

Alternatives to In-Feed Antibiotics in Animal Feed: A Healthy Gut Microbiota Approach

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ABSTRACT

Sub-therapeutic doses of antibiotics are added to animal feed to treat and prevent infections and to improve growth and production. Intensive animal breeding for food production has led to a substantial increase in the use of antibiotics in the recent decades. Exposing bacteria to low doses of antimicrobial agents over a long period of time lead to selection of antimicrobial resistant genes in bacteria. This overuse of sub-therapeutic antibiotics in animal feeds can contribute to antibiotic resistance development in bacteria creating health dangers to human. It has been shown that the antibiotic avoparcin, when removed from animal feeds in Sweden, resulted in a significant reduction in vancomycin resistance in human clinical isolates.

The European Union has banned all in-feed use of antibiotics from 2006 and the use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. Moreover consumer requests for antibiotic free meat products are increasing and countries exporting meat to the European Union are required to follow suit. It is also clear that the complete withdrawal of feed antibiotics has a detrimental effect on production and animal health to the extent that more antibiotics may ultimately be subscribed for therapeutics purposes to control disease conditions. The removal of antibiotics from animal feed will also lead to an increase in the proportions of harmful microbes like *E. coli*, *Salmonella* and *Campylobacter* in the gut microflora of farm animals challenging human food safety. Therefore alternatives to sub-therapeutic antibiotics are urgently needed, but to make this transition, a better understanding and a more fundamental knowledge about the role of microorganism in gut function is required.

This report will outline how the native intestinal microbial population can be harnessed to improve animal health and performance. Moreover the recent findings in the search for alternatives to in-feed antibiotics that hold potential to successfully manipulate this ecosystem will be discussed.

INTRODUCTION

The inclusion of sub-therapeutic (low) levels of antibiotics in animal feed has become a mainstay of animal agriculture (Gaskins et al, 2002). For several decades, antibiotics have been used in animal feed as growth promoters to improve animal welfare and to obtain economic benefits in terms of improved animal performance and reduced medication costs. Antibiotics were discovered more than 50 years ago and initially their use in dietary formulations was restricted due to its high cost (Yan et al 2004). However by the mid 1960's the cost has decreased to a tenth of the original price and the inclusion of antibiotics in animal diets has increased in parallel with the decline in cost.

There are increasing concerns about the risk of developing cross-resistance and multiple-antibiotic resistance in pathogenic bacteria both in human and livestock, linked to the therapeutic uses of antibiotics in human and veterinary medicine and as growth promoters in animal feed (Gibson and Roberfroid, 1995). A continuous exposure of bacteria to antibiotics (i.e. in the intestinal tract) sets a selection pressure that allows resistant bacteria to survive and proliferate while eliminating the susceptible bacteria. Antibiotic resistant genes are present in the mobile genomic elements of bacteria such as plasmids and can be transferred between bacteria by three main routes of gene transfer, conjugation, transduction and transformation. When animals become colonized with resistant organisms, these organisms can eventually reach humans through the food chain, direct contact, or contamination of water or crops from animal excreta making humans vulnerable to infections that are difficult to be treated by the same antibiotics.

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According to the understanding at present, the benefits to animal health due to the action of sub-therapeutic antibiotics can be summarized as follows: i) reduction of the bacterial load in the intestinal tract and inhibition of sub-clinical infections, ii) reduction of the microbial use of nutrients in the gut and iii) enhanced uptake and use of nutrients through the intestinal wall (Chio et al, 1994). Antibiotics may increase the supply of net energy to the animal by sparing carbohydrates from bacterial fermentation. Nitrogen and amino acid availability may also be improved by the reduction of bacterial proteolytic activity and production of toxic amines (Visek, 1978). These changes are frequently associated with a decreased rate of food passage through the gut allowing more time for digestion and improving nutrient availability and absorption. It has been shown that improvements in daily weight gain of animals ranging from 4-16% and feed utilization of 2-7% has been achieved due to the use of antibiotic growth promoters (Yan et al, 2004). The suggested modes of action of feed grade antibiotics on bacteria are inhibition of their growth, modification to their carbohydrate and protein metabolism, damage to cell wall formation and disruption to their nucleic acid synthesis. After the complete ban of using antibiotics in animal feed by the European Union in January 2006, the negative consequences for animal health and welfare and for food safety on a multi-national scale have become particularly evident in the food chain. For example the incidence of *Campylobacter jejuni*, one of the most common pathogens in livestock, has increased both in Europe and in North America during the period from 2006 to date (EFSA, 2009, Fernando et al, 2007). This is in agreement with the idea that the use of antibiotics resulted, besides the improvement of nutrient absorption, enhancing feed intake and weight gain, in the inhibition of widely spread pathogens at the primary production level. Therefore in the research of antibiotic alternatives, new strategies must be found not only to improve animal performance but also to

