MANIPULATING INTESTINAL MICROFLORA THROUGH NUTRITION

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Introduction

Scientific, political and market developments are forcing animal production to reduce the use of antibiotics. However, in order not to impact or at least to minimize negative effects on animal well-being, food safety and production efficiency alternative measures have to be taken to maximize animal health when reducing the use of antibiotics.

A healthy digestive system is crucial for optimal animal performance. However, due to its large surface area and the heavy microbial load the gut is a vulnerable site for pathogen entry into the body. The large surface is necessary to optimize nutrient absorption. To allow an efficient transfer of nutrients to the blood the gut is only protected with one layer of epithelial cells. Unfortunately, this thin layer does not only facilitate nutrient transfer, but also weakens the gastro intestinal (GI) tract in keeping pathogens from entering the body.

Therefore, an array of additional protection systems is in place to minimize the risk of intestinal disease. Mucins and glycoproteins associated with the intestinal brush border serve as important barriers protecting the delicate absorptive surface from the abrasive action of feedstuffs, bacteria colonization, and toxins. Endogenous acids, digestive enzymes and bile reduce bacterial growth. Digestive flow and peristaltic movements transport the digesta through the digestive tract, and with it bacteria, thus limiting bacterial development. To further optimize gut protection the animal has devoted more than half of its immune cells to protecting the digestive tract. In addition, the GI microflora plays a crucial role in gut defense. Through different complex mechanisms beneficial bacteria limit the growth of pathogens, trying to exclude them from the system (Rolfe, 1991).

Profound knowledge of the development and composition of the GI microflora and its regulatory forces is essential to understand the dynamic of the GI microflora as well as interactions with feedstuffs and feed additives.

Competitive Exclusion

Competitive exclusion (CE) implies the prevention of entry or establishment of one bacterial population into the GI tract because a competing bacterial population already occupies potential attachment sites. To be able to succeed, the latter population must be better suited to establish or maintain itself in that environment or must produce compounds inhibitory to its competition (Bailey, 1987). Nurmi and Rantala (1973) were the first to apply the CE concept to domestic animals. The mechanisms, which are involved in CE, are very complex. The mechanisms can be grouped in direct and indirect mechanisms. Indirect mechanisms are the result of the normal microbial flora altering the physiologic response of the host, which in turn affects the interaction between the host and microorganism (Rolfe, 1991). Direct mechanisms are exerted by different bacterial populations on each other.

Two basic nutritional approaches can be applied to promote the beneficial GI microflora. First, by feeding beneficial bacteria (probiotics) we can support and complement the endogenous microflora. The effect of probiotics is well documented in the literature. Hollister *et al.* (1999) reduced salmonella colonization in chicks by feeding a live cecal culture from salmonella-free poultry. Fedorka-Cray *et al.* (1999) has shown similar response to microbial cultures in young pigs. Gram-positive bacteria, including *Lactobacillus, Enterococcus, Pediococcus, Bacillus,* and *Bifidobacteria*, and fungi of the *Saccharomyces* (yeast) genus are often fed after antibiotic therapy as a means of re-introducing a beneficial flora to the gut of affected animals.

A second possibility to influence the outcome of bacterial competition comes through influencing specific mechanisms, which have an impact on CE, such as:

- GI environment
- Substrate / nutrient supply
- Substances with antimicrobial properties
- Inhibiting of bacterial adhesion
- Stimulation of gut peristalsis
- Modulation of the immune response

All those mechanisms can be influenced through specific modifications of the diets.

Substrate availability controls proliferation of the GI microflora

Gut health and enteric disease resistance is often dependent upon the digestibility of feed components and feed formulation. Poorly digested protein meals cause the proliferation of putrefying bacteria in the hindgut, which increases toxic metabolites (ammonia and biogenic amines) that compromise gut health. In general, antibiotics are most effective in birds fed diets containing high levels of indigestible protein (Smulders *et al.*, 2000). Similarly, poultry fed diets containing high levels of poorly digested non-starch polysaccharides from wheat, barley or rye are more susceptible to enteric disease, such as necrotic enteritis (Riddell and Kong, 1992; Kaldhusdal and Skjerve, 1996). Langhout *et al.* (1999) observed that dietary NSP significantly increases gut populations of pathogenic bacteria at the expense of beneficial bacteria. However, the digestibility of wheat, barley, rye, triticale and even corn-based diets can be significantly improved through use of exogenous enzymes including xylanases, phytases and ß-glucanases (Rosen, 2001). Because supplemental enzymes mediate their beneficial effects primarily by enhancing feed digestibility and nutrient availability to the host, they also influence the gut microbial ecosystem. The use of enzymes has been shown to alter the gut microflora populations in the small intestine and caeca (Choct *et al.*, 1996; Hock *et al.*, 1997; Bedford, 2000a) and reduce mortality rates (Rosen, 2001). Such a benefit is brought about by a more rapid digestion and absorption of starch, protein and fat from

the small intestine, which effectively limits available substrate for the resident flora.

