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Update on Feed Additives and Enteric Health

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Feed additives, for which enteric health benefits are claimed, have been developed largely on an empirical basis. Nevertheless, they can be subdivided in a few large classes according to their putative or documented mode of action. Most probiotic feed additives are composed of lactic acid producing live bacterial strains. Inulin, oligofructose and a number of related prebiotics are considered to support the growth of lactic acid producing endogenous microbiota strains. Mannanoligosaccharides additionally interact with the gut mucosal immune system. Medium chain fatty acids act through anti-microbial effects. Butyrate has documented anti-inflammatory activity. Xylanases and glucanases can prevent the viscosity increasing effects of non-starch polysaccharides present in cereals. Crucial to enteric health, however, is the maintenance of oral tolerance. The syndrome described by poultry practioners in the field as “dysbacteriosis” probably is the practical reflection of a breach in oral tolerance. Understanding the mechanisms of oral tolerance by studying the complex interactions between the gut microbiota, the feed and the host mucosa (including its immune system), may be the key to a more rational design of enteric health promoting feed additives.

Keywords: inflammation, feed additives, oral tolerance, microbiota

Introduction

On the feed market for poultry, one can find a wide range of different feed additives for which beneficial effects on intestinal health and performance are claimed. Many of these additives do not always give consistent results when tested under various conditions in feeding trials. The major reason is that many of these products are not just nutrients that are either absorbed or not. In many cases they induce a more or less pronounced change in the equilibrium of the intestinal ecosystem. The complexity of this gut ecosystem is often underestimated. In general there are three players in this system, namely the feed (nutritive as well as indigestible components), the host (mucosa and immune system) and the intestinal microbiota. These three players intimately interact with one another in many different ways. Understanding the gut ecosystem will be crucial for making further progress in poultry nutrition.

Feed additives affecting the intestinal microbiota

Animals as well as humans live in a bacterial world. Microbes from the environment are in permanent contact with the mucosal surfaces of the body, including the gastrointestinal mucosa. Rapidly after hatching, especially the distal segment of this gastrointestinal mucosa (in the case of the chicken the caeca and colon) becomes colonized by the microbes. As a consequence there are more bacterial cells in the indigenous microbiota of the lower gut than the body’s own eukaryotic cells (Blaser and Muser, 2001). The caeca and colon lumen indeed harbours up to 10^{12} bacteria / ml. The microbiota is diverse, with up to 1000 or more different bacterial species (Salzman et al., 2007). These include both resident organisms, which occupy anatomical and physiological niches within the gut, as well as ingested organisms in transit through the intestines.

The intestinal microbiota can be subdivided into auxotrophic, heterotrophic and hypotrophic species. The first group can survive or even multiply on the nutrients present in the intestinal lumen. The second commonly requires certain nutrients derived from living cells of the host, while the third group often can only replicate inside living cells of the host. Fortunately, the vast majority of bacteria in the gut are harmless. Some even have documented beneficial effects on the host. These include a number of bacterial species belonging to the genera *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Bifidobacterium*. These beneficial species commonly are called probiotics (Fuller, 1989). It is generally accepted that the “normal” microbiota fosters growth and development, aids digestion and protects host cells from pathogenic bacteria. The latter phenomenon is known as “colonization resistance” (Stecher and Hardt, 2008). A number of probiotic strains, in particular strains known to produce lactic acid, have been developed for use in poultry. These strains are expected to support the endogenous beneficial microbiota components. Also dietary mannanoligosaccharides, supplemented at high concentrations (2.5%) have been shown to increase the *Bifidobacterium* population in the chicken gut (Fernandez et al., 2002). Some lactobacilli and bifidobacteria have been shown to possess the ability of suppressing the growth of enteric pathogens including *Salmonella* (Hütt et al., 2006). Feed additives, indigestible for the host, but supporting the growth of beneficial gut microbiota components, by definition are prebiotics. Inulin and oligofructose e.g. have well documented prebiotic effects in various animal models. In broilers, supplementation of inulin or oligofructose has been shown to improve weight gain and feed conversion (Yusrizal and Chen, 2003).

