Effect and interaction between wheat bran and zinc oxide on productive performance and intestinal health in post-weaning piglets

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Abstract

The inclusion of fibre has been studied as an alternative to antimicrobials in weaning pig diets, while ZnO is used as an effective method to prevent post-weaning diarrhoea. However, it has not been investigated to what extent these two strategies interact with each other. The present study was designed to evaluate the effects of including wheat bran (WB) and ZnO alone or combined in the diet of early-weaning pigs on productive performance and microbial activity in the gastrointestinal tract (trial 1). A total of sixty-four piglets were distributed in a 2 × 2 factorial combination of two levels of WB (0 v. 40 g/kg) and ZnO (0 v. 3 g/kg) in the diet. The inclusion of ZnO in the diet improved the feed intake and growth of the animals and reduced the incidence of diarrhoea. The inclusion of WB increased SCFA concentrations and decreased Escherichia coli counts. However, simultaneous incorporation of WB and ZnO increased E. coli counts. Two in vitro trials were also designed to clarify hypotheses derived from the in vitro test: (1) the ability of WB and other fibre sources to bind E. coli in vitro (trial 2) and (2) the in vitro interactions between WB and ZnO with respect to E. coli growth (trial 3). We can conclude that incorporation of WB in the diet improved gut health by modulating the activity and composition of the microbial population. The negative interaction between WB and ZnO raises the interest of considering the inclusion of phytase enzymes to reduce the therapeutic levels of ZnO in post-weaning diets.

Key words: Colibacillosis; Microbiota; Piglets; Post-weaning diets

Post-weaning colibacillosis is a diarrhoeal disease of young pigs, strongly influenced by the drastic changes faced by the piglets immediately after weaning. Reduced feed intake, intestinal villous atrophy and reduced enzymatic and absorptive capacity of the gut may result in impaired digestion and absorption of nutrients and in the overgrowth of bacteria such as enterotoxigenic Escherichia coli(1). The main aetiological agents are those enterotoxigenic E. coli expressing fimbrial antigens, which mediate adhesion to complementary carbohydrates located in the brush borders of villous enterocytes(2,3).

So far, the most common strategy used to prevent enterotoxigenic E. coli growth in the intestine has been the addition of in-feed antimicrobial agents(4). In those situations when the use of sub-therapeutic doses of antimicrobial agents has been suppressed (i.e. in the European Union in 2006(5)), the prescription of therapeutic antimicrobial agents has considerably increased(6). In some European Union countries, pharmacological doses of ZnO (2500–3000 parts per million) are now extensively used in feed during the first 2 weeks after weaning to reduce the incidence of post-weaning E. coli diarrhoea(7). Besides having antimicrobial properties, some authors have proposed that high doses of Zn may increase feed intake by promoting an increase in the synthesis of ghrelin in the digestive tract(8) and may also reduce the inflammation of the intestinal mucosa(9). Although the use of pharmacological doses of ZnO does not have the downside of selecting microbial resistances with its subsequent implications in human medicine (as opposed to the use of antibiotics), its use has raised environmental concerns about the low retention of Zn by pigs and soil toxicity.

Considerably, efforts are being made in the search of an efficient replacer of antimicrobial agents. One of the possible alternatives would be to modify the main ingredients of the feed formula to promote fermentation in the hindgut, which would protect animals from opportunistic bacterial proliferation. Several reports support that low-crude protein diets(10,11), diets including protein of animal origin(7) or diets supplemented with fermentable carbohydrates (such as high lactose levels(12) or wheat bran (WB) and sugarbeet

Abbreviations: CT, control diet; OD, optical density; PA, phytic acid; TRF, terminal restriction fragments; WB, wheat bran.

