# Nutritional tools to control gut health.

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## Introduction:

Gut health has become a major issue in livestock production in general and in broiler and pig production in particular, especially following the ban on antimicrobial growth promoters (2006) and efforts to reduce therapeutic antibiotic use in Europe. Also in the U.S. poultry producers are currently making a major shift towards 'zero antibiotic' production under pressure from major retailers (Newman L., pers. commun.). In experimental feeding trials comparing the same feed formula with and without antimicrobial growth promoter supplements, it has been clearly demonstrated that antibiotics alter the microbiota composition in the lower intestinal tract, even at concentrations which, in vitro, are considered to be far below the minimal inhibitory concentrations for most culturable intestinal microorganisms (Teirlynck et al., 2009). These data support the common belief that, in the past, the routine use of antimicrobial growth promoters in feed has masked intestinal health problems in animals which are selected for rapid growth (Onrust et al., 2015).

The intestinal health issues associated with high feed intake, rapid weight gain and the ban of antibiotics from the feed, particularly in broilers and in pigs, are grouped under the common denominator of 'dysbiosis' or 'dysbacteriosis'. Dysbiosis can be defined as a nutritionally induced shift in the microbiota composition in the lower intestinal tract, resulting in inflammation. The inflammation in the intestinal wall compromises nutrient absorption and leads to leakage of body fluids into the intestinal lumen, which ultimately results in increased moisture content of the faeces. In broilers, the phenomenon may become manifest from 2 to 3 weeks of age onwards, as from this age on, the animals tend to ingest large amounts of feed. With thousands of animals in a chicken house and limited available floor space, this may lead to serious problems of wet litter, soiling of the feathers and foot pad lesions (hyperkeratosis and bumble foot). The latter are considered as important welfare problems.

## Beneficial microbes and their metabolites:

It is commonly accepted that dysbacteriosis has a negative effect on weight gain and feed conversion in young animals. This implies that different groups of microorganisms in the intestinal tract may differentially affect intestinal health. Following-up on this, attempts have been made to identify and characterize performance-related gut microbiotas in broilers across feeding trials (Torok et al., 2011). Such studies inevitably go back to the ancient concept that certain species, genera, families or even complex consortia of microorganisms would be critically important to protect the intestinal tract from inflammation and also from colonization by specific pathogens such as *Salmonella*. The concept was originally launched by Metchnikov in the early twentieth century and further developed by Nurmi in the nineteen

seventies (Nurmi and Rantala, 1973). In the early days the focus was on lactobacilli and bifidobacteria, since these were the organisms used for fermentative preservation of different foods and feeds. Various health promoting effects were conferred on them. Numerous strains are on the market as probiotics. Lactobacilli are naturally present in the lower intestinal tract of animals and man in relatively high numbers, to the point that they form the dominant population in the ileum (Lan et al., 2005). Attempts to try and identify a mode of action explaining the putative beneficial effects of these microbes have focused mostly on the antibacterial activity of the bacteriocins which they produce. However, the main metabolite produced by both lactobacilli and bifidobacteria is lactate. Lactate can be used as an energy source by Firmicutes belonging to the Clostridium cluster XIVa, as well as by sulfate reducing bacteria such as *Desulfobrio piger*. Bacteria produce both the D and the L isomer of lactate. While L-lactate can be used as energy source by various tissues of the host after absorption, D-lactate cannot be used by the host tissues. Thus it remains enigmatic to some extent whether or not, and how, lactobacilli and bifidobacteria might be beneficial for intestinal health.

Other intestinal bacteria that have received particular attention as potential candidates for protection of intestinal health are *Bacillus* spp., including a.o. *Bacillus subtilis* and *Bacillus amyloliquefaciens*. These bacteria are notorious for their production of various antibacterial substances (including lipopeptides) that can kill pathogenic microorganisms. Numerous bacillus strains have been developed and are commercially available as probiotics.

In order to make further progress in our understanding of dysbiosis and of the protective role of beneficial microbes in the intestinal tract, more metagenomics studies, taking into account the entire microbiome and analyzing the shifts in the microbiome, like the one of Torok et al. (2011) are needed. It can be speculated that microbes affect the inflammatory status of the host intestinal mucosa, probably mainly through the release of specific end products of their metabolism or certain cell fragments that become available after the death of the bacterial cells. Indeed, taking into account the enormous numbers of bacteria present ($10^{11}$ per gram) and their very fast replication rate, dying off of bacteria must be a very important phenomenon. One of the important compounds that are released following death of gram negative bacteria, is the lipopolysaccharide (LPS) of the outer cell membrane. LPS is a very stable molecule and one of the most powerful triggers of inflammation through binding on TLR-4.

