

Supplementary Materials

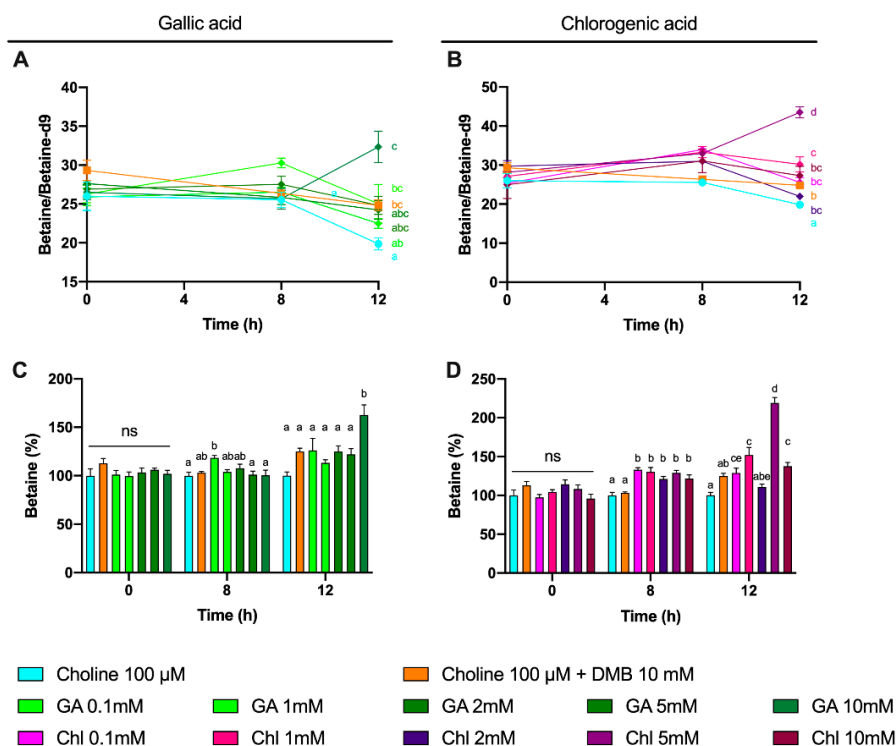


Figure S1: Effect of gallic acid and chlorogenic acid (A,B) in the peak area of betaine relativized to its internal standard betaine-d₉. Relative changes in betaine/betaine-d₉ versus choline 100 μ M conditions (C,D). All fermentations were carried out at optimal conditions (fecal slurry 20 % and choline 100 μ M). Results are expressed as % \pm SEM ($n=6$). Different letters indicate statistical differences ($p<0.05$) by One-way (C,D) or Two-way (A,B) ANOVA (Tukey's post hoc test). Factors for Two-way ANOVA were gallic acid or chlorogenic acid concentration and time. Abbreviations: DMB, 3,3-dimethyl-1-butanol; GA, gallic acid; and Chl, chlorogenic acid.

Table S1. Molecular weight (MW), retention time (RT) and optimized MRM condition for TMA and related compounds.

Compound	MW	RT (min)	MS/MS Transition	CV (V)	CE (eV)
Choline	103.16	1.01	104.2>60.0	38	16
Choline-d ₉	112.16	1.02	113.3>69.1	40	16
L-carnitine	161.20	2.17	162.3>85.0	34	20
L-carnitine-d ₉	170.25	2.17	171.3>85.0	34	20
Betaine	117.15	1.07	118.2>59.4	44	18
Betaine-d ₉	126.15	1.07	127.3>68.1	46	18
γ -Butyrobetaine	145.20	2.26	146.3>87.0	26	16
TMAO	75.11	1.35	76.2>58.9	40	10
TMAO-d ₉	84.12	1.39	85.2>68.1	40	12
TMA ^a	59.11	0.76	146.3>118.2	34	16
TMA-d ₉ ^a	68.17	0.75	155.3>127.2	34	20

Abbreviations: TMAO, trimethylamine *N*-oxide; TMA, trimethylamine; MW, molecular weight; RT, retention time CV, cone voltage; and CE, collision energy. ^a Molecular weight listed is for the original molecules, and MS/MS transitions listed for derivatives (ethyl betaine and ethyl betaine-d₉).

Table S2: Parameters for the quantification of TMA and TMA-related compounds in spiked fecal fermentation samples by UPLC-MS/MS.

Compound	Calibration curve	R ²	Working linearity range (μM)	LOD (μM)	LOQ (μM)	MDL (μM) ^a	MQL (μM) ^a
Choline	$y = 0.765x + 3.003$	0.997	1.93 – 247.10	0.488	1.628	0.977	3.256
L-carnitine	$y = 5.932x - 4.723$	0.997	0.24 – 497.72	0.024	0.081	0.049	0.162
γ-Butyrobetaine	$y = 0.197x + 0.181$	0.998	0.27 – 37.92	0.032	0.105	0.063	0.210
TMAO	$y = 0.355x - 0.978$	0.997	0.43 – 228.71	0.011	0.037	0.075	0.075
TMA	$y = 58.301x + 13.776$	0.999	0.84 – 462.73	0.194	0.648	0.387	1.291

Abbreviations: TMAO, trimethylamine N-oxide; TMA, trimethylamine; R², determination coefficient; LOD, limit of detection; LOQ, limit of quantification; MDL, method detection limit; and MQL, method quantification limit. ^a MDL and MQL for 25 μL of fecal fermentation media.