Maternal supplementation with *Bacillus altitudinis* spores improves porcine offspring growth performance and carcass weight.

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Complete List of Authors:	Crespo-Piazuelo, Daniel; TEAGASC, Pig Development Department Gardiner, Gillian; Waterford Inst. of Technology, Department of Science Ranjitkar, Samir; TEAGASC, Pig Development Department Bouwhuis, Meike; TEAGASC, Pig Development Department Ham, Rebecca; Waterford Inst. of Technology, Department of Science Phelan, John; Waterford Inst. of Technology, Department of Science Marsh, Alan; Waterford Inst. of Technology, Department of Science Lawlor, Peadar; TEAGASC, Pig Development Department
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Maternal supplementation with *Bacillus altitudinis* spores improves porcine offspring growth performance and carcass weight

Daniel Crespo-Piazuelo[1]†, Gillian E. Gardiner[2]†*, Samir Ranjitkar[1], Meike A. Bouwhuis[1], Rebecca Ham[2], John P. Phelan[2], Alan Marsh[2], and Peadar G. Lawlor[1]

[1]Pig Development Department, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland.
[2]Eco-Innovation Research Centre, Department of Science, Waterford Institute of Technology, Waterford, Ireland.

†These authors contributed equally to this work.

*Corresponding author: Gillian Gardiner, +353 51 302626, ggardiner@wit.ie

Running head: Maternal and post-weaning supplementation with probiotic spores

Key words: probiotic, sow, pig, swine, small intestinal morphology, colostrum

Abstract

The objective of this study was to evaluate the effect of feeding *Bacillus altitudinis* spores to sows and/or offspring on growth and health indicators. On day (D) 100 of gestation, 24 sows were selected and grouped as: control (CON), fed with a standard diet; and probiotic (PRO), fed the standard diet supplemented with *B. altitudinis* WIT588 spores from D100 of gestation until weaning. Offspring (n=144) from each of the two sow treatments were assigned to either a CON (no probiotic) or PRO (*B. altitudinis*-supplemented) treatment for 28 days post-weaning (pw), resulting in four treatment groups: 1) CON/CON, non-probiotic supplemented sow/non-probiotic supplemented piglet; 2) CON/PRO, non-probiotic supplemented sow/probiotic-supplemented piglet; 3) PRO/CON, probiotic-supplemented sow/non-probiotic supplemented piglet; 4) PRO/PRO, probiotic-supplemented sow/probiotic-supplemented piglet. *Bacillus altitudinis* WIT588 was detected in the faeces of probiotic-supplemented sows and their piglets, and in the faeces and intestine of probiotic-supplemented piglets. Colostrum from PRO sows had higher total solids (P=0.02), protein (P=0.04), and true protein (P=0.05), and lower lactose (P<0.01) than colostrum from CON sows. Maternal treatment improved offspring feed conversion ratio at D0-14 pw (P<0.001) and increased offspring bodyweight at D105 and D127 pw (P=0.01), carcass weight (P=0.05) and kill-out percentage (P<0.01). It also increased small intestinal absorptive capacity and impacted the haematological profile of sows and progeny. Little impact of post-weaning treatment was observed on any of the parameters measured. Overall, the lifetime growth benefits in the offspring of *B. altitudinis*-supplemented sows offer considerable economic advantages for pig producers in search of alternatives to in-feed antibiotics/zinc oxide.

Introduction

Stress at weaning can negatively impact piglet immunity and gut health, impairing growth and feed efficiency and often resulting in diarrhoea[1]. Along with the stress of weaning, passive immunity of the piglets is also reduced at this time, while active immunity is not fully developed. This makes weaned pigs more prone to disease[2], in particular post-weaning diarrhoea which can be caused by enterotoxigenic *Escherichia coli*[3] or other pathogens[4]. To reduce the incidence of these pathogens and the occurrence of post-weaning diarrhoea and to prevent the weaning-associated growth check, in-feed antibiotic and/or zinc oxide treatments are frequently used[5]. However, following the ban of in-feed antibiotic growth promoters in the European Union (EU) in 2006, a ban on the preventive use of antibiotics in groups of animals and via medicated feed will enter into force in the EU in 2022. In the same year, the use of pharmacological levels of zinc oxide will also be banned. As a result, alternative treatments, such as probiotics will be of increased importance in the future. Probiotics not only control pathogens, but they can also improve pig growth and feed efficiency[5,6].

Bacteria from *Bacillus* spp. are commonly used as probiotics in pig production[7–9]. Species from this genus form spores, which increases their resistance to hostile conditions such as those encountered in the gastrointestinal tract and during feed manufacture[10,11]. In addition, the vegetative cells of *Bacillus* spp. produce extracellular enzymes, which can increase nutrient availability in the diet and improve digestibility[12], and *Bacillus* are well-known for the production of antimicrobials[13–15]. On this basis, many studies which administered spores of *Bacillus* spp. to weaned pigs found improved growth performance and feed conversion[16–18], while the incidence of post-weaning diarrhoea was also reduced in some cases[19]. Nevertheless, commencing the administration of *Bacillus* spores to pigs post-weaning may not be the most effective strategy. Firstly, it may be too late, as evidence suggests that early-life gut microbiota interventions are more effective[20–25]. Secondly, the spores may not germinate in the gastrointestinal tract[26]; and lastly, which may/may not be related to lack of germination, *Bacillus* administered as spores do not usually persist for more than one week after ceasing administration[12].

A cheaper and potentially more effective alternative to probiotic supplementation of post-weaning diets is the inclusion of *Bacillus* spores in the diet of gestating and/or

lactating sows. Vertical transmission of the probiotic from sows to their offspring then occurs between birth and weaning[27,28], although this is sometimes limited[29]. Maternal administration can also benefit the sow, minimizing weight loss during lactation and improving reproductive performance and milk quality[18,20,30,31]. These maternal benefits sometimes increase the number of piglets weaned per sow[30], although some studies did not find any significant effects on sow productivity[22,24,27,28]. Probiotic administration to sows also leads to improved weight gain and feed efficiency in the offspring post-weaning[18,20,22,28]. However, the mechanisms by which maternal probiotic supplementation benefits offspring growth are not fully understood. Probiotic administration stimulates the immune system of sows, which confers passive immunity to offspring through colostrum and milk[32]. Stimulation of the immune system of the piglets may even start before the piglets are born, as piglets become immunocompetent *in utero* and their active immunity depends on maternal antibody levels[33]. Furthermore, the faecal bacterial community of the sows, including any administered probiotic and/or probiotic-modulated taxa, can be transferred to their litter through the intake of maternal faeces[28].

However, most studies that administer probiotics to gestating/lactating sows do not follow the growth of offspring beyond the weaner stage, as they are usually focused on the incidence of post-weaning diarrhoea[7,18,20,24,27–29,31]. The aim of the present study was therefore to evaluate the efficacy of a novel *Bacillus altitudinis* probiotic delivered as spores to sows and/or their offspring on sow health, reproductive performance and colostrum quality, as well as on lifetime growth and health and carcass characteristics of the offspring.

Materials and Methods

Ethical approval

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC148/2017) and the project was authorised by the Health Products Regulatory Authority (project authorisation no. AE19132/P066). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

Experimental design and diets

A total of 24 sows (Large White × Landrace; Hermitage Genetics, Sion Road, Co. Kilkenny, Ireland) were selected on day 100 (D) 100 of gestation and blocked by parity, body weight (BW) and back fat (BF) depth, following which they were individually housed and randomly assigned to one of two experimental treatment groups as follows: 1) control (CON, *n*=12), fed with a standard gestation diet from D100 of gestation to farrowing, followed by a standard lactation diet for 26 days until litters were weaned; and 2) probiotic (PRO, *n*=12), fed the standard gestation/lactation diet supplemented with *B. altitudinis* WIT588 spores (~4×10^9 spores daily from D100 of gestation to farrowing and ~1.2×10^{10} spores daily during lactation for 26 days until weaning of litters, administered as outlined below). Cross-fostering of piglets was performed between 24 and 48 h *post-partum* to equalize litter size (14 piglets/litter) if necessary, but only within the same treatment group.

At weaning (at D26 ± 1.5 of age), a total of 144 piglets from these sows (n=72/sow treatment) were selected across all litters, blocked by sow treatment, sex, BW and litter origin and randomly assigned to dietary treatments. Offspring from each of the two sow treatments were assigned as same gender pairs of pigs to either a CON (no probiotic) or PRO (probiotic-supplemented) treatment for 28 days post-weaning (pw), resulting in four treatment groups (n=36 piglets/treatment) as follows: 1) piglets weaned from CON sows, fed a CON diet (CON/CON); 2) piglets weaned from CON sows, fed a probiotic-supplemented diet (CON/PRO); 3) piglets weaned from PRO sows, fed a CON diet (PRO/CON); and 4) piglets weaned from PRO sows, fed a probiotic-supplemented diet (PRO/PRO). Probiotic supplementation consisted of ~1×10^9 CFU of *B. altitudinis* WIT588 spores administered daily, as outlined below. Probiotic supplementation ceased at D28 pw, but pigs were monitored until the end of the finisher period (~D127 pw). A starter/link diet was fed for the first 28 days pw, followed by a weaner diet until D55 pw, and thereafter a finisher diet was fed until slaughter at D127 pw.

The ingredient composition and nutrient content of all sow and offspring diets are shown in **Table 1**. The diets were manufactured in the Teagasc feed mill (Moorepark, Fermoy, Co. Cork) and were formulated to meet or exceed National Research Council recommendations (NRC, 2012)[34] for pigs at the relevant stage of the production cycle. All starter/link diets were formulated with 10.74 MJ/kg net energy and 14.0 g/kg standardised ileal digestible lysine (SID Lys) using the same ingredients. Similarly, the

weaner diet was formulated with 10.55 MJ/kg net energy and 11.49 g/kg SID Lys. The finisher diet was formulated with 9.80 MJ/kg net energy and 9.97 g/kg SID Lys. All diets were fed in 3 mm pellet form. Sows were fed 2.7 kg/day of feed up to the day of farrowing and thereafter were provided with *ad libitum* access to feed from a trough using a computerised feed delivery system (DryExact Pro, Big Dutchman, Vechta, Germany). Water was available on an *ad libitum* basis to sows during gestation and lactation from a single-bite drinker in the feed trough and to suckling piglets from a bowl in the farrowing pen. Suckling piglets were offered creep feed in pelleted form from D12 of age to weaning. At all stages post-weaning, pigs were provided with *ad libitum* access to feed from a 30 cm wide stainless-steel feeder (O'Donovan Engineering, Coachford, Co. Cork, Ireland) and to water from one nipple-in-bowl drinker (BALP, Charleville-Mezieres, Cedex, France). Representative samples were taken from all diets and analysed for dry matter, ash, crude protein, total oil, crude fibre, and neutral detergent fibre by Sciantec Analytical Services Limited, Cawood, UK.

Preparation and administration of probiotic spores

Bacillus altitudinis WIT588 is a rifampicin resistant variant of a seaweed-derived isolate (WIT572; NCIMB 43558) characterized, both *in vitro* and *in vivo* as a probiotic for pigs, used to facilitate enumeration in the porcine gastrointestinal tract[26,35,36]. The strain was first referred to as *B. pumilus* on the basis of sequencing of the *gyrB* and *pyrE* genes[26], but has since been identified as *B. altitudinis* on the basis of whole genome sequencing (unpublished data). The *B. altitudinis* WIT588 spore suspension used in the current feeding trial was prepared according to the nutrient exhaustion method described by Prieto *et al*. (2014)[36] and the spores were suspended in sterile water. The concentration was then determined using a haemocytometer and adjusted to ~10^9 spores/ml. Aliquots of this spore suspension were stored at -20°C until use. Probiotic spores were administered once daily in the morning to the respective treatment groups. The doses used for sows and weaned pigs, as outlined above, were calculated based on data from previous experiments and doses used for comparable commercially available probiotics. The amount of spore suspension required each day was thawed over night at 4°C. On the morning of administration, spore suspensions were diluted in distilled water to the required dose and top-dressed onto the feed in a final volume of 4 ml for

gestating sows and weaned pigs and 12 ml for lactating sows. The same volume of sterile water was top-dressed onto the feed of CON pigs not administered probiotic.

