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Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Madame UERLINGS Julie,

Titulaire d'un diplôme de master en bioingénieur : sciences agronomiques, à finalité spécialisée,

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 14 juillet 2020, à 9h00 précises, en visioconférence :

https://uliege.webex.com/join/julie.uerlings

Cette dissertation originale a pour titre :

« Agro-industrial feed ingredients, a novel approach to enhance intestinal health in weaned piglets: from in vitro to in vivo perspectives ».

Le jury est composé comme suit :

Président : Prof. Y. BECKERS, Professeur ordinaire,
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Abstract

Weaning is a critical transition period in pig production with major dietary, behavioural and environmental challenges leading to tremendous physiological, immunological and microbiological changes in the piglet's intestines. This multifactorial process predisposes the piglets to subsequent intestinal disturbances and infections, resulting in poor performance and diarrhoea. To tackle the current problematic expansion of antibiotic resistance, nutritional management is yet another promising strategy to alleviate intestinal disorders around weaning. By-products from the agro-industrial sector and, to a lesser extent, whole cereals and roots, are major sources of dietary fibres suitable for livestock feeding. These native sources of ingredients could represent potential prebiotic candidates due to the heterogeneous dietary fibre components embedded in their cell wall matrix. These feed ingredients, regarded as environmental-friendly, socially accepted and more economic and sustainable than antimicrobials and isolated fibre fractions, are therefore of upmost interest. However, among potential dietary strategies, mainly purified fibre fractions have been accepted and promoted, while the prebiotic capacities of several cereals, roots and corresponding by-products remain to be investigated. We therefore hypothesised that the prebiotic effects of these feed ingredients could reach or overcome the ones of isolated fibre fractions.

For this purpose, 36 fibre-rich feed ingredients were first tested in a three-step *in vitro* model of the piglet's gastro-intestinal tract for their prebiotic potential (**objective 1**), measuring the gas kinetics, short-chain fatty acid and microbiota profiles in the fermentation broth as indicators of prebiotic activity. Several feed ingredients such as chicory pulp, citrus and orange by-products, rye bran and soybean hulls, with complex structural composition varying in soluble and insoluble dietary fibres and distinct constituent sugars, positively modulated health-related microbiota communities in the fermentation broths, reaching or overcoming the prebiotic effect of their corresponding purified fraction and/or inulin. Purified fractions were ranked as highly fermentable, with inulin displaying the most interesting prebiotic profile as butyrogenic ingredient.

Subsequently, the six most promising ingredients were tested via a functional *in vitro* fermentation – cell culture assay (**objective 2**) combining the *in vitro* batch fermentation model with cultured intestinal porcine epithelial cells (IPEC-J2), for their barrier-enhancing and immunomodulatory effects. Inulin and its fermentation metabolites promoted the intestinal barrier function via up-regulated expressions of tight and adherens junction genes. Chicory pulp fermentation supernatant enhanced the intestinal barrier integrity and seemed to induce anti-inflammatory and pro-apoptotic regulations in comparison to inulin, while the fermentation supernatants of chicory root, citrus pulp, rye bran and soybean hulls only showed minor immunomodulatory effects.

These *in vitro* assays led to preliminary conclusions that allowed the selection of the most promising ingredients for the **third objective** of the thesis, i.e. evaluating the inclusion of citrus pulp (0.2 % and 2 %) and inulin (0.2 %) in the feed of newly weaned piglets *in vivo*. The aim was to provide insights into their capacities to modulate intestinal fermentation, ecology, morphology, inflammation and permeability, with the purpose of improving gut health at weaning, and hence, reducing the incidence of infections. We observed that citrus pulp improved gut morphology and promoted colonic fermentation at weaning without depressing growth performance or impairing the inflammatory response and the intestinal barrier function. Colonic health-associated microbiota communities were notably enhanced in both the early and late post-weaning stages under citrus pulp supplementation at 2 %. A decline in colonic branched-chain fatty acids, concomitant with greater acetate proportions, was also observed and could account for a possible reduction of proteolytic fermentation in newly weaned piglets following citrus pulp inclusion at 2 %. Moreover, greater villus height to crypt depth ratios were observed following citrus pulp (0.2 % and 2 %) and inulin supplementations, which might suggest the capacity of the ingredients to cope with epithelial damage during the weaning transition. Citrus pulp could therefore be considered as a valuable nutritional strategy for future inclusion in the weaner diet.

In summary, this thesis improved our understanding of dietary fibre management around weaning using whole cereals, roots and by-products from the agro-industrial sector. The heterogeneous sources of endogenous dietary carbohydrates present in these feed ingredients, causing a more gradual degradation, could effectively maintain fermentation throughout the entire hindgut. Several types of feed ingredients may therefore be promising alternatives to antimicrobials and pure fibre fractions, to reduce the prevalence of post-weaning diarrhoea. Nevertheless, a challenge trial could better recreate the conditions encountered in commercial production at weaning and might provide a clearer picture of the preventive or curative effects of these feed ingredients against pathogen infection at weaning. Alternatively, using the functional fermentation – cell culture model, other feed ingredients and their fermentation metabolites may be tested for their bioactive properties for future inclusion in young mammalian diets.