Lu et al. (2003) analyzed the composition of the gut microbiota of chickens using random sequencing. They found that in the broiler ileum, nearly 70% of sequences were from *Lactobacillus*, 11% were from *Clostridium*, 6.5% from *Streptococcus* and 6.5% from *Enterococcus*. In the broiler caeca however, 65% were Clostridiaceae-related sequences. Similarly in the human large intestine, a large proportion of the microbiota consists of *Clostridium*-related anaerobic bacteria (Flint et al., 2007). These to a large extent belong to Clostridial cluster IV and Clostridial cluster XIVa, many of which are butyrate producers (Louis and Flint, in press).

The intestinal microbiota can be considered as a postnatally acquired organ composed of a large diversity of bacterial cells that can perform different functions for the host. This organ is highly exposed to environmental influences and thus modulated in its composition and functions by external factors, such as nutrition (Schiffrin and Blum, 2002). The traditional "in feed growth promoting antibiotics" generally were considered to exert their effects by suppressing components of the gut microbiota that may be disadvantageous to the host, although Niewold (2007) suggested that they might also have an anti-inflammatory effect. It is therefore not surprising and probably fortunate that the few Clostridiaceae belonging to clusters IV and XIV tested so far, are intrinsically resistant to a wide range of antibiotics used in the past as growth promoters in Europe (Eeckhaut et al., unpublished results).

Some of the newer feed additives, commonly not considered under the heading of traditional antibiotics, including the medium chain fatty acids (MCFA), also have fairly powerful anti-microbial effects at concentrations used in the feed, affecting the growth and the fenotype of certain pathogens like *Salmonella* (Van Immerseel et al., 2006).

Protection against pathogens versus oral tolerance

Many pathogens infect their hosts via the mucosal surfaces of the gastrointestinal tract. These mucosal surfaces are protected against infection by several mechanical and immunological barriers, including the colonization resistance afforded by the microbiota (see above). The intestinal immune system has the difficult and apparently conflicting task of mounting protective immune responses against harmful intestinal pathogens while preventing excessive responses to harmless microbiota and nutrients. In contrast with the classical immune response which is induced by e.g. administration by the parenteral route, administration of an antigen by the oral route induces hyporesponsiveness to subsequent challenge with the same antigen given in an immunogenic form. This phenomenon is known as oral tolerance (Faria and Weiner, 2005). Under the conditions of normal oral tolerance, potential immunogenic substances in the gut lumen are continuously sampled by the gut mucosal immune system through the villous epithelium. Two mechanisms then contribute to the induction of oral tolerance. First of all, a specific subset of T-lymphocytes, the regulatory T-lymphocytes, mediates active immune suppression following repeated contact with low doses of the substance (Hanson and Miller, 1982). Second, T cell hyporesponsiveness is induced through anergy and/or deletion of antigen specific T cells after feeding high doses of antigen (Friedman and Weiner, 1994). Recent studies suggest that these two phenomena might be closely related to one another, with regulatory T cells playing an important part in both (Von Boehmer, 2005). Some feed additives, including mannanoligosaccharides (MOS), have been reported to influence the intestinal immune system. Their mode of action in many cases is, however, partly unknown.

The constant exposure to intestinal microbiota seems to play an important part in creating a unique microenvironment in the gut associated lymphoid tissue (GALT) that is prone to regulatory T cell differentiation (Dubois et al., 2005). In support of this statement, it has been shown that oral tolerance can be restored in germ-free mice following administration of gut microbiota including *Bifidobacterium infantis* (Sudo et al., 1997). Both gram-positive and gram-negative bacteria seem to contribute to the oral tolerance phenomenon. With respect to gram-negative bacteria, there are indications that the lipopolysaccharide might be involved (Dubois et al., 2005). Under conditions of normal gut homeostasis, the lipopolysaccharide is in contact only with the apical side of the epithelial cells, where there is little lipopolysaccharide sensing through TLR4 / MD-2 (Abreu et al., 2003). When there is a breach in the gut mucosal barrier, e.g. caused by pathogenic micro-organisms, the lipopolysaccharide can reach the basolateral side of the epithelial cells, where interaction with TLR4 can activate the inflammatory cascade through activation of NF-kappaB. A normal gut mucosal barrier requires the presence of well differentiated epithelial cells connected to one another with fully developed tight junctions and an active expression of multidrug resistance (MDR1) gene at the apical plasma membrane. It has indeed been shown that deficient expression of MDR1 in the presence of microbiota leads to spontaneous colitis in a mouse model (Ho et al., 2005).

