

Microfloral Rehabilitation: Normalisation of Gut Function



Intestinal Health

Gut health and its management is an intricate and complex area governed by numerous factors, including nutrition, microbiology, immunology and physiology. When gastrointestinal health is compromised, nutrient digestion and absorption are affected, feed conversion becomes reduced, susceptibility to disease is heightened and, ultimately, these issues result in a negative economic impact.

The comprehensive community of microorganisms in the gut is referred to as the microbiome and is recognised as a very diverse community of bacteria, fungi, protozoa and viruses. Its diversity varies along the different regions of the gastrointestinal (GI) tract, with some regions having less tolerable conditions and therefore containing reduced microbial diversity in comparison to regions more favourable to microbial growth. The gastrointestinal microbiome plays a pivotal role in nutritional, physiological and immune functions. Poor intestinal health is associated with increased pathogen colonisation and susceptibility to infectious disease, which can ultimately lead to poor weight gain and increased mortalities.

In general, the development of the adult gut microbiota begins on hatching or on birth, whereby microbes are picked up predominantly from the post-hatch/delivery environment and from feed or drinking water. From a management point of view, humans can also be a source of microbes, and care needs to be taken not to inadvertently introduce pathogenic species. The time it takes for the establishment of the stable adult microbiota will depend on many factors, including the size of species, type of feeding regime and management practices in place.

Within the GI tract, there are multiple interactions between the host, intestinal environment and microbial cells, in addition to feed components. These interactions underline the critical role of the microbiota in the health and wellbeing of the host, although the exact way in which this is achieved is not yet fully understood.

The diversity within the microbiome plays a critical role in gut health, with beneficial microbes forming a protective barrier lining the gut that prevents the growth of pathogenic bacteria such as *Salmonella*, *Campylobacter*, *Clostridia* and *Escherichia*, amongst others. There are numerous theories on how the beneficial microbes prevent pathogen colonisation. Some theories suggest that potential attachment sites on the gut cells become occupied, thereby reducing the opportunity for attachment and colonisation by pathogens. Another proposed mechanism is that the intestinal microbiota secrete compounds such as volatile fatty acids (VFAs), organic acids and natural antimicrobials that either inhibit the growth of or make the environment unsuitable for less favourable bacteria.

Studies using germ-free animals have further demonstrated that the intestinal microbiome is also critical to the stimulation, development and maturation of the

immune system. In addition, the intestinal microbiome can influence host growth rates by producing extra nutrients through the breakdown of less digestible nutrients.

Profiling and understanding the role of intestinal microbial communities is important for the development and understanding of new and existing feed additives, thus allowing the manipulation of diets to improve performance, health and welfare. Dietary supplements, which focus on rehabilitating or repairing the gut microfloral diversity in order to aid intestinal health and decrease the animal's susceptibility to disease, can be broadly classed as either prebiotics or probiotics. Through the use of techniques based on molecular sequencing technologies, difficulties associated with cultivating intestinal bacteria have been overcome, providing detailed insights into the malleable nature of the microbiome. Over the last number of years, a focus has been placed on identifying the population-modulating effects of dietary supplementation with yeast cell wall-based prebiotics (such as mannose rich fractions (MRF)). In particular, the role of overall microbial diversity in influencing health and performance has come under increased scrutiny. Ultimately, the goal with nutritional intervention is to normalise gut function through a process of microbial repair and rehabilitation.

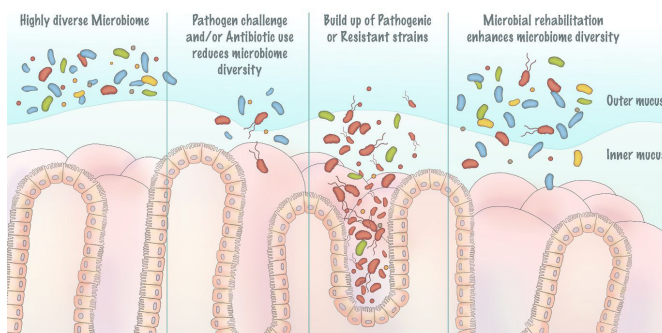


Figure 1. Microbial diversity: the key to gut health

Rehabilitation of the Microbiome

From a nutritional standpoint, there are many feed supplements focused on stabilising the gut microflora to aid intestinal health and decrease the animal's susceptibility to disease. These include products that are designed to regulate and support the gut environment and its microflora:

- Coccidial vaccines
- Probiotics
- Competitive exclusion products
- Feed enzymes
- Functional nutrients, such as nucleotides
- Organic acids and feed hygiene products
- Organic minerals
- Plant-based products, such as herbs, spices and essential oils
- Prebiotics, such as yeast MRF

Of the functional ingredients currently in use for microbial control, MRFs isolated from the yeast cell wall (MRFs) are widely used in animal nutrition and have been

shown to improve animal performance in a manner similar to antibiotic-like growth promoters, suggesting they are a viable non-antibiotic alternative. MRF products have been commercially available since the early 1990s. Since 1999, the use of MRF in animal feed has become more prominent, mainly due to the European ban on prophylactic antibiotic growth promoters in animal feed.