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Materials and methods

Trial 1: in vivo experiment

Animals and diets. This experiment was performed at the Animal Facilities of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review Committee of this institution. The treatment, management, housing, husbandry and slaughtering conditions conformed to the European Union Guidelines(21). A total of sixty-four commercial crossbred piglets ((Large White × Landrace) × Pietrain), which had been excluded from receiving creep feed, were weaned at 21 d of age with an average body weight of 6·7 (SEM 0·37) kg. Pigs were transported from the water and some or all of the oil from fish by heating at low temperature (70 °C) and percolation of their emergence. The experiment was performed on a farm to the University facilities and were allocated into thirty-two pens (two animals/pen) based on the litter origin and body weight. Pens were allotted to four dietary treatments (eight replicates/treatment; Table 1) in a 2 × 2 factorial arrangement that included two levels of WB (0 v. 40 g/kg, control diet (CT) v. WB, respectively) and two levels of ZnO (0 v. 3 g/kg, CT v. ZnO diet, respectively) in the diet. The diets were formulated to be isoenergetic (averaging 16·1 MJ/kg) and isoproteic (averaging 182 g crude protein/kg) based on ground maize, barley and soyabean protein concentrate.

Experimental procedures and sampling. Animals received the diets from day 1 to 12 of the experiment. Individual body weight and pen feed consumption were recorded on days 0, 3, 6, 9 and 12 after weaning. Physical and behavioural examination of the animals was done daily to evaluate their health status. Samples of fresh faeces were collected from the rectum of one animal/pen for microbial counts on days 3, 6, 9 and 12 after weaning. At the end of the experimental period, the faecal samples were kept in tubes and immediately frozen at −80 °C for analysing the microbial structure, quantifying the lactobacilli counts and determining the SCFA concentration.

Analytical procedures. Chemical analyses of the diets (Table 1) were performed according to the Association of Official Analytical Chemists(22) standard procedures.

Traditional culture methods were used to determine some bacterial groups. The faecal samples were diluted 1:10 in PBS (Sigma, St Louis, MO, USA) immediately after collection, and subsequently homogenised. Viable counts of enterococci

Table 1. Composition and chemical analysis of pre-starter diets*

<table>
<thead>
<tr>
<th>Ingredients (g/kg DM)</th>
<th>CT</th>
<th>WB</th>
<th>ZnO</th>
<th>WB – ZnO</th>
</tr>
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<tbody>
<tr>
<td>Maize</td>
<td>414·0</td>
<td>367·0</td>
<td>414·0</td>
<td>364·0</td>
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<tr>
<td>Barley</td>
<td>200·0</td>
<td>200·0</td>
<td>200·0</td>
<td>200·0</td>
</tr>
<tr>
<td>Whey</td>
<td>112·0</td>
<td>102·0</td>
<td>112·0</td>
<td>102·0</td>
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<tr>
<td>High-fat whey</td>
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<td>90·0</td>
<td>69·0</td>
<td>90·0</td>
</tr>
<tr>
<td>Soyabean protein concentrate</td>
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<td>52·0</td>
<td>55·0</td>
<td>52·0</td>
</tr>
<tr>
<td>Spray-dried porcine plasma</td>
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<td>50·0</td>
<td>50·0</td>
<td>50·0</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>30·0</td>
<td>30·0</td>
<td>30·0</td>
<td>30·0</td>
</tr>
<tr>
<td>Fishmeal LT†</td>
<td>40·0</td>
<td>40·0</td>
<td>40·0</td>
<td>40·0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>–</td>
<td>40·0</td>
<td>–</td>
<td>40·0</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>10·0</td>
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<tr>
<td>Dicalcium phosphate</td>
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<tr>
<td>Benzoic acid</td>
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<td>5·0</td>
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<tr>
<td>Synthetic amino acids‡</td>
<td>11·2</td>
<td>11·2</td>
<td>11·2</td>
<td>11·2</td>
</tr>
<tr>
<td>Vitamin and mineral premix§</td>
<td>3·7</td>
<td>3·7</td>
<td>3·7</td>
<td>3·7</td>
</tr>
<tr>
<td>ZnO</td>
<td>–</td>
<td>3·0</td>
<td>–</td>
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</tr>
<tr>
<td>Chemical analysis (g/kg DM)</td>
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<td>903·0</td>
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<tr>
<td>Gross energy (MJ/kg)</td>
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<td>17·9</td>
<td>17·8</td>
<td>17·9</td>
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<tr>
<td>Crude protein (N × 6·25)</td>
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<td>200·0</td>
<td>203·0</td>
<td>200·0</td>
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<tr>
<td>Neutral-detergent fibre</td>
<td>79·0</td>
<td>92·0</td>
<td>79·0</td>
<td>92·0</td>
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<tr>
<td>Acid-detergent fibre</td>
<td>23·0</td>
<td>23·0</td>
<td>23·0</td>
<td>23·0</td>
</tr>
<tr>
<td>Diethyl ether extract</td>
<td>63·0</td>
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</tr>
<tr>
<td>Ash</td>
<td>56·0</td>
<td>57·0</td>
<td>56·0</td>
<td>57·0</td>
</tr>
</tbody>
</table>

