

Supplementary Information

The combined use of B vitamins and probiotics promotes B vitamin absorption and gut microbiota restoration

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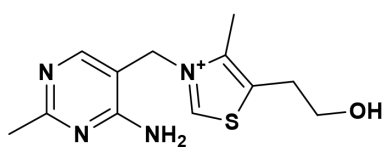
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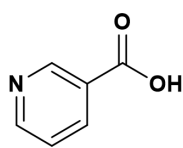
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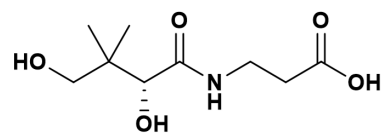
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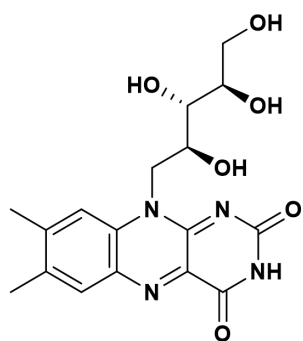
B1



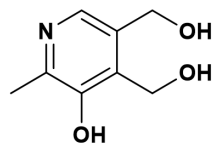
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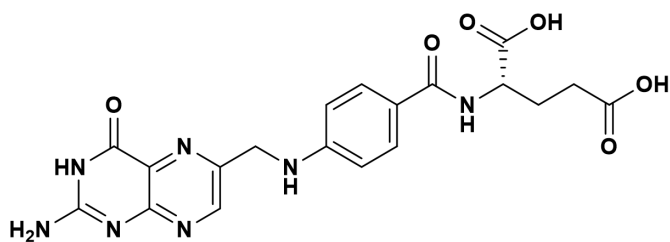
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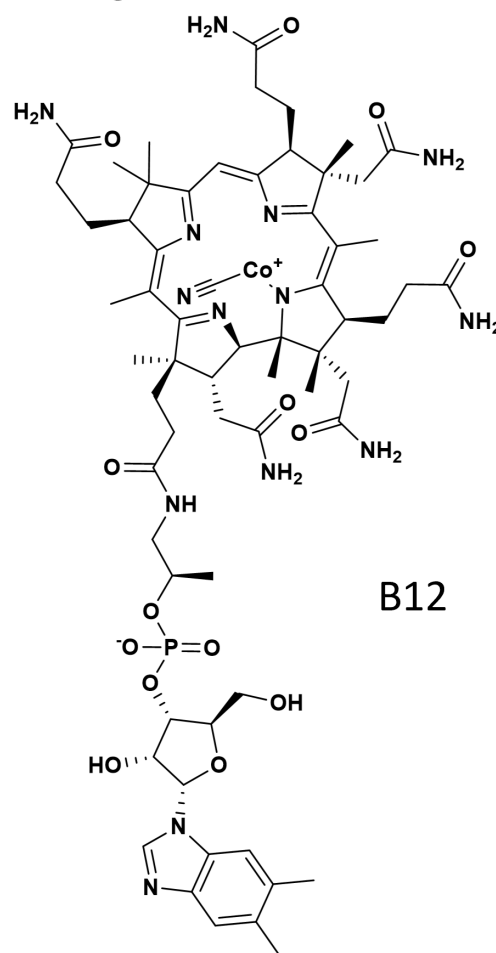
B2



B6



B9



B12

Fig.S1 Molecule structures of B vitamins.

