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Influence of diet type on gut microbiome, nutrient assimilation in GIFT tilapia

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A low-cost feed alternative could benefit small-scale fish farmers in Papua New Guinea

Results of this study demonstrated that the development of a low-cost feed alternative could improve the success of small-scale GIFT tilapia farmers in Papua New Guinea, increasing both food and income security within the region. Photo by Darryl Jory.

Tilapia are amongst the most important aquaculture species of the 21st century, accounting for 10 percent of the world's finfish production. Nile tilapia (*Oreochromis niloticus*) is the most commonly farmed tilapia species accounting for 8 percent of tilapia production through both commercial and small-scale aquaculture.

WorldFish (http://www.worldfishcenter.org) selectively bred *O. niloticus* to develop the Genetically Improved Farmed Tilapia (GIFT). The global impact and success of GIFT has been particularly evident in developing nations where it has helped to improve food and income security.

Inland aquaculture in Papua New Guinea (PNG) is mostly small-scale subsistence farming with growth limited by infrastructure, the high cost and limited availability of commercial feed, the poor economic status of people and a lack of fish husbandry skills in the farming communities. The farming of GIFT has been a greater success in PNG compared to other fish species, such as trout, because it is a lower maintenance species and easy to breed. Nevertheless, the ruggedness of PNG's interior, where GIFT is mostly farmed, makes fish farming a challenge.

As for many farmed species, commercial fish feed pellets are widely considered the best option to increase GIFT farm productivity; however, only 10 percent of the small-scale fish farmers in PNG use commercial fish feed. Although the nutritional needs of farmed tilapia can be met through a variety of natural food sources, a poor diet can negatively impact their growth and overall health.

Knowledge of the composition and microbial diversity within the gastrointestinal tract is vital because of the influence these symbionts have on the host's growth and survival. For aquatic species, including finfish, farming practices can heavily influence the gastrointestinal microbiota, impacting digestion and the assimilation of essential nutrients. Recent studies on tilapia microbiomes (the collective genomes

of the microorganisms that reside in an environmental niche or the microorganisms themselves) have generated data on the effects of dietary supplementation and rearing conditions; however, little is known on the effects of locally sourced feeds.

This article – adapted and summarized from the **original publication** (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0237775) – determined how different feeding practices affect the condition, nutrient assimilation patterns and the gastrointestinal microbiome of GIFT reared in earthen ponds.

Study setup

O. niloticus (GIFT strain) individuals were collected from six different fish farms within the Aiyura Valley in the Eastern Highlands of Papua New Guinea. The stocked GIFT were from the same family line acquired from the Highlands Aquaculture Development Centre (HAQDEC) breeding program. The sampled farms all stock GIFT in earthen ponds, and represent one of two different feeding practices; a locally sourced raw vegetable-based diet (mostly sweet potato, banana leaves and garden waste), hereafter referred to as a "vegetable" diet, and a mixed diet consisting of both the occasional supplementation of raw vegetables (mostly sweet potato, banana leaves and garden waste) and regular commercial feed pellets, hereafter referred to as a "pellet" diet.

The commercial fish feed pellets were all from a single imported source from Vietnam and included 30 percent crude protein, 5 percent crude fat, 16 percent ash, 6 percent crude fiber and 11 percent moisture with raw ingredients including fishmeal, wheat flour, soybean meal, fish oil, rice bran and vitamins and minerals. Parameters such as feeding frequency and pond size were recorded on site during sample collection.

Fish of similar size were collected from each farm using handheld nets and immediately euthanized. All fish were devoid of any gross or clinical signs of disease. Standard measurements of length and weight were recorded prior to dissection. Dissections were undertaken at HAQDEC within two hours of collection. White dorsal tissue samples were aseptically removed, scaled and skinned before being rinsed with distilled water and stored at minus-20 degrees-C. In addition, the gastrointestinal tract was aseptically removed with a combined hindgut content and hindgut wall sample collected and frozen (initially at minus-20 degrees-C) and stored at minus-80 degrees-C prior to microbial analysis.

For detailed information on the experimental design and sampling; microbial analyses carried out including stable isotope analysis and 16S rRNA gene amplicon sequencing and analysis; and statistical analyses, refer to the original publication.

Results and discussion

The aim of this research was to determine how different feeding practices affect the condition, nutrient assimilation patterns and the gastrointestinal microbiome of GIFT reared in earthen ponds. Stable isotope analysis of carbon and nitrogen was used to identify differences in diets and therefore the trophic status of GIFT. Additionally, specific microbial assemblages were associated with different conditions, assimilation patterns and feeding practices to support the development of more effective farming practices for small-scale fish farmers.

The isotopic values (isotopes are variants of a particular chemical element that differ in neutron number; all isotopes of a given element have the same number of protons but different numbers of neutrons in each atom) of a consumer are related to its diet; therefore, stable isotope analysis can be used to accurately identify a consumer's dietary profile and trophic status. The carbon-13 (δ13C)

isotopic values for both the vegetable only and pellet-fed GIFT indicates some similarities in dietary carbon sources. Assessing dietary carbon is important, as it encompasses essential nutrients such as carbohydrates and lipids, vital for fish health as they play an important role in growth and metabolism.

