Efficacy of Feed Additives for the Manipulation of Gut Physiology and Microbiota in Weanling Pigs

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1.- Introduction

The time around weaning is one of the most stressful moments in the early life of a pig. During this period, the piglets faces multiple challenges such as the removal from the mother into a new environment, shift from mothers milk to cereal based (less palatable) diets and mixing with piglets from other litters. In the intestinal tract, the pancreatic and brush border enzymes are not fully adapted to digest complex cereal-based diets instead of milk-based nutrients (Lallés et al., 2009). The intestinal microbiota, which also relies mostly on nutrients taken up with the diet, has to adapt as well and undergoes dramatic changes in the time (1 to 2 weeks) after the weaning (Janczyk et al., 2007; Pieper et al., 2008). In addition, the gut associated and general immune system are still immature and have to deal with both, microbial, dietary and environmental antigens. The consequences are local inflammation and loss of intestinal barrier function. Together with the environmental, social and nutritional stressors, this sets the scene for overgrowth of opportunistic pathogens, digestive disorders, and the post-weaning growth check with respective economic losses for the farmer. Of course, the on-farm conditions (sow and weaning management, hygiene, feed presentation, temperature, stocking density, general pathogen pressure) are important risk factors which can influence the post-weaning problems (e.g. Madec et al., 1998). For many decades, in-feed antibiotics were used to...
overcome health problems in weaning piglets and promote performance. However, the excessive use of antibiotics can promote the development of (multi-)resistant bacteria – a threat also for human health. The European ban of antibiotics as feed additives by 2006 was a logical consequence. It has also enforced a search (and opened a huge market) for possible alternatives during the past decade. This does not mean that these additives will act similar as antibiotics, but should help to maintain health and productivity during the post-weaning phase.

Besides the use of feed additives to promote health and productivity in young pigs, there are of course numerous other strategies including feeding fermented liquid diets, reduced dietary protein concentration, hydrothermal treatment of feeds or feed ingredients (starch and/or protein sources) or the use of industrial by-products as functional feeds. All of these strategies have their advantages and justification in successful management systems. Similarly, the motivation to use feed additives to promote gastrointestinal functionality and health might differ from farm to farm. The current overview will focus on selected feed additives to manipulate intestinal physiology and microbiota in weaning piglets.

2. FEED ADDITIVES USED FOR WEANING PIGLETS

As indicated above, the gastrointestinal tract of the pig is still underdeveloped and highly susceptible at the time of weaning. Strategies to help the animal through this serious time with the use of feed additives can be therefore grouped into several aspects such as the promotion of feed intake, promotion of nutrient digestion through exogenous enzymes or stimulation of intestinal enzyme activity, induction of gut maturation and barrier function, stimulation of the immune system or stimulation of beneficial bacteria and/or reduction of potential pathogens.

To accomplish these goals, there are currently numerous products of different origin on the market or under investigation as potential feed additives (Table 1). It is not possible to cover the mode of action of these groups of products within one short overview paper. However, a number of excellent reviews have been published during the past years regarding some of these additives in weaning piglets (e.g. see Taras et al., 2007; Lallés et al., 2009; de Lange et al., 2010; Zentek et al., 2011; Heo et al., 2013). Following, the use of probiotics and zinc oxide in weaning piglets will be discussed a bit more in detail.
Table 1.- Functional feedstuffs and possible feed additives used for pigs

<table>
<thead>
<tr>
<th>Group of additives</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids and their salts</td>
<td>Benzoic-, lactic-, formic-, propionic</td>
</tr>
<tr>
<td>Botanicals</td>
<td>Essential oils, herbal parts or extracts, algae</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Phytase, Xylanase, β-glucanase, Proteinase</td>
</tr>
<tr>
<td>Fats</td>
<td>Unsaturated fats, medium chain fatty acids</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Oligosaccharides, non-starch polysaccharides</td>
</tr>
<tr>
<td>Probiotics</td>
<td>Live bacteria, yeasts</td>
</tr>
<tr>
<td>Symbiotics</td>
<td>Combination of selected prebiotics and probiotics</td>
</tr>
<tr>
<td>Proteins</td>
<td>Immunized egg products, milk protein, spray dried plasma, bovine colostrum</td>
</tr>
<tr>
<td>Trace elements/rare earth elements</td>
<td>Zinc, copper and others</td>
</tr>
<tr>
<td>Others</td>
<td>Nucleotides, betaine, amino acids, yeast cell walls</td>
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</tbody>
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3. - PROBIOTICS

