of DON, a mycotoxin produced by Fusarium species (Magan and Aldred, 2007). The presence of DON in animal feed has been associated with reduced feed intake and growth rates, as well as adverse effects on intestinal barrier function and immune responses in animals (Weaver et al., 2014; Lessard et al., 2015). Additionally, the dried barley demonstrated elevated levels of OTA and HT-2 toxin compared to the preserved barley. Although a recent assessment deemed OTA to be a low risk to animal health [EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2023], exposure to even low levels of OTA has been shown to negatively impact pigs, affecting their performance and causing immunosuppression (Malagutti et al., 2005; Bernardini et al., 2014; Malir et al., 2016). Although the mycotoxin levels in both preservation methods remained below the European Union's regulatory limits and guidelines for cereal grains destined for pig feed (EU Commission, 2003,

Table 9
The effect of grain preservation method on the % bacterial abundance at day 35 post-weaning at family level (least square mean with their SEM).

Family	Treatments		SEM	P-value
Preservation method	Dried	Preserved		
Ileum				
Clostridiaceae	9.29	15.02	1.121	**0.003**
Lactobacillaceae	75.84	79.69	2.863	0.356
Streptococcaceae	14.47	5.00	0.988	**<0.001**
Colon				
Clostridiaceae	5.00	4.56	0.631	0.631
Coriobacteriaceae	0.87	2.35	0.356	**0.012**
Erysipelotrichaceae	1.17	0.70	0.276	0.248
Eubacteriaceae	2.13	1.09	0.361	0.062
Hungateiclostridiaceae	1.30	1.39	0.335	0.851
Lachnospiraceae	19.22	18.62	1.256	0.737
Lactobacillaceae	24.07	22.52	1.393	0.440
Oscillospiraceae	0.94	1.09	0.299	0.706
Peptococcaceae	0.09	0.14	0.098	0.728
Prevotellaceae	1.67	1.35	0.354	0.531
Ruminococcaceae	28.95	32.29	1.597	0.154
Streptococcaceae	7.84	9.02	0.838	0.332

SEM, standard error of the mean.

Table 10
The effect of grain preservation method on the % bacterial abundance at day 35 post-weaning at genus level (least square mean with their SEM).

Genus	Treatments		SEM	P-value
Preservation method	Dried	Preserved		
Ileum				
Clostridium	9.29	16.69	1.189	**0.001**
Lactobacillus	75.84	77.43	2.918	0.705
Streptococcus	14.47	5.55	1.027	**<0.001**
Colon				
Agathobacter	1.02	1.16	0.301	0.757
Anaerobacterium	0.96	1.08	0.292	0.775
Blautia	3.00	3.93	0.536	0.235
Butyricicoccus	1.89	1.72	0.386	0.756
Catenibacterium	0.49	0.38	0.190	0.678
Clostridium	3.03	2.83	0.494	0.776
Collinsella	0.89	2.34	0.357	**0.014**
Coprococcus	3.91	5.71	0.916	0.059
Dorea	8.36	5.28	0.749	**0.009**
Eubacterium	2.20	1.08	0.364	**0.048**
Faecalibacterium	13.70	18.07	1.148	**0.014**
Gemmiger	8.44	9.12	0.855	0.581
Holdemanella	0.50	0.31	0.183	0.481
Lactobacillus	24.26	22.45	1.395	0.370
Methanosphaera	0.16	0.37	0.146	0.346
Oscillibacterum	0.95	0.88	0.277	0.857
Peptococcus	0.09	0.14	0.098	0.729
Phascolarctobacterium	0.22	0.22	0.136	0.980
Prevotella	1.09	0.91	0.288	0.657
Pseudobutyrivibrio	0.34	0.56	0.192	0.423
Roseburia	1.63	0.62	0.298	**0.034**
Ruminococcus	2.17	3.00	0.463	0.226
Streptococcus	7.93	8.94	0.838	0.404

SEM, standard error of the mean.

2006, 2013), the observed differences may partially explain the differences in pig performance between the dietary treatments.

