# Carbohydrate-boosted control of intestinal health



**DOI:**
10.26481/dis.20240118mo

# Impact

Poultry, is one of the most widely consumed meats globally, providing a significant source of animal protein that is culturally accepted in many societies. However, broiler chickens are highly susceptible to developing an intestinal disease called coccidiosis caused by the protozoa *Eimeria*. Coccidiosis is one of the most prevalent and economically significant diseases in poultry. To reduce the impact of *Eimeria* infections, antimicrobial drugs are applied as feed additives throughout broilers' lives. However, objections against antimicrobial use in feed of food producing animals and resistance development to these drugs demand alternative control measures.

Coccidiosis has been linked to the overgrowth of *Clostridium perfringens* (*C. perfringens*), a bacterium that contributes to necrotic enteritis. Necrotic enteritis is an inflammatory condition of the intestine that disrupts the gut microbiota and can induce inflammation and damage to the gut lining, impairing its ability to function as a protective barrier and proper digestion. This can lead to the entry of harmful pathogens and undigested food particles into the system, causing severe health issues and even death. When necrotic enteritis is recognized, entire flocks are often needed to be treated with antibiotics. Both coccidiosis and necrotic enteritis impact broiler health and welfare and predispose for other diseases. They can significantly impact public health and food safety, by increasing the risk of foodborne disease by e.g, *Clostridium*, and of development of antimicrobial resistance. *C. perfringens* is also associated with necrotizing enterocolitis in humans, a life-threatening disease characterized by severe intestinal inflammation and bacterial overgrowth throughout the small and large intestines, primarily affecting preterm-born infants.

The intestinal microbiota has been shown to play a role in many physiological processes including digestion, metabolism, and immune function, in both humans and animals. The development of the intestinal microbiota starts at birth, and undergoes rapid and dynamic changes, with the establishment of a diverse and stable microbiota. A balanced microbiota composition is known to protect against pathogenic overgrowth, and in view of the above, could protect against *C. perfringens* overgrowth.

One way to modulate the microbiota, or increase its resilience, is by diet or specific dietary additives, thereby boosting intestinal health. Some carbohydrates are resistant to digestion by the host and absorption in the small intestine and can be fermented by the intestinal microbiota, conferring a health benefit, which is referred to as prebiotics. Prebiotics, besides modulation of the microbiota, can influence the immune system and intestinal barrier function.

The research presented in this thesis was carried out within the Carbohydrate Competence Center – Dutch Research Council "CarboBiotics: pre/probiotics mitigating the antibiotics burden" (CCC NWO CARBOBIOTICS: https://www.cccresearch.nl/ccc-nwo-carbobiotics/), where the overall aim was to study the health benefits of certain dietary non-digestible carbohydrates. Our research aimed to investigate the impact of specific non-digestible carbohydrates on the gut microbiota (composition and activity) and the effect of the modulation of the microbiota on the intestinal protective barrier and immune responses of the host. To achieve this, we developed and used a pipeline of various *in-vitro* tools. In general, *in-vitro* tools have long been instrumental in understanding biology and disease processes. The pursuit of novel *in-vitro* models is driven by the need for more accurate and cost-effective methods to study complex biological systems and test potential interventions. Moreover, it is important to minimize the use of animals in research as much as possible, therefore we performed experiments for which animal trials were not needed. In this thesis, fermentation *in-vitro* tools for humans, as well as chickens, optimized chicken intestinal organoids, and the use of a co-culture containing human intestinal epithelial cells in combination with immune cells, provide a valuable platform for future investigations into the complex interactions between nutrients, the gut microbiome, and cellular/immune processes.

The knowledge gained from this research has significant implications for the scientific community and beyond. It has the potential to shape future studies in this field, enabling scientists to gain a deeper (mechanistic) understanding of the complexity of the digestive/fermentative system, including its microbiota, and develop targeted interventions to improve health outcomes. Specifically, our findings shed light on the effects of the non-digestible carbohydrates tested, such as Isomalto/malto-polysaccharide (IMMP) derived from potatoes, three different citrus pectins, for broilers and fermentation products of proven prebiotics, namely Galacto-oligosaccharides (GOS), and 2'-fucosyllactose (2'-FL) for infants.

IMMP and the pectins showed promising effects on the chicken microbiota composition and the production of metabolites during fermentation. The identified potential prebiotics can now be further tested in a co-culture of chicken intestinal organoids and immune cells, and in a later stage in field studies to validate their effects on gut health and explore their potential preventive and therapeutic applications in broiler flocks. This will contribute to a decrease in the occurrence of gut-related diseases in chickens, such as necrotic enteritis, leading to a drastic reduction in mortality, and thus costs and economic loss. Besides, the purported increase in health is of course beneficial for animal welfare.

The fermentation products of GOS and 2'-FL did not substantially improve the barrier function of the human intestinal cell cultures. These results highlight the complexity of the interactions between complete *in-vitro* large intestine-derived fermentation products in this *in-vitro* co-culture model combining intestinal epithelial cells and immune cells. We are one step closer to unraveling the effects of these non-digestible carbohydrate-derived fermentation products, but further studies are needed. It can be that a combination of microbiota-derived signals and direct (non-microbiota-derived) effects of the prebiotics in the host work in synergy to provide (intestinal) health benefits. In the current experimental *in-vitro* pipeline, these have not been combined, but both have helped to gain some insight into the separate processes related to the health benefits of dietary ingredients.

In summary, the implications of this research extend beyond the scientific community to e.g., poultry farmers, clinicians, and the general public. If the positive results observed in our models are confirmed through field trials, the implementation of the non-digestible carbohydrates studied in this thesis in the chicken diet can significantly contribute to improving the health of broiler chickens. This, in turn, will lead to healthier poultry products for consumers and reduce the economic burden associated with coccidiosis and necrotic enteritis in the poultry industry. Similarly, if we can increase the health of the infant microbiota, this can prevent necrotizing enterocolitis in preterm-born infants, but may also extend to benefits later in life, for instance, dysbiosis of the gut microbiota in infants has been linked to obesity at a later stage in life.

In conclusion, our research highlights the importance of understanding the impact of non-digestible carbohydrates on gut health and disease prevention in infants and broiler chickens. By employing a pipeline of innovative *in-vitro* models, we have contributed to the growing body of knowledge in this field and provided a platform for further investigations. The potential preventive and therapeutic applications of the non-digestible carbobiotics tested hold promise for the human health and poultry industry, with the aim of promoting a healthy gut.