CT, control diet; WB, wheat bran diet.

† Fishmeal LT, fishmeal low temperature: product obtained by removing most of the fish oil by heating at low temperature (70 °C) and percolation.

‡ Synthetic amino acids: L-Lys 0·099, L-Thr 0·099, L-Ile 0·10, L-Trp 0·08.

§ Supplied per kg of feed: 12 000 IU (3900 μg) vitamin A, 1800 IU (45 μg) vitamin D₃, 60·0 mg vitamin E, 3·0 mg vitamin K₃, 2·0 mg vitamin B₁₂, 6·0 mg vitamin B₆, 3·0 mg vitamin B₁, 0·02 mg vitamin B₂, 35·0 mg niacin, 15·0 mg calcium pantothenate, 0·12 mg biotin, 1·0 mg folic acid, 20·0 mg Fe, 120·0 mg Cu, 110 mg Zn, 45·0 mg Mn, 0·30 mg Se, 0·10 mg Co, 1·0 mg I and 2·5 mg ethoxyquin as an antioxidant (Capsuquin; Itpsa, Barcelona, Spain).
were done by plating serial tenfold dilutions onto Chromo
cult™ Enterococci-Agar (Merck K GaA, Darmstadt, Germany)
and incubating the plates for 24 h at 37°C. For the enumeration
of E. coli and coliforms, 1 ml of solution of the corre-
sponding dilution was pipetted onto an E. coli–coliform count
plate (3M Petrifilm, Europe Laboratories 3M Santé, Cergy-Pontoise, France) with Violet Red Bile gel as an indi-
cator of glucuronidase activity. The plates were incubated
for 48 h at 35°C, and the colonies were counted following
the manufacturer’s instructions. DNA from faeces was
extracted and purified using the commercial QIAamp DNA
Stool Mini Kit (Qiagen, West Sussex, UK), and the lactobacilli
population was quantified by real-time PCR using SYBR
Green dye, following Castillo et al. (25). The terminal restriction
fragment length polymorphism analysis of bacterial commu-
nity was performed following the procedure described by
Hojberg et al. (24) and adapted by Castillo et al. (25). Finally,
SCFA concentrations were determined by GC, after an
acid–base treatment followed by diethyl ether extraction
and derivatisation (26).

**Trial 2: in vitro adhesion test**

**Fibrous ingredients.** Seven different fibrous ingredients, WB,
rice hulls, soybean hulls, oat hulls, pea hulls, sugarbeet pulp
and cereal straw, were selected as test products. Bovine serum
albumin (Sigma) served as the reference (negative control),
following the protocol described by Becker et al. (27).

**Bacterial strains.** Two different E. coli strains were used in
this experiment to elucidate the interaction between fibre
substrates and bacterial fimbriae. The first one was an E. coli
K88 (enterotoxigenic E. coli, strain FV12048) isolated from
a colibacillosis outbreak in Spain (28), the second one was an E. coli
K88 (enteroxigenic E. coli, strain FV12048) isolated from
a colibacillosis outbreak in Spain (28), the serotype
(O149:K91:H10, F4+, LT1+, STb+) was provided by the E. coli Reference Laboratory, Veterinary Faculty of Santiago
de Compostela (Lugo). The other strain was a non-fimbriated
E. coli (F4−, F6−, F18−, LT1−, ST1−, ST2+, Stx2e−) isolated from the faeces of post-weaning piglets and kindly donated by the Departamento de Sanitat i Anatomia Animal from the Universitat Autònoma de Barcelona.

