Improving Gut Health Of Production Animals; Looking For Ways To Optimise Functional Feed Additives

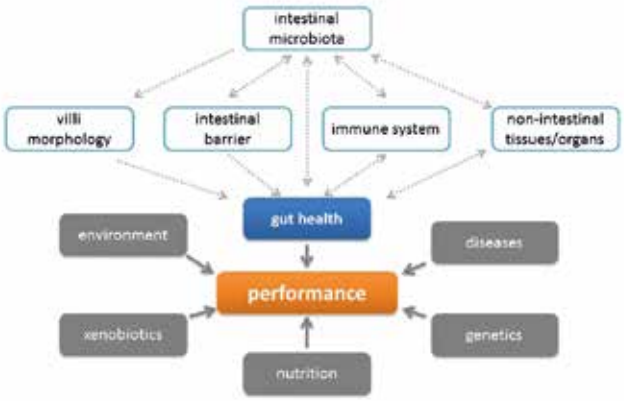
PEER REVIEWED

Gut Health and Feed Additives

One of the most important changes in livestock production over the past two decades has been the recognition of gut health as a key driver of animal performance. This phenomenon has been accelerated by increasing awareness of the impact of good livestock management profitability and the importance on disease prevention, as well as the search for means to reduce the use of antibiotics.

How can the rather general term "gut health" in this context be defined? One could describe it as the intestinal status that ensures the effective digestion and absorption of nutrients, an optimal mucosal development and functioning, the presence of a normal and stable endogenous microbiota, and an effective gut-associated immune status. As such, gut health itself is the result of many physiological, cell biological and microbiological parameters (Figure 1).

Figure 1



It therefore comes as no surprise that many functional feed additives, i.e. components included in feed at low concentrations to trigger responses beyond their nutritional value, have been developed to specifically influence the outcome of these factors in a positive way. Additives that specifically support gut health include, among others, (salts of) organic acids, yeast and yeast cell wall products, botanical components and pre- and probiotics.

The application of many of these additives often originated once their effectiveness in the field was demonstrated empirically, rather than that they were the end result of comprehensive basic research of the potential cell biological or physiological effects of their constituents. In recent years, however, the scientific knowledge on intestinal function and health has increased tremendously, and although much of this information is often too fundamental to be readily translated into the development of novel additives in the short term, recent scientific insights and methods are being used by feed additive researchers in an effort to optimise the efficacy of their products.

In this article, I want to exemplify this for three classes of functional feed additives: butyrate products, yeast cell walls and botanical products.

Butyrate: Where to Deliver in the GIT?

Sodium butyrate is a salt of the short-chain fatty acid butyric acid, which is the end product of fermentation of carbohydrates by anaerobic bacteria in the hindgut. It is a molecule that is well known for its ability to elicit many effects at the cellular or microbiological level in several tissues. For example, it can be used as an energy source by epithelial cells lining the intestinal tract, and it is known to reduce production of pro-inflammatory cytokines, to induce the production of enteric hormones and to strengthen cellular junctions between enterocytes, to name only a few responses. Many of these effects are related to an increased health status of the gastro-intestinal tract (GIT), thereby supporting the use of butyrate as performance-enhancing supplement in the feed of production animals.

Figure 1: Gut health is a key factor in determining the zootechnical performance of livestock animals. Gut health in itself is the result of several physiological, cell biological and microbiological parameters, such as intestinal integrity and the composition of the gut microbiota.

Several different cell types and bacteria that can be found along the entire GIT are responsive to butyrate. Which part of this wide range of butyrate-dependent effects will be triggered, will therefore depend heavily on the enteric location where butyrate is delivered after oral ingestion. For instance, butyrate, when unprotected, will be easily absorbed and metabolised in the first part of the digestive tract, the stomach. The part that is not readily metabolised by the gastric mucosa, will be transported via the porta vena to the liver and subsequently to hepatic veins.

As it can be argued that animals could also benefit from butyrate delivered more directly to the intestinal mucosa (e.g. to bind the luminal receptors of entero endocrine cells, thereby stimulating the release of enteric hormones), feed additive producers have tried to create products in which butyrate is protected from gastric absorption. To do so, they typically relied on one of two approaches: the coating of butyrate and the use of

butyrate derivatives such as butyrins.

