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BE-03 Effects of Dietary Iron on Taxonomic Composition and Function of the Zebrafish Gut Microbiome

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The Effects of Dietary Iron on Taxonomic Composition and Function of the Zebrafish Gut Microbiome

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INTRODUCTION

A healthy gut microbiota is essential to promote host health and well-being, therefore, effects of dietary components on the gut microbiome are important to investigate as the gastrointestinal tract can be a major route of infection. Iron-an essential component of heme and iron-sulfur proteins plays a central role in many biological activities, including oxygen transport and cellular respiration. In particular, the iron homeostasis system is one of the best characterized due to iron's causative relationship with iron-deficiency anemia. Dietary iron supplementation is a commonly used treatment for iron deficiency anemia; however, the known direct impacts of iron on the gut microbiome functional potential remain limited.

In the present study, using Zebrafish (Danio rerio) as a model organism, we sought to determine if increases in dietary iron would cause changes in taxonomic composition and gut microbiome function. Based on our analysis, an increase in dietary iron significantly altered the zebrafish microbiome taxonomic composition with specific increases in Firmicutes and Proteobacteria. Analysis of taxa for functional potential suggested that iron enriches physiological functions such as aerobic respiration. These results will be further explored through a spectroscopic analysis of primary metabolites and lipids.

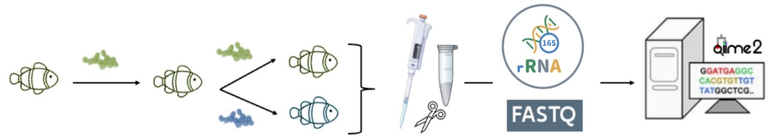
OBJECTIVE

Using Zebrafish as a model organism to elucidate the effects of an increase in dietary iron on gut taxonomic composition and functional potential.

METHODS

Zebrafish of the same age and genotype (AB wildtype) were initially fed Ziegler Brothers Adult Zebrafish Food with 309.00 ppm of dietary iron for two weeks. After two weeks, the fish were separated into the control and experimental groups. The control group continued to receive the initial feed while the experimental group received feed supplemented with 366 ppm of ferrous sulfate to give an elevated iron level (a 19% increase in iron supplementation). After four weeks, the fish were sacrificed, dissected, and gut contents harvested for DNA extraction and analysis.

The suspended gut contents were sent to Mr. DNA for 16s rRNA amplicon sequencing for identification of bacterial species within the hypervariable regions. Sequenced DNA was analyzed computationally using QIIME2 (Quantitative Insights Into Microbial Ecology) to translate raw sequence data into statistical results and phenotype predictor BugBase was utilized to analyze functional potential. After another replicate zebrafish trial, gut contents were suspended and sent to Clemson University's Multi-User Analytical Lab for further analysis of primary metabolites and lipids.



ACKNOWLEDGEMENTS

SCINBRE
P20GM103499-20
MR DNA
MUAL
Multi-User Analytical Lab and Metabolomics Core
Clemson University

RESULTS AND DISCUSSION

1. Effect of dietary iron on taxonomic composition at the phylum level

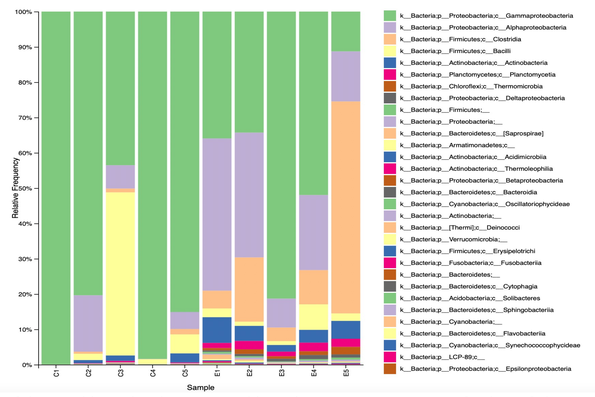
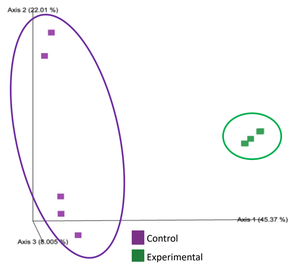


Figure 1: Zebrafish control (C1-C5) and experimental (E1-E5) taxonomic composition after being fed respective diet. Sequenced 16s amplicons were grouped into class Operational Taxonomic Units (OTUs).

Based on the control and experimental groups, diet is the biggest factor that influences the diversity of bacteria in the gut. The most noticeable taxonomic increases within the experimental group is that of Kingdom proteobacteria (purple) and firmicutes (orange).

2. Beta diversity: Principal Coordinates



Beta diversity is a measure of diversity between two biological communities. The Jaccard PCoA plot compares two communities based on the presence or absence of species. There is less similarity among the control group and more similarity within the experimental group, comparing each of the samples to each other. More similarity exists among the samples within the groups rather than between the groups themselves.

