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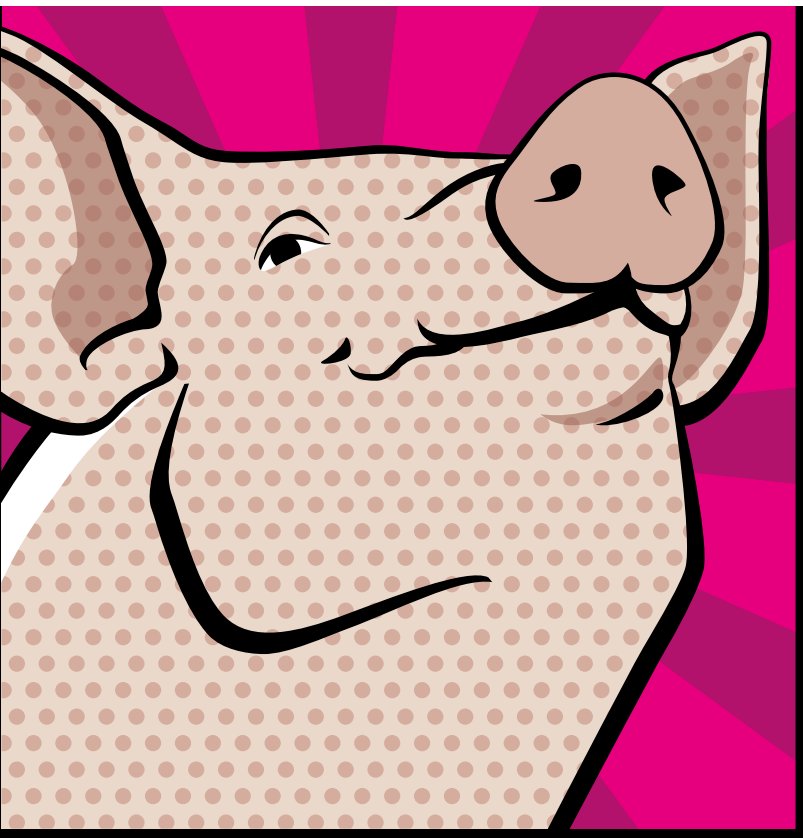
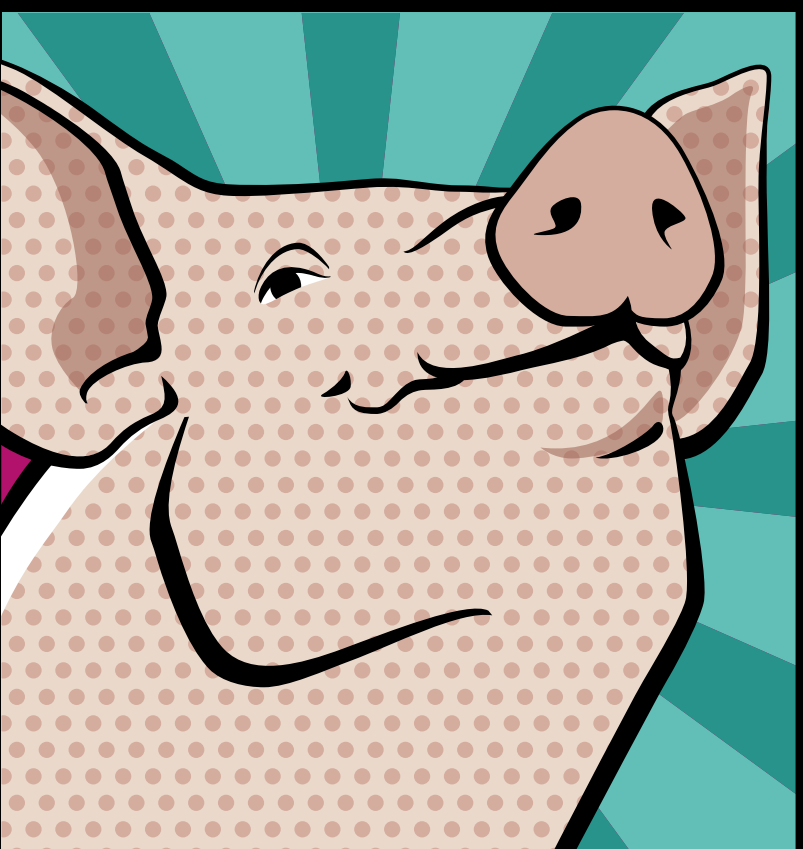
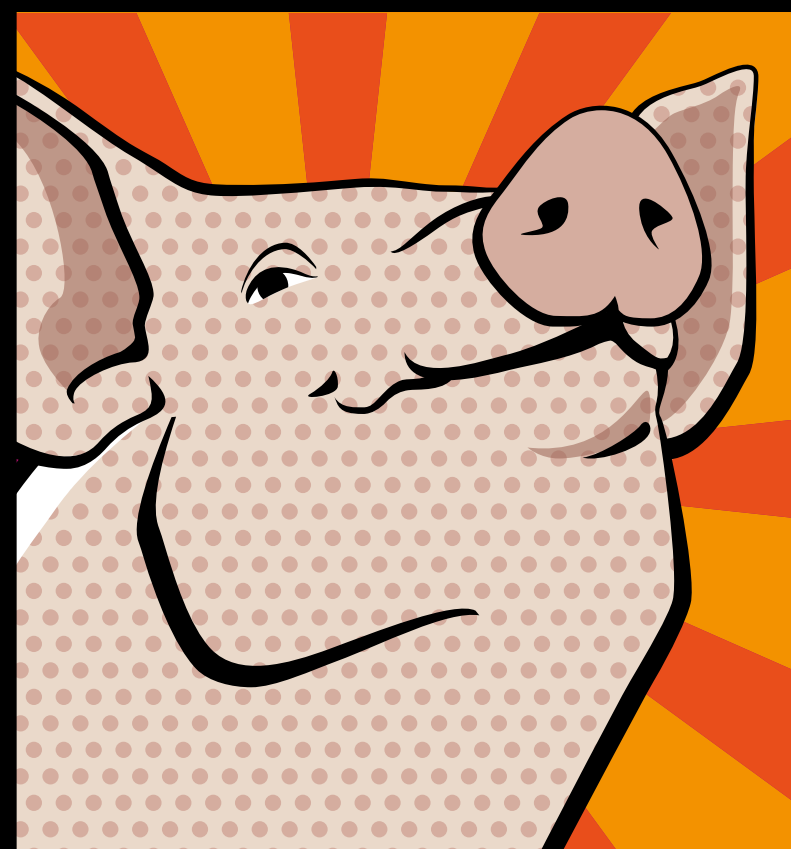






