## Short Chain Fatty Acid (SCFA) Analysis by Gas Chromatograph tandem mass spectrometry

Instrumentation: Agilent 8890 gas chromatograph coupled with an Agilent 7010B triple quadrupole mass spectrometer with CTC PAL autosampler equipped with headspace and SPME options and a split/splitless injector.

The column used is an Agilent DB FATWAX UI 30 meter column, 0.25mm diameter and 0.25um film thickness with Helium as carrier gas at a flow of 1.2ml/min.

Cecal or stool samples and standard curves of underivatized SCFA were analyzed by headspace injection after incubation in acidified water at 95C for 40 minutes and utilized a 0.5ml injection and a 10:1 split. The column oven was temperature programmed from 90C to 230C over 18 minutes with baseline separation of all SCFA. Calibration curves utilized authentic standards and deuterium labeled internal standards of acetic, propionic, butyric and caproic, all purchased from Millipore Sigma or CDN isotopes. The GCMS was operated in SRM mode and regression lines were calculated using quadratic fits with correlation coefficients of 0.995 to 0.9995.