FEEDING BROODSTOCK NILE TILAPIA (*Oreochromis niloticus*) WITH HIGH LEVEL OF CARBOHYDRATES: IMPACT ON METABOLISM AND EPIGENETICS OF OFFSPRING

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อาหารคาร์โบไฮเดรตสูงในพ่อแม่พันธุ์ส่งผลต่อเมตาบอลิซึมและอีพิเจเนติกส์ในลูกพันธุ์ปลานิล (*Oreochromis niloticus*)

นางสาวลิลลี่ หลัว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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ลิลลี่ หลัว : อาหารคาร์โบไฮเดรตสูงในพ่อแม่พันธุ์ส่งผลต่อเมตาบอลิซึมและอีพิเจเนติกส์ในลูกพันธุ์ปลานิล (*Oreochromis niloticus*) (FEEDING BROODSTOCK NILE TILAPIA (*Oreochromis niloticus*) WITH HIGH LEVEL OF CARBOHYDRATES: IMPACT ON METABOLISM AND EPIGENETICS OF OFFSRPING) อาจารย์ที่ปรึกษา: ศาสตราจารย์ ดร. สุรินทร บุญอนันธนสาร, 137 หน้า.

คำสำคัญ: เมตาบอลิซึม/อิทธิพลระยะยาว/ประสิทธิภาพการใช้อาหาร/โปรแกรมทางโภชนาการ/พันธุศาสตร์ด้านกระบวนการเหนือพันธุกรรม

โปรแกรมการส่งเสริมโภชนาการ (Nutritional programing; NP) ในพ่อแม่พันธุ์ปลานิลด้วยอาหารคาร์โบไฮเดรตสูง (Hyperglucidic stimuli) ส่งผลต่อการปรับเปลี่ยนเมแทบอลิซึมของคาร์โบไฮเดรตในลูกพันธุ์ปลา การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์อิทธิพลระยะยาวของโปรแกรมการส่งเสริมโภชนาการในพ่อแม่พันธุ์ด้วยอาหารคาร์โบไฮเดรตสูง (HC) ต่อการปรับเปลี่ยนเมแทบอลิซึมของคาร์โบไฮเดรตและอีพิเจเนติกส์ในลูกปลาระยะวัยรุ่นและระยะโตเต็มวัย ตลอดจนประสิทธิภาพการใช้ประโยชน์จากอาหารที่ใช้วัตถุดิบโปรตีนจากพืช การทดลองนี้แบ่งเป็น 2 กลุ่มทดลอง คือ กลุ่มพ่อแม่พันธุ์ปลาที่ได้รับการกระตุ้นด้วยอาหารคาร์โบไฮเดรตสูง/โปรตีนต่ำ (HC/LP) และ กลุ่มพ่อแม่พันธุ์ปลาที่ได้รับการกระตุ้นด้วยอาหารคาร์โบไฮเดรตต่ำ/โปรตีนสูง (LC/HP) นำลูกปลาจากพ่อแม่พันธุ์ทั้ง 2 กลุ่ม มาเลี้ยงจนถึงระยะปลาวัยรุ่นเพื่อทดสอบด้วยอาหาร HC/LP (การทดลองที่ 1) และระยะปลาโตเต็มวัย เพื่อทดสอบด้วยอาหาร HC/LP (การทดลองที่ 2) นอกจากนี้การทดลองที่ 3 เป็นการทดสอบลูกปลาทั้ง 2 กลุ่มด้วยอาหารที่ใช้วัตถุดิบโปรตีนจากพืช

ในการทดลองที่ 1 การกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการปรับเปลี่ยนเมแทบอลิซึมของคาร์โบไฮเดรตในลูกพันธุ์ปลาระยะวัยรุ่น (สัปดาห์ที่ 17) ได้แก่ เพิ่มค่าดัชนีตับ เพิ่มไตรกลีเซอไรด์ในตับ และกระตุ้นการแสดงออกของยีนที่เกี่ยวข้องกับกระบวนการไกลโคไลซิส (*pfkma* และ *pfkmb*) ที่กล้ามเนื้อ และยับยั้งการแสดงออกของยีนที่เกี่ยวข้องกับกลูโคนีโอเจเนซิส (*g6pca1*) และการสลายกรดอะมิโน *(alat*) ที่ตับ เมื่อลูกปลาได้รับการทดสอบด้วยอาหาร HC/LP เป็นระยะเวลา 4 สัปดาห์ (สัปดาห์ที่ 18 - 21) พบการเปลี่ยนแปลงของกระบวนการสังเคราะห์การใช้ประโยชน์และการย่อยสลายของสารโมเลกุลเล็ก และการตอบสนองเมแทบอลิซึมของคาร์โบไฮเดรต ได้แก่ 1) ระดับกลูโคสและไตรกลีเซอไรด์เพิ่มขึ้น และโปรตีนลดลงในพลาสมา 2) เพิ่มการสะสมไขมันไกลโคเจนและไตรกลีเซอไรด์ในตับและกล้ามเนื้อ 3) กระตุ้นการแสดงออกของยีนที่เกี่ยวข้องกับไกลโคไลซิส (*gck* และ *pklr*) และการสร้างไขมัน (*fasn* และ *g6pd*) ที่ตับ และการขนส่งกลูโคส (*glut4*) และ ไกลโคไลซิส (*hk1* และ *hk2*) ที่กล้ามเนื้อ 4) ยับยั้งกลูโคนีโอเจเนซิส (*g6pca2* และ *pck2*) และการสลายกรดอะมิโน (*asat*) ที่ตับ นอกจากนี้การกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการ

เปลี่ยนแปลงของเอ็มอาร์เอ็นเอของเอนไซม์ที่เกี่ยวข้องกับกระบวนการเพิ่มและลดหมู่เมทิลของดีเอ็นเอและการเพิ่มและลดหมู่เมทิลและอะเซทิลของฮิสโตน ที่ตับและกล้ามเนื้อในลูกปลาระยะวัยรุ่น

การทดลองที่ 2 พบอิทธิพลของ NP ในลูกปลาระยะโตเต็มวัย (สัปดาห์ที่ 25) การกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการเพิ่มค่าดัชนีตับ กระตุ้นการแสดงออกของยีนที่เกี่ยวข้องกับไกลโคไลซิส (*pfkma* และ *pfkmb*) ที่กล้ามเนื้อ และยับยั้งการสลายกรดอะมิโน *(alat*) ที่ตับ เมื่อลูกปลาได้รับการทดสอบด้วยอาหาร HC/LP เป็นระยะเวลา 4 สัปดาห์ (สัปดาห์ที่ 26 - 29) ลูกปลาของพ่อแม่พันธุ์ที่ได้รับอาหาร HC มีสมรรถนะการเจริญเติบโตที่ดีขึ้น และพบการเปลี่ยนแปลงของเมแทบอลิซึมของคาร์โบไฮเดรต เช่น การกระตุ้นไกลโคไลซีสและการสร้างไขมันที่ตับ และการขนส่งกลูโคสที่กล้ามเนื้อ และพบการยับยั้งกลูโคนีโอเจเนซิสและการสลายกรดอะมิโนที่ตับ นอกจากนี้การกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการเปลี่ยนแปลงของยีนที่สร้างเอนไซม์ที่เกี่ยวข้องกับกระบวนการเพิ่มและลดหมู่เมทิลของดีเอ็นเอและการเพิ่มและลดหมู่เมทิลและอะเซทิลของฮิสโตน ที่ตับและกล้ามเนื้อ แสดงให้เห็นว่ากระบวนการอีพิเจเนติกส์น่าจะเกี่ยวข้องกับอิทธิพลของ NP ของคาร์โบไฮเดรตระยะยาวในปลานิล