The weaning process introduces abrupt nutritional, environmental, and psychological changes for piglets, often resulting in reduced feed intake and transient growth setbacks (Lallès et al., 2007). These challenges frequently coincide with the onset of PWD (Rhouma et al., 2017). Historically, pharmacological levels of ZnO were added to PW pig diets to mitigate these issues (Sales, 2013). In the current study, the inclusion of ZnO in both dried and preserved grain diets reduced faecal scores and diarrhoea incidence during the first 21 days PW. However, pigs offered the preserved grain diet exhibited lower faecal scores and diarrhoea incidence compared to those offered the dried grain diet but not to the same extent as those supplemented with ZnO. As a result, this technology may be more applicable after 21 days PW. While a wide variety of nutritional strategies have been researched to replace in-feed antimicrobials and

Table 11
The effect of grain preservation method on total VFA concentrations in ileal and colonic digesta at day 35 post-weaning (least square mean with their SEM; mmol/g digesta).

	Treatments		SEM	P-value
Preservation method	Dried	Preserved		
Ileum (mmol/ g digesta)				
Total	22.78	29.60	5.130	0.359
Acetate	19.28	25.68	4.266	0.302
Propionate	1.71	0.52	0.387	**0.042**
Butyrate	1.26	2.98	0.928	0.206
Iso-butyrate	0.10	0.05	0.017	**0.049**
Iso-valerate	0.30	0.32	0.070	0.837
Valerate	0.13	0.05	0.008	**<0.001**
Branched-chain fatty acids[a]	0.52	0.42	0.081	0.377
Colon (mmol/ g digesta)				
Total	263.62	246.99	17.752	0.515
Acetate	132.71	124.71	10.916	0.609
Propionate	72.31	70.36	5.494	0.804
Butyrate	39.74	36.13	3.425	0.464
Iso-butyrate	3.39	2.94	0.365	0.395
Iso-valerate	6.74	3.83	0.894	**0.032**
Valerate	8.74	6.99	1.029	0.244
Branched-chain fatty acids[a]	18.86	13.66	1.680	**0.041**

SEM, standard error of the mean.
[a] Branched-chain fatty acids were calculated by the sum of iso-butyrate, iso-valerate and valerate.

ZnO (de Lange et al., 2010; O'Doherty et al., 2017), no alternative has been identified to match the growth performance and diarrhoea reduction provided by in-feed antimicrobials and ZnO (O'Doherty et al., 2021). These nutritional strategies are no longer considered alternatives but will be essential to improve digestive health in the absence of ZnO in combination with other strategies.

Throughout the experimental period, pigs fed the preserved grain diet showed higher ADFI compared to those fed the dried grain diet, with intake levels similar to those supplemented with ZnO. Some studies have reported variable differences in dietary feed intake (Ettle et al., 2004) and concerns in relation to the palatability of OA-supplemented diets (Eisemann and Van Heugten, 2007). However, recent studies have shown that supplementing with blends of OA can enhance ADFI and palatability (Kuang et al., 2015; Xu et al., 2016; Nowak et al., 2021). This improvement in ADFI supports the increased ADG, final BW and tendency of enhanced feed efficiency observed in pigs fed the preserved grain diet. These findings align with previous research, which also reported similar growth improvements with dietary OA supplementation during feed manufacture (Luise et al., 2017; Long et al., 2018; Xiang et al., 2021).

The inclusion of ZnO in the dried grain diet positively impacted ADFI and BW throughout the experimental period, leading to increased ADG in pigs during the first 21 days PW. The benefits of ZnO supplementation on growth parameters were most pronounced during the initial 2–3 weeks PW, which is a critical period for maximising feed intake (O'Doherty et al., 2017). The CATTD of nutrients in the dried grain diet was also enhanced with the addition of ZnO. As a cofactor for digestive enzymes, ZnO improves the breakdown and absorption of proteins, carbohydrates, and lipids (Hu et al., 2012; Pluske, 2013; Lei and Kim, 2018). Additionally, the antimicrobial properties of ZnO positively affect the gut microbiota, by reducing pathogenic bacteria, promoting a healthier gut environment and improving diet digestibility (Bonetti et al., 2021). However, similar to the current study, excessive or prolonged ZnO supplementation can negatively affect growth (Brugger and Windisch, 2017; Conway et al., 2022). This was particularly evident when ZnO was added to the preserved grain diet, where ZnO inclusion reduced final BW. The reduction in performance can be partially explained by the decrease in CATTD of nutrients, observed when ZnO was added to the preserved grain diet.