The use of probiotics in the pig industry has increased during the past years and there are several commercial products registered under Regulation (EC) No 1831/2003 as feed additives for pigs. According to Ohashi and Ushida (2009), probiotics should meet also the following criteria: (i) can be prepared on a large scale; (ii) remain viable and stable during storage; (iii) can colonize or at least survive the intestinal passage; (iv) the host gains benefit from the probiotics, and (v) their safety should be evident. The classical definition of probiotics states that it is ‘A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’ (Fuller 1989). It is difficult to define a balanced intestinal microbiota and thus, to show clear health promoting effects of probiotics in pigs. Regarding the benefits of the host, several modes of actions have been hypothesized or claimed for probiotics, which go even beyond this definition regarding the microbial balance. This includes (as summarized by Chaucheyras-Durand and Durand, 2010):

- Production of organic acids (e.g. lactate, acetate) and reduction of the luminal pH
- Competitive exclusion of other bacteria by occupation of their ecological niche
- Production of bactericidal compounds (e.g. bacteriocins)
- Stimulation of other bacteria through cross-feeding (e.g. butyrate producers)
- Stimulation of the immune system and epithelial barrier function
- Stimulation of intestinal secretion and nutrient absorption
- Detoxification of harmful compounds (e.g. amines, nitrates)
Some of the above-mentioned modes of action on the host may also be the result of the interaction of probiotics with other intestinal bacteria (e.g. immune response or physiological effects). Nowadays we know also that the effects depend largely on the probiotic strain used (not only the bacterial species) and likely also the age, physiological and immune status of the animal and the hygienic conditions of the farm. Following, a few examples for probiotics in pigs will be discussed. The list is by far not complete and many other bacilli spp., lactic acid bacteria, clostridia and yeasts have been successfully applied in pigs during the past years.

3.1. *Enterococcus faecium* NCIMB 10415

One of the main aims when feeding probiotics is the reduction of diarrhoea and improvement of performance. Some studies showed that diarrhoea was reduced and performance increased with *E. faecium* feeding (Taras et al., 2006; Zeyner and Boldt, 2006) whereas others did not (Broom et al., 2006; Martin et al., 2012). Similarly, the picture regarding the influence on intestinal physiology is not consistent (Martin et al., 2012). During the past 10 years, the mode of action of *E. faecium* NCIMB 10415 in weaning piglets has been studied quite intensively and also within a large research consortium in Berlin. These studies in Berlin included all types of measurements from zootechnical response in sows and their piglets to immune signalling in the pig intestine. The results have been published in numerous papers (see website www.sfb852.de for some examples). Following, a few details should be highlighted. It was shown that the probiotic strain can be transferred already from the mother to the piglets during the early postnatal period, although the absolute and relative numbers compared to total enterococci in the intestinal tract were low (Macha et al., 2004). Feeding the strain to sows and their piglets showed effects on the intestinal microbial community composition but also showed some animal-specific variability with so-called “responders” and “non-responders” (Starke et al., 2013a). In vitro co-culture experiments further showed that the probiotic may reduce the growth of other enterococci and pathogenic *E.coli* but no other enterobacteria (Starke et al., 2015). Similarly, data from in vivo feeding trials showed that *E. faecium* NCIMB 10415 does not affect the diversity and number of luminal enterobacteria but reduces the abundance of *E.coli* virulence factors and the number of pathogenic *E.coli* that adhere to the intestinal mucosa (Taras et al., 2006; Bednorz et al., 2013a). In addition, challenge models with *Salmonella* showed no protective effect of the probiotic on shedding of the pathogens in weaned pigs (Szabo et al., 2009; Kreuzer et al., 2012). An explanation could be that feeding *E. faecium* NCIMB 10415 seems to influence the immune system of piglets during early postnatal period, which is intensified during the weaning time and opens a so-called “window of opportunity” for the pathogens to colonize and persist in the host (Siepert et al., 2014). Further studies suggest that this immune stimulating effect may be
promoted not only through the transfer of the probiotic itself but also through immunooactive compounds from the probiotic-fed mother via the milk (Scharek-Tedin et al., 2015). However, the effect of *E. faecium* NCIMB 10415 on the sow-piglet interaction needs further clarification and is a promising field of future research.