การทดลองที่ 3 พบอิทธิพลของ NP ในลูกปลาโตเต็มวัยในระยะเก็บเกี่ยว (สัปดาห์ที่ 56) โดยการกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการยับยั้งการแสดงออกของยีนที่เกี่ยวข้องกับกลูโคนีโอเจเนซิส (*g6pca1* และ *g6pca2*) และการสลายกรดอะมิโน (*asat* และ *alat*) ที่ตับ และกระตุ้นไกลโคไลซิสที่กล้ามเนื้อ (*pfkma*) เมื่อลูกปลาได้รับการทดสอบด้วยอาหารที่ใช้วัตถุดิบโปรตีนจากพืช ผลการศึกษาพบว่าอาหารที่ใช้วัตถุดิบโปรตีนจากพืชส่งผลให้ปลามีสมรรถนะการเจริญเติบโตลดลง และเพิ่มการสะสมไขมัน ไกลโคเจน และไตรกลีเซอไรด์ที่ตับและกล้ามเนื้อ แต่การกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการเพิ่มสมรรถนะการเจริญเติบโต เช่นเดียวกันการกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลต่อการเปลี่ยนแปลงของยีนที่เกี่ยวข้องกระบวนการอีพิเจเนติกส์

โดยสรุปการกระตุ้นพ่อแม่พันธุ์ปลาด้วยอาหาร HC ส่งผลดีต่ออิทธิพลของ NP ในระยะยาวในลูกพันธุ์ปลา สามารถเพิ่มประสิทธิภาพการใช้ประโยชน์ของคาร์โบไฮเดรตในอาหารและอาหารที่ใช้วัตถุดิบโปรตีนจากพืชในลูกพันธุ์ปลา

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LINLI LUO : FEEDING BROODSTOCK NILE TILAPIA (*Oreochromis niloticus*) WITH HIGH LEVEL OF CARBOHYDRATES: IMPACT ON METABOLISM AND EPIGENETICS OF OFFSRPING. THESIS ADVISOR: PROF. SURINTORN BOONANUNTANASARN, Ph. D., 137 PP.

Keyword: CARBOHYDRATE METABOLISM/LONG-TERM EFFECTS/EFFECTIVE UTILIZATION/ NUTRITIONAL PROGRAMMING/EPIGENETICS

Nutritional programming (NP) using hyperglucidic stimuli through high carbohydrate (CHO) feeding in broodstock may lead to modulation of CHO metabolism in the offspring. This study, therefore, aimed to analyze the long-term effects of NP induced by high CHO (HC) feeding in broodstock on CHO metabolism and epigenetic modulation in juvenile and adult offspring, as well as the effectiveness of plant-based diet utilization. The study included two experimental broodstock groups: one fed a high CHO/low protein (HC/LP) diet and the other fed a low CHO/high protein (LC/HP) diet. Offspring from both experimental groups were cultured through the juvenile stage for dietary challenge with the HC/LP diet (Experiment I) and through the adult stage for dietary challenge with the HC/LP diet (Experiment II). In addition, Experiment III involved dietary challenging the offspring of both groups with a plant-based diet.

In Experiment I, broodstock feeding with an HC diet could modulate CHO metabolism and related metabolic pathways in their offspring at the juvenile stage (week 17), including increased hepatosomatic index (HSI) and hepatic triglyceride levels, induction of muscular glycolysis (*pfkma* and *pfkmb*), and suppression of hepatic gluconeogenesis (*g6pca1*) and amino acid catabolism (*alat*). When juvenile offspring were challenged with the HC diet for 4 weeks (week 18–21), more pronounced intermediary metabolism and CHO metabolic responses were observed, including: 1) increased plasma glucose and triglyceride levels and decreased plasma protein levels, 2) increased hepatic and muscular fat, glycogen, and triglyceride contents, 3) induction of hepatic glycolysis (*gck* and *pklr*), lipogenesis (*fasn* and *g6pd*), muscular glucose transport (*glut4*), and glycolysis (*hk1* and *hk2*), and 4) suppression of hepatic gluconeogenesis (*g6pca2* and *pck2*) and amino acid catabolism (*asat*). In addition, HC feeding in broodstock led to modulation of mRNA expression levels of enzymes associated with hepatic and muscular DNA (de)methylation, histone (de) methylation,

and (de)acetylation in juvenile offspring.

In Experiment II, the long-term NP effects of dietary HC stimulus in broodstock on the modulation of CHO metabolism persisted into adulthood (week 25). Parental HC stimulation increased HSI, induced muscular glycolysis (*pfkma* and *pfkmb*), and decreased hepatic amino acid catabolism (*alat*). Furthermore, during the dietary challenge phase (weeks 26–29), parental HC stimulation improved growth performance. The NP effects of parental HC stimulation also modulated several CHO metabolic responses in adult offspring, including the induction of hepatic glycolysis and lipogenesis, as well as muscular glucose transport, and the suppression of gluconeogenesis and amino acid catabolism in the liver. At the molecular level, parental HC stimulation modulated several enzymes related to DNA and histone (de)methylation and histone de(acetylation) in the liver and muscle, suggesting that epigenetic modifications are involved in the long-term NP effects of CHO in Nile tilapia.

In Experiment III, the long-term NP effects of dietary HC stimulus in broodstock on the modulation of CHO metabolism persisted into harvestable adult offspring (week 56), including the suppression of gluconeogenesis (*g6pca1* and *g6pca2*) and amino acid catabolism (*asat* and *alat*) in the liver, and the induction of glycolysis (*pfkma*) in muscle. After the offspring were exposed to high plant-based diets, irrespective of the NP effects of CHO in broodstock, the plant-based HC diet inhibited growth performance and increased hepatic and muscular fat, glycogen, and triglyceride levels, as well as whole-body fat content in fish. However, a history of broodstock HC stimulation improved growth performance and modulated CHO metabolism. Again, at the molecular level, parental HC stimulation modulated several enzymes related to epigenetics.

In conclusion, dietary HC stimulation in broodstock could exert long-term beneficial NP effects that improve the adaptability of the offspring to HC diets. This study suggests that parental nutritional regulation of CHO could serve as a tool to improve the efficient use of dietary CHO and plant-based diets in Nile tilapia.

School of Animal Technology and Innovation Student's Signature Linli Luo
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tired and anxious, it was your encouragement that lifted me up. Your selfless efforts have enabled me to devote myself to research. Without your support, I could not have come to this day.