Animal housing and management

PRO sows were housed separately from CON sows, with two farrowing rooms for PRO sows, each with 7 pens per room, and one room for CON sows with 14 pens per room. Farrowing pens (2.5 m × 1.8 m) had a farrowing crate on a partially slatted floor with a heated floor pad for piglets. The temperature of the farrowing rooms was maintained at ~24°C at farrowing and gradually reduced to 21°C by D7 of lactation. Each room was illuminated by daylight and artificial light. The temperature inside the building was automatically controlled. Ventilation was via punched ceiling ventilation with air exhausted via a variable speed fan linked to a thermostat and controlled automatically via a controller (135-L2 Pro climate computer; Big Dutchman, Vechta, Germany) outside each room.

At weaning, piglets were housed in same gender pairs in 72 pens (n=2 pigs/pen) across 4 rooms. Each room contained 24 pens (1.2 m × 0.9 m), with treatments distributed equally across rooms. Pens were fully slatted with plastic flooring (Faroex, Manitoba, Canada). Empty pens were left between treatments to minimise probiotic cross-contamination and strict hygiene procedures were followed. Pigs were penned as pairs for the first 7 days post-weaning. A total of 40 pigs (n=10/treatment; one pig from each of 10 pen pair replicates per treatment) were sacrificed by captive bolt stunning followed by exsanguination on D8 pw to facilitate sampling of digesta and intestinal tissue. To coincide with this, one pig from each of the remaining pairs of pigs was removed from the trial at this time also and the remaining piglets (n=72) were individually penned until slaughter at D127 pw. The temperature of the weaner rooms was maintained at 28°C for the first 7 days pw, gradually reduced to 22°C by D28 pw and maintained at 22°C until D56 pw. Temperature and ventilation were controlled by a hot air heating system and an exhaust fan drawing air from under slat level connected to a controller (Stienen PCS 8400; Stienen BV, Nederweert, the Netherlands). At D56 pw, pigs were moved to one of 4 finisher rooms, each with 18 pens/room, where they were individually penned in fully slatted pens (1.81 m × 1.18 m) until the end of the experimental period (D127 pw). Pigs were kept in the same order as in the weaner rooms but without the empty pen between treatments. Finisher rooms were ventilated

with fans and air inlets controlled by a Stienen PCS 8200 controller (Stienen BV). Air temperature was maintained at 20 to 22°C. Sows and piglets were observed closely at least twice daily. Any pig showing signs of ill health was treated as appropriate and this was recorded. All veterinary treatments were recorded, including identity of pig, symptom, medication used and dosage.

Data recording and sampling

During sampling and weighing of sows and offspring, strict hygienic measures were taken to prevent cross-contamination between treatments. CON pigs not receiving probiotic were handled first, followed by PRO treatment groups. Gloves were changed between pigs, and fresh disposable overalls were worn by all personnel prior to commencing sampling of each treatment group. All equipment, such as weighing scales and the cradle used for collection of blood samples was disinfected thoroughly with 1% Virkon® after use to prevent cross contamination at subsequent weighings/samplings. In both CON and PRO farrowing rooms and beside both PRO and CON pens within the weaner rooms, settle plates containing agar medium selective for the probiotic strain (see below) were exposed for 30 min at faecal sampling time points, and incubated with the faecal sampling plates as outlined below in order to check for the presence of the probiotic strain in the air.

Sow body weight and back fat thickness

Feed intake of sows was recorded daily between D100 of gestation and D28 of lactation. Body weight and BF were recorded at the start of the experiment (D100 of gestation), on the expected farrowing date (D114 of gestation), and again at weaning of litters (~D26 of lactation). Sow BW was recorded using an electronic sow scales (EziWeigh 7i, O'Donovan Engineering, Co. Cork, Ireland). Sow BF was measured using a digital BF indicator (Renco LEAN-MEATER, Renco Corporation, Golden Valley, Minneapolis, USA) by placing the probe of the digital indicator on the back of the sow at the level of the second last rib, 6.5 cm from the side of the backbone. A reading was taken from the right and left side of the sow's back and the average of both readings was recorded.

Colostrum and milk sampling

Colostrum samples (n=12 sows/treatment) were collected by manual milking of the first four teats immediately distal to the sow's head on one side of the udder within 12 h of farrowing. On D14 of lactation, milk samples were collected from sows (n=12 sows/treatment) in the same way but this time following administration of a 1 ml (10 IU) intramuscular injection of oxytocin (Eurovet 247 Animal Health, Bladel, Netherlands) to induce milk let-down. All samples were stored at -20°C until analysis.

Litter data at birth and pre-weaning piglet growth performance

Reproductive parameters were recorded per litter i.e. number of piglets (total born, born alive, stillborn). The weight and sex of each piglet was recorded at birth, and each piglet was tagged for identification purposes. Thereafter, piglets were individually weighed at birth (D0), D14 and D26 *post-partum* and these data was used to determine pre-weaning piglet average daily gain (ADG). Piglet mortality between birth and weaning was also recorded.

Post-weaning growth performance, faecal scoring and carcass measurements

Growth performance of piglets was measured by weighing pigs individually and monitoring individual feed intake in order to calculate ADG, average daily feed intake (ADFI), and feed conversion ratio (FCR). Feed disappearance was recorded weekly and pigs were individually weighed at weaning (D0 pw), D14 pw, the changeover to weaner feed (D28 pw), changeover to finisher feed (D56 pw), D105 pw and immediately before slaughter (D127 pw). Pigs were fasted for 12 h prior to pre-slaughter weighing.

The incidence of post-weaning diarrhoea was assessed by daily faecal consistency scoring between weaning and D28 pw. The scoring system used was as follows: 0 for dry pelleted faeces; 1 for soft faeces with shape; 2 for very soft or viscous liquid faeces (mild diarrhoea); and 3 for severe diarrhoea with or without blood.

Pigs were slaughtered at ~123.5 ± 1.38 kg SEM live-weight by CO_2 stunning followed by exsanguination. Carcass weight was estimated by multiplying the weight of the hot eviscerated carcass 45 min after slaughter by 0.98. Kill out percentage was calculated as carcass weight/live-weight at slaughter. Back fat thickness and muscle depth measured at 6 cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland,

New Zealand). Lean meat content was estimated according to the following formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm)[37].

Faecal sampling

Faecal samples were collected from sows (*n*=24) directly from the rectum using gentle digital stimulation on D100 and D115 of gestation, ~D13 of lactation and at weaning of litters (~D26 of lactation). Pre-weaning, rectal swabs were taken from offspring on ~D13 of lactation (*n*=12 pig replicates per treatment) and faecal samples were obtained by digital rectal stimulation at weaning (*n*=10 pig replicates per treatment), D27 pw and D56 pw (*n*=10 pig replicates per treatment). Faeces were collected into sterile containers and, together with swabs, were put on ice and stored at 4°C until analysis for the administered probiotic strain (within 12 h), as outlined below.

Blood sampling

Blood samples were taken from sows (n=24) by anterior vena cava/jugular venepuncture on D100 and D114 of gestation and at weaning of litters (~D26 of lactation). Piglets (*n*=10 pig replicates per treatment) were blood sampled by anterior vena cava/jugular venepuncture on D0 pw, D28 pw and D57 pw. Blood samples were also collected from piglets sacrificed at D8 pw (*n*=10 pig replicates per treatment) following exsanguination. In all cases, ~1-2 ml of whole blood was collected in a Vacutainer® tube containing EDTA (Becton-Dickson Ltd, Plymouth, UK) (except at sacrifice when the volume was ~9 ml) and immediately inverted a number of times to prevent clotting. Samples were kept at room temperature and haematological analysis performed within 6 h, as outlined below.

Intestinal sampling

After euthanasia of piglets on D8 pw (*n*=10 pig replicates per treatment) the entire intestinal tract was immediately removed. Digesta samples from the ileum (15 cm proximal to the ileo-caecal junction), cecum (terminal tip) and rectum were collected aseptically into sterile containers, put on ice and stored at 4°C until analysis for the administered probiotic strain (within 12 h), as outlined below. Samples (~2 cm) of tissue were excised from the duodenum (15 cm distal to the pyloric junction), jejunum

(1.5 m distal to the pyloric junction) and the ileum (15 cm proximal to the ileo-caecal junction). Tissue samples were rinsed in phosphate buffered saline (PBS) immediately post-harvest and placed in No-Tox, an alcohol/aldehyde fixative (Scientific Device Lab, Des Plaines, IL, USA) on a shaker for 48 h prior to histological analysis, as outlined below.

Compositional analysis of sow colostrum and milk

Colostrum and milk samples were defrosted at room temperature. When fully thawed, samples were mixed by inverting several times to disrupt settled solids and mixed well. The volume of each sample was recorded prior to decanting into 50 ml tubes on ice. Sterile water was added to bring the volume up to 40 ml. Tubes were mixed thoroughly and kept on ice. Each sample was analysed in duplicate for total solids, lactose, fat, protein, true protein and casein B content by near-infrared absorption using a Bentley Dairyspec FT (Bentley Instruments, Inc., Chaska, MN, USA). Data were recorded as % (g/100g), taking the dilution factor into account.

Immunoglobulin A and Immunoglobulin G quantification in colostrum

Immunoglobulin A (IgA) and Immunoglobulin G (IgG) concentrations in colostrum were determined using ELISA kits (Pig IgA and IgG ELISA Kits, Bethyl Laboratories Inc., Texas, USA). First, 200 µl of colostrum was diluted 1:2 with PBS (1X, pH 7.4) and centrifuged at 10,000 rpm for 20 min at 4°C. The fat was then removed and the supernatant was collected and diluted 1:100,000 and 1:500,000 with 1X Dilution Buffer B (Bethyl Laboratories Inc.) for IgA and IgG analyses, respectively. The rest of the analysis was performed according to the manufacturer's protocol. All colostrum samples were analysed in duplicate. Absorbance was measured at 450 nm using a plate reader (ELx808 Absorbance Microplate Reader, BioTek, Vermont, USA). The IgA and IgG concentrations in the colostrum were obtained by reading absorbance values from standard curves prepared using standard solutions containing 1,000.0, 333.3, 111.1, 37.0, 12.3, 4.1 and 1.4 ng/ml of IgA and 500.0, 250.0, 125.0, 62.5, 31.3, 15.6 and 7.8 ng/ml of IgG.

Small intestinal histology

Duodenal, jejunal, and ileal tissue samples were removed from the No-Tox fixative and dehydrated through a graded alcohol series, cleared with xylene and embedded in paraffin wax. Tissue samples were sliced into 5 micrometre sections using a microtome (Leica RM2135, Wetzlar, Germany), mounted on microscope slides and stained with hematoxylin and eosin for determination of gross morphological parameters of intestinal structure (villus height and width and crypt depth and width). For each pig, 10 villi and 10 crypts were measured on five fields of view, where villi were attached to the lumen, and the means were utilised for statistical analysis. The goblet cell number was determined by periodic acid-Schiff staining. Positively stained periodic acid-Schiff cells were enumerated on 10 villi/sample, and the means were utilised for statistical analysis.

Microbiological analysis of faecal and digesta samples

Faecal and digesta samples and rectal swabs were homogenized and subsequently diluted in maximum recovery diluent (MRD; Merck, Darmstadt, Germany) as described by Gardiner *et al*. (2004)[38]. Appropriate dilutions were spread-plated in duplicate on brain heart infusion agar containing 3.5% NaCl, 200 µg/ml rifampicin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), and 50 U/ml nystatin (Sigma-Aldrich) in order to enumerate the administered probiotic strain. Plates were incubated aerobically for 2 days at 37°C, the colonies were counted and the counts averaged and presented as $\log_{10}$ CFU/g of the original sample or $\log_{10}$ CFU/swab.

Haematological analysis of blood samples

Haematological analysis was performed on whole blood using an Abbot Cell-Dyn 3700 analyser (GMI-Inc, Minnesota, USA). The following parameters were measured; white blood cell (WBC) number, lymphocyte number and percentage, monocyte number and percentage, granulocyte number and percentage, eosinophil number and percentage, basophil number and percentage, red blood cell (RBC) number, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, platelets and packed cell volume.

