

Potential Antibacterial and Synergistic Effects of Optimized Fermented *Azolla* with Lactic Acid Bacteria

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Structured Abstract

Background: Broiler farms have utilized antibiotics with hygiene and biosecurity to control bacterial diseases. However, antimicrobial resistance against antibiotics has raised concern in the broiler industry. There are many strategies to improve broiler health management, such as phytogetic feed additives and probiotics. Hence, this project utilized *Azolla* plants, which have been proven to contain rich nutrients and have been developed as an alternative for cost-effective broiler feeds. The purpose of this study is to investigate the potential antibacterial and synergistic effects of fermented *Azolla* with lactic acid bacteria fermentation.

Methods: The dried *Azolla* plants were fermented using lactic acid bacteria isolated from probiotic drinks and optimized through pH modulation. In this study, the effect of the varying inoculum sizes ranging from 1×10^8 to 3×10^8 CFU/g and moisture levels ranging from 55% to 85% were employed to observe their fermentation activity. Next, the actual fermentation of *Azolla* was carried out using optimized conditions, and the fermented *Azolla* was extracted using ethanol. The fermented extract was used to observe potential antibacterial and combinatorial effects with tetracycline and ampicillin using a well diffusion assay against three different bacteria: *S. aureus*, *E. coli*, and *S. enteritidis*.

Results: The fermentation was effectively optimized the lactic acid bacteria fermentation on *Azolla* by using 3×10^8 CFU/g and with 75% moisture content for optimal fermentation efficiency. The result shows that the potential antibacterial activity was observed only against gram-positive *S. aureus* ($p < 0.05$). Furthermore, primary screening on combinatorial effects suggests that the combination between fermented *azolla* with tetracycline is effective against gram-negative *E. coli*, whereas the combination between fermented *Azolla* with ampicillin does not show any combinatorial effects.

Conclusion: To conclude, the fermented *Azolla* pot potentially produces antibacterial activity against gram-positive bacteria and synergistic effects with tetracycline to support broiler health management. The use of lactic acid bacteria for *Azolla* fermentation has the potential for producing antibacterial activity and synergistic effects to reduce reliance on antibiotics and provide a sustainable alternative for broiler feeds.

Keywords: Fermented *Azolla*, Lactic Acid Bacteria, Fermentation, Antibacterial, Broiler

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