Antibacterial effects of zinc oxide in weaned piglets

By Valérie Kromm and Agathe Roméo, Animine

Dietary zinc oxide is known to increase growth and reduce post-weaning diarrhoea in piglets. But why do other forms of zinc do not manage to perform as well as zinc oxide? Let's focus on the antimicrobial activity of zinc oxide.

ZnO in diets of weaned piglets

At weaning, when the intestinal microbiota changes and the immune system is not fully developed, pathogenic bacteria like enterotoxigenic *E. coli* (ETEC) find a favourable environment to multiply and colonise in the gastrointestinal tract of piglets. This manifests in the diarrhoea that we frequently observe post-weaning, combined with reduced growth. Among all the additives available to the feed industry, zinc in the form of zinc oxide is a very popular solution. It is well established that zinc oxide exerts antimicrobial activity and recent studies with combined deep sequencing and PCR amplification offer new insights into the effect of the pharmacological dosage (3 kg/T) on the intestinal microbiota of weaned piglets. *In vitro*, the minimal inhibitory concentration (MIC) of ZnO against 75 strains from pig intestine showed that it is not possible to classify zinc sensitivity of bacteria according to their taxonomic origin. However, strict anaerobic strains generally showed more diverse MIC than lactic acid bacteria or enterobacteria. Instead of an overly simple separation between Gram+ and Gram-, the effect of zinc oxide seems to be species specific. Under *ex vivo* conditions, it has been shown that high levels of ZnO leads to bacterial growth depression in the stomach and jejunum of weaned piglets, lactobacilli being more susceptible to high zinc dosage than other bacteria. When piglets are fed 3000 mg/kg zinc oxide, enterobacterial diversity increases, with reduced Enterobacteriaceae, Escherichia group and Lactobacillus species (Figure 1)

