

Supplement 1. Methodology of the primers and optimised quantitative real-time PCR (QPCR) conditions applied to faecal samples of participants after follow a their habitual diets and provided low FODMAP and typical Australian diets.

All QPCR was performed in triplicate on a CFX 384™ real-time PCR Detection System (Bio-Rad, CA, USA) in a total volume of 10  $\mu$ L. Each reaction consisted of 3  $\mu$ L (1 ng/ $\mu$ L) DNA template and 7  $\mu$ L of PCR mixture containing 5  $\mu$ L SsoFast™ EvaGreen® Supermix, 0.5  $\mu$ L bovine serum albumin, forward and reverse primers, and PCR-grade water (Sigma-Aldrich, MO, USA). The QPCR cycling conditions were: hot-start 98 °C for 3 min followed by 35 cycles of two-step QPCR with denaturing at 98 °C for 15 s and annealing/elongation time and temperature as in the Table. This was followed by fluorescence acquisition after each cycle. A final melt-curve analysis was performed after completion of all cycles with fluorescence acquired at 0.5 °C intervals between 55 and 95 °C to verify specificity of amplification. An eight-series of 10-fold dilutions of a sample derived standard containing the target amplicon was analysed in parallel with DNA samples for estimation of PCR efficiency and absolute abundance. Data were analysed with Bio-Rad CFX Manager software (Version 2.1) for absolute abundances<sup>1</sup>. Data were presented as absolute abundance and as a proportion of total bacteria.

Table. Real-time PCR primers used and their amplification conditions.

Target	Primers	Sequence (5'-3')	Conc. (nM)	Annealing		Reference
				Temp (°C)	Time (Sec.)	
Total bacteria	UnivF UnivR	TCCTACGGGAGGCAGCAGT GGACTACCAGGGTATCTATCCTGTT	500	60	45	<sup>2</sup>
<i>Clostridium</i> cluster IV	sg-Clept-F sg-Clept-R	CTTTGAGTTTCATTCTTGCGAA GCACAAGCAGTGGAGT	500	56	20	<sup>3</sup>
<i>F. prausnitzii</i>	FPR-1F FPR-2R	AGATGGCCTCGCGTCCGA CCGAAGACCTTCTTCCTCC	500	60	20	<sup>4</sup>
<i>Clostridium</i> cluster XIVa	g-Ccoc-F g-Ccoc-R	AAATGACGGTACCTGACTAA CTTTGAGTTTCATTCTTGCGA A	500	60	20	<sup>3</sup>
<i>Roseburia</i> spp.	RosF RosR	TACTGCATTGGAAACTGTCTG CGGCACCGAAGAGCAAT	700	58	45	<sup>5</sup>
<i>Lactobacilli</i> group	Lacto-F Lacto-R	AGCAGTAGGGAATCTTCCA CACCGCTACACATGGAG	600	56	20	<sup>6,7</sup>
<i>Bifidobacterium</i> spp.	Bif-F Bif-R	TCGCGTC(C/T)GGTGTGAAAG CCACATCCAGC(A/G)TCCAC	600	56	20	<sup>8</sup>
<i>A. muciniphila</i>	AM1 AM2	CAGCACGTGAAGGTGGGGAC CCTTGCGTTGGCTTCAGAT	350	63	30	<sup>9</sup>
<i>R. gnavus</i>	RgnaF RgnaR	GGACTGCATTTGGAAGTGTGAG AACGTCAGTCATCGTCCAGAAAG	500	58	20	<sup>10</sup>

<i>R. torques</i>	RtorF	GCTTAGATTCTTCGGATGAAGAGGA	500	58	40	10
	RtorR	AGTTTTTACCCCCGCACCA				

Microbial diversity of the *Clostridium* cluster XIVa, which includes a large number of mainly butyrate-producing bacteria, was determined by denaturing gradient gel electrophoresis (DGGE) using the INGENYphorU-2 gel electrophoresis system (Ingeny International, NL, USA) according to the manufacturer's method for perpendicular gels. These analyses were carried out using *Clostridium* cluster XIVa-specific primers, Erec688F and Erec841R-GC, as previously described<sup>11</sup>. Briefly, bacterial PCR products were separated on a gel with a 40-65% denaturing gradient and 6% acrylamide. Gels were loaded with 15 µl of PCR product and run at 110V for 16 h at 60 °C. They were then cooled and stained for 30 min using 10 µl of 1x Sybr-gold nucleic acid stain (Molecular Probes, Eugene, USA). Gels were de-stained in 100 mL of MilliQ water and digitally photographed using a Dark Reader transilluminator (DR195M, Clare Chemical Research, USA) equipped with a DigiDoc System (Bio-Rad Laboratories, CA, USA) with an attached Syber-gold filter. The DGGE banding patterns for each specimen were analysed using the GelCompar II version 6.5 (Applied Maths Inc., Texas, USA) software package by normalised and standardised all gels using four DGGE standards run on every gel. The total banding intensities were standardised between lanes and individual band intensities were expressed as a fraction of total peak height for that sample. The Shannon index<sup>12</sup> was calculated for each DGGE profile, also taking into account relative band intensities, to estimate bacterial diversity.

Lactate and succinate were measured as previously published<sup>13</sup>. Briefly, lactate and succinate were measured in duplicate digesta samples by L-lactate and succinic acid kits from Megazyme (Ireland, UK). Digesta samples of approximately 0.5 g were diluted three fold in distilled water, mixed thoroughly and centrifuged at 2,000 g for 10 min. The samples were then clarified by mixing 500 µL of supernatant with 300 µL water, 50 µL of each Carrez solution and 100 µL NaOH. The mixed solution was then filtered using a 0.45 µm syringe filter. The lactate and succinate assays were then conducted separately in 96 well plates following the kit instructions. Lactate and succinate concentration was determined using a spectrophotometer (SpectroMax, Molecular Devices Corporation, Sunnyvale, CA) at an absorbance of 340 nm. The data are reported as g per 100 g and the lower detection limit is 0.016 g/100g.

## References:

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