### 3.2. *Bacillus toyonensis (Bacillus cereus var. toyoi)*

*Bacillus toyonensis*, previously known as *Bacillus cereus* var. *toyoi* (Jimenez et al., 2013), has been used for many years as probiotic in pigs and has been shown to reduce the incidence of diarrhoea and improve feed efficiency (Taras et al., 2005). Similar to results obtained with *E. faecium* NCIMB 10415, studies suggest that feeding of *Bacillus toyonensis* to sows changed their intestinal microbiota and the probiotic is transferred to the neonatal piglets (Jadamus et al., 2002). Significant effect on the intestinal fermentation patterns during the early suckling period and in weaning piglets have been reported (Kirchgessner et al., 1993; Jadamus et al., 2002). One of the main modes of action of *Bacillus spp.* in pigs might be through their immunomodulating properties. Spore formers such as *Bacillus toyonensis* evoke a strong response of the gut associated immune system in pigs (Scharek et al. 2007; Schierack et al., 2009). There seems to be an up regulation of the gut associated immune reaction, indicated by shifting of immune cell populations towards more pro-inflammatory phenotypes. The underlying regulatory mechanisms are still unclear but this may protect the animal from other bacterial infections. We recently performed an infection trial with *Salmonella* Typhimurium in piglets fed the probiotic. The data show that piglets responded to *S.* Typhimurium challenge with reduced growth and high incidence of diarrhoea, which was less pronounced in piglets fed with the probiotic (Scharek-Tedin et al., 2013).

### 3.3. *Lactobacillus spp.*

Several different *Lactobacillus spp.* and strains have been used in past studies in pigs showing modulating effects on the intestinal microbial communities or beneficial effects under challenge conditions. This includes *L. plantarum* (Pieper et al., 2009; 2010; Guerra-Ordaz et al., 2014), *L. sobrius/amylovorus* (Konstantinov et al., 2008) or *L. reuteri* (Hou et al., 2015; Yang et al 2015a,b). For example, *L. plantarum* DSM 8862/8866 strains or the *L. sobrius/amylovorus* DSM 16698 strain have been shown to reduce diarrhoea in enterotoxigenic *E.coli* (ETEC) challenged piglets (Konstantinov et al., 2008; Pieper et al., 2010). Fermentation of feed for piglets using a *L. reuteri* strain which produces bioactive compounds (levan, reutericyclin, reuteran) was shown to modulate the developing intestinal microbial communities and reduce the abundance of enterobacteria or the number of genes encoding for *E.coli* virulence factors (Yang et al., 2015ab). This shows
that fermented liquid feeding (under controlled conditions using defined lactic acid bacteria as inoculants) is a promising strategy combining the use of a probiotic with other positive effects of liquid diets.

4. – ZINC OXIDE

Zinc is an important trace element and involved in many processes in the body. The recommendations for the inclusion of zinc into the diets of young pigs are between 80 to 100mg/kg, which already covers the uncertainties regarding the bioavailability of zinc in the diet. The upper allowance in the EU is 150 mg/kg. In the late 80s and early 90s, first reports showed positive effects of very high inclusion rates of zinc oxide (2000 to 3500 mg/kg) on pig performance and the reduction of diarrhoea (Hahn and Baker, 1993). These effects have been confirmed in many studies in during the past decades although the mechanisms are yet not fully understood. Nowadays, the inclusion of high concentrations of zinc oxide is a common practice and recommended alternative to in-feed antibiotics in young pigs in many countries of the world, sometimes bypassing the official upper allowances in the feed. This picture may change again in the future as discussed below.

There are several assumptions about the mode of action of high dietary zinc oxide levels in the pig:

1. Influence on the gastrointestinal microbiota and reduction of pathogens
2. Influence on nutrient digestion and absorption
3. Improved intestinal barrier function
4. Improved immune response
5. Influence on metabolism of the animal (energy, amino acids)

The truth is likely something of everything within the above-mentioned list. This is not surprising since zinc is an important trace element involved in manifold metabolic processes in the body and, on the other hand, a toxic heavy metal at higher doses. Interestingly, some studies reported an improved animal performance during the first 2 weeks of high zinc oxide feeding whereas no or even opposite effects were observed thereafter. Following, a few interesting points regarding the mode of action of zinc oxide in pigs should be discussed a bit more in detail.

