Applied Microbiology and Biotechnology

Supplementary Material

Fecal tryptophan metabolite profiling in newborns in relation to microbiota and antibiotic treatment

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Table S1. Characteristics of individual neonates participating in the study.

Sample	Gestation age	Mode of delivery	Medication	Anamnesis of mother	Anamnesis of neonate	Sex	Length (cm)	Weight (g)	Apgar score
1	40+2	Vaginal	---	---	---	Male	51	3600	[9, 10, 10]
2	39+6	Vaginal	Epidural	---	Erythema	Male	50	3240	[10, 10, 10]
3	40+4	Vaginal	---	---	Intrauterine hypoxia, jaundice	Female	52	3410	[6, 9, 10]
4	40+4	Vaginal	---	Anemia	Erythema	Male	49	3240	[9, 9, 10]
5	41+0	Vaginal	---	---	Jaundice, traumatism	Female	50	3760	[8, 9, 9]
6	39+6	C-Section	---	---	Erythema	Female	47	3220	[10, 10, 10]
7	39+3	Vaginal	Oxytocin	Anemia	Intrauterine hypoxia	Male	51	3230	[3, 7, 9]
8	40+1	Vaginal	---	---	---	Male	52	3630	[10, 10, 10]
9	40+1	Vaginal	---	Anemia, diabetes	---	Female	49	3320	[10, 10, 10]
10	39+1	Vaginal	---	Asthma	---	Male	50	3240	[10, 10, 10]
11	40+0	Vaginal	---	Anemia	Erythema	Female	49	3820	[10, 10, 10]
12	41+3	C-Section	---	---	Erythema	Male	49	3250	[9, 9, 10]
13	39+3	C-Section	---	---	Jaundice	Male	46	2490	[5, 8, 8]
14	40+2	Vaginal	Oxytocin	---	---	Female	49	3320	[10, 10, 10]
15	41+0	Vaginal	Oxytocin	*Streptococcus*	---	Female	49	3690	[9, 10, 10]
16	40+2	Vaginal	Epidural	---	Intrauterine hypoxia	Male	50	3360	[10, 10, 10]
17	40+5	C-Section	Epidural	Asthma	---	Male	51	3900	[7, 7, 8]
18	40+4	Vaginal	---	---	---	Male	54	3910	[10, 10, 10]
19	40+5	Vaginal	---	Anemia, *Streptococcus*	---	Female	50	3600	[9, 10, 10]
20	39+5	C-Section	---	---	---	Female	51	3790	[9, 10, 10]
21	40+0	Vaginal	---	---	Intrauterine hypoxia	Male	52	3800	[10, 10, 10]
22	38+5	Vaginal	---	---	Intrauterine hypoxia, erythema	Female	48	2750	[9, 9, 9]
23	39+3	Vaginal	---	---	---	Male	52	3950	[9, 9, 10]

Sample	**Gestation age**	**Mode of delivery**	**Medication**	**Anamnesis of mother**	**Anamnesis of neonate**	**Sex**	**Length (cm)**	**Weight (g)**	**Apgar score**
24	40+0	Vaginal	---	---	---	Male	48	3200	[9, 10, 10]
25	39+3	Vaginal	Epidural	Anemia	Jaundice	Female	52	3520	[9, 10, 10]
26	38+6	Vaginal	---	---	Erythema, traumatism	Male	49	3030	[9, 10, 10]
27	39+5	Vaginal	---	---	---	Male	53	4100	[10, 10, 10]
28	39+4	Vaginal	---	---	Jaundice	Female	48	3040	[10, 10, 10]
29	40+4	Vaginal	Epidural	---	Intrauterine hypoxia, jaundice	Male	51	3730	[9, 10, 10]
30	40+3	Vaginal	Entonox	Anemia	---	Male	60	3390	[9, 10, 10]
31	40+3	Vaginal	---	Anemia	Erythema, jaundice	Male	51	3770	[9, 10, 10]
32	39+2	Vaginal	Epidural	Asthma	Erythema, traumatism	Male	48	2960	[10, 10, 10]
33	39+4	Vaginal	Epidural	---	---	Female	49	3210	[10, 10, 10]
34	40+2	Vaginal	---	---	---	Male	49	3370	[9, 10, 10]
35	40+5	Vaginal	Oxytocin	---	---	Female	49	3200	[9, 10, 10]
36	39+4	Vaginal	---	---	Intrauterine hypoxia	Male	50	3360	[9, 10, 10]
37	39+5	Vaginal	Epidural	Anemia, *Streptococcus*	Intrauterine hypoxia	Female	50	3650	[8, 10, 10]
38	38+6	Vaginal	---	Anemia	---	Male	49	3260	[9, 10, 10]
39	40+2	Vaginal	---	---	---	Male	52	3540	[10, 10, 10]
40	39+6	Vaginal	Epidural	---	Jaundice	Female	51	3550	[9, 9, 9]
41	40+2	Vaginal	---	Anemia	Congenital pneumonia	Male	51	3590	[8, 9, 9]
42	40+2	Vaginal	Epidural	Anemia	---	Female	50	3700	[9, 9, 10]
43	39+5	Vaginal	---	---	Jaundice	Male	51	2900	[9, 10, 10]
44	39+5	Vaginal	Epidural	Asthma	Erythema, jaundice	Male	53	3540	[8, 10, 10]
45	39+3	C-Section	---	*Streptococcus*	---	Female	50	2880	[10, 10, 10]
46	39+1	Vaginal	---	Asthma	Jaundice	Male	51	3740	[9, 10, 10]
47	40+6	Vaginal	---	---	Erythema	Male	51	4180	[5, 8, 9]
48	39+0	C-Section	Oxytocin	Anemia	---	Female	50	3000	[10, 10, 10]

Sample	Gestation age	Mode of delivery	Medication	Anamnesis of mother	Anamnesis of neonate	Sex	Length (cm)	Weight (g)	Apgar score
49	40+4	Vaginal	Epidural	---	---	Female	51	3460	[10, 10, 10]
50	40+1	Vaginal	---	---	Traumatism	Female	50	3120	[10, 10, 10]
51	40+0	C-Section	---	---	---	Male	49	3780	[9, 10, 10]
52	39+3	Vaginal	Epidural	Asthma	Erythema, traumatism	Male	51	2870	[9, 10, 10]
53	40+3	Vaginal	---	Anemia	Erythema	Male	52	3550	[10, 10, 10]
54	40+2	Vaginal	---	---	Traumatism	Female	47	2810	[9, 10, 10]
55	40+2	Vaginal	---	Anemia	---	Female	51	3380	[10, 10, 10]
56	39+6	Vaginal	Epidural	---	Intrauterine hypoxia, erythema	Female	52	3340	[9, 10, 10]
57	40+4	Vaginal	Epidural	---	---	Male	55	4190	[9, 10, 10]
58	40+5	Vaginal	Epidural	---	---	Female	49	2670	[10, 10, 10]
59	40+1	Vaginal	---	---	---	Male	52	3420	[9, 10, 10]
60	41+1	Vaginal	---	---	Erythema	Female	55	4290	[9, 9, 10]
61	40+6	Vaginal	Epidural	*Streptococcus*	Intrauterine hypoxia	Female	50	3220	[9, 9, 10]
62	40+3	C-Section	Oxytocin	---	Intrauterine hypoxia	Male	53	4900	[10, 10, 10]
63	40+5	Vaginal	---	---	---	Male	49	3300	[10, 10, 10]
64	38+5	Vaginal	---	Anemia	Jaundice	Female	49	2750	[9, 9, 10]
65	40+6	Vaginal	---	---	---	Female	52	3860	[9, 9, 9]
66	40+3	Vaginal	Epidural	---	Intrauterine hypoxia, erythema	Male	54	3740	[7, 9, 9]
67	40+3	Vaginal	Entonox	*Streptococcus*	---	Male	50	3030	[10, 10, 10]
68	39+4	Vaginal	---	---	Erythema	Male	50	3380	[9, 9, 9]
69	38+5	C-Section	Epidural	---	Intrauterine hypoxia, jaundice	Male	49	4070	[9, 9, 9]
70	40+5	Vaginal	Epidural	---	Intrauterine hypoxia	Female	51	3300	[9, 10, 10]
71	40+1	Vaginal	Epidural	---	Intrauterine hypoxia, jaundice	Male	49	3280	[9, 9, 9]

