An *in vitro* approach to quantify protein fermentation using ileal digesta of pigs

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Metabolites released during the process of protein fermentation by the microbiota in the gut are brought in relationship with health problems in pigs and humans. Therefore, an increased understanding on the process of protein fermentation and how to affect this is needed. It was hypothesised that the protein composition that flows into the hindgut, including amino acid (AA) composition and matrix in which these are present, influences protein fermentation. To gain insight in this process, a combination of an *in vivo* and *in vitro* studies was performed in pigs. Pigs, equipped with a simple T-cannula at the distal ileum, were fed 10 different protein sources in test diets containing 100 g CP/kg DM feed and a N-free diet. Due to a difference in protein quality and the variety in matrices in which the protein are build, large differences in the amount and composition of protein in ileal effluents were expected between diets. Intestinal effluents were collected via the simple T-cannula and used as substrates in three *in vitro* batch culture studies, to simulate hindgut fermentation of the ileal effluents under controlled conditions. Ileal effluents were incubated for 48 hours using fresh faecal samples from 5 pigs that received a commercial diet. In a first run ileal effluents were incubated as is. Gas production was measured to assess the microbial activity. After 12, 24 and 48 hours of incubation the batch cultures were sampled for analysis on metabolite profiles. In a second run ileal effluents were incubated in standardized C:N ratios, by adding calculated amounts of CHO. Gas production and metabolite profiles were measured identically to the first run. In a third run, the accessibility of the proteins from the ileal effluents was tested for microbial use. Equal quantities of nitrogen from all ileal effluents were incubated in the presence of an excess of carbohydrates to make nitrogen limiting for growth. Accessibility of the protein for microbial growth was assed via cumulative gas production measurements. Multivariate analysis will be performed to find out how differences in protein amount, composition and accessibility together with other macronutrients in the ileal effluents explains the variation in metabolite profiles. The data obtained will be used to understand and predict protein fermentation profiles in the gut.