4.1. - Influence of ZnO on the gastrointestinal microbiota in pigs

Since the established positive effects of high dietary zinc oxide in weaning piglets, one of the main hypotheses about the mode of action was its stabilizing influence on the gastrointestinal microbiota (Katouli et al., 1999). Although the general idea to manipulate
the intestinal microbiota in a positive way is to promote lactobacilli and decrease the abundance of enterobacteria, the first studies on zinc oxide showed a rather opposite effect. The counts of coliforms in general and the number of different enterobacterial species was higher and under the influence of high zinc (Høyberg et al., 2005; Vahjen et al., 2011; Pieper et al., 2012). The number of lactobacilli and other gram positive bacteria such as clostridia was lower as well (Høyberg et al., 2005). This leads of course also to changes in the fermentation activity and lower concentration of short chain fatty acids or ammonia can be found in the gut lumen (Pieper et al., 2012). Further analyses showed that many other bacteria are affected by high zinc concentrations and some of these bacteria are more susceptible to zinc than others (Vahjen and Liedke, 2012). There are several possibilities by which some bacteria can resist high concentrations of heavy metals in the environment or in the cell. This results in a strong shift in the intestinal ecosystem when high levels of zinc oxide are fed. After 4 weeks of zinc oxide feeding, the bacteria have adapted to high zinc concentration in the digesta and show normal fermentation and growth (Starke et al., 2013b). One important new finding regarding the mode of action of zinc is that it mainly acts in the proximal digestive tract. Zinc oxide is nearly insoluble at neutral pH but solubility increases at acidic pH. A high concentration of free zinc ions can be found in the stomach of piglets and most of the changes in bacterial ecology can be detected in the proximal part of the gut. Only few effects were observed in the large intestine (Starke et al., 2014). Thus, zinc oxide acts mainly on microbial communities in the upper part of the gut, and protecting zinc oxide (e.g. through coating) from dissociation in the stomach or small intestine appears to be rather counterproductive.

4.2. - Influence of high dietary ZnO on animal physiology

Under normal physiological conditions, the zinc concentration in the body is kept in relatively narrow margins. This homeostasis is somehow outbalanced when high concentrations of zinc oxide are fed and zinc accumulates in different organs when very high zinc oxide is fed for longer periods (Davin et al., 2013; Martin et al., 2013). One may speculate that the enhanced barrier function of the intestinal epithelium und high dietary zinc influence is a side of the “protection” of the body against zinc ions. Similarly, several changes in pancreatic enzyme activity and hormonal status of piglets have been shown due to high zinc oxide feeding (Hedemann et al., 2006; Li et al., 2006; Yin et al., 2009). The zinc accumulation is reversible when normal zinc concentrations are fed afterwards (Zetzsche et al., 2015). New studies show that zinc accumulation in the liver or pancreas of weaning piglets after feeding high dietary zinc oxide for 4 weeks changes the expression of genes and proteins related to energy and amino acid metabolism but increases also oxidative stress reactions in these organs (Bondzio et al., 2013; Pieper et al., 2015). Thus, one may therefore speculate that the observed opposite effect on animal performance
during longer feeding of high zinc oxide levels are due to systemic overload of the animal with zinc.

4.3. – Does ZnO promote antibiotic resistant bacteria?

In recent years, reports suggested a link between the development of multi-resistant bacteria and high concentration of zinc and copper in the pig feed and manure from pig farms (Hölzel et al., 2012; Yazdankhah et al., 2014). Some of our own results support this hypothesis showing that the prolonged use of high levels of zinc oxide (up to 4 weeks) promotes the increase of multi-resistant *Escherichia coli* in the intestine of piglets (Bednorz et al., 2013b). In addition, new results show that some of the antibiotic resistance genes (e.g. against sulphonamides and tetracycline) increase in the intestinal tract of weaned piglets after 2 weeks of zinc oxide feeding (Vahjen et al., 2015). These are just first results and the mechanisms behind such as horizontal gene transfer and co-selection for antibiotic and metal resistance (Baker-Austin et al., 2006) are yet not clear.

5. - CONCLUSION

Feed additives such as probiotics may help to influence the intestinal microbial communities (balance) in pigs. However, there is a high variability due to strain-specific effect, the animal status and the experimental conditions under which the effects were observed. Thus, data generated under experimental conditions cannot generally be transferred into each management system and, in turn, experimental setups with high hygienic conditions may also mask some effects. Unravelling mechanisms behind the observed effects is a challenge for scientists but will help to generate individualized and more targeted recommendations for the use of probiotics in the swine industry. Zinc oxide has a well-established positive effect on piglet performance and reduces diarrhoea. High levels of dietary zinc oxide have manifold effects in piglets including changes in the intestinal microbiota and host physiology. However, longer exposure of piglets to such high zinc levels may lead to adverse effects on performance and (antibiotic) resistance in bacteria. Based on the current knowledge, the prolonged use (>2 weeks) of high amounts of zinc oxide as alternative to in-feed antibiotics currently bears the risk of just “replacing one evil with another”.

6. – REFERENCES

STARKE, I. et al. (2013b) PLoS ONE 8: e56405.