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Minerals favor bacterial resistance

»ADDITIVES

Zinc, copper favor bacterial resistance

Fighting antibiotic resistance in swine production requires not only a reduction of antibiotic use, but also a better understanding of the effects of zinc and copper supplementation in pig diets.

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»Antibiotics have been added to pig diets for some decades. They are not only used to treat sick animals and to prevent bacterial infections, but also to enhance the performance of the animals; their growth-promoting effect has been known since the 1950s.

Antimicrobials substances have been used worldwide in large quantities. For example, in 1994, antimicrobial consumption in Danish animal production was 200 tons; more than half of these 200 tons were supplemented as growth promoter in animal feed. Occurrence of antibiotic-resistant bacteria has become an important concern in recent years and has led to the ban of non-medicinal use of antibiotics in livestock production in the European Union.

Other substances are known for their antimicrobial properties and for their growth promoting effect. Zinc (especially zinc oxide) and copper are supplemented in excess in pig diets to improve intestinal health, but their impact in the fight against antimicrobial resistances is not entirely positive.

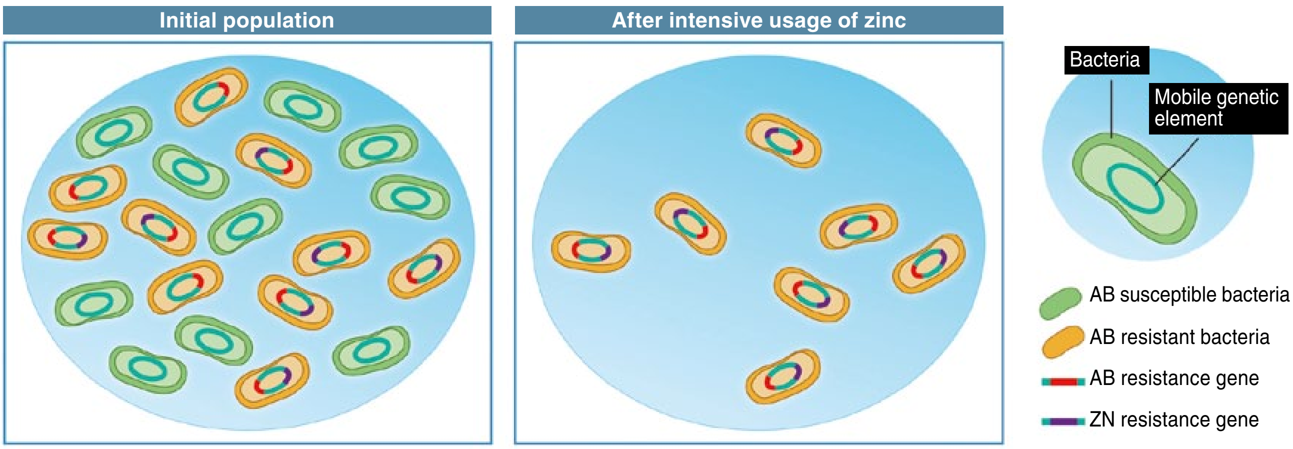
Current situation

Zinc is an essential trace element for the life of numerous organisms. Its positive effect on growth performance has long been recognized in swine production: zinc deficiency causes depression of feed consumption, reduction of growth, weakening of the immune system and, in severe cases, skin lesions (parakeratosis). According to the National Research Council, pig requirements are between 50 and 100 ppm in relation to the age and the weight of the animal. As the zinc content in feedingstuffs is low, zinc is usually supplemented in pig diets. In the EU, the maximum content in monogastrics feeds is 150 ppm, but some countries authorize pharmacological dosages of zinc (3000 ppm), in medicated premix, during the post-weaning period.

Like zinc, copper is an essential trace element. It is a cofactor for numerous enzymes and a famous growth promoter. A deficiency can result in physical problems: depression of growth, anaemia, malformations of the legs, etc. Pig requirements are low (4 to 10 mg copper per kg dietary dry matter), but copper is often supplied in excess. Maximum copper contents in European feeds are 170 ppm for piglets and 25 ppm for pigs.

These trace elements are used

FIGURE 1: SELECTION OF ANTIBIOTIC (AB) RESISTANT BACTERIA AFTER INTENSIVE USAGE OF ZINC (ZN)



After prolonged feeding of diets rich in zinc, bacteria that are resistant to certain antibiotics may emerge.

at high levels for their antimicrobial properties. As a result of the common use of drugs in livestock production, bacteria have developed four main strategies against antibiotics:

- ✔Reduction of membrane permeability (lower porin expression)
- ✔Drug inactivation (production of enzymes)
- ✔Alteration of cellular targets (modification of binding sites of the antibiotics)
- ✔Efflux of toxic elements (via efflux pumps)

In Denmark, a glycopeptide named avoparcin was used as growth promoter until 1995; at that time, 21 percent of the *Enterococcus faecium (E. faecium)* obtained from pig carcasses were resistant to avoparcin. Fully aware of the problem, Denmark banned the non-medical use of antibiotics in swine production in 1998 for adult pigs and in the 1999 for piglets. In 2006, the withdrawal of the antibiotics used as a growth promoter was implemented in the EU. Copper and zinc can regulate the intestinal microflora and reduce diarrhea associated to post-weaning period: they are therefore able to partly replace antibiotics. However, excess of copper and excess of zinc have a negative impact on the environment and select copper- and zinc-resistant bacteria. In addition, we can observe a strong correlation between resistance to metals and resistance to antibiotics.