Statistical analysis

Power calculations were performed to determine the minimum number of observations required to detect effect sizes, using a statistical power of 80%, an α level at 5% and standard deviation of variables of interest from 7 previously published studies. The power calculation indicated that 12 sows per treatment were required to see a difference of 2.5 mm in BF depth, 10 piglets were required to see a 2 $\log_{10}$ CFU/g difference in selected microbial counts between treatments and that 18 piglets were required to see a 1.5 $\log_{10}$ CFU/g difference in microbial counts between treatments.

The experiment was a 2 × 2 factorial arrangement, with the factors being maternal treatment (control or probiotic supplementation) and post-weaning treatment (control or probiotic supplementation). All data were analysed using the MIXED procedure in SAS® 9.4 (SAS Institute, Inc., Cary, NC, US), unless otherwise stated. The model included maternal treatment and post-weaning treatment as fixed effects and their interaction. Where required, data were analysed as a repeated measure with sampling day as the repeated variable and the appropriate covariance structure, as indicated by the model fit statistics, was fitted to the data. Simple main effects were obtained using the 'slice' option in SAS.

The sow/litter was the experimental unit for sow performance, sow haematology, sow probiotic count data, colostrum and milk composition and colostrum IgA and IgG. The individual pig was the experimental unit for analysis of pre- and post-weaning pig growth performance, carcass characteristics, haematology, small intestinal morphology and probiotic count data. The normality of scaled residuals was investigated using the Shapiro-Wilk and Kolmogorov-Smirnov tests within the UNIVARIATE procedure of SAS. Differences in least square means were investigated using the t-test after Tukey adjustment for multiple comparisons. Degrees of freedom were estimated using Satterthwaite adjustment.

For sow performance, litter size, and pre-weaning mortality data, block was included as a random effect. The initial value (D100 of gestation) was included as a covariate in the analysis when significant in the model. Pre-weaning performance was analysed as repeated measures, including sex (male, female) as a fixed effect and block as a random effect. Birth weight was included as a covariate when significant in the model. Post-weaning performance was analysed as repeated measures, including sex (male, female) as fixed effect and weaning weight as a covariate, when significant in the model. For carcass characteristics, sex (male, female) was included as a fixed effect and BW at

weaning was included as a covariate when significant in the model. Counts of *B. altitudinis* WIT588 were analysed as repeated measurements. For the faecal counts of *B. altitudinis* WIT588 in the sows, block was included as a random effect. For the faecal counts of *B. altitudinis* WIT588 in the post-weaned piglets, the count at weaning was included as a covariate in the analysis, when significant. Haematological parameters were analysed including the initial value (D100 of gestation for sows or D0 pw for the offspring) as a covariate in the analysis when significant in the model. In addition, block was included as a random effect for the haematological values of sows. The haematological parameters that were not normally distributed were further analysed to find the best fitting distribution using the GLIMMIX procedure in SAS, using a gamma distribution. For these variables, the ilink function was used to back-transform the data to the original scale. The small intestinal morphology data were analysed using sex (male, female) as a fixed effect.

The results are presented in the text and tables as the least square means together with the pooled standard errors of the mean. Differences between treatments were considered significant for $P \leq 0.05$, while $0.05 < P \leq 0.10$ was considered as a tendency.

Results

Sow reproductive performance and tissue mobilisation

The effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from D100 of gestation to weaning (D26 of lactation) on sow weight, BF depth, feed intake and reproductive performance is presented in **Supplementary Table S1**. There was no treatment × day interaction for any of the variables of interest. Sows from the CON group were heavier than those in the PRO group at weaning (257.0 vs 248.7 ± 2.71 kg; $P=0.03$). However, gestation length (114.8 vs 114.6 ± 0.33 days), total born per litter (14.62 vs 15.49 ± 1.253), live born per litter (13.50 vs 13.97 ± 1.170), percentage of piglets live born per litter (93.3 vs 90.8 ± 3.25 %), stillbirths per litter (1.15 vs 1.51 ± 0.592) and the numbers of piglets suckling per litter at 48h *post-partum* (14.3 vs 14.2 ± 0.40) were not affected by sow treatment ($P>0.1$). Although not significant, there was a numerical reduction in pre-weaning mortality (15.6 vs 10.1 ± 2.82 %; $P=0.18$) when the probiotic was fed and because of this a numerical increase in the number of piglets

weaned per litter (11.8 vs 12.6 ± 0.55; *P*=0.29) in response to probiotic supplementation of sows.

Recovery of *Bacillus altitudinis* WIT588 from the faeces of sows and their litters during lactation

Faecal counts of the administered probiotic (*B. altitudinis* WIT588) from the faeces of sows during gestation and lactation and from their offspring during lactation are shown in **Table 2.** Prior to commencing probiotic treatment (D100 of gestation), *B. altitudinis* WIT588 was not detected in the faeces of either CON or PRO sows. There was a treatment × day interaction for faecal counts of *B. altitudinis* WIT588 in sows. Counts of *B. altitudinis* WIT588 increased over time in PRO sows from D100 of gestation until D13 of lactation, declining slightly on D26 of lactation (*P*<0.001). Faecal counts of *B. altitudinis* WIT588 were higher in PRO than in CON sows at all time points during probiotic administration (D115 of gestation, and D13 and D26 of lactation; *P*<0.001), as the administered probiotic was essentially undetectable in CON sows. Although not administered the probiotic themselves, most of the offspring from PRO sows shed *B. altitudinis* WIT588 by D13 of age. There was a treatment × day interaction for faecal counts of *B. altitudinis* WIT588 in the offspring of PRO sows, with probiotic counts increasing over time (*P*<0.001). However, counts are not comparable, as the D13 count is presented as CFU/swab and the D26 count as CFU/g faeces. Similar effects were observed in the offspring as in the sows, in that piglets born to PRO sows had higher faecal counts of *B. altitudinis* WIT588 at D13 and D26 of age than piglets born to CON sows (*P*<0.001), again due to lack of probiotic detection in the offspring from CON sows.

Haematological parameters of sows during gestation and lactation

The full results for all haematological parameters measured in sows are presented in **Supplementary Table S2**. Only results for haematological parameters where there were significant treatment differences are reported in **Table 3**. There was a tendency for a treatment × day interaction for mean corpuscular haemoglobin concentration (*P*=0.09), which decreased on D114 of gestation in CON sows, increasing again at weaning (D26 of lactation). The only treatment difference found for blood cell counts

was for basophils. Overall, PRO sows had a higher basophil count than CON sows (P<0.01). This was also found on D114 of gestation (P=0.04) and a tendency for this effect was found on the day of weaning (D26; P=0.07). Similar results were found for the overall percentage of basophils, where PRO sows had higher levels than CON sows (P=0.001). This was also found on D114 of gestation (P=0.05) and on the day of weaning (D26; P<0.01). Regarding the other parameters measured, treatment differences were also observed for mean corpuscular volume and mean corpuscular haemoglobin. Overall, CON sows had higher mean corpuscular volume than PRO sows (P<0.001) and this was also found on D114 of gestation (P=0.001) and on the day of weaning (D26; P<0.01). Overall, CON sows had greater mean corpuscular haemoglobin levels than PRO sows (P =0.001) and this was also found on D114 of gestation (P=0.01) and at weaning (D26; P=0.001). In addition, the mean corpuscular haemoglobin concentration was higher for PRO sows than for CON sows on D114 of gestation (P=0.04).

Colostrum and milk composition

The effect of supplementing sow diets with *B. altitudinis* WIT588 spores from D100 of gestation to weaning of litters (D26 of lactation) on the composition of sow colostrum and milk is shown in **Table 4.** Colostrum composition was impacted by maternal treatment for all of the parameters measured, with the exception of fat percentage (P=0.75) and IgA and IgG concentrations (P=0.46 and P=0.34, respectively). The colostrum from PRO sows had a higher percentage of total solids (P=0.02), protein (P=0.04), true protein (P=0.05) and casein B (P=0.05), and had less lactose (P=0.01) than the colostrum from CON sows. However, milk composition was not affected by sow treatment (**Table 4**).

Pre-weaning and post-weaning pig growth performance

Pig weights and average daily gains while suckling the sow were not affected by treatment (**Supplementary Table S3;** P>0.05). Birth weight averaged 1.47 ± 0.029 kg and weaning weight averaged 7.27 ± 0.168 kg for piglets from both treatments.

The effects of *B. altitudinis* WIT588 spore supplementation to sow and piglet diets on post-weaning growth and carcass characteristics are shown in **Table 5.** No maternal

503 treatment × post-weaning treatment × day interaction was found. A maternal treatment
504 × post-weaning treatment interaction was found for BW on D127 pw (P=0.05) with a
505 tendency for the same on D105 pw (P=0.07) and overall (P=0.09). On D105 pw,
506 PRO/PRO pigs tended to be heavier than CON/PRO pigs and on D127 pw, PRO/PRO
507 pigs were heavier than pigs born to CON sows. At D105 pw BW was 91.7 and 95.2 ±
508 0.98 kg (P=0.01), while at D127 pw it was 121.0 and 124.5 kg ± 0.97 (P=0.01) for pigs
509 born to CON and PRO sows, respectively. Overall, pigs born to PRO sows were
510 heavier than pigs born to CON sows (P=0.01). Average daily gain from D0 to D127 pw
511 was 890 and 922 ± 10.9 g/day (P=0.04) for pigs born to CON and PRO sows,
512 respectively. Overall, pigs born to PRO sows had higher ADG than pigs born to CON
513 sows (P=0.04). A maternal treatment × post-weaning treatment interaction was found
514 for FCR from D0 to D14 pw (P<0.001), where PRO/CON pigs had better FCR than
515 CON/PRO pigs. During this period (D0-D14 pw), pigs born to PRO sows had better
516 FCR than those born to CON sows (1.28 vs 1.45 ± 0.030 g/g; P<0.001). A maternal
517 treatment effect for FCR was also observed for the overall period (P=0.02). A post-
518 weaning treatment effect was observed from D0 to D14 pw, where CON pigs had better
519 FCR than PRO pigs (1.30 vs 1.43 ± 0.030 g/g; P<0.01). A tendency for a post-weaning
520 treatment effect was also observed from D57 to D105 pw and during the entire post-
521 weaning period (D0-127 pw), but this time with PRO pigs having a better FCR than
522 CON pigs [2.21 vs 2.13 ± 0.032 g/g (P=0.06) and 2.07 vs 2.04 ± 0.014 g/g (P=0.07),
523 respectively].

524 There was no maternal treatment × post-weaning treatment interaction for carcass
525 weight or carcass quality parameters (P>0.05). Carcass weight and kill out percentage
526 were 90.9 and 94.4 ± 1.22 kg (P=0.05) and 75.0 and 75.9 ± 0.187 % (P<0.01) for pigs
527 born to CON and PRO sows, respectively. There was no effect of post-weaning
528 treatment on carcass weight or carcass quality parameters (P>0.05).

529

530 **Recovery of *Bacillus altitudinis* WIT588 from the faeces and intestinal digesta of**
531 **pigs post-weaning**

532 Counts of the administered *B. altitudinis* probiotic in the faeces and ileal, caecal and
533 rectal digesta of the offspring post-weaning are shown in **Table 6**. No maternal
534 treatment × post-weaning treatment × day interaction was found. A maternal treatment
535 × post-weaning treatment interaction was found at D27 pw (P<0.001) and a tendency

for this effect was also found at weaning (P=0.08). At weaning *B. altitudinis* WIT588 counts tended to be higher in the faeces of PRO/CON than PRO/PRO piglets. A maternal treatment effect was observed at weaning, where piglets born to PRO sows had higher *B. altitudinis* WIT588 counts than those born to CON sows (4.70 vs 3.00 ± 0.088 $\log_{10}$ CFU/g faeces; P<0.001), due to lack of detection in the latter. At D8 pw, post-weaning treatment affected counts in the intestinal digesta. *Bacillus altitudinis* WIT588 counts were higher in the ileal, caecal and rectal digesta of PRO compared to CON piglets (P<0.001), as the administered strain was undetectable in the latter. *Bacillus altitudinis* WIT588 counts were also higher in the faeces of PRO versus CON piglets on D27 pw (5.93 vs 3.00 ± 0.021 $\log_{10}$ CFU/g faeces; P<0.001) and there was a tendency for this effect at weaning (3.96 vs 3.74 ± 0.088 $\log_{10}$ CFU/g faeces; P=0.08).