Sample	Gestation age	Mode of delivery	Medication	Anamnesis of mother	Anamnesis of neonate	Sex	Length (cm)	Weight (g)	Apgar score
72	40+0	Vaginal	Nalbuphine	---	Intrauterine hypoxia, erythema	Female	48	2840	[9, 9, 10]
73	40+6	Vaginal	---	---	---	Male	51	3570	[8, 9, 10]
74	39+2	Vaginal	---	---	---	Male	50	3150	[10, 10, 10]
75	41+2	Vaginal	---	---	Erythema, jaundice	Male	51	3580	[5, 8, 9]
76	40+5	Vaginal	---	---	Jaundice	Female	50	3120	[5, 7, 9]
77	40+2	Vaginal	Oxytocin	*Streptococcus*	Erythema	Male	52	4350	[8, 9, 10]
78	41+0	Vaginal	Entonox	---	---	Male	50	3420	[9, 10, 10]
79	40+1	Vaginal	Oxytocin	*Streptococcus*	Intrauterine hypoxia	Male	52	3290	[5, 7, 8]
80	40+5	C-Section	Epidural	*Streptococcus*	Intrauterine hypoxia	Male	52	3700	[9, 10, 10]
81	39+4	Vaginal	---	Anemia	Intrauterine hypoxia	Female	50	4140	[9, 10, 10]
82	40+5	Vaginal	Epidural	*Streptococcus*	---	Female	52	3440	[8, 10, 10]
83	41+1	Vaginal	Epidural	Anemia	Traumatism	Female	52	3930	[9, 9, 10]
84	40+5	Vaginal	Epidural	---	Erythema	Male	51	3790	[10, 10, 10]
85	40+3	Vaginal	---	Anemia	Erythema	Female	52	3760	[10, 10, 10]
86	40+4	Vaginal	---	---	Erythema	Female	52	3640	[10, 10, 10]
87	40+0	Vaginal	---	---	Erythema	Male	50	2850	[9, 9, 10]
88	39+2	C-Section	---	---	Intrauterine hypoxia	Male	50	3810	[1, 6, 8]
89	38+6	Vaginal	---	---	---	Male	50	3530	[9, 10, 10]
90	38+6	Vaginal	---	Anemia	Erythema	Female	50	3430	[10, 10, 10]
91	40+0	Vaginal	---	*Streptococcus*	---	Female	47	3230	[10, 10, 10]
92	40+4	Vaginal	Epidural	---	Erythema	Male	52	3810	[9, 10, 10]
93	39+2	Vaginal	---	---	---	Female	50	3250	[10, 10, 10]
94	39+2	Vaginal	---	---	---	Male	50	3900	[9, 10, 10]
95	39+2	C-Section	---	---	---	Male	49	3130	[9, 9, 10]
96	41+3	Vaginal	---	---	---	Female	50	3700	[9, 10, 10]
97	38+6	C-Section	Remifentanil	---	Erythema, jaundice	Female	50	3950	[10, 10, 10]

Sample	Gestation age	Mode of delivery	Medication	Anamnesis of mother	Anamnesis of neonate	Sex	Length (cm)	Weight (g)	Apgar score
98	39+3	Vaginal	Oxytocin	---	---	Female	51	3510	[10, 10, 10]
99	40+5	Vaginal	Epidural	Anemia, diabetes	Intrauterine hypoxia	Male	50	3750	[9, 9, 9]
100	39+6	Vaginal	Oxytocin	---	Jaundice	Male	50	3200	[9, 10, 10]
101	39+3	Vaginal	---	---	---	Female	54	3820	[9, 10, 10]
102	40+1	Vaginal	---	Diabetes	Intrauterine hypoxia, jaundice	Female	51	4010	[9, 10, 10]
103	41+0	Vaginal	---	*Streptococcus*	Erythema	Male	51	3730	[9, 10, 10]
104	40+2	Vaginal	Epidural	---	Erythema	Male	50	3300	[8, 9, 10]
105	40+1	Vaginal	---	---	Jaundice	Male	51	3590	[10, 10, 10]
106	39+1	C-Section	---	Anemia	Intrauterine hypoxia	Male	47	2960	[1, 0, 0]
107	40+2	Vaginal	Epidural	---	---	Male	50	3000	[10, 10, 10]
108	40+0	C-Section	Epidural	---	Erythema	Female	47	2930	[9, 10, 10]
109	39+4	C-Section	---	---	---	Male	47	3300	[9, 10, 10]
110	39+0	Vaginal	---	Anemia	Erythema	Male	47	2470	[9, 10, 10]
111	41+3	Vaginal	Epidural	---	---	Male	51	4190	[10, 10, 10]
112	41+0	Vaginal	---	Asthma	---	Female	53	3550	[8, 8, 9]
113	39+5	Vaginal	Oxytocin	Anemia, asthma, diabetes	---	Female	50	3730	[9, 10, 10]
114	39+3	C-Section	---	---	---	Male	50	2960	[9, 9, 9]
115	41+1	Vaginal	---	Anemia	Intrauterine hypoxia	Male	52	3170	[8, 10, 10]
116	40+3	Vaginal	---	Diabetes	---	Female	51	3680	[9, 10, 10]
117	40+4	Vaginal	---	---	---	Male	50	4130	[10, 10, 10]
118	40+4	Vaginal	---	Anemia	Erythema	Female	51	4110	[9, 9, 10]
119	39+2	Vaginal	---	---	---	Male	48	3180	[9, 10, 10]
120	40+0	Vaginal	---	*Streptococcus*	---	Female	51	3820	[9, 9, 9]
121	40+6	Vaginal	---	---	---	Female	53	4450	[9, 10, 10]
122	39+0	Vaginal	---	---	---	Male	48	3230	[9, 9, 9]

Sample	**Gestation age**	**Mode of delivery**	**Medication**	**Anamnesis of mother**	**Anamnesis of neonate**	**Sex**	**Length (cm)**	**Weight (g)**	**Apgar score**
123	38+2	C-Section	Epidural	Anemia	---	Male	49	3230	[9, 10, 10]
124	41+1	C-Section	---	---	---	Female	53	3930	[10, 10, 10]
125	40+2	Vaginal	---	---	---	Male	51	4000	[10, 10, 10]
126	40+6	Vaginal	---	---	Erythema, jaundice	Male	53	4130	[7, 9, 10]
127	40+1	Vaginal	---	---	---	Female	52	3620	[9, 9, 10]
128	39+4	Vaginal	---	---	Jaundice	Male	49	3450	[7, 8, 9]
129	40+6	Vaginal	Epidural	---	Jaundice, traumatism	Female	52	3690	[10, 10, 10]
130	40+2	Vaginal	Entonox	---	---	Male	50	3380	[9, 9, 9]
131	40+4	Vaginal	Oxytocin	---	Intrauterine hypoxia	Male	54	4560	[9, 10, 10]
132	39+6	Vaginal	Oxytocin	Anemia	Erythema, jaundice, traumatism	Male	50	3090	[9, 10, 10]
133	39+5	Vaginal	---	---	Erythema	Female	49	2980	[9, 10, 10]
134	40+1	Vaginal	---	Diabetes	Intrauterine hypoxia, jaundice	Male	52	3430	[10, 10, 10]

Table S2. List of primer pairs targeting the hypervariable V4 region (515F-806R) of the bacterial 16S rRNA gene