Beside modifying nutrient availability from raw ingredients for bacterial fermentation, the addition of specific carbohydrates such as lactose or FOS, which are preferably fermented by beneficial microorganisms, can positively influence the composition of the GI microflora. The effects of dietary FOS on the intestinal microflora are well documented (Mitsuoka *et al.,* 1987; Hidaka *et al.*, 1991; Patterson *et al.* 1997; Hidaka and Hirayama, 1991). Hidaka *et al.* found that consumption of 8 g FOS/day increased numbers of bifidobacteria, improved blood lipid profiles and suppressed putrefactive substances in the intestine of humans. However, Waldroup and coworkers (1993) found that supplementing broilers with 0.375% FOS had few consistent effects on production parameters or carcass *Salmonella* concentrations. These authors also caution of possible antagonism between FOS and BMD.

Recent evidence suggests that novel oligosaccharides with improved anti-pathogen effects in probiotic microorganisms can be synthesized (Rastall, personal communication). When the relationship between sugar structure and those effects are better understood, it should be possible to design novel prebiotics to maximize the protective effect. Another dimension to influence animal health through carbohydrates is to exploit the involvement of carbohydrates in cell-to-cell interactions. Carbohydrates are important surface entities of animal and bacterial cells that function in a variety of ways to influence cell-to-cell communication, impact the immune system and allow bacterial attachment to the host. The science of understanding the sugars, which make up the cells and their structures is known as glycomics.

Carbohydrates, cell-to-cell communications and defense against pathogens

Carbohydrates project from the cell surface and form the antigenic determinants of certain cell types. One of the classical examples of this antigenicity is blood type in humans. The ABO blood group antigens are glycoproteins on red blood cells. Small differences in the terminal sugar residues distinguish the A and B blood-group antigens (Kuby, 1994) Mannose binding protein (MBP) is an integral part of the immune system. MBP in the serum can bind to terminal mannose groups on the surface of bacteria and interact with two serine proteases (MASP and MASP2), which ultimately lead to antibody independent activation of the classical pathway of the immune system (Roitt *et al.,* 1998). Bacterial infection is due in many cases to the ability of the bacteria to recognize host cell surface sugars and use specific receptors that allow them to attach, colonize, and in the case of pathogens, cause disease in the animal. Mannose-specific adhesins (the binding entity on the surface of bacterial cells) are utilized by many gastrointestinal pathogens as a means of attachment to the gut epithelium. One way to prevent pathogens from causing disease is to prevent them from attaching to the epithelial cells in the gut. Early studies using mannose in the drinking water of broiler chicks demonstrated that this therapy could reduce colonization rate of *Salmonella typhimurium*.

Purified mannose and a complex sugar called mannan oligosaccharide (MOS) have been successfully used to prevent bacterial attachment to the host animal by providing the bacteria a mannose-rich receptor that serves to occupy the binding sites on the bacteria and prevent colonization in the animal.

Several studies have been conducted examining the role of mannans and their derivatives on binding of pathogens to epithelial cells in the gastrointestinal tract. *E. coli* with mannose-specific lectins did not attach to mammalian cells when mannose was present (Salit and Gotschlich, 1977). Spring and coworkers (2000) used a chick model to demonstrate that MOS could significantly reduce the colonization of *Salmonella* and *E. coli*. Animal trials in other species show similar benefits in reducing pathogen concentrations. In dogs, as well as in poultry, reductions in fecal clostridial concentrations have also been noted with MOS supplementation (Finucane *et al.,* 1999; Strickling, 1999). Different researchers also found improved performance with MOS (Miguel *et al.,* 2002, Hooge, 2004a,b). It has been suggested that improvements in the GI microflora are a main factor leading to improved performance. While inhibition of mannose receptors are commercially exploited, adhesions which are specific for other sugars are currently being investigated.

Glycomics also plays a vital role in viral diseases. The influenza virus infects by first attaching to a cell surface carbohydrate called sialic acid. This attachment 'opens the door' of the cell and allows the virus to replicate within. The commercial drugs Tamiflu and Relenza shorten the duration of the flu by binding to the active site of an enzyme produced by the virus that frees the virus from the sialic acid. By tying up this enzyme, the virus cannot easily spread and infect other cells (Schmidt, 2002). There are also data examining a novel anti-human immunodeficiency virus (HIV) protein. This protein, called actinohivin, binds to a glycoprotein on various HIV strains and simian immunodeficiency virus (SIV) inhibiting viral entry into cells by binding to this envelope glycoprotein. Further investigation showed that only yeast mannan can inhibit the binding of actinohivin to these viruses. These results demonstrate that the mannose saccharide chains of the virus glycoprotein are the molecular targets of the anti-HIV activity of actinohivin (Chiba *et al.,* 2004). Sulfated galactomannans also demonstrate *in vitro* and *in vivo* activity against the flaviviruses, yellow fever virus and dengue virus (Ono *et al.,* 2003)..

Conclusions

The intestinal microflora itself is a unique protection system, as beneficial bacteria are continuously competing with pathogens through competitive exclusion (CE). Nutrition offers an array of approaches to influence different bacterial control mechanisms that play a role in CE. While mannan oligosaccharide is currently being used to improve health and production of animals, there are enormous possibilities to use other sugars as possible agents against pathogen infections.

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