Faecal scoring of pigs post-weaning

Statistical analysis of the probiotic effect on post-weaning diarrhoea prevalence could not be conducted, as the occurrence of faecal consistency scores higher than 0 was rare. Out of 504 faecal consistency scores given to each one of the four treatments up to D28 pw, a score of 1 (soft faeces with shape) was given 45 times to the CON/CON treatment group, 28 times to the CON/PRO treatment group, 38 times to the PRO/CON treatment group and 27 times to the PRO/PRO treatment group. No scores higher than 1 were given at any time to any animal.

Haematological parameters of pigs post-weaning

The effects of *Bacillus altitudinis* WIT588 supplementation to sow and piglet diets on the haematological parameters of pigs post-weaning are shown in **Table 7**. No maternal treatment × post-weaning treatment × day interactions were found for any of the parameters measured, except for mean corpuscular volume (P=0.08) and mean corpuscular haemoglobin (P=0.09) which tended to decrease with increasing age in the pigs.

Pigs on the post-weaning PRO treatment had higher WBC counts on D57 pw than CON pigs (14.62 vs 11.68 ± 0.962 $\times 10^3$ cells/µL; P=0.04). There was a tendency for a maternal treatment × post-weaning treatment interaction for the total lymphocyte count on D57 pw (P=0.10). An effect of post-weaning treatment was found for the total

number of lymphocytes and lymphocyte percentage at D57 pw, where PRO pigs had a higher lymphocyte count and percentage than CON pigs [10.97 vs 7.29 ± 1.145 $\times 10^3$ cells/μL (P=0.03) and 68.03 vs 59.33 ± 2.954 % (P=0.04), respectively]. Similarly, the overall lymphocyte count and lymphocyte percentage tended to be higher in PRO compared to CON pigs [10.61 vs 8.42 ± 0.822 $\times 10^3$ cells/μL (P=0.06) and 68.95 vs 61.11 ± 2.135 % (P=0.01), respectively].

A maternal treatment × post-weaning treatment interaction was found on D8 pw for monocyte count (P<0.01), with counts lower in the CON/CON group than in the PRO/CON group. Likewise, a tendency for a maternal treatment × post-weaning treatment interaction was also found for the percentage of monocytes on D8 pw (P=0.09), with piglets from the CON/CON group having a lower percentage than their PRO/CON counterparts. This led to offspring from PRO sows having a higher monocyte percentage than pigs born to CON sows at D8 pw (6.65 vs 4.76 ± 0.667 %; P=0.05). In addition, pigs on the post-weaning probiotic treatment had a lower percentage of monocytes than CON pigs on D57 pw (7.95 vs 10.65 ± 0.873 %; P=0.03) and overall (6.36 vs 8.28 ± 0.631 %; P=0.04).

A maternal treatment × post-weaning treatment interaction was observed at weaning for the neutrophil count (P=0.05), where pigs from the CON/PRO group had a higher count than PRO/PRO pigs. A tendency for a post-weaning treatment effect was observed overall for the neutrophil percentage, where probiotic-supplemented pigs had a lower percentage of neutrophils than CON pigs (21.90 vs 26.90 ± 1.877 %; P=0.07).

There was a maternal treatment × post-weaning treatment interaction for both the eosinophil count (P=0.01) and percentage (P=0.001) on D57 pw, with pigs from the PRO/CON group having a higher eosinophil count and percentage than pigs from the CON/PRO and PRO/PRO groups. A post-weaning treatment effect was also observed, with probiotic-supplemented pigs having lower eosinophil counts than CON pigs on D8 pw (0.11 vs 0.16 ± 0.017 $\times 10^3$ cells/μL; P=0.03), D57 pw (0.15 vs 0.22 ± 0.019 $\times 10^3$ cells/μL; P<0.01), and overall (0.15 vs 0.19 ± 0.014 $\times 10^3$ cells/μL; P=0.050). Similarly, probiotic-supplemented pigs had a lower eosinophil percentage than CON pigs on D57 pw (0.95 vs 1.89 ± 0.140 %; P<0.001) and overall (0.97 vs 1.47 ± 0.102 %; P=0.001).

A maternal treatment × post-weaning treatment interaction was found for basophil count (P=0.001) and percentage (P=0.02) on D8 pw, with CON/CON pigs having a lower basophil count and percentage than pigs from CON/PRO and PRO/CON groups. In addition, pigs born to CON sows had a lower basophil count than those born to PRO sows at weaning (0.07 vs 0.12 ± 0.012 $\times 10^3$ cells/μL; P=0.05) and D8 pw (0.04 vs 0.06 ± 0.006 $\times 10^3$ cells/μL; P=0.02). This led to offspring from CON sows having a lower basophil percentage than those from PRO sows at weaning (0.58 vs 1.16 ± 0.108 %; P=0.01) and D8 pw (0.37 vs 0.55 ± 0.058 %; P=0.03). An effect of post-weaning treatment was also observed for basophil percentage overall, where probiotic-supplemented pigs had a lower percentage than CON pigs (1.56 vs 2.07 ± 0.179 %; P=0.05).

At weaning, tendencies for a maternal treatment effect were observed for RBC count (7.82 vs 6.98 ± 0.318 $\times 10^6$ cells/μL; P=0.07), haemoglobin (15.08 vs 13.64 ± 0.594 g/dL; P=0.10) and haematocrit (0.50 vs 0.45 ± 0.018 L/L; P=0.05), with offspring from CON sows having higher levels than those from PRO sows. A tendency for a maternal treatment × post-weaning treatment interaction was observed for mean corpuscular haemoglobin at weaning (P=0.10), D57 pw (P=0.08) and overall (P=0.07), and for mean corpuscular haemoglobin concentration overall (P=0.06). On D8 pw, PRO-supplemented pigs tended to have a higher mean corpuscular haemoglobin concentration than CON pigs (28.88 vs 28.43 ± 0.186 g/dL; P=0.10).

Regarding platelet counts, a significant maternal effect was found on D8 pw, with the offspring from CON sows having a lower platelet count than those from PRO sows (224.25 vs 332.28 ± 22.892 $\times 10^3$ cells/μL; P<0.01).

Intestinal morphology of piglets post-weaning

There was no maternal treatment × post-weaning treatment interaction (P>0.05) for any of the intestinal morphological parameters investigated (**Supplementary Table S4**). In addition, there was little effect of post-weaning treatment, except for an increase in villous height:crypt depth ratio in the jejunum (1.9 to 2.1 ± 0.06; P=0.03) and an increase in villous area in the ileum (36786 to 42443 ± 1724.3 μm^2; P=0.03) in response to feeding the probiotic post-weaning. For this reason, only the main effects of maternal treatment are presented in **Table 8**.

631 Pigs born to PRO sows had longer villi (P<0.01), greater villous area (P<0.01), deeper
632 crypts (P=0.04) and a tendency for greater crypt area (P=0.06) in the duodenum than
633 pigs born to CON sows (**Figure 1**). The offspring from PRO sows also had deeper
634 crypts (P=0.04) and a greater crypt area (P<0.01) in the jejunum than those from CON
635 sows. Ileal villous height (P=0.06) and area (P=0.10) tended to be greater in pigs born
636 to PRO sows than in the offspring from CON sows.

637

638 **Discussion**

639 This study assessed the effect of supplementing *B. altitudinis* WIT588 spores to
640 transition and lactating sows and/or their offspring on the growth and health of sows
641 and their offspring. While a number of probiotic supplementation studies with a similar
642 design have been published, piglet growth has rarely been determined after the early
643 post-weaning period[18,20,24,27–29]. The novelty of this study lies in the fact that the
644 offspring of probiotic-supplemented sows were followed from birth to slaughter. To
645 our knowledge, this is the first study to date that conclusively demonstrates lifetime
646 growth benefits in the offspring of probiotic-supplemented sows.

647 Maternal probiotic supplementation improved FCR of offspring during the first 14 days
648 post-weaning. Improved FCR early post-weaning is considered a good indicator of
649 improved intestinal health at this critical period[39]. This was corroborated in the present
650 study when increased villous height was found at D8 pw in the small intestine of pigs
651 born to probiotic-supplemented sows. This indicates increased absorptive capacity
652 which may account for the increased lifetime growth in these animals. In fact,
653 improved FCR early post-weaning has previously been shown to correlate well with
654 increased lifetime growth[40]. This held true in the current study. Incremental increases
655 in growth in offspring due to maternal probiotic supplementation were observed, with
656 the initial increases in pig live-weight at D14, D28 and D56 pw not being statistically
657 significant. It was only in the late finishing period (D105 and 127 pw) when increased
658 live-weight in pigs in response to feeding probiotic to the sows became significant. The
659 improvement in live-weight at the end of the finishing period resulted in a 3.5 kg
660 increase in carcass weight in offspring from probiotic-supplemented versus control
661 sows.

Interestingly, there was no additive effect of post-weaning supplementation of the offspring from probiotic-supplemented sows, nor were there any benefits of probiotic supplementation of weaned pigs alone. This agrees with the findings from a previous study from our group in which growth benefits in weaned pigs supplemented with this strain were only found when compared with a medicated diet containing apramycin and pharmacological levels of zinc oxide, and not when compared with the negative control[26]. The lack of effect in weaned pigs may be because commencing supplementation to pigs post-weaning might be too late to see an effect, as it is understood that there is a critical window early in life during which gut microbiota modulation is more impactful[41]. Probiotic supplementation of sow diets offers an effective means of early-life (prior to weaning) probiotic administration, as litters do not consume appreciable amounts of creep feed until ~D14 of age and oral dosing of individual piglets prior to this is not feasible on a commercial pig unit.

In the present study, *B. altitudinis* WIT588 was detected as early as D13 of age in suckling piglets born to sows fed this probiotic strain, even though the probiotic had not been administered to the piglets themselves. This demonstrates probiotic transfer from sows to offspring. Although, the use of *Bacillus* strains as probiotics in pig production is well documented, whether administered to weaned piglets[8,9] or to gestating sows and their offspring[18,20,24,27–29], few studies have reported probiotic transmission from the sow to the piglet[27,28]. Although the mechanisms by which the probiotic is vertically transmitted in the present study are not fully understood, it is most likely via the faecal-oral route[42]. In fact, we hypothesise that *Bacillus* spores excreted in the sow's faeces germinate in the farrowing house environment and, due to the relatively high gastric pH in suckling piglets[43], survive gastric transit as vegetative cells in the piglets leading to early colonization of the gut. This early colonization may also help to explain why beneficial effects are observed in these animals and not in piglets to which the probiotic spores are administered post-weaning, as it appears from our previous work that the spores do not germinate in the gut[26]. Another mechanism by which the probiotic could be vertically transmitted to the piglets is that the spores might be transferred to the piglets in dust from the sow feed or indeed via direct contact with the feed, hence bypassing faecal transplantation from the mother. However, this potential mechanism leaves little opportunity for the spores to germinate outside the pig and become

694 metabolically active and so is not considered by the authors to be as important as faecal-
695 oral transfer.

696 Similar to the lack of persistence found in weaned piglets, which no longer shed *B.*
697 *altitudinis* WIT588 one month after ceasing probiotic administration, this early
698 colonization in suckling piglets was also transient. This is evidenced by the fact that *B.*
699 *altitudinis* WIT588 was not detected in the intestinal digesta of piglets from the
700 PRO/CON group on D8 pw, i.e. one week after contact with the probiotic-supplemented
701 mothers had ceased. This lack of persistence post-administration is not uncommon with
702 probiotics[12]. In addition, this early colonization in suckling piglets was not at as high a
703 level or as consistent as when the probiotic was directly administered to weaned piglets.
704 Not all of the piglets born to probiotic-supplemented sows shed *B. altitudinis* WIT588
705 at both time points prior to weaning, and some of those that shed the probiotic at D13
706 were no longer doing so at D26. However, the probiotic was recovered from all of the
707 piglets at some point prior to weaning, and the differences in shedding may be due to
708 variations in the level of probiotic to which the piglets were exposed and also to
709 variations in gastric pH[43] or coprophagic behaviour[44].