Primer name		5´-sequence-3´	Reference of primers
EMP16S-1	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCCTTCGTCGCGTGTGYCAGCMGCCGCGGTAA	**16S Metagenomic Sequencing Library protocol**; Illumina, San Diego, California, USA (EMP515-806)
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTAACGGTCCACCGGACTACNVGGGTWTCTAAT	
EMP16S-2	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCATACCGGAAGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGCGCCTTAAACCCGGACTACNVGGGTWTCTAAT	
EMP16S-3	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCCCTGCTACAGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATGGTACCCAGCCGGACTACNVGGGTWTCTAAT	
EMP16S-4	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGAGACCCTACAGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTCTACGTCGCCGGACTACNVGGGTWTCTAAT	
EMP16S-5	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTTGGTGTAAGGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTACTGAGGATCCGGACTACNVGGGTWTCTAAT	
EMP16S-6	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTACGTATCATGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATTCACCTCCTCCGGACTACNVGGGTWTCTAAT	
EMP16S-7	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACGCAGTCTACGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGTATAAATGCGCCGGACTACNVGGGTWTCTAAT	
EMP16S-8	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTGCACGCCATGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGCTGCAACACCCGGACTACNVGGGTWTCTAAT	
EMP16S-9	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCGGACAAGAAGGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTCGCTCGCTGCCGGACTACNVGGGTWTCTAAT	
EMP16S-10	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGCTGGACGCTGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTCCTTAGTAGTCCGGACTACNVGGGTWTCTAAT	
EMP16S-11	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTACTAACGCGGTGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGTCCGTATGAACCGGACTACNVGGGTWTCTAAT	
EMP16S-12	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCGATCACACCTGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGTGAGGAACGCCGGACTACNVGGGTWTCTAAT	
EMP16S-13	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAAACGCACTAAGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTTGCCCTGTACCGGACTACNVGGGTWTCTAAT	
EMP16S-14	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGAGGGTTGAGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATATAGCCCGACCGGACTACNVGGGTWTCTAAT	
EMP16S-15	F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGAGTGGTCTGTGTGTGYCAGCMGCCGCGGTAA	
	R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTATGAGATCCCGGACTACNVGGGTWTCTAAT	

Mass Spectrometry Data Processing. The area under a chromatographic peak (AUC) was used to calculate concentration (nM and mg/L). The AUC and the known concentration of labelled internal standards were used to calculate the LOD (Equation 2) and LOQ (Equation 3) using the slope of the calibration curve.

$$LOD = \frac{SD * 3}{slope}$$

Equation S-1: Calculation for the Limit of Detection for labelled standard.

$$LOQ = \frac{SD * 10}{slope}$$

Equation S-2: Calculation of the Limit of Quantification for labelled standards.

For non-labelled standards, the response factor determined for each analyte was used to calculate respective LOD (Equation 4) and LOQ (Equation 5).

$$LOD = \frac{LOD_{labelled\ metabolite}}{RF_{labelled\ metabolite}}$$

Equation S-3: Calculation of Limit of Detection for non-labelled metabolites.

$$LOQ = \frac{LOQ_{labelled\ metabolite}}{RF_{labelled\ metabolite}}$$

Equation S-4: Calculation of Limit of Quantification for non-labelled metabolites.

Linearity. The measured metabolites were normalized through the ratio between an unlabelled and a labelled metabolite peak area. The normalized metabolite area was plotted against the metabolite concentration of non-labelled metabolites. The area measured for each dilution was plotted against the concentration of the non-labelled metabolite. For every labelled standard in the linearity curve, the Coefficient of variation (CV) was calculated (Equation 6).

$$\text{Coefficient of Variation} = \frac{\bar{x}_{Area\ of\ sample}\ x\ 100}{s_{Standard\ deviation\ of\ each\ dilution\ step}}$$

Equation S-5: Calculation of Coefficient of Variation (CV)

Table S3. SRM assay library for positive ion detection mode. Compounds marked with a *are transitions used for quantification in the stool matrix.

Compound	**Precursor m/z**	**Product m/z**	**RT [min]**
L-Kynurenine*	209.09	192	1.6
L-Kynurenine	209.09	94	1.6
[$^{2}D_{4}$] L-Kynurenine*	215.13	198	1.6
[$^{2}D_{4}$] L-Kynurenine	215.13	96.1	1.6
L-Tryptophan	205.1	188	2.9
L-Tryptophan*	205.1	91	2.9
[$^{13}C_{11}$] [$^{15}N_{2}$] L-Tryptophan	218.1	200	2.9
[$^{13}C_{11}$] [$^{15}N_{2}$] L-tryptophan*	218.1	98.1	2.9
Anthranilic acid	138.06	120	7.2
Anthranilic acid*	138.06	65	7.2
[$^{13}C_{6}$] Anthranilic acid	144.08	126.1	7.2
[$^{13}C_{6}$] Anthranilic acid*	144.08	70.2	7.2
N-Acetyl-tryptophan*	247.11	188.1	7.8
N-Acetyl-tryptophan	247.11	130	7.8
N-Acetyl-tryptophan	247.11	118.1	7.8
Indole-3-lactic acid	206.06	188	7.8
Indole-3-lactic acid	206.06	130.1	7.8
Indole-3-lactid acid*	206.06	117.9	7.8
Indole-3-aldehyde*	146.06	118.1	8
Indole-3-aldehyde	146.06	117.1	8
Indole-3-aldehyde	146.06	91.1	8
Indole-3-aldehyde	146.06	65.1	8
Indole-3-aldehyde	146.06	39.2	8

Table S4. List of all metabolite standards generated for absolute quantification.

Analyte	Short name	Average molecular weight (MW)	Spiked metabolite concentration (nM)	Quantified selected reaction monitoring (SRM) transition	Retention time (min)	Calibration		
						LOD (nmol/L)	LOQ (nmol/L)	R^2
Anthranilate	ATA	137.1	50	138.1 → 120	7.1	3	8	0.9997
Indole-3-acetic acid	IAA	175.2	200	176.1 → 130.1	8.2	20	67	0.9993
Indole-3-aldehyde	IAld	145.2	500	146.1 → 118.1	8.1	18	62	-
Indole-3-lactic acid	ILA	205.2	500	206.1 → 117.9	7.9	2	8	-
L-Kynurenine	KYN	208.2	50	209.1 → 192	1.6	2	8	0.999
N-Acetyl-tryptophan	NAceTRP	246.3	500	247.1 → 159	8.2	25	83	-
Tryptophan	TRP	204.2	1000	205.1 → 188	2.9	10	16	0.9999

Table S5. Linear calibration range [nM] for isotope labelled metabolites

Compound	Linear calibration range [nM]
Anthranilate	8 - 500
Indole-3-acetic acid	67 - 1000
Kynurenine	8 - 250
Tryptophan	16 - 10000

