EXPLORATORY STUDY TO IDENTIFY POTENTIAL METABOLOMIC BIOMARKERS TO FORESEE THE IMPACT OF HEARD HEALTH STATUS ON MEAT MICROBIAL QUALITY

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I. INTRODUCTION

When animals are healthy, muscles, with the exception of lymph nodes, harbour limited numbers of microorganisms. It is through carcass dressing and breaking that meat surfaces get contaminated by microorganisms that are present in different parts of the animal including content of the digestive tract and faeces [1]. Hence, the sanitary/health status of the herd is important to monitor to secure meat microbial quality and safety. The aim of this study is to investigate if metabolomic analysis of faeces coming from pigs ready for slaughter can reveal potential biomarkers to foresee microbial quality and safety of meat and meat products.

II. MATERIALS AND METHODS

Two commercial farms out of 126 were selected by experienced veterinarians based on their sanitary status, the gastrointestinal health of the animals and the medical history associated with the farms; one farm with a lower sanitary status (farm-L) and one with a higher status (farm-H). Both were finishing farms of similar housing size, with 1200 and 1600 animals, respectively. Animals (115 ± 7 kg) came from the same production lot where three out of the five shipments were followed. For each shipment, 3 days before the swine were transported to the slaughterhouse, freshly defecated faeces were collected: one sample per pen collected from 16 pens for a total of 48 samples per farm. Untargeted metabolomics was performed on the individual lyophilized (Lyovapor L-300, BÜCHI Labortechnik AG, Flawil, Suisse) faeces samples using a reverse phase liquid chromatography coupled to a mass spectrometer (LC-MS; Vanquish ultra-high-performance liquid chromatograph and Fusion Tribrid, Thermo Fisher Scientific, Waltham, MA, USA). Mass spectrometry (MS) acquisitions were performed on an orbitrap at a resolution of 120000 in profile mode using Easy-IC for mass correction. A quality control (QC) pool sample was injected every nine injections. Data analysis, including statistics, was performed with Compound Discovered 3.2. Putative metabolite identification was further confirmed with MetFrag Web 2.1 [2].

III. RESULTS AND DISCUSSION

Metabolomic data were strictly filtered to keep reproducible and stable metabolites (presence in all QC-pool injections, % CV < 20 % in at least one sample group, MS^2 spectra acquired). Metabolomics data analysis revealed a clear distinction of the animals from the two farms based on the first principal component of a PCA score plot for both positive (27.1% variability) and negative ions (28.5%; data not shown). Discriminant analysis through Volcano plots (Log_2-fold change > 1 and P value < 0.05) provided also clear indication of the different metabolites characterizing each farm (Fig. 1). A total of 85 and 16 ions were significantly more abundant under positive and negative ionization, respectively. Putative identification based on molecular mass and fragmentation spectra was obtained for 21 metabolites in positive ionization and six metabolites in negative ionization. Of those identified metabolites, three were

revealed with a high level of confidence and were more abundant with samples from farm-L: 2-hydroxyquinolin (quinoline degradation product naturally found in some plants) and methyl jasmonate (phytohormone with bactericidal activity) in positive and 3-methyl-dodecanedioic acid (medium chain fatty acid found in plants) in negative ionization, respectively. The animals were under the same feeding program, but a month prior to sampling, farm-L experienced a salmonellosis outbreak, necessitating a switched to mash feed to control the diarrhoea caused by the outbreak; mash feed is known to induce higher levels of short chain fatty acids (SCFA) in faeces [3]. This change could have contributed to the differences observed.

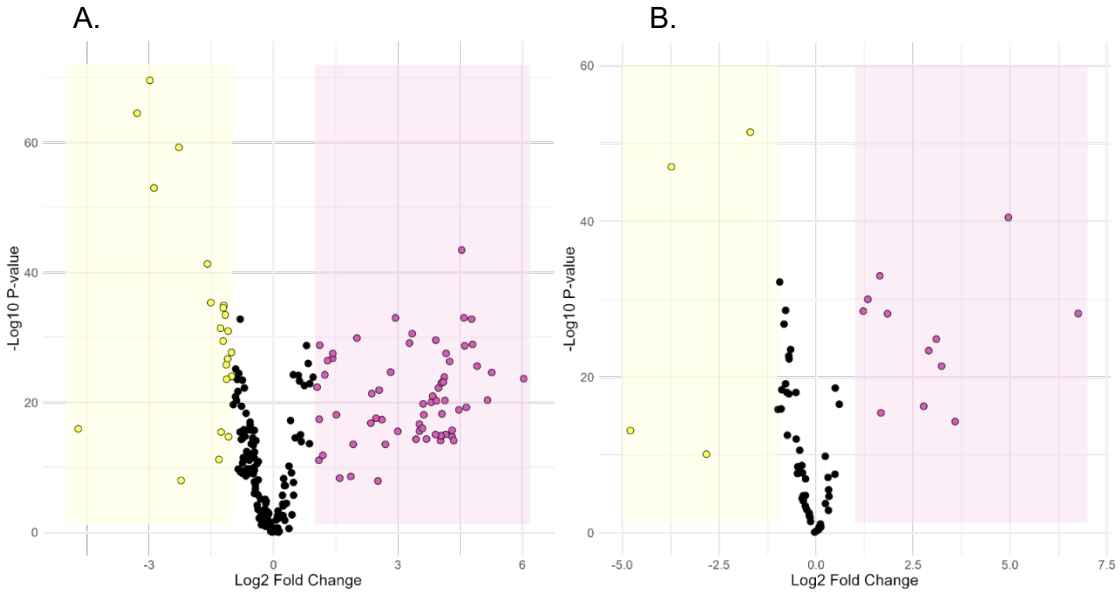

Figure 1. Volcano plot comparing metabolite abundance from farm-L and farm-H under positive (A) and negative (B) ionization. Dots in the yellow section are ions that are significantly more abundant in farm-H samples and dots in the pink section are significantly more abundant in farm-L samples. The highlighted sections represent a Log_2-fold change > 1 and a P-value less than 0.05.

IV. CONCLUSION

Our results clearly indicate that a subset of the faeces metabolites were unequivocally different between the two farms. Three metabolites (2-hydroxyquinolin, methyl-jasmonate and 3-methyl-dodecanedioic acid) were significantly more abundant in faeces samples from farm-L. Since only two farms were under investigation, we cannot confidently ascribe them as biomarkers of the herd health status. Nonetheless, they represent potential candidates for further studies that should include more farms with different health statuses.

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