Feed additives as alternatives to zinc oxide to reduce post-weaning diarrhea

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Introduction

Several feed additives are proposed as potential substitutes for zinc oxide (ZnO) to reduce the incidence of post-weaning diarrhea and provide protection against intestinal injury. Any reduction of dietary zinc will result in less excretion of zinc to the feces, and thereby in reduced contamination of the agricultural soil. The aim of this abstract is to evaluate the potential of commonly suggested feed additives for their effect on improving gut health, and thereby, for reducing post-weaning diarrhea (PWD) in pigs.

Feed additives versus ZnO - Gut health versus post weaning diarrhea

Post-weaning diarrhea is a multifactorial disease, and its pathogenesis is still unclear. Frequently described, this is a condition of weaned pigs characterized by frequent discharge of watery faeces during the first two weeks after weaning. This condition is typically associated with faecal shedding of haemolytic enterotoxigenic *E. coli* (ETEC), proliferating in the ileum. The pathogenesis of ETEC-PWD can briefly be described as follows: 1) adherence of ETEC to specific receptors in the small intestine, 2) ETEC proliferation, 3) production of enterotoxins, and 4) hypersecretion of water and electrolytes to the lumen of the small intestine.

It is important to keep in mind that ZnO is approved in Denmark as a medicinal drug (not as a feed additive) by the Ministry of Environment and Food. Currently, ZnO can, upon veterinary prescription, be used up to a level of 2,500 ppm for treating piglet PWD the first 14 days post-weaning. Contrary to medicinal drugs, feed additives are applied to improve the performance of healthy animals or to cover the animals' physiological requirements. According to the Danish authorities, common feed additives for use in pig production belong either to groups of *nutritional* (such as vitamins, trace elements, and amino acids) or *zootechnical* feed additives (such as digestibility enhancing substances, gut microbiota stabilizers, environment-improving substances). Therefore, the use of feed additives should not, as such, be considered alternatives to medicinal ZnO for treating PWD, but rather as a strategy to enhance gut health, hence preventing pigs from developing diarrhea.

While it is relatively easy to assess the efficiency of a given intervention with regard to its effect on PWD, it is more challenging to establish the effect with regard to gut health, as several analytical tools are required to assess whether a gut is healthy or not. There is, in general, a lack of in vivo studies on the impact of feed additives using PWD as the primary outcome. So far, lowering the level of dietary crude protein is probably the most clearly documented feeding strategy to reduce the incidence of PWD in piglets (Lauridsen et al., 2017).

The various possible mode of action by which a given feed additive or a bioactive feed component may prevent progression of ETEC-infection of pigs are (1-3 related to gut microbiota, 4-5 to host immunology):

1) Protecting the gut from ETEC adhesion and colonization by reducing the sensitivity of fimbrial receptors on the porcine enterocytes and/or blocking the fimbriae of ETEC (e.g. specific immunoglobulins).

2) Inhibiting the growth of ETEC in the gut due to bactericidal or bacteriostatic effects (e.g., organic acids, medium-chain fatty acids, fermented liquid feed, antimicrobial peptides, bacteriophages, lysozomes).

3) Maintaining a balanced intestinal microbiota (e.g., probiotics, prebiotics).

4) Improving the host immune functions (systemically and locally) including prevention of excessive inflammation (e.g., immunoglobulins, fatty acids, vitamins, trace minerals).

5) Preventing disruption of intestinal mucosal integrity and/or improving the morphology of the small intestinal epithelium (e.g., specific egg yolk antibodies, bacteriophages).

Although the mechanisms behind the success of high dietary ZnO levels in terms of limiting PWD are still not clear, they are considered to be related both to an impact on the gut microbiota and to the physiological zinc status of the animal. Thus, medicinal levels of ZnO have direct antimicrobial effects, potentially targeting specific pathogens, like certain *E. coli* strains, but also, and maybe more so, reducing the microbial load in general, similar to the suggested mechanism of in-feed growth promoting antibiotics (Højberg et al., 2005; Vahjen et al., 2016). Moreover, ZnO presumably exerts an indirect effect on host-immunity and epithelial barrier functions.

Mode of action of feed additives

In the following, we summarize some examples of feed additives considered capable of promoting gut health of pigs and thereby prevent ETEC infection.

One of the main parameters influencing bacterial growth is pH, wherefore reducing the luminal pH, i.e. by addition of organic acids or other acidifiers, has an antibacterial effect (Canibe et al., 2005). Lactic acid bacteria are able to grow at relatively low pH, and are therefore more resistant to organic acids than, for example, enterobacteria, including *E. coli* (Knarreborg et al 2002). Besides the antibacterial effect, organic acids have a variety of other effects on the host considered to contribute to the positive output (de Lange et al., 2010). Among organic acids, butyric acid has been in focus, especially in human but also in pig research, due to the multiple ways by which this compound affect the host (Bedford and Gong, 2018). The impact of other acids and their combinations have also been intensively studied in pigs (Canibe et al., 2001), showing positive effects on gut health and growth performance, mainly when added at higher levels (Maribo, SEGES unpublished). Another feeding strategy to reduce the luminal pH (of the stomach) is fermented liquid feed, prepared by mixing water or another liquid, e.g., whey, with feed and incubating the mixture (for a certain period of time, at a certain temperature). As fermentation progresses, lactic acid bacteria proliferate, resulting in high concentration of, mainly, lactic acid and low pH, leading to reduced numbers of coliform bacteria (Canibe and Jensen, 2012). This implies a reduced load of *E. coli* reaching the small intestine, and protection from proliferation of pathogens, in order to maintain a stable bacterial community (Canibe and Jensen, 2003, 2012).

