

# CHAPTER I

## INTRODUCTION

### 1.1 Introduction

In recent years, the growing market demand for aquatic products has led to overfishing of marine life, resulting in a significant decline in marine biodiversity. To meet this demand, global aquaculture production has increased substantially. In the aquaculture industry, feed costs account for 60% to 70% of total expenses, with fish meal being the primary protein source, primarily derived from marine organisms. Tilapia, the second most widely farmed freshwater species globally after carp, requires 30-40% protein during its growth and development, and fish meal was the main source of protein for tilapia diet, which contributes significantly to high feed costs. Therefore, reducing feed costs has become a research focus. As a cheapest resource in aquatic feed, carbohydrate (CHO) was widely to study on replace partly of protein to decrease the feed cost (Kamalam et al., 2017a). However, a short/long-term (45 days/40 weeks) study on feeding Nile tilapia with varying carbohydrate and protein levels found that the optimal growth performance was achieved with a diet containing 33% carbohydrates and 46% protein. When the diet contained 50% carbohydrates and 27% protein, the body weight was significantly lower than that fish in other experimental groups. Interestingly, despite fed with the high-carbohydrate diet, the fish did not exhibit post-prandial hyperglycemia, and the hepatic and muscular intermediary CHO metabolism were modulated by high-CHO (HC) diet, indicated that tilapia was a good user of dietary HC (Boonanuntanasarn et al., 2018a, b). This study suggested that reducing protein content and increasing carbohydrate content in tilapia diet could reduce feed costs, but the key was to solve the problem of low carbohydrate utilization, which may lead to loss of fish body mass. Therefore, improving efficient of CHO utilization could be used to improve the benefits of Nile tilapia aquaculture, at the same time, research on the intermediate CHO metabolism might be a scheme.

In this regard, since nutritional programming could be used as a strategy for tailoring metabolism in fish in long-term (Hou and Fuiman, 2020), application of nutritional programming would be able to improve more efficient use of CHO as energy source. Nutritional programming (NP) refers to the long-term impact of changes in the quality or quantity of nutrients intake during the critical window period of early development on young children (Lucas, 1998). Several studies of NP of high CHO (HC) in fish were demonstrated and showed that NP effects could modulated CHO metabolism and improve the utilization of CHO in later life. For instance, in zebrafish, HC stimulus in larvae decreased the plasma glucose and regulated hepatic glycolysis and gluconeogenesis in adult fish receiving the HC feed (Fang et al., 2014). Similar studies also demonstrated in rainbow trout, Siberian sturgeon (*Acipenser baerii*) and gilthead bream, and NP concept of CHO in their early life adjusted the glucose metabolism and homeostasis in later when challenged with the HC diet (Geurden et al., 2007; 2014; Rocha et al., 2016a, b; Gong et al., 2015). In Nile tilapia, NP of CHO was revealed to be an effective approach to improve the utilization of CHO through juvenile and adult stages. For instance, NP of glucose injection into yolk of larvae and HC feeding in fry could improve the growth performance in juvenile and adult fish feeding with high CHO diet (Kumkhong et al., 2020b, 2020a; Srisakultiew et al., 2022). In addition, NP of CHO in Nile tilapia showed several modulatory effects of CHO and its related metabolism including 1) increased plasma glucose and lipids, 2) increased fat, glycogen and triglyceride content in liver and muscle, 3) induction hepatic glycolysis and lipogenesis, 4) suppression hepatic gluconeogenesis and amino acid catabolism, 5) induction muscular glucose transport and glycolysis in juvenile and adult fish when they were fed with HC diet again (Kumkhong et al., 2020a, b, 2021; Srisakultiew et al., 2022). Moreover, the mechanism of NP of CHO effects was proposed to be related to epigenetics regulation (Skjærven et al., 2023), and more investigations are needed to clarify.

Epigenetics is the study of the genetic information of related traits by DNA methylation and histone modifications without changing the DNA sequence, which is preserved and persists for a long term (Villota-Salazar et al., 2016). It was demonstrated that specific chromatin modifications have been identified during persistent metabolic alterations in tissues caused by dietary glucose and lipids (Keating and El-Osta, 2015).