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LIST OF ABBREVIATIONS

CHO	=	Carbohydrate
HC	=	High carbohydrate
NP	=	Nutritional programming
HC	=	High carbohydrate
LP	=	low protein
LC	=	Low carbohydrate
HP	=	high protein
PBMC	=	plant-based medium carbohydrate
PBHC	=	plant-based high carbohydrate
BUN	=	Blood urea nitrogen
IW	=	Initial body weight
FW	=	Final body weight
WG	=	Weight gain
ADG	=	Average daily gain
SGR	=	Specific growth rate
FCR	=	Feed conversion ratio
HSI	=	Hepatosomatic index
gck	=	Glucokinase
pfklr	=	Phosphofructokinase
pklr	=	Pyruvate kinase
g6pca1	=	Hepatic glucose-6-phosphatase 1
g6pca2	=	Hepatic glucose-6-phosphatase 2
pck1	=	Phosphoenolpyruvate carboxykinase cytosolic
fasn	=	Fatty acid synthase
g6pd	=	Glucose-6-phosphate dehydrogenase
gdh	=	Glutamate dehydrogenase
alat	=	Alanine aminotransferase
asat	=	Aspartate amino transferase

LIST OF ABBREVIATIONS (Continued)

glut4	=	Glucose transporter
hk1	=	Hexokinase I
hk2	=	Hexokinase II
pfkma	=	Phosphofructokinase a
pfkmb	=	Phosphofructokinase b
pkma	=	Pyruvate kinase
dnmt	=	DNA methyltransferases
tet	=	TET methyl cytosine dioxygenases
H3K4me3	=	histone 3 lysine 4 trimethylation
H3K9me3	=	histone 3 lysine 9 trimethylation
H3K36me3	=	histone 3 lysine 36 trimethylation
H3K9ac	=	histone 3 lysine 9 acetylation
setd	=	SET domain-containing
kmt	=	lysine methyltransferase
kdm	=	lysine demethylase
riox1	=	lysine-specific demethylase and histidyl hydroxylase
suv39h1b	=	histone lysine N-methyltransferase
kat	=	lysine acetyltransferase
gtf3c4	=	general transcription factor IIIC subunit 4
sirt	=	sirtuin
µL	=	Microliter
g	=	Gram
°C	=	Degree Celsius
h	=	Hour

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 programing 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 programing 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.

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CHAPTER II
LITERATURE REVIEW

Tilapia exhibits high adaptability to aquaculture conditions and is widely cultured in tropical and subtropical regions. Currently, it is one of the most important freshwater fish species for scientific research and breeding globally and is considered a key future source of animal protein. Presently, feed costs account for approximately 70% of total tilapia farming expenses, with protein comprising about 40% and carbohydrates about 30% of the feed composition. As omnivores, tilapias consume both plant matter and organic debris in water, making them efficient users of carbohydrates. Enhancing carbohydrate utilization can lead to protein-sparing effects.

Figure 2.1 Tilapia (*Oreochromis niloticus*).

2.1 Carbohydrate and protein

Carbohydrate is one of seven essential nutrients of animal body: protein, vitamin, carbohydrate, mineral, water, dietary fiber and lipid. It is the main component of life cell structure and the main energy supply material, and has the important function of regulating cell activity. There are three main forms of carbohydrates in the body, glucose, glycogen and sugar-containing complexes. The physiological function of carbohydrates is related to the type of carbohydrates consumed and the form of carbohydrates in the body. 1) Dietary carbohydrates are the most economical and main source of energy for human beings, which can provide and store heat energy; 2)

Carbohydrate is an important substance in body tissue, essential energy for maintaining brain function, and participates in cell composition and various activities; In addition, it can save protein, regulate fat metabolism, provide dietary fiber, antiketo, detoxify and enhance intestinal function (Guo et al. 2006). Previous studies have confirmed that the 20% carbohydrate group has the highest weight gain rate, specific gain rate and protein efficiency when feeding juvenile carp for 8 weeks with diets equal to protein (45%), fat (8%) and carbohydrate levels of 0%, 5%, 10%, 15%, 20% and 25%, respectively (Hua et al. 2011). Tilapia were fed six diets with 6% to 46% cornstarch (in 8% increments). It was found that weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FET) and protein efficiency ratio (PE) of fish fed with 22% starch were significantly higher than those fed with 6% or 14% starch (Wang et al. 2005). Atlantic salmon fed a diet containing 5% to 30% carbohydrate (CHO) found that fish fed a diet containing 10% CHO had the lowest mortality. Different dietary CHO levels have a slight effect on Atlantic salmon immunity and resistance to bacterial infections (Waagbø et al.1994).

Protein is an important component of all cells and tissues in human body. All important components of the body require the participation of proteins. In the process of fish feeding, the protein content has an important impact on the growth of fish, Juvenile Nile tilapia was fed with three compound diets with different protein content (23.1%, 37.6% and 47.9%) for 78d. The results showed that with the increase of dietary protein content from 23.1% to 47.9%, the average daily gain increased from 0.30g/d to 0.74g/d, and there were significant differences in daily gain and body weight among the three groups (Hu et al.2006). Protein also had a positive effect on the activities of digestive enzymes in the intestinal tract of fish. Six isoenergetic and isolipid diets with protein levels of 37.52%, 41.80%, 46.52%, 49.84%, 56.80% and 61.48% were used to feed juvenile yellow drum. The results showed that the intestinal protease activity of fish firstly increased and then stabilized with the increase of dietary protein level (Lu et al.2015). At the same time, adding protein in the diet can improve the disease resistance of fish. Three kinds of protein diets were prepared by adding shrimp, tilapia or krill hydrolysate (named SH, TH and KH respectively) in the low fish meal (LFM) diet to feed red sea bream larvae. Superoxide dismutase activity and total immunoglobulin levels were significantly increased in fish fed diets containing protein hydrolysates (Khosravi et al. 2015).

Nowadays, price of fish feed has trend to increase in the aquaculture feed markets, feed is the major operational cost-item, accounting for 50-70% of total production cost. However, protein content accounts for about 30%~40% of feed composition. In order to reduce the cost of aquaculture and maximize economic benefits, it is very important to research and develop low protein, low cost and higher quality feed. In the current research, the use of appropriate proportion of high carbohydrate, low protein fish feed to save protein, saving feeding cost is a research hotspot. 2-years old male and female rainbow trout (Oncorhynchus mykiss) were either fed a diet containing no carbohydrates (NC) or a 35%-carbohydrate diet (HC) for an entire reproductive cycle, the result show that broodstock consumed the HC diet, and in contrast to what is commonly observed in juveniles, they were able to grow normally and they did not display postprandial hyperglycemia (Callet, et al. 2020). Sparus aurata L. were fed 93 days three diets containing 63% protein and 5% gelatinized cornstarch (LC diet), 54% protein and 18% GCS (MC diet) or 47% protein and 26% GCS (HC diet), find that fish on MC diet registered higher fresh weight than fish on LC and HC, and higher specific growth rate (SGR) than fish on HC, conclude that when carbohydrates levels below 20% could replace dietary protein, enhance growth rate (Fernández, et al. 2017). Five groups of purified diets were fed to juvenile tilapia with carbohydrate levels of 20%, 27%, 34%, 41%, 48% (corresponding protein levels were 48%, 41%, 34%, 27%, 20%), respectively. The results showed that with the increase of dietary carbohydrate level, the mass gain rate and specific growth of juvenile tilapia firstly increased and then decreased, reached the highest level in the 41% group, indicating that the dietary carbohydrate in juvenile tilapia had a saving effect on protein, and the appropriate level of carbohydrate was 34%-41% (Wu, et al. 2011). These results are highly promising and suggest that dietary carbohydrates can at least partially replace proteins in broodstock aquafeed. The above studies found the effects of short-term high carbohydrate low protein (HC/LP) diet stimulation on the growth performance and carbohydrate metabolism of fish. In order to explore the effects of short-term HC/LP diet stimulation on the long-term life of fish, the term nutrition planning was proposed.