Most MRF products are derived from the cell wall of the yeast *Saccharomyces cerevisiae*. Given their ability to bind and limit the colonisation of gut pathogens, MRF have proven to be an effective solution for antibiotic-free diets as well as providing support for immunity and digestion. The effects of MRF supplementation on health and performance have been studied comprehensively, and they have proven effective at improving weight gain and feed conversion efficiencies while also protecting against pathogen colonisation of the gut.

While the effects of MRF supplementation on bird health and performance have been studied comprehensively, newer studies have focused on the effects on the overall bacterial community of the poultry gut – not just specific bacteria – and such work has shown that supplementation with MRF can significantly alter the intestinal microflora, the so-called microbiome (Corrigan *et al.*, 2015). This analysis, which was carried out using so-called deep-sequencing technology, allows for the effects of MRFs in modulating the microbial population at varying phylogenetic levels to be studied. One specific study (Corrigan and Murphy, 2016b) focused on a number of commercial broiler trials and identified consistent alterations in the cecal microbiota of broilers, demonstrating increases in the phylum *Bacteroidetes* and decreases in the phylum *Firmicutes* (Figure 2).

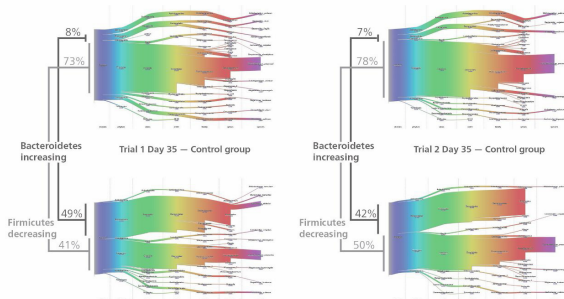


Figure 2. Consistent alterations in broiler cecal diversity following supplementation with MRF (Corrigan *et al.*, 2016b)

Of particular interest in this study was the noted effect of supplementation on the overall microbial diversity of the cecal microbiota, with a significant increase in overall phylum-level diversity being consistently noted in poultry receiving MRF in the diet. Such increases in microbial diversity are documented in the literature as being associated with an increased resistance to pathogen colonisation. On further examination, the studies also showed a consistent reduction in the overall prevalence of a specific phylum known as the Proteobacteria, which includes such well-known pathogens as *Escherichia* and *Salmonella*.

Additional analysis and studies have focussed on the microbiome-potentiating effects of MRFs to reduce the prevalence of a pathogen with particular resonance for consumer health: *Campylobacter*. A recent research trial demonstrated the additional benefits accruing from enhancing microbial diversity and the potential for reducing the *Campylobacter* load in the cecum (Figure 3, Corrigan and Murphy, 2016).

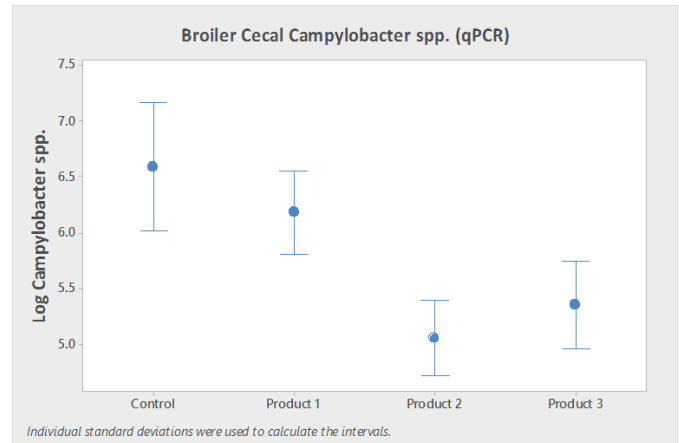


Figure 3. Modulation of *Campylobacter* colonisation by MRF

In essence, the use of MRF aids in the repair and rehabilitation of the gut microflora by increasing the overall diversity, reducing pathogen load and enhancing the gut's resistance to pathogen colonisation. These studies are the first of their kind and represent a breakthrough in our understanding of how nutrition is intricately linked with the overall health and welfare of poultry. Considering how human health is intimately linked with the food produce we consume, one can also appreciate the additional benefits accruing from pathogen control in poultry production.

Anti-microbial Resistance

Antibiotic resistance has the potential to become one of the greatest problems of our generation, given the ever-increasing rise in bacterial strains that are less and less sensitive to existing treatments. Currently, there is political pressure worldwide to restrict the use of antibiotics in animals to therapeutic use only, following the 2006 European Union ban on the use of antibiotics for all non-therapeutic use, such as antibiotic growth promoters (AGPs). The public is also aware of the increasing problem of antibiotic resistance, and this has led to heightened awareness regarding bacterial prevalence in meat products.