Dietary OA supplementation has been recognised for its ability to enhance gastrointestinal acidity, proteolysis, and nutrient digestibility (Wang et al., 2022). However, preserving grains with the OA mould inhibitor in the current study did not affect gastric pH and had minimal effects on the expression of a panel of key genes in the fundic and pyloric gland regions of the stomach. The gastrin receptor gene, *CCKBR*, had increased expression in the pyloric gland region of pigs offered the preserved grain diet. The *CCKBR* gene is involved in the process of acid stimulation in the stomach. Nevertheless, given the absence of differential expression of other genes involved in the process of acid secretion and the fact that the pyloric gland region is not the predominant site for *CCKBR* expression in the stomach (Kiernan et al., 2023), it is difficult to interpret this finding with any major significance. However, pigs offered the preserved grain diet showed significant improvements in the CATTD and CAID of nutrients compared to pigs offered the dried grain diet.

Preserving wheat and barley with the OA mould inhibitor reduced the starch content in both grains during storage and in their respective diets after diet manufacture on an as-fed basis, in contrast to their dried counterparts. This reduction in starch content is consistent with findings from Xu et al. (2016), where corn preserved with an OA preservative was fed to grow-finisher pigs. These authors also observed lower concentrations of resistant starch in the preserved corn compared to dried corn. Increasing levels of resistant starch can impact the digestibility of nutrients in pigs PW (Morel et al., 2005; Sun et al., 2006; Gerritis et al., 2012; Cervantes-Pahm et al., 2014), which may partially explain the depression in CAID of nutrients in pigs fed the dried grain diet. Unfortunately resistant starch was not measured in this study. The OA have also been shown to directly affect the breakdown of fibre (Mosier et al., 2001), which may explain the improvements in the CAID of aNDF in pigs offered the preserved grain diet.

The gut microbial composition can be positively influenced by nutritional interventions to enhance pig performance and health, with OA playing a significant role in inhibiting the growth of undesirable microorganisms (Ngoc et al., 2020) and potentially limiting dysbiosis. In the current study, the abundance of ileal *Clostridium* and colonic *Faecalibacterium* and *Collinsella* were increased in pigs offered the preserved grain diet. Dietary supplementation of *Clostridium* probiotics can improve growth performance (Yang et al., 2012; Zhang et al., 2018), reduce diarrhoea (Chen et al., 2018) and enhance nutrient digestibility (Han et al., 2020). *Faecalibacterium* is considered to be one of the most promising next-generation probiotics due to its strong anti-inflammatory properties and positive effects against gastrointestinal diseases (Sokol et al., 2008, Cao et al., 2014; Lopez-Siles et al., 2018). The abundance of *Faecalibacterium prausnitzii* increases with weaning age, and its presence has been associated with increased resilience in pigs (Massacci et al., 2020). Additionally, *Collinsella* is positively associated with feed intake (Kubasova et al., 2018; Vigors et al., 2020), nutrient digestibility (Niu et al., 2019) and immune function in pigs (Wu et al., 2017). Under the conditions of this study, these findings suggest an overall improvement in intestinal health and highlight the potential of preserved grain on pig performance, particularly after the first 21 days PW.