In fish, epigenetics was found to be involved in the CHO metabolic response which induced by the NP effects of CHO, such as in zebrafish, Chinese perch (*Siniperca chuatsi*), rainbow trout and gibel carp (*Carassius gibelio*) (Song et al., 2019; Callet et al., 2021; Lu et al., 2022; Xiao et al., 2020; Geng et al., 2023). Also, in Nile tilapia, NP of glucose injection history led to epigenetics change in adult fish by detected global DNA methylation (Kumkhong et al., 2020a). At present, the main methods used to determine epigenetic modifications include global methyl cytosine level, DNA bisulfite modification, Western blot and the detection of related modifying enzymes (Fraga and Esteller, 2002; Ma et al., 2019). So far, two main enzyme families including DNMT and TET enzymes were essential for the dynamic regulation of DNA methylation (Horii and Hatada, 2016). For histone modifications, histone lysine methyltransferases such as SET domain, KMT2 and Suv39h1 and histone lysine demethylases including KDM subfamily 4 and 5 related enzymes were identified (Park et al., 2020; Pavlenko et al., 2022; Rice et al., 2003; Xiao et al., 2003). In addition, histone acetyltransferases (KAT and Gtf3c4) and deacetylases (Sirt) which catalyze acetyl groups adding and removing, respectively, to protein lysine residues (Hyndman and Knepper, 2017).

To further extend scientific understanding of NP concept, this study investigated the effects of NP of dietary HC in Nile tilapia broodstock on growth performance and intermediary CHO metabolism in juvenile offspring at before and after challenged HC diet. In addition, to explore whether the NP effects of CHO in broodstock could persist into adult offspring. This study determined the CHO and its related metabolism in adult offspring and fish were subjected to challenge the HC diet to evaluate the capacity of CHO utilization. To explore the optimizing formula from high-quality and low-cost CHO resources, combined with the protein retention effect provided by the NP strategy. we designed two challenge diets with more plant-based sources including plant-based medium CHO (PBMC) and plant-based high CHO (PBHC), fed to harvestable adult offspring for 4 weeks (week 57-60) which obtained from parental LC/HP and HC/LP stimuli history. The growth performance and intermediary CHO metabolism were determined before and after adult offspring challenged the diets. To explore whether the NP effects of broodstock could persist into harvestable offspring and their efficient utilization of high plant-based diets. The genes expression of the enzymes associated with DNA methylation and histone modifications were determined in

juvenile, early adult and harvestable offspring when they were challenged HC diet and high plant-based diets to measure whether the epigenetics were involved in NP concept.

## **1.2 Research objectives**

The objectives of this study were:

1.2.1 To investigate the effect of nutritional programming of dietary carbohydrate stimulus in broodstock on intermediary metabolism and epigenetics regulation in Nile tilapia offspring during juvenile stage.

1.2.2 To investigate whether the NP of CHO effects in broodstock could persist its impact on offspring through early adult stage.

1.2.3 To investigate whether the NP of CHO effects in broodstock could improve the utilization of high plant-based diets in harvestable adult offspring.

## **1.3 Research hypotheses**

It was demonstrated that there is nutritional programming of CHO in Nile tilapia. Both glucose injection and early dietary CHO feeding stimulus could exert nutritional programming effects in CHO metabolism in Nile tilapia at later in life (Kumkhong et al 2020). In addition, dietary high CHO level in broodstock also exerted modulation of intermediate CHO metabolism in offspring at early stage. This study hypothesizes that this stimulus broodstock by dietary high CHO level could also exert nutritional programming effects to modulate CHO metabolism in offspring at later stage including juvenile, early adult and harvestable adulthood. In addition, epigenetic modification might be involved in the nutritional programming impact in subsequent offspring at long term.

## **1.4 Scope of the study**

In previous experiment, broodstock was fed with high dietary CHO which showed to modulate CHO metabolism in their offspring at early stage. This study therefore will use the offspring from previous experiment to compare the impact of CHO intervention in offspring in long-term. This study will investigate whether the history of CHO

intervention in broodstock could affect CHO metabolism during juvenile stage. Since the metabolism of Nile tilapia varies according to growth phase, this study will also investigate whether the history of CHO intervention in broodstock could continue through early adult and harvestable adult stage. This study will explore whether epigenetics would involve in the nutritional programming effects by determining the genes expression of enzymes related to epigenetic modifications.

### 1.5 Expected benefits

Information of how nutritional programming of CHO in broodstock improves the CHO utilization in offspring would enable to modulate carbohydrate metabolism which is benefit for formulation of new diet with least cost and high quality. In addition, information of epigenetics regulation would provide for the mechanism of nutritional programming of CHO.

### 1.6 References

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