Quantitatively important end products of bacterial metabolism other than lactate include acetate, propionate, butyrate, methane and hydrogen sulfide. In low amounts, hydrogen sulfide is considered as a beneficial signaling molecule with an important physiological role in the intestine (Kimura, 2014). It has been suggested, however, that high concentrations of hydrogen sulfide may be an important primary cause of oxidative stress (Stroot, 2014). Oxidative stress in turn is a powerful trigger of inflammation. This suggests that excessive bloom of hydrogen sulfide producing microorganisms, such as Desulfovibrio spp. or Enterobacteriaceae, might trigger inflammation. Acetate can be used as a substrate by numerous (beneficial as well as harmful) bacteria, and also by the host tissues. Thus it could be considered more of an intermediate metabolite for the microbiome. However, it has been shown that acetate does trigger the expression of virulence genes in *Salmonella* Enteritidis, leading to more invasiveness and more severe infection (Gantois et al., 2006; Van Immerseel et al., 2003). This suggests that excessive accumulation of acetate in the intestinal lumen may

favor inflammation. Methane is the major metabolite produced by Archaea. These archaea indeed derive their metabolic energy from reducing methanol into methane, using hydrogen formate as electron donor (Carbonero et al., 2012). Methane has documented anti-inflammatory effects at low physiological concentrations, to the point that it can protect from ischemia-reperfusion injury (Boros et al., 2012). Nevertheless, excessive methane production in the intestinal tract has been associated with a range of intestinal pathologies, including IBS associated constipation in humans (Jahng et al., 2012). Propionate and especially butyrate have documented beneficial effects on the intestinal mucosa, protecting against intestinal inflammation. For an overview of the various beneficial effects of butyrate in the intestine and beyond, we refer to the review by Guilloteau et al. (2010).

## Nutritional tools:

In livestock production, steering the intestinal microbiota for the benefit of intestinal health through feed formulation is the ambition of numerous research groups. In order to do so, it is critically important to know which are the beneficial microbes and how these microbes protect the intestine from inflammation.

Both in human and in animal nutrition, it has become clear that diet in all its aspects can heavily influence the microbiota composition in the lower intestinal tract (Walker et al., 2011).

In humans, it has been suggested that long-term dietary patterns lead to specific patterns of the microbiome, called enterotypes (Wu et al., 2011). Diet is considered also as a key factor in the pathogenesis of inflammatory bowel disease (IBD) in humans, although published data on the association between specific foods and the development of the disease(s) show inconsistent results (Serban, 2015). As IBD is associated with reduced abundance of butyrate producing Firmicutes (Frank et al., 2007), it has been suggested that foods favoring the development of butyrate-producing Firmicutes could have a protective role in IBD (Li et al., 2015). A high meat-containing diet has been associated with excessive hydrogen sulfide production in the colon, due to increased activity of sulfate reducing bacteria (Serban, 2014). At these high concentrations, hydrogen sulfide is toxic for the epithelial cells and inhibits butyrate production. However, studies associating specific foods to specific aspects of intestinal health are difficult to perform. Indeed, observational studies comparing cohorts of people often are hampered by confounding factors, while experimental studies with human volunteers suffer from problems such as differences in genetic background and variable adherence of individuals to their diet.

In animals, little information is available on the role of the feed formula in intestinal health. A number of studies have tried to stimulate butyrate production in the lower intestinal tract through feed formulation (for review see Onrust et al., 2015). Also studies are ongoing in pigs to identify specific feed ingredients that may affect intestinal health. We recently set up a number of experimental feeding trials in broilers. Advantages of the broiler model are the homogenous genetic background and the inevitable perfect adherence of the animals to the diet. In an initial approach, we compared two feed formulas with wheat and some rye as major energy sources, and 2 different protein sources (one easily digestible and another slow digestible). We showed that this relatively minor difference in protein source led to a shift in

the metabolic pathways used by the caecal microbiota, with a relative reduction in the butyrate production pathway and an increase in the hydrogen sulfide production pathway. Next, we showed that adding low amounts of xylo-oligosaccharides to the diet (0.2% in the starter and 0.5% in the grower) could revert the harmful effects of the above mentioned feed formula on performance. This beneficial effect was associated with an increase in Lactobacillaceae in the colon and an increase in *Clostridium* cluster XIVa in the caeca. In each of these groups the change in abundance could be attributed to a significant change in just one species, namely *Lactobacillus crispatus* and *Anaerostipes butyraticus*. In vitro culture and coculture of these microorganisms showed that *Lactobacillus crispatus* used the xylo-oligosaccharides to produce lactate and *Anaerostipes butyraticus* used the lactate to produce butyrate, thus demonstrating the cross-feeding of two species (De Maesschalck et al., 2015). This phenomenon may explain at least in part the beneficial effects of the xylo-oligosaccharides in this model. Furthermore, it confirms the important role of butyrate in intestinal health These results show that specific feed ingredients and feed additives can specifically modify the intestinal microbiota and, by doing so, influence intestinal health.

## Conclusion:

Modern intensive livestock production is characterized by large homogeneous groups of animals fed a nutrient dense diet in order to achieve optimal performance. Genetic selection has focused on high feed intake, thus putting pressure on the function of the digestive system. While in the past, microorganisms in the gastro-intestinal tract were largely considered as a burden, and in feed antibiotics were used widely to suppress microbial growth in the intestine, it is now generally accepted that the microbiome which colonizes the intestinal tract is of critical importance for the health and welfare of the animals. Health and welfare go hand in hand with growth and performance. In order to achieve optimal (intestinal) health and performance, we need to learn how to feed the microbes so as to achieve a better symbiosis.

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