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Supplementary material

Vitamin B6 deficiency impairs gut microbiota and its metabolites in rats

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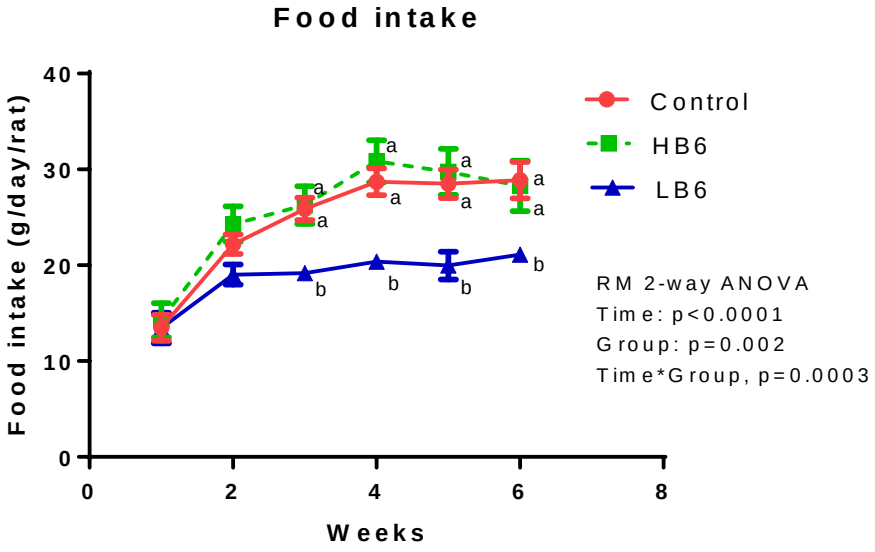
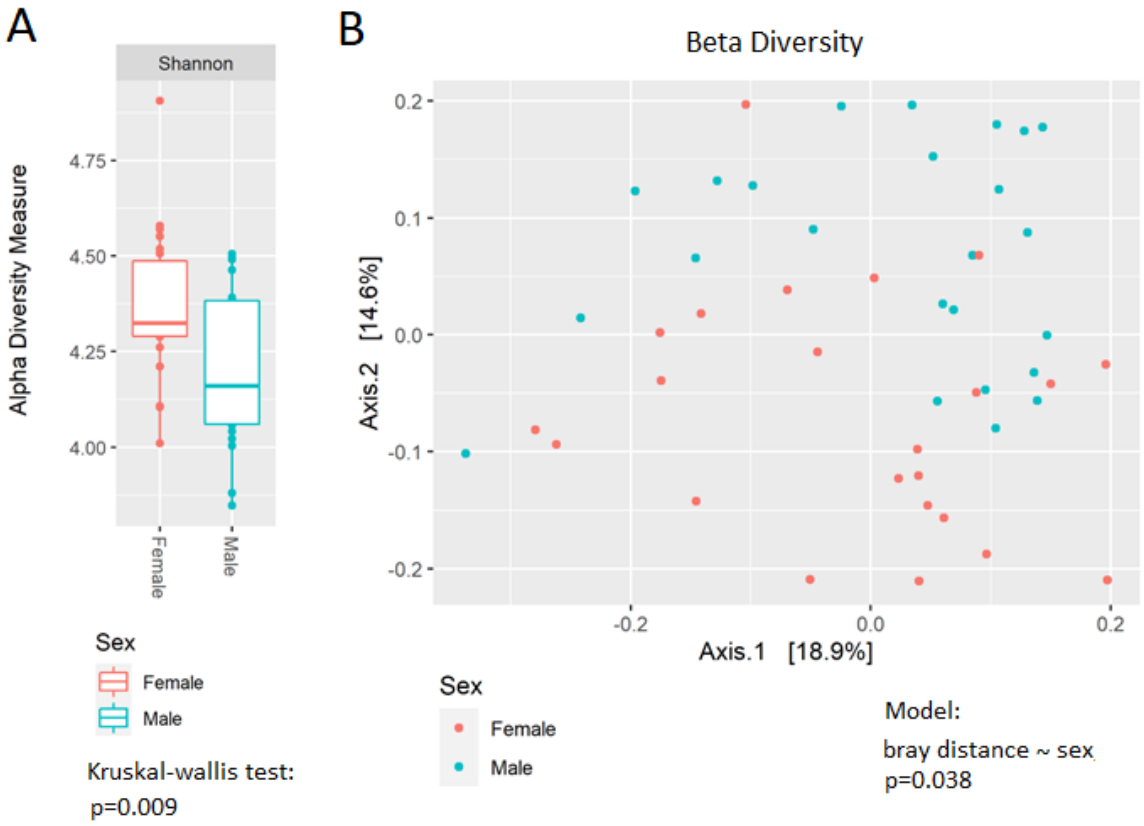