Bacteria were cultured in unshaken Luria broth (Sigma) at
37°C and serially passaged every 48 h, at least five times.
Bacterial cells from the culture were harvested and processed
as described earlier (27).

**Adhesion test methodology.** E. coli K88 and the non-
fimbriated strain were allowed to adhere to different fibre
components supplied as well coatings in microplates in a
miniaturised adhesion test, following the protocol described
by Becker et al. (27). Briefly, fibre ingredients were suspended in PBS to a final concentration of 4 % (w/v), and the soluble fraction was extracted for coating the flat-bottom wells of high-binding polystyrene microtitration plates. After blocking the non-specific sites with BSA, bacteria were added to a final concentration of 1·20 ± 10⁸ colony-forming units/ml. Bacteria were allowed to adhere by incubation at room
temperature for 30 min. Afterwards, the wells were washed
to remove non-adherent bacteria, and bacteria were allowed
to grow in Luria broth media by incubation in a microplate
reader (SPECTRAmax 384 Plus; Molecular Devices Corpor-
ation, Sunnyvale, CA, USA) at 37°C. Bacterial growth was
monitored as optical density (OD) at 650 nm at intervals of
10 min. The test principle, as described by Becker et al. (27),
is based on an inverse relationship between initial cell den-
sities and the appearance of growth defined as the duration
(h) needed for the cultures to reach an OD of 0·05 at
650 nm (t₀.⁵ = 0·05): the higher the adhering cell numbers,
the shorter the detection times of growth.

**Trial 3: in vitro wheat bran and zinc oxide interaction test**

**Sample preparation.** In order to elucidate the interaction
between WB and ZnO and the likely role of phytates, eight
different samples were prepared in a 4 × 2 factorial design,
which included four different buffered solutions (a negative
treatment: 4 % WB, 4 % WB + 0·02 % phytase enzyme (5000 IU/g; Ronozyme™; P500; DSM Nutritional Products Limited, Basel,
Switzerland); and 4 % WB + 0·02 % xylanase and glucanase
enzyme mixture (22 000 visco units/g xylanase and
2000 visco units/g glucanase; Rovabio™; Excel AP, Adisseo,
France)), and two levels of ZnO (0 v. 0·3 %, w/v). Samples
of buffered solutions were adjusted to a pH of 5·1 with
HCl and incubated for 4 h at room temperature. Then,
the suspensions were sonicated three times for 30 s each
and then centrifuged at 460 g for 5 min. The supernatant was
adjusted to a pH of 7·0 with NaOH and ZnO added
appropriate to the specific treatment.

**Bacterial strains.** Two different E. coli strains (E. coli K88
and a non-fimbriated E. coli strain) were used in this experi-
ment as described earlier.

**In vitro test.** E. coli K88 and the non-fimbriated E. coli
strains were centrifuged (1700 g) and adjusted to a final
concentration of approximately 3·5–3·9 × 10⁸ colony-forming
units/ml in Luria broth. Subsequently, 750 μl of each bacterial
suspension were incubated with 750 μl of each experimental
treatment. Thereafter, 300 μl of each suspension were added
to polystyrene microtitration plates, and the growth of the
bacteria was measured in a microplate reader at 37°C following
the protocol described by Becker et al. (27). Bacterial
growth was monitored as OD at 650 nm at intervals of
10 min for 10 h. All readings were done in two independent
assays and in triplicate per assay.

