

Electronic Supplementary Information captions:

Table S1: List of identified metabolites shown on the IDEOM spreadsheet and comparison of their concentration with 0-hour time-point after intramammary challenge with *Streptococcus uberis*. This spreadsheet is an output of the IDEOM software.¹ This includes details about the identified metabolites such as their mass, retention time, chemical formula and pathway mapping. For each metabolite, its change in post-challenge time-points compared to the pre-challenge time-point (0-hour) is given as ratio (Time0 to Time312) along with the results from T-test. Other statistical measures like mean, standard deviation, relative standard deviation are also given for each metabolite. This spreadsheet includes macros, but the links may not work in isolation. So, please ignore the Excel dialogue while opening the spreadsheet.

Table S2: ANOVA table comparing the quantified putative metabolites with 0-hour time-point after intramammary challenge with *Streptococcus uberis*. This also includes the list of differentially expressed metabolites at 36, 42, 57, 81 and 312 hours after intramammary challenge with *Streptococcus uberis* in separate sheets within the workbook. The ANOVA table includes the putative metabolites, their mass, retention time, chemical formula, mapped pathway major class, mapped pathway, identified base peak, p-value, FDR-step-up p-value, change ratio, and fold-change for each time-point compared with 0-hour time-point post-challenge. The list of metabolites differentially expressed at each time-point compared with the 0-hour post-challenge were generated from the ANOVA table using a threshold of fold-change > 2 and FDR-step-up p-value < 0.05.

Tables S3 – S7: Results of metabolic pathway representation in the metabolites using Pathos web-tool at 36, 42, 57, 81 or 312 hours after intramammary challenge with *Streptococcus uberis*. The intensity ratio for each metabolite at each post-challenge time-point compared with the 0-hour post-challenge time-point was used in the Pathos web-tool to identify the represented metabolic pathways, and their direction and severity of change. The results show the number of metabolites in the dataset mapping to a particular pathway, total number of metabolites included in the pathway and the number of metabolites changing at that particular comparison. The metabolites mapped to the pathway are listed with colour coded tags showing their direction and severity of change.

Figure S1: Base peak chromatograms of samples at 0, 36, 42, 57, 81 and 312 hours after intramammary challenge with *Streptococcus uberis*. The raw data obtained from the LC-MS runs were explored by generating various plots using MZmine. This file includes base peak chromatograms generated from raw files obtained from both positive and negative modes from each sample at a given time-point. The samples from each time-point show a similar chromatographic profile without significant drift.

Figure S2 – S4: Visualization of pathway mappings on iPath web-tool. The identified metabolites were uploaded to iPath web-tool to generate pathway maps of KEGG metabolic pathways (Figure S2), KEGG regulatory pathways (Figure S3) and KEGG pathways involved in biosynthesis of secondary metabolites (Figure S4).

1. D. J. Creek, A. Jankevics, K. E. Burgess, R. Breitling and M. P. Barrett, *Bioinformatics*, 2012, **28**, 1048–1049.