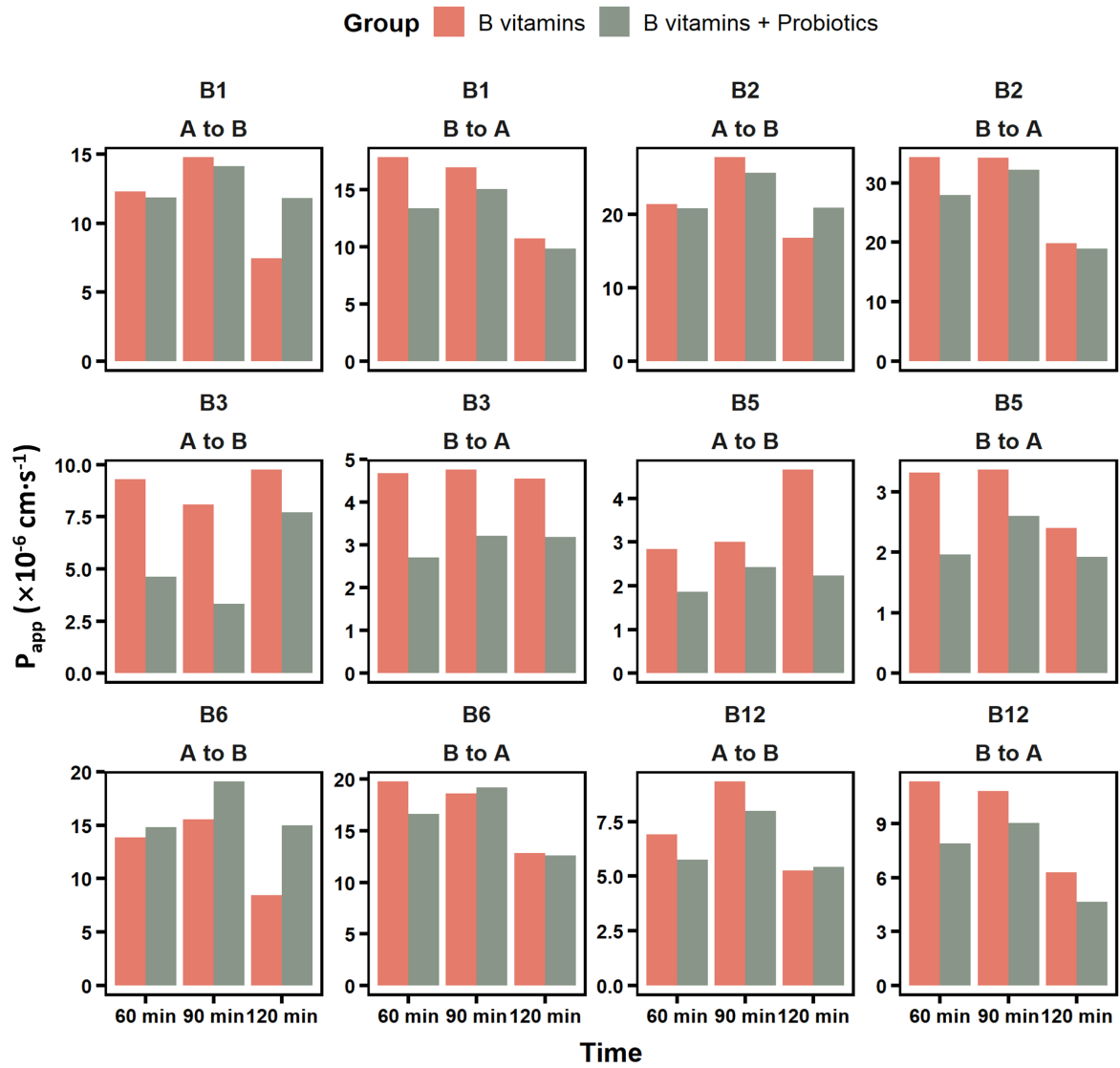


Fig. S2 The impact of probiotics on the permeability of B vitamins in caco-2 cells. A indicates the apical side, while B indicates the basal side. Data are expressed as mean (n = 2).

Table S1. The calibration curve and MS transitions for each analyte.