**Statistical analyses**

The OD data from the in vitro experiments were processed by
non-linear regression analysis using the non-linear P-NNLIN
(Gauss–Newton method) procedure (29) following the
equations described by Becker et al. (27) in order to obtain
the OD for each treatment. The OD for each treatment was
subjected to ANOVA using the generalised linear model
procedure (29). Classification factors included in each model
were WB, ZnO level and their interaction for trial 1, fibrous
ingredient, bacterial strain and their interaction for trial 2
and buffered solution, ZnO inclusion and their interaction
for trial 3. Means were calculated as least-squared means, and multiple mean comparisons were done using Tukey’s correction. The α level for the determination of significance was 0.05, and tendencies for 0.05 < P < 0.15 were also presented.

Results

Trial 1: in vivo experiment

Animal performance and health status. The effects of WB and ZnO on the average daily feed intake and average daily gain of the animals as well as the incidence of diarrhoea are shown in Table 2. The inclusion of ZnO in the diet increased the average daily feed intake of the animals from day 6 to 12 (P = 0.006) and from day 0 to 12 (P = 0.035). This resulted in an increased average daily gain of the animals for the same periods (P = 0.008 and 0.036, respectively) and a higher body weight at the end of the experiment (P = 0.044) compared with the animals not receiving ZnO in the feed. The inclusion of ZnO in the diet also reduced the incidence of diarrhoea (P = 0.009).

Metabolic activity and composition of faecal microbiota. Concentrations of total and individual SCFA in the faecal samples and also the counts of major bacterial groups using culturing methods or quantitative PCR are given in Table 3. Significant differences were observed for the SCFA concentration associated with the incorporation of WB and ZnO in the diet. Moreover, the interaction between WB and ZnO was also significant for the total SCFA (P = 0.048), propionic acid (P = 0.018) and butyric acid concentrations (P = 0.007) and also tended to be significant (P = 0.120) for acetic acid. Thus, the WB diet increased the total SCFA, propionic acid and butyric acid concentrations in comparison with the CT, ZnO and WB–ZnO diets. The incorporation of WB (WB and WB–ZnO diets) increased the concentration of isoacids (P = 0.001), and the inclusion of ZnO (ZnO and WB–ZnO diets) diminished the concentrations of acetic acid (P = 0.024) and isoacids (P = 0.001).

A significant interaction was observed between the ZnO and WB supplementation on the counts of E. coli and coliform after weaning. The simultaneous incorporation of ZnO and WB in the diet increased the E. coli and coliform counts compared with the ZnO diet on day 6 after weaning (P = 0.026) and compared with the WB diet on day 9 after weaning (P = 0.024). On day 12, animals fed the WB diet showed lower counts of E. coli (P = 0.054) and coliforms (P = 0.028) than those fed the CT diet, but no significant differences were observed with the ZnO or WB–ZnO diet. On day 12, the incorporation of ZnO in the diets (ZnO and WB–ZnO) also tended to increase the enterococci population (P = 0.064) and to reduce the lactobacilli counts (P = 0.084) in the faeces of pigs compared with that in the animals that did not receive ZnO.

The terminal restriction fragment length polymorphism method was employed to evaluate global changes in the microbial ecosystem. Fig. 1 shows the analysis focused on two of the diets: the WB and ZnO diets. It shows the microbial profiles of all pens except one from which we were unable to take faecal samples. The effect of the diet on the composition of faeces was clearly observed as most of the animals were grouped into two separate clusters. Microbial profiles of pigs fed the WB diet were more similar (50–75 %) than those of pigs fed the ZnO diet, which showed more heterogeneous microbial profiles (52–90 %). In silico restriction, using Ribosomal Database Project II and MiCA software (version 3; Department of Biological Sciences, University of Idaho; http://mica.ibest.uidaho.edu)(50), was used to deduce potential ecological changes in the samples. Results are presented as potential compatible bacterial species. The WB dietary treatment showed higher diversity in compatible terminal restriction fragments (TRF) than the ZnO or the WB–ZnO treatment exposed by the following results: four animals of the WB treatment showed a TRF of 105–106 bp that was not found in any of the other three treatments (CT, ZnO and WB–ZnO). Possible bacteria compatible with this fragment size are Bacteroides fragilis (102 bp), Prevotella ruminicola.

