**NMR Metabolomics Methods for Blaser Cecal contents samples**

Frozen cecal contents (cecal) samples were weighed into labeled homogenizer bead tubes. D20 was added to each tube (500 µL if less than 50 mg and 1000 µL if more than 100 mg of tissue). The samples were homogenized on a Spex Geno/Grinder for two 30 second pulses at 1750 rpm. Samples were centrifuged at 12000 rcf for 5 min. Cecal supernatants were transferred (450/700 µL) into 2.0 mL 0.2 µm nylon filter tubes and centrifuged at 16000 rcf until all the homogenate was filtered. The 200 µL remaining aliquot from each 1000 µL cecal sample was combined in a 10 mL tube and vortexed for 30 seconds to generate pooled samples for QC during analysis. Three “low” QC pools were generated by transferring 450 µL of the pooled homogenate into 2.0 mL 0.2 µm nylon filter tubes; and three “high” QC pools were generated by transferring 700 µL of the pooled homogenate into 2.0 mL 0.2 µm nylon filter tubes and also centrifuged at 16000 rcf until all the homogenate was filtered. The volume of homogenate needed to analyze 50 mg/sample and volume of D20 needed to bring the total volume to 630 µL was calculated. The calculated volumes of filtered supernatant and D20 were then transferred into BSI-labeled tubes. Chenomx Internal Standard solution (Chenomx ISTD, Edmonton, Alberta, Canada) contains 5mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Chemical Shift Indicator), 100 mM Imidazole (pH indicator), and 0.2% NaN3 (to inhibit bacterial growth) was added (70 µl) to the tubes. Samples were vortexed for 30 seconds and centrifuged at 12000 rcf for 5 minutes. A 600 µL aliquot of the supernatant was then transferred into 5mm NMR tubes (Bruker-BioSpin, Switzerland) which were kept on ice until data acquisition.

1H NMR spectra of cecal samples were acquired on a Bruker Avance III 700 MHz NMR spectrometer (located at the David H. Murdock Research Institute at Kannapolis, NC, USA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25℃. A 1D NOESY presaturation pulse sequence (noesypr1d, [recycle delay (RD)-90°-t1-90°-tm-90°-acquire free induction decay (FID)]) was used for data acquisition. For each sample 64 transients were collected into 64k data points using a spectral width of 14.01 kHz (20.14 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 2.324 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. NMR spectra were processed using ACD NMR software (Advanced Chemistry Development, Toronto, ON, Canada). NMR bins (0.50-9.00 ppm) were made after excluding water (4.70-4.90 ppm) and imidazole (7.22-7.34 ppm) using Intelligent Bucketing Integration with a 0.04 ppm bucket width and a 50% looseness factor. Integrals of each of the bins were normalized to total integral of each of the spectrum.