SUPPLEMENTARY MATERIALS AND METHODS

DNA extraction

DNA was extracted from faecal samples using the PowerFecal™ DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA concentration and quality were measured by NanoDrop 2000 (Thermo Scientific) and Qubit 2.0 fluorometer (Invitrogen).

Amplification and High-Throughput Sequencing

Briefly, the V4 and V5 region of the bacterial 16S rRNA gene was amplified from extracted DNA with universal primers (U515F: 5'-GTGYCAGCMGCCGCGGTA and U927R: 5'-CCCGYCAATTCMTTTRAGT). Forward fusion primers consisted of the GS FLX Titanium primer A and the library key (5' -CATCTCATCCCTGCGTGTCTCCGACTCAG) together with one of a suite of sixteen 10-base multiplex identifiers (MIDs 1–16) (Roche Diagnostics Ltd, UK). Reverse fusion primers included the GS FLX Titanium primer B and the library key (5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG). Amplification was performed with FastStart HiFi Polymerase (Roche Diagnostics Ltd, UK) using the following cycling conditions: 94°C for 3 min; 30 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1 min; followed by 72°C for 8 min. Ampure XP magnetic beads (Beckman Coulter) were used for purification of amplicons. Amplicon concentration was assessed using the fluorescence-based Picogreen assay (Invitrogen) and concentrations normalized before pooling. Amplicon pools were immobilized and amplified on beads by emulsion PCR using Lib-L emPCR kits (Roche Diagnostics Ltd, UK). Unidirectional sequencing from the forward primer was performed on the 454 GS FLX Titanium platform according to the manufacturer's instructions (Roche Diagnostics Ltd, UK).

Real-time quantitative PCR (qPCR)

We quantified *Bifidobacterium* using real time qPCR as it has been reported that primers used for the high throughput sequencing may underestimate *Bifidobacterium* abundance (Sim *et al.*, 2012). Confirming this, *Bifidobacterium* was only detected in 45% of the samples using metagenomics approach whereas *Bifidobacterium* was detected in all samples by real-time qPCR.

Bacteria groups in the samples were quantified by real-time qPCR on a QuantStudio 7 Flex Real-time system (Life technologies, USA). Amplification data was analysed using the QuantStudio 7 Flex Real-time system software v1.0. Primers used in the reactions are as previously described and represented in Table 1.

Table 1: Primers used for qPCR.

Target	Primer Name	Sequecnce	Product bp	Reference
***Clostridium leptum* subgroup**	C-leptF	GCACAAGCAGTGGAGT	239	(Matsuki *et al.*, 2002)
	C-leptR	CTTCCTCCGTTTTGTCA		
***Clostridium coccoides* subgroup**	C-cocF	AAATGACGGTACCTGACTAA	440	(Matsuki *et al.*, 2002)
	C-cocR	CTTTGAGTTTCATTCTTGCGAA		
Roseburia	RosF	TACTGCATTGGAAACTGTCG	230	(Larsen *et al.*, 2010)
	RosR	CGGCACCGAAGAGCAAT		
***Lactobacillus* group**	LacF	AGCAGTAGGGAATCTTCCA	341	(Penders *et al.*, 2005)
	LacR	CACCGCTACACATGGAG		
Bifidobacterium	BifF	GCGTGCTTAACACATGCAAGTC	126	(Rinttilä *et al.*, 2004)
	BifR	CACCCGTTTCCAGGAGCTATT		
All bacteria	UnivF	TCCTACGGGAGGCAGCAGT	466	(Walter *et al.*, 2001)
	UnivR	GACTACCAGGGTATCTAATCCTGTT		

Enterobacteriaceae	EcoF	CATTGACGTTACCCGCAGAAGAAGC	190	(Frank *et al.*, 2007)
	EcoR	CTCTACGAGACTCAAGCTTGC		

A typical 20 µl qPCR reaction contained 0.3µM of each (forward and reverse) primer, 10 µl GoTaq qPCR master mix, 7.8 µl of nuclease-free water and 5 – 20 ng of template genomic DNA extract. Each reaction mix is summarised in table 2.