THERE IS A STRONG CORRELATION between resistance to metals and resistance to antibiotics

Cross-resistance

Multi-resistances can be explained by cross-resistance. In this case, a single genetic determinant leads to resistance to several elements (metals and antibiotics). For example, bacteria may synthesize transport proteins that carry both antibiotics and metals. In the zinc-resistant microorganisms, efflux pumps decrease intracellular zinc from the cell; these pumps can be specific to zinc or can accept other molecules. Consequently, zinc-resistant bacteria can be also resistant to one or more antibiotics.

Co-resistance

Multi-resistance can also be associated to co-resistance. In this case, different resistance genes take place in the same genetic element, in general in a mobile element like plasmid. Some association's metal-antibiotics are studied in the literature; for example, zinc-resistance linked to methicillin-resistance in *Staphylococcus aureus (S. aureus)* or copper-resistance linked to macrolide and glycopeptide resistances in *E. faecium*.

Zinc

A gene named czrC confers cadmium and zinc resistance in ***S. aureus***. A study on pig isolates from different countries demonstrated that the gene czrC was found in 95 percent of the zinc-resistant isolates. In contrast, less than 1 percent of the isolates involving crzC were susceptible to zinc. A strong correlation between zinc-resistance and methicillin-resistance was observed; among the methicillin-susceptible isolates, all were susceptible to zinc chloride, and among the methicillin-resistant isolates, more than 90 percent were resistant to zinc chloride.

In another study, with Danish pig isolates, none of the methicillin-susceptible isolates and 74 percent of the methicillin-resistant isolates were resistant to zinc. In average, the minimum inhibitory concentration (MIC) of zinc for these resistant bacteria is 4 times higher than the normal MIC: 8 mM for the methicillin-resistant *S. aureus* (MRSA) versus 2 mM for the methicillin-susceptible *S. aureus* (MSSA). In a word, selecting zinc-resistant bacteria means selecting antibiotic-resistant bacteria (Figure 1).

In Europe, in Canada and the U.S. (Midwest), emergence of MRSA with a similar genetic profile (complex clonal 398) is facilitated by use of tetracycline and by use of zinc oxide. This strain has also been found recently in Australia and in New Zealand. Experiments on pig nasal colonization with MRSA were conducted and higher MRSA counts were observed in pigs treated with zinc or with tetracycline. Spread of resistant bacteria was rapid; after three weeks, all MRSA-negative animals housed with MRSA-positive animal became positive.

Copper

A similar phenomenon is observed with copper. Copper exerts a co-selective pressure in favor of bacteria resistant to macrolides and to glycopeptides. Acquired transferable resistance to copper is generally observed in gram-negative bacteria, rarely in gram-positive bacteria. It is linked to the gene tcrB (transferable copper resistance homologous to copB). A study on Danish *E. faecium* isolates suggests that the presence of this gene is directly correlated to level of copper in diet. Resistance to copper was usual in pig isolates (45 among 59 isolates), quite common in poultry isolates (10/29) and rare in isolates from calves (5/32), sheep (0/22) and human (3/29). Higher results in pigs could be explained by higher supplementation of copper. In addition, more than half of the pig isolates were resistant to macrolides and almost a quarter to glycopeptides.

In this study, all glycopeptites-resistant strains were resistant to both macrolides and copper and all macrolides-resistant were resistant to copper; only 4 copper-resistant isolates (less than 10 percent of this group) were susceptible to antibiotics. Genes tcrB, responsible to copper resistance, and erm(B), responsible to macrolides resistance, seem to be located in the same plasmid in pig isolates; gene for glycopeptides resistance would be also physically linked to gene tcrB.

As pig manure can be used as fertilizer, resistant-antibiotic can be spread in soils. A study showed a correlation between antiobiotic resistances and copper resistance in microorganisms from soils; for example, occurrence of ampicillin resistance was significantly higher in copper-resistant bacteria than in copper-susceptible bacteria. In addition, frequency of multiples resistances in copper-resistant bacteria is more than twice as high as in copper-susceptible bacteria.

Resistant strains could spread rapidly in the animal, between the animals and in the environment. Over the generations the antibiotics become less and less effective when zinc or copper selects resistant strain. Horizontal transfers are involved in this phenomenon.

Spread of the resistance

Susceptible bacteria may become resistant on contact with resistant bacteria. Genetic exchanges are possible between microorganisms. Resistance genes are generally located in mobile genetic elements (plasmid, transposons), which facilitate horizontal transfers. Bacteria may be able to accept plasmids or transposons of other cells, by mating (conjugation); they could also to incorporate exogenous genetic material on their own genome (transformation). Specifically, DNA may be transferred from resistant bacteria to another by a phage (transduction). Next, bacteria that become resistant may transfer their genes to daughter cells during cell division. Consequently, the use zinc or copper in excess creates a new generation of metal- and antibiotic-resistant bacteria. **PIGI**