The statistics behind the rise in resistance are startling, with almost 23,000 people dying in the US each year due to infections caused by antibiotic-resistant bacteria. Even more alarming is that since 1998, the US Food and Drug Administration (FDA) has only approved two new classes of antibiotics, with the vast majority of today's antibiotics developed before the 1970s.

Antimicrobial resistance arising in agriculture can negatively impact public health. Treatment of animals with antimicrobials important in human medicine or drugs of the same family or class can select for drug resistance in zoonotic pathogens (e.g., *Salmonella*, *Campylobacter*) that can be transmitted to humans through direct contact or indirectly through food or water. Resistant bacteria from animals or plants are part of a larger antimicrobial-resistant ecosystem, and their resistant genes could find their way through a variety of poorly understood, indirect pathways to human pathogens.

Bacteria from animals can be spread to food products during slaughter and processing, and this spread has been extensively documented for conventional foodborne pathogens such as *Salmonella*, *Campylobacter* and *E. coli*. More recent studies have indicated the emergence of enterococci resistant to antimicrobials, with direct transmission of resistant enterococci between animals and farm workers also being identified. More importantly, these studies have found identical or closely related subtypes in animals, food and humans, supporting the hypothesis that

the foodborne route of antibiotic resistance transmission is of significance.

An increase in food safety concerns resulting from extensive antibiotic use has seen the poultry and livestock industries challenged in recent years, as meat free from antibiotics and disease has become a requirement within the European Union. Consumer demand for antibiotic-free meat has also increased within the US and other antibiotic-using countries as a result of concerns about the rise in antibiotic resistance, making it necessary for producers to find suitable replacements for antibiotic growth promoters. Globally, it is recognised that there is no so-called “silver bullet” to replace antibiotic use in animal production, and producers will also have to focus on incremental improvements in hygiene and husbandry to address the issue.

One drawback of the use of antibiotics is their non-specific effects on the gut microbiome and the reduction in overall gut microfloral diversity (Vrieze *et al.*, 2014). Without intervention, the use of antibiotics can lead to a vicious cycle in which their use can reduce overall microfloral diversity and select for the expansion of resistant species to the detriment of non-resistant commensal strains. This reduced diversity allows for the continued proliferation of resistant species and without intervention can have negative impacts on health and performance. One strategy to combat the negative consequences of antibiotic use is to repair and rehabilitate the microfloral diversity after their administration. Given the ability of MRF to enhance the overall gut microfloral diversity, it is an ideal solution to combat the negative consequences of diversity reduction. While there is still much to be learned – in particular, the mode of action – in the long term, such findings will have significant practical value in commercial production settings and will also have beneficial impacts on consumer health and wellbeing.

The Microbiome and Metabolism

Profiling microbial diversity within the gut is a key tool for studies trying to understand these microbial communities, but it does not provide direct evidence of the microbiome’s functional capabilities. The development of advanced computational approaches in the last couple of years has begun to allow us to predict what the effect of altering the bacterial community structure is on the overall function of the gut. This approach was recently used (Corrigan *et al.*, 2015), and the study highlighted that numerous biological metabolic pathways were found to be affected as a result of supplementation with MRF (Figure 4). Some of the

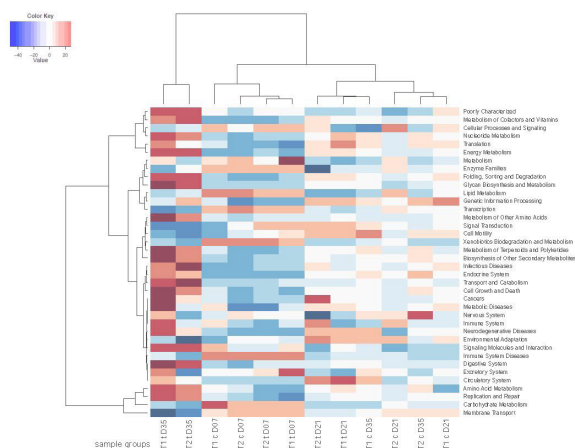


Figure 4. Heat map reflecting hierarchical clustering of samples classified into clusters of orthologous groups of proteins metabolic pathways (Corrigan *et al.*, 2015)

pathways that were predicted to be enriched as a result of MRF supplementation included those associated with energy metabolism and were attributed to the observed increases in the *Bacteroidetes* group.

In a general sense, we can now begin to attribute cause and effect when considering the way in which nutrients affect changes in the gut microflora, which are ultimately responsible for digestion and metabolite production.

Summary

This work provides new insights into the specific and reproducible effects of MRF supplementation on the composition of the bacterial community as a whole, rather than looking at specific beneficial or detrimental bacterial species. By making changes in the overall microbial diversity within the gut, we can aim to repair and rehabilitate the gut microflora, which can lead to reductions in pathogen load, enhance the gut’s resistance to pathogen colonisation and reduce the abundance of antibiotic-resistant strains. Improving the understanding of these comprehensive changes in bacterial community composition and how they might contribute to host health and performance is now key in the poultry industry.

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