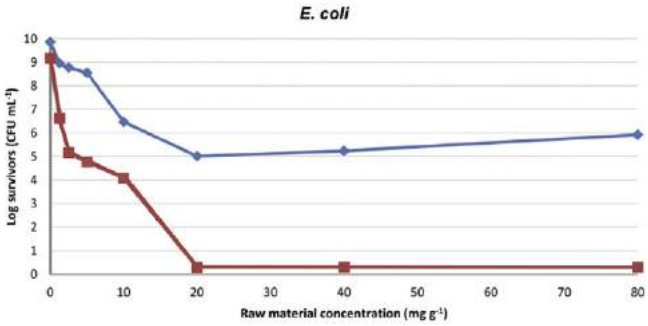
Even though we have gained much understanding on the effect of ZnO on gut microbiota of weaned piglets, some questions remain:

- how does zinc oxide impair bacterial metabolism and health?
- is the positive action of ZnO on piglet gut health only due to antibacterial activity?
- do all sources of zinc oxide perform equally?

Mechanisms of ZnO

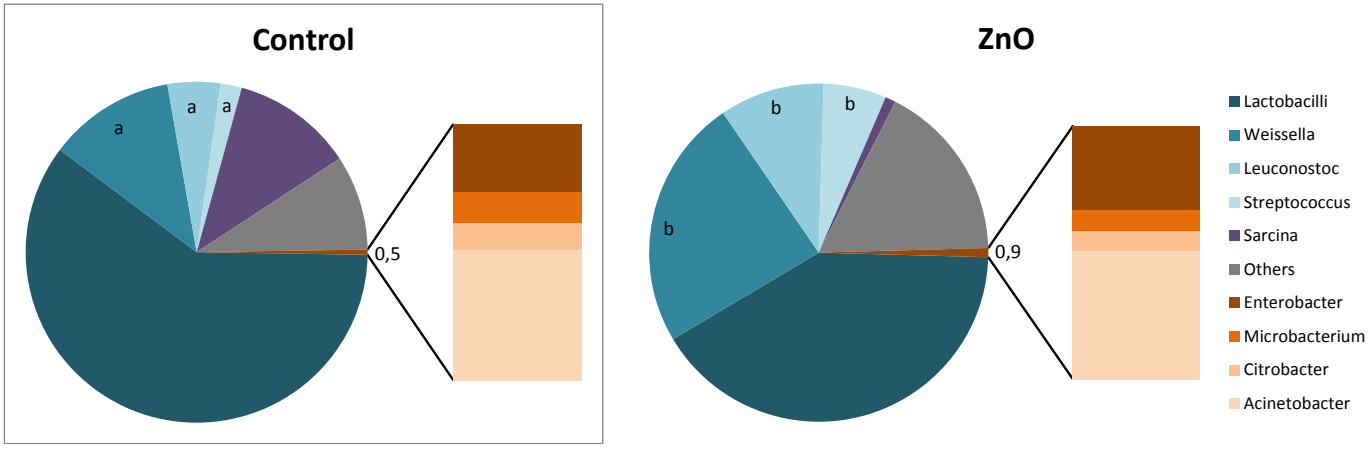
Zinc oxide is a non-water soluble compound which can dissociate at low pH. The gastric environment of young pigs is not acidic enough for full dissociation with immature digestive system and too high buffer capacity of the feed. Once dissociated, solubilized Zn^{2+} are released into the gastro-intestinal tract. It is generally thought that the antimicrobial properties of ZnO are only attributed to these ions, which interact with bacterial amino acids and play a role in the inhibition of active transporters. However, if the antibacterial activity was limited to Zn^{2+}, water soluble zinc sources like zinc sulphate would be more efficient. Recent studies have confirmed a specific effect from the ZnO molecule. The contribution of soluble zinc ions to the antimicrobial activity of ZnO differed among bacterial species and can be very low: for example, only 15% for *Escherichia coli* (Figure 2).