minimize or eliminate the pathogen load in the livestock as well as in the human food chain.

Understanding the Role and Importance of Intestinal Microbiota

The gastrointestinal (GI) tract of vertebrate animals contains a species-diverse group of microflora, although bacteria, and particularly gram-positive bacteria, predominate (Beckhead et al., 2005). As many as 500 bacterial species exist in the GI microflora, with numbers up to 10^{10} to 10^{12} bacterial cells/g of colonic content or feces (Jensen, 2001). The bacterial population influences a variety of immunological, physiological, nutritional, and protective processes of the GI tract and exerts profound effects on the overall health, development, and performance of animals. Experiments comparing conventionally reared versus germ-free (sterile) animals have demonstrated that commensal bacteria play important roles in organ, tissue, and immune system development, as well as providing a variety of nutritional compounds (Gaskins, 2001). The major benefit provided by commensal microbiota is resistance to colonization by pathogenic and other non indigenous microbes, a phenomenon also known as competitive exclusion (Gaskins, 2001; Kelly and King, 2001). Many studies have demonstrated that germ-free animals are far more susceptible to colonization by pathogens than are conventionally grown animals (Koopman et al., 1984). The specific mechanism(s) by which this protection occurs have yet to be demonstrated, but many hypotheses have been proposed. It is widely believed that the resident flora suppresses colonization by secreting antimicrobial compounds such as organic acids, by direct stimulation of the immune system, and by competing for nutrients and attachment to the mucosal surfaces (Kelly and King, 2001). Also the microflora-secreted nutrients become available for use by the host. These include short chain fatty acids (SCFA), amino acids as well as vitamins B and K (Snel et al.,

2002). Short chain fatty acids, such as acetate, butyrate and propionate are highly prevalent anions in the colon produced by anaerobic species that ferment dietary fiber (Kelly and King, 2001). Likewise, the commensal bacteria also generate lactate, acetate, propionate and butyrate (van der Wielen et al., 2000) and these fatty acids contribute significantly to the energy supply of the animal. Butyrate has been shown to be the preferred source of energy for the epithelial cells of the lower intestinal tract, affecting cell proliferation, differentiation and apoptosis (Dalmaso et al., 2008). Butyrate reinforces the mucosal defence barrier in the lower intestinal tract by increasing the production of mucins and antimicrobial peptides (Barcelo et al., 2000).

The resident microflora of the gut stimulate development of intestinal host defenses, including the mucus layer; the epithelial monolayer; and the lamina propria, with its system of immune cells that underlie the epithelium (McCracken and Gaskins, 1999; Kelly and King, 2001). The mucus layer segregates both normal and pathogenic microbes away from the animal tissues, and the underlying network of immune cells provides antibodies, cytotoxic and helper T cells, and phagocytic cells. These immune cells combat not only pathogenic bacteria and their toxins but also the overgrowth of or inappropriate attachment by the normal microflora. Studies of germ-free animals, have shown that they exhibit delayed lymphocyte and other immune cell development in the lamina propria and far fewer IgA-producing cells when compared to conventionally reared animals (Gordon and Pesti, 1971). The majority of evidence supports the notion that the intestinal immune system develops in parallel with the development of the normal microflora. Introduction of even a single species of commensal bacteria into germ-free animals can stimulate the development of the secretory IgA system (McCracken and Gaskins, 1999).

