Antimicrobial 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. Now, more than ever, alternative strategies are required to further reduce antibiotic use on-farm and to restrict antibiotic use to therapeutic uses 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 of the prevalence of bacteria in meat products; retailers are also putting more pressure on their suppliers to change their management practices to find ways to limit the use of antibiotics on-farm, with many retailers including antibiotic usage data as a key metric on audits.

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, meaning that the vast majority of today's antibiotics were developed before the 1970s. The story continues in the EU, with estimates showing that close to one in five infections are due to antibiotic-resistant bacteria, and direct treatment costs and the problems associated with these bacteria cost the healthcare systems of these countries 1.1 billion Euros annually.

Antimicrobial resistance arising in agriculture can negatively impact public health. Treating animals with antimicrobials that are important in human medicine or drugs of the same family or class can select for drug resistance in zoonotic pathogens (e.g., *Salmonella, Campy-lobacter*), which 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 these resistant genes could find their way through a variety of poorly understood, indirect pathways to huma n 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 that are resistant to antimicrobials, with the direct transmission of resistant enterococci between animals and farm workers also being identified. More importantly, these studies have found identical or closely related subtypes of enterococci in animals, food and humans, supporting the hypothesis that the foodborne route of antibiotic resistance transmission is significant.

An increase in food safety concerns resulting from extensive antibiotic use has been a challenge for the poultry and livestock industries in recent years, as meat being 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 this issue. Since the European ban on the use of prophylactic antimicrobial growth promoters in animal feed, the use of MOS has become more prominent. With its ability to bind and limit the colonisation of gut pathogens, MOS has proven to be an effective solution for antibiotic-free diets, as well as providing support for immunity and digestion. Further refinements of yeast MOS have led to the isolation of a mannose-rich fraction (MRF) with enhanced benefits for intestinal health.

One drawback to the use of antibiotics is their non-specific effects on the gut microbiome and the reduction in overall gut microfloral diversity that they can cause (Vrieze *et al.*, 2014). Without intervention, the use of antibiotics can lead to a vicious cycle in which their use reduces the overall microfloral diversity and selects 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 a negative impact on health and performance.

One strategy to combatting the negative consequences of antibiotic use is to repair and rehabilitate the microfloral diversity after their administration. Multiple studies looking at the means of promoting microbiota diversity showed that MRF can alter the composition of the bacterial community. Corrigan *et al.* (2015) found that with MRF supplementation, the microbiota shifted, with *Bacteroidetes* replacing *Firmicutes*, although *Firmicutes*, *Bacteroidetes*, *Proteo-bacteria, Actinobacteria* and *Tenericutes* remained the predominant phyla. The bacterial species and the overall interaction with the internal GI (gastrointestinal) structure play an important role in the physiological capabilities of the GI tract. In poultry, the fermentation of undigested feed occurs in the caeca, with the microbial population contributing to the functionality of the fermentation process. As such, alterations of the microbiota through supplementation with MRF are also likely to alter the functional capability of the caeca and have been shown to improve performance.

Given the ability of MRF to enhance the overall gut micro-floral diversity, it was believed to be an ideal solution to combat the negative consequences of diversity reduction and to break the cycle of resistant strain expansion whilst helping producers move toward antibiotic-free production systems. Further investigation into the alteration of microbial populations in the gut identified that MRF could not only alter the bacterial profiles but could also reduce the growth rate of antibiotic-resistant strains. Smith *et al.* (2017) looked at the influence of MRF on *E. coli* and *Salmonella* carrying plasmids that conferred antibiotic resistance. Enterobacteria, when grown in the presence of MRF, showed a reduction in the growth of antibiotic-resistant strains – but another interesting observation was that when enterobacteria were grown in the presence of both MRF and antibiotics, a greater reduction in

the bacterial growth rate was observed than with antibiotics alone (Figure 1). This finding demonstrated that resistant organisms became more susceptible to antibiotics when in the presence of MRF. The possible field applications for this finding are significant. If antibiotics can be administered alongside an MRF programme, then bacterial sensitivity should increase, and this could provide a means of looking at lower doses. Furthermore, as discussed above, one of the major issues with antibiotic usage is that it promotes a reduction of the diversity of the GI tract and allows resistant strains to expand at the expense of commensals. However, due to MRF's ability to promote diversity and improve the sensitivity of bacterial organisms to antibiotics, it will also help prevent the vicious cycle of usage.