Table 2. Body weight (BW; g), average daily feed intake (ADFI, g/animal per d), average daily gain (ADG, g/animal per d) and diarrhoea incidence (no. of animals) in early-weaned pigs*  
(Mean values with their standard errors, n 8)

<table>
<thead>
<tr>
<th></th>
<th>Diets</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>BW 0</td>
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<td>Diarrhoea incidence 0–12</td>
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<td>16/16</td>
<td>11/16</td>
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</table>

CT, control diet; WB, wheat bran diet; WB–ZnO, wheat bran and ZnO diet; WB × ZnO, effect of WB and ZnO inclusion in the diet.

* Trial 1: in vivo experiment.
(102 bp) and numerous uncultured rumen bacteria (102–104 bp). Analysing the final part of the electropherograms, animals that received the CT and WB diets showed eight and thirteen TRF of 522–582 bp, respectively, whereas only one TRF (102 bp) and numerous uncultured rumen bacteria were found related to the adhesion of some E. coli strains. Previous studies have shown that ZnO supplementation showed antimicrobial effects when supplemented into the WB + phytase treatment. However, when it was added to the WB or WB + xylanase treatment, ZnO did not reduce the growth of E. coli.

**Trial 3: in vitro analysis of interaction between wheat bran and zinc oxide**

Table 5 presents the results related to trial 3 as detection times of growth for E. coli K88 and the non-fimbriated E. coli. A significant (P=0.0001) interaction between the ZnO and buffered solutions was found related to the growth of the two E. coli strains. The incorporation of ZnO in the buffered solution inhibited (P=0.0001) the bacterial growth for both E. coli strains in comparison with the negative control. Also, the ZnO supplementation showed antimicrobial effects when supplemented into the WB + phytase treatment. However, when it was added to the WB or WB + xylanase treatment, ZnO did not reduce the growth of E. coli.

**Discussion**

**The influence of zinc oxide and wheat bran on the adaptation of piglets after weaning**

The present results showed that a dietary supplementation with a high level of ZnO (3 g/kg) increased the feed intake and average daily gain of the animals and reduced the onset of diarrhea in weaning piglets during the first days after weaning. These results are in accordance with observations from animal performance studies, in which a larger number of animals were used. Since ZnO is known to possess antimicrobial properties, it has been usually assumed that it enhances growth by controlling pathogenic bacteria. In the
present study, ZnO reduced the fermentation activity and the counts of lactobacilli and increased the counts of enterococci, as described by Hojberg et al.\textsuperscript{(24)}. On the other hand, some studies have also demonstrated that high doses of ZnO are effective in increasing the feed intake related to modulations of gene expression\textsuperscript{(9,33)}. Yin et al.\textsuperscript{(8)} observed that ZnO supplementation increased plasma levels of ghrelin in early-weaned piglets. Ghrelin is a hormone released by the stomach, which is involved in the secretion of growth hormone and insulin-like growth factor 1 and in the stimulation of the feed intake and muscle growth.

The present results also revealed that ZnO supplementation decreased the incidence of diarrhoea and the counts of \textit{E. coli} in faeces, as observed by Cardinal et al.\textsuperscript{(17)}. Diarrhoea in piglets is an important problem, which is associated, in some cases, with an overproliferation of enteropathogenic \textit{E. coli}. However, animals in the present study presented diarrhoea without a pathological picture of fever, dehydration or apathy. Ou et al.\textsuperscript{(25)} demonstrated that Zn is also able to ameliorate intestinal inflammation due to inadequate feed intake after weaning by reducing the number of mast cells in the small-intestinal mucosa and submucosa and by inhibiting histamine release from mast cells.