Analyte	T _R (min)	Calibration Curve	r ²	Linear range (ng/mL)	MRM	
					Transition	CE (V)
B1	1.27	y=4.96779x+0.0043820	0.9964	7.5-1500	265.1>122.0	15
B2	8.62	y=0.215987x+0.0021384	0.9948	10-1000	377.0>243.0	24
B3	2.73	y=0.618146x+0.095806	0.9936	50-20000	124.1>80.0	24
B5	4.74	y=1.16982x+0.0056099	0.9953	50-1000	220.1>90.0	14
B6	1.77	y=1.78348x+0.148824	0.9922	300-15000	170.1>152.0	13
B9	8.70	y=0.617695x-0.0017542	0.9972	10-1000	442.1>295.2	17
B12	8.43	y=0.0395913x-0.0004553	0.9948	20-1000	678.0>147.0	46
IS	4.57				127.1>84.0	24

Table S2. The non-compartmental analysis (NCA) parameters of each B vitamin in healthy rats (n = 8).

Parameters	AUC _{0-∞}	AUC _{0-t}	T _{max}	C _{max}	CL _z /F	MRT _{0-∞}	MRT _{0-t}	t _{1/2z}	V _z /F	
Unit	mg/L·h	mg/L·h	h	mg/L	L/h/kg	h	h	h	L/kg	
B1	VB	0.85 ± 0.36	0.8 ± 0.34	0.69 ± 0.26	0.26 ± 0.08	104.36 ± 65.13	3.39 ± 1.13	2.89 ± 0.9	2.05 ± 1	286.73 ± 191.28
	V + P	2.34 ± 0.82	2.29 ± 0.88	0.88 ± 0.23	0.52 ± 0.18	33.53 ± 12.12	6.75 ± 3.2	5.3 ± 1.23	4.63 ± 5.88	256.28 ± 418.18
	FC	2.76	2.84	1.27	2.05	0.32	1.99	1.83	2.26	0.89
	P val	0.000	0.000	0.161	0.003	0.000	0.002	0.001	0.180	0.195
B2	VB	2.41 ± 0.77	0.92 ± 0.08	1.12 ± 0.74	0.06 ± 0.01	4.88 ± 2.79	50.8 ± 19.92	10.86 ± 0.48	39.63 ± 8.59	216.97 ± 26.84
	V + P	4.91 ± 6.08	0.81 ± 0.12	2.12 ± 1.79	0.06 ± 0.01	5.41 ± 4.67	128.54 ± 171.71	11.19 ± 0.66	89.36 ± 120	233.47 ± 44.15
	FC	2.04	0.89	1.89	0.97	1.11	2.53	1.03	2.25	1.08
	P val	0.608	0.064	0.202	0.610	0.608	0.448	0.267	0.760	0.470
B3	VB	3.75 ^a	5.32 ± 2.31	0.5 ^a	8.04 ± 4.28	2.66 ^a	3.66 ^a	2.14 ^a	2.38 ^a	9.15 ^a
	V + P	NA ^b	7.31 ± 1.34	0.38 ± 0.17	10.78 ± 2.22	NA ^b	NA ^b	NA ^b	NA ^b	NA ^b
	FC	NA ^c	1.37	0.75	1.34	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
	P val	NA ^c	0.050	0.075	0.104	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
B5	VB	2.08 ± 1.01	1.28 ± 0.72	3.25 ± 0.71	0.17 ± 0.06	12.1 ± 6.56	15.46 ± 8.44	5.96 ± 1.94	10.84 ± 6.76	154.04 ± 91.39
	V + P	2.02 ± 0.7	1.9 ± 0.71	3.25 ± 0.71	0.26 ± 0.07	10.98 ± 3.67	8.26 ± 2.56	6.87 ± 1.78	4.37 ± 2.95	64.43 ± 40.49
	FC	0.97	1.48	1.00	1.52	0.91	0.53	1.15	0.40	0.42
	P val	0.894	0.089	1.000	0.012	0.894	0.054	0.353	0.036	0.010
B6	VB	8.37 ± 1.97	8.37 ± 1.99	0.56 ± 0.18	5.63 ± 1.76	2.62 ± 1.12	1.4 ± 0.22	1.37 ± 0.18	1.26 ± 0.61	5.61 ± 6.37
	V + P	8.9 ± 0.72	8.89 ± 0.71	0.62 ± 0.23	6.43 ± 1.05	2.26 ± 0.18	1.02 ± 0.09	1.02 ± 0.09	0.7 ± 0.74	2.19 ± 2.11
	FC	1.06	1.06	1.11	1.14	0.86	0.73	0.75	0.56	0.39
	P val	0.443	0.441	0.587	0.251	0.443	0.000	0.000	0.014	0.015
B9	VB	2.04 ± 0.21	1.38 ± 0.42	1.19 ± 0.53	0.41 ± 0.07	4.96 ± 0.54	4.64 ± 1.46	2.64 ± 0.1	2.74 ± 1.08	19.36 ± 7.22
	V + P	2.33 ± 0.66	1.71 ± 0.2	2.25 ± 0.89	0.4 ± 0.08	4.57 ± 1.13	6.83 ± 4.52	3.58 ± 0.8	4.28 ± 3.65	23.8 ± 13.46
	FC	1.14	1.24	1.89	0.97	0.92	1.47	1.35	1.56	1.23
	P val	0.327	0.126	0.022	0.772	0.327	0.276	0.004	0.452	0.582
B12	VB	0.17 ± 0.08	0.08 ± 0.02	0.56 ± 0.18	0.05 ± 0.01	132.73 ± 41.2	3.81 ± 3.41	1.15 ± 0.34	2.58 ± 2.37	387.63 ± 118.7
	V + P	0.19 ± 0.05	0.1 ± 0.03	0.48 ± 0.26	0.09 ± 0.02	108.95 ± 26.69	2.41 ± 1.48	0.9 ± 0.04	1.57 ± 1.08	222.39 ± 103.87
	FC	1.12	1.21	0.85	1.64	0.82	0.63	0.79	0.61	0.57
	P val	0.351	0.213	0.369	0.000	0.349	0.236	0.055	0.207	0.018

