

Table S1. Influence of HMO interventions and age on diversity of fecal microbiota in artificially reared suckling rats. Values for observed features and evenness, and weighted UniFrac distances represent the structural and compositional differences of fecal microbiota in pups grouped by HMO interventions or age. Significant difference was set as p -value <0.05.

Factor	Group1	Group 2	p -values		
			Observed features	Evenness	Weighted UniFrac distances
Diet	CTR	2' FL	0.612	0.653	0.873
		2' FL+3' SL	0.945	0.795	0.490
		3' SL	0.975	0.962	0.376
	2' FL	2' FL+3' SL	0.815	0.594	0.420
		3' SL	0.831	0.503	0.641
	2' FL+3' SL	3' SL	0.940	0.777	0.068
Age	W1	W2	0.989	0.692	0.048
		W3	0.000	0.024	0.002
	W2	W3	0.000	0.000	0.002

Table S2. Relative abundance of bacterial genera in fecal microbiota of suckling rats fed with rat milk substitutes with or without 2'-FL and 3'-SL. Data were determined by sequencing of 16S rRNA tags and analyzed by QIIME2. Values are mean± SEM (%) and results in the same row that do not share a common superscript are significantly different ($P < 0.05$). **CTR**: basal rat milk substitute; **2'FL**: CTR +1.2 g/L 2'-fucosyllactose; **3'SL**: CTR +1.2 g/L 3'-sialyllactose; **2'FL+3'SL**: CTR +0.6 g/L 2'-fucosyllactose +0.6 g/L 3'-sialyllactose.

