Fibre degradation by pig microbiota

Impact

It is complicated and costly to investigate the effect of feed fermentation by the gut microbiota in pigs *in vivo*, due to the interaction with other feed-components (such as other fibers) in the swine feed, the ethical considerations (such as using pathogens, toxic compounds, and animal experiments itself) and other uncontrollable factors (such as infectious disease or death during the experiment), which are important for reproducibility of results. *In vitro* fermentative models are considered excellent alternatives to study the effect on the composition and/or activity of the gut microbiota without ethical constraints. They allow the reasonable high throughput screening of a large number of substances such as dietary ingredients and components thereof such as enzyme-, chemical- or physically-treated recalcitrant fibres. In this thesis, a Swine Large Intestinal *in vitro* Model (SLIM) was developed, which was developed based on the human, computer-controlled, dynamic TNO *in vitro* model of the colon, nick-named TIM-2. Therefore, the new developed SLIM can be used by researchers in both universities and feed-related companies to study the scientific questions which cannot be answered *in vivo* as mentioned previously. In this thesis, the SLIM system was used to study the (increased) fermentation of (enzyme- or chemically-processed) recalcitrant fibres in rapeseed meal (RSM), with the aim to increase energy extraction from the diet, through liberation of short-chain fatty acids (SCFA) produced by the pig gut microbiota.

The European Union (EU) is the second-largest importer of raw material for feed (especially protein-rich feed ingredients) in the world, after China. Therefore, in order to have a more sustainable supply of responsible protein-rich ingredients, the European livestock sector needs an alternative local protein feed ingredient to fill the “protein gap”. Rapeseed meal (RSM), a byproduct from rapeseed oil production, is not only a suitable protein source for animal feed but also a potential energy source. RSM contains a high amount of cell wall polysaccharides, even higher when compared to soybean meal commonly used in the feed industry. However, the high amount of pectins in RSM can also reduce the absorption of other nutrients in the gut. Therefore, the aim of the project was to improve utilization of recalcitrant fibre of by-product of rapeseed oil in pigs. Enzymatic and chemical treatment on the RSM were performed in the project to improve the digestibility and fermentability of these by-product in pigs.

The current thesis provides the first detailed analysis of changes in the swine intestinal microbiota due to RSM processed by enzymatic and chemical treatment using the newly developed SLIM sytem. The present studies clearly demonstrated that both enzymatic (cellulase or two different pectinases) and chemical (6 N sodium hydroxide; alkaline) pretreatment on RSM shifted its cell wall polysaccharide structure, subsequently altering microbial community composition and functional profile compared to untreated RSM, and eventually increased fibre degradability as evaluated by SCFA production in SLIM. Moreover, it was validated in pigs by the mobile nylon bag technique that cellulase and alkaline treatment on RSM improved the overall degradation of RSM. Our findings that the specific treatments increased fibre degradation in RSM could help to guide feed additive strategies to improve efficiency and productivity in swine industry. The current study gave insight into how feed enzymes may modulate microbial status, which provides good opportunity to develop novel carbohydrase, particularly in swine feed. In particular, the genomes of microorganisms that were stimulated by the processed RSM can be mined for novel carbohydrases, that may be used as novel enzymes to pretreat RSM to increase the use of its recalcitrant fibres by the pig gut microbiota.

In order to develop new methods to improve the degradability of plant cell wall

polysaccharides, a better understanding of the cell wall polysaccharide composition and structure and their interactions are essential. In the thesis, a high-throughput technique was developed to screen composition and structure of plant cell wall polysaccharides. The technique (glycome profiling of plant cell wall polysaccharides, or glycoprofiling) was developed with the use of carbohydrate binding modules (CBMs) with unique specificities for plant cell wall polysaccharides, linked to a green fluorescent protein (GFP) as reporter. This toolbox may become essential in the study of the dynamics of breakdown of dietary fibres by the (pig) gut microbiota.

Researchers from both academia and industry can access our results from scientific journals. The result of SLIM model is published in Beneficial Microbes, the results of RSM degradation in pigs are published in Journal of Agricultural and Food Chemistry and Frontiers in Microbiology, and the results of the glycoprofiling technique are prepared for publication. The results call for additional research into recalcitrant fibre fermentation by the (pig) gut microbiota, e.g., by combining multiple enzymes for pretreatment. Both tools (SLIM and glycoprofiling) in our view are pivotal for studying the increased use of feed components by the gut microbiota, leading to increased energy extraction for the host. The use of the tools can be extended to other fibre-sources and other hosts (beyond pigs).