^a Standard deviation was not available due to the limited number of detectable samples.

^b The mean value of parameter and standard deviation were not available due to the limited number of detectable samples.

^c Fold change and p value were not available due to the limited number of detectable samples.

All NCA parameters except T_{max} were log transformed prior to analysis. All NAC parameters except T_{max} were analyzed by Student's t test. T_{max} was analyzed by Wilcoxon test. Data of all NCA parameters are expressed as mean±SD. VB, B vitamins. V + P, B vitamins + Probiotics. FC, fold change. NA, not available.

Table S3. The non-compartmental analysis (NCA) parameters of each B vitamin in pseudo-germ-free rats (n = 6).

Parameters	AUC _{0-∞}	AUC _{0-t}	T _{max}	C _{max}	CL _z /F	MRT _{0-∞}	MRT _{0-t}	t _{1/2z}	V _z /F	
Unit	mg/L·h	mg/L·h	h	mg/L	L/h/kg	h	h	h	L/kg	
B1	VB	6.15 ± 1.36	3.63 ± 0.2	0.92 ± 0.23	0.82 ± 0.06	11.86 ± 2.2	9.51 ± 4.89	3.18 ± 0.06	6.9 ± 3.84	106.79 ± 31.07
	V + P	20.22 ± 32.26	3.8 ± 0.73	0.53 ± 0.24	0.85 ± 0.2	10.87 ± 5.24	37.52 ± 66.03	3.3 ± 0.18	26.37 ± 45.99	109.93 ± 20.66
	FC	3.29	1.05	0.58	1.05	0.92	3.94	1.04	3.82	1.03
	P val	0.451	0.739	0.033	0.798	0.451	0.465	0.206	0.462	0.755
B2	VB	1.7 ± 0.15	0.88 ± 0.15	1.56 ± 1.17	0.18 ± 0.04	5.98 ± 0.55	10.81 ± 3.06	3.55 ± 0.24	7.36 ± 2.36	63.42 ± 20.12
	V + P	1.64 ± 0.93	0.91 ± 0.22	1.17 ± 0.62	0.17 ± 0.05	7.87 ± 3.32	10.49 ± 8.5	3.59 ± 0.21	9.33 ± 5.48	65.22 ± 27.09
	FC	0.96	1.03	0.75	0.96	1.32	0.97	1.01	1.27	1.03
	P val	0.444	0.919	0.673	0.974	0.443	0.339	0.954	0.999	0.875
B3	VB	NA ^b	7.88 ± 0.87	0.39 ± 0.16	10.68 ± 1.23	NA ^b	NA ^b	NA ^b	NA ^b	NA ^b
	V + P	NA ^b	7.65 ± 1.72	0.39 ± 0.16	11.64 ± 2.94	NA ^b	NA ^b	NA ^b	NA ^b	NA ^b
	FC	NA ^c	0.97	1.00	1.09	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
	P val	NA ^c	0.677	1.000	0.615	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
B5	VB	3.46 ± 0.81	2.12 ± 0.36	1.67 ± 1.11	0.38 ± 0.08	6.06 ± 1.19	8.49 ± 2.92	3.67 ± 0.15	5.39 ± 2.2	44.59 ± 10.82
