ALTERNATIVES TO IN-FEED ANTIBIOTICS: OPTIONS, LIMITATIONS AND OPPORTUNITIES

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Introduction

Increasing public concern regarding antimicrobial resistance and regulatory actions to eliminate non-therapeutic uses of antibiotics has heightened interest to find alternatives that provide similar benefits as in-feed antimicrobials. Those benefits have included, but are not limited to, improving feed efficiency, growth rate, and carcass quality, reducing manure output and lowering pathogen loads and disease incidence (Roth and Kirchgessner,1993; Kyriakis *et al.* 1996; Ebner and Mathew, 2000; Phillips *et al.* 2004; Andreasen *et al.* 2005; Mathew *et al.* 2007). Ideally, alternatives to in-feed growth promoting antibiotics would provide all of the above benefits without risking the efficacy of therapeutics and medical interventions important for human and animal health, or presenting risks of contaminants that might compromise food safety and public health. Importantly for the economic sustainability of pork production, the costs of alternative strategies, including the cost of the product(s) and additional labor or equipment for product delivery, must not exceed the economic benefits provided.

The challenges of meeting all of the above criteria with a single product or strategy are considerable, and that goal may not be possible with the current state of knowledge and science. More likely pork producers will need to implement multi-faceted approaches, perhaps specific to their own production systems, to achieve similar benefits previously possible with growth promoting antimicrobials. However, to advance our knowledge and increase the possibility of identifying or creating alternatives that match the benefits of in-feed antibiotics, we will likely need to focus on the intestinal microbiota and gut health, where growth promoting antibiotics demonstrate their primary effects. This presentation will thus focus on current and potential alternatives that may beneficially impact gut health, including through promotion of a "healthy" gut microbiota.

The notion that the gut microbiome is intimately involved in the production benefits realized by antimicrobials stems from early studies that noted the growth promoting effects observed in livestock were not evident when antibiotics were fed to gnotobiotic or "germ-free" animals (Coates, 1955). Such animals are derived from and raised in artificial environments devoid of microbes and thus do not develop a gut microbiota. But more than six decades and hundreds of studies since, we still do not fully understand all of the mechanisms of growth promoting antimicrobials, or how to mimic those mechanisms to produce similar positive outcomes through other strategies. Such speaks to the high complexity of the intestinal microbiome, the many biophysical and biochemical interfaces between the microbiota, intestinal tissues, gut contents and nutrients, and their interplay with the hosts' immune function and metabolic, nutritional and physiological states.

A number of hypotheses regarding the modes of action of non-therapeutic antimicrobials have been suggested, most with good evidence. Among the frequently cited reviews is that of Dibner and Richards (2005), who suggested antimicrobial modes of action likely include a reduction in total gut microbes thus reducing host competition for nutrients as well as reducing the level of deleterious microbial metabolites, a thinning of intestinal villi and reduction of gut wall thickness and total mass, perhaps due to lower bacterial fermentation and reduction of short chain fatty acids that promote gut tissue proliferation, and reduction of pathogen loads. In combination these changes result in significant metabolic and immunological savings for the host resulting in higher productivity. To date, no single alternative product has been reported to promote all of the above, although some hold hope to modulate one or more related factors, and/or promote other microbiota or gut changes to provide similar production benefits.

As it is not possible to provide an in-depth overview of all current and potential alternatives in this report, the goal here is to summarize current strategies for non-antibiotic alternatives and provide a few examples. Those strategies can be defined within three broad categories including:

1) promoting a healthy and stable gut microflora that excludes pathogens and promotes optimal gut function and animal health,

2) killing or inhibiting potential pathogens or detrimental microbes, and

3) enhancing nutrient availability, digestion and uptake.

A number of reviews provide in-depth information for various non-antibiotic alternatives that fall within these strategies, including Thacker (2013), Seal *et al.* (2013), and Czaplewski *et al.* (2016). However, admittedly the above categories are not mutually restrictive or exclusive and some potential alternatives may fall into more than one category. The above categories also may not capture some underlying fundamental aspects or modes of action that may be key for gut health and/or animal productivity. Such include the ability to maintain a healthy intestinal mucosal barrier that excludes pathogens and/or toxins, optimizing innate immune function to maximize resistance to disease or directly or indirectly enhancing appetite and intake, particularly during critical stages such as early postweaning. To adhere to the theme of the conference session, this paper will focus on Strategy 1, and to a lesser degree Strategy 2 above.

Alternatives for promoting a healthy and stable gut microflora

Alternative products that fall into this category include probiotics, prebiotics, essential oils, organic acids, and some enzyme products that degrade complex carbohydrates or other complexes, providing smaller substrates that may favor specific beneficial microbes.

Among the most well-studied alternatives are probiotics, which are live microorganisms intended to promote a healthy/positive gut microbial profile (Gaggia *et al.* 2010, Kenny *et al.* 2011). To be effective for their intended mode of action, the organisms must survive storage and/or any feed processing, and remain viable in transit through the gastric portion of the digestive tract where the pH may range from 1.5 to 3. Numerous commercial products containing single or multiple microbial strains are available as in-feed probiotics. And many organisms have been investigated and/or included in probiotic products, including numerous species of the bacteria genera *Lactobacillus, Bacillus, Bifidobacterium*, and *Streptococcus*, as well as several species of the yeast genus *Saccharomyces*. While studies of probiotics have not provided consistent results and positive production benefits are not always realized, a recent meta-analysis of 61 randomized controlled studies in pigs focusing on feed efficiency or average daily gain (Zimmerman *et al.* 2016) indicates that growth performance is more likely than not to be enhanced by probiotics. Prebiotics (Gaggia *et al.* 2010, Patel and Goyal, 2012) are products that nutritionally support microbes considered beneficial to gut health, and thus might act as complimentary additives to probiotics, or for already-established beneficial bacteria gut microbes. Prebiotics are typically complex oligosaccharides or plant fibers that are not readily digestible by the host animal, but are digestible by specific microbes considered as beneficial for gut health.