2.2 Carbohydrate metabolism pathways

Carbohydrate metabolism refers to the process of digestion, absorption, synthesis, storage and utilization of carbohydrates. Carbohydrates are the main source of energy, and their metabolic pathways can be divided into glycolysis and gluconeogenesis. When there is too much glucose, the body converts the excess glucose into fat (Lipogenesis); When the body does not have enough glucose to supply energy, the body converts non-sugar substances (such as amino acids) to produce energy for energy supply (Amino acid catabolism) (Figure 2.2, Figure 2.3).

Glycolysis is the process by which glucose is broken down into pyruvate in the cytoplasm in the absence of oxygen. The steps of glycolysis: 1. the phosphorylation of C6 of glucose catalyzed by hexokinase (gck, hk) to form glucose 6-phosphate; 2. Hexose phosphate isomerase catalyzes the isomerization of glucose 6-phosphate to fructose 6-phosphate; 3. Phosphofructokinase (pfk) catalyzes the phosphorylation of fructose 6-phosphate to fructose 1, 6-diphosphate; 4. Phosphoenolpyruvate is generated from fructose 1, 6-diphosphate through a series of other enzymes; 5. Under the catalysis of pyruvate kinase (pk), the high-energy phosphate groups of phosphoenolpyruvate molecules are transferred to ADP to generate ATP and pyruvate (Chandel, 2021).

Gluconeogenesis refers to the process by which organisms convert various non-sugar substances (such as lactate, pyruvate, amino acids and glycerol) into glucose or glycogen. The steps of gluconeogenesis: 1. phosphoenolpyruvate carboxykinase(pck) is used to convert all kinds of gluconeoplasts (except glycerol) into phosphoenolpyruvate. 2. Phosphoenolpyruvate is converted to glucose 6-phosphate; 3. glucose 6-phosphate is converted into glucose under the action of glucose-6-phosphatase(g6pca) (Exton, 1972).

Lipogenesis: glucose from excess dietary carbohydrate undergoes glycolysis in liver and is eventually converted into fatty acids (FA) to be esterified to TAG for VLDL secretion. The process of converting glucose to fatty acids, de novo lipogenesis (DNL), is tightly controlled by hormones and nutritional status (Wang et al 2015).

Amino acid catabolism: mino acids in muscle transfer amino acids to pyruvate to produce alanine, which is transported to the liver through the blood circulation and then deaminated. The generated pyruvate is then transported to the muscle through the blood circulation to synthesize glucose after gluconogenesis and decompose again to produce pyruvate. Through this cyclic reaction process, amino acids in the muscle can be

transferred to the liver for processing. This cycle is called the alan-glucose cycle. Ammonia from the muscle is transported to the liver in the form of non-toxic alanine and the liver provides glucose to the muscle (Torres et al 2023).

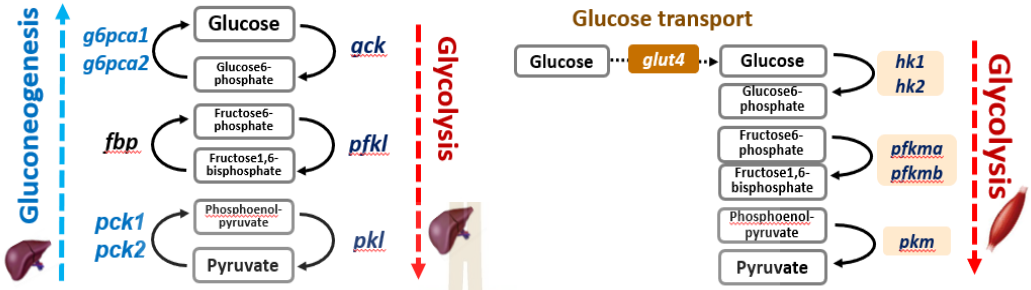

Figure 2.2 The process of glycolysis and gluconeogenesis (Tongchaitriwat et al., 2024). Liver, *g6pca*: glucose-6-phosphatase 1; *fbp*: phosphofructokinase1; *pck*: phosphoenolpyruvate carboxykinase 1; *gck*: glucokinase; *pfkl*: phosphofructokinase1; *pkl*: pyruvate kinase; muscle, *glut4*: glucose transporter 4; *hk2*: hexokinase 2; *pfkm*: hosphofructokinase; *pkm*: pyruvatekinase.

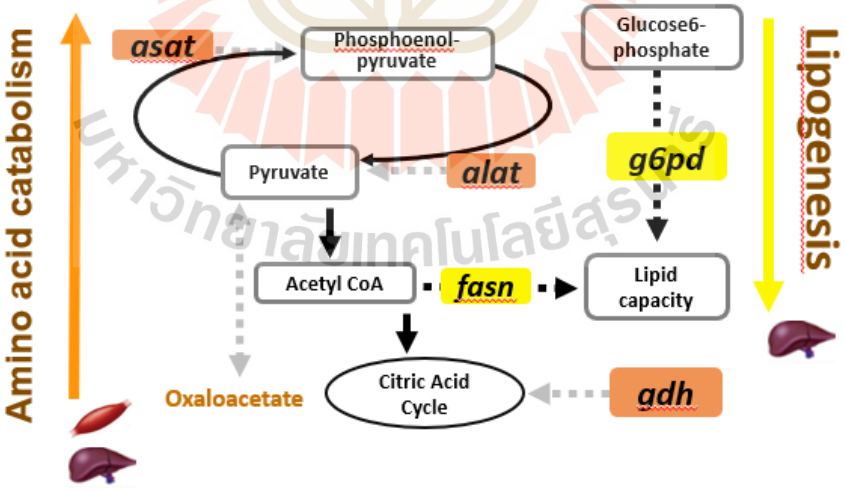

Figure 2.3 The process of lipogenesis and amino acid catabolism (Tongchaitriwat et al., 2024). *asat*: aspartate transaminase; *alat*: alanine aminotransferase; *gdh*: glutamate dehydrogenase; *fasn*: fatty acid synthase; *g6pd*: glucose-6-phosphate dehydrogenase

2.3 Carbohydrate utilization in fish

Carbohydrates are the main components of life cell structure and main energy supply substances and have important functions in regulating cell activities. The main carbohydrate resources include Wheat starch, Corn starch, Dextrin, Maltose, Glucose and Cellulose, etc. However, different species of fish have different ability to use carbohydrates and are also affected by the source of carbohydrates.

In herbivorous fishes, Grass carp (Ctenopharyngodon idellus) has a maximum acceptable content of maize starch and wheat starch in the diet of 38% (Li et al. 2014a) and 47% (Tian et al. 2012); Rohu (Labeo rohita) has a maximum acceptable content of starch and dextrin in the diet of 51% (Mohapatra et al. 2003) and 43% (Erfanullah and Jafri 1998a); Blunt snout bream (Megalobrama amblycephala) has a maximum acceptable content of cassava starch and dextrin in the diet of 45% (Zhou et al. 2013) and 42% (Li et al. 2013), respectively.

In omnivorous fishes, Common carp (Cyprinus carpio) has a maximum acceptable content of α-maize starch and α-potato starch in the diet of 38% (Kheyyali et al. 1990) and 55% (Shimeno et al. 1995); Nile tilapia (Oreochromis niloticus) has a maximum acceptance level of 48% in maize grain and wheat bran diets (Ali and Al-asgah 2001); Walking catfish (Clarias batrachus) have a maximum dietary acceptance of dextrin at 43% (Erfanullah and Jafri 1998b).