	V + P	3.08 ± 0.75	1.92 ± 0.25	2.17 ± 0.37	0.34 ± 0.06	6.9 ± 1.67	8.01 ± 1.71	3.57 ± 0.07	5.26 ± 1.37	49.3 ± 4.21
	FC	0.89	0.91	1.30	0.90	1.14	0.94	0.97	0.98	1.11
	P val	0.420	0.333	0.116	0.489	0.420	0.844	0.259	0.957	0.362
B6	VB	17.02 ± 1.37	16.39 ± 1.24	0.75 ± 0.25	10.25 ± 1.04	1.18 ± 0.09	1.1 ± 0.1	0.99 ± 0.05	0.56 ± 0.13	0.95 ± 0.18
	V + P	13.35 ± 1.29	12.39 ± 1.33	0.44 ± 0.12	9.69 ± 1.29	1.51 ± 0.17	1.03 ± 0.15	0.86 ± 0.13	0.58 ± 0.12	1.24 ± 0.19
	FC	0.78	0.76	0.59	0.95	1.28	0.94	0.87	1.03	1.30
	P val	0.002	0.001	0.054	0.434	0.002	0.372	0.080	0.857	0.032
B9	VB	1.78 ± 0.55	1.37 ± 0.3	1.17 ± 0.37	0.4 ± 0.1	6.21 ± 2.07	5.15 ± 2.53	2.77 ± 0.4	3.67 ± 1.71	29.45 ± 9.4
	V + P	4.06 ± 4.06	1.48 ± 1.48	1 ± 1	0.44 ± 0.44	5.88 ± 5.88	14.15 ± 14.15	2.71 ± 2.71	10.35 ± 10.35	33.32 ± 33.32
	FC	2.28	1.08	0.86	1.11	0.95	2.75	0.98	2.82	1.13
	P val	0.532	0.843	0.389	0.726	0.532	0.554	0.771	0.558	0.829
B12	VB	NA ^b	0.01 ± 0.02	0.17 ± 0.24	0.07 ^a	NA ^b	NA ^b	NA ^b	NA ^b	NA ^b
	V + P	1.27 ^a	0.15 ± 0.26	0.61 ± 0.3	0.12 ± 0.08	15.75 ^a	3.57 ^a	1.42 ^a	2.43 ^a	55.27 ^a
	FC	NA ^c	13.78	3.67	1.73	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c
	P val	NA ^c	0.412	0.034	0.153	NA ^c	NA ^c	NA ^c	NA ^c	NA ^c

^a Standard deviation was not available due to the limited number of detectable samples.

^b The mean value of parameter and standard deviation were not available due to the limited number of detectable samples.

^c Fold change and p value were not available due to the limited number of detectable samples.

All NCA parameters except T_{max} were log transformed prior to analysis. All NAC parameters except T_{max} were analyzed by Student's t test. T_{max} was analyzed by Wilcoxon test. Data of all NCA parameters are expressed as mean±SD. VB, B vitamins. V + P, B vitamins + Probiotics. FC, fold change. NA, not available.

Table S4. Multiple-group comparison of Chao index among five groups (n = 5).