Antimicrobial lipids such as fatty acids and monoglycerides are promising antibacterial agents that destabilize bacterial cell membranes, causing a wide range of direct and indirect inhibitory effects. Besides, medium-chained fatty acids (MCFA) are an immediate energy source for the host and its immune cells, and improve intestinal integrity during inflammatory conditions. Combining MCFA with organic acids are some of the most recent initiatives to identify alternatives to antibiotics (Ferrara et al., 2017), however, more in vivo studies are needed to document the efficacy of these feed additives for preventing PWD as well as solving the challenges of reduced palatability of some MCFA. In this context, encapsulation can be used to allow the organic acids (including short-chain fatty acids) to reach the distal small intestine without being absorbed and thereby exert their antibacterial effect at the site of interest. Mono- and diglycerides are produced upon enzymatic hydrolysis of dietary triglycerides, and research has suggested that these fatty acids and monoglycerides exert antibacterial effects against infectious pathogens (Zentek et al., 2011). Using these agents as therapeutics requires improvement of the delivery in order to be applied in vivo.

Bacteriophages are bacteria-targeting viruses, common in all natural environments and very specific as each type generally attacks specific bacterial species. Several studies have evaluated the antimicrobial ability of phages targeting *E. coli*, including the fimbriae types F4 and F18, most commonly associated with piglet PWD. The use of phages is still limited in controlling feed borne pathogens in pigs, and more knowledge is needed to understand essential challenges, including phage resistance, phage-host interactions as well as unwanted perturbations of the gut microbiota.

Probiotics are, by definition, live microorganisms that, when administered in adequate amounts, confer health benefits to the host, and are one of the functional foods that link diet and health. Prebiotics are defined as selectively fermentable components, inducing specific changes in composition and/or activity of the gastrointestinal microbiota and conferring host well-being and health benefits. During the past years, many studies on pre- and probiotics (or their combination also known as synbiotics) have been carried out in pigs. These studies have shown a broad range of beneficial effects in terms of pathogen inhibition and its consequences, including immunological development and fortification of intestinal barrier functions. Because prebiotics are readily available substrates for probiotics, prebiotics may improve the survival of concurrently administered probiotic strains. Studies have shown potential capacity of probiotics in terms of immunomodulatory activities, but contrasting effects can also be obtained, which is probably due to differences with respect to the probiotic strain used, experimental settings, diets, initial microbiota colonization, administration route, time and frequency of administration of the probiotic strain and sampling for analysis (Roselli et al., 2017).

Passive immunization, i.e. the administration of antibodies (immunoglobulins) for protecting the host against infections, is not a new idea and seems to constitute a real and widely applicable alternative to antibiotics in modern animal production (Hedegaard and Heegaard, 2016). Oral administration of specific chicken Igϒ has been shown to be effective against a variety of intestinal pathogens, including ETEC (Diraviyam et al., 2014). Use of blood plasma or purified porcine immunoglobulin G from pooled natural pig plasma is also of interest as an immune-enhancing technology. Yeast derivatives based on *Saccharomyces cerevisiae*, where the bioactive components are mannans and β-glucans, may be immunomodulatory and can prevent colonization of pathogenic *E. coli* (Jensen et al., 2013). However, verification is needed on the exact composition and dosage of the bioactive components of the yeast derivatives, as well as use of antibodies, specifically when to apply and dose required.

Other active ingredients such as antimicrobial peptides (AMPs), vitamins, and trace elements have a major influence on the immune system. Once the immune system is activated, the nutrient partitioning is altered and partly directed to produce immune molecules and inflammatory responses. Reduction of inflammatory responses (for instance by acetylsalicylic acid and vitamin E) and oxidative stress reactions (by using antioxidative enzymes and vitamins) during the activation of the immune cells may protect against severity of infectious disease, including disruption of epithelial barrier function and mucosal injury, and eventual subsequent septic shock due to toxins entering the system. In this context, clay, some fibers, and vitamins exert specific actions on intestinal barrier integrity by influencing (size and/or activity) of goblet cells of the ileum (Moran, 2017).

During recent years, blends or combinations of the above-mentioned feed additives have been investigated in relation to gut health effects in pigs, which seems a reasonable strategy as one molecule cannot prevent e.g. progression of *E. coli* infection at all steps. In this context, algae, plant components (e.g. dried plant material), plant extracts and essential oils, berries and fruit extracts have gained interest in terms of potential benefits on gut health in pigs. These 'food'-related components may encompass a natural cocktail of antibacterial and immunomodulatory agents. However, the proposed bioactivity of the given cocktail, and its stability in feeds during should be investigated further.

Conclusion and perspectives

There is probably not a single feed additive, which can replace ZnO for its capability to prevent diarrhea. However, many potential feed additives share several of the mechanisms by which ZnO may influence the PWD outcome. Thus, the ideal feed additive for enhancing gut health of pigs is probably a cocktail of various antibacterial and immunomodulatory agents targeting specific challenges as *E. coli* infection progresses in piglets post-weaning. Further, starting nutritional interventions already during the suckling period may be necessary in order to efficiently influence the interaction between the microbiota and the host.

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