Age Diet	Week1				Week2				Week3			
	CTR	2'FL	3'SL	2'FL+3'SL	CTR	2'FL	3'SL	2'FL+3'SL	CTR	2'FL	3'SL	2'FL+3'SL
Actinobacteria												
<i>g_Bifidobacterium</i>	ND	ND	ND	ND	0.12±0.08	ND	ND	0.03±0.03	0.04±0.03	0.02±0.02	ND	0.19±0.19
<i>g_Rothia</i>	0.01±0.01	0.02±0.02	0.01±0.01	0.02±0.02	ND	0.03±0.02	0.01±0.01	0.08±0.07	0.06±0.02	0.06±0.03	0.15±0.05	0.08±0.03
Firmicutes												
<i>g_Staphylococcus</i>	0.17±0.06	0.45±0.38	0.05±0.03	0.26±0.12	0.18±0.12	0.07±0.02	0.05±0.03	0.1±0.09	0.15±0.04	0.12±0.05	0.13±0.05	0.05±0.02
<i>g_Enterococcus</i>	59±3.68	48.5±14.7	51±9.1	39.8±10.6	52.2±10.2	51.6±8	52.6±12.9	45.8±15.2	11.5±4.19	23±8.79	11.5±3.51	32.3±9.04
<i>g_Lactobacillus</i>	1.51±0.52	0.72±0.48	1.28±0.53	4.2±2.54	2.85±0.68	2.68±1.29	2.5±1.34	3.36±0.7	2.51±0.38	1.98±0.54	1.61±0.32	1.4±0.4
<i>g_Ligilactobacillus</i>	8.33±1.95	10.2±5.41	6.02±3.11	19±7.21	14.5±4.58	12.6±3.82	11.5±4.46	17.7±6.46	22.5±3.02	17.7±3.82	23.4±3.95	13.8±2.86
<i>g_Limosilactobacillus</i>	0.03±0.02	0.02±0.02	0.01±0.01	0.06±0.04	0.03±0.02	0.01±0	0.03±0.02	0.04±0.04	0.08±0.04	0.01±0	0.05±0.03	0.05±0.05
<i>g_Lactococcus</i>	0.34±0.13	0.43±0.3	0.21±0.1	1.63±1.21	0.07±0.06	0.23±0.08	0.03±0.01	0.29±0.23	0.32±0.15	0.27±0.11	0.5±0.23	0.22±0.11
<i>g_Streptococcus</i>	1.19±0.43	1.1±0.74	0.64±0.28	3.47±0.6	1.04±0.17	1.43±0.59	0.95±0.43	3.49±2.81	0.58±0.09	1.0±0.58	0.66±0.1	0.31±0.08
<i>g_Clostridium sensu stricto 1</i>	0.16±0.07	0.27±0.16	0.15±0.09	ND	1.27±0.66	1.58±0.69	0.36±0.22	0.65±0.58	4.89±0.9	4.82±1.57	5.23±2.07	3.95±0.67
<i>g_Clostridium sensu stricto 13</i>	0.07±0.06	0.09±0.09	0.05±0.05	ND	0.76±0.3	0.3±0.19	0.17±0.17	0.15±0.1	1.95±0.69	0.99±0.43	0.62±0.14	0.63±0.14
<i>g_Blautia</i>	0.15±0.14	ND	ND	0.03±0.03	ND	0.43±0.3	0.27±0.27	0.02±0.02	3.27±0.92	3.3±1.35	8.7±2.53	3.67±1.42
<i>f_Lachnoclostridium</i>	0.01±0	0.09±0.08	ND	ND	0.37±0.21	1.11±0.82	0.24±0.24	1.38±1.17	8.09±2.88	6.28±1.91	6.75±2.46	9.21±3.56
<i>f_Lachnospiraceae</i>	ND	ND	ND	ND	ND	ND	ND	ND	0.61±0.61	0.01±0.01	ND	0.96±0.96
<i>g_Terrisporobacter</i>	ND	0.03±0.03	ND	0.03±0.03	0.47±0.18	0.3±0.23	0.17±0.1	0.85±0.78	2.14±0.25^a	1.64±0.31^a	0.97±0.17^b	0.97±0.2^b
<i>f_Ruminococcaceae</i>	ND	ND	ND	ND	ND	ND	ND	ND	0.15±0.15	0.61±0.27	0.1±0.09	0.01±0.01
<i>g_Clostridium</i>	0.09±0.04	0.05±0.04	0.05±0.03	ND	0.95±0.58	1.19±0.44	0.35±0.21	1.2±1.13	7.33±0.99	4.79±1.27	6.01±1	5.85±1.9
Proteobacteria												
<i>g_Enterobacter</i>	1.54±1.08	5.88±4.82	7.04±3.99	7.06±3.46	0.02±0.01	0.33±0.17	1.09±1.09	ND	0.44±0.25	ND	ND	0.06±0.05
<i>g_Escherichia-Shigella</i>	20.6±3.79	18.7±6.46	21±8.98	9.17±9.1	22.7±3.83	22.6±4.8	26.3±9.14	22.7±4.17	31.7±1.91	31.7±4.48	32.1±3.16	23.6±1.97
<i>g_Morganella</i>	ND	0.5±0.5	ND	ND	0.15±0.15	0.38±0.23	0.39±0.3	1.02±0.99	0.11±0.11	0.76±0.28	0.36±0.13	0.61±0.33
<i>g_Proteus</i>	3.27±2.9^a	1.25±0.57^a	0.02±0.02^b	ND ^b	1.89±1.56	2.02±1.46	0.24±0.23	0.17±0.14	0.77±0.23	0.39±0.11	0.57±0.21	1.51±0.63
<i>f_Enterobacteriaceae</i>	2±1.15	6.23±4.89	8.63±5.08	8.06±3.04	0.12±0.06	0.71±0.37	1.25±1.23	0.29±0.15	0.53±0.27	0.15±0.05	0.36±0.26	0.39±0.21
<i>g_Haemophilus</i>	0.38±0.19	0.78±0.76	0.35±0.32	2±0.99	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.03	ND	ND	0.01±0	ND
<i>g_Acinetobacter</i>	0.45±0.21	1.89±1.61	0.43±0.35	0.77±0.28	0.08±0.03	0.14±0.07	1.11±0.84	0.15±0.06	0.01±0	0.03±0.01	0.08±0.03	0.04±0.03
<i>g_Pseudomonas</i>	0.25±0.2	0.68±0.59	0.01±0	1.84±1.66	0.02±0.01	0.02±0.02	0.06±0.03	0.02±0.01	0.01±0.01	0.01±0.01	0.02±0.02	0.01±0
<i>g_Stenotrophomonas</i>	0.47±0.23	2.07±1.3	3.02±2.85	2.54±1.53	0.19±0.1	0.18±0.1	0.31±0.15	0.41±0.17	0.12±0.05	0.05±0.01	0.08±0.02	0.04±0.01