

Evaluation of Dual Culture (LAB & *Monascus* sp.) Fermented *Azolla*-Rice Bran (ARB) Mix as a Diet Substrate for the Juvenile *Pangasius* sp.

Nur Khairani Ayuni Adnan, Rohana Mat Nor

Structured Abstract

Background: Aquaculture production has expanded dramatically, now accounting for more than 50% of global aquatic protein production for human consumption. This rapid expansion has caused fishmeal prices to skyrocket and threatens the sustainability of aquaculture, which has prompted the hunt for a sustainable alternative protein source. While plant-based proteins have proven potential as alternative protein sources, their inclusion in fish diets may impair fish digestibility and growth due to the presence of insoluble fibres and antinutritional factors (ANFs). Thus, this research aims to explore the efficiency of solid-state fermentation (SSF) using a lactic acid bacterium (LAB), *Lacticaseibacillus paracasei* Shirota and the red fungus *Monascus* sp. on *Azolla* and rice bran (ARB) mixture to enhance the functional and nutritional quality of these plant-based substrates.

Methods: The study compares the effects of single and dual culture fermentation on the nutritional enhancement of the ARB diet. The fermented substrates from the SSF method were analysed for their proximate composition using the AOAC standard methods and gas chromatography-mass spectrometry (GC-MS) analysis. The formulated feed was then subjected to a feeding trial on juvenile *Pangasius* sp. to evaluate its growth performance, feed efficiency, and overall nutritional value under controlled conditions.

Results: Findings suggest that dual culture fermentation of ARB substrates proves superior in improving nutrient content, particularly by increasing lipid, ash, and moisture while reducing crude fibre by nearly 50% within 5 days ($p < 0.05$). GC-MS analysis further validated the nutritional enhancement across all treatments by identifying key metabolites, most notably linoleic acid, an essential fatty acid component for animal growth and health, prominently detected in *Monascus* sp. incorporated SSF. However, due to time constraints, the feeding trial remained incomplete.

Conclusion: In conclusion, dual culture SSF using LAB and *Monascus* sp. shows strong potential to enhance the nutritional quality of plant-based substrates, offering a promising and cost-effective solution for sustainable aquafeed development.

Keywords: Dual culture, Solid-state fermentation, *Azolla* plant, Rice bran, *Pangasius* sp.

*Correspondence: rohana_micro@uitm.edu.my

School of Chemistry & Environment, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia