

I. INTRODUCTION

1.1. Background

Aquaculture is one of the most promising sectors related to increasing food security and human nutrition (Nunes *et al.*, 2024). In 2020, global aquaculture production reached 214 million tonnes, including 178 million tonnes of aquatic animals such as marine and freshwater fish, mollusks, and crustaceans (FAO, 2020). Freshwater ecosystems are among the most important ecosystems that support human survival, as they provide essential resources. One of the most valuable commodities of freshwater fishes is Nile tilapia (*Oreochromis niloticus*) (Miao *et al.*, 2020). This fish species is widely cultivated around the world because it has several advantages, such as fast growth, adaptability, and low-cost production (Prabu *et al.*, 2019). Asia is the largest contributor to global production, producing nearly 70 percent of the total. Southeast Asian countries such as Indonesia and Thailand are among the largest producers in Asia. In 2018, Indonesia's Nile tilapia production reached 1.171.698 tons (FAO, 2020). Nile tilapia (*Oreochromis niloticus*) is a species that has the second rank below seaweed and is number one in the fin fish categories, especially in Indonesian aquaculture production (Gustiano *et al.*, 2023).

Diseases are major challenges in aquaculture industries, including in Nile tilapia. One of the most common diseases is Motile Aeromonas Septicemia (MAS) caused by *Aeromonas spp.*, including *Aeromonas*

hydrophila. This infection is one of the most severe bacterial diseases that leads to significant economic losses in the global freshwater aquaculture sector. Currently, antibiotics such as oxytetracycline, tetracycline, erythromycin, and enrofloxacin are used to prevent this disease and maintain fish health. However, antibiotics that are used continuously and inappropriately can lead to many problems, such as antimicrobial residues and the development of antimicrobial resistance (AMR). Studies have shown that these bacteria exhibit resistance to antibiotics such as ampicillin, tetracycline, kanamycin, erythromycin, and clindamycin (Chandrarathna *et al.*, 2018). Therefore, alternative prevention is needed, such as probiotics applications.

Probiotics contribute to gut colonization and produce profitable metabolites, quorum quenching, and antagonistic activities against pathogenic bacteria. The lactic acid bacteria (LAB) group, including *Lacticaseibacillus casei*, represents the most extensively applied probiotics in the aquaculture industry. Research has shown that giving 15% *L. casei* as a supplemented feed resulted in the highest level of LAB in the intestines of the Catfish (*Clarias gariepinus*) compared to the control group, which received no probiotics in the feed (Aini *et al.*, 2024). Another study also showed that *L. casei* can enhance the expression of immune-related genes, such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), in Zebrafish (*Danio rerio*), thereby increasing resistance to *Aeromonas hydrophila* (Alavinejad *et al.*, 2023). Dietary administration of probiotics into fish has been proven to increase growth, immune response, and resistance to pathogenic bacteria (Rohani *et al.*,

2022). Therefore, this research aims to assess the efficacy of *Lacticaseibacillus casei* as a feed supplement in Nile tilapia (*Oreochromis niloticus*) by observing growth performance, gut colonization, fish survival, and histopathological changes.

1.2. Research Questions

- 1.2.1. Does *Lacticaseibacillus casei* feed supplementation affect the growth performance of Nile tilapia (*Oreochromis niloticus*)?
- 1.2.2. Does *Lacticaseibacillus casei* feed supplementation affect disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*?
- 1.2.3. Can *Lacticaseibacillus casei* supplemented in the fish feed colonize the Nile tilapia (*Oreochromis niloticus*) gastrointestinal (GI) Tract?
- 1.2.4. Does dietary supplementation with *Lacticaseibacillus casei* affect histopathological changes in Nile tilapia (*Oreochromis niloticus*) following infection with *Aeromonas hydrophila*?

1.3. Objectives

- 1.3.1. To determine the effect of *Lacticaseibacillus casei* feed supplementation on the growth performance of Nile tilapia (*Oreochromis niloticus*)
- 1.3.2. To determine the effect of *Lacticaseibacillus casei* feed supplementation on the disease tolerance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*

- 1.3.3. To determine the ability of *Lacticaseibacillus casei* feed supplementation to colonize in the gastrointestinal (GI) tract of Nile tilapia (*Oreochromis niloticus*)
- 1.3.4. To determine the effect of *Lacticaseibacillus casei* in reducing histopathological changes or severity in the tissue of Nile tilapia (*Oreochromis niloticus*) after infection

1.4. Benefits

- 1.4.1. Providing broader knowledge regarding the application of probiotics in the field of aquaculture, especially in tilapia farming
- 1.4.2. Enhancing knowledge on the application of probiotics to improve growth and disease resistance in Nile tilapia
- 1.4.3. Providing valuable insight into the potential of *Lacticaseibacillus casei* to colonize the GI tract of Nile tilapia
- 1.4.4. Supporting tissue-level understanding through the histopathological observation