

Non-medicinal excipients in infant vitamin D drops, fecal microbiota and their metabolites at 3 months of age

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Introduction: Health Canada recommends exclusively breastfed, healthy, term infants be supplemented with vitamin D. Vitamin D liquid formulations contain non-medicinal excipients, such as glycerin (glycerol) or propylene glycol (PG). Digested glycerin and PG are fermentable by gut microbiota, producing metabolites likely influencing the host. In this respect, we sought to assess infant fecal samples for the presence of glycerin and PG levels in relation to vitamin D supplementation and their association with gut microbiota and metabolites.

Methodology: Fecal samples, information on infant vitamin D supplementation and breastfeeding status were collected at 3 months of age from 575 infants in the Canadian Healthy Infant Longitudinal Development (CHILD) Cohort Study. Fecal metabolites and microbiota were quantified using Nuclear Magnetic Resonance Spectroscopy and 16S rRNA sequencing, respectively. Logistic regression was used to determine the association between vitamin D supplementation and fecal levels of glycerol and PG. Spearman's rank correlation was used to correlate fecal microbiota and metabolites with glycerol or PG.

Results: Seventy-eight percent of exclusively breastfed infants were supplemented with vitamin D drops. Controlling for breastfeeding status, introduction to solids and maternal education, infants supplemented with vitamin D had 1.6 times higher odds of high-level fecal PG ($p < 0.05$), and 41% lower odds of high-level fecal glycerol ($p < 0.05$) compared to the control group. Fecal PG levels were positively correlated with the relative abundance of *Lactobacillus* spp. ($r = 0.3$, $p < 0.05$), Enterobacteriaceae ($r = 0.2$, $p < 0.05$), *Bifidobacterium* spp. ($r = 0.2$, $p < 0.05$) but inversely related to Lachnospiraceae ($r = -0.3$, $p < 0.05$). Accordingly, a higher fecal PG concentration was correlated with higher acetate ($r = 0.1$, $p < 0.05$) but lower butyrate ($r = -0.4$, $p < 0.05$) and propionate ($r = -0.4$, $p < 0.05$) levels.

Conclusions: Infants supplemented with vitamin D exhibit higher fecal PG levels. The novel link between fecal PG and probiotic microbiota, notably *Bifidobacterium*, and with microbial acetate, butyrate and propionate may have implications for infant health.