Figure 2: Decrease of microbial populations with respect to zinc concentration for ZnO-1 suspension (red squares) or its supernatant (blue diamonds)



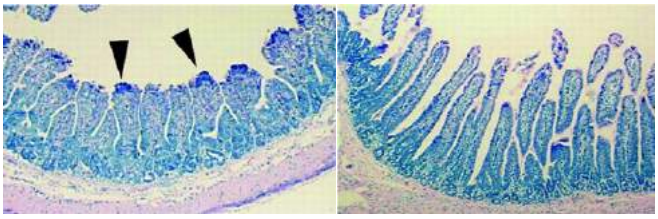
This would explain why piglet diets are only supplemented with pharmacological doses of zinc oxide and not with other zinc compounds. It is still debated if the general positive effect of ZnO on piglet gut health

Figure 1: Effect of the pharmacological dosage of ZnO on the bacteria distribution in the intestine



can only be attributed to antibacterial activity. Among the most likely hypotheses concerning the specific effect of ZnO, this zinc source would be able to generate reactive oxygen species (ROS), under UV exposure but also in darkness, and consequently causes severe damage to the cells. ROS induce oxidative stress and can cause the oxidation of bacterial lipid membrane; this could lead to an alteration of the membrane composition and partially explain the reduced adherence of the pathogenic bacteria like *E. coli* to host cells. This reduction of the bacteria attachment to the epithelium would avoid the secretion of enterotoxins, leading to diarrhoea. Variable effects have been observed from ZnO supplementation on gut morphology (Figure 3).

Figure 3: *E.coli* ileal adherence without (left) and with (right) zinc supplementation



Currently, mechanisms of the antimicrobial activity of ZnO are not totally identified and could vary with the target cells, but it is recognised that specific mechanisms exist. The mechanisms by which zinc oxide products will improve piglet intestinal health can also depend on the chemical features of the particles in the powder.

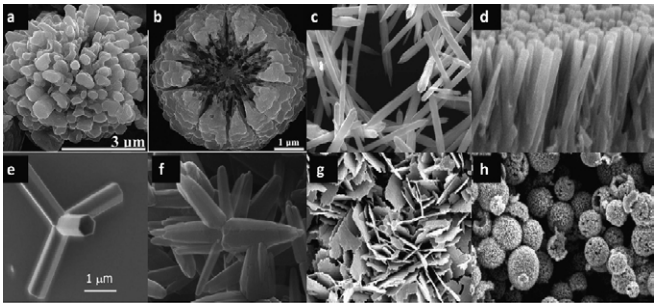
Not all zinc oxides are equal

There are many different zinc oxide sources. Any result obtained with analytical grade trace minerals, which are standardized, cannot be fully extrapolated to feed grade sources, which are highly variable. Zinc oxide can be manufactured with various raw materials and manufacturing processes. Physico-chemical properties of ZnO products used in animal nutrition show strong differences (Figures 4 and 5), which explain variable bioavailability values and antibacterial strength.

Figure 4: Various feed grade ZnO sources (left) and potentiated ZnO (right)

Manufacturing zinc oxide at the nano size can elicit significant differences compared to the bulk equivalent and may be of potential interest in livestock feeding. However, before testing engineered nano ZnO particles in animal diets, certain challenges will need to be addressed: product characterization with appropriate analytical methods, industrial scaling from laboratory samples, ADME (Absorption, Distribution, Metabolism, Excretion) and toxicity studies on animals, safety for workers and for the environment, and compliance with local regulation.

Figure 5: SEM images of different ZnO products



Potentiated zinc oxide

A patented manufacturing technology is at the origin of a potentiated form of zinc oxide, HiZox®, which has been developed for animal nutrition. Physico-chemical properties are modified: specific particle size and shape with increased (10 to 15 times higher than conventional sources) specific surface area drastically increases the surface of contact with bacteria. The high porosity of the powder amplifies the antibacterial activity of this potentiated zinc oxide. Scientific experiments have confirmed its efficacy to manipulate intestinal and faecal microbiota of weaned piglets.