Table 2: qPCR Reaction mix.

	Volume (µl)	**Final concentration**
GoTaq qPCR master mix, 2x	10	1x
Nuclease-free water	7.8	n/a
Forward primer	0.6	0.3 (µM)
Reverse primer	0.6	0.3 (µM)
DNA template	1	Approx. 5 – 20 ng
Total	20	n/a

The qPCR cycling protocol are as described below;

- 1x initial denaturation cycle at 95^{o}C for 2 minutes
- 40x (Denaturation at 95^{o}C for 15 seconds, annealing at 55^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds) – fluorescence measured after each cycle
- 1x dissociation – 60 – 95^{o}C

Quantitation of each target in the samples was determined based on a standard curve of each target using purified target DNA template. A 10-fold dilution series ranging from $1x10^{4}$ to $1x10^{8}$ copies of each target gene was prepared in nuclease-free water and analysed in triplicate. The test samples were analysed in 96-well plates (MicroAMP Optical plates, Life Technologies, USA), along with the standard. The qPCR software generated the standard curve (based on the average of each standard) and computed the template concentrations. The

amplification of a single product by the primer sets used was confirmed by analysis of the dissociation profile of each target and agarose gel electrophoresis of a standard PCR reaction using each primer set, the same cycling conditions and DNA template.

Preparation of template DNA

Isolated DNA was diluted 1 in 50 in nuclease-free water to reduce the concentration of putative PCR inhibitors carried-over from the DNA isolation process. The resulting material was quantified using NanoDrop and subsequently used as PCR template.

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SUPPLEMENTARY DATA

Table S1: Lipids and inflammatory markers before and after 12 weeks of supplementation[a].

	Prebiotic		Placebo		
	Pre	**Post**	**Pre**	**Post**	**P-value[c]**
NEFA (mmol/L)	0.61 ± 0.06	0.61 ± 0.06	0.71 ± 0.07	0.68 ± 0.06	0.865
TAGs (mmol/L)	1.03 ± 0.10	1.02 ± 0.13	0.91 ± 0.11	0.97 ± 0.09	0.534
Total cholesterol (mmol/L)	3.40 ± 0.20	3.33 ± 0.16	3.44 ± 0.26	3.65 ± 0.21	0.068
HDL cholesterol (mmol/L)	1.03 ± 0.08	1.09 ± 0.09	1.00 ± 0.07	1.09 ± 0.07	0.798
LDL cholesterol (mmol/L)	1.91 ± 0.18	1.77 ± 0.16	2.02 ± 0.20	2.11 ± 0.17	0.051
LPS (EU/mL)[b]	0.63 (0.49-1.27)	0.60 (0.315-1.125)	1.00 (0.435-1.370)	1.10 (0.375-2.590)	0.229
LBP (µg/mL)	11.43 ± 0.81	12.39 ± 1.89	10.10 ± 1.21	11.2 ± 1.30	0.671
sCD14 (µg/mL)	1.26 ± 0.17	1.08 ± 0.11	0.95 ± 0.08	0.99 ± 0.08	0.811
hsCRP (mg/L)	1.31 (0.72 - 1.69)	1.26 (0.82-3.18)	1.65 (0.45-3.58)	0.92 (0.49-1.86)	0.444
IL-6 (pg/mL)	12.17 ± 1.68	12.90 ± 2.58	11.53 ± 2.08	8.21 ± 1.06	0.103
TNF-α (pg/mL)	17.7 (5.0-22.3)	13.0 (5.0-23.4)	8.2 (5.0-13.0)	5.0 (5.0-13.0)	0.640

[a]Means ± SEM or median (interquartile ranges) presented. n = 14 in the prebiotic group and n =15 in placebo group unless otherwise stated. There are no differences in baseline (Pre) values between groups ($P > 0.05$, unpaired t-test or Mann-Whitney test). [b]n= 13 per group. [c]The P-value is for the comparison of the change between groups with Pre value as covariate (ANCOVA). NEFA: Non-esterified fatty acids. TAG: triglycerides. LPS: lipopolysaccharide. LBP: Lipopolysaccharide binding protein. sCD14: soluble CD14. hsCRP: high sensitivity C-reactive protein. IL-6: Interleukin 6. TNF-α: Tumour necrosis factor α.