Supplementary Figure 1. Effect of vitamin B6 levels on food intake of rats fed control, HB6 and LB6 for 6 weeks. Values are mean ± SD (n=15-16/group). Values with different superscripts indicate significant differences between groups at the same time points ($p < 0.05$). HB6, high vitamin B6; LB6, low vitamin B6.



Supplementary Figure 2. Cecal microbiota showing significant sex effect for alpha diversity (Shannon index, p=0.009) and beta diversity (p=0.038). n=7-8/group of each sex.

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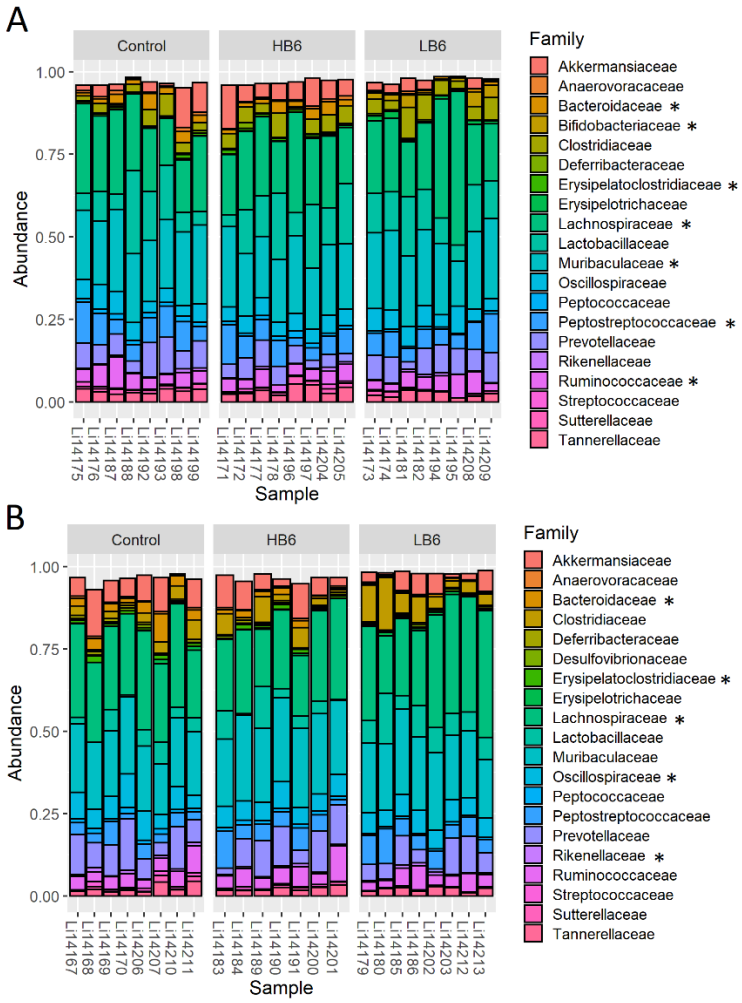
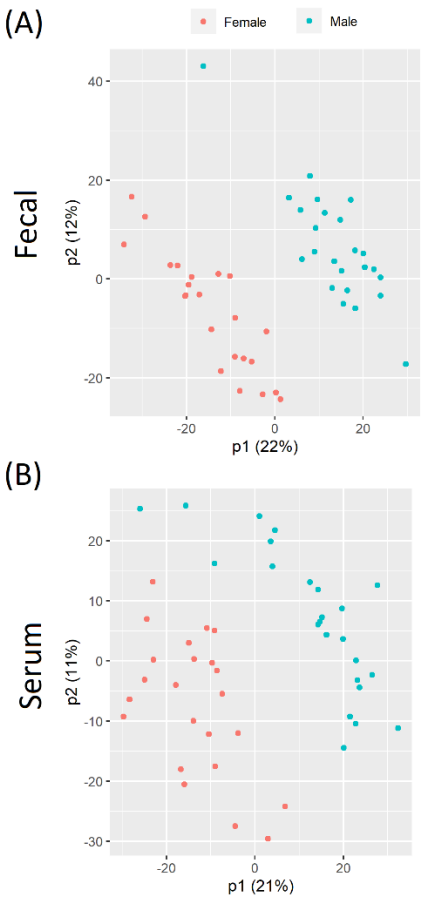
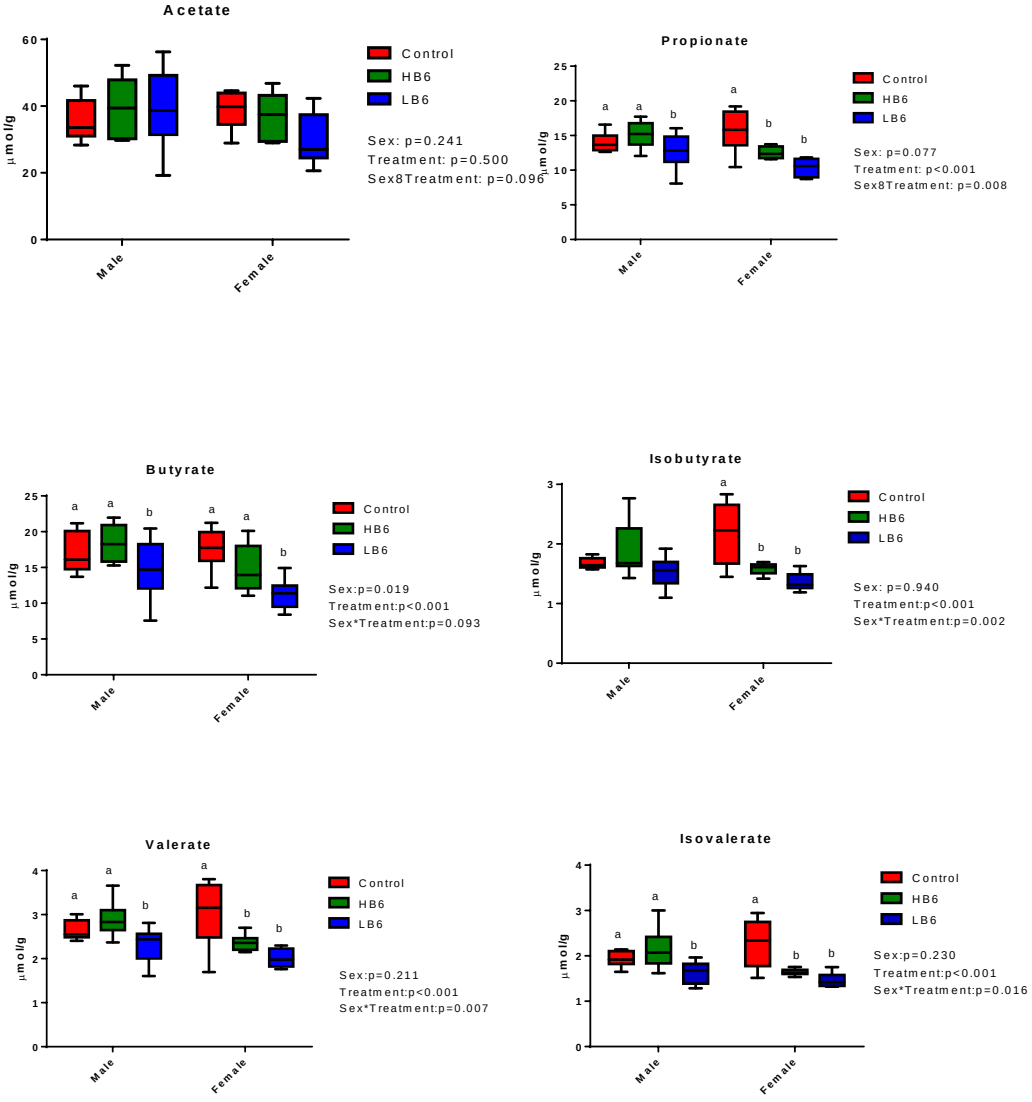


Supplementary Figure 3. The effect of vitamin B6 on the relative abundance of the top 20 family levels for male (A) and female (B) rats. * indicates the taxa that differed between the control and LB6 rats at fdr p-value <0.05 (n=7-8/group of each sex). HB6, high vitamin B6; LB6, low vitamin B6.



