

The Effects of Cosequin and Omega-3 Supplementation on the Gut **Microbiome of Penguins using DNA Metabarcoding** Alexander Pietraru, Samuel Wen, Ava Weiss

Abstract:

This project examines how Cosequin and Omega-3 supplements affect the gut microbiome of African Black-Footed Penguins (Figure 1) using DNA barcoding. Fecal samples from one supplemented and two non-supplemented penguins were sequenced using Oxford Nanopore Technology. Microbial diversity and species composition were compared using 16S rRNA analysis. Results suggest that supplementation may influence gut microbial communities, with lower diversity observed in the supplemented penguin. These findings provide insight into how dietary supplements may impact gut health in captive birds.

Introduction:

The gut microbiome is vital to animal health, influencing digestion, immunity, and behavior. In African Black-Footed Penguins (Spheniscus demersus), it supports nutrient absorption and disease resistance, especially in captive settings (Clavere et al. 2017). This study examines penguins at the Long Island Aquarium, focusing on one receiving Cosequin and Omega-3 supplements for arthritis, compared to two control birds. Cosequin contains glucosamine, chondroitin, and manganese ascorbate, which support cartilage health and reduce joint stress (Hoffman, Jessica M., et al. 2018). Omega-3s like EPA and DHA reduce inflammation and promote tissue repair (Hoffman et al. 2020). Though little is known about how such supplements affect avian microbiomes, research in mammals shows they can shift microbial composition—sometimes increasing diversity, but often promoting specific anti-inflammatory taxa while reducing potentially pathogenic ones (Shmagel, Anna, et al. 2010). These changes may enhance digestive efficiency or immune response. Penguins typically eat fish like herring and sardines, which may interact with supplements and further influence gut microbial communities. Using Oxford Nanopore Technologies (ONT) sequencing and metabarcoding, this study will analyze fecal samples to explore how supplements influence microbiome diversity and composition, potentially improving captive penguin care and informing broader avian health strategies.

Materials and Methods:

1. We collected fecal samples from African Black-Footed Penguins at the Long Island Aquarium



3. PCR and indexing were done using the ONT 16S Barcoding Kit 24 V14



2. DNA was extracted using the Qiagen Powersoil Pro Kit.



4. Sequencing was done using the MinION sequencer with a Flongle flow cell.



Jennifer Newitt, Vijay Suthar, Jeffry Petracca **Results:**





Figure 1: African Black-Footed

Fecal samples from three African Black-Footed Penguins—Kramer, Little, and Yoshi—were analyzed to study the effects of Cosequin and Omega-3 supplementation on gut microbiome composition using DNA barcoding. DNA was extracted, amplified using PCR, and sequenced with the Oxford Nanopore MinION platform. The number of sequencing reads varied across samples, with Kramer (supplemented) having 12,511 reads, Yoshi 10,158 reads, and Little 6,976 reads (Figure 2). Clostridium perfringens was the most abundant bacterium in all three, with the highest proportion in Kramer (66.99%), followed by Little (59.21%) and Yoshi (45.67%). *Eubacterium tenue* was the second most abundant in Kramer (16.61%) and Little (17.8%), whereas Yoshi showed a different pattern with Ligilactobacillus aviarius as the second most abundant (31.2%), followed by E. tenue (11.47%) (Figure 4). Shannon's Diversity Index was lowest in Kramer (1.37), suggesting reduced microbial diversity associated with supplementation, compared to Yoshi (1.70) and Little (1.84) (Figure 5). PCR amplification was successful, as indicated by clear, distinct bands in gel electrophoresis (Figure 3) Additionally, Sample 24, a negative control, unexpectedly yielded 41 reads, indicating low-level contamination or barcode cross-talk and highlighting the importance of quality controls in microbiome sequencing.



Figure 4: Species rank and abundance in each sample.



Cold Spring Harbor Laboratory DNA LEARNING CENTER

Discussion:

Although we hypothesized that supplementation would increase microbial diversity, Shannon's Diversity Index revealed that the supplemented penguin (Kramer) had the lowest microbial diversity among the three. This suggests a potential link between supplement use and reduced microbial richness. Clostridium perfringens dominated Kramer's gut microbiome, making up over 66% of reads, while the two control penguins showed more varied microbial compositions. Unexpected reads in our negative control (Sample 24) indicate low-level contamination or barcode misassignment, emphasizing the importance of robust quality controls and careful interpretation of sequencing data. Our small sample size limits the ability to generalize findings, but these results highlight the need for further studies to understand how supplements may affect gut microbiomes in captive animals.

ner	Yoshi	Little
ed)	(Unsupplemented)	(Unsupplemented)
	1.70	1.84

Figure 5: Shannon's Diversity Index for each penguin's Microbiome.

Acknowledgements:

We would like to express our sincere gratitude to Jennifer Newitt, Vijay Suthar, Dr. Jeffry Petracca, and Maggie Seiler for their invaluable guidance, support, and expertise throughout our project. Their mentorship greatly enhanced both our research process and hands-on experimental work.



References & Report