In carnivorous fishes, Rainbow trout (Oncorhynchus mykiss) has a maximum dietary acceptance of gelatinized potato starch and gelatinized maize starch of 36% (Yamamoto et al. 2001) and 50% (Suárez et al. 2002); European sea bass (Dicentrarchus labrax) has a maximum acceptable dietary content of 31% for precooked starch and wheat shorts (Pérez et al. 1997); European eel (Anguilla anguilla) has a maximum acceptable level of 50% gelatinized maize starch in the diet (Suárez et al. 2002).

Carbohydrates is cheapest source on fish feed, if can improve CHO utilization enable protein sparing effects, perform least cost feed with high quality, the cost of artificial feeding will be saved, and the economic benefit will be improved. In previous studies, nutritional programming can be one approach.

2.4 Nutritional programming

Nutritional programming (NP) is the process through which variation in the quality or quantity of nutrients consumed during pregnancy exerts permanent effects upon the developing fetus (Langley; Evans, 2008) (Figure 2.3). Fish nutrition is one of the most important perspectives for developing sustainable fish farming, which has become an important food-producing sector for global food security. In order to improve fish nutrition, it is scientifically challenging to not only search for potential alternative feed ingredients and feed supplementation but also perform research into understanding fish metabolism and how it can be modulated. Suboptimal nutritional status during early life and predisposition to metabolic diseases later, such as permanent growth retardation and impairment of neural development and key metabolic pathways, this phenomenon, termed nutritional programming or metabolic programming, is beginning to be studied in fishes. In a study of Senegalese sole (Solea senegalensis), diets containing protein with different degrees of hydrolysis were given at first feeding followed by a 1-month common feeding. Fish that were fed intact protein (vs.protein hydrolysates composed of peptides) showed greater dry weight as juveniles (Canada et al. 2018). In another study, while no short-term changes were observed after 4 weeks on a vitamin-supplemented diet, the first feeding diet modified muscle gene expression in juveniles after a 4-month common feeding period. Specifically, metabolic genes involved in nutrient (lipid, glucose, and amino acid) catabolism (hoad, pk-m, gdh32) and mitochondrial energy metabolism (qcr2, cox43) were upregulated in the juveniles that were given the vitamin supplementation at first feeding (Panserat et al. 2017). European seabass larvae were given either a high or a low HUFA5 diet [expressed as eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA) at 2.2% or 0.8% on dry matter basis, respectively from first feeding, followed by a HUFA-rich common diet (2.7%) for 3 months. When juveniles were challenged with a HUFA-depleted diet (0.5%) for about 2 months, those that had been fed the low HUFA diet as larvae had higher DHA content in polar lipids (Vagner et al. 2007).

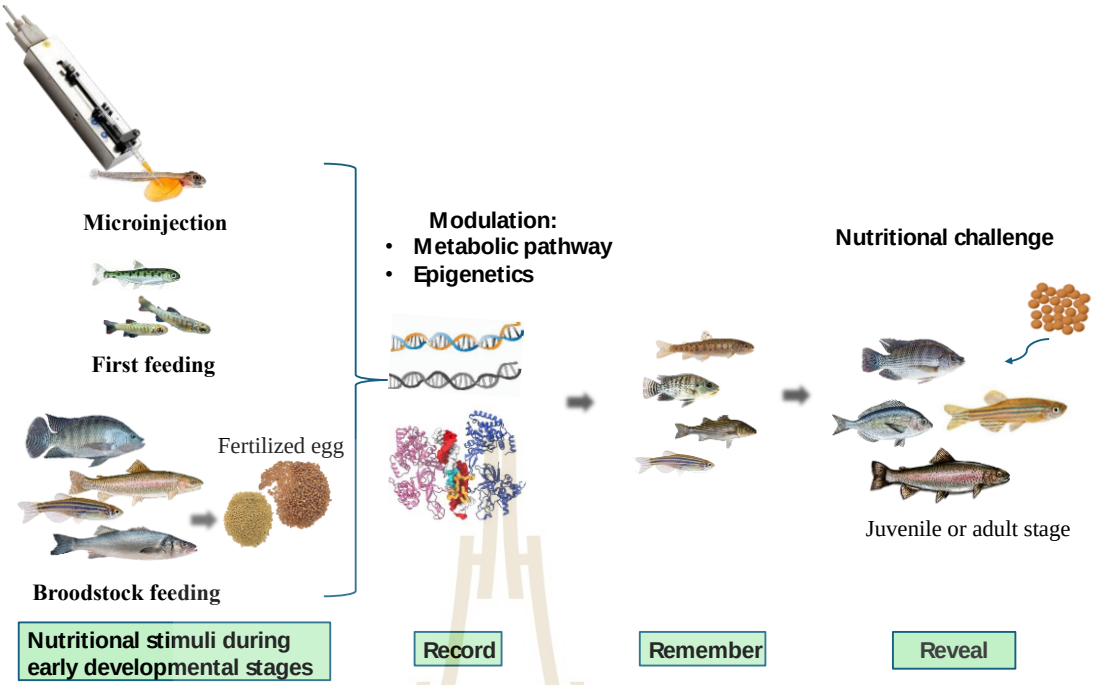

Figure 2.4 The principle of nutritional programming.

2.5 Nutritional programming of dietary carbohydrate on fish

2.5.1 Nutritional programming of dietary carbohydrate on fish early stage

Several studies have provided evidence of enhanced utilization of dietary carbohydrates in fishes by means of programming. High-carbohydrate diets were fed to zebrafish (*Danio rerio*) during four ontogenetic stages: from the first-feeding stage to the end of the yolk-sac larval stage; from the first-feeding stage to 2 d after yolk-sac exhaustion; after yolk-sac exhaustion for 3 or 5 d, find that it is possible to permanently modify carbohydrate digestion, transport and metabolism of adult zebrafish through early nutritional programming (Fang et al. 2014). Rainbow trout alevins (around 100 mg) were fed for 5 days with the two experimental hyperglucidic (40% gelatinized starch + 20% glucose) and hypoproteic (20%) diet (VLP diet) or a high-protein (60%) glucose-free diet (HP diet, control), following a common 105-day period on a commercial diet, both groups were then challenged (65 days) with a carbohydrate-rich diet (28%), in whole alevins (short term), diet VLP relative to HP rapidly increased gene expressions of glycolytic enzymes, while those involved in gluconeogenesis and amino acid catabolism decreased, by contrast, muscle of challenged juveniles subjected

previously to the VLP stimulus displayed downregulated expression of markers of glycolysis and glucose transport (not seen in the short term), in summary, the data show that a short hyperglucidic–hypoproteic stimulus during early life may have a long-term influence on muscle glucose metabolism in trout (Geurden et al. 2014). During early development, a group of gilthead seabream (*Sparus aurata*) larvae (control, CTRL) were kept under a rich-protein-lipid feeding regime whereas another group (GLU) was subjected to high-glucose stimuli, delivered intermittently over time. At juvenile stage, triplicate groups (IBW: 2.5 g) from each fish nutritional background were fed a high-protein (59.4%) low-carbohydrate (2.0%) diet before being subjected to a low-protein (43.0%) high-carbohydrate (33.0%) dietary challenge for 36-days, find that GLU juveniles showed higher absorption of starch-derived glucose in the gut, suggesting an enhanced digestion of carbohydrates, while amino acid use was not affected, suggests that the early glucose stimuli may alter carbohydrate utilization in seabream juveniles (Rocha et al. 2016). A nutritional stimulus was accomplished by microinjecting 2M glucose into yolk reserves during the alevin stage in Nile tilapia (*Oreochromis niloticus*), and the glucose stimulus history were examined in fish fed with two different dietary carbohydrate/protein levels (medium-carbohydrate diet, CHO-M; high-carbohydrate diet, CHO-H) in juvenile (during weeks 20–24) and adult (during weeks 32–37) fish, the result show that early glucose stimuli were found to be clearly associated with a positive metabolic programming effect later in life, improving the growth performance of the fish (Kumkhong et al., 2020; 2021).