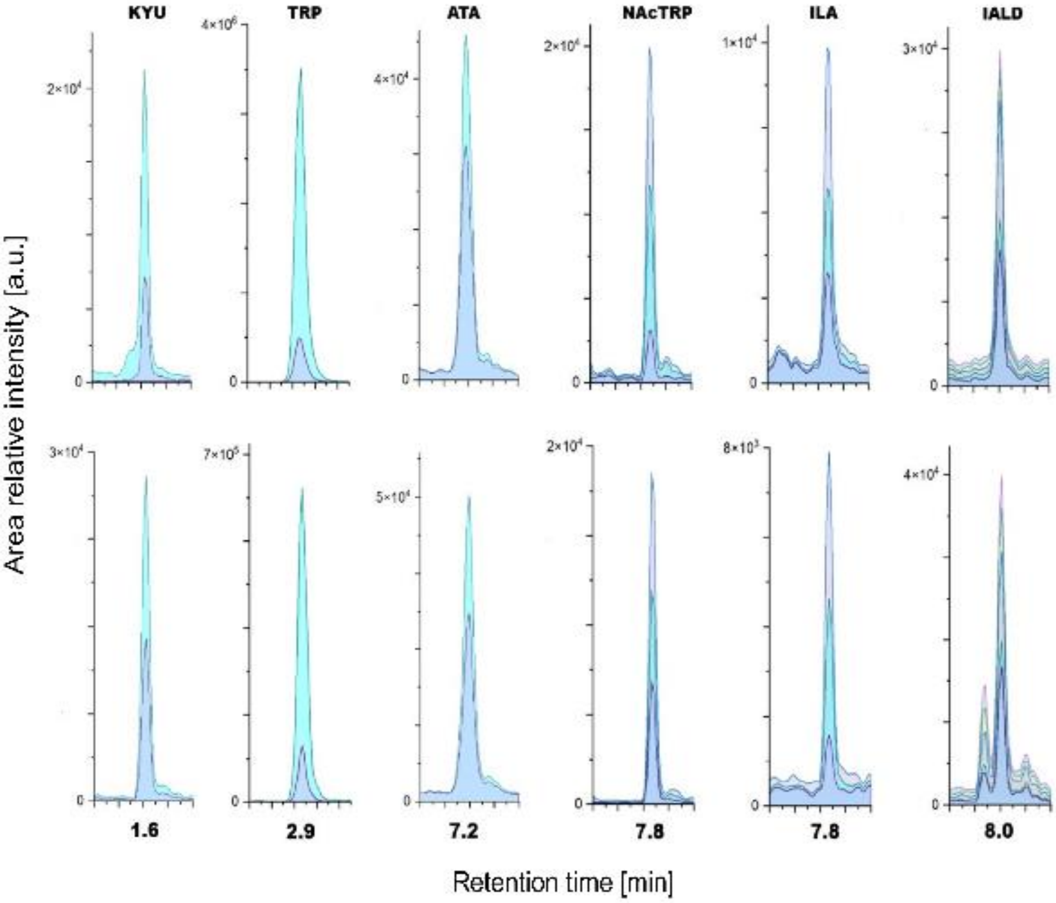

Figure S1: Typical chromatograms of tryptophan catabolites in meconium and stool samples. The upper panel shows the conformity with SRM signature and retention time of measured standards, the lower panel in measured meconium and stool samples. The relative intensity is given as Area relative intensity [a.u.], a.u. = arbitrary units.

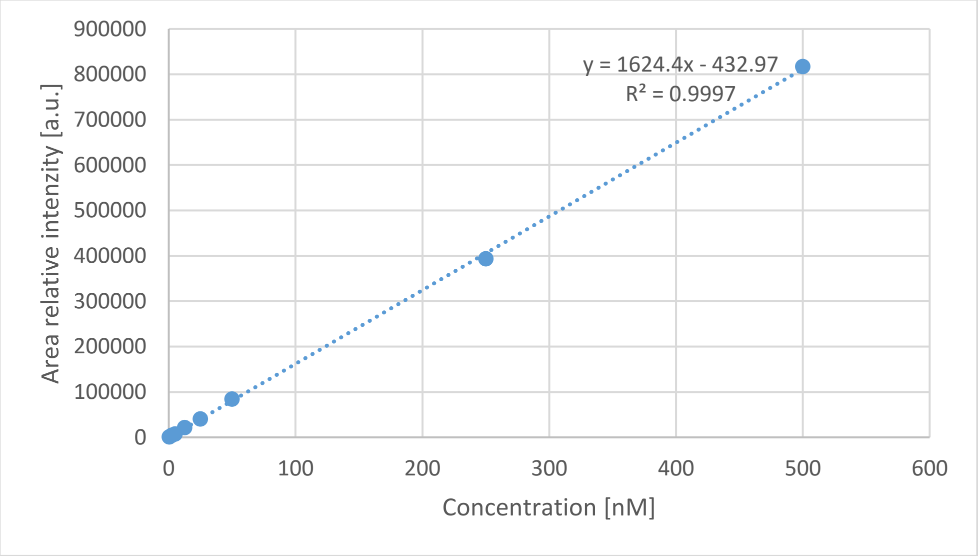

Figure S2: Calibration curve of ATA.

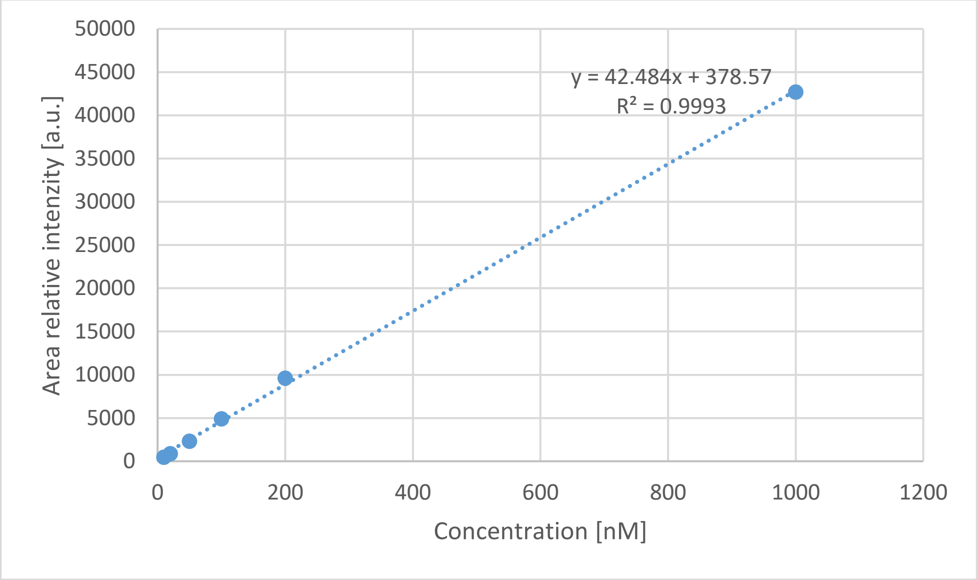

Figure S3: Calibration curve of IAA.

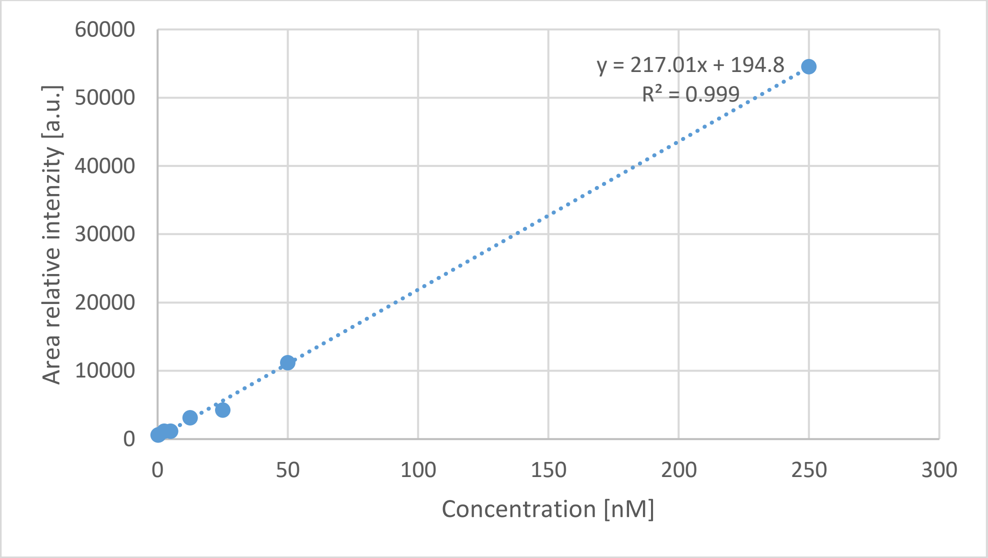

Figure S4: Calibration curve of KYN.

