Revisiting prebiotics

What is a prebiotic?

Prebiotics are, typically, complex carbohydrates that are not digested by host enzymes but are "selectively utilized by host microorganisms to confer a health benefit" (Gibson et al., 2017). Oligosaccharides, which are short polymers of monosaccharides, are probably the best studied prebiotics and include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS) and xylo-oligosaccharides (XOS). Other dietary fibres that may be considered prebiotics are β-glucans, inulin (fructan), pectins and resistant starch. The degree of polymerisation and molecular structure of the particular prebiotic compound will determine its solubility, fermentability and viscosity affects, all of which, including concentration, will influence gut transit rate, nutrient digestion and absorption dynamics, and location and extent of fermentation by gut microbes (Holscher, 2017). Generally, soluble substrates increase viscosity and are fermented more proximally in the gastrointestinal (GI) tract. Fermentation of prebiotic compounds produces short-chain fatty acids (SCFAs), including acetic, propionic, and butyric acids.

Uses and benefits

In humans, prebiotics are considered to enhance intestinal and wider health through modulating the composition and activity of the gut microbiota, as well as increasing digesta viscosity and slowing glucose absorption to help glycaemic control and reduce any associated oxidative stress and inflammation (Lunde et al., 2011). Typical daily recommendations for prebiotics in adults are around 5 – 8 g per day.

In monogastric farm animals, the situation is a little less clear. For example, the cereal grains comprising their diets include various prebiotic substances (e.g. β-glucan), which have been considered anti-nutritional factors, primarily due to their effect on digesta viscosity and digestive dynamics, which can impede nutrient acquisition, and compromise high performing animals. The residual nutrients may also cause intestinal dysbiosis. Suitable enzymes (e.g. β-glucanase) have been employed in monogastric diets to hydrolyse these structures and mitigate their effects. More recently, prebiotic substrates are being investigated for their prebiotic and immunomodulatory potential in farm animals, including a focus on the use of exogenous enzymes to generate prebiotic compounds *in vivo* from the parent fibre structure (Ribeiro et al., 2018). In some of these studies, the benefit of prebiotic (XOS) supplementation is observed at very low levels (e.g. 0.1 g per kg feed or ~0.01 g (average) per day (broiler chickens)), which is intriguing. We should note that effects in different species could be different due to physiology and microbiome differences.

Action

Retention times in the oral cavity and oesophagus make these sites unlikely locations for notable prebiotic activity. However, in chickens, the crop, a diverticulum of the oesophagus, stores ingested feed and is estimated to harbour 10^8 to 10^9 CFU of bacteria per gram (Shang et al., 2018). Crop retention time in *ad libitum* fed chickens has been reported at between 7 and 25 minutes, although it's predicted that much ingested food passes straight to the proventriculus (Classen et al., 2016). This suggests some opportunity for ingested prebiotics to influence the microbial composition and activity, and for interactions with immune-related cells of the crop. The crop is considered to have competent immune function (Classen et al., 2016) and various immune cells express receptors for prebiotic substrates. For example, Dectin-1 (a C-type lectin receptor that recognises β-glucan) is expressed by chicken heterophils and peripheral blood mononuclear cells (Nerren and Kogut, 2009) and various porcine gut-associated tissues (Sonck et al., 2009). Around the entrance of the proventriculus in chickens is the oesophageal tonsil, which could potentially play a role in sensing ingested feed components or pathogens (Zmrhal and Slama, 2020). The stomach, and proventriculus and gizzard in chickens, are hostile environments with relatively low microbial numbers dominated by acid-tolerant taxa, such as lactobacilli. The retention time in these segments is relatively short (<1 hr) (Wilfart et al., 2007), thus, collectively, the potential for prebiotics to shape the microbiome here is limited but interactions can still occur with immune-related cells. Moving through the small intestine segments to the caeca and large intestine, retentions times, microbial populations and diversity, and host-defence related tissue increases, providing greater opportunity for prebiotics to modulate the microbiome and for metabolised and non-metabolised components to be sensed by, and interact with, the host. However, in the chicken, total tract retention time can be as little as ~3.5 hrs, although small and/or soluble particles can enter the caeca and be retained for longer periods (~12 hrs) (Svihus et al., 2013). Many prebiotic studies typically report increases in bifidobacteria and/or lactobacilli, and reductions in taxa within the *Enterobacteriaceae* family. As more is understood and appreciated about the gut microbiota of different host species, it is probable that the importance of other taxa will become more evident. The effects of SCFAs generated by the gut microbiota on the host, including immunomodulation, are well established but the nuances of optimal, and relative, quantities remain to be determined. The more direct immunomodulatory effects of prebiotics are generally less well characterised, although there is better understanding for some (e.g β-glucan). As mentioned, β-glucans can be sensed by dectin-1 and/or other pattern recognition receptors, which activates signalling pathways within the respective cells, influencing both innate and adaptive immune responses (Jin et al., 2018). There are suggestions that β-glucans are typically immunostimulatory and can reverse LPS-induced immunological tolerance (Ifrim et al., 2014), and have been considered as vaccine adjuvants (Jin et al., 2018), although the effects could be context dependent (Teng and Kim, 2018). Certain prebiotics (e.g. MOS) have also been reported to bind certain pathogens via mannose specific type-I fimbriae and thus may affect the microbiome and host-pathogen/microbiome interactions (Ganner et al., 2013), although more investigation is required to better understand the significance of this *in-vivo*.

Conclusion

Various compounds qualify as prebiotics and others are emerging as further data are generated. The chemical structure of a prebiotic determines its individual characteristics and, along with concentration, impact on digestive physiology and probable intestinal regions for fermentation. Lower

microbial numbers and diversity in the upper GI tract indicate that prebiotic utilisation and impact will be greater more distally, while interactions with the gut-associated lymphoid tissue (GALT) (or any pathogen binding) can begin shortly after ingestion. As key health- or performance-enhancing microbial taxa are identified and confirmed, prebiotics can be better tailored to optimise these populations. Similarly, better understanding the consequences of how, where and the frequency with which prebiotics (or metabolites) engage the GALT would help us to better utilise their immunomodulatory potential. Moreover, structure-function is important here too. For example, β-glucans from yeast and fungi have been reported to show superior immunomodulatory potential but cereal β-glucans have also been demonstrated to possess such properties, which was governed by the cellotriosyl:cellotetraosyl ratio and thus solubility and aggregation in solution (Mikkelsen et al., 2014). Some prebiotic application differs between humans and highly productive animals, where increased digesta viscosity is proposed as positive in the former and negative in the latter. Similarly, in humans the daily recommended prebiotic intake is around 5-8 g but much lower intakes have been shown to be beneficial in chickens suggesting a) mechanisms aside from straightforward microbial utilisation, and b) the potential to better utilise prebiotic substrates that are intrinsic in standard diets.

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