2.5.2 Nutritional programming of dietary carbohydrate on broodstock zebrafish

Lu et al (2022) fed HS (CHO 53.6%) to zebrafish larvae at 3-5 dph (FF) and 6-10 dph (YE), then hatched their offspring F0, and collected offspring F1 of F0, which were challenged to carbohydrate diet HC (CHO 35.36%) one week at F0 and F1 adulthood (15weeks), respectively, then, the expression levels of glycolysis and gluconeogenesis genes in livers of F0 and F1 after HC diet were analyzed. The results showed that in the glycolysis process of F0 (Figure 2.4), the expression levels of *gck* gene were significantly increased in FF and YE groups, and the expression levels of *pfkla* and *pfklb* were the highest in FF group. During gluconeogenesis, the expression levels of *fbp1a*, *fbp1b* and *pck1* genes in FF and YE groups were significantly lower

than those in the control group. In the process of F1 (Figure 2.5) glycolysis, the expression levels of *gck*, *pfkla* and *pfklb* were not significantly different among all groups. However, in the process of gluconeogenesis, the expression levels of *pfkla* and *pfklb* in YE group were significantly lower than those in control group and FF group, and pck1 in FF and YE group Gene expression was significantly lower than the control group.

The increased expression of key genes and decreased expression of major genes involved in gluconeogenesis in the offspring during glycolysis indicated that the H-CHO nutritional programming on broodstock had long-term effects on the offspring. These results indicate that H-CHO nutrition program for species is feasible, and tilapia has a higher tolerance to carbohydrates, which provides a reliable theoretical basis for the study of carbohydrate nutritional programming for tilapia species.

However, the mechanism of nutritional programming in broodstock fish effect on offspring is not yet known well. Epigenetics could be one regulation factors.

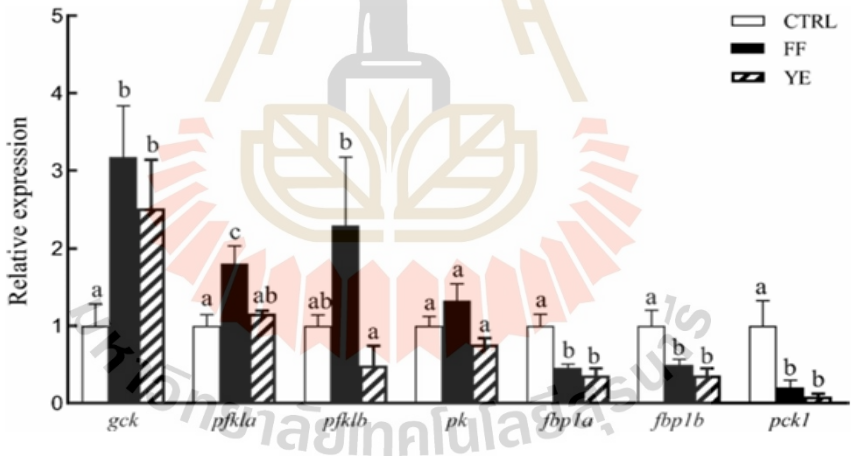

Figure 2.5 The long-term effect of early high-carbohydrate treatment on hepatic gene expression in adult zebrafish F0 (1-week HC challenge) (Lu et al 2022). CTRL, fish fed with control diet; FF, fish fed with HS from the first-feeding stage to the end of the yolk-sac larval stage; YE, fish fed with HS after yolk sac exhaustion for 5 days. Different letters in the bar graph indicate significant differences ($P < 0.05$, $n = 6$)

2.6 Epigenetics modification

2.6.1 Main research contents of epigenetics

The main research contents of epigenetics include DNA methylation, Histone modification and Non-coding RNA (ncRNA). Different from diseases caused by DNA sequence changes, epigenetic modifications are easily affected by the environment, and many of their changes are reversible.

2.6.1.1 DNA methylation

DNA methylation in mammals occurs mainly at cytosines of CpG dinucleotides. DNA methylation is catalyzed by DNA methyltransferase (Dnmt). In this process, sadenylymethionine is the donor of methyl groups, and Dnmt transfers the methyl group to the 5-position carbon atom of cytosine to form 5-methylcytosine. CpG islands refer to Cpg-rich DNA fragments in the genome, which are usually about 1 to 2 kb in length.

In general, DNA hypomethylation in the promoter region of a gene means the activation of the gene, while hypermethylation means the silencing of the gene. Study have shown that DNA methylation inhibits gene expression through two main mechanisms: One is that methylated DNA is not recognized by some transcription factors.

2.6.1.2 Histone modifications

Histone modification is another important way of epigenetic modification. The nucleosome is the basic building block of chromatin, which is formed by 146 bp of DNA surrounding the histone octamer. Histone octamers are formed from two molecules each of histone H2A, H2B, H3, and H4. The N-terminal tail of histone H3 and H4 can be post-translational modified by methylation, acetylation, SUMOylation, phosphorylation, ubiquitination and other modifications (Strahl; Allis 2000), which can change the loose or condensed state of chromatin, or by recruiting other regulatory proteins to participate in DNA processing (Taverna et al. 2007). At present, much research is focused on histone methylation and acetylation.

Histone methylation modification is mainly accomplished under the action of Histone methlytransferase (HMT). According to the number of methylation groups on the modified residues, lysine methylation can be divided into single methylation (me1), double methylation (me2) and triple methylation (me3). Arginine methylation can

be classified as mono-methylation, symmetric dimethylation, and asymmetric dimethylation. After histone methylation, the activation or inhibition of gene expression is determined by the modified amino acid residue.

Histone acetylation modification is carried out under the coordinated action of Histone acetyltansferase (HAT) and Histone deacetylase (HDAC). HAT can catalyze lysine acetylation at the N-terminus of histone proteins, which neutralizes the positive charge carried by lysine and changes the structure of chromatin, making it loose and easy to bind to transcription related proteins, thereby promoting gene transcription. HDAC can remove the acetyl modification of histone N-terminal lysine residues, condensing chromatin and inhibiting gene transcription (Kouzarides 2007).

2.6.1.3 Non-coding RNA

Recently, non-coding RNA have been playing an increasingly important role in epigenetic modifications. Non-coding RNA refers to functional RNA that does not encode proteins. These RNA still contain genetic information and have corresponding functions and participate in the translation process of proteins. Studies have shown that in 90% of the sequenced human genome, only 1.5% of RNA encodes proteins; the rest are ncRNA (Skreka, 2007). The role of non-coding RNA in regulating gene expression is shown in Table 2.1.

Table 2.1 Roles of non-coding Rnas in regulating gene expression (Skreka 2007).

Species	Length	Function
mi RNA	~21~25	Regulation of histone modification causes chromatin remodeling (Tuddenham et al. 2006).
si RNA	~21~25	Mediate DNA methylation and histone modifications, leading to transcriptional gene silencing (Kawasaki et al. 2004).
pi RNA	~24~31	Formation of transposon methylation, triggering the silent regulation of transposons in germ cells (Aravin et al. 2007).
Inc RNA	>200	Genomic imprinting and X-chromosome inactivation (Yang et al. 2007).