Figure 2: Beta Diversity Jaccard PCoA Plot generated by Qiime2 visualizing community dissimilarity. The circled areas depict that there is more similarity within the control and experimental groups rather than between the groups.

3. Beta diversity: ANOSIM box plot analysis

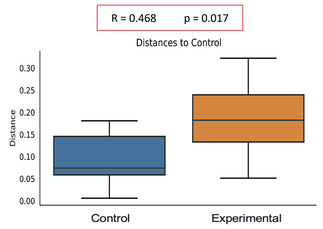
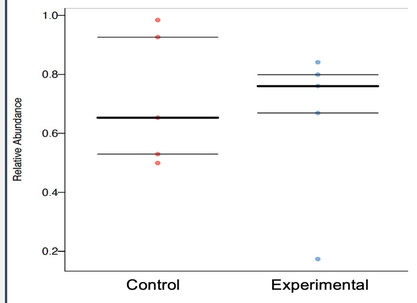


Figure 3: Qiime2 generated Analysis of Similarities is a non-parametric test, or assumption-of-distribution-free test, of significant difference between two or more groups to estimate distance distribution with 999 permutations.

Weighted-Unifrac Analysis of Similarities Box Plot takes into account the relative abundance of species/taxa shared between samples. An R value of 0.468 suggests that there are equal amounts of similarity and dissimilarity; however, the p proves significant changes in beta diversity due to the new species present between groups.

4. Bugbase functional potential analysis of aerobic respiration

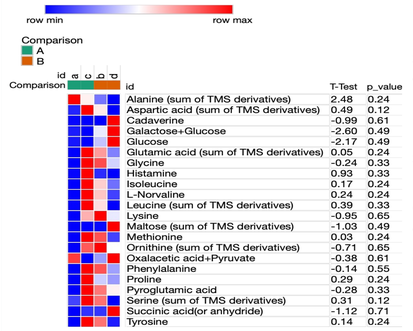


In the presence of increased iron, aerobic respiration is enriched, allowing more ATP to be consumed via aerobic respiration [relative to anaerobic]. Median statistical analysis was used to prevent outliers from skewing mean values.

The downfall about utilizing a phenotype predictor is that functional potential is simply predicted, not yet physiologically demonstrated.

Figure 4: Bugbase predicted phenotype of aerobic respiration. Relative abundance median distribution is 65.3 for control compared to 76.0 for experimental.

5. Primary metabolite analysis among control group



id	T-Test	p_value
Alanine (sum of TMS derivatives)	2.48	0.24
Aspartic acid (sum of TMS derivatives)	0.49	0.12
Cadaverine	-0.99	0.61
Galactose+Glucose	-2.60	0.49
Glucose	-2.17	0.49
Glutamic acid (sum of TMS derivatives)	0.05	0.24
Glycine	-0.24	0.33
Histamine	0.93	0.33
Isoleucine	0.17	0.24
L-Norvaline	0.24	0.24
Leucine (sum of TMS derivatives)	0.39	0.33
Lysine	-0.95	0.65
Maltose (sum of TMS derivatives)	-1.03	0.49
Methionine	0.03	0.24
Ornithine (sum of TMS derivatives)	-0.71	0.65
Oxalacetic acid+Pyruvate	-0.38	0.61
Phenylalanine	-0.14	0.55
Proline	0.29	0.24
Pyroglutamic acid	-0.28	0.33
Serine (sum of TMS derivatives)	0.31	0.12
Succinic acid(or anhydride)	-1.12	0.71
Tyrosine	0.14	0.24

Four samples within the control group were subdivided into groups A and B. Despite the varying levels of metabolites depicted, the p-values dictate that none of the means between the t-tests are statistically significant. Examining the control group holistically, the samples are consistent with each other and not statistically different, which is to be expected. Upon analysis completion comparing the control to experimental group, the p-values are expected to be significant.

Figure 5: Metabolic analysis performed Clemson's Multi-User Analytical Lab. Heat map generated from Morpheus Broad of relative metabolomic levels of four samples of within the control group.

CONLUSIONS AND FUTURE DIRECTIONS

Changes in levels of dietary iron play a large role in altering the microbial makeup within the dynamic gut microbiome. There were substantial changes in alpha diversity based on the numerical increase in species richness in the experimental group. The significant p-value demonstrates the impact on beta diversity due to the new species present between groups. The most notable taxonomic increase was with Firmicutes and Proteobacteria known to cause inflammation (Stojanov et al., 2020). Physiologically, aerobic respiration is enriched as a result of excess iron. For future directions, metabolomic and lipidomic expression will be examined through spectroscopical analysis. Based on PCR bias, total metagenomic data will be examined and compared to determine genomic effectiveness.

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