# Exploring quorum sensing to optimise gut health and performance

Gut health is one of several factors important to obtaining optimum efficiency and profitability in livestock production and there is increasing evidence of the central role played by gut microbiota in animal health and disease. Many feed additives are therefore designed to specifically support gut health.

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Numerous phytochemicals, supplemented as dried herbs, plant extracts or essential oils, have been described to have favourable effects on myriad parameters, such as digestion, blood pressure, anti-inflammation and hepatic protection. It is therefore a challenge to rationally develop a botanical feed additive mixture: how does one select ingredients from a plethora of plant-derived components, each triggering several physiological responses, with the objective of supporting animal health and performance as much as possible?

## Gut microbiota

Several phytogenic feed additives that aim to support gut health and performance in livestock animals are therefore trying to target the composition and activity of the gut microbiota. This can be explained by the fact that, in recent years, evidence has been accumulating for a pivotal role of the gut microbiota in maintaining the health status of several organs and tissues, including the digestive tract. While in the past, the intestinal microbial composition was considered to mainly reflect the health status of humans and animals, it is becoming ever more clear that this bacterial community can directly and indirectly affect the development and function of several tissues and organs, including the enterocytes, the gut-associated lymphoid tissue, the liver and the brain.

When selecting for ingredients affecting gut bacteria, such as botanical components, many feed additive producers rely on in vitro experiments that demonstrate their bacteriostatic effect. However, the active ingredients of these botanicals will end up in the digestive tract of production animals at concentrations far below the minimal concentration needed to inhibit growth of (pathogenic) bacteria.

It might therefore be a more reliable approach to select feed additive ingredients by focusing on the effects botanical components can have at much lower concentrations, and that are likely to be of relevance for controlling bacterial activity in vivo and improving gut health.

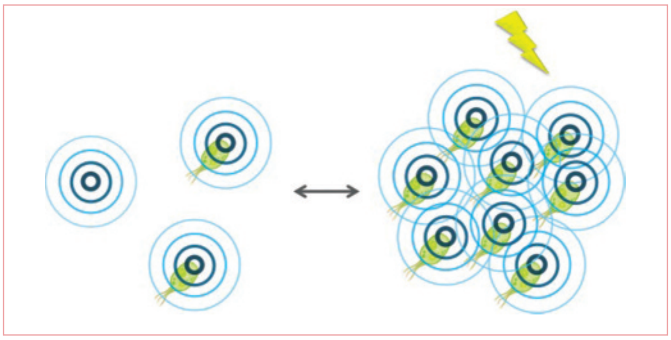

**Fig. 1. Bacteria continuously produce and secrete QS signals in the environment, as depicted by concentric circles around bacteria. If the concentration of a bacterial species is low (as shown on the left), the QS signals will get rapidly diluted in the environment. On the contrary, if the number of bacteria increases (on the right), so will the concentration of QS signal molecules in the environment. If a certain threshold of these molecules is reached, QS signalling will become activated inside the bacteria (shown as a lightning bolt).**

## Quorum sensing

One of the potential mechanisms of botanical feed additives that can be placed in the picture is their effect on quorum sensing (QS).

Bacteria continuously secrete QS signals, which allows them to synchronise their behaviour. More specifically, when the number (the quorum) of a certain bacterial species or group in an environment increases, so will the concentration of their secreted QS signals (Fig. 1).

If a specific threshold of these molecules is reached, it will activate QS-dependent signalling pathways inside the bacteria, resulting in biochemical responses that are often associated with pathogenicity, such as the production of toxins.

