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Review

Supplementation of pigs diet with zinc and copper as alternative to conventional antimicrobials

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Abstract

Modern commercial pig farming systems inflict increased stress in animals, which often leads to various negative changes in the gastro-intestinal tract, especially in the case of piglets. Ban of antibiotics, used as growth promoters, has caused a need for alternatives to conventional antimicrobials in swine diets. Use of pre-/or probiotics, organic acids and plant extracts is often recommended, but it seems that zinc oxide and copper salts, which were traditionally included in high doses to piglets diet, possess the highest efficacy. In commercial conditions feeding piglets with high doses of Zn and/or Cu stimulates piglets daily gain and decreases feed conversion factor. However, as heavy metals Zn and Cu tend to accumulate in soil and cause serious environmental pollution of soil and tap-water. Furthermore, high zinc concentrations (2500–3000 mg/kg feed) in feed may have an impact on development of antimicrobial resistance, and may regulate the expression of genes that modify piglets' immune response. Therefore, the use of high doses of ZnO and/or Cu salts, as growth promoter, has always been a subject of discussion, and caused different legal status of such treatment in various EU countries. This short review describes current European Food Safety Authority (EFSA) point of view on the use of ZnO in medicated feed. The higher bioavailability of recently introduced new sources or forms of these metals allows for substantial reduction of dietary inclusion rate, which should have a positive outcome for pigs health and the environment.

Key words: ZnO, pig, dietary supplement, alternative antimicrobials, growth promoters

Conditions of modern commercial pig farming inflict different type of stress which is associated with significant negative impact (physiological, microbiological and immunological) on the gastro-intestinal tract in housed animals. These stress-related changes lead to sub-optimal growth, decrease feed efficiency and increase incidences of intestinal disturbances, including diarrhoea. Post-weaning diarrhea is one of the

most frequent causes of economic losses dealing with pigs. To minimize all these negative changes antibiotics were extensively used in pig farming, which, in turn, caused a development of increased antibiotic resistance in numerous bacterial strains. As a consequence, in 2003 the use of in-feed antibiotics in livestock diets was banned in the EU (Regulation No. 1831/2003).

Some minerals and/or pre- and probiotics, acidifiers, and plant extracts may serve as alternative to antibiotics used as growth promoters in weaning piglets (Vondruskova et al. 2010, Thacker 2013).

Zinc oxide (ZnO) has been traditionally included in diets for weaning pigs since early 1990's. However, use of ZnO, as growth promoter, has always been a subject of discussion because although it promotes piglets' health and performance, it is a compound containing heavy metal (Zn), which may be toxic in concentrations used. Feeding high concentrations of ZnO has been demonstrated to reduce diarrhoea incidences and improve piglets' growth. Based on data published by Moreno (2012) in Spain 57% of piglets were given ZnO during pre-weaning stage of life and 73% during the growing stage (27-75 days).

Numerous studies have demonstrated growth-promoting effect of ZnO (Mavromichalis et al. 2000, Grela and Pastuszek 2004, Li et al. 2006a, Cho et al. 2015). Use of different Zn sources, including ZnO and Zn-chelates, in commercial pig farming, shows positive response in growth and feed conversion rate. Mullan et al. (2007) demonstrated significant differences in average daily gain (ADG) (day 0-39) of piglets which did not receive any Zn supplementation (367g) in comparison to those fed diets containing Zn (389-427g, depending on the type of supplementation). Furthermore, the authors observed a reduction in feed conversion ratio (FRC) from 1.58 (control) to 1.36-1.38 (experimental groups). Another study showed that microbial fecal score was improved during 21 day of life in piglets treated with organic Zn (Castillo et al. 2008).