Significant reductions in the abundance of ileal *Streptococcus* and colonic *Dorea* and *Eubacterium* were also observed in pigs offered the preserved grain diet. *Streptococcus* has been associated with various health issues in pigs, including pneumonia, meningitis, septicaemia, and arthritis (Su et al., 2008). In contrast, *Dorea* is typically considered part of a healthy gut microbiota; however, some studies have suggested that *Dorea* can metabolise mucin and induce pro-inflammatory cytokines, potentially having adverse effects on pig health (Crost et al., 2013; Shahi et al., 2017). *Eubacterium* members are known for producing butyrate, which has an important role in suppressing gut inflammation, modulating the immune system, and regulating energy balance. This unexpected reduction may have been due to an adaptive mechanism, where pigs offered the preserved diet had ingested sufficient OA, suppressing the growth of *Eubacterium* (Tugnoli et al., 2020). Consistent with this finding, Xiang et al. (2021) also reported a decrease in *Dorea* and *Eubacterium* in pigs supplemented with OA through their drinking water. These authors found that OA supplementation led to decreased pro-inflammatory cytokines and increased host defence peptides and tight junction gene expression in the jejunum of pigs compared to non-supplemented pigs. Although intestinal gene expression was not measured, the results from this study suggest further investigation is required to evaluate the impact of feeding preserved grain on the expression of genes related to nutrient transport and gut immune parameters, particularly during the critical first two weeks PW when pigs experience the most significant nutritional, environmental, and physiological stress (Campbell et al., 2013).

Although no significant impact on the total VFA concentrations were observed on day 35 PW, pigs offered the preserved grain diet exhibited reductions in specific VFA such as iso-butyrate and valerate in the ileum, as well as iso-valerate and total BCFA in the colon. These findings align with Piva et al. (2002) who reported a similar decrease in BCFA when grower pigs were supplemented with a blend of dietary OA. These BCFA are unique metabolites generated through microbial fermentation of specific amino acids. They can significantly affect the intestinal mucosa by altering the acid-base balance necessary for water absorption in the large intestine (Jha and Berrocoso, 2016). Imbalances in this equilibrium can trigger PWD and contribute to growth retardation (García et al., 2014). The reduction in these metabolites further explains the observed improvements in faecal scores, reduced diarrhoea incidence, and enhanced performance in pigs offered the preserved grain diet.

5. Conclusion

In conclusion, this study highlights the promising potential of the organic acid mould inhibitor used to maintain grain quality, especially under challenging weather conditions. The preserved grains not only exhibited minimal chemical composition discrepancies compared to traditionally dried grains but also maintained mycotoxin levels well within safe levels. Significantly, the use of grains preserved with the OA mould inhibitor in post-weaned pig diets was associated with enhanced growth performance, including increased daily feed intake, daily gain, and body weight. These results could be due to the improvements in nutrient digestibility observed in pigs offered the preserved grain diet or the beneficial impact the preserved grain diet had on the gut microbiome. These findings are pivotal for the pig sector, offering insights into sustainable and efficient grain preservation methods that not only preserve quality but also contribute positively to animal health and growth, in the absence of ZnO, thereby potentially revolutionising grain processing and animal nutrition strategies.

Funding

This research was supported by the Science Foundation Ireland (BiOrbic, Dublin, Ireland) and Adesco Nutricines (Dungarvan, Waterford, Ireland) [Grant no. 16/RC/3889].

Author Statement

We would like to thank the editor and reviewers for the thorough review of our manuscript.

CRediT authorship contribution statement

Shane Maher: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Torres Sweeney:** Writing – review & editing, Resources. **Dillon P. Kiernan:** Data curation. **Marion T. Ryan:** Data curation. **Vivian Gath:** Data curation. **Stafford Vigors:** Writing – review & editing, Formal analysis. **Kathryn Ruth Connolly:** Data

curation. **John O'Doherty:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors want to thank Bernie Flynn, Maria Markiewicz-Keszycka, Denise Cunningham and Kevin Thornton for their help with the analysis of feed, faecal and tissue samples. The authors wish to express sincere thanks to John Ryan for his assistance with sample collections.

Declaration of competing interest

None of the authors had a financial or personal conflict of interest concerning the current study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2024.116078.

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