I. INTRODUCTION

1.1 Background

Aquaculture has experienced remarkable growth globally, emerging as a key contributor to the global food production system (Leong et al., 2023). According to the FAO (2022), aquaculture's share in global fish production increased from 25.7% in 2000 to 46% in 2018. By 2017, fish comprised 7% of total protein intake and 17% of animal protein consumption globally. Nile tilapia (*Oreochromis niloticus*) is a freshwater fish found in many places and of high economic importance in global aquaculture widely distributed freshwater fish known for its global commercial significance (El-Sayed & Fitzsimmons, 2023). It is especially prominent in Asia, including Indonesia and Thailand, as both are among the world's top five tilapia producers (FAO, 2019). The increasing demand for Nile tilapia led the farms to use the semi-intensive and intensive systems to get higher yields of fish with low costs for production (FAO, 2022; Novita et al., 2021). However, inadequate management of this system can cause stress in the fish due to high stocking density, lack of oxygen, and poor water quality, leading to compromised immune function. These factors could potentially increase the spread of disease, resulting in an outbreak (Cabello, 2006; FAO, 2022; Nugroho et al., 2024; Quesada et al., 2013; Wambua et al., 2021).

Flavobacterium oreochromis (*F. oreochromis*) is the causative agent of columnaris disease in the tilapia genus (*Oreochromis* spp.) (LaFrentz et al., 2022). Columnaris disease significantly impacts fish production due to the extremely high mortality rates (Sebastião et al., 2011; Welker et al., 2005), reaching up to 70% during fry to fingerlings phase (Dong et al., 2016; Hai et al., 2020). The bacteria infect the fish easily under stressed conditions mentioned above (Kubilay et al., 2008). The transmission can occur either through direct contact with infected fish or indirectly through the environment, such as contaminated water from the upstream rivers (Austin & Austin, 2019; Groff & LaPatra, 2000; Kunttu et al., 2012; Wakabayashi, 1991).

Antibiotics, such as tetracycline, penicillin, erythromycin, neomycin, aminoglycosides, polypeptides, cephalosporin, and nitrofurans, have been used to treat columnaris disease outbreaks (El-Tawab et al., 2020; Kubilay et al., 2008). However, the appropriate use of antibiotics in freshwater fish presents a significant challenge due to insufficient monitoring and varying conditions in each region (Pelić et al., 2024; Mo et al., 2017). Improper doses of antibiotics can leave residue and lead to antibiotic-resistant bacteria (Pelić et al., 2024). The residue can disrupt gut microbiota and accumulate in muscle tissue, potentially harming the fish's health, the environment, and the consumers (Lihan et al., 2021; Yang et al., 2021). Antibiotic resistance among bacteria occurs due to the high frequency of antibiotic use to combat disease on the farm, which leads to chromosomal changes or plasmid-mediated mechanisms, leading to horizontal gene transfer (Bondad-Reantaso et al., 2005; FAO, 2022; Ogbonne et al., 2019).

Probiotics offer an alternative approach to reducing the negative impacts of antibiotics in aquaculture (Leong et al., 2023). Natto is a food made from soybeans and fermented by probiotic bacteria, *Bacillus subtilis* var. *natto* (BSN) (Nishito et al., 2010). The utilization of BSN as a supplement has a potential impact due to its safety (categorized as GRAS) in humans (Nishito et al., 2010). BSN produces degradative enzymes to help nutrient absorption, helps balance the gut microbiota, and acts as a competitive bacterium to inhibit pathogens within the host (Lin et al., 2021; Sella et al., 2021; Zhang et al., 2021a; Zhu et al., 2023). In brief, they have a role in enhancing growth and immune responses (Subedi & Shrestha, 2020).Thus, probiotics serve as a suitable choice for aquaculture production (Hai et al., 2020; Leong et al., 2023). A study by Mohamed et al. (2011) shows that using *B. subtilis* in fish feed and water has proven effective in preventing lesions caused by *F. columnare* in Egyptian freshwater fish. According to the research by Abarike et al. (2018); Adorian et al. (2019); Kazuń et al., (2018), the *B. subtilis* group can enhance immune responses in aquatic animals.

Therefore, the research aims to evaluate the effects of *B. subtilis* var. *natto* feed supplementation in Nile tilapia (*Oreochromis niloticus*) on growth performance, survival, and lysozyme gene expression after *F. oreochromis* infection.

1.2 Research Questions

1.2.1 Can *Bacillus subtilis* var. *natto* feed supplementation enhance Nile tilapia growth performance?

1.2.2 Can *Bacillus subtilis* var. *natto* feed supplementation reduce Nile tilapia mortality from *F. oreochromis* infection?

1.2.3 Does *Bacillus subtilis* var. *natto* feed supplementation increase or upregulate lysozyme gene expression in Nile tilapia after *F. Oreochromis* infection?

1.3 Objectives

1.3.1 To evaluate the effect of *Bacillus subtilis* var. *natto* feed supplementation on Nile tilapia growth.

1.3.2 To assess the effect of *Bacillus subtilis* var. *natto* feed supplementation on Nile tilapia mortality after *F. oreochromis* infection.

1.3.3 To investigate the impact of *Bacillus subtilis* var. *natto* feed supplementation on Nile tilapia lysozyme gene expression after *F. oreochromis* infection.

1.4 Benefits

1.4.1 Provide valuable information regarding the application of probiotics in fish feed to enhance fish growth and decrease mortality due to *F. oreochromis* infection.

1.4.2 Improve the understanding of lysozyme gene expression in Nile tilapia following infection with *Flavobacterium oreochromis*.