In contrast to the results observed with the ZnO treatments, the incorporation of WB did not improve the productive performance, as was also shown in earlier studies\textsuperscript{(16,17)}, but increased the concentration of fermentation products in the faeces, especially the concentration of butyrate. These results confirm that the early-weaned pigs were able to increase carbohydrate fermentation with the inclusion of WB in the diet. It is accepted that starch and bran from wheat or oats stimulate the formation of butyrate\textsuperscript{(26)}, which is considered the principal oxidative fuel for colonocytes and may have beneficial trophic effects on the inflamed caeco-colonic mucosa\textsuperscript{(27)}. As observed previously with other insoluble fibre sources\textsuperscript{(17,35)}, the addition of WB in the diet decreased the \textit{E. coli} and coliform bacteria counts in faeces. Previous results from our group have also indicated that the incorporation of WB in the diet also decreased the enterobacteria counts in the caecal digesta\textsuperscript{(27)} and the K88 \textit{E. coli} attachment to the ileum mucosa after an experimental infection\textsuperscript{(18)}. The main mechanism involved in these changes could be an increased fermentation of carbohydrates, which may reduce protein fermentation\textsuperscript{(13)}, or likely changes in the physico-chemical properties of digesta, which can increase its water-binding capacity or the ability of some long-chain NSP to block the attachment of \textit{E. coli} to the intestinal tract\textsuperscript{(20)}.

However, a significant two-way interaction was observed between the WB and ZnO supplementation. The administration of medicated feed (ZnO) decreased the concentration of SCFA, increased coliform bacteria counts in faeces and reduced some of the microbial groups that were growth promoted when WB was included in the diet. It could be suggested that the antimicrobial effect of ZnO may have reduced the fermentation of fibre, and the main microbial changes were promoted by the WB supplementation. However, it is also intriguing that the combination of WB and ZnO also reduced the effect of therapeutic doses of ZnO in the counts of \textit{E. coli} and coliform in faeces. The following two trials were designed to test hypotheses evidenced in the \textit{in vitro} trial: (1) the ability of WB to bind \textit{E. coli} in the intestinal digesta (trial 2) and (2) the likely mechanisms by which WB may affect the antimicrobial activity of the ZnO on the \textit{E. coli} growth \textit{in vitro} (trial 3).

### Potential of different fibrous substrates to bind \textit{Escherichia coli}

Different studies have shown the promising effects of glycoconjugates from different origins such as cranberry and...
blueberry extracts\(^{56}\), mannan-oligosaccharides\(^{37,38}\), palm kernel extracts\(^{39}\) or soya and fermented soyabean products\(^{40}\) to inhibit the adhesion of different pathogens such as \textit{E. coli} or \textit{Salmonella} to the intestinal mucosa of different animal species. Dietary fibre from plants may provide an alternative adhesion matrix to enteropathogenic bacteria because of their carbohydrate nature similar to the intestinal microbiota, enhancing the fermentation and blocking the attachment of \textit{E. coli} strains as \textit{ZnO} and the combination of \textit{WB}–phytase and \textit{ZnO} did. Therefore, it is suggested that a negative interaction between phytic acid (PA) and \textit{ZnO} modifies the antimicrobial properties of therapeutic doses of \textit{ZnO} in \textit{vivo} and \textit{in vitro}. Champagne & Fisher\(^{44}\) suggested that PA, primarily found in the pericarp of cereal grains, may form a rather stable complex with bivalent cations, such as Cu\(^{2+}\) and Zn\(^{2+}\). These complexes are known not to affect Zn bioavailability in chicks\(^{45}\) and also in humans\(^{46}\). Procedures that degrade phytate have been studied as a means to increase the bioavailability of Zn and other cations in the diet\(^{47,48}\). It is known that fermentation of feed may reduce the PA:Zn ratio, promoting a better Zn absorption\(^{49}\). Other authors, namely Gaetke \textit{et al}.\(^{50}\), also have shown that yam (both active and heat-treated) protects against growth retardation in weanling rats fed high PA. In animals, feeding PA has been regarded as an anti-nutrient, which reduces P availability, and most research in this field has been aimed at eliminating PA from the animal feed by adding exogenous phytase to it. The term phytase is defined as a class of phosphatases with the \textit{in vitro} capability to release at least one phosphate from PA\(^{31}\). Some authors\(^{51,52}\) have suggested that phytase supplementation may increase the amount of Zn absorbed, even when pharmacological doses of Zn are included in the diet. Thus, Martinez \textit{et al}..\(^{52}\) suggested that present pharmacological doses of Zn (2000 mg/kg) fed to pigs could be reduced to 1000 mg/kg by adding phytase. The present study confirms a negative interaction between the WB and therapeutic doses of \textit{ZnO}, which appears to be related to the high levels of PA in WB. This study shows that the incorporation of WB in the diet of early-weaning piglets may improve their gut health by modulating the activity of the intestinal microbiota, enhancing the fermentation and blocking the attachment of \textit{E. coli} K88 to the intestinal mucosa. A negative interaction observed \textit{in vivo} and \textit{in vitro} between WB (rich in phytate) and \textit{ZnO} raises the interest of considering the inclusion of phytase enzymes to reduce the required levels of \textit{ZnO} in post-weaning diets.