In fish, fluctuations in dietary carbon are often reflected by their gastrointestinal microbiota. In our study, an overlap in dietary carbon may be due to the occasional provision of vegetables to pellet-fed fish; however, a clear separation in nitrogen-15 (δ15N) suggests distinct dietary nitrogen sources. In aquatic systems, including aquaculture ponds, significantly enriched δ15N values can be indicative of anthropogenic nitrogen input such as fertilizers. While the remoteness of our study site and absence of intensive agricultural farming reduces the potential for such anthropogenic inputs, some farmers do use chicken manure to fertilize crops. And small-scale swine production and vegetable cropping also occur in the catchment of the farms, and urban activities in the nearby town of Kainantu, may also be sources of nutrients; however, their contribution to the nutrient budgets of the farms is likely to be negligible.

Within pond cannibalism can negatively affect a farm's ability to produce table-sized fish and can therefore negatively affect productivity and profitability. While fish cannibalize for many reasons, stress, limited food availability and low dissolved oxygen are considered major drivers. Previous studies on tilapia have reported filial (both egg and fry) cannibalism, with stunted individuals, or progeny from the initial stocked fish, more likely to become prey. In our study, vegetable-fed GIFT averaged 6 cm smaller and 100 grams lighter than their pellet-fed counterparts, further supporting the possibility of within pond cannibalism.

It should be noted that these differences may also be attributed to age, as fish reproduction in farms is not traditionally controlled and was therefore not considered. While no direct evidence of cannibalism was observed, the organic material in the hindgut of the gastrointestinal tract of fishes is usually in an advanced stage of digestion, thus making it difficult to visually identify what was consumed.

The ability of a fish to effectively absorb nutrients and digest foods depends on its gastrointestinal microbiota. The gastrointestinal microbiota can impact a fish's weight and overall health. Overall, we identified significant differences in the gastrointestinal microbiota of GIFT in response to feeding practice. This result is in accordance with previous studies that have reported changes in the microbiome of fishes in response to changes in diet, and dietary supplementation.

An operational taxonomic unit, or OTU, is an operational definition used to classify groups of closely related individuals, while zOTUs are valid operational taxonomic units that are corrected to achieve more reliable sequence clustering to group related biological sequences. In our study, a larger number of zOTUs were identified as significantly associated with commercial pellet-fed GIFT than those fed vegetables (Fig. 1), likely reflecting diet stability as has been seen for humans and ants. Farmers using a commercial pellet-based diet for their GIFT consistently source imported tilapia feeds directly from the National Fisheries Authority (NFA). In contrast, the vegetable-only diet is inconsistent and largely determined by harvesting season and the availability of vegetable garden waste.

Fig. 1: Bacterial taxa that are significant indicators of diet. Differentially abundant log-transformed zOTUs (identified to the lowest taxonomic level possible) (P-Adj <0.05) that represent the taxa that were found to be significantly indicative or associated with either the pellet, or vegetable-fed GIFT. zOTU abundances have been z-score transformed and thus show the number of standard deviations a zOTUs abundance is from the mean abundance of that zOTU.

The presence of nine bacterial taxa across most (90 percent) of the fish in our study is of interest as it implies that these taxa may have been acquired from the hatchery prior to distribution. This finding is significant because the microbial composition of larval and juvenile fish has a significant influence of

the microbiome of adults]. Three of these “hatchery-associated” taxa were negatively correlated with relative fish condition and δ13C. These results suggest that depleting the availability of δ13C could decrease the abundance of Fusobacteria, and subsequently improve the relative fish condition of GIFT.

Our study has shown that farming practices incorporating commercial-feed pellets increase relative fish condition of GIFT. The promotion of a commercial-feed supplemented farming strategy would need to be incorporated not only by the fish farms, but also the hatcheries because microbial symbionts attained from the rearing water during the early stages of ontogeny can be maintained into adulthood.

Fusobacteria are commonly identified as a major constituent of freshwater fish microbiomes. In our study, Fusobacteria correlated with poor fish condition which was predominately the case for vegetable-fed GIFT. Previous studies have reported that high abundances of Fusobacteria are often associated with carnivorous species, likely due to their ability to metabolize protein derived amino acids. Therefore, the possibility that vegetable-fed GIFT are turning to cannibalism could explain the presence of Fusobacteria in fish with poor condition.

Perspectives

The results of our study contribute to a growing body of work on the influence of diet on the microbiota, trophic status and condition of freshwater fishes. We showed that trophic level is not always indicative of a good diet and can represent poor farming practices. Specifically, our results demonstrate how poor feeding practices can negatively impact the success of GIFT farms.

We found that fish fed an insufficient vegetable-based diet were in a relatively poor condition and while yet to be confirmed, possibly supplementing their diet through filial cannibalism. These results further highlight the extent of challenges faced by low income, small-scale subsistence farmers in developing nations. Small-scale fish farms account for the majority of inland freshwater finfish aquaculture and play a fundamental role in enhancing not only food and income security but also quality of life.

For GIFT to contribute to human nutrition and livelihoods in PNG, they need to be farmed productively and profitability. Further research in rural communities in developing nations is needed to improve farming practices through the education of farmers and increased availability of suitable feeds. The introduction of new farming practices such as the use of mono-sex fingerlings stocked by size class could help boost local production of GIFT in PNG.

Furthermore, the development of a low-cost feed alternative that better suits the nutritional needs of GIFT would reduce the costs involved with accessing commercial feed pellets, and further increase the farming success and profits of small-scale GIFT farmers in Papua New Guinea.

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