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**“Fecal Nitrogen and Proteolytic Fermentation Shape Phenolic Metabolite Profiles:
Evidence for Bacterial Origin Over Direct Dietary Signal”**

Background: Diet is one of the strongest drivers of gut microbial composition, and the bacteria in the colon determine which compounds get produced there. When dietary protein escapes digestion and reaches the colon, bacteria ferment phenylalanine and tyrosine into phenolic and aromatic acid metabolites, including phenylacetylglutamine (PAG) and p-cresol, linked to cardiovascular disease and uremic toxicity, respectively. Fecal nitrogen is a proxy for how much protein reaches the colon and the degree of proteolytic fermentation there. Whether fecal phenolic metabolites reflect dietary intake or microbial activity remains unclear. This analysis used fecal GC-MS metabolomics and dietary recall to test that distinction.

Methods: Fecal samples and dietary recall data from N = 37 healthy adults came from an existing cohort study. Untargeted GC-MS metabolomics identified 96 fecal metabolites across 10 chemical classes; the phenolic/aromatic acid class (n = 9) was investigated further because these compounds can arise from dietary polyphenol breakdown or bacterial amino acid fermentation. Each phenolic metabolite was correlated with fecal nitrogen via Spearman rank correlation ($p < 0.05$, uncorrected). Four metabolites with confirmed identities, syringic acid, 2-coumaric acid, DOPAC, and gentisic acid, were correlated against ASA24 dietary variables. A Wilcoxon rank-sum test on gentisic acid isolated diet from microbial activity.

Results: Across all 96 metabolites, the dominant pattern was an inverse association with fecal nitrogen, consistent with active fermentation of substrates into downstream products. In the phenol group, three phenylalanine-pathway intermediates; phenylpyruvic acid ($\rho = -0.43$), mandelic acid ($\rho = -0.37$), and alpha-phenylbutyric acid ($\rho = -0.36$); decreased together as fecal nitrogen rose, consistent with bacterial processing toward PAG, linked to platelet activation and cardiovascular risk. A similar decline in the tyrosine pathway (4-HPAA, $\rho = -0.42$) suggests processing toward p-cresol. Two plant-derived phenolics, syringic acid ($\rho = +0.35$) and 2-coumaric acid ($\rho = +0.33$), trended positively, pointing to a more dietary origin. Of the four metabolites tested against dietary recall, only gentisic acid showed a significant association: an inverse correlation with total plant food intake ($\rho = -0.298$, $p = 0.038$). A follow-up Wilcoxon test found no difference between consumers and non-consumers of gentisic acid-rich foods ($p = 0.925$; $p = 0.960$), indicating a microbial rather than dietary origin.

Conclusions: Proteolytic fermentation, driven by protein reaching the colon, appears to be the primary force shaping fecal phenolic metabolite profiles in this cohort. Coordinated depletion of phenylalanine and tyrosine intermediates as fecal nitrogen rises suggests bacterial flux toward PAG and p-cresol, which are compounds that have been associated cardiovascular and renal health risks. The gentisic acid result shows a dietary correlation can mask an underlying microbial explanation, supporting fecal phenolic profiling as a tool for studying colonic protein exposure.