710 One possible mechanism by which the probiotic strain improved lifetime growth of the
711 progeny of the sows to which it was administered is via modulation of colostrum
712 composition. Although all of the measured colostrum and milk compositional values
713 fell within reference ranges[45], the colostrum from probiotic-fed sows had a higher
714 protein content than that from control sows, indicating that it was of higher nutritional
715 value[46]. In previous studies, protein, together with fat content, of milk was also
716 increased as a result of *Bacillus* supplementation of sows[9,30], although others reported
717 only an increase in fat content[18]. The higher protein content of the colostrum from the
718 probiotic-supplemented sows in the current study may have resulted from increased
719 mobilisation of the sows' body reserves as these sows were lighter than control sows on
720 the weaning day and lost more weight (numerically) during the lactation period.
721 However, probiotic-supplemented sows also had to produce more milk during lactation,
722 as they suckled more piglets to weaning. Furthermore, we do not know the exact
723 mechanism by which probiotic supplementation increased colostrum protein content.
724 Another avenue that we explored was that higher concentrations of immunoglobulins in
725 the colostrum of probiotic-supplemented sows would confer increased immune
726 protection to offspring, thereby helping to explain the observed growth benefits, the

numerical reduction in pre-weaning mortality and the improved intestinal morphology found in piglets born to probiotic-supplemented sows. However, maternal probiotic supplementation did not have a significant effect on the concentrations of IgA or IgG in the colostrum.

Interestingly, some of the haematological parameters measured in sows indicate a possible inflammatory response after the first 2 weeks of probiotic treatment (D114 of gestation) which persisted throughout the suckling period. Basophil counts in probiotic-supplemented sows were higher than those in control sows, although all values were within reference ranges, except the basophil percentage at weaning (the upper limit is 2.0% and the value in probiotic-supplemented sows was 2.32%)[47]. Probiotic-supplemented sows also had lower mean corpuscular volume and less mean corpuscular haemoglobin than control sows from farrowing to weaning but values were within the normal ranges, being indicative of subtle anaemia or possible inflammation. This possible immune modulation in the sow could have affected the pigs *in utero* (despite swine placenta being epitheliochorial), which may also help to explain the improved gut health early post-weaning and the subsequent growth benefits. It has previously been reported that *Bacillus* spores can trigger immune responses in the gut[48,49], which may protect against external pathogens. However, specific immune assays in intestinal cells are required in order to further investigate the probiotic-mediated immunomodulation hypothesised in the current study.

It is interesting to note that some of the haematological effects found in the sows were mirrored in the offspring. For example, piglets born to probiotic-fed sows had higher basophil counts and percentages than the offspring from control sows on the day of weaning and at D8 pw. This may have been caused by an *in utero* effect or it could be indicative of immune stimulation during the early stages of suckling due to early-life probiotic exposure. Nonetheless, this effect diminished after D8 pw and was not observed thereafter. There was no effect of post-weaning treatment with the probiotic on the haematology of pigs; however, piglets that were never exposed to *B. altitudinis* WIT588 had the lowest levels of basophils. Other significant differences of note were the effects on WBC populations found due to probiotic administration post-weaning. These included elevated total WBC and lymphocyte counts and reduced monocyte and eosinophil levels, albeit all were within reference values[50]. Interestingly, all were observed two months post-weaning (D57 pw). However, it is difficult to explain these

differences, because at this stage the piglets were no longer shedding *B. altitudinis* WIT588. The effects may however be residual. In any case, these post-weaning treatment-related haematological effects did not translate into improved growth, highlighting the fact that maternal supplementation is the preferred route of administration to pigs for this probiotic strain.

Conclusions

The data presented in this study indicate that *B. altitudinis* WIT588 dietary supplementation to sows during late gestation and lactation is more beneficial than post-weaning administration to piglets. Piglets born to sows supplemented with the probiotic displayed faecal shedding of the administered strain while suckling. This vertical transmission is rarely reported for other probiotics and demonstrates that maternal supplementation is an effective means of early-life probiotic administration. Maternal treatment improved feed efficiency in the early post-weaning period in progeny and increased live-weight at the end of the finishing period, which resulted in increased carcass weight at target slaughter age. Possible mechanisms of action are improved colostrum quality in sows, maternal immunomodulation, which was mirrored to a certain extent in the offspring, and increased small intestinal absorptive capacity in offspring early post-weaning. However, further analyses are needed to elucidate the mechanism(s) of action, including serum immunoglobulin measurements. In summary, the novelty of this study lies in the fact that the offspring of probiotic-supplemented sows were followed from birth to slaughter. The lifetime growth benefits observed offer considerable economic advantages for commercial pig producers in search of alternatives to in-feed antibiotics and pharmacological levels of zinc oxide. Work is ongoing to develop a product containing spray/freeze dried spores to facilitate formulation of the probiotic strain into commercial pig diets.

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Conflict of Interest

Gillian Gardiner, Peadar Lawlor and Alan Marsh have a patent "An isolated *Bacillus altitudinis* strain and its use as a probiotic" pending.

Authorship

G.E.G. and P.G.L. conceived the study and, together with A.M. and S.R., designed the experiment. P.G.L and G.E.G directed the study. S.R. and P.G.L. conducted the animal experiment. G.E.G., A.M. and R.H. performed laboratory analyses together with J.P., who also interpreted the haematology data. D.C.-P., M.A.B., S.R. and P.G.L. statistically analysed the data. D.C.-P., G.E.G. and P.G.L. interpreted the data and drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

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Table 1. Composition of experimental diets (on an air-dry basis; kg/tonne unless otherwise stated).

Item	Dry Sow	Lactating Sow	Starter/link	Weaner	Finisher
Barley	753.02	269.81	62.86	257.58	384.67
Wheat	0	429.6	112	433.57	400
Maize	0	0	300	0	0
Soybean meal	89.62	196.65	255	187.92	183.01
Soya hulls	121.8	0	0	0	0
Full fat soya	0	0	70	50	0
Lactoflo[1]	0	0	100	0	0
Skim milk powder	0	0	25	0	0
Soya oil	11	66	40	40	9.69
Lysine HCl	2.19	4.47	5.14	5.02	3.75
DL-Methionine	0.58	1.35	2.62	1.85	0.93
L-Threonine	0.6	2.45	2.55	2.09	1.7
L-Tryptophan	0	0.71	0.97	0.27	0.15
L-Valine	0	2.34	0.26	0	0
Vitamin and mineral mix	1.5[2]	1.5[2]	3[3]	3[3]	1[4]
Salt feed grade	4	5	3	3	3
Mono di-calcium phosphate	6.49	8.5	9.5	4.6	1
Limestone flour	9.08	11.5	8	11	11
Phytase[5]	0.1	0.1	0.1	0.1	0.1
Analysed chemical composition					
Dry matter	875	898	891	897	876
Crude protein	129	164	190	193	171
Fat	36.6	102.8	65.1	72.1	43.5
Crude fibre	72	26	30	27	31
Neutral detergent fibre	162	82	88	84	103
Ash	40	48	48	45	43
Lysine	8.2	11.5	15.0	13.0	11.0
Methionine	2.7	3.8	5.8	4.6	3.5
Methionine and cysteine	5.4	7.0	9.1	7.9	6.7
Threonine	5.5	8.3	10.1	8.6	7.7
Tryptophan	1.7	2.8	3.4	2.6	2.3
Calculated chemical composition[6]					
Standardised ileal digestible lysine	6.60	10.67	14.00	11.49	9.97
Calcium	7.20	8.32	8.00	7.25	6.59
Digestible phosphorus	3.45	3.88	4.44	3.32	2.55
Digestible energy (MJ/kg)	13.2	15.2	15.0	14.5	13.8
Net energy (MJ/kg)	8.9	10.9	10.74	10.55	9.80

[1]Lactoflo 70 contains 70% lactose, 11.5% protein, 0.5% oil, 7.5% ash and 0.5% fibre (Volac, Cambridge, UK).

[2]Premix provided per kg of complete diet: Cu, 15 mg; Fe, 70 mg; Mn, 62 mg; Zn, 80 mg; I, 0.6 mg; Se, 0.2 mg; vitamin A, 1000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 2 mg; vitamin B_{12}, 15 µg; riboflavin, 5 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 500 mg; biotin, 200 mg; folic acid, 5 g; vitamin B_1, 2 mg; vitamin B_6, 3 mg.

[3]Premix provided per kg of complete diet: Cu, 155 mg; Fe, 90 mg; Mn, 47 mg; Zn, 120 mg; I, 0.6 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin D_3, 1000 IU; vitamin E, 100 IU; vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; choline chloride, 250 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg; Endox, 60 g.

[4]Premix provided per kg of complete diet: Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg; I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg.

[5]The diet contained 500 phytase units (FYT) per kg feed from RONOZYME HiPhos (DSM, Belfast, UK).

[6]Calculated from tabulated ingredient values(51).

Table 2. Effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from day (D) 100 of gestation to weaning (D26 of lactation) on faecal counts (log_{10} CFU/g) of sows and their piglets[1].

	Treatment			*P*-value		
Days	CON[2] (No. pigs in which probiotic detected/ No. pigs sampled)	PRO[3] (No. pigs in which probiotic detected/ No. pigs sampled)	SEM[1]	Treatment	Day	Treatment × Day
Sows						
N	12	12				
D100 Gestation	3.00[4] (0/12)	3.00 (0/12)	-	-		-
D115 Gestation	3.08 (1/12)	5.93 (12/12)	0.047	**<0.001**		
D13 Lactation	3.00 (0/12)	6.39 (12/12)	0.047	**<0.001**		
Weaning (D26 Lactation)	3.00 (0/12)	6.17 (12/12)	0.047	**<0.001**		
Overall			0.034	**<0.001**	**<0.001**	**<0.001**
Piglets during lactation						
N	20	20				
D13[5]	3.00 (0/20)	3.47 (12/20)	0.075	**<0.001**		
D26[6]	3.00 (0/20)	4.79 (16/20)	0.080	**<0.001**		
Overall			0.055	**<0.001**	**<0.001**	**<0.001**

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]The limit of detection of the assay for *B. altitudinis* WIT588 was 1000 CFU/g faeces or /swab. Values below the limit of detection were recorded as 3.00 log_{10} CFU/g faeces or /swab.
[5]Counts are from rectal swabs and are presented as log_{10} CFU/swab.
[6]A rectal swab was taken from three pigs in the probiotic treatment group due to insufficient faecal sample. Probiotic was detected in these animals but the counts were excluded from the statistical analysis.

Table 3. Effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from day (D) 100 of gestation to weaning (D26 of lactation) on haematological parameters of sows[1].

		Treatment			*P*-value		
Blood parameters	Day	CON[2]	PRO[3]	SEM	Treat-ment	Day	Treat-ment × Day
N		12	12				
Basophils (×10³ cells/µL)	G100	0.10	0.11	0.013	0.54		
	G114	0.11	0.17	0.024	**0.04**		
	W26	0.17	0.22	0.022	*0.07*		
	Mean	0.14	0.20	0.018	**<0.01**	**<0.01**	0.72
Basophils (%)[4]	G100	1.11	1.36	0.127	0.19		
	G114	1.24	1.81	0.207	**0.05**		
	W26	1.58	2.32	0.188	**<0.01**		
	Mean	1.41	2.06	0.155	**0.001**	**0.02**	0.61
Mean corpuscular volume (fL)	G100	63.52	62.77	0.601	0.25		
	G114	66.18	63.88	0.474	**0.001**		
	W26	65.01	63.23	0.431	**<0.01**		
	Mean	65.60	63.55	0.357	**<0.001**	**0.03**	0.51
Mean corpuscular haemoglobin (pg/cell)	G100	19.90	19.57	0.197	0.13		
	G114	20.47	19.93	0.154	**0.01**		
	W26	20.20	19.54	0.139	**0.001**		
	Mean	20.34	19.74	0.113	**0.001**	**0.02**	0.65
Mean corpuscular haemoglobin concentration (g/dL)	G100	31.33	31.14	0.220	0.56		
	G114	30.89	31.23	0.122	**0.04**		
	W26	31.02	31.00	0.111	0.91		
	Mean	30.96	31.12	0.093	0.14	0.62	*0.09*

G100: Day 100 of gestation; G114: day 114 of gestation; W26: weaning (day 26 of lactation).
[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]Percentages are based on the differential count of white blood cells.

Table 4. Effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from day (D) 100 of gestation to weaning (D26 of lactation) on the composition of sow colostrum and milk[1].