In vitro bacterial growth

Studies supervised by the University of Berlin have compared the inhibitory effects of HiZox® and regular zinc oxide on the growth of pathogenic strains of *Escherichia coli* (fimbriated *E. coli* PS79/K88/F4 and PS7/K81/F18). HiZox® showed stronger inhibitory action on *E.coli* in neutral (pH 6.5) and acidic conditions (pH 4.6) which mimic the gastro-intestinal pH environment (Figures 6 and 7).

Figure 6: Effects of regular ZnO and HiZox on the growth kinetics of pathogenic strains of *E.coli* in neutral conditions

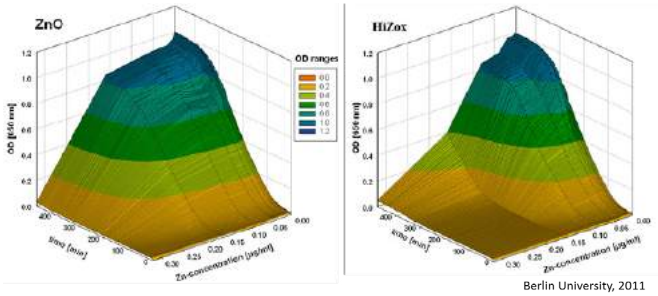
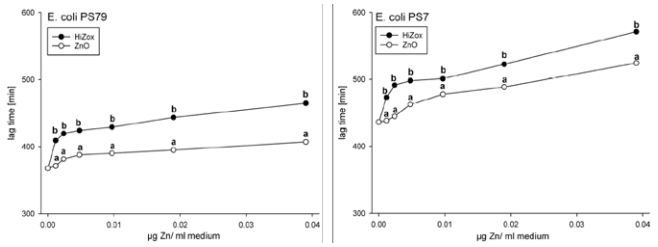


Figure 7: Effects of regular ZnO and HiZox on the lag times of pathogenic strains of *E.coli* in acidic conditions



Ex vivo bacterial growth

Optimal manipulation of gut microbiota in piglets not only reduces pathogenic bacteria, but also decreases bacterial load in the small intestine. An experiment (Vahjen et al, JAS 2012) compared the growth repressing effect of HiZox® and regular zinc oxide on bacteria from stomach and jejunum samples from weaned piglets. Differences in the small intestine (Figure 8) are likely to originate in the stomach (Figure 9).

Figure 8: Bacterial growth inhibition of ZnO and HiZox, in the jejunum

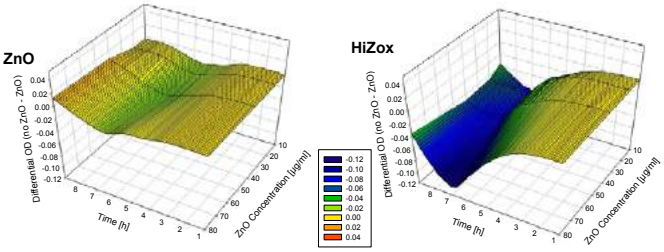
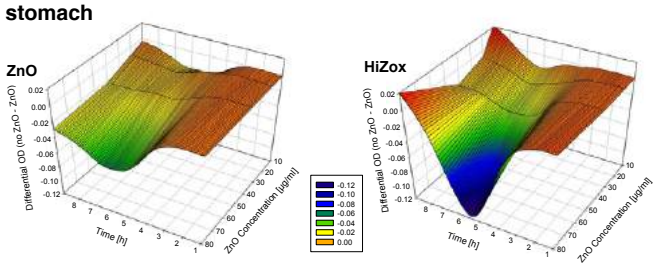


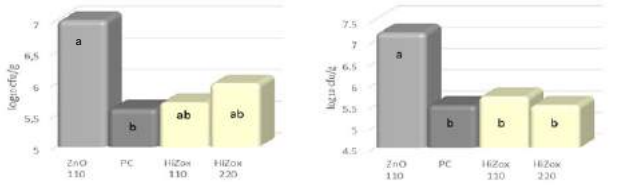
Figure 9: Bacterial growth inhibition of ZnO and HiZox, in the stomach



Intestinal microbial populations

A study performed in Ghent University (Michiels, 2016) measured bacterial counts of *E.coli* and coliforms in the small intestine of weaned piglets, which were fed one of the four experimental diets: negative control (110 mg/kg Zn from standard ZnO); positive control (3 kg/T ZnO); HiZox® at EU legal dosage (110 mg/kg Zn) and higher dosage (220 mg/kg Zn). Replacing the conventional zinc oxide by HiZox® decreased microbial concentrations in the small intestine (Figure 10).