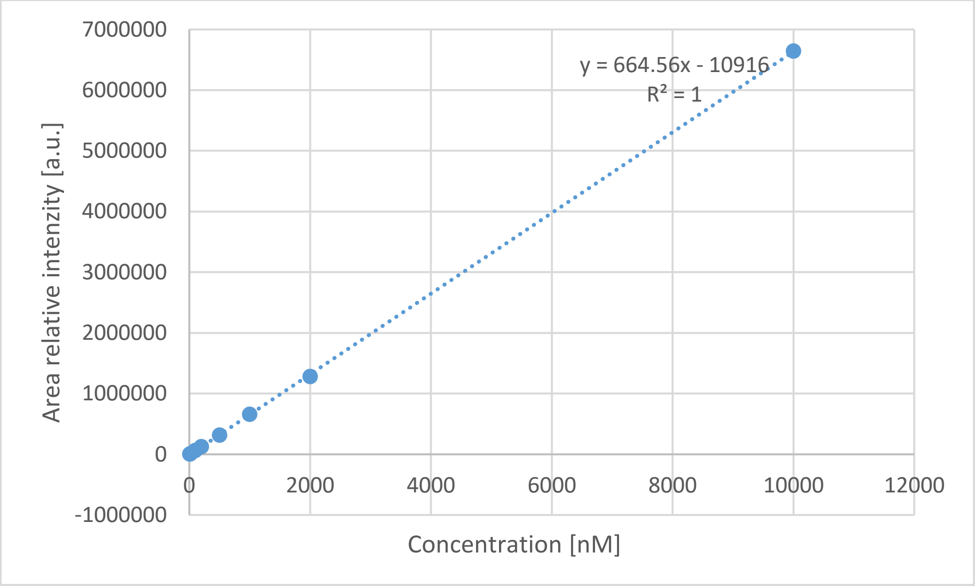

Figure S5: Calibration curve of TRP.

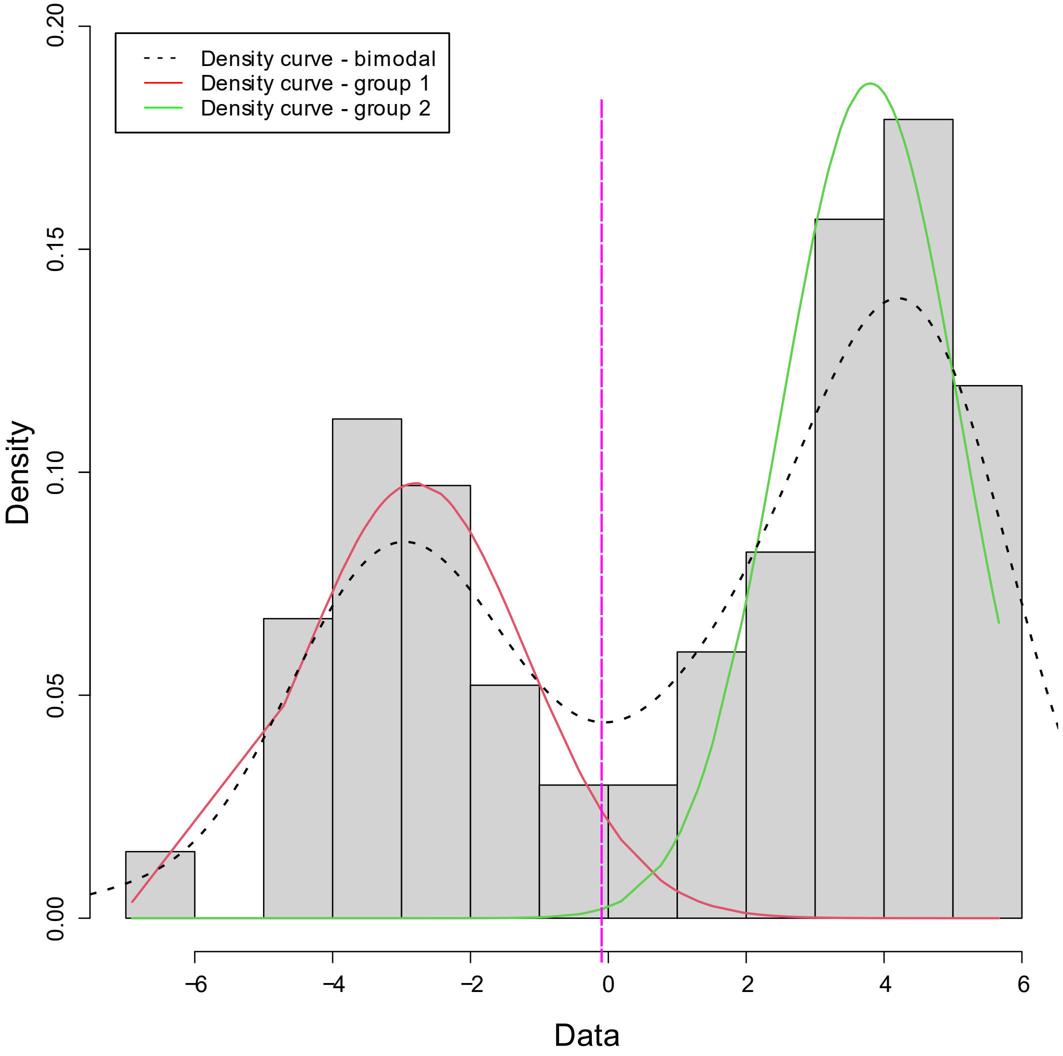

Figure S6: Identification of meconium and stool samples based on distribution of log concentration of IGHA. Red density curve represents distribution of samples determined as meconium. Green density curve represents the distribution of samples determined as stool. Dashed curve shows the bimodal distribution. Purple line indicates the local minimum used for the threshold to define stool or meconium.

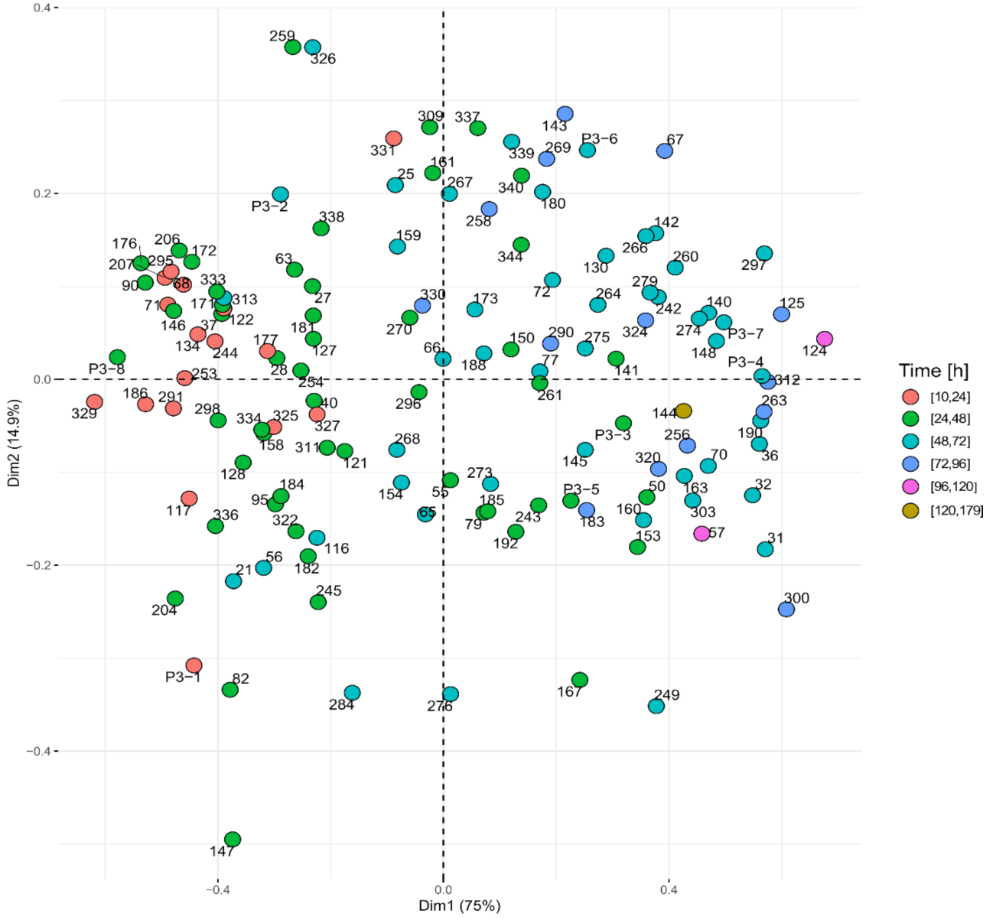

Figure S7: PCA plot of metabolites (TRP, ILA and IAld) concentrations in meconium and stool samples – conversion of metabolites composition in time after delivery. Red dots represent meconium or stool passed by within 24 hours, green 24 – 48 hours, turquoise 48 – 72 hours, blue 72 – 96 hours, pink 96 to 120 hours and brown 120 hours and brown 120 – 179 hours after delivery.