Feed additives affecting the anti-inflammatory effects of the gut microbiota

The gut is continuously exposed to a tremendous amount of dietary and environmental antigens (Dubois et al., 2005). Disturbances of oral tolerance can lead to inflammatory pathologies such as Crohn's disease in humans (Garside et al., 1999). Deficiencies in the oral tolerance mechanism also may lead to allergic inflammatory responses to food proteins in humans (Strobel and Mowat, 2006).

Under normal conditions of gut homeostasis, inflammation is prevented a.o. through inhibitory signalling by commensal bacteria belonging to the normal microbiota (Strobel and Mowat, 2006). There are two documented pathways of anti-inflammatory signalling. One is through inhibition of the nuclear histone deacetylase activity and as a consequence suppression of NF-kappaB activation in the host intestinal mucosal cells (Place et al., 2005). The other may be through stimulation of Nod2(CARD 15), leading to increased expression of IL-10 (Hedl et al., 2007).

Several histone deacetylase inhibitors can be present in the diet (Dashwood and Ho, 2007). Amongst these, butyrate is the most common inhibitor, which is naturally produced in the lower intestinal tract. It is commonly used as a feed additive in poultry. Butyrate is the preferred source of energy for the epithelial cells of the lower intestinal tract, affecting cell proliferation, differentiation and apoptosis (Dalmasso 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; Schauber et al., 2003). Finally, butyrate decreases intestinal epithelial permeability by increasing the expression of tight junction proteins (Peng et al., 2007).

Inflammation in the chicken gut

The indigestible non-starch polysaccharide fraction, present in various cereals, is known to negatively affect performance in broilers (Daniche et al., 1999). This is explained usually by an increase in viscosity of the intestinal contents. It is unclear however why increased viscosity of intestinal contents should have such negative effect on the host. In order to better understand the mechanisms underlying the observed effects on performance, we set up a study comparing broilers on a wheat/rye based diet with broilers on a corn based diet. We investigated the effects on gut wall functional morphology and gut microbiota composition. Both diets were formulated to fulfil the nutrient requirements (energy, protein, essential amino acids etc). As expected, birds on a wheat/rye had poorly digested viscous lower intestinal contents. Especially in the starter period, it was found that the diet had a profound effect on the morphology of the gut wall, particularly at the level of the jejunum. The wheat/rye based diet resulted in fusion of villi, thinning of the tunica muscularis, increased numbers of apoptotic epithelial cells at the villous tips and in the crypts and, most remarkably, massive infiltration of T lymphocytes in the propria mucosae (Teirlynck et al., 2008). Also throughout the rearing period, there was a gradual increase in the number of goblet cells. Using T-RFLP, a shift in the composition of the intestinal microbiota at the level of the caeca was found. Using immunohistochemistry, we were able to show that in the mucosal regions infiltrated with T-lymphocytes, there was a reduced expression of MDR-1 in the apical plasma membrane of the enterocytes. Thus indigestible non-starch polysaccharides induce a shift in the intestinal microbiota, which in turn appears to lead to a breach in the oral tolerance and consequent inflammation. In a recent follow-up experiment we also showed that this inflammatory condition renders the birds more susceptible to *Salmonella*.

It is well known that feed additives based on xylanases and glucanases can help to overcome the adverse effects of non-starch polysaccharides (Choct, 2006). In line with this, we recently showed that a feed additive based on partially predigested non-starch polysaccharides can be beneficial to help protect the birds against *Salmonella* (Eeckhaut et al., 2008a).

Conclusion

In the past various feed additives have been developed for which a beneficial effect on intestinal health in poultry is claimed. Unfortunately, it has been enigmatic for a long time, how intestinal health should be defined, let alone that the exact mode of action of these feed additives would be known. It is becoming clear nowadays that intestinal health to a large extent depends on optimal functioning of the oral tolerance mechanism.

Oral tolerance is an active state of suppression of immune and inflammatory response against normal components in the intestinal lumen, including feed components as well as microbiota components. Oral tolerance appears to be essential also in the chicken. A profound understanding of oral tolerance mechanisms will be required for further progress to be made in poultry nutrition. The role of butyrate producing microbiota components belonging to Clostridial clusters IV and XIV in poultry merits to be investigated, since butyrate may play an important role in maintaining oral tolerance. Recently, we have been successful in isolating and characterizing the first such strain from poultry (Eeckhaut et al., 2008b).

Acknowledgements

The authors would like to thank the Contract Research Office of the Belgian Ministry of Public Health, Food Safety and the Environment for financial support.

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