Figure 10: *E.coli* count (left) and coliforms count (right) in distal small intestine

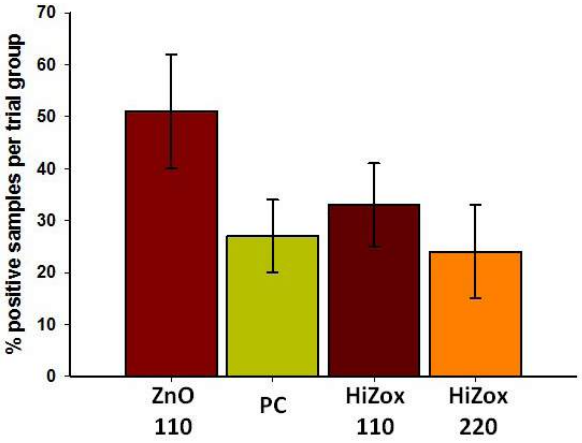


P<0.05

Faecal *E. coli* pathogenic factors

An experiment conducted by Berlin University (Vahjen, JAS in press) analysed via PCR assays total enterobacteria, the Escherichia group and *E. coli* pathogenic factors in faecal samples from weaned piglets which were fed one of the four experimental diets: negative control (110 mg/kg Zn from standard ZnO); positive control (3 kg/T ZnO); HiZox® at EU legal dosage (110 mg/kg Zn) and higher dosage (220 mg/kg Zn). More than half of the samples in the negative control were positive for at least one

Figure 11: Percentage of faecal samples positive for E.coli pathogenic factor

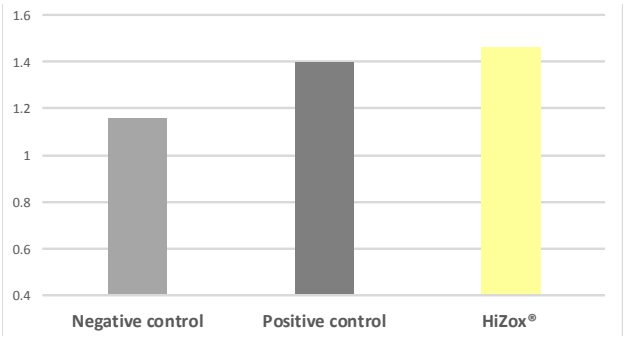


of the analyzed *E. coli* pathogenic factors, but the other treatment groups showed much lower percentages of positive samples (Figure 11).

Faecal microbial populations

One study supervised by Dankook University (Cho et al, Anim. Sci. Journ. 2015) measured the effect of zinc oxide dose and source on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding and faecal score of weaned piglets which were fed one of the four experimental diets: negative control (110 mg/kg Zn from the premix); positive control (3 kg/T ZnO); HiZox® at two supplementation levels (200 mg/kg and 300 mg/kg). It was concluded that HiZox® modified the faecal *Lactobacilli/E. coli* ratio (Figure 12), thus improving faecal score and growth performance.

Figure 12: Ratio of faecal Lactobacilli/E.coli at 14 days post weaning



Conclusion

There is growing pressure on the reduction of antibiotics and pharmacological dosage of zinc oxide in piglet diets. Excessive and prolonged used of ZnO are known for negative consequences: risk of heavy metal contaminations, nutritional antagonisms, environmental accumulation, bacterial resistance. Feed formulators are seeking feed ingredients and feed additives which can reduce the risk of digestive disorders in critical phases like the post-weaning period. HiZox® can be used, in combination with alternatives to AGPs, in a preventive way to improve intestinal health and reduce the need for non-sustainable practices.

(References available on request)

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