As a consequence, compounds able to disrupt QS are being increasingly investigated in human medical

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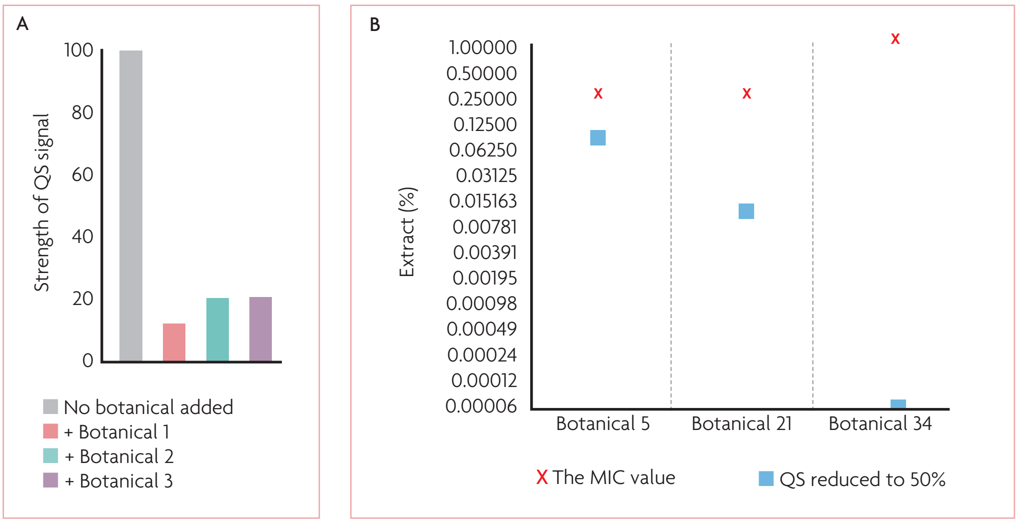
**Fig. 2. Examples of results from a quorum sensing (QS) screening. In a first experiment (A), the effect of a series of extracts from single compounds and mixes were tested for their capacity to inhibit a specific QS signal. In a second experiment (B), (mixes of) selected botanical extracts were further tested in a dilution experiment. The MIC value (red X signs) and the concentration at which the QS is reduced to 50% (blue squares) are given for three test products.**



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research as potential alternatives to antibiotics due to their efficacy at low concentrations and the low chance of bacteria developing resistance against these non-lethal molecules.

Apart from the role of QS signalling in human diseases, knowledge about its relevance for veterinary pathogens, such as Clostridium perfringens, Yersinia pseudotuberculosis and Salmonella enterica, is also expanding. In addition, modulating QS in the gastro-intestinal tract has sparked interest as an approach to control gut microbial activity and composition, thereby influencing animal health and zootechnical performance.

## QS as a tool to select ingredients

We have used QS as a tool to select for highly active bioactive compounds when developing APEX 5, a new feed additive for broilers.

Starting from botanicals and other raw materials for which we already had evidence that they have a positive effect on digestion, anti-oxidation and immunomodulation, we were still left with a long list of potential ingredients to choose from. As we wanted these new additives to have a significant activity on gut microbial activity and composition as well, we relied on QS assays to define the final composition of APEX 5.

In a first step (Fig. 2), we screened botanical ingredients, individually and in mixes, for their capacity to inhibit two types of QS. This was achieved by making extracts of these ingredients, determining their MIC values for the different reporter strains, and adding them to a culture of these strains at concentrations well below their MICs.

Subsequently, the QS-dependent readout of the reporter strain (a fluorescent signal that is produced when QS is active) was compared with the readout of the strain after they had been incubated with the extracts.

In a next step, we selected the most performant extracts and made a dilution series for further testing of QS inhibition at even lower concentrations.

Of note, we observed that the capacity to inhibit QS was not correlated with the MIC values of these substances.

As can be seen in Fig 2B, 'botanical 34' had the highest MIC compared to the other two test compounds, but the lowest concentration at which the QS signal was reduced by half.

Based on these results, we selected a botanical mixture to test it in a simple in vivo model: microscopic roundworms (C. elegans) that were infected with Salmonella typhimurium. It is important to note that we did not do this to evaluate the potential of the botanical prototype to reduce salmonella colonisation in broilers.

Rather, we exploited the knowledge that salmonella is able to colonise the digestive tract of C. elegans, culminating in QS-dependent production of toxins that affect viability of these roundworms.