Coated products are typically composed of beads containing butyrate that is embedded in a protective matrix of vegetable fat. Products with only a small amount of fat (typically around 30 %) offer no significant protection to the butyrate, although they will have a less pungent smell compared to uncoated butyrate. The rationale of highly-coated products with a fat content of around 70 %, is that a significant part of the butyrate content will only be released from the moment lipase is secreted in the duodenum, breaking down the protective lipid matrix. The time needed to fully degrade the latter underlies the hypothesis that this type of protection results in a more gradual release of butyrate throughout the intestinal tract, which is often referred to as "target release" or "slow release".

Mono-, di- and tributyrins, on the other hand, are composed of a glycerol backbone to which one, two or three butyrate units are bound, respectively. It can be hypothesised that these molecules, as short-chain triglycerides, will not be absorbed in the stomach, but that the bonds on the outside end of glycerol will be cleaved off by pancreatic lipase, thereby releasing butyrate in the proximal part of the small intestine. As these bonds are readily accessible to the lipase molecules, this release can be expected to be more rapid compared to the breakdown of fat-coated particles.

The presumptive butyrate release locations of all these products can therefore be summarised as in Figure 2.

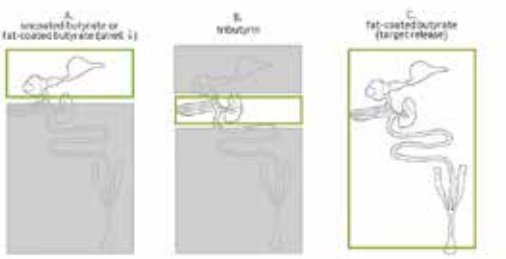


Figure 2: Proposed release butyrate release location of different product, such as (A) butyrate products that are not coated, or only coated to reduce the odour of the product, (B) tributyrin and other butyrins and (C) products with a matrix that will protect part of the butyrate from gastric absorption and release butyrate gradually throughout the GIT.

In a next step, these assumptions on butyrate release kinetics need to be tested *in vitro* and *in vivo*. *In vitro* evaluation is mostly done by incubation products in a solution that mimics gastric or intestinal environments. Gastric simulation is done at low pH and in the presence of pepsin, whereas intestinal incubation occurs at higher pH and in the presence of pancreatin or lipase. The amount of butyrate that is set free from the product is then measured over time, and is an approximation of the release profile of that product *in vivo*.

Subsequently, the product needs to be validated *in vivo*. This can be done by supplementing the feed of test animals with butyrate product, euthanising the animals after a few days of feeding, and analysing intestinal content, sampled at different parts of the GIT, for its butyrate concentration. A true target release product, for example, is then expected to be able to increase the concentration of butyrate in the hindgut, when compared to animals fed no or unprotected butyrate.

A last step would be to investigate which butyrate release profiles could then be linked to a maximal effect on specific benefits related to gut health, such as improvement of digestion, healing of intestinal wounds caused by pathogens, etc. This knowledge will be critical to be able to assess which type of butyrate products prove to be superior for use in animal production, or perhaps more likely, which products are to be preferred in specific situations, such as intestinal development of young animals, raising animals in stress conditions, occurrence of pathogenic challenges, etc.

There certainly is still a long way to go before we reach that goal. At the moment, feed additive producers are building up their own expertise in comparing and evaluating different types of butyrate products, but people involved in animal production or the animal health sector would benefit from more independent studies, published in peer-reviewed journals.

The few comparative academic studies that have been published already show interesting differences between butyrate products. Van Immerseel and colleagues, for example, have demonstrated *in vitro* that butyrate can downregulate genes in *Salmonella* that are important for invasion of intestinal epithelial cells, one of the important steps of *Salmonella* pathogenesis in birds and other animals. In a follow-up study, they showed that for butyrate to be effective in reducing caecal colonisation and shedding of Salmonella in broilers *in vivo*, it needs to be well-protected. As indeed only a target release butyrate product is expected to be able to deliver butyrate where Salmonella colonisation occurs *in vivo*, i.e. in the hindgut, these findings are in line with the model as depicted in Figure 2.

Yeast Cell Walls: Which Components Determine their Efficacy?

Cell walls from yeast, such as *Saccharomyces cerevisiae*, have been extensively studied for their immunomodulating properties. The mechanism by which yeast cell walls (YCWs) exert their beneficial function is typically being attributed to two of their main components: β-1,3-glucans and manno-oligosaccharides (MOS). Binding of β-1,3-glucan molecules to macrophage receptors such as CR3 has been demonstrated to induce a series of signal cascades, resulting in macrophage

activation and stimulation of B- and T-cells. MOS, on the other hand, is known to inhibit intestinal attachment of gut pathogens that have a certain class of appendages or fimbriae. Bacteria with type 1 fimbriae have an affinity for mannose sugars located on intestinal host cells and are therefore able to bind and colonise the gut epithelium and cause disease. MOS, being a polymeric structure of mannose, provides a substrate for these type 1 fimbriae, thereby preventing bacterial attachment.

