

Evaluation Of Dual Culture Solid State Fermentation of *Azolla*-Rice Bran (ARB) Mix Substrate in Diets for The Freshwater Catfish, *Pangasius* sp.

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Structured Abstract

Background: The aquaculture industry is the fastest growing sector in animal protein production, but is increasingly facing challenges due to rising cost of aquafeed, which significantly impacts overall operational expenses and profitability, making the industry economically unsustainable for many, particularly small-scale farmers. Plant-based proteins have emerged as sustainable and viable alternative for aquafeed ingredient in many aquatic species. However, their application is limited by the presence of high insoluble fibre and antinutritional factors (ANFs), which hinder digestibility and negatively affect animal growth. The study explores the potential application of microbial treated plant-based diets for the freshwater fish, *Pangasius* sp.

Methods: A mixture of *Azolla* and rice bran (ARB), two sustainable plant-based substrates, was subjected to solid state fermentation (SSF) using lactic acid bacteria and a fungal strain, *Rhizopus* sp. The study compares the effects of single and dual culture fermentation on the nutritional enhancement of the feed. The proximate and compositional profiles of the fermented substrates were determined using AOAC standard methods and gas chromatography mass spectrometry (GC-MS) analysis. The formulated feed was then subjected to a feeding trial on *Pangasius* fingerlings to evaluate feed efficiency.

Results: Significant increases in moisture, ash, total protein and total lipid were observed across all treatments, notably in dual culture SSF, whereby protein and lipid contents increased by 41% and 164% respectively, along with 9% reduction of crude fibre. GC-MS further confirmed the nutritional enhancement in all treatments by revealing the presence of beneficial metabolites in substantial amounts, notably oleic and linoleic acids, both polyunsaturated fatty acids (PUFA) are known for promoting fish health and growth. However, due to time limitation, the feeding trial remained incomplete.

Conclusion: These findings highlight the potential of SSF method, particularly dual culture fermentation as a promising strategy to enhance the nutritional value of plant-based aquafeed ingredients as a sustainable and cost-effective alternative feed, reducing dependency on expensive conventional feed components and supporting more economically viable freshwater aquaculture

Keywords: *Azolla* sp., Rice Bran. *Rhizopus* sp., Lactic Acid Bacteria, Solid State Fermentation

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