Zn is used to decrease fermentation of digestible nutrients in intestines, improve nutrients digestibility and to improve appetite. Dietary Zn supplementation stimulates feed intake probably caused by increased ghrelin secretion (Yin et al. 2009). It was observed that it caused an increase in insulin-like growth factor expression in the small intestine mucosa (Li et al. 2006a). Increased Zn concentration in the intestines influences their structure and function. Increased height of small intestine villi in comparison with the depths of crypts was observed by Castillo et al. (2008) in ZnO treated pigs. Pharmacological doses of Zn affect not only colonic morphology but also host defense mechanisms. However, in piglets used in experiments by Liu et al. (2014) the highest colonic crypt area was found in piglets treated with much lower dose of ZnO (164 mg/kg feed). Also higher colonic crypt area and mRNA expression of cytokines and composition of intestinal mucins in such piglets was found (Liu et al. 2014). All these observations suggest that the local level of Zn plays a significant role in the mechanism of ZnO action, especially since it was

shown that Zn bioavailability is not a significant factor influencing its efficacy (Mavromichalis et al. 2000). The 2000 mg/kg feed dose of ZnO caused 3 to 5 fold increase in Zn concentration along the gastrointestinal tract, and a slight increase of copper concentration in the blood, compared to those determined in unsupplemented piglets (Davin et al. 2012).

Dose-dependent effects of ZnO on composition of bacterial communities and metabolic properties were observed in the ileum of weaned piglets (Pieper et al. 2012). The number of food borne pathogens such as *Salmonella typhimurium* and *Staphylococcus aureus* was significantly reduced, suggesting that ZnO might be used as antimicrobial agent and even to reduce antibiotic-resistant strains of bacteria present in the digestive tract (Thati et al. 2010). The results of specific viral pathogens challenge of pigs indicated that the use of high concentrations of dietary zinc could enhance protection of the treated animals (Chai 2013). However, it was also found that prolonged (over 4 weeks) use of such high Zn supplemented diets caused growth reduction and could increase some forms of resistance in intestinal microbes. It was also observed that very high level of maternal dietary zinc (5 g/kg feed) reduces copper body stores in newborn piglets (Hill et al. 1983).

Zn mechanism of action against diarrhea is still not well understood. Zhang and Guo (2009) have speculated that high concentrations of dietary Zn decreases intestinal permeability preventing translocation of pathogenic bacteria through the intestinal barrier. It was also shown that weaning creates Zn deficiency in piglets (Davin et al. 2013). However, in diarrhea treatment only high pharmacological doses of ZnO are efficient (2000-3000 mg/kg feed). Such concentrations are very high because maximum tolerable concentration for pigs was estimated to be 1000 mg/kg feed (NRC 2005). Treatment with Zn using such high doses of ZnO is beneficial to piglets only during early phases of nursery period. The feeding period of high ZnO doses should be limited to 2-3 weeks after weaning.

The use of ZnO at high concentrations (≥ 2500 mg/kg feed) in medicated feed is allowed in EU countries except Germany, France and The Netherlands. In Poland, Portugal, Spain, Sweden and UK ZnO is allowed in treatment of piglets diarrhea, administered at the concentration of 3000 mg/kg feed for 2-6 weeks. In Poland medicated premix Suibicol is registered, which contains ZnO in final feed concentration of 3000 mg/kg feed and sulphaguanidin in final feed concentration 2000 mg/kg feed. This premix is recommended for use in weaned piglets for 10 to 14 days. Medicated feed containing this product requires veterinary prescription. ZnO has been allowed as feed

additive in Europe, and EFSA considered this additive as safe for target animals and consumers in feed at maximum concentration of 150-200 mg/kg feed. The newly proposed maximum total Zn concentration in complete feed for piglets and sows is 150 mg/kg feed (EFSA 2014).