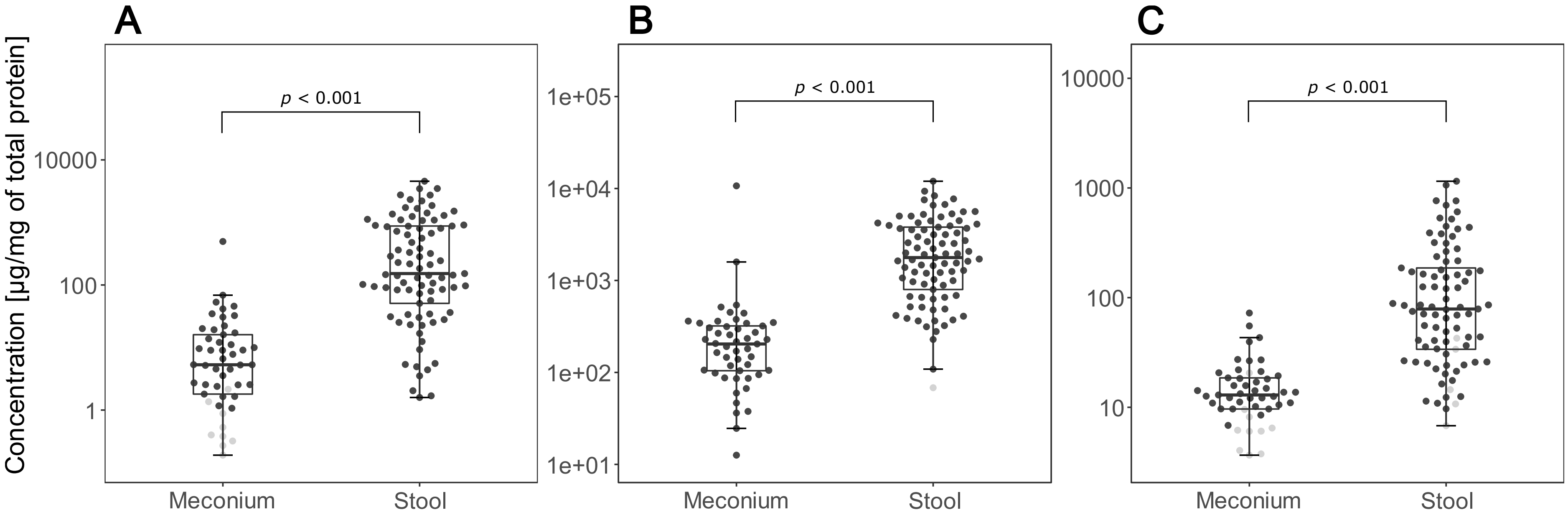

Figure S8: Box plots of metabolites quantified in meconium and stool of 134 newborns. Determined statistical difference of ILA (A), TRP (B) and IAld (C) in meconium and stool. Grey spots indicate values <LOQ. The box represents the middle 50%, the bold horizontal line inside the box represents the median (Q2), the bottom of the box is at the first quartile (Q1; 25th percentile), the top of the box is at the third quartile (Q3; 75th percentile). Whiskers extend to the smallest and largest values within 1.5 times the IQR (1.5 * IQR) from Q1 and Q3, respectively (IQR = inter-quartile range). Points plotted individually beyond the whiskers represent outliers.

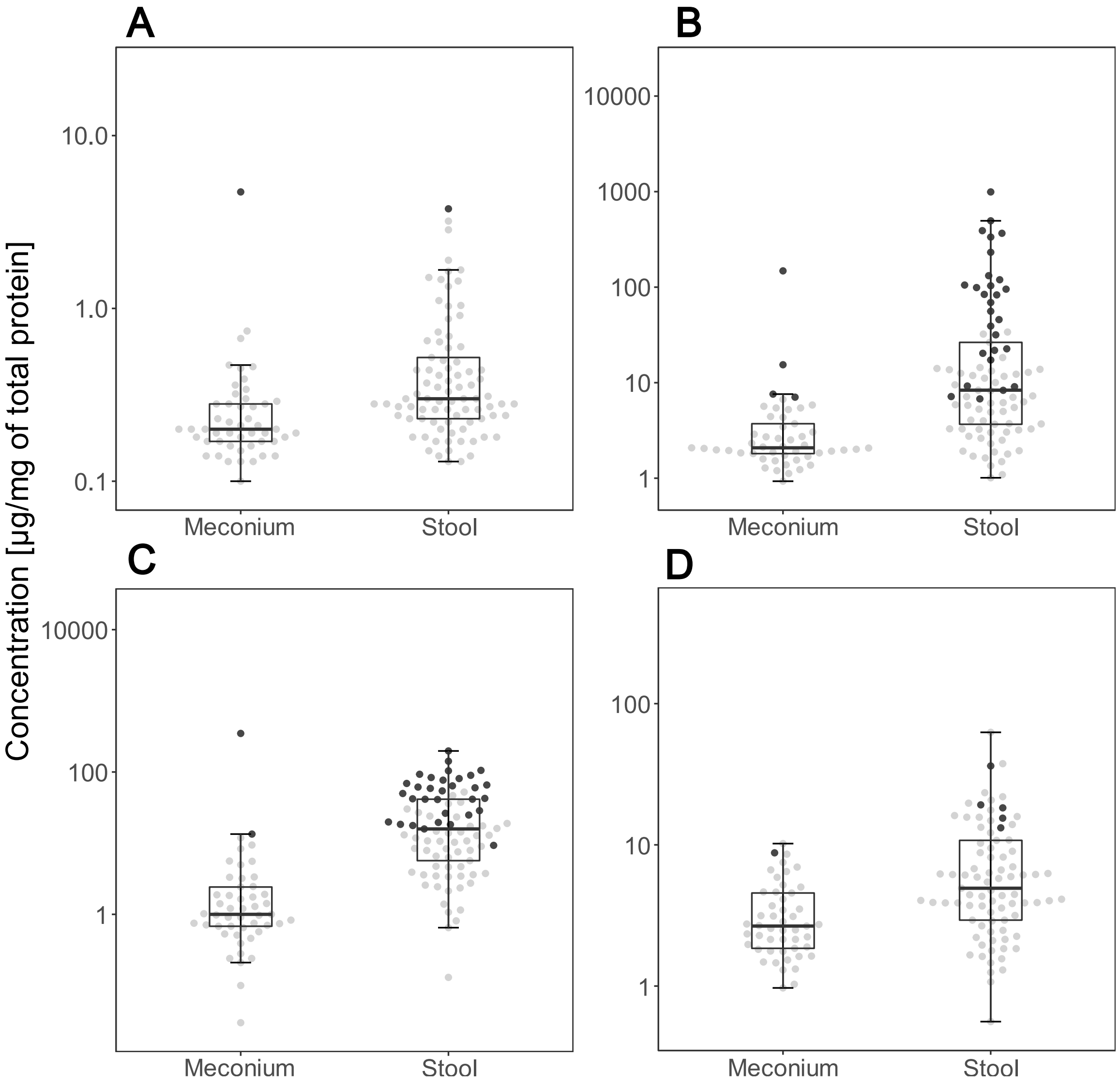

Figure S9: Comparison of metabolites concentrations determined in meconium and stool samples from 134 newborns. Box plots represent groups of concentrations of ATA (A), IAA (B), NAceTRP (C) and KYN (D). Black spots represent quantified values. Grey spots represent values <LOQ.