	Treatment			
	CON[2]	PRO[3]	SEM	*P*-value
N	12	12		
Colostrum				
Total solids (%)	21.97	24.01	0.581	**0.02**
Lactose (%)	2.06	1.52	0.128	**<0.01**
Fat **(%)**	3.94	4.14	0.430	0.75
Protein (%)	14.25	16.56	0.759	**0.04**
True protein (%)	13.83	16.18	0.791	**0.05**
Casein B (%)	11.98	14.08	0.717	**0.05**
Immunoglobulin A (mg/ml)	18.06	21.40	3.120	0.46
Immunoglobulin G (mg/ml)	79.79	96.42	12.157	0.34
Milk[4]				
Total solids (%)	18.91	18.56	0.500	0.63
Lactose (%)	5.23	5.22	0.130	0.93
Fat (%)	7.42	6.74	0.524	0.37
Protein (%)	4.78	4.89	0.116	0.49
True protein (%)	4.25	4.41	0.115	0.34
Casein B (%)	3.34	3.47	0.111	0.45

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]Milk was sampled 14 days *post-partum*.

Table 5. Effect of *Bacillus altitudinis* WIT588 spore supplementation to sow and piglet diets on post-weaning growth and carcass characteristics[1].

Maternal Post-weaning (pw)		Control Control	Control Probiotic	Probiotic Control	Probiotic Probiotic		*P*-value				
	Day (pw)	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
N		18	18	18	18						
Mortality[6]		0	1	0	0						
Off trial[7]		2	2	2	0						
Body weight (kg)	0‡	8.1	8.7	8.1	8.4	0.36	0.62	0.16	0.72		
	14	11.8	10.9	11.8	11.3	1.31	0.89	0.57	0.95		
	28	18.8	17.4	18.9	18.6	1.32	0.62	0.50	0.84		
	56	44.4	40.5	42.9	43.1	1.34	0.68	0.16	0.23		
	105	92.7^{AB}	90.8^{A}	95.1^{AB}	95.4^{B}	1.39	**0.01**	0.55	*0.07*		
	127	121.1^{A}	120.9^{A}	123.4^{AB}	125.6^{B}	1.38	**0.01**	0.47	**0.05**		
	Overall					0.60	**<0.01**	0.27	*0.09*	**<0.001**	0.88
ADG (g/day)	0-14	229	200	232	210	24.9	0.80	0.31	0.77		
	15-28	502	465	509	519	25.1	0.22	0.60	0.47		
	29-56	910	818	862	874	25.5	0.87	0.13	*0.10*		
	57-105	1019	1030	1065	1067	26.5	0.12	0.80	0.46		
	106-127	1303	1365	1365	1375	26.3	0.17	0.17	0.19		
	Overall					11.5	**0.04**	0.55	0.40	**<0.001**	0.26
	0-127	897	883	921	924	15.5	**0.04**	0.73	0.60		
ADFI (g/day)	0-14	303	282	284	271	42.0	0.72	0.68	0.96		
	15-28	641	600	648	637	42.3	0.61	0.54	0.86		
	29-56	1353	1193	1259	1288	43.0	0.99	0.13	*0.08*		
	57-105	2293	2170	2288	2300	44.7	0.17	0.21	0.14		
	106-127	3230	3273	3309	3336	44.3	0.11	0.43	0.35		
	Overall					19.4	0.15	0.19	*0.08*	**<0.001**	0.38

Maternal Post-weaning (pw)		Control Control	Control Probiotic	Probiotic Control	Probiotic Probiotic		*P*-value				
	Day (pw)	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
	0-127	1874	1795	1884	1883	33.7	0.15	0.24	0.26		
Feed conversion ratio (g/g)	0-14	1.37[ab]	1.53[a]	1.22[b]	1.33[ab]	0.042	**<0.001**	**<0.01**	**<0.001**		
	15-28	1.28	1.31	1.28	1.23	0.042	0.35	0.78	0.63		
	29-56	1.49	1.45	1.47	1.47	0.043	0.95	0.69	0.94		
	57-105	2.26	2.09	2.16	2.16	0.045	0.68	*0.06*	*0.07*		
	106-127	2.51	2.39	2.46	2.46	0.044	0.91	0.18	0.33		
	Overall					0.019	**0.02**	0.70	0.28	**<0.001**	0.22
	0-127	2.09	2.03	2.05	2.04	0.019	0.41	*0.07*	0.19		
Carcass characteristics											
Carcass weight (kg)		91.7	90.1	93.0	95.9	1.73	**0.05**	0.71	0.21		
Kill out (%)		75.1	75.0	75.5	76.3	0.27	**<0.01**	0.15	0.13		
Lean meat (%)		53.8	54.6	54.6	54.0	0.47	0.86	0.81	0.15		
Muscle (mm)		47.7	48.7	51.8	49.7	1.61	0.12	0.73	0.34		
Fat (mm)		16.0	15.1	15.8	16.0	0.60	0.51	0.58	0.33		

ADG: average daily gain; ADFI: average daily feed intake.
[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON/CON, non-probiotic supplemented sow/non-probiotic supplemented piglet; [3]CON/PRO, non-probiotic supplemented sow/probiotic-supplemented piglet; [4]PRO/CON, probiotic-supplemented sow/non-probiotic supplemented piglet; [5]PRO/PRO, probiotic-supplemented sow/probiotic-supplemented piglet.
[6]Mortality: Due to polyserositis and septicaemia (*Streptococcus suis* infection).
[7]Off trial: Pigs were removed from the trial due to lameness (PRO/CON, N=1), pneumonia (CON/CON, N=1 and CON/PRO, N=1), bloody diarrhoea (CON/CON, N=1 and PRO/CON, N=1) and abdominal hernia (CON/PRO, N=1).
[a-b]Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A-B]Values within a row that do not share a common superscript tended to differ ($P \leq 0.10$).
‡Day 0 pw is the day of weaning.

Table 6. Effect of *Bacillus altitudinis* WIT588 spore supplementation to sow and piglet diets on ileal, caecal and rectal digesta counts (log_{10} CFU/g)[1] of piglets euthanized on day (D) 8 post-weaning and on faecal counts at D0, D27 and D56 post-weaning.

Maternal	Control	Control	Probiotic	Probiotic		*P*-value				
Post-weaning (pw)	Control	Probiotic	Control	Probiotic						
	CON/CON[2] (No. pigs in which probiotic detected/ No. pigs sampled)	CON/PRO[3] (No. pigs in which probiotic detected/ No. pigs sampled)	PRO/CON[4] (No. pigs in which probiotic detected/ No. pigs sampled)	PRO/PRO[5] (No. pigs in which probiotic detected/ No. pigs sampled)	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
N	10	10	10	10						
Ileum (D8 pw)	3.00[6] (0/10)	5.13 (10/10)	3.00 (0/10)	5.13 (9/10)	0.153	0.99	**<0.001**	0.99		
Caecum (D8 pw)	3.00 (0/10)	5.48 (10/10)	3.00 (0/10)	5.37 (10/10)	0.114	0.62	**<0.001**	0.62		
Rectum (D8 pw)	3.00 (0/10)	5.97 (10/10)	3.00 (0/10)	6.07 (10/10)	0.065	0.44	**<0.001**	0.44		
N	10	10	10	10						
Weaning (D0 pw)	3.00^{A} (0/10)	3.00^{A} (0/10)	4.47^{B} (8/10)	4.93^{C} (8/10)	0.124	**<0.001**	*0.08*	*0.08*		
D27 pw	3.00^{a} (0/10)	5.95^{b} (10/10)	3.00^{a} (0/10)	5.91^{b} (10/10)	0.033	0.85	**<0.001**	**<0.001**		
D56 pw	3.00 (0/10)	3.00 (0/10)	3.00 (0/10)	3.00 (0/10)	0.033	0.63	0.96	0.97		
Overall					0.025	0.87	**<0.001**	0.57	**<0.001**	0.52

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON/CON, non-probiotic supplemented sow/non-probiotic supplemented piglet; [3]CON/PRO, non-probiotic supplemented sow/probiotic-supplemented piglet; [4]PRO/CON, probiotic-supplemented sow/non-probiotic supplemented piglet; [5]PRO/PRO, probiotic-supplemented sow/probiotic-supplemented piglet.
[6]The limit of detection of the assay for *B. altitudinis* WIT588 was 1000 CFU/g faeces. Values below the limit of detection were recorded as 3.00 log_{10} CFU/g faeces.
[a-b]Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A-C]Values within a row that do not share a common superscript tended to differ ($P \leq 0.10$).

Table 7. Effect of *Bacillus altitudinis* WIT588 spore supplementation to sow and piglet diets on haematological parameters of piglets at weaning and day 8, 28 and 57 post-weaning[1].

Maternal		Control	Control	Probiotic	Probiotic		*P*-value				
Post-weaning (pw)		Control	Probiotic	Control	Probiotic						
	Day (pw)	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
White blood cells (×10³/µl)	0‡	10.51	13.37	11.94	9.83	1.390	0.47	0.85	*0.08*		
	8	12.76	11.92	13.63	10.22	1.555	0.74	0.17	0.40		
	28	15.66	15.40	13.33	13.55	1.522	0.18	1.00	0.60		
	57	10.34	14.05	13.20	15.23	1.363	0.13	**0.04**	*0.08*		
	Mean	12.73	14.71	13.26	14.37	1.159	0.92	0.19	0.70	0.11	0.43
Lymphocytes (×10³ cells/µL)	0	5.12	6.56	6.11	5.76	1.121	0.94	0.63	0.43		
	8	7.75	6.98	7.68	5.88	1.331	0.63	0.33	0.67		
	28	10.44	10.75	8.67	9.76	1.667	0.41	0.68	0.81		
	57	5.99	10.40	8.59	11.54	1.619	0.25	**0.03**	*0.10*		
	Mean	8.21	10.57	8.63	10.65	1.162	0.83	*0.06*	0.89	0.51	0.63
Lymphocytes (%)[6]	0	50.58	47.86	54.11	52.73	6.077	0.49	0.79	0.91		
	8	57.96	53.29	57.04	55.48	5.473	0.90	0.57	0.78		
	28	65.49	71.52	60.29	68.23	4.348	0.34	0.11	0.29		
	57	59.30	66.80	59.37	69.26	4.180	0.76	**0.04**	0.22		
	Mean	62.39	69.16	59.83	68.74	3.027	0.63	**0.01**	0.72	0.37	0.97
Monocytes (×10³ cells/µL)	0	0.71	0.81	0.88	0.63	0.130	0.89	0.54	0.17		
	8	0.45[a]	0.77[ab]	1.00[b]	0.58[ab]	0.123	0.16	0.96	**<0.01**		
	28	0.83	0.67	0.76	0.69	0.135	0.85	0.42	0.86		
	57	1.10	1.17	1.20	1.05	0.130	0.91	0.78	0.84		
	Mean	0.96	0.92	0.98	0.87	0.094	0.83	0.44	0.72	**<0.001**	0.41
Monocytes (%)[6]	0	6.44	6.32	7.06	7.49	1.063	0.41	0.90	0.80		
	8	3.78[A]	5.99[AB]	7.08[B]	6.24[AB]	0.955	*0.05*	0.32	*0.09*		

