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# **Abstract**

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# Complementary foods modulate the colonic microbiota of New Zealand weaning infants: an in vitro study

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The transition from breastmilk to solid foods (weaning) is a critical stage in infant development and plays a decisive role in the maturation of the complex microbial community inhabiting the human colon. Diet is a major factor shaping the colonic microbiota, which ferments nutrients reaching the colon unabsorbed by the host to produce a variety of microbial metabolites influencing host physiology(1). Therefore, making adequate dietary choices during weaning can positively  $modulate \ the \ colonic \ microbiota, \ ultimately \ contributing \ to \ health \ in \ in fancy \ and \ later \ life^{2)}. \ However, our \ understanding$ of how complementary foods impact the colonic microbiota of weaning infants is limited. To address this knowledge gap, we employed a metagenome-scale modelling approach to simulate the impact of complementary foods, either combined with breastmilk or with breastmilk and other foods, on the production of organic acids by colonic microbes of weaning infants<sup>(3)</sup>. Complementary foods and combinations of foods with the greatest impact on the *in silico* microbial production of organic acids were identified. These foods and food combinations were further tested in vitro, individually or in combination with infant formula. Fifty-three food samples were digested using a protocol adapted from INFOGEST to mimic infant digestion and then fermented with faecal inoculum from 6 New Zealand infants (5-11 months old). After 24h of fermentation, the production of organic acids was measured by gas chromatography. Differences in organic acid production between samples were determined using the Tukey Honestly Significant Difference test to account for multiple comparisons. The microbial composition was characterised by amplicon sequencing of the V3-V4 regions of the 16S bacterial gene. Taxonomy was assigned using the DADA2 pipeline and the SILVA database (version 138.1). Bioinformatic and statistical analyses were conducted using the R packages phyloseq and ANCOM-BC2, with the Holm-Bonferroni adjustment to account for false discovery rates in differential abundance testing. Blackcurrant and raspberries increased the production of acetate and propionate (Tukey's test, p<0.05) and the relative abundance of the genus Parabacteroides (Dunnett's test, adjusted p<0.05) compared to other foods. Raspberries also increased the abundance of the genus Eubacterium (Dunnett's test, adjusted p<0.05). When combined with infant formula, black beans stood out for increasing the production of butyrate (Tukey's test, p<0.05) and the relative abundance of the genus Clostridium (Dunnett's test, adjusted p<0.05). In conclusion, this study provides new evidence on how complementary foods, both individually or in combination with other dietary compounds, influence the colonic microbiota of weaning infants in vitro. Insights generated by this research can help design future clinical trials, ultimately enhancing our understanding of the relationship between human nutrition and colonic microbiota composition and function in post-weaning life.

Keywords: infant; food; gut microbiota

Ethics Declaration: Yes

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