2.6.2 The role of epigenetic modifications in glucose metabolism

Studies have shown that epigenetic modifications such as DNA methylation, histone modification and non-coding RNA are closely related to glucose and lipid metabolism. Epigenetic modifications can regulate the development and differentiation of pancreatic islets, insulin secretion, and pathways related to glucose metabolism, thereby affecting glucose metabolism (Figure 2.5).

Similarly, histone modifications are also closely related to glucose metabolism. Experiments have shown that the distribution of HDAC4, HDAC5, and HDAC9 of I1a class HDAC in β and 8 cells has significant spatial specificity, and they play a very important role in regulating the development and differentiation of islet cells. High expression of HDAC4 and HDACS decreases the number of β and 8 cells (Lenoir et al. 2011). SIRT6 is a member of the HDACu family and plays an important role in regulating metabolism, DNA damage and lifespan.

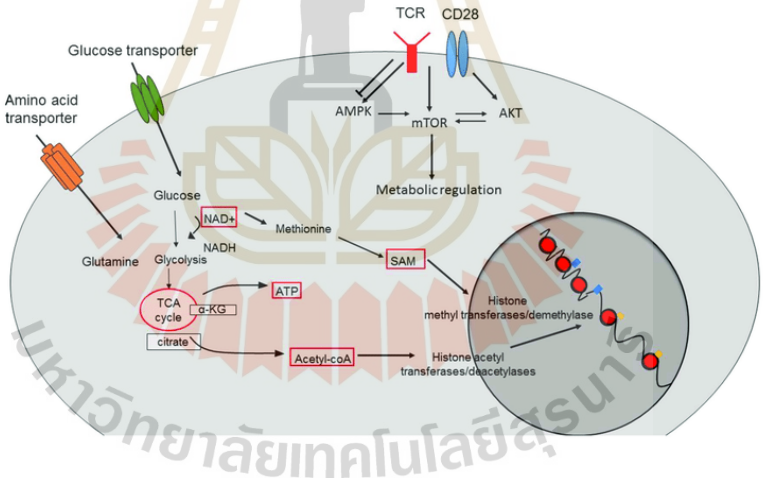

Figure 2.6 The role of epigenetic modifications in glucose metabolism (Yerinde et al.2019).

2.6.3 The role of epigenetic modifications in lipid metabolism

Epigenetic modifications affect the growth and development of adipose tissue and pathways related to lipid metabolism, thereby regulating the balance of lipid metabolism (Figure 2.6).

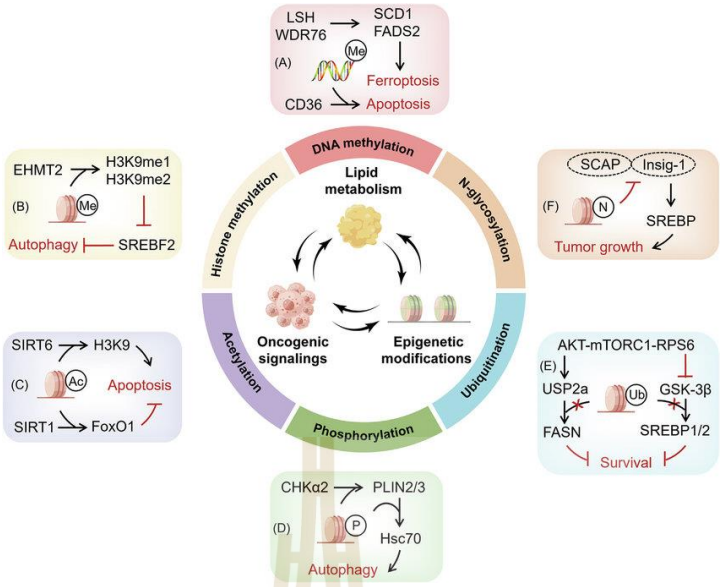

Figure 2.7 The role of epigenetic modifications in lipid metabolism (Zhang et al. 2022).

2.6.3.1 DNA methylation and lipid metabolism

Peroxisome proliferator activated receptor-y (PPARy) is associated with adipocyte differentiation, glucose regulation and insulin resistance. Studies have found that Leptin promoter demethylation is closely related to the process of adipocyte precursor differentiation into adipocytes. Leptin is mainly secreted by fat cells. As a protein hormone, it can inhibit the appetite of animals and regulate the body's energy metabolism.

2.6.3.2 Histone modification and lipid metabolism

Jhdm2a (also known as Jmjdla and Kdm3a) is a specific demethylase for H3K9. Wang et al (2013) found that histone methyltransferase G9a could inhibit the expression of PPARy through H3K9me2, thereby inhibiting adipogenesis. Sterol regulatory elementbinding proteins (SREBPs) can regulate the production of fatty acids and cholesterol, and are important transcription factors for lipid metabolism, including SREBP-1 and SREBP-2 isoforms.

2.6.3.3 Non-coding RNA and lipid metabolism

Lin et al (2009) found that the high expression of miR-27 could inhibit the formation of adipocytes. Other studies have shown that miR-27 can inhibit Peroxisome proliferatoractivated receptor-y, PPARy), CCAAT/Enhancer-binding protein alpha (C/EBPa), Retinoid Xreceptor alpha (retinoid Xreceptor alpha, C/EBPa), RXRa),

Adiponection (ADIPOQ), CD36 molecule (CD36). miR-122 accounts for 70% of adult mouse liver miRNAs.

2.7 Effect of dietary high carbohydrate on fish epigenetics modification

Previous study of NP in Nile tilapia was stimulus high carbohydrate at alevin and have a strong impact at later in life, improve carbohydrate utilization as much as 60% CHO level (Kumkhong et al., 2020), NP of CHO is possible method to modulated CHO metabolism.

The continued effects of nutritional programming in later life may be due to epigenetic modifications. Callet et al (2021) stimulated rainbow trout female and male fish with a low protein/high carbohydrate diet (LP/HC) and a no carbohydrate diet (NC) for 10 months, then fertilization was carried out and four sets of offspring were obtained: NN, NH, HN, HH. By examining the metabolic level of offspring, it was found that the average SMR and RMR of NN and NH were significantly higher than those of HN and HH (Figure 2.7), which indicated that the paternal LP/HC diet reduced the metabolic rate of offspring. The Global DNA Methylation of offspring was detected, and it was found that the 5-mC level of HN and HH was significantly lower than that of NN and NH, and the C level of HH was significantly higher than that of other groups (Figure 2.8), indicating that the paternal LP/HC diet induced DNA hypomethylation in offspring.

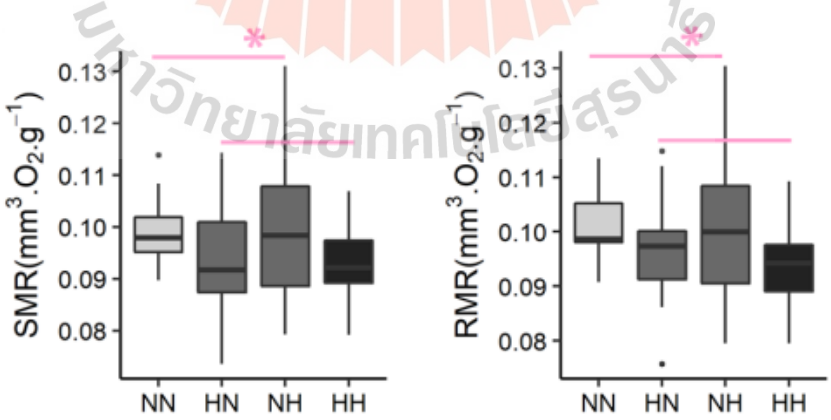

Figure 2.8 Effect of parental LP/HC diet on fry metabolic rates (Callet et al 2021).