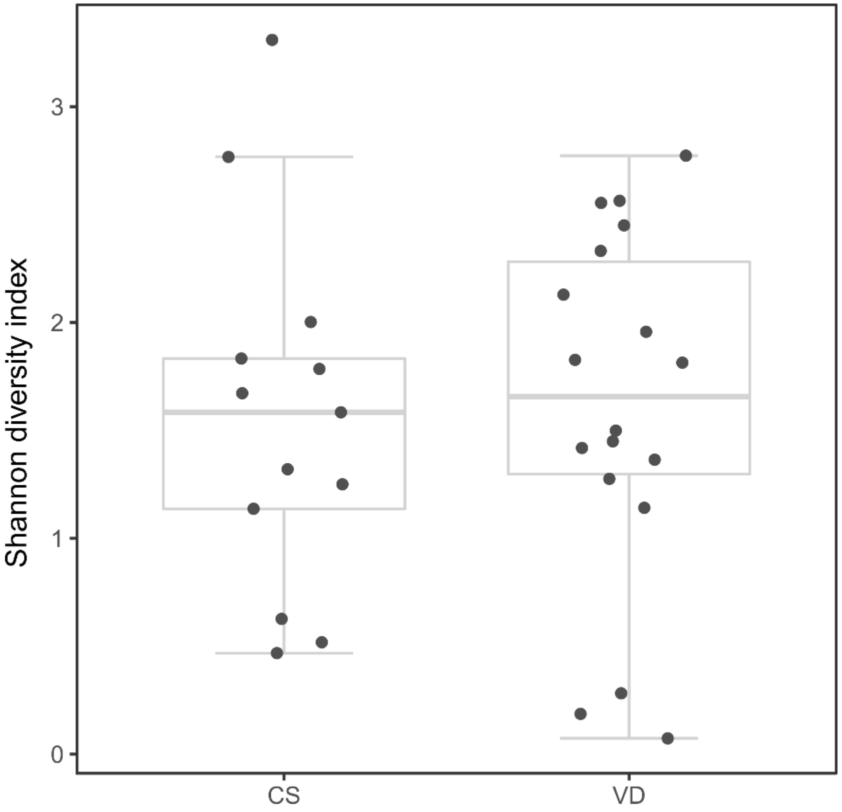

Figure S10: The comparison of concentrations of microbial diversity in stool of VD and CS newborns of ATB-treated mothers. The box represents the middle 50%, the bold horizontal line inside the box represents the median (Q2), the bottom of the box is at the first quartile (Q1; 25th percentile), the top of the box is at the third quartile (Q3; 75th percentile). Whiskers extend to the smallest and largest values within 1.5 times the IQR (1.5 * IQR) from Q1 and Q3, respectively (IQR = inter-quartile range). Points plotted individually beyond the whiskers represent outliers.

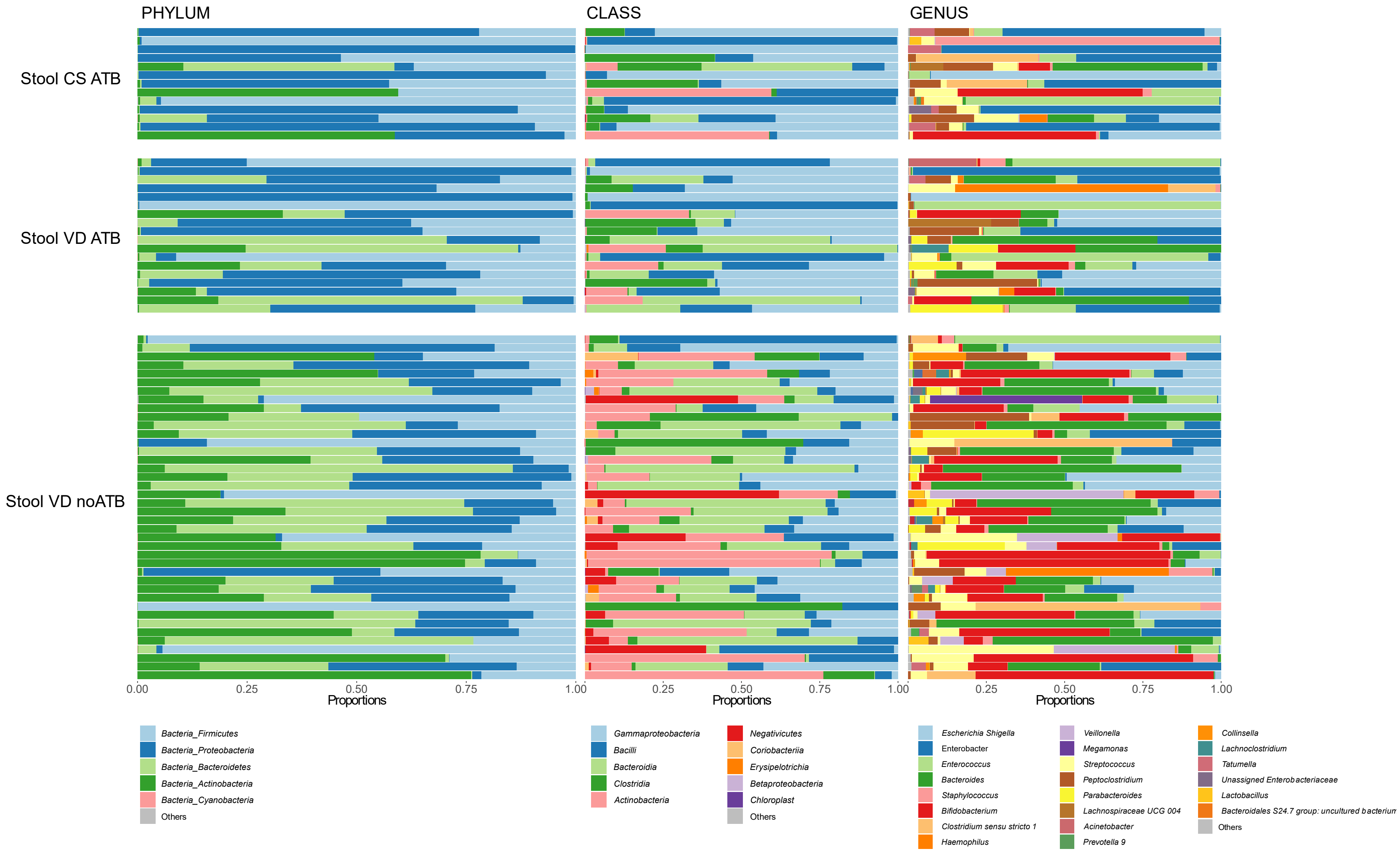

Figure S11: Relative abundance of bacteria at various taxonomic levels in stool samples of CS and VD newborns of ATB-treated/untreated mothers. The "others" means the sum of taxa with relative abundance in the sample < 0.01.

