Electronic Supplementary Material (ESI) for Food & Function. This journal is © The Royal Society of Chemistry 2023

Supplementary Figure legends



Fig S1. Vitamin D deficiency in early life effect on serum 1,25-dihydroxyvitamin D₃, calcium and PTH in adult and offspring rats. 1,25-dihydroxyvitamin D₃ levels in the serum of (A) F1 rats at 8 week, (B) F1 rats at 18 week, (C) F2 rats at 8 week. Serum calcium ion levels in (D) F1 rats at 8 week, (E) F1 rats at 18 week, (F) F2 rats at 8 week. PTH levels in the serum of (G) F1 rats at 8 week, (H) F1 rats at 18 week, (I) F2 rats at 8 week, (I) F2 rats at 8 week. Data were presented as the means \pm SDs, Early-VDD vs. Control group * P < 0.05.



Fig S2. The effect of early vitamin D deficiency on fasting blood glucose, blood insulin concentration and HOMA-IR in adult and offspring rats. Fasting blood glucose levels of (A) F1 rats 8 week, (B) F1 rats 18 week and (C) F2 rats 8 week. Blood insulin concentration of (D) F1 rats 8 week, (E) F1 rats 18 week and (F) F2 rats 8 week. HOMA-IR levels of (G) F1 rats 8 week, (H) F1 rats 18 week, (I) F2 rats 8 week. Data were presented as the means \pm SDs, Early-VDD vs.Control group * P < 0.05.



Fig S3.The effect of early vitamin D deficiency on serum lipid metabolism in F1 rats. Lipid metabolism of F1 rats at 8 week (A-H). (A)ALT, (B)AST, (C)ALB, (D)TP, (E) TG,(F)CHO, (G)HDL, (H)LDL. Lipid metabolism of F1 rats at 18 week (I-P).(I) ALT, (J) AST, (K) ALB,(L) TP, (M) TG, (N) CHO, (O) HDL, (P) LDL. Data were presented as the means \pm SDs, Early-VDD vs. Control group * P < 0.05.



Fig. S4. Vitamin D deficiency in early life alters the composition of gut microbiota in F1 male rats at 8 week. (A) Comparison of Alpha Diversity of gut microbiota between different groups using Shannon index. (B) Comparison of Beta Diversity of gut microbiota between different groups using non-linear model PCoA method based on OTUs information in samples. (C) Relative abundance of microbiota at the genus level. (D) Cladogram generated from LEfSe analysis. (E) A histogram of the log 10 transformed Linear discriminant analysis (LDA) scores was computed for features that showed differential abundance between F1 Male Early-VDD and F1 Male Control at 8 week. (F) The top ten genera with a rich difference between F1 Male Early-VDD and F1 Male Control at 8 week.



Fig. S5. Vitamin D deficiency in early life alters the composition of gut microbiota in F1 female rats at 8 week. (A) Comparison of Alpha Diversity of gut microbiota between different groups using Shannon index. (B) Comparison of Beta Diversity of gut microbiota between different groups using non-linear model PCoA method based on OTUs information in samples. (C) Relative abundance of microbiota at the genus level. (D) Cladogram generated from LEfSe analysis. (E) A histogram of the log 10 transformed Linear discriminant analysis (LDA) scores was computed for features that showed differential abundance between F1 Female Early-VDD and F1 Female Control at 8 week. (F) The top ten genera with a rich difference between F1 Female Early-VDD and F1 Female Control at 8 week.



Fig. S6. Vitamin D deficiency in early life effects on the composition of gut microbiota in F1 male rats at 18 week. (A) Comparison of Alpha Diversity of gut microbiota between different groups using Shannon index. (B) Comparison of Beta Diversity of gut microbiota between different groups using non-linear model PCoA method based on OTUs information in samples. (C) Relative abundance of microbiota at the genus level. (D) Cladogram generated from LEfSe analysis. (E) A histogram of the log 10 transformed Linear discriminant analysis (LDA) scores was computed for features that showed differential abundance between F1 Male Early-VDD and F1 Male Control at 18 week. (F) The top ten genera with a rich difference between F1 Male Early-VDD and F1 Male Control at 18 week.



Fig. S7. Vitamin D deficiency in early life effects on the composition of gut microbiota in F1 female rats at 18 week. (A) Comparison of Alpha Diversity of gut microbiota between different groups using Shannon index. (B) Comparison of Beta Diversity of gut microbiota between different groups using non-linear model PCoA method based on OTUs information in samples. (C) Relative abundance of microbiota at the genus level. (D) The top ten genera with a rich difference between F1 Female Early-VDD and F1 Female Control at 18 week.



Fig. S8. Vitamin D deficiency in early life alters the composition of gut microbiota in F2 male rats at 8 week. (A) Comparison of Alpha Diversity of gut microbiota between different groups using Shannon index. (B) Comparison of Beta Diversity of gut microbiota between different groups using non-linear model PCoA method based on OTUs information in samples. (C) Relative abundance of microbiota at the genus level. (D) Cladogram generated from LEfSe analysis. (E) A histogram of the log 10 transformed Linear discriminant analysis (LDA) scores was computed for features that showed differential abundance between F2 Male Early-VDD and F2 Male Control at 8 week. (F) The top ten genera with a rich difference between F2 Male Early-VDD and F2 Male Control at 8 week.