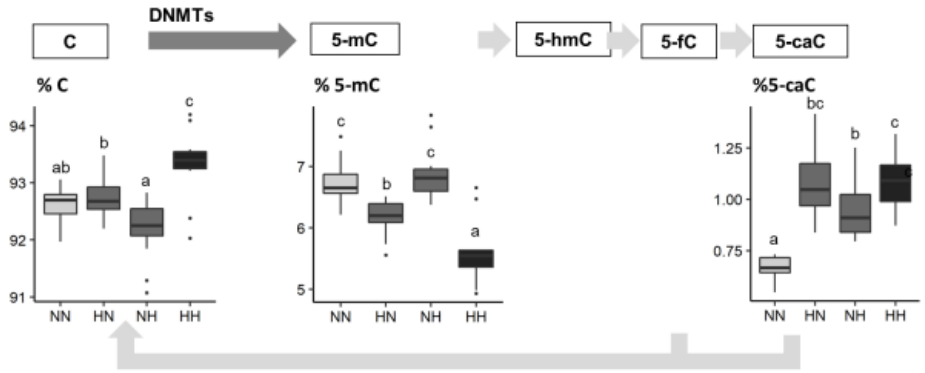

Figure 2.9 Effect of parental LP/HC diet on fry DNA methylation (Callet et al 2021).

Lu et al. (2022) through the zebrafish (Danio rerio) early larvae are high in carbohydrates (53.66%), respectively, FF (from first feeding to the end of the yolk sac), YE (5 days after the yolk sac failure) and the CTRL (control diet) feed group, the larvae (F0) and their offspring (F1) were then both fed the control diet (22.69%) until adulthood (15 weeks). By examining the expression level of gluconeogenesis related gene *pck1* in the liver, it was found that the high carbohydrate diet reduced the expression level of *pck1* gene in F0 (Figure 2.9). At the same time, it also had the same effect on its offspring: gene-specific DNA hypomethylation of *pck1* promoter region in F0 liver after high-carbohydrate stimulation, and the same result was observed in F1 (Figure 2.10). This suggests that the possibility of nutritional programming of carbohydrates is related to DNA hypomethylation.

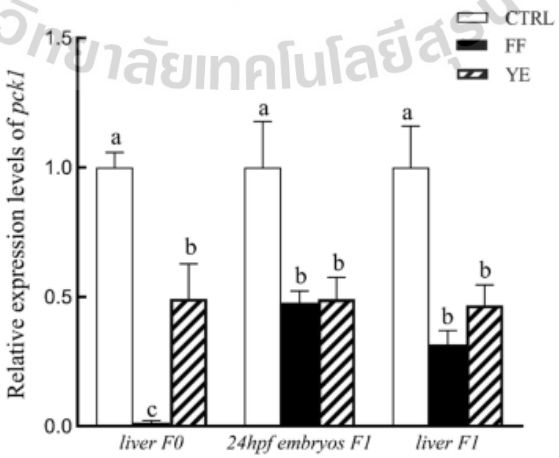

Figure 2.10 The pck1 expression levels in FF, YE, and the control group in F0 and F1. pck1, phosphoenolpyruvate carboxykinase 1 (Lu et al. 2022).

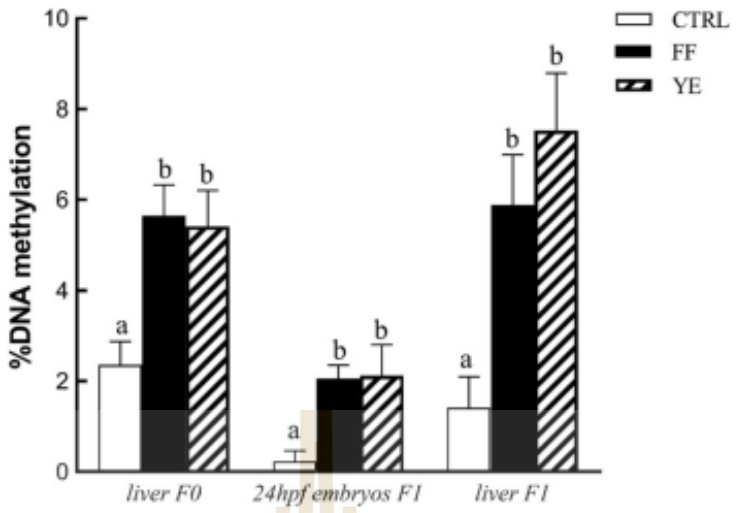

Figure 2.11 Gene-specifc DNA methylation in the promoter region of pck1 (Lu et al. 2022).

Song et al (2019) use HP (no carbohydrate / high protein) diet and LP (high carbohydrate/low protein) diet stimulus Rainbow trout during first feeding until 4weeks, and fed fish HP diet every other day as another stimulus named HPR, then challenge with LP diet at 20–30 weeks, the result indicated that the level of DNA CmCGG methylation in the muscle of juvenile trout with a history of LP diet was significantly lower than that of those with a history of HP diet.

Callet et al (2021) fed with either the NC diet (no carbohydrates) or the HC/LP (high carbohydrates/low protein) diet on female and male rainbow trout broodstock, then through fertilization to obtain four group of offspring (NN, NH, HN and HH), and fed with a commercial diet 7 months, after that challenged during three months with a complete plant-based diet, the hepatic epigenetic landscape result show that the level of cytosine in the liver of the HN and HH groups was significantly higher than that of the control group. However, the level of 5-mdc in the liver of the HN and HH groups was significantly lower than that of the control group, and the level of 5-hmdC in the liver was significantly lower in the NH, HN and HH groups.

Jingwei Liu et al (2021) fed the rainbow trout with the HP-NC, MP-HC diets, and the MP-NC, MP-HC, LP-NC diets by 4 days fasting and refeeding, the result in fig. A show that when fed HP-NC and MP-HC diet, the hepatic 5-mdC and 5-hmdC concentration significant lower than control group, and the dC concentration higher than control group, 5-fdC and

5-cad concentration in MP-HC group were significant different low than other group. In fig. B, the hepatic dC concentration in MP-HC and LP-NC group were significant different low than FD and MP-NC group, 5-mdC was opposite, the 5-fdC concentration in FD group was significant different high than the other group, 5-hmdC in MP-NC and MP-HC ware significant different high than FD and LP-NC group.

Marandel et al (2016) fed the rainbow trout with fasted for four days and refed with either the no CHO or the high CHO diet, find that whatever fed with no CHO, high CHO or fasted, there was no significant effect on H3K4me3/H3 and H3K36me3/H3 levels, but the H3K9me3/H3 was significant different low in fasted when compare with another two group, and H3K9ac/H3 in no CHO group was significant different high than other group.

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CHAPTER III

HIGH CARBOHYDRATE FEEDING IN NILE TILAPIA BROODSTOCK AFFECTS METABOLISM AND EPIGENETICS OF JUVENILE OFFSPRING

3.1 Abstract

Hyperglucidic stimuli by high carbohydrate (CHO) feeding in broodstock would be provided as a practical