Methods:

Sample preparation:

Mouse fecal samples were extracted and derivatized as described previously (J.C. Chan, D.Y. Kioh, G.C. Yap, B.W. Lee, E.C. Chan A Novel LCMSMS Method for Quantitative Measurement of Short-Chain Fatty Acids in Human Stool Derivatized With 12 C- And 13 C-labelled Aniline.J. Pharm. Biomed Anal., 138 (2017), pp. 43-53). Briefly, ice-cold extraction solvent (1:1 v/v ACN/water) was added to fecal sample at a ratio of 2uL:1 mg sample and internal standard mix to a final concentration of 100 uM. and subjected to vortex mixing for 3 min at room temperature and sonicate for 15 min. The suspension was then centrifuged at 18,000g for 15 min at 4◦C. An aliquot of 100 uL was subsequently derivatized using a final concentration of 10 mM aniline and 5 mM EDC for 2 h at 4◦C. Derivatization reaction was quenched using a final concentration of 18 mM succinic acid and 4.6 mM 2-mercaptoethanol for 2 h at 4◦C. All samples were stored at 4◦C until analysis on the same day.

Mixed calibrators of acetic acid, propanoic acid, butyric acid and isobutyric acid (10nM- 10×103nM) together with single- and double- blanks, spiked with internal standard mix (Acetic acid-d3, propanoic acid-d2, butyric acid-d2) to a final concentration of 100 µM were prepared and subjected to the same sample preparation procedure as fecal samples.

Liquid Chromatography Mass Spectrometry (LC-MS):

Derivatized samples were analyzed using an ultra-high performance liquid chromatography (UHPLC) system 1290 connected to a quadrupole time of flight (Q-TOF 6545) mass spectrometer from Agilent Technologies (Santa Clara, CA, USA) equipped with an orthogonal DUAL AJS-ESI interface. Samples were subjected to reverse phase C18 separation (Phenomenex Scherzo SS-C18 100 x 2 mm and data were collected in positive ion mode. Data were acquired from 50 to 750 m z-1 at 2 spectra s-1. Electrospray ionization (ESI) source conditions were set as follows: gas temperature 325 °C, drying gas 9 L min-1, nebulizer 35 psi, fragmentor 125 V,
sheath gas temperature 350 °C, sheath gas flow 8 L min-1, nozzle voltage 1000V. For reverse phase C18 chromatographic separation, a two-solvent gradient running at 0.3 mL min-1 (Mobile Phase: A: 100:0.1 Water: Formic Acid, B: 100:0.1 Isopropanol: Formic Acid) was used. Column was equilibrated at 15% B for 1 min and a sample was introduced. The solvent ratio was then increased from 15% B to 100% B over 13 min and then reduced back to 15% B over 2 min. Injection volume is 5 μL and column temperature of 45 °C.   The LC-MS/MS data acquired using Agilent Mass Hunter Workstation (.d files) were processed in quantitative analysis software (Agilent Technologies) for quantitative analysis of samples. The linear calibration plots for acetic acid, propanoic acid, butyric acid and isobutyric acid were constructed using peak area ratios of each analyte to the IS versus the concentrations of calibrators (x) with 1/x weighting, and the least squares linear regression equations were obtained as the calibration equations for individual analytes.