In contrast the commensal bacteria compete with the host for nutrients, secrete toxic compounds, and induce an ongoing

immune/inflammatory response in the GI tract. All of these have an impact on animal health and performance. Two important areas for future research are, i) to determine the optimal microflora for animal health and performance under commercial growth conditions (in other words, to discover the microflora that maximize the benefits while minimizing the costs and, ii) to develop dietary and other interventions to foster development of the beneficial microflora.

Competitive Enhancement Strategy

The bacterial species best adapted to occupy a particular niche within the GI tract will become the most successful and will eventually dominate the niche. An established, mature, gastrointestinal microbial population fills all available environmental niches making an animal more resistant to colonization by opportunistic bacteria, especially pathogens (Fuller, 1989). This natural anti-pathogen activity has been described as 'bacterial antagonism, or 'Competitive exclusion' (CE) (Nurmi and Rantala, 1973). It is known that beneficial and pathogenic bacteria co-exist in the gastrointestinal tract in an inverse relationship that is ruled by CE. When an animal undergo a period of stress or disease challenge, the intestinal population of beneficial and commensal bacteria declines. This contingency can allow certain opportunistic pathogens to multiply to a threshold which can lead to clinical signs of diarrhoea and other serious infections. Such diarrhoea causing pathogenic bacteria usually consist of *Clostridia*, *Salmonella* and *E. coli*. Competitive enhancement techniques that have been developed mainly include: (i) addition of a microbial supplement called a probiotic or a direct fed-microbial (DFM), that improves gastrointestinal health and the diversity of the intestinal microbial ecology (Collins and Gibson, 1999); and, (ii) providing a specific nutrient (a 'prebiotic') that allows an existing commensal microbial species or population to expand its current niche or to occupy a new niche in the gastrointestinal tract. No matter

which of these strategies is employed, the overall goal is to fill all ecological niches within the gut, thereby preventing colonization by a pathogen or displacing an established pathogenic bacterial population in the gut.

Alternatives to Antibiotic Growth Promoters

Probiotics

Probiotic preparations for use in animals are typically comprised of individual species or mixtures of lactic acid bacteria (LAB), yeasts, or their end products and are not species-specific, or even necessarily originally isolated from animals. Probiotics often fall into the categories of: (1) live cultures of yeast or bacteria, (2) heat-treated (or otherwise inactivated) cultures of yeast or bacteria, or (3) fermentation end products from incubation of yeast or bacteria. The most commonly used probiotic bacterial strains remain *Bifidobacteria* and *Lactobacillus*, and many studies demonstrate that these types of products can enhance production efficiency of farm animals. However, the development of probiotics in animal feed is limited by certain restraining factors including stringent European legislation on the use of probiotics and a wide range of alternate ingredients. Furthermore, animal feed products are often treated at high temperatures and probiotics (being microbial in nature) cannot survive such temperatures.

Prebiotics

A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or more of beneficial bacteria. The non-digestibility of prebiotics ensures that they can reach the colon and act as an energy source for bacteria, unlike normal sugars, which get digested directly by the host (Gibson et al, 1995). As a result the composition and/or the activity of the microbiota is altered, leading to secondary effects such as

increased gas production (short chain fatty acids - SCFA, lactic acid, hydrogen, carbon dioxide, hydrogen sulphide) and a drop in pH which may also be translated into macro-effects like an increased resistance against pathogens or an improved growth performance. Prebiotics can also have other beneficial effects, as they can prevent the adhesion of pathogens to the mucosa, by competing with its sugar receptors and several studies have shown that supplementing animal feed with various oligosaccharides have led to reduced susceptibility to *Salmonella* and *E.coli* colonization (Bailey et al 1991). From about 1980s the potential effects of prebiotics in animal feeds has already been recognized. Since then, the interest in the use of prebiotics in animal feed and pet food has resulted in an extensive research activity, For example, Iji & Tivey (1998, 1999), Flickinger & Fahey (2002) and Patterson & Burkholder (2003) have documented the use of prebiotics in diets for farm animals and pets. Some prebiotics provide a competitive advantage to specific members of the native microflora (e.g. *Bifidobacteria* and *Butyrivibrio*) that can act as a natural CE culture against pathogens. Coupling the use of CE with prebiotics is a technique known as 'synbiotics'; and this can induce a synergistic reduction of pathogens and disease (Collins and Gibson, 1999).