Supplementary Figure 4. PLS-DA score plot for metabolite features showing a significant sex effect on cecal matter (A): R2Y=0.94, Q2Y=0.89, $p<0.05$; and serum (B): R2Y =0.83, Q2Y=0.67, $p<0.05$; n=23-24/group.



Supplementary Figure 5. Effect of vitamin B6 on cecal short-chain and branched-chain fatty acid concentrations in rats fed control, HB6, LB6 for 6 weeks. Values are mean ± SD (n=7-8/group of each sex). Values with different superscripts indicate a significant difference between the treatment group within sex ($p<0.05$). HB6, high vitamin B6; LB6, low vitamin B6.

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Supplementary Table 1. Effect of high and low vitamin B6 on body composition at the end of a 6 week feeding period.

	Male			Female		
	Control	HB6	LB6	Control	HB6	LB6
BMC	12.4±0.7[a]	13.2±1.0[a]	9.7±0.5[b]	9.6±0.3[a]	8.9±0.6[a]	7.8±0.4[b]
BMD	0.16±0.0	0.16±0.0	0.16±0.0	0.16±0.0	0.15±0.01	0.15±0.01
Fat mass	94.9±19.3[a]	110.8±30.5[a]	34.9±4.2[b]	56.8±4.9[a]	46.7±3.6[b]	21.1±3.4[c]
Lean mass	395.5±32.3[a]	422.9±30.2[a]	313.3±30.3[b]	257.5±29.6[a]	259.2±7.2[a]	224.7±11.8[b]
HOMA-IR	4.2±1.4	4.2±2.2	2.3±1.1	1.6±0.4	1.6±0.7	1.1±0.7

Values are mean ± SD (n=7-8/group of each sex). Values with different superscripts indicate significant differences between groups within the same sex ($p<0.05$). BMC, bone mineral content; BMD, bone mineral density; HB6, high vitamin B6; LB6, low vitamin B6.

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Supplementary Table 2. Putatively identified metabolites involved in the significant pathways identified in cecal and serum-linked metabolism with their adducts and mass differences.

Source	mz	KEGG	Adducts	Mass diff	Pathway	Putative metabolites
Cecal	136.040500	C00049	M(S34)+H[1+]	0.000084483770	Arginine biosynthesis	L-Aspartate
	177.104780	C00437	M(Cl37)+H[1+]	0.000139290770	Arginine biosynthesis	N-Acetylornithine
	154.049741	C00624	M-H4O2+H[1+]	0.000058133770	Arginine biosynthesis	N-Acetyl-L-Glutamate
	349.235792	C01595	M+HCOONa[1+]	0.000885565230	Biosynthesis of unsaturated FA	Linoleate
	301.212653	C06427	M+Na[1+]	0.001203370770	Biosynthesis of unsaturated FA	Linolenate
	426.153402	C00415	M-H2O+H[1+]	0.001393594230	Folate biosynthesis	Dihydrofolate
	358.184051	C00504	M-HCOOK+H[1+]	0.001607141770	Folate biosynthesis	Folate
	269.114849	C00921	M-HCOOH+H[1+]	0.000233989230	Folate biosynthesis	Dihydropteroate
Serum	84.080494	C00408	M-HCOOH+H[1+]	0.000361257770	Lysine degradation	L-Pipecolate
	146.117586	C01181	M[1+]	0.000517661000	Lysine degradation	Butyro-betaine
	162.112812	C00487	M+H[1+]	0.000345233230	Lysine degradation	Carnitine
	153.065839	C05843	M+H[1+]	0.000014876770	Nicotinate & nicotinamide metabolism	1-Methyl-4-pyridone-3-carboximide
	175.048303	C05842	M+Na[1+]	0.000449023230	Nicotinate & nicotinamide metabolism	N1-Methyl-2-pyridone-5-carboxamide
	168.065362	C00250	M+H[1+]	0.000157225770	Vitamin B6 metabolism	Pyridoxal
	285.988022	C00018	M+K[1+]	0.000672164230	Vitamin B6 metabolism	Pyridoxal 5 phosphate