Organic acids have also been well-studied as feed additives (Suiryanrayna and Ramana, 2015). These weak acids that include propionic acid, citric acid, lactic acid, fumaric acid and others, can modulate gut microbes, primarily by reducing pH of gastric and/or intestinal contents, favoring beneficial bacteria such as *Lactobacilli* over potentially detrimental bacteria such as *E. coli*, which can proliferate under higher pH environments. Organic acids may also increase digestion of proteins by promoting increased activity of the digestive enzyme pepsin in the stomach.

Essential oils are plant-derived products, some of which are aromatic and distinctive of the plant from which they were derived (Zeng *et al.* 2015). Extracts from cinnamon, oregano, clove, thyme are among many products that have been studied or used in pig diets, some with promising results. The mode(s) of action of essential oils are poorly understood at this time, and are likely compound-specific. Some essential oils are said to facilitate intake by enhancing flavor and/or palatability, others may have an antibacterial effect on some types of intestinal microbes, with some studies indicating increased *Lactobacilli*, and/or *Lactobacilli* to coliform ratios in weaned pigs (Li *et al.* 2012a, Li *et al.* 2012b).

A wide range of exogenous enzymes are used in swine and poultry diets Among the most commonly used in swine are phytase and variety of carbohydrases including xylanase, ß-glucanase and ß-mannanase (Adeola and Cwieson, 2011). In particular, carbohydrases may impact the gut microflora, potentially by altering transit time of gut contents and providing additional substrates to facilitate bacterial fermentation and the production of volatile fatty acids which help maintain a healthy gut pH. Through recombinant technologies, the array of enzymes available for in-feed application is expanding rapidly. And while much of the focus for these products is on enhancing nutrient availability and uptake by the host, research is needed to also understand their potential effects on the gut microbiota so greater health benefits can be derived through strategic enzyme development and use.

Inhibiting pathogenic or detrimental gut organisms

As a primary mode of action of growth promoting antibiotics appears to be related to reduction of specific pathogens, and thus alternative products that provide similar protection are important additions to the arsenal of alternatives to feed-based antimicrobials. Zinc oxide and copper sulfate are commonly used in pig feeds to help control bacteria associated with diarrhea and other gut disorders. Both products have shown similarities to the mode of action of some antibiotics in reducing deleterious bacteria. Thus these products have been included in this section; but it could be argued that they might also be included the category of alternatives that foster a healthy microbiota and gut. Recent studies suggest that one mode of action for both Zn and Cu is to reduce bacteria responsible for the production of bile salt hydrolase (BSH), an enzyme that has a negative effect on fat digestion and absorption by the host, similar to some antibiotics which also reduce BSH production (Lin 2014). As such these commonly-used products would appear to be valuable alternatives to in-feed antibiotics. However, there may be challenges ahead for continued use of these products on two fronts. Concerns are increasing over environmental contamination resulting from continual application of swine manure containing high levels of copper and zinc, particularly in countries that rely heavily on these products to control diarrhea in young pigs (Jensen *et al.* 2016). Perhaps even more concerning are recent reports the selection pressures imparted by copper sulfate and zinc oxide are not only promoting bacterial resistance to Zn and Cu, but are also co-selecting for resistance to some antibiotics (Amachawadi *et al.* 2011; Yazdankhah, *et al.* 2014; Argudin and Butaye, 2016). Combined, these concerns may present challenges for wide-spread continued use of these two important alternatives for the swine industry.

Other research to date has focused on a number of other technologies including active bacteriophages, purified bacteriophage lysins, in-feed antibodies, and antimicrobial peptides, all which by design target specific groups or species of microbes considered to be pathogenic or significantly detrimental (Czaplewski *et al.* 2016). Most of these approaches target unique molecular receptors, complexes and/or antigens on the surface of the organism, thus compromising either the function or integrity of the cell structure, or the growth and replicating capability of the bacterium. Key to success of these strategies is the identification of appropriate targets on the organisms of interest. Focusing on agents that target complexes essential to the bacterium will be important as those sites typically have a lower risk or rate of mutation, and thus there is less likelihood of the development of resistance, similar as can occur with antibiotics.

Evolving technologies to aid in identifying effective antibiotic alternatives

Very important for the success of identifying effective antibiotic alternatives are newer and evolving molecular and bioinformatics technologies that provide an increasingly in-depth understanding of the gut microbiota, effects of dietary and other treatments, and the impact on animal health and productivity. These newer technologies enable researchers to characterize subtle changes in bacterial families, genera and even species under various treatments and/or nutritional regimes, and characterize how those changes may impact gut and host health (White *et al.* 2016). Through application of these newer tools researchers are better able to find potential clues for the development of new strategies and products, or better evaluate and refine current alternatives to antimicrobials, to optimize food animal production and health.

Summary

Much research continues in an effort to find and develop cost-effective alternative products that match or exceed the production benefits associated with in-feed antibiotics. As of yet, no single product, or combination of products, appears to consistently provide all of the benefits or multiple modes of action as do antibiotics. Effects of current alternatives are likely to be more subtle, often encouraging a healthy gut microbiota, but not as likely to overcome significant pathogen loads or pre-existing diseases, in contrast to antimicrobial products. Producers must think of alternatives as preventative agents that work in concert with optimal management, nutrition, housing and other husbandry factors to optimize growth and performance.

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