CHAPTER I
INTRODUCTION

1.1 Background and Significance

Recently, the price of feed ingredients has begun an unprecedented escalation, leading to an unprecedented rise in feed costs. Using alternative feed materials, particularly food and feed by-products, would enable the reduction of feed costs. Food-derived bioactive peptides could provide feed ingredients and/or feed additives. In Thailand, the duck meat production yield was approximately 74,700 metric tons in 2022 (Statista Search Department, 2023), with duck blood being one of the main byproducts. Duck blood contains a richness of essential amino acids, heme iron, and other macro- and micronutrients and can be utilized as an inexpensive protein source with a high percentage of protein when compared to chicken and bovine sources (Sorapukdee and Narunatsopanon, 2017). For these reasons, duck blood byproduct is considered a beneficial protein source whose value increases through hydrolysis as a feed additive in animal diets.

Protein hydrolysates derived from animal byproducts have been reported to be a feasible alternative source of high-quality protein in the diets of the livestock and aquaculture industry sectors (Hou et al., 2017; Nikoo et al., 2023). In addition to providing essential nutrient sources and growth factors, protein hydrolysate is also recognized as a value-added product due to its functionalities. The hydrolysis process breaks down proteins into peptides of varying sizes, making them easier to absorb compared to native proteins. Peptides are indeed common products of protein digestion that can enter enterocytes through the peptide transport system according to metabolic physiology. In addition, some peptides also generate a bioactive activity by stimulating the gastrointestinal tract and the immune system to further exert a broad spectrum of functions, including immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial properties depending on their sequence and amino acid composition (Kiewiet et al., 2018; Siddik et al., 2020).

Typically, bioactive peptides refer to low molecular weight peptides ranging in size from 2 to 20 amino acid residues, although they can sometimes be larger. In general, it is commonly reported in the literature that potent bioactive peptides typically have a molecular weight below 10 kDa. Since protein hydrolysate consists not only of valuable functional ingredients but also possesses health-enhancing properties, it has been well-demonstrated that it boosts productivity and performance, disease resistance, and immune responses of many fish species, such as common carp (Carvalho et al., 1997), Japanese sea bass (Liang et al., 1997), large yellow croaker (Tang et al., 2008), Japanese flounder (Zheng et al., 2014), barramundi (Chaklader et al., 2020), Gilthead sea bream (Gisbert et al., 2021), and Nile tilapia (Rahman et al., 2023). Although the hydrolysis process increases the price of value-added protein hydrolysate products, it still reached the break-even point for the ornamental fish business due to the high market value of the ornamental fish industry.

The global ornamental fish market is valued at approximately USD 15–30 billion each year and is expected to increase continuously (Evers et al., 2019). Consequently, demand for feed additives for ornamental fish has increased. Among ornamental fish species, the flowerhorn fish has emerged as one of the most popular aquarium ornamental fish in the world since it first appeared in 1996 (Lin et al. 2008). The price of flowerhorn fish is determined by the uniqueness of the type of fish regarding its size, color, attributes, rarity, individual consumer preferences, healthiness, and its demand in different regional markets. Generally, flowerhorn fish are highly territorial and aggressive, especially in confined spaces. They are known to attack other fish, making them difficult to keep in a community tank. Indeed, flowerhorn fish often suffer from disease, and the requirement of dietary prophylaxis in flowerhorn fish is necessary.

Recently, numerous studies have investigated the effects of protein hydrolysate on the health of fish, as well as its effect on gut microbiota. Some research has reported that protein hydrolysates can interact with the microbiota in the fish gut, thus enhancing the epithelial barrier and nutrient absorption, and exerting antimicrobial activity by promoting the release of mucus in the intestinal tract (Kiewiet et al., 2017; Gao et al., 2023). Moreover, the administration of protein hydrolysate can improve the development of the immune system, augmenting immunoglobulin and cytokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-1β, and IL-10 production in

juvenile barramundi Lates calcarifer (Siddik et al., 2020). To date, several scientific studies have demonstrated that antimicrobial and antioxidant activities were found in animal blood protein hydrolysates, such as those from bovine, chicken, and porcine sources (Chang et al., 2007; Wang et al., 2008). Research on low molecular weight duck blood protein hydrolysate and their application in the ornamental aquaculture field is required. The development of duck blood byproducts as a source of protein and bioactive peptides in the ornamental fish industry through the application of protein hydrolysate technology is a promising and reliable strategy for promoting the health and immunity of fish.

Therefore, this study aimed to investigate the optimal level of DBPH as a feed additive in a commercially practical diet and its effects on the intestinal microbiome, humoral immune response, and antioxidant activity in flowerhorn fish. In addition, after one month of feeding trials, the fish were intraperitoneally injected with *S. agalactiae*, a significant bacterial pathogen affecting various freshwater fish species, particularly under stress conditions. The expression of antioxidant and inflammatory genes was then analyzed to evaluate the immune response's effectiveness in combating the harmful bacterial infection.

1.2 Research objectives

The objectives of this study were:

1.2.1 To investigate the effects of low molecular weight duck blood protein hydrolysate as a feed additive on growth, innate immune responses, antioxidant status, inflammatory response, and gene expression in flowerhorn fish.

1.2.2 To investigate the effects of low molecular weight duck blood protein hydrolysate as a feed additive on resistance to *Streptococcus agalactiae* infection in flowerhorn fish.

1.2.3 To investigate the effects of low molecular weight duck blood protein hydrolysate as a feed additive on the intestinal microbiome in flowerhorn fish.

1.3 Research hypothesis

1.3.1 Dietary supplementation with low molecular weight DBPH enhances innate immune responses, antioxidant activity, and modulates gene expression related

to immunity and inflammation in flowerhorn fish.

1.3.2 Dietary supplementation with DBPH improves resistance to *Streptococcus agalactiae* infection in flowerhorn fish.

1.3.3 Dietary supplementation with DBPH positively alters the composition and diversity of the intestinal microbiome in flowerhorn fish, promoting beneficial bacterial populations and enhancing gut health.

1.4 Scope and limitation of this study

This research focuses on increasing the value of underutilized poultry byproducts, specifically duck blood from the poultry processing industry. The study explored the development of duck blood protein hydrolysate as a valuable feed additive in aquaculture, aiming to enhance the value of this resource and promote zero-waste practices. Flowerhorn fish were fed a commercial diet + 0.85% NaCl as a control group, a commercial diet + DBPH at levels of 0.5%, 1%, and 2%, the fish were challenged with *S. agalactiae*. Immune-related parameters were examined to understand the genes associated with crucial immune responses and the antioxidant activity of fish.

1.5 Expected benefits

This study is expected to demonstrate that dietary supplementation with 2% low-molecular-weight duck blood protein hydrolysate (DBPH) can enhance the overall health of flowerhorn fish. Anticipated benefits include improved growth performance, stronger immune and antioxidant responses, and beneficial changes in gut microbiota specifically increased *Cetobacterium* and *Romboutsia*. The fish are also expected to show greater resistance to *S. agalactiae* infection. These results would support the application of DBPH as a functional feed additive for promoting health and disease resistance in ornamental aquaculture species.