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POTENTIAL OF METABOLOMICS FOR INNOVATIVE CHEMICAL RISK ASSESSMENT

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1. Introduction

Food safety has become a major issue worldwide and in particular, detecting the presence of toxins, contaminants or residues of chemical substances along the food chain and in fine in food items constitutes a strong consumers demand. Characterizing the effects associated to the exposure is also considered a challenge although constituting a key step of the risk assessment workflow. Generally all these substances and corresponding metabolites of interest are analysed using efficient targeted methodologies. However, in some cases these targeted approaches do not allow the detection of either those substances or emerging compounds/practices and therefore new approaches and strategies are demanded to efficiently assess the exposure to chemical contaminants. Thereby the study of physiological perturbations induced upon exposure to a given chemical substance has emerged as an interesting alternative approach to be applied in chemical food safety.

Metabolomics in particular is considered as an analytical strategy of interest in the field of chemical food safety for risk assessment purposes. It is recognized as an innovative tool to predict the likely occurrence and nature of risks, together with improving detection methods, in the aim of answering global safety issues and anticipating human health problems.

In the present work, slow growing broiler chickens have been exposed through their feed to two different doses of polychlorobiphenyls (PCBs) mixture over the last 8 weeks of their 12 weeks of growth. It was hypothesized that the serum lipids profile will be disrupted following exposure to PCBs, therefore, the serum collected has been characterized using a lipidomics workflow for better insight into this phenomena. Till recently, analytical strategies to investigate these fine changes thoroughly were not available, but technological advances in liquid chromatography, mass spectrometry technologies and omics strategies are now well suited to address this issue; The current research presents a combination of hydrophilic interaction liquid chromatography (HILIC) and Reverse-Phase Liquid Chromatography (RPLC) both coupled to high resolution mass spectrometry (HRMS) for characterizing lipid profile disruption in serum of broiler chickens exposed to PCBs.

2. Materials and methods

The experiment was approved by the relevant ethics committee (Agreement from Comité d'éthique du Val de Loire n°19 ref 01012.01). It was conducted in an appropriate facility (Agreement C37-175-1) with cages allowing feed ingestion of individual broilers to be monitored. Thirty three chickens (JA 657), 28 days old, were placed in individual cages for 8 weeks. While 15 chickens were assigned to the control group and were given the control feed, 18 chickens were divided into two groups of 9 chickens to be exposed to PCBs through contaminated feed (Doses 1 and 2). Doses 1 and 2 have been prepared with 20 and 100 µg Aroclor 1260 diluted in hexane and added to 1 kg feed, resp. Animals have been fed with increasing feed quantities over the 8 weeks of the experiment, starting from 68 g/day/animal till 153g/day/animal. Before slaughter (84 days old), blood samples were taken on each animal, while muscle was collected after. Serum and muscle were stored -80°C and -20°C, resp., before analysis.

NDL PCBs concentrations in feed and muscle were measured using ISO 17025 accredited method as implemented within the French National Reference Laboratory.

For lipidomics investigations, the extraction procedure firstly reported by Bligh and Dyer (1959)[1] and further re-adapted by Bird et al. (2011)[2] was employed to extract the lipids. The procedure consisted of a liquid-liquid extraction with MeOH:CHCl3:H2O (1:2:0.63). The CHCl3 extract was evaporated to dryness at 25°C under gentle nitrogen stream, subsequently reconstituted in mobile phase and injected the same day onto the LC-HRMS system. A pooled quality control (QC) samplecomposed of all the

study' subject population was prepared, extracted along with each sample batch and analysed throughout the analytical run, in order to provide robust quality assurance for each metabolic feature detected. Lipid chromatographic separation was performed using two complementary stationary phases. Lipid species separation was performed on a reverse phase CSH C18 column (Waters Corporation, Milford, MA), while lipid classes were separated using a BEH HILIC column from Waters. Both LC chromatographic separations were coupled to an Exactive Orbitrap instrument equipped with a heated electrospray (H-ESI II) ionization source. When reverse phase chromatography was used, a polarity switching ion mode (positive/negative) and "all ion fragmentation" (AIF) MS/MS mode (mass range m/z 100 – 2000). When HILIC separation was applied a full scan acquisition mode (mass range m/z 100 – 2000) operating at a mass resolving power of 100,000 FWHM was selected [3]

3. Results and discussion

- Contaminated feed was analysed for NDL PCBs. Feed contaminated with dose 1 (10 µg Aroclor 1260/ kg feed) and dose 2 (100 µg Aroclor 1260/kg feed) led to NDL PCBs concentrations of 8.2 µg/kg feed and 46.3 µg/kg feed, resp.
According to Directive 2002/32/CE, dose 1 led to compliant feed below the 10µg/kg limit, while dose 2 led to non compliant feed.

- Muscle meat was analysed also for NDL PCBs. Mean value of 4.7 µg/kg lw was measured in control animals, while mean concentrations of 49 and 248 µg/kg lw have been measured in samples collected from chickens fed respectively with doses 1 and 2.
Most muscle samples analysed presented concentrations above the 40 µg/kg lw established threshold (Reg 1259/2011/CE), even when compliant feed (dose 1) was used.

- Using unsupervised data analysis (PCA), lipidomics study enabled discriminating serum from control and PCBs exposed animals (Fig. 1).Further supervised analysis (OPLS) enabled investigating lipids associated to the observed difference (Fig 2).

Chickens exposed through their feed to two different doses of NDL PCB presented similar modified lipids profiles compared to control animals. These observations have been performed using both RP and HILIC chromatographic separations. Statistical models have been validated and lipids involved in the fingerprint differences have been further investigated. This preliminary study suggests that PCBs disturb the lipid fraction, in particular the plasmalogen one, which is associated to cell membrane integrity and anti-oxidative properties. Although the functions of plasmalogens have not yet been fully elucidated, it has been demonstrated that they can protect cells against the damaging effects of reactive oxygen species. The oxidative stress associated to PCB exposure may be hypothesized as the reason of this observed lipid profile disruption.

4. Ackowledgements

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5. References

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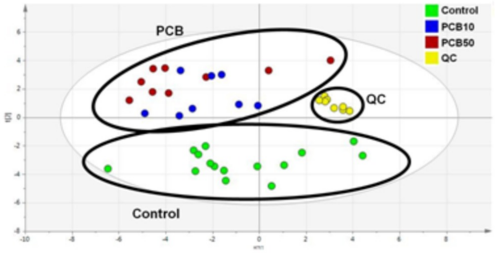

Figure 1: PCA analysis of the lipid fraction obtained from chickens serum samples (RP-LC ESI+)

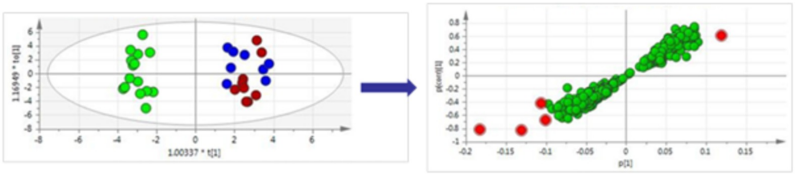

Figure 2: OPLS analysis of the lipid fraction obtained from serum samples of chickens (RP-LC ESI+) (green= control, blue = PCB dose 1 and red = PCB dose 2) (left) and associated S-plot (Y)=0,958 ; Q2(Y)=0,900 et p-value = 4,29 E^{-13}) (right)