Supplemental Material

Use of Shotgun Metagenomics and Metabolomics to Evaluate the Impact of Glyphosate or Roundup MON 52276 on the Gut Microbiota and Serum Metabolome of Sprague-Dawley Rats

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Figure S1. Preparation of metabolomics technical replicates. A small aliquot of each sample (colored cylinders) is pooled to create a CMTRX technical replicate sample (multi-colored cylinder), which is then injected periodically throughout the platform run. Variability among consistently detected biochemicals can be used to calculate an estimate of overall process and platform variability (Table S2 to S4). Figure provided by Metabolon Inc. as part of their results package.

Figure S2. Correlations between the serum metabolome and the caecum metabolome. The Mantel.test function in the cultevo package of R was used to calculate the Mantel statistic for a Spearman's rank correlation coefficient between the log-transformed abundance values of the caecum metabolome and the serum metabolome datasets. The figure shows the density distribution of 1,000 permuted Spearman's rank correlation coefficients (Spearman's ρ, x-axis) from permutations of the samples. The blue arrow shows the Spearman's rank correlation coefficient for the unpermuted samples, with the empirical p-value of the Mantel test calculated as from a Monte-Carlo procedure described in North, et al. 2002, The American Journal of Human Genetics, 71 (2): 439—41.

Figure S3. Chromatograms following HPLC-MSMS for the detection of shikimic acid. Optimisation of the shikimic acid quantification assay were done in serum (top panel), water (middle panel) and in a 5% bovine serum albumin solution (lower panel). Y-axis: peak height (relative units).

Figure S4. Proteins interfere with the detection of shikimic acid in serum. Although a dose dependent increase in peak area (arbitrary units) was detected after spiking of samples with shikimic acid, the presence of proteins severely interfered with the detection of the signal and limited the reproducibility and sensitivity of this assay. BSA, bovine serum albumin.

Figure S5. The results of the analysis of 16S rRNA abundance correlates well with the results of the analysis of the complete shotgun metagenomics dataset. Statistical significance of the effects of the treatments was assessed with aldex2 using the complete shotgun metagenomics dataset (taxonomy inferred with the RefSeq database on the metagenomics RAST server) or a subset of 16S rRNA reads isolated from the shotgun metagenomics datasets using the metagenomics RAST server with a cut-off of 70% identity to ribosomal sequences from a reduced version of M5RNA with SortMeRNA. Statistical analysis was performed on a dataset corrected for asymmetry (uneven sequencing depths) using the inter-quartile log-ratio method, which identifies features with reproducible variance. We assessed statistical significance using a Kruskal–Wallis test. The p-values for statistical significance from these both analyses were transformed (- log10). The resulting -log10 p-values for each of the taxonomic group detected in both the complete shotgun metagenomics dataset and its 16S rRNA gene subset were correlated (p = 0.003) and plotted.

Table S1. Experimental design and sampling (N = number of animals measured).

Table S2. Description of Metabolon quality control (QC) Samples.

Table S3. Metabolon QC Standards.

Table S4. Serum and caecum metabolomics quality control.

Table S5. Determination of Shikimic Acid in serum by LCMSMS. Version 201023.

Table S6. Composition of the de Man, Rogosa et Sharpe (MRS) broth used in the *in vitro* study of bacterial growth.

Additional File- Excel Document

Contaminant Screening.pdf File – Feed analysis to identify possible contaminants.