Also copper was shown to stimulate growth rate of piglets when included into diet at high levels (125-250 mg/kg feed). Armstrong et al. (2004) observed that the concentration of copper at the level of 125mg/kg feed have similar effect on growth of weanling piglets, regardless the copper source. The inclusion of Cu to starter diets for weaning pigs is a common practice in swine industry. Cu is mostly used as copper sulfate but Cromwell et al. (1998) showed similar efficacy in the case of tri-basic copper chloride. The growth-stimulating properties of dietary copper have been attributed to its antimicrobial action however, it was shown that also intravenous injection of copper to weanling piglets stimulate their growth (Zhou et al. 2014). It seems that the growth-promoting properties of high dietary concentrations of copper are in addition to its antimicrobial action (Jacela et al. 2010). Hojberg et al. (2005) found that administration of 175 mg/kg feed CuSO_4 reduced amounts of lactic acid bacteria, lactobacilli and streptococci in the gastrointestinal tract of pigs. It is interesting that in copper treated piglets a reduced number of lactic acid bacteria was observed because this group of commensal bacteria are considered very important for stabilization of the gut ecosystem. However, decreased level of these commensals in the proximal part of the gastrointestinal tract may benefit by allocation of increased feed amount for growth performance. Carlson et al. (2004) suggest that observed reduction of coliforms in the large intestine of Cu-supplemented piglets may be a part of mechanism influencing the suppression of growth and causing reduced number of the specific pathogens in the digestive tract.

Nutritional requirement of Cu is about 5 mg/kg feed, but the mechanism of action of high pharmacological Cu doses (125-250 mg/kg feed), is not recognized. In the majority of experiments, piglet diets were supplemented with 250 mg/kg feed. However, the European Commission (2003) extrapolated Cu concentration, acting as growth promoter, at the level of 175 mg/kg feed, based on Cu inclusion levels currently allowed in EU countries. It seems that copper given in such a dose modifies the composition of bacterial population in the gastro-intestinal tract. There are significant differences in the bioavailability of Zn and Cu originating from different sources; that is why only the recommended sources of these minerals can be used in pigs for growth promotion. A response of piglets to high doses of Cu and Zn seems to be independent of

other antimicrobials in the diet (Cromwell et al. 1997). Copper and zinc are efficacious even in the presence of antibiotics, suggesting their different modes of action. It was observed that high dietary level of copper may cause increased content of unsaturated fatty acids, which resulted in softer pork fat, but this effect could be minimized by reduction in Cu dosage before slaughter.

High dose ZnO – side effects

Environmental issue

As a heavy metal Zn tends to accumulate in soil and may cause serious environmental pollution, especially in areas with intensive pig farming. Environmental concern is related to groundwater, drainage and Zn run-off to the surface water. Sandy soils are most vulnerable to these processes. Another point is accumulation of Zn in topsoil to concentrations potentially toxic to organisms living there. The use of high doses of ZnO in pig farming causes increased Zn excretion which might be a real threat to the environment (Heo et al. 2013). Increased excretion of heavy metals (Zn, Cu) could be a potential problem in off-site nurseries where weanling piglets are farmed year around. Use of high doses of Zn and Cu cause visible changes of sows feces e.g. increased fecal firmness was observed. Addition of ZnO causes disappearance of characteristic black color of feces in copper supplemented piglets. Zinc and copper are not degradable in the slurry and their increased consumption in modern pigs farming cause accumulation of Zn and Cu in the slurry. Roof and Mahan (1982) reported that piglets supplementation with CuSO_4 at the level of 250 mg Cu/kg feed caused 14 times increase of fecal copper excretion. National monitoring study performed in Denmark has revealed that current use of zinc and copper for pig production may pose environmental pollution and a serious risk to aquatic animals (Jensen et al. 2016). However, ZnO provided feasible alternative to in-feed antibiotics and its reduction of inclusion level might be obtained by introduction of new, more efficient Zn compounds and/or forms. Increased heavy metals emission has adverse environmental effects, like impairment of plant production, accumulation of these metals in water supply chain and induction of antimicrobial resistance (Brugger and Windisch 2015).

Antimicrobial resistance

Development of antibiotic-resistant bacterial strains and their potential impact on human health elicits an increasing concern. Studies have shown

that Zn and Cu used at high concentrations for a longer period might promote the spread of antimicrobial resistance of gut microflora in pigs (Holzel et al. 2012). It was demonstrated that prolonged exposure to pharmacological doses of Zn can increase the resistance in some form of microbes e.g. increased number of multi-resistant *Escherichia coli* in piglets treated for 4 weeks with 2500 mg/kg feed of ZnO (Bednorz et al. 2013). Slifierz et al. (2014) observed increased prevalence and persistence of methicillin-resistant *Staphylococcus aureus* in post weaning piglets treated with high doses of dietary ZnO. The results of a study by Agga et al. (2015) obtained on weaned pigs fed 3 weeks copper sulfate (125 mg Cu/kg feed) suggest that Cu supplementation may have significant impact on certain genes expression. The prolonged use of heavy metals such as Zn and Cu provides selective pressure on antimicrobial-resistant bacteria and this is a reason why implementation of high doses of these metals may play a role in maintaining of antimicrobial resistance (Holman and Chenier 2015).

