EXPLORATORY STUDY TO IDENTIFY POTENTIAL EFFECT OF DEAMINASE INHIBITOR ON *CAMPYLOBACTER* AND CECAL MICROBIOTA SUPPLEMENTED WITH PEPTIDES

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I. INTRODUCTION

Characterizing the microbiota involved in metabolite cross-feeding with *Campylobacter*, particularly, the preferences for amino acids and peptides may help identify key metabolic interactions and offer non-*Campylobacter* bacterial targets for interventions that "starve" *Campylobacter*. Diphenyleneiodonium chloride (DIC) is a known nicotinamide adenine dinucleotide phosphate (NADPH)/nicotinamide adenine dinucleotide (NADH) oxidase inhibitor and flavoenzymes inhibitor [1]. Early work indicated that DIC reduced *C. jejuni* growth and amino acid catabolizing activity [2]. In the current study, we hypothesized that DIC would inhibit the growth of *C. jejuni* and affect the overall composition of a cecal microbial community incubated *in vitro*.

II. MATERIALS AND METHODS

The current study utilized a poultry cecal *in vitro* model where the treatments were administered via randomized complete block design where eight cecal contents were used as a block for each treatment and samples were collected at 0 and 18 h time points (N = 128, k = 8, n = 8). Cecal contents were diluted to 0.1% in anaerobic dilution solution (ADS) for each chicken. The treatments consisted of 1) a mix of structurally different peptides (intact casein, casein peptides, and casein amino acid hydrolysate) (mix), 2) casein acid hydrolysate (acid), 3) casein peptides (peptide), 4) intact casein (casein), 5) mix + DIC, 6) acid + DIC, 7) peptide + DIC, 8) casein + DIC. The treatments were administered to anaerobic batch cecal cultures at 7% per volume and 1 µmol of DIC and inoculated with 10^5 colony forming units (CFU/ml) of *C. jejuni*. The vials were incubated at 42°C under microaerophilic conditions. Genomic DNA was extracted and V4 region of the 16S rRNA gene was amplified using custom dual-indexed primers and sequenced on an Illumina MiSeq. Sequencing data were analyzed in QIIME2-2021.11 using Casava 1.8 paired-end demultiplexed format. Demultiplexed sequences were filtered and denoised for quality in dada2. Pairwise comparisons for alpha and beta diversity were determined using Kruskal-Wallis and ANOSIM. Linear mixed effect model (LME) was utilized to assess the effect of time on treatment. Differential abundance for each treatment was assessed via ANCOM. Core microbiota analysis was performed using *core members* and *phyloseq* package in RStudio Version 1.3 for eight treatments. The core microbiota was defined as amplicon sequencing variants (ASV) that represented at least 5% of the population and were abundant in 50% of the samples.

III. RESULTS AND DISCUSSION

Phylogenetic differences were observed between most treatments, such as peptide and peptide DIC and acid and acid DIC (Faith PD, $P < 0.05$). The phylogeny of the community was also significantly different at 18 h for peptide, mix, and casein groups based on LME results ($P < 0.05$; Fig. 1).

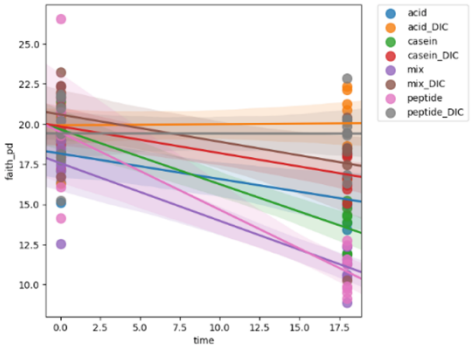

Figure 1. Linear mixed effects model for Faith PD representing the phylogenetic change of microbial communities from 0 to 18h

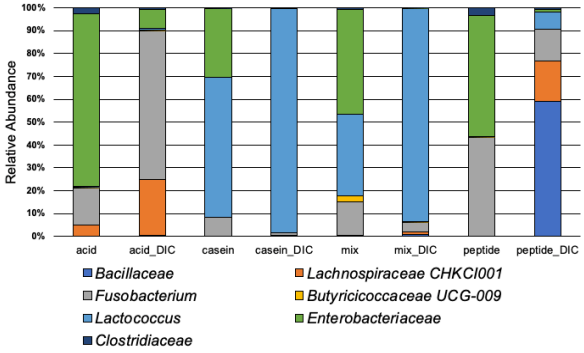

Figure 2. Significantly different taxa on genera level via ANCOM ($P < 0.05$) is visualized based on the median of relative abundance for each treatment.

From 226 identified taxa in treatments, six taxa significantly differed in relative abundance based on ANCOM analysis at the genus level. Based on core microbiota results, *Campylobacter* was a core member of the microbial community in each treatment except for casein DIC. *Synergistes* was present in all DIC groups at 18 h except for casein + DIC. *Victivallis, Megamonas,* and *Clostridia* UCG-014 were only present in casein DIC at 18 h, not other treatments. DIC effects on *Campylobacter* abundance differed from Anderson [2] findings that utilized enrichment media for *Campylobacter*. The findings indicated that adding DIC in peptide supplements seemed to recover certain microbial populations compared to the treatments without DIC (Fig. 1). Since DIC primarily acts on flavoproteins it can potentially affect energy conservation pathways such as oxidative phosphorylation and pathways dependent on the electron transport chain (ETC) [3, 4]. Enterobacteriaceae utilizes such pathways, which may be why DIC affected their abundance. In contrast, bacteria that ferment amino acids and peptides and utilize substrate-level phosphorylation, such as *Fusobacterium*, *Clostridia*, and *Lactococcus* were abundant in DIC treatments based on the core microbiota results and ANCOM (Fig. 2). These metabolic differences could explain the differences in taxa abundances in DIC treatments at 18 h.

IV. CONCLUSION

Utilization of DIC inhibitor altered phylogeny of the cecal microbial communities and did not reduce *Campylobacter* as a core member of the community, except for casein DIC treatment. The unique genera that were enriched by the casein DIC treatment can indicate bacterial associations that prevent the growth and abundance of *Campylobacter*.

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REFERENCES

1. Thakare, R., Kaul, G., Shukla, M., Kesharwani, P., Srinivas, N., Dasgupta, A., & Chopra, S. (2020). Chapter 5— Repurposing nonantibiotic drugs as antibacterials. In P. Kesharwani, S. Chopra, & A. Dasgupta (Eds.), Drug discovery targeting drug-resistant bacteria (pp. 105–138). Academic Press.
2. Anderson, R., Krueger, N., Byrd, R., Harvey, T., Callaway, T., Edrington, T., & Nisbet, D. (2009). Effects of thymol and diphenyliodonium chloride against *Campylobacter* spp. during pure and mixed culture *in vitro*. Journal of Applied Microbiology 107: 1258-1268.
3. Ratz, J., McGuire, J., Anderson, D., & Bennet, B. (2000). Effects of the flavoprotein inhibitor, diphenyleneidonium sulfate, on ex vivo organic nitrate tolerance in the rat. Journal of Pharmacology and Experimental Therapeutics, 293(2): 569-7. 4. Zavadskis, S., Weidinger, A., Hanetseder, D., Banerjee, A., Schneider, C., Wolbank, S., Marolt Presen, D., et al. (2020). Effect of diphenyleneiodonium chloride on intracellular reactive oxygen species metabolism with emphasis on NADPH oxidase and mitochondria in two therapeutically relevant human cell types. Pharmaceutics, *13*(1): 10.