Chao index		
Groups for comparison	P value*	Q value*
Model <i>vs</i> Control	0.002	0.023
Probiotics <i>vs</i> Control	0.001	0.013
B vitamins <i>vs</i> Control	0.000	0.000
B vitamins + Probiotics <i>vs</i> Control	0.000	0.000
Probiotics <i>vs</i> Model	0.806	1.000
B vitamins <i>vs</i> Model	0.060	0.597
B vitamins + Probiotics <i>vs</i> Model	0.000	0.002
B vitamins <i>vs</i> Probiotics	0.096	0.961
B vitamins + Probiotics <i>vs</i> Probiotics	0.000	0.003
B vitamins + Probiotics <i>vs</i> B vitamins	0.018	0.180

* Statistical analysis was conducted using Conover's all-pairs rank comparison test with Bonferroni correction.

Table S5. Multiple-group comparison of the relative abundance of the gut microbat at the phylum level (n = 5).

Groups for comparison	Bacteroidetes		Firmicutes		Verrucomicrobia		Proteobacteria	
	P value*	Q value*	P value*	Q value*	P value*	Q value*	P value*	Q value*
Model <i>vs</i> Control	0.000	0.000	0.013	0.134	0.073	0.731	0.001	0.013
Probiotics <i>vs</i> Control	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.003
B vitamins <i>vs</i> Control	0.000	0.001	0.000	0.001	0.001	0.014	0.003	0.027
B vitamins + Probiotics <i>vs</i> Control	0.176	1.000	0.000	0.000	0.000	0.000	0.000	0.000
Probiotics <i>vs</i> Model	1.000	1.000	0.000	0.000	0.020	0.203	0.558	1.000
B vitamins <i>vs</i> Model	0.562	1.000	0.048	0.476	0.085	0.849	0.769	1.000
B vitamins + Probiotics <i>vs</i> Model	0.001	0.007	0.000	0.000	0.000	0.000	0.003	0.027
B vitamins <i>vs</i> Probiotics	0.562	1.000	0.003	0.034	0.486	1.000	0.382	1.000
B vitamins + Probiotics <i>vs</i> Probiotics	0.001	0.007	0.431	1.000	0.009	0.085	0.010	0.104
B vitamins + Probiotics <i>vs</i> B vitamins	0.003	0.029	0.001	0.005	0.002	0.017	0.001	0.013

* Statistical analysis was conducted using Conover's all-pairs rank comparison test with Bonferroni correction.

Table S6. Multiple-group comparison of the relative abundance of the gut microbat at the genus level (n = 5).

Groups for comparison	<i>Akkermansia</i>		<i>Bacteroides</i>		<i>Blautia</i>		<i>Flavonifractor</i>	
	P value*	Q value*	P value*	Q value*	P value*	Q value*	P value*	Q value*
Model vs Control	0.073	0.731	0.000	0.000	0.000	0.000	0.000	0.005
Probiotics vs Control	0.000	0.003	0.000	0.000	0.005	0.048	0.000	0.000
B vitamins vs Control	0.001	0.014	0.000	0.000	0.000	0.000	0.015	0.152
B vitamins + Probiotics vs Control	0.000	0.000	0.051	0.510	0.543	1.000	0.000	0.000
Probiotics vs Model	0.020	0.203	0.879	1.000	0.011	0.114	0.006	0.057
B vitamins vs Model	0.085	0.849	1.000	1.000	0.543	1.000	0.148	1.000
B vitamins + Probiotics vs Model	0.000	0.000	0.001	0.015	0.000	0.000	0.003	0.025
B vitamins vs Probiotics	0.486	1.000	0.879	1.000	0.043	0.425	0.000	0.002
B vitamins + Probiotics vs Probiotics	0.009	0.085	0.002	0.021	0.019	0.189	0.727	1.000
B vitamins + Probiotics vs B vitamins	0.002	0.017	0.001	0.015	0.000	0.001	0.000	0.001

* Statistical analysis was conducted using Conover's all-pairs rank comparison test with Bonferroni correction.