Nutritional interactions of zinc/copper with products reducing use of antibiotics

Zinc/copper interaction

Administration of Zn and Cu together usually does not cause additive effects, however, Perez et al. (2011) observed appearance of additive effect of supplementation with pharmacological amounts of ZnO and CuSO₄ on weaning piglets growth. Shelton et al. (2011) have shown advantages of inclusion of Zn and Cu in the pigs diet when Zn was added from 0 to 14 days postweaning and Cu from 14 to 42 days postweaning. Jongbloed et al. (2011) quantified the dose-response relationship of supplemented Cu on growth performance and they found the highly significant relation in piglets weighted from 5 to 25 kg BW.

Another strategy to minimize a transient drop in feed intake, growth and increased sensitivity to digestive diseases (diarrhea) is the use of various nutritional approaches. Enrichment of the feed with substances that show various properties, such as: anti-microbial and/or immune stimulating properties or appetite stimulation may exert a positive effect on the health status of farmed piglets.

Probiotics

Probiotics are living cultures of microorganisms which administered in adequate amounts exerting

beneficial effect on host, improving the indigenous microbial balance. Function of probiotics in the digestive tract is suggested to consist in: competing with pathogenic bacteria for nutrients and binding sites on the intestinal wall, stimulation of the immune system and production of compounds that are toxic for pathogens (Cho et al. 2011). *Enterococcus faecium* NCIMB 10415 is authorized in the EU as a probiotic feed additive for pigs and Wang et al. (2014) have shown that the use of this probiotic bacteria have the same beneficial effect on weight gains as supplementation of piglets with ZnO at the level of 2500mg/kg feed.

Prebiotics

Prebiotics defined as non-digestible feed components that positively affect animal health by selective stimulation of beneficial intestine bacteria. Example of such substances are yeast derivative – different kinds of polysaccharides are able to modulate immune functions in piglets. It has been shown that β -glucans possess anti-inflammatory properties enhancing anti-inflammatory cytokine production in pigs (Li et al. 2006). In some situations, glucans may stimulate growth and feed intake but also depress piglet's performance (Hahn et al. 2006).

Plant extracts

Seaweed extract containing complex polysaccharides added to piglets feed was found to possess growth promoting properties similar to those of ZnO (McAlpine et al. 2012). Lipinski et al. (2014) observed that use blend of organic acids, plant extracts with mineral clays (B-Safe supplement) or ZnO in weaning pigs improved the digestibility of crude protein and nitrogen retention. The lowest feed conversion ratio was found in piglets fed zinc oxide and in the pigs fed with nutritional supplement B-Safe containing plant extracts. Several studies have reported that essential oils of labiate plants (oregano, thyme, rosemary) stimulate growth performance of piglets but other scientists have not observed such influence. Most studies showing bactericidal or bacteriostatic effect on several pathogenic intestinal microorganisms were performed only *in vitro*. It is possible that labiate oils mode of action is connected with their high anti-oxidative potential.

Acidifiers

Another group of substances used in pig farming with proven efficiency providing positive influence on