Fig. S9. Vitamin D deficiency in early life alters the composition of gut microbiota in F2 female rats at 8 week. (A) Comparison of Alpha Diversity of gut microbiota between different groups using Shannon index. (B) Comparison of Beta Diversity of gut microbiota between different groups using non-linear model PCoA method based on OTUs information in samples. (C) Relative abundance of microbiota at the genus level. (D) Cladogram generated from LEfSe analysis. (E) A histogram of the log 10 transformed Linear discriminant analysis (LDA) scores was computed for features that showed differential abundance between F2 Female Early-VDD and F2 Female Control at 8 week. (F) The top ten genera with a rich difference between F2 Female Early-VDD and F2 Female Control at 8 week.



Fig. S10. Changes of metabolites and metabolic pathways in feces of F1 male rats at 8 week. (A) Heatmap of changed fecal metabolites in F1 Male Early-VDD and F1 Male Control by LC-MS analysis at 8 week. (B) KEGG enriched pathways of changed fecal metabolites by LC-MS in F1 Male Early-VDD vs. F1 Male Control at 8 week. (C) Heatmap of changed fecal metabolites in F1 Male Early-VDD and F1 Male Control by GC-MS analysis at 8 week. (D) KEGG enriched pathways of changed fecal metabolites by GC-MS in F1 Male Early-VDD vs. F1 Male Control at 8 week.



Fig. S11. Changes of metabolites and metabolic pathways in feces of F1 female rats at 8 week. (A) Heatmap of changed fecal metabolites in F1 Female Early-VDD and F1 Female Control by LC-MS analysis at 8 week. (B) KEGG enriched pathways of changed fecal metabolites by LC-MS in F1 Female Early-VDD vs. F1 Female Control at 8 week. (C) Heatmap of changed fecal metabolites in F1 Female Early-VDD and F1 Female Control by GC-MS analysis at 8 week. (D) KEGG enriched pathways of changed fecal metabolites by GC-MS in F1 Female Early-VDD vs. F1 Female Control by GC-MS in F1 Female Early-VDD vs. F1 Female Control at 8 week.



Fig. S12. Changes of metabolites and metabolic pathways in feces of F1 male rats at 18 week. (A) Heatmap of changed fecal metabolites in F1 Male Early-VDD and F1 Male Control by LC-MS analysis at 8 week. (B) KEGG enriched pathways of changed fecal metabolites by LC-MS in F1 Male Early-VDD vs. F1 Male Control at 8 week. (C) Heatmap of changed fecal metabolites in F1 Male Early-VDD and F1 Male Control by GC-MS analysis at 8 week. (D) KEGG enriched pathways of changed fecal metabolites by GC-MS in F1 Male Early-VDD vs. F1 Male Control at 8 week. (D) KEGG enriched pathways of changed fecal metabolites by GC-MS in F1 Male Early-VDD vs. F1 Male Control at 8 week.



Fig. S13. Changes of metabolites and metabolic pathways in feces of F1 female rats at 18 week.

(A) Heatmap of changed fecal metabolites in F1 Female Early-VDD and F1 Female Control by LC-MS analysis at 18 week. (B) KEGG enriched pathways of changed fecal metabolites by LC-MS in F1 Female Early-VDD vs. F1 Female Control at 18 week. (C) Heatmap of changed fecal metabolites in F1 Female Early-VDD and F1 Female Control by GC-MS analysis at 18 week. (D) KEGG enriched pathways of changed fecal metabolites by GC-MS in F1 Female Early-VDD vs. F1 Female Control at 18 week.



Fig. S14. Changes of metabolites and metabolic pathways in feces of F2 male rats at 8 week. Heatmap of changed fecal metabolites in F2 Male Early-VDD and F2 Male Control at 8 week. (B) KEGG enriched pathways of changed fecal metabolites in F1 Male Early-VDD vs. F1 Male Control at 8 week. (C) Correlation of fecal metabolites and gut microbiota.



Fig. S15. Changes of metabolites and metabolic pathways in feces of F2 female rats at 8 week. (A) Heatmap of changed fecal metabolites in F2 Female Early-VDD and F2 Female Control by LC-MS analysis at 8 week. (B) KEGG enriched pathways of changed fecal metabolites by LC-MS in F2 Female Early-VDD vs. F2 Female Control at 8 week. (C) Heatmap of changed fecal metabolites in F2 Female Early-VDD and F2 Female Control by GC-MS analysis at 8 week. (D) KEGG enriched pathways of changed fecal metabolites by GC-MS in F2 Female Early-VDD vs. F2 Female Control by GC-MS in F2 Female Early-VDD vs. F2 Female Control at 8 week.



Fig S16 Redundancy analysis between intestinal flora and the indicators of glucose tolerance at the genus level. Notes: fasting blood glucose levels (GLU1), blood insulin (Amount of Insulin), insulin resistance (IR), AUC Area under the curve of glucose tolerance test.