Maternal Post-weaning (pw)		Control Control	Control Probiotic	Probiotic Control	Probiotic Probiotic		*P*-value				
	Day (pw)	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
	28	5.89	4.55	5.93	4.99	1.286	0.85	0.38	0.83		
	57	11.34	8.59	9.97	7.30	1.236	0.29	**0.03**	0.13		
	Mean	8.62	6.57	7.95	6.15	0.895	0.55	**0.04**	0.89	**<0.001**	0.93
Neutrophils (×10^3 cells/µL)	0	4.43^{AB}	5.75^{A}	4.66^{AB}	3.21^{B}	0.714	0.10	0.72	*0.05*		
	8	4.36	3.99	3.85	3.62	0.519	0.40	0.58	0.92		
	28	4.13	3.43	3.47	2.98	0.407	0.19	0.15	0.30		
	57	2.85	3.07	3.21	2.78	0.392	0.93	0.79	0.86		
	Mean	3.49	3.25	3.34	2.88	0.285	0.38	0.22	0.70	*0.07*	0.45
Neutrophils (%)[6]	0	40.69	43.98	36.20	36.98	5.059	0.26	0.69	0.81		
	8	36.81	38.02	34.10	36.84	4.676	0.68	0.67	0.86		
	28	25.63	22.04	30.54	23.98	3.393	0.33	0.14	0.31		
	57	25.15	21.09	26.27	20.49	3.270	0.94	0.14	0.51		
	Mean	25.39	21.57	28.41	22.24	2.671	0.50	*0.07*	0.66	0.26	0.88
Eosinophils (×10^3 cells/µL)	0	0.17	0.19	0.16	0.14	0.035	0.38	0.98	0.65		
	8	0.16	0.13	0.17	0.09	0.024	0.42	**0.03**	0.29		
	28	0.19	0.17	0.13	0.15	0.028	0.22	0.99	0.59		
	57	0.19^{AB}	0.14^{A}	0.26^{B}	0.15^{A}	0.027	0.14	**<0.01**	**0.01**		
	Mean	0.19	0.15	0.20	0.15	0.019	0.888	**0.05**	0.71	0.19	0.19
Eosinophils (%)[6]	0	1.63	1.31	1.47	1.63	0.291	0.78	0.77	0.41		
	8	1.20	1.14	0.94	0.93	0.151	0.13	0.82	0.90		
	28	1.15	1.09	0.94	0.89	0.206	0.34	0.78	0.81		
	57	1.72^{ab}	0.89^{a}	2.06^{b}	1.02^{a}	0.198	0.23	**<0.001**	**0.001**		
	Mean	1.43	0.99	1.50	0.95	0.145	0.903	**0.001**	0.72	**<0.01**	0.71

Maternal Post-weaning (pw)		Control Control	Control Probiotic	Probiotic Control	Probiotic Probiotic		*P*-value				
	Day (pw)	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
Basophils (×10^3 cells/μL)	0	0.09	0.06	0.16	0.10	0.024	**0.05**	0.11	0.70		
	8	0.03^a	0.06^b	0.08^b	0.05^{ab}	0.009	**0.02**	0.69	**0.001**		
	28	0.23	0.15	0.26	0.21	0.039	0.27	0.13	0.27		
	57	0.27	0.28	0.24	0.22	0.038	0.22	0.88	0.63		
	Mean	0.25	0.22	0.25	0.22	0.027	0.95	0.23	0.98	0.13	0.57
Basophils (%)[6]	0	0.66	0.51	1.15	1.16	0.230	**0.01**	0.64	0.61		
	8	0.26^a	0.54^b	0.60^b	0.51^{ab}	0.085	**0.03**	0.11	**0.02**		
	28	1.76	1.13	2.02	1.46	0.369	0.44	0.11	0.34		
	57	2.56	2.10	1.92	1.56	0.355	0.11	0.24	0.26		
	Mean	2.16	1.61	1.97	1.51	0.259	0.59	*0.05*	0.87	*0.08*	0.98
Red blood cells (×10^6 cells/μL)	0	7.94	7.71	7.02	6.93	0.450	*0.07*	0.72	0.88		
	8	7.10	7.03	7.25	7.15	0.190	0.48	0.64	0.94		
	28	7.03	7.20	7.09	7.16	0.173	0.96	0.48	0.90		
	57	7.19	7.22	7.01	7.19	0.169	0.56	0.53	0.81		
	Mean	7.11	7.21	7.05	7.18	0.151	0.77	0.45	0.93	0.68	0.44
Haemoglobin (g/dL)	0	15.36	14.79	13.38	13.90	0.840	*0.10*	0.98	0.52		
	8	12.92	12.89	12.96	13.03	0.307	0.77	0.95	0.87		
	28	12.10	12.45	12.26	12.72	0.280	0.45	0.15	0.47		
	57	12.99	12.45	12.31	12.84	0.270	0.59	0.99	0.25		
	Mean	12.55	12.45	12.29	12.78	0.195	0.86	0.31	0.13	0.18	0.22
Haematocrit (L/L)	0	0.51	0.49	0.44	0.46	0.024	*0.05*	0.98	0.55		
	8	0.45	0.45	0.46	0.45	0.011	0.90	0.61	0.76		
	28	0.40	0.42	0.41	0.42	0.010	0.48	0.15	0.48		
	57	0.43	0.42	0.41	0.43	0.010	0.67	0.83	0.58		

Maternal Post-weaning (pw)		Control Control	Control Probiotic	Probiotic Control	Probiotic Probiotic		*P*-value				
	Day (pw)	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	Maternal × pw	Day	Maternal × pw × Day
	Mean	0.42	0.42	0.41	0.43	0.009	0.87	0.33	0.53	0.20	0.15
Mean corpuscular volume (fL)	0	63.79	63.87	63.19	66.08	0.904	0.39	0.11	0.13		
	8	64.02	64.34	63.04	62.72	1.151	0.27	0.99	0.78		
	28	57.19	58.17	58.10	59.18	0.762	0.22	0.19	0.37		
	57	59.81	57.71	58.64	59.39	0.747	0.73	0.37	0.23		
	Mean	58.48	57.94	58.37	59.28	0.651	0.35	0.78	0.27	*0.07*	*0.08*
Mean corpuscular haemoglobin (pg/cell)	0	19.38	19.25	19.12	20.12	0.328	0.37	0.20	*0.10*		
	8	18.22	18.42	17.86	18.26	0.325	0.43	0.36	0.75		
	28	17.16	17.28	17.25	17.75	0.233	0.24	0.20	0.31		
	57	18.08	17.25	17.50	17.83	0.230	0.98	0.28	*0.08*		
	Mean	17.61	17.26	17.38	17.79	0.204	0.49	0.89	*0.07*	**<0.01**	*0.09*
Mean corpuscular haemoglobin concentration (g/dL)	0	30.37	30.15	30.29	30.45	0.307	0.72	0.92	0.54		
	8	28.50	28.65	28.37	29.12	0.264	0.53	*0.10*	0.27		
	28	29.98	29.70	29.70	29.93	0.191	0.90	0.90	0.60		
	57	30.25	29.87	29.87	30.04	0.184	0.57	0.58	0.42		
	Mean	30.12	29.78	29.78	29.99	0.137	0.64	0.64	*0.06*	0.18	0.96
Platelets ($\times 10^3$ cells/µL)	0	318.10	351.50	426.70	406.80	50.627	0.11	0.90	0.60		
	8	228.06	220.50	386.20	285.89	32.594	**<0.01**	0.16	0.26		
	28	362.07	371.50	349.61	345.03	34.682	0.58	0.95	0.94		
	57	289.40	262.96	285.10	349.16	27.984	0.16	0.58	0.21		
	Mean	323.70	312.55	315.71	347.09	25.040	0.61	0.70	0.41	**<0.01**	0.15

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON/CON, non-probiotic supplemented sow/non-probiotic supplemented piglet; [3]CON/PRO, non-probiotic supplemented sow/probiotic-supplemented piglet; [4]PRO/CON, probiotic-supplemented sow/non-probiotic supplemented piglet; [5]PRO/PRO, probiotic-supplemented sow/probiotic-supplemented piglet.

[6]Percentages are based on the differential count of white blood cells.
[a-b]Values within a row that do not share a common superscript are significantly different ($P \leq 0.05$).
[A-B]Values within a row that do not share a common superscript tended to differ ($P \leq 0.10$).
‡Day 0 pw is the day of weaning.

Table 8. Effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from day (D) 100 of gestation to weaning (D26 of lactation) on small intestinal morphology of piglets at D8 post-weaning[1].

	Maternal treatment			
	CON[2]	PRO[3]	SEM	*P*-value
N	20	20		
Duodenum				
Goblet cells	13.8	14.9	1.14	0.52
Villous height (µm)	351.8	392.7	8.61	**<0.01**
Crypt depth(µm)	177.0	190.5	4.43	**0.04**
VH:CD ratio[4]	2.1	2.1	0.07	0.62
Villous area (µm^2)	40888	48962	1814.2	**<0.01**
Crypt area (µm^2)	6739	7485	269.3	*0.06*
Jejunum				
Goblet cells	8.7	10.2	0.85	0.20
Villous height (µm)	346.3	362.8	8.07	0.16
Crypt depth(µm)	175.9	189.1	4.44	**0.04**
VH:CD ratio[4]	2.0	2.0	0.06	0.37
Villous area (µm^2)	38947	42105	1961.2	0.26
Crypt area (µm^2)	6731	8075	343.7	**<0.01**
Ileum				
Goblet cells	13.7	15.9	1.27	0.22
Villous height (µm)	325.7	345.8	7.39	*0.06*
Crypt depth(µm)	183.1	187.0	3.98	0.50
VH:CD ratio[4]	1.8	1.9	0.05	0.41
Villous area (µm^2)	37552	41677	1724.3	*0.10*

Crypt area (μm^2)	7211	7659	290.6	0.28

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]VH:CD ratio, villous height:crypt depth ratio.

Figure legends

Figure 1. Intestinal morphology of duodenum sections taken on day 8 post-weaning from piglets born to sows receiving the *B. altitudinis* WT588-supplemented diet (**A**) or a control diet (**B**). The black line shows the villous height measurement. Boxplots show the significant effects of the maternal treatment on the crypt depth (**C**) and villous height (**D**) of the duodenum of the offspring. Significant differences between treatments are indicated as ** ($P \leq 0.01$) and * ($0.01 < P \leq 0.05$).

Supplementary Tables

Supplementary Table S1. Effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from day (D) 100 of gestation to weaning (D26 of lactation) on tissue mobilisation and reproductive performance of sows[1].

		Treatment			*P*-value		
Item	Days	CON[2]	PRO[3]	SEM	Treat-ment	Day	Treat-ment × Day
N		12	12				
Body weight (kg)	D100 Gestation	257.10	258.61	9.337	0.84		
	D114 Gestation	283.47	278.77	2.769	0.23		
	D26 Lactation[4]	256.97	248.68	2.709	**0.03**		
	Overall			1.991	**0.02**	**<0.001**	0.51
Back fat (mm)	D100 Gestation	17.29	18.61	0.784	0.24		
	D114 Gestation	17.01	17.38	0.413	0.53		
	D26 Lactation	14.80	14.35	0.405	0.43		
	Overall			0.300	0.92	**<0.001**	0.31
Feed intake (kg)	Gestation	2.89	2.90	0.096	0.97		
	Lactation	5.75	5.84	0.096	0.51		
	Overall			0.068	0.62	**<0.001**	0.66
Body weight reduction (%)[5]		9.89	11.48	1.292	0.24		
Back fat reduction (%)[5]		6.38	8.21	3.923	0.62		
Reproductive performance							
Gestation length (days)		114.76	114.59	0.331	0.689		
Total born		14.62	15.49	1.253	0.560		
Live born		13.50	13.97	1.170	0.735		
Live born (%)		93.32	90.76	3.247	0.543		
Stillborn		1.15	1.51	0.592	0.648		
Piglets suckling at 48h *post-partum*		14.26	14.17	0.398	0.871		
Mortality (%)[6]		15.61	10.14	2.815	0.183		
Weaned piglets		11.75	12.58	0.548	0.294		

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]Day 26 of lactation was the day that litters were weaned.
[5]Body weight reduction and back fat reduction were calculated for the entire lactation period.
[6]Mortality percentage was calculated for the entire pre-weaning period.

Supplementary Table S2. Effect of supplementing sow diets with *Bacillus altitudinis* WIT588 spores from day (D) 100 of gestation to weaning (D26 of lactation) on haematological parameters of sows[1].