digestive microbiota are organic acids commonly used in animal nutrition. Acidic growth promoters act by lowering the pH of the digestive tract content and/or by effective antibacterial function. However, zinc oxide acid binding capacity is very high (similar to limestone flour) and in case of high ZnO diet enrichment it appears to be antagonistic with such feed acidifiers like organic acids. Lactic, fumaric and citric acids reduce bacterial population indirectly by lowering stomach pH. Another group of acids consist of formic, acetic, propionic and sorbic acid. These acids are directly acting on gram-negative bacteria by lowering the pH, thus preventing their replication (Castro 2005). A combination of different organic acids leads to suppression of *E. coli*, *Salmonella* and *Enterococcus sp.* in pigs' digestive tract causing growth improvement (Missotten et al. 2009). However, as alternative to antibiotics, organic acids not fully ameliorate negative effects of microbial challenges e.g. using *Salmonella enterica* or *Escherichia coli* KCTC 2571 on growth performance and immunity of pigs (Ahmed et al. 2014). Lampromsuk et al. (2012) based on results of experiment with the use of ZnO (2300 mg Zn/kg feed), Zn acetate (Zn 500 mg/kg feed) or acidifier based on sodium benzoate (3000 mg/kg feed) have concluded that piglets supplementation of organic zinc between 24 and 45 day of life with addition of acidifiers at 24-73 day of life may increase their growth performance.

Future perspectives of using minerals as alternatives to conventional antimicrobials

Different mixtures of phytochemical substances may have additive effects increasing antioxidant status and/or improving immune status, or beneficially modulating gut microbiota composition. These positive changes should improve growth performance and health status of piglets. Zinc oxide supplied at pharmacological level evidently improves weaned pig performance and is cheap what may led to inappropriate usages, environmental Zn accumulation and increase of bacterial resistance.

Hernandez et al. (2008) have demonstrated that the use of dietary Cu and Zn organic complexes, which show higher bioavailability, enabled to obtain increased improvement of the performance of pigs in comparison to that observed in the animals receiving supplementation with inorganic sources of these metals. For example, Zn in TM bioplexes may be used in concentrations of 100 and 250 mg/kg feed still showing some positive impact on piglets growth (Mullan et al. 2007).

Another way to reduce the amount of ZnO added to the feed is increasing the active surface area of ZnO by the use of high porosity product or introduction of nanopreparats. Initial trials with this form of ZnO have been shown to be very promising. Nano-grinding of ZnO has several times larger surface of interaction with the gastrointestinal tissue and microbial population. The introduction of new generation of Zn compounds e.g. zinc-doped copper oxide nanoparticles (Malka et al. 2013) seems also very promising. Tayel et al. (2011) observed very strong antibacterial properties of ZnO powder given in a form of nanoparticles. Genotoxicity of these particles is rather low. However, *in vitro* studies on cell lines have shown that induction of genotoxic cell damage and increased frequency of micronuclei is also observed at ZnO concentrations below 100 µg/ml (Demir et al. 2014). Antibacterial properties and toxicity mechanism of zinc oxide nanoparticles were described by Sirelkhatim et al. (2015). In methicillin-resistant *S. epidermidis* strains ZnO nanoparticles caused decreased bacterial growth (Ansari et al. 2012). There is however, a concern dealing with the use of high doses of ZnO due to its potential impact on the expression of genes encoding proteins associated with inflammatory response in piglets (Sargeant et al. 2010). This property of ZnO indicates possible modifications of host's immune response to compounds responsible for initiation of inflammatory processes.

Recently microencapsulated ZnO was released on the market with intended dose of Zn decreased to only 100 mg/kg feed. Results obtained by Kim et al. (2010) have shown beneficial effects of this lipid-coated ZnO, which efficiently suppressed the incidence of post-weaning diarrhea caused by enterotoxigenic *E. coli*. New forms of Zn supplementation could provide an effective replacement of traditional ZnO (added at high concentrations) by reduction of dietary inclusion, but with maintained beneficial effects of ZnO. New potentiated form of ZnO, known as HiZox, was used by Morales et al. (2014) and in inclusion level of 110 mg Zn/kg feed it caused growth performance of piglets similar to that observed during regular application of ZnO given at the dose of 3000 mg/kg feed.

Furthermore, in the case of organic sources it was possible to lower the level of supplementation with these elements. Very often promising new products are not properly described and the only information concerns e.g. particle size or fact that they are chelated. Nevertheless, it is worth noting that data presented in the literature are very often conflicting and need reevaluation. The majority of the mentioned studies were performed by pharmaceutical companies or were funded by producers of specific supplements.

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