Therefore, in an attempt to select the most effective YCW, feed additive suppliers have initially focused on selecting YCW with maximal concentrations of glucan and MOS. However, subsequent research has revealed that a model linking the activity of YCW to their glucan and MOS content is not consistent with results from *in vitro* analyses of YCW-activated immune responses, and that other YCW characteristics must have key effects on their activity.

The amount of glucans and MOS, for example, does not predict the immune-modulating effects of YCW *in vitro*. In Figure 3 (unpublished data), it is shown that in assays optimised at the University of Ghent, the phagocytic activity of white blood cells by YCW *in vitro* is not related to the glucan content of these YCW. Similarly, the capacity of YCW to inhibit binding of *E. coli* to porcine villi is not directly proportional to their MOS content.

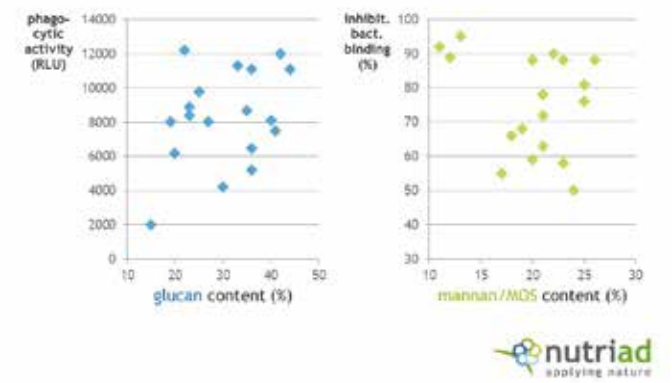


Figure 3: The phagocytic activity (left) and inhibitory capacity of bacterial binding to porcine villi (right) of different commercially available YCW products with distinct glucan and mannan content as measured using in vitro assays. Note that there is no significant correlation between the glucan/MOS content of the YCW and their biological activity in vitro.

Moreover, β-1,3-glucans from different sources were shown to have distinct effects on immunoactivation of white blood cells, which might be attributed to the specific characteristics of their molecular structure, such as the occurrence and length of the glucan side chains. In addition, other fractions of YCW, such as chitin, have been implicated in immune-responses as well.

Results from *in vitro* assays, measuring the effect of YCW on the activity of immune cells, such as phagocytosis, production of interleukins and antibody production, might therefore be a better way to evaluate the activity of these YCW *in vivo*. Ideally, YCW that are extensively analysed by such assays, should be tested in different *in vivo* experiments, where animals are given an immune challenge and are supplemented with YCW, to reveal the potential of *in vitro* readouts to predict *in vivo* biological responses.

Botanical Products: How to Look for Effect on Microbial Activity

Compared to butyrate and YCW, the study of botanical feed additives is even more complex, as these products are often composed of a mix of several components. 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?

In this article, I want to limit myself to the effect of these botanical products on endogenous intestinal microbiota in animals, as this is an area of research that has attracted considerable attention over the last two decades. 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 GIT. 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.

Several 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. 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 and improving gut health.

One of the potential mechanisms of botanical feed

additives that can be placed in the picture is their effect on quorum sensing (QS), which is a form of bacterial communication. Bacteria secrete QS signals, which are molecules that are secreted in their immediate environment. As bacteria also produce their own receptors capable of receiving these molecules, these QS signals can be used as a language by which bacteria are able to interact with each other and to synchronise their behaviours. More specifically, it allows bacteria to perceive the presence of neighbours: when the number (the *quorum*) of a certain bacterial species or group in an environment increases, so will the concentration of their respective secreted QS signals (Figure 4). If a specific threshold of these molecules is reached, it will activate QS-dependent signalling pathways inside the bacteria, resulting in biochemical responses. These can include the production of several virulence factors, such as the formation of biofilms, production of exoproteases, enterotoxins, haemolysin and cellular invasion factors. QS has been detected in a variety of ecological niches in which bacteria reside, including the skin and the intestines of humans and animals, oceans and soils. As a consequence, compounds able to disrupt QS are being increasingly investigated in human medical 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.

