P-11-21

Post slaughter metabolomics analysis by comprehensive GCxGC-MS to identify genotypic and sex-specific differences in pigs (#655)

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Introduction

Meat quality is a highly complex trait resulting from multiple endogenous as well as exogenous factors. In addition to endogenous factors such as genotype, sex or fitness, pre-slaughter animal stress represents a major issue impacting meat quality due to its effects on metabolic processes in muscle tissues.

In order to investigate the effects of stress before and during slaughter on blood and muscle metabolism we apply non-targeted metabolite analyses by comprehensive 2D GC-MS. Beforehand, we generated a fundamental data set on blood, muscle and liver tissues from pigs of different genotypes and sex, reared under controlled conditions. These data represent the basis to identify independent stress profiles and to avoid overestimation of changes due to endogenous factors.

Here, we present our preliminary results gained from pig blood profiling to evaluate metabolic differences that are induced by genotype and sex.

Methods

Pigs were reared for 120 days in groups of 12 animals under the same feeding conditions. Four groups were kept per stable compartment. Approximately one hour before starting the slaughtering pigs were transported to the waiting pens (2 minutes transport distance). During the electrical stunning, the pigs were fixed in a stunning box and were subsequently bled within 5-10 seconds after stunning. The stunning was done by head-only followed by cardiac arrest stunning and the bleeding was done in a lying position. Blood from the bleeding process was immediately frozen in liquid nitrogen and stored at -80°C. Before extraction, the blood was lyophilized and homogenized.

Table 1: Pig sample set for GCxGC-MS analysis comparing sex and genotypic differences.

Abbreviations are: m – male (boar), f – female (guilt), c – castrate, DE – Large White, DLS – German Landrace, PI - Pietrain

	DExDLS	DLS	DLSxDE	PI	PIx(DExDLS)	PIxDLS	Sum
m	3	10	1	0	6	11	31
f	1	0	1	11	12	10	35
c	10	14	11	0	0	0	35
Sum	14	24	13	11	18	21	101

Extraction was performed in two steps. A polar extraction using 80% methanol was followed by a non-polar extraction using methanol:chloroform (v/v 2:1), both in a 1:30 ratio (w/v). From the combined extract 100 µl were dried in GC-vials. Before GCxGC-MS measurement, samples were derivatized by methoxyamination followed by silylation with MSTFA (including 1% TMCS). The GCxGC-MS system consisted of a gas chromatograph combined with a fast-scanning quadrupole mass spectrometer (Shimadzu GCMS QP2010 Ultra). For chromatographic separation a non-polar Rxi-5SilMS (Restek, Bellefont, USA) was chosen for 1D and the 2D column was a medium-polar BPX50 (SGE, Milton Keynes, UK).

For data analysis an in house developed workflow was conducted (Weinert et al. 2015) consisting of data pre-processing steps and peak alignment. For statistical analysis and visualization the software tools SIMCA (Umetrics, Malmö, Sweden) and SigmaPlot (Systat, Erkrath, Germany) were used.

Results

The investigated pig genotypes represent common German crossbred and purebred lines. Preliminary results from metabolite profiling indicated that the individual variations have a stronger impact on total metabolic patterns than genotypic or sex-specific differences (PCA not shown). To analyze genotype and sex specific variations OPLS-DA was applied.

The OPLS-DA model calculation including the genotype information indicated a group separation between pigs with PI background (purebred and crossbred lines) and DE/DLS background (Fig 2a). As exemplarily been shown in Fig 2b differences mainly occur between purebred PI and purebred DLS. In crossbreeds the individual metabolites show either intermediate values or correlate to one of the parents.

Application of OPLS-DA including the sex information (Fig 3a) revealed higher variations between males and castrates than between males and females. A closer analysis of selected signals confirmed these patterns but also indicated the presence of signals that are stronger female related (examples shown in Fig 3b).

Conclusion

The initial results of pig blood profiling revealed that from non-supervised metabolomic analysis neither the sex nor the genotype could be clearly dif

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ferentiated, which confirms that multiple factors play a role in the metabolomic variability. By including genotype and sex information in statistical analysis (OPLS-DA) differences between the applied groups were observed. Especially in the purebred lines DLS and PI several blood metabolites were affected. These results indicate that for the analysis of metabolic changes following stress treatments endogenous factors like the genetic and sex background of animals has to be taken into account.

Literature:

Weinert C, Egert B, Kulling S (2015) On the applicability of comprehensive two-dimensional gaschromatography combined with a fast-scanning quadrupole massspectrometer for untargeted large-scale metabolomics. Journal of Chromatography A, 1405 (2015) 156–167.

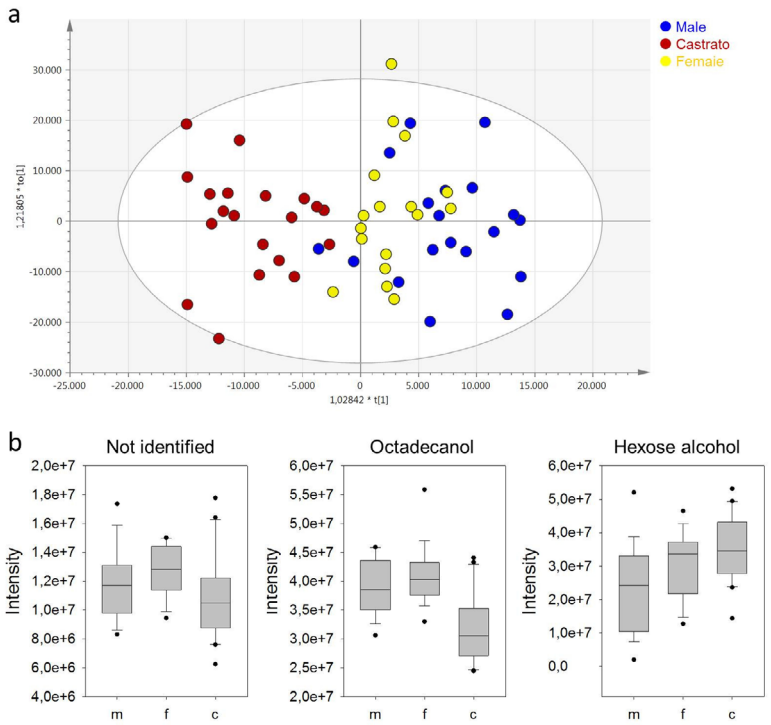

Fig.3:
Comparison of pig sex by OPLS-DA (a) and for selected metabolites (b).

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