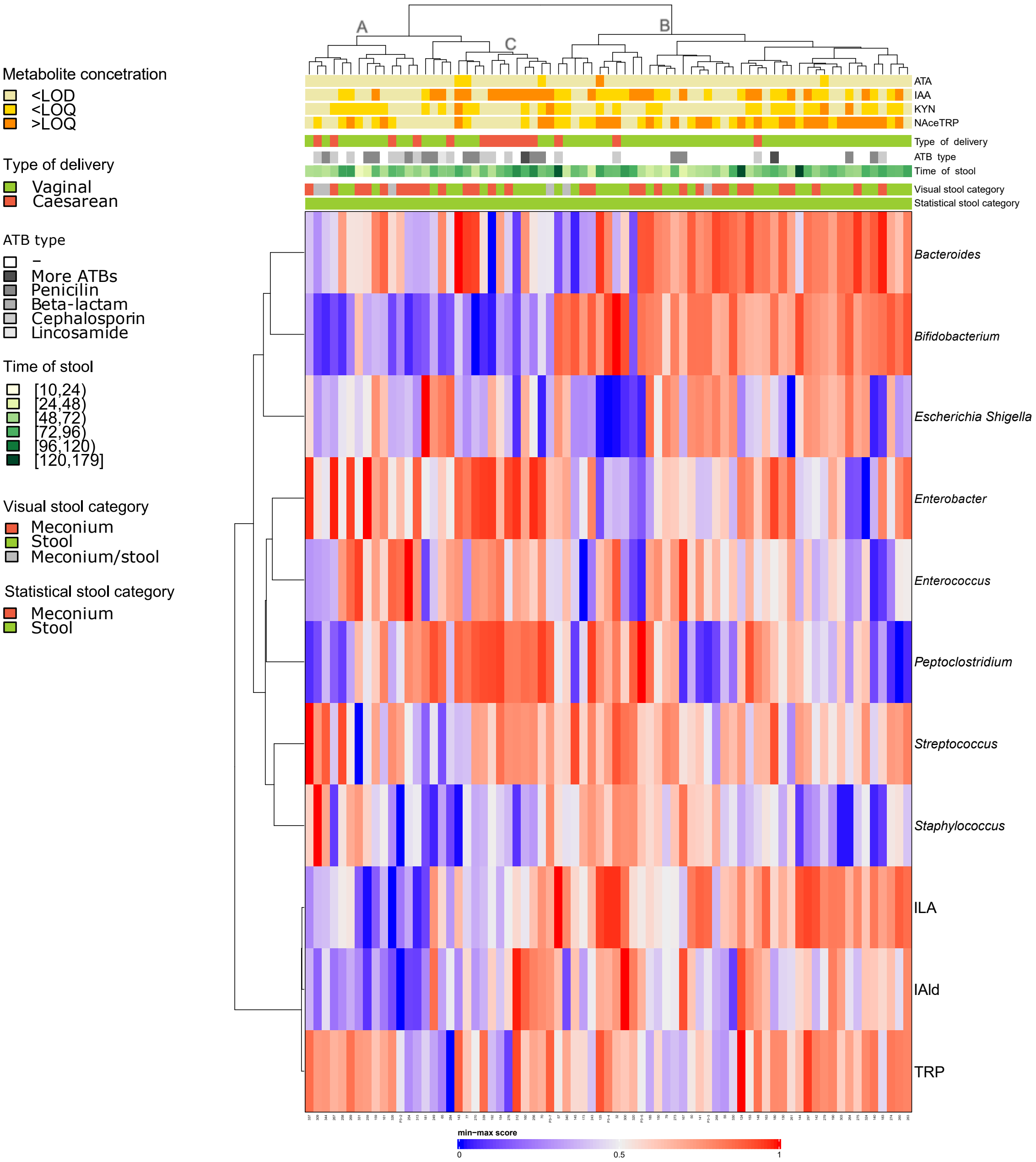

Figure S12: Heat map of selected bacteria and metabolites in stool. The darker the red/blue colour, the higher is amount of bacteria o concentration of ILA, IAld and TRP in the sample. The yellow/orange coloured plot indicates the metabolites below/above LOD/LOQ (NAceTRP, KYN, IAA, ATA). Red/green/grey coloured inserted plots indicate type of delivery or categorization of sample based on visual identification. Shades of green coloured plot indicate the hours when sample was collected after delivery. Shades of grey coloured plots indicate ATB intake. Letters A, B and C indicate resulting clusters.

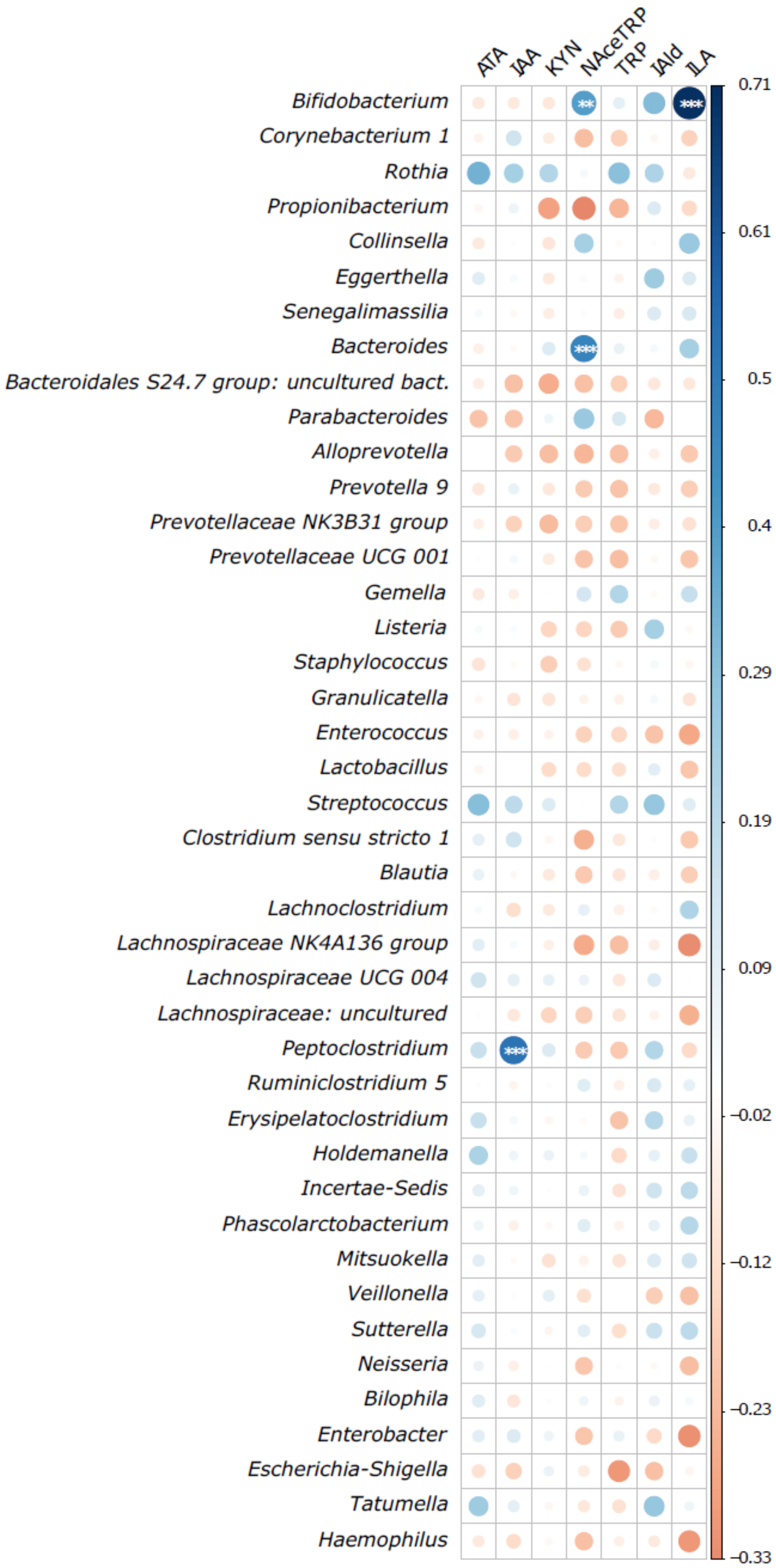

Figure S13: Spearman correlation of TRP metabolites and microbiome abundance in the stool. The colour indicates Spearman correlation coefficients. Statistical significance is marked with * for FDR<0.1, ** for FDR<0.05 and *** for FDR<0.01.