Table S2: Dietary intakes (mean± SEM) before supplementation (Pre) and during the last week of supplementation (post) as assessed by seven day food diaries[a].

	Prebiotic		Placebo		
	Pre	**Post**	**Pre**	**Post**	**P-value[c]**
Energy (kJ/day)	8929 ± 538	9099 ± 601	8683 ± 581	8285 ± 375	0.170
Total carbohydrate (g/day)	225.2 ± 19.0	223.0 ± 13.4	208.0 ± 17.4	198.3 ± 12.3	0.197
Sugars (g/day)	79.4 ± 11.9	79.6 ± 9.3	76.2 ± 9.8	71.0 ± 7.2	0.296
Protein (g/day)	82.1 ± 4.6	83.9 ± 6.1	85.1 ± 4.9	88.1 ± 3.3	0.682
Total fat (g/day)	87.3 ± 6.6	90.1 ± 8.1	87.6 ± 6.8	81.7 ± 4.9	0.176
Saturated fat (g/day)	30.2 ± 2.8	30.6 ± 2.7	28.0 ± 2.0	27.2 ± 2.1	0.443
Dietary fibre (g/day)	22.5 ± 1.5	21.9 ± 1.6	21.9 ± 1.5	20.8 ± 1.5	0.674
Alcohol (g/day)	12.7 ± 3.6	15.4 ± 5.7	12.6 ± 3.3	10.6 ± 3.3	0.202
Total carbohydrate (E%)	42.1 ± 2.5	42.1 ± 2.2	40.0 ± 1.5	40.1 ± 1.7	0.847
Sugars (E%)	14.5 ± 1.7	15.2 ± 1.8	14.3 ± 1.0	14.0 ± 1.1	0.352
Protein (E%)	15.7 ± 0.9	15.5 ± 0.7	16.8 ± 0.8	18.0 ± 0.5[b]	0.004
Total fat (E%)	36.6 ± 1.5	36.7 ± 1.2	37.7 ± 1.5	37.0 ± 1.4	0.819
Saturated fat (E%)	12.5 ± 0.8	12.6 ± 0.6	12.1 ± 0.4	12.2 ± 0.8	0.907
Alcohol (E%)	4.2 ± 1.2	4.5 ± 1.8	4.2 ± 1.0	3.7 ± 1.1	0.457

[a]n = 14 for prebiotic group and n = 15 for placebo group.. There are no differences in baseline (Pre) values between groups ($P > 0.05$, unpaired t-test).[b]Significant within group change ($P < 0.05$, paired t-test). [c]The P-value is for the comparison of the change between groups with Pre value as covariate (ANCOVA). E%: percentage of energy.

Table S3: Diversity, richness and evenness indices based on OTU data set (mean ± SEM, prebiotic group: *n* = 7, placebo group: *n* =9).

	Prebiotic		Placebo		
	Pre	**Post**	**Pre**	**Post**	**P-value[b]**
Diversity (Shannon)	3.22 ± 0.11	3.59 ± 0.17[a]	3.49 ± 0.15	3.51 ± 3.51	0.324
Diversity (Simpson)	0.92 ± 0.01	0.94 ± 0.02	0.93 ± 0.01	0.93 ± 0.01	0.509
Diversity (Inverse Simpson)	13.59 ± 1.69	22.33 ± 3.46[a]	17.63 ± 2.80	17.96 ± 2.44	0.133
Richness	25.98 ± 3.03	38.83 ± 5.21[a]	35.85 ± 4.80	35.17 ± 3.82	0.261
Evenness	0.70 ± 0.02	0.76 ± 0.03	0.73 ± 0.03	0.74 ± 0.01	0.397

[a]$P < 0.05$, within group comparison (paired t-test). There were no significant differences between groups in any of the indices at baseline. [b]The *P*-value is for the comparison of the change between groups with Pre value as covariate (ANCOVA).