The non-digestible inulin-type fructans are found widely in many vegetable feed and food ingredients and are perhaps the best studied and documented prebiotics in domesticated animals (Flickinger et al. 2003a). However, in the recent past emphasis has widened towards a complete range of oligosaccharides including galacto-oligosaccharides, beta-glucans, soybean oligosaccharides and xylo-oligosaccharides. The structure of the prebiotic substance largely determines its effect. Inulin is known to be fermented faster (resulting mainly in the production of butyric acid) than xylo-oligosaccharides, which are slowly fermented, generating acetic, propionic and lactic acids. Research has shown that adding indigestible

oligosaccharides to the feed based on either fructose or mannose sugars derived from yeast-cell wall can be used to attract pathogenic bacteria to attach to these dietary particles rather than the intestinal cells. This competitive exclusion principle is now in commercial practice. However it was also shown that supplementation of poultry feed with mannanoligosaccharides (MOS) resulted in an improvement in intestinal morphology and intestinal enzyme activity, yet the growth performance of the broilers was not up to the level of including an antibiotic growth promoter to the feed (McCann et al 2006).

Cereal grains such as barley and oat are interesting sources of potential prebiotic carbohydrates, especially mixed-linked β -glucan and resistant starch (Pieper et al, 2008). Recent research has shown that increased levels of β -glucans and altered amylopectin/amylose ratios in the pig diet seemed to selectively favour butyrate-producing bacteria, possibly capable of degrading complex carbohydrates, which would have beneficial effects on the host. Furthermore, other beneficial microorganisms such as *bifidobacteria* and *lactobacilli* were influenced by the choice of cereal variety, i.e. whether it was hullless or hulled barley or oats (Pieper et al 2008).

Feed enzymes

Many feed ingredients contain chemical constituents that are not readily digested by the endogenous enzymes in the intestine. These are primarily non-starch polysaccharides, such as cellulose, glucans and xylans. These can increase the viscosity of the gastrointestinal contents and reduce digestive efficiency. Exogenous enzyme additions can be used to decrease gut viscosity and enhance diffusion of enzymes and acids into the food mass, resulting in more complete digestion and absorption with less residual substrate for bacterial growth. Addition of specific enzymes to young animal diets can augment and complement those enzymes secreted endogenously. Amylase, protease, and lipase additions have been shown to

improve post-weaning growth and minimize digestive disturbances (Kitchen, 1997). The use of beta-glucanase and xylanase are beneficial with high fibre grains e.g. wheat, barley and their by-products (Chesson, 1987). Alpha-galactosidase is used to breakdown the galactose units in raffinose and stachyose found in soybean. The benefits of these enzymes are firmly established in poultry feeding, and their commercial use is already widespread. Also, they can reduce the available substrates for microbial proliferation in the ileum and caecum, while stimulating the more beneficial organisms, as a result of the oligosaccharides and/or sugars they are able to release (Bedford, 2000). Moreover, these enzymes allow for greater flexibility and thus lower costs in feed formulation, especially when maize is scarce and/or very expensive compared to other cereals. Also other enzyme activities such as α -1,6 galactosidases and β -1,4- mananases, are used to hydrolyze flatus-causing oligosaccharides of soybeans and other legume grains. In a recent review of 14 trials of soybean-based diets for pigs, supplemented with α -1,6 galactosidases, β -1,4-mananases, or enzyme complexes, Kim and Baker (2003) concluded that the enzymes had positive effects on growth performances and digestibility in 70% of the cases.

CONCLUSIONS

The increased concern about the potential for developing antibiotic resistant strains of bacteria within the food chain has compelled the researchers to test for other non therapeutic alternatives to be used as feed additives in animal production. The research on alternatives to antibiotic growth promoters needs to be focused on strategies to develop the optimal microflora for animal health and performance under commercial growth conditions and to find dietary and other interventions to foster development of the beneficial microflora. The primary tools for utilizing the native microbial population against diseases of

farm animals described here are probiotics, prebiotics and feed enzymes. All of these can improve animal health through a variety of mechanisms that are still not completely understood. However, by enhancing our knowledge on the effects of microbial population on animal growth, we can further enhance growth efficiency, productivity, food safety and animal health.

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