**Possible mechanism involved in the interaction between wheat bran and zinc oxide**

Negative interactions between WB and \textit{ZnO} have been reported in the present study \textit{in vivo} and \textit{in vitro}. In the \textit{in vitro} trial, the WB–\textit{ZnO} combination did not reduce \textit{E. coli} and the coliform counts as \textit{ZnO} or WB did. In the \textit{in vitro} trial, the WB–\textit{ZnO} combination did not have the same antimicrobial effect on the \textit{E. coli} strains as \textit{ZnO} and the combination of WB–phytase and \textit{ZnO} did. Therefore, it is suggested that a negative interaction between phytic acid (PA) and \textit{ZnO} modifies the antimicrobial properties of therapeutic doses of \textit{ZnO} in \textit{vivo} and \textit{in vitro}. Champagne & Fisher\(^{44}\) suggested that PA, primarily found in the pericarp of cereal grains, may form a rather stable complex with bivalent cations, such as Cu\(^{2+}\) and Zn\(^{2+}\). These complexes are known not to affect Zn bioavailability in chicks\(^{45}\) and also in humans\(^{46}\). Procedures that degrade phytate have been studied as a means to increase the bioavailability of Zn and other cations in the diet\(^{47,48}\). It is known that fermentation of feed may reduce the PA:Zn ratio, promoting a better Zn absorption\(^{49}\). Other authors, namely Gaetke \textit{et al}.\(^{50}\), also have shown that yam (both active and heat-treated) protects against growth retardation in weanling rats fed high PA. In animals, feeding PA has been regarded as an anti-nutrient, which reduces P availability, and most research in this field has been aimed at eliminating PA from the animal feed by adding exogenous phytase to it. The term phytase is defined as a class of phosphatases with the \textit{in vitro} capability to release at least one phosphate from PA\(^{31}\). Some authors\(^{51,52}\) have suggested that phytase supplementation may increase the amount of Zn absorbed, even when pharmacological doses of Zn are included in the diet. Thus, Martinez \textit{et al}..\(^{52}\) suggested that present pharmacological doses of Zn (2000 mg/kg) fed to pigs could be reduced to 1000 mg/kg by adding phytase. The present study confirms a negative interaction between the WB and therapeutic doses of \textit{ZnO}, which appears to be related to the high levels of PA in WB. Taking into account these results, phytase supplementation may be proposed as a good approach to increase the effectiveness or to reduce the levels of \textit{ZnO} in post-weaning diets.

### Conclusion

Based on the results of the present study, we conclude that the incorporation of WB in the diet of early-weaning piglets may improve their gut health by modulating the activity of the intestinal microbiota, enhancing the fermentation and blocking the attachment of \textit{E. coli} K88 to the intestinal mucosa. A negative interaction observed \textit{in vivo} and \textit{in vitro} between WB (rich in phytate) and \textit{ZnO} raises the interest of considering the inclusion of phytase enzymes to reduce the required levels of \textit{ZnO} in post-weaning diets.
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