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Metagenomic analysis of the effect of coconut milk on the colon microbiota

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The main source of fat in the diet affects the gut microbiome composition. Coconut milk (CM) has a high percentage of medium chain fatty acids (MCFA). A portion of MCFA reaches the colon and is fermented by the microbiota. This study was conducted with Wistar rats to study the effect of CM on colonic microbial diversity. Twelve-week-old female Wistar rats were randomly assigned to two experimental groups (12 rats/group). Ad libitum access to water and food was provided throughout the study. The control group was fed with a WHO-recommended diet containing 4.2 % total fat; of that 3% fat from soybean oil (SOD). The other group was fed a diet in which the fat component was replaced with CM (CMD). After 28 days, six rats from each group were fasted for 10–12 h and treated with ethanol (20%, 6 g/kg body weight) by oral gavage (SODE and CMDE). Mean ± standard deviation (SD) feed Intakes were 900.50±4.93 g, 899.50±9.31 g, 818.00±6.57 g, 820.00±6.57 g and body weight gains were 52.83±1.83 g, 52.33±1.75 g, 45.50±2.43 g, 47.33±2.34 g in CMD, CMDE, SOD and SODE groups respectively. Feed conversion rates were approximately equal in the four groups (average 0.0580±0.002). At the end of the feeding experiments, animals were subjected to barbiturate euthanasia and a transverse abdominal incision was made. The cecal wash samples with phosphate-buffered saline (pH 7.4) were stored at -80 °C. All experimental procedures were approved by the Ethics Review Committee, University of Kelaniya. Microbial DNA was isolated from cecal wash samples using DNeasy blood and tissue kit (Qiagen). The 16S rRNA gene libraries were prepared and sequenced according to the protocols recommended by Ion Torrent (Ion GeneStudio S5 prime system, Thermo Fisher Scientific). Trimmed sequences were clustered into operational taxonomic units (OTUs) with a hierarchical cutoff of 97.0% similarity using Ion Reporter v5.16. Taxonomic annotation was conducted against Curated MicroSEQ 16S Reference Library v2013.1 and Curated Greengenes v13.5 databases. Alpha-diversity and beta-diversity analyzes were performed using the QIIME2 platform. Bacteroidetes and Firmicutes phyla represent 90% of the cecal bacterial community across dietary groups. Other microbial phyla in the cecal wash samples were Actinobacteria, Proteobacteria, and Tenericutes. The cecal microbiota of CMD-fed rats was characterized by a significant increase ($P<0.05$) in the relative abundance of Desulfovibrionaceae, Eubacteriaceae, Erysipelotrichaceae, Lachnospiraceae Porphyromonadaceae, Ruminococcaceae bacterial families, and a decreased relative abundance of Bacteroidaceae, Clostridiaceae and Lactobacillaceae compared to the control diet. Studies have shown that alcohol promotes both dysbiosis and bacterial overgrowth. According to the two factor ANOVA, there was a significant difference ($P<0.05$) in colonic-microbiota between the four groups. Family level rarefaction plots were varied CMD>CMDE>SOD>SODE and CMD>SODE>CMDE>SOD according to Chao1 index and Simpson's indexes respectively. Principle component analysis revealed four distinct clusters, suggesting that both diet and alcohol-induced oxidative stress affected gut microbiota. The elevated bacterial families have an impact on microbial-mediated saccharolytic functions, lipophilic functions, vitamin synthesis, and protection against intestinal infections. Thus, the intestinal microbiota in Wistar rats varies significantly with dietary fat source and oxidative stress conditions.

Keywords: Gut Microbiome, 16S rRNA, Coconut milk, Wistar rats, Medium-chain fatty acids

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