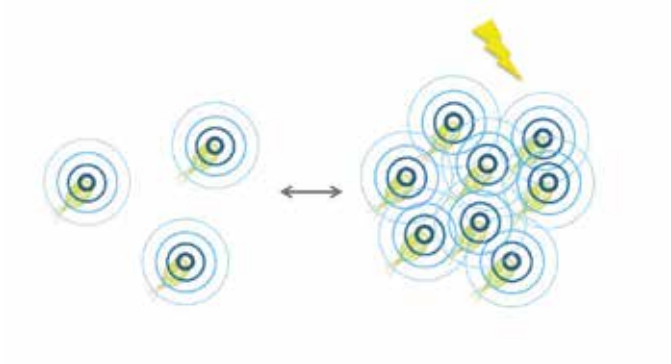


Figure 4: Bacteria continuously produce and secrete QS signals in the environment, as depicted by concentric circles around bacteria in this figure. If the concentration of a bacterial species is low (as shown on the left), the QS signals will get rapidly diluted in the environment. On the contrary, 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).

But QS has also been implicated to be of importance for veterinary and zoonotic pathogens, including *Clostridium perfringens, Yersinia pseudotuberculosis, Campylobacter jejuni* and *Salmonella enterica* subspecies enterica. Given that botanical feed additives have already been accepted in the agricultural sector as a means to enhance gut health and animal performance, screening phytogenic compounds for their capacity to inhibit QS will most likely be added to the toolbox of feed additive

producers to develop products that are highly active at low concentrations.

The concept of QS can therefore be said to clearly hold promise for future applications in human and veterinary medicine. However, caution is still warranted when it is used as a tool to compose feed additives. Especially for agricultural production animals, the relevance of QS and the true potential of QS inhibitors in improving gut health remain to be established.

That being said, I argue that if feed additive researchers are to increase the chances to select for the most performant bioactive components *in vivo*, the study of effects these substances can exert at low concentrations, such as QS-inhibition, will be of vital importance.

Conclusion

Gut health is of vital importance to the wellbeing and performance of production animals. Several feed additives, such as butyrates, YCW and botanical products, have therefore been commercialised to support intestinal development and function. Recent insights in digestive physiology, microbiology and immunology have revealed some of the cell biological mechanisms that are likely underlying their beneficial effects. Moreover, they have provided tools to assess the biological activity of these products *in vitro*. These assays can be used to rationalise the future development of these feed additives, thereby increasing the probability of their effectiveness *in vivo*. However, future *in vivo* experiments will be crucial to further evaluate the predictive and explanatory power of these *in vitro* tests.

References

1. Boisen, S. and Fernández, J. A. (1997). Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Animal Feed Science and Technology 68, 277-286.
2. Boyen, F., Eeckhaut, V., Van Immerseel, F., Pasmans, F., Ducatelle, R. and Haesebrouck, F. (2009). Quorum sensing in veterinary pathogens: mechanisms, clinical importance and future perspectives. Veterinary Microbiology 135, 187-95.
3. Cox, C. M., Stuard, L. H., Kim, S., McElroy, A. P., Bedford, M. R. and Dalloul, R. A. (2010). Performance and immune responses to dietary beta-glucan in broiler chicks. Poultry Science 89, 1924-33.
4. Gareau, M. G., Sherman, P. M. and Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol 7, 503-14.
5. Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R. and Van Immerseel, F. (2010). From the gut to the peripheral tissues: the multiple effects of butyrate. Nutrition Research Reviews 23, 366-84.
6. Trevisi, P., Priori, D., Gandolfi, G., Colombo, M., Coloretti, F., Goossens, T. and Bosi, P. (2012). In vitro test on the ability of a yeast cell wall based product to inhibit the Escherichia coli F4ac adhesion on the brush border of porcine intestinal villi. Journal of Animal Science 90 Suppl 4, 275-7.
7. Van Immerseel, F., Boyen, F., Gantois, I., Timbermont, L., Bohez, L., Pasmans, F., Haesebrouck, F. and Ducatelle, R. (2005). Supplementation of coated butyric acid in the feed reduces colonization and shedding of Salmonella in poultry. Poultry Science 84, 1851-6.

Tim Goossens got a Master's degree in Biotechnology at Ghent University, and subsequently worked as an academic researcher in the Laboratory of Molecular Biotechnology at the University of Antwerp. After working 4 years on the characterization of a gene family involved in bone development, he moved to the KU Leuven, where he took up a doctoral project on neurodevelopment in the Laboratory of Developmental Genetics. After obtaining his Ph.D. in Biomedical Sciences, he joined Nutriad to work as an R&D Engineer, and later as a Business Development Manager, focusing on the development of the Digestive Performance range of feed additives.
Email: t.goossens@nutriad.com