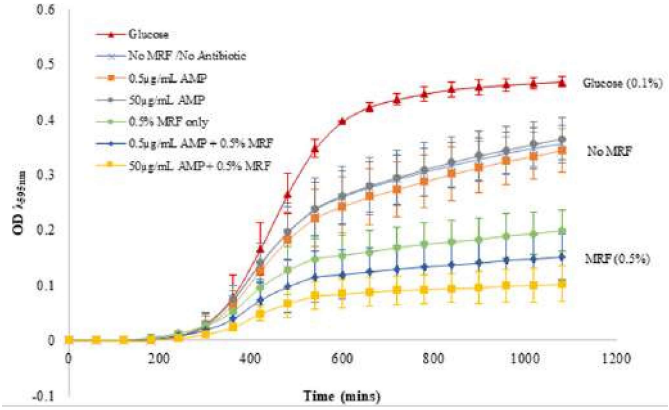

Figure 1

The kind of work described above is a solid starting point for further research, but understanding the underlying mechanisms of antibiotic resistance is crucial if this work is to be applied in a commercial setting. To fully utilise alternative approaches, we must first understand how we can impact the phenotypic antibiotic outcome. Antibiotics, both bactericidal and bacteriostatic, are known to impact cellular energetics, which affect metabolism and energy output, resulting in cell growth reduction or the induction of apoptosis (Smith *et al.*, 2020). It had long been suspected that MRF was likely

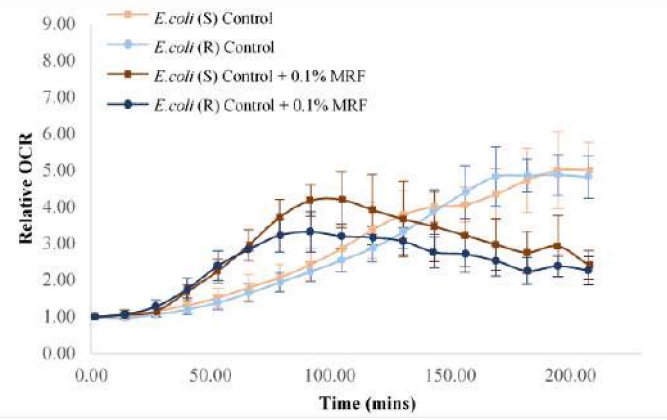

Figure 2

to be impacting cellular energetics while enhancing the susceptibility of bacteria to antibiotics.

Work that was recently published in Nature's Scientific Reports (Smith *et al.*, 2020) shows that MRF from *Saccharomyces* cerevisiae modulates the growth of antibiotic-susceptible and -resistant *E. coli* and potentiates the bactericidal antibiotic efficiency through the modulation of bacterial cellular respiration. Figure 2 shows the impact of MRF on both resistant and sensitive organisms. Effectively, MRF is having a classical prebiotic effect in stimulating the metabolism of cells. This is because they are driven to a higher state of metabolic activity, so the bacterial cells are metabolising faster. As cells respire, however, they are producing a larger number of internal by-products, including toxic ones, and these toxins stress the bacterial cells, leading to an increased sensitivity to antibiotics. Initially, this work has been done on *E. coli*, but as MRF has also been shown to increase the sensitivity of Salmonella to antibiotics, it is likely that its mode of action is similar.

New research such as this has exciting implications for antibiotic usage in commercial settings. The historic work completed by the same group mentioned earlier showed that, through interacting with the microbial community in the gut, dietary MRF can help alter the profile of organisms

and, in doing so, can reduce the prevalence of potentially pathogenic species. When looking at microbial populations *in vivo* taken from poultry caeca, it is shown again that when changing the way that bacteria metabolise or grow through the supplementation of MRF, the diversity of the bacteria alters, too. In commercial settings, this showed as a decrease in resistant enterococci. In addition to this, altering the bacterial metabolism to increase sensitivity to antibiotics is thought to impact their prevalence as well, and research that is soon to be published is expected to show that an increase in sensitivity to antibiotics can decrease the bacterial prevalence.

Information such as this is critical when devising new programmes for producers looking to reduce both their antibiotic usage and the level of antimicrobial resistance on their farms. New classes of antibiotics are notoriously hard to find, with no new drugs being brought to market in recent decades. If new antibiotics are not being found and direct alternatives are also slow to be introduced, then alternative approaches to improve the use of what we already have available become extremely important. If we can change the way we need to use antibiotics as a result of the antibiotics that we do use becoming more effective, then overall, the industry levels of utilisation will drop. This, in turn, will prevent a contribution to the rise in antimicrobial resistance at a bacterial population level and should, therefore, reduce our requirements for usage.

The traditional overuse of antibiotics has led to the widespread evolution of antimicrobial resistance. Antibiotics are crucial for promoting animal welfare when used therapeutically, and consequently, alternative strategies are needed to reduce the antibiotic load on-farm. Antibiotic usage has a non-specific impact on the modulation of cellular proliferation and the metabolism of the gut microbiota, often leading to a significant reduction in both commensal and pathogenic strains. Understanding the molecular epidemiology associated with resistant organisms allows for the modulation of said mechanisms to enhance the susceptibility of all organisms, especially resistant ones, to antimicrobials — without providing any selection pressure that could contribute to the emergence of resistance. Reducing antibiotic use through dosages and by reducing the necessity for treatment may be possible by gaining a better understanding of the mechanisms behind the interaction between MRF and antibiotics. This, in addition to the improvements in animal performance and natural immunity seen as a result of MRF supplementation, provides producers an excellent means of improving both their production targets and their profitability in an easily applicable manner, all whilst reducing their antibiotic usage.

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