C. elegans worms were grown on medium, in presence or absence of salmonella, and with or without supplementation of the botanical extract under study (Fig. 3).

We observed that the botanical prototype, when supplemented at low concentrations that did not affect salmonella growth, was able to significantly increase survival of the roundworms.

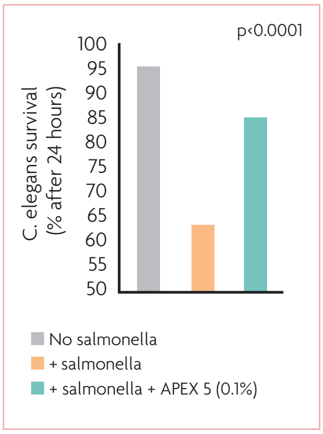


**Fig. 3. C. elegans roundworms were grown in absence (grey) or in presence (orange) of salmonella bacteria. After intestinal colonisation, salmonella will activate QS signals, culminating in the production of toxins, thereby decreasing C. elegans survival. When a botanical extract was added to the infected roundworms at a concentration not affecting salmonella growth, it significantly reduced the salmonella-induced mortality.**

Given that the mortality resulting from salmonella infection is QS-dependent, and that the botanical prototype is capable of inhibiting QS in vitro, it can be hypothesised that this result can be explained by the QS inhibiting effect of the prototype.

## Effect on gut health and performance

Subsequently, field trials were set up to evaluate the effect of supplementing broilers with the botanical prototype.

The microbial composition of birds fed APEX 5 shifted toward a profile that is typically associated with an increased gut health (Fig. 4).

In line with that, their zootechnical performance was better than that of control birds (Fig. 5).

## Conclusion

Intestinal bacteria are of vital importance to the well being and performance of production animals. Several feed additives, including botanical products, have therefore been commercialised to modulate gut microbial bacteria and to support intestinal health.

In vitro tests are valuable tools to develop such botanical products. Apart from screening for bacteriostatic effects, it can be argued that biological activities playing a role at much lower concentrations, such as the modulation of gut bacterial QS, should be considered.

The importance of QS in production animals is currently not fully clear and the potential and consequences of inhibiting QS in the digestive tract remain to be further investigated.

However, it is important that the effects that botanical substances can exert at low concentrations are explored; as such, QS is a potential promising tool to be considered for such investigations.

We have used QS assays as tools to develop a botanical feed additive, which in subsequent analyses has been shown to improve gut health parameters and animal performance. ■

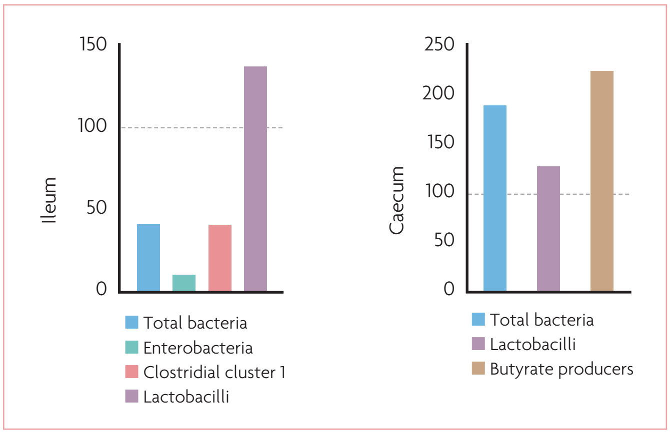


**Fig. 4. Quantification of microbial classes in ileum and caecum of broilers fed APEX 5, relative to their abundance in control birds (set at 100%, dashed line). A low ratio of total bacteria and enterobacteriaceae over lactobacilli in the ileum is generally regarded as reflecting a healthy microbial balance. In the caeca, a rich microbial population, with a substantial population of butyrate-producing bacteria, is seen as beneficial.**

**Fig. 5. Broilers supplemented with APEX 5 outperform birds from the negative control group in average daily weight gain, final weight and FCR, corrected at 1500g (FCR-1500).**