		Treatment			*P*-value		
Blood parameter	Day	CON[2]	PRO[3]	SEM	Treat-ment	Day	Treat-ment × Day
N		12	12				
White blood cells (×10³ cells/µl)	G100	9.08	9.77	0.754	0.53		
	G114	8.37	8.90	0.659	0.56		
	W26	11.00	9.93	0.571	0.18		
	Mean	9.68	9.42	0.454	0.66	**<0.01**	0.19
Lymphocytes (×10³ cells/µL)	G100	4.32	4.15	0.386	0.76		
	G114	3.31	3.88	0.255	0.11		
	W26	3.86	3.51	0.229	0.26		
	Mean	3.58	3.69	0.182	0.64	0.70	*0.05*
Lymphocytes (%)[4]	G100	45.83	46.74	2.370	0.79		
	G114	39.02	43.08	2.586	0.28		
	W26	36.27	36.37	2.319	0.98		
	Mean	37.65	39.73	1.739	0.41	*0.06*	0.43
Monocytes (×10³ cells/µL)	G100	0.66	0.80	0.087	0.20		
	G114	0.69	0.65	0.058	0.55		
	W26	0.67	0.71	0.051	0.62		
	Mean	0.68	0.68	0.042	0.89	0.70	0.43
Monocytes (%)[4]	G100	8.04	8.63	0.516	0.43		
	G114	8.22	7.57	0.563	0.40		
	W26	6.40	7.36	0.506	0.17		
	Mean	7.31	7.46	0.405	0.77	*0.05*	0.12
Neutrophils (×10³ cells/µL)	G100	3.45	3.32	0.355	0.79		
	G114	3.99	3.67	0.571	0.70		
	W26	5.67	4.64	0.505	0.17		
	Mean	4.83	4.16	0.384	0.23	**0.02**	0.52
Neutrophils (%)[4]	G100	37.28	37.05	2.602	0.95		
	G114	47.04	40.74	2.990	0.15		
	W26	51.00	49.05	2.646	0.61		
	Mean	49.02	44.90	2.001	0.16	**0.04**	0.45
Eosinophils (×10³ cells/µL)	G100	0.44	0.50	0.045	0.32		
	G114	0.35	0.37	0.073	0.82		
	W26	0.47	0.40	0.066	0.45		
	Mean	0.41	0.39	0.052	0.74	0.26	0.50
Eosinophils (%)[4]	G100	4.59	5.58	0.439	0.13		
	G114	4.08	4.11	0.639	0.97		

	W26	4.29	3.90	0.571	0.63		
	Mean	4.19	4.01	0.429	0.77	1.00	0.73
Basophils ($\times 10^3$ cells/µL)	G100	0.10	0.11	0.013	0.54		
	G114	0.11	0.17	0.024	**0.04**		
	W26	0.17	0.22	0.022	*0.07*		
	Mean	0.14	0.20	0.018	**<0.01**	**<0.01**	0.72
Basophils (%)[4]	G100	1.11	1.36	0.127	0.19		
	G114	1.24	1.81	0.207	**0.05**		
	W26	1.58	2.32	0.188	**<0.01**		
	Mean	1.41	2.06	0.155	**0.001**	**0.02**	0.61
Red blood cells ($\times 10^6$ cells/µL)	G100	7.36	6.99	0.534	0.64		
	G114	5.88	5.87	0.128	0.94		
	W26	5.56	5.83	0.115	*0.10*		
	Mean	5.72	5.85	0.089	0.29	0.15	0.24
Haemoglobin (g/dL)	G100	14.55	13.61	1.081	0.56		
	G114	12.00	11.67	0.244	0.32		
	W26	11.27	11.37	0.219	0.73		
	Mean	11.64	11.52	0.175	0.61	**0.03**	0.32
Haematocrit (L/L)	G100	0.47	0.44	0.032	0.51		
	G114	0.39	0.37	0.008	0.14		
	W26	0.36	0.37	0.007	0.67		
	Mean	0.38	0.37	0.005	0.40	*0.05*	0.16
Mean corpuscular volume (fL)	G100	63.52	62.77	0.601	0.25		
	G114	66.18	63.88	0.474	**0.001**		
	W26	65.01	63.23	0.431	**<0.01**		
	Mean	65.60	63.55	0.357	**<0.001**	**0.03**	0.51
Mean corpuscular haemoglobin (pg/cell)	G100	19.90	19.57	0.197	0.13		
	G114	20.47	19.93	0.154	**0.01**		
	W26	20.20	19.54	0.139	**0.001**		
	Mean	20.34	19.74	0.113	**0.001**	**0.02**	0.65
Mean corpuscular haemoglobin concentration (g/dL)	G100	31.33	31.14	0.220	0.56		
	G114	30.89	31.23	0.122	**0.04**		
	W26	31.02	31.00	0.111	0.91		
	Mean	30.96	31.12	0.093	0.14	0.62	*0.09*
Platelets ($\times 10^3$ cells/µL)	G100	120.80	161.51	27.197	0.31		
	G114	152.77	164.59	18.432	0.64		
	W26	240.59	239.14	16.473	0.95		
	Mean	196.68	201.87	13.474	0.76	**<0.001**	0.68

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]Percentages are based on the differential count of white blood cells.

Supplementary Table S3. Pre-weaning growth performance of piglets born to sows fed a control or a probiotic-supplemented diet[1].

		Treatment			*P*-value		
Item	Day (D)	CON[2]	PRO[3]	SEM	Treatment	Day	Treatment × Day
N		153	154				
Mortality[4]		24	15				
Off trial[5]		6	3				
Body weight (kg)	Birth (D0)	1.48	1.47	0.029	0.90		
	D14	3.89	3.91	0.167	0.90		
	Weaning (D26)	7.24	7.30	0.168	0.69		
	Overall			0.150	0.71	**<0.001**	0.84
Average daily gain (g)	D0-14	181.97	183.52	10.785	0.86		
	D15-26	293.68	305.09	10.863	0.21		
	Overall			9.854	0.31	**<0.001**	0.44
	D0-26	233.27	236.13	11.207	0.70		

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON: non-probiotic supplemented sows; [3]PRO: probiotic-supplemented sows.
[4]Mortality: In CON group, mortality was due to overlay (N=12), starvation (N=11), and pot belly (N=1). In PRO group, mortality was due to overlay (N=5) and starvation (N=8).
[5]Off trial: Runt piglets (CON, N=6 and PRO, N=3) that were removed from the trial.

Supplementary Table S4. Effect of *Bacillus altitudinis* WIT588 spore supplementation to sow and piglet diets on small intestinal morphology of piglets at day 8 post-weaning[1].

Maternal	Control	Control	Probiotic	Probiotic		*P*-value		
Post-weaning (pw)	Control	Probiotic	Control	Probiotic				Maternal
	CON/CON[2]	CON/PRO[3]	PRO/CON[4]	PRO/PRO[5]	SEM	Maternal	pw	× pw
N	10	10	10	10				
Duodenum								
Goblet cells	14.2	13.5	14.3	15.5	1.62	0.52	0.88	0.54
Villous height (µm)	340.9	362.7	400.7	384.7	12.18	**<0.01**	0.81	0.13
Crypt depth(µm)	175.2	178.9	192.7	188.3	6.27	**0.04**	0.96	0.52
VH:CD ratio[6]	2.0	2.1	2.1	2.1	0.09	0.62	0.78	0.69
Villous area (µm^2)	38819	42958	49822	48103	2565.7	**<0.01**	0.64	0.26
Crypt area (µm^2)	6531	6946	7636	7335	380.9	*0.06*	0.88	0.35
Jejunum								
Goblet cells	9.9	7.4	10.3	10.1	1.19	0.20	0.26	0.32
Villous height (µm)	346.5	346.1	345.7	379.9	11.41	0.16	0.15	0.14
Crypt depth(µm)	182.5	169.2	190.8	187.4	6.29	**0.04**	0.19	0.44
VH:CD ratio[6]	2.0	2.1	1.9	2.0	0.08	0.37	**0.03**	0.69
Villous area (µm^2)	38151	39743	38426	45784	2773.5	0.26	0.12	0.31
Crypt area (µm^2)	6918	6544	7615	8535	486.1	**<0.01**	0.58	0.19
Ileum								
Goblet cells	11.8	15.6	15.9	15.9	1.79	0.22	0.30	0.30
Villous height (µm)	317.9	333.6	334.4	357.2	10.46	*0.06*	*0.08*	0.74
Crypt depth(µm)	180.2	186.0	182.1	191.8	5.62	0.50	0.18	0.73
VH:CD ratio[6]	1.8	1.9	1.9	1.9	0.07	0.41	0.48	0.85

Villous area (μm^2)	36571	38533	37001	46354	2438.5	*0.10*	**0.03**	0.14
Crypt area (μm^2)	7116	7307	7006	8312	411.0	0.28	*0.08*	0.18

[1]Least square means and pooled standard errors of the mean (SEM).
[2]CON/CON, non-probiotic supplemented sow/non-probiotic supplemented piglet; [3]CON/PRO, non-probiotic supplemented sow/probiotic-supplemented piglet; [4]PRO/CON, probiotic-supplemented sow/non-probiotic supplemented piglet; [5]PRO/PRO, probiotic-supplemented sow/probiotic-supplemented piglet.
[6]VH:CD ratio, villous height:crypt depth ratio.

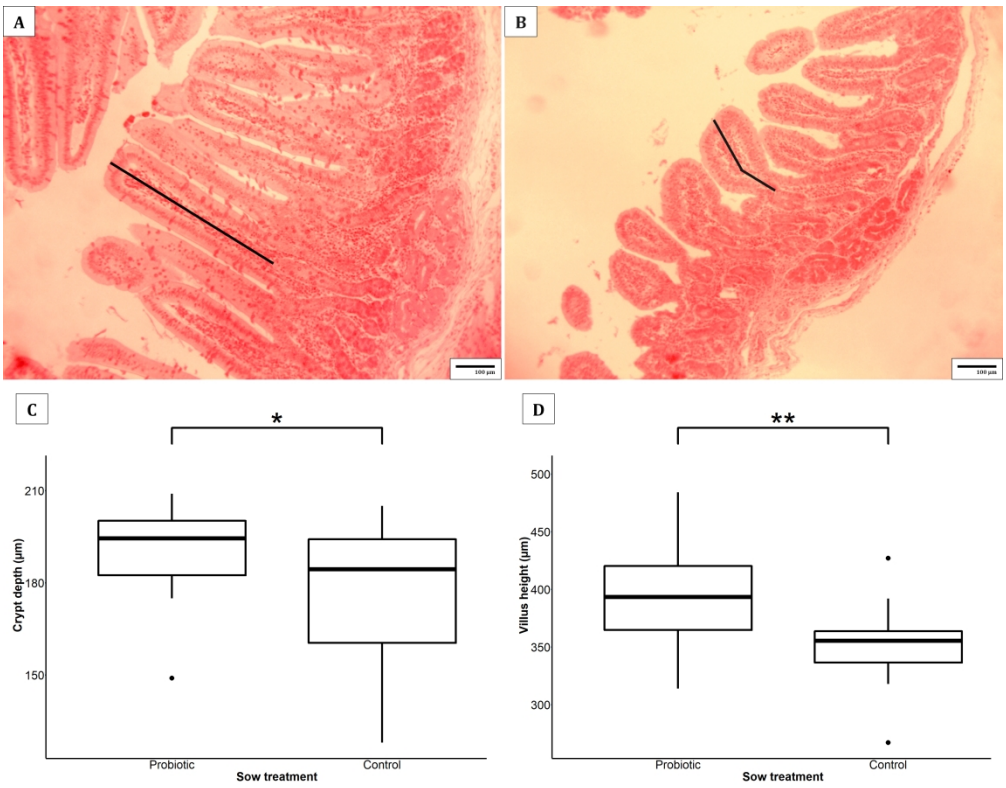

Figure 1. Intestinal morphology of duodenum sections taken on day 8 post-weaning from piglets born to sows receiving the *B. altitudinis* WT588-supplemented diet (**A**) or a control diet (**B**). The black line shows the villous height measurement. Boxplots show the significant effects of the maternal treatment on the crypt depth (**C**) and villous height (**D**) of the duodenum of the offspring. Significant differences between treatments are indicated as ** ($P \leq 0.01$) and * ($0.01 < P \leq 0.05$).

The ARRIVE Essential 10: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Sows: L.113-121 Pigs: L.128-136 Lines 375-378
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Sows: L.113-121 Pigs: L.128-136 Lines 358-364
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Lines 215-218 Table 5 Tables
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Sows: L.113-121 Pigs: L.128-130 Sows: L.113-121 Pigs: L.128-130 Pig rooms: L195-197
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	No blinding was applied
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Results section Lines 361-364
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Lines 365-408 Lines 365-408
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Sows: L.113-115 Pigs: L.128-130 Were born in farm
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Sows: L.113-121 Pigs: L.128-136 Same as above Lines 185-218 Lines 99-102
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Results section and Tables NA