

Applying a metabolomics approach to investigate the effects of *Escherichia Coli* LPS and citrus dietary supplementation on broiler chickens

3 Untargeted metabolomics studies (MS-LS based), 2 pilot studies (liver and plasma) 1 complete study (plasma), were performed to look at the effects of *Escherichia Coli* LPS challenge and dietary effect (citrus and cucumber) on the metabolome of broilers at T0h pre challenge and T12, T24, T48h post challenge.

A dietary trial (12birds x4replicates x3dietary treatments) was performed to test the effects of citrus diet (CTS) and cucumber diet (CMB) on the metabolome of broilers. Birds were challenged at 15 days old with *E.Coli* LPS and plasma and liver samples collected pre and post challenge at 4 time points (T0,12,24,48h).

In the pilot studies, 48 samples were extracted and pooled based on pen, diet and time points (T0,T12,T24,T48h) while in the complete study, unpooled samples (N=92) of control diet (CTL) and CTS diet were used. The workflow used for the two untargeted metabolomics studies was as follow: samples were extracted using chloroform/methanol/water (1:3:1) and separated by liquid chromatography (LC) before the MS/MS identification. Data obtained from MS were investigated using Polyomics integrated Metabolomics Pipeline (Pimp) and Metaboanalyst software.

In the datasheet deposited the data referred to the following pairwise comparisons (T-tests) depending on dietary treatment and LPS challenge are shown:

CTS_T0 relative to CTL_T0
CTS_T12 relative to CTL_12
CTS_T24 relative to CTL_T24
CTS_T48 relative to CTL_T48

CTL_T12 relative to CTL_T0
CTL_T24 relative to CTL_T0
CTL_T48 relative to CTL T0

Data includes the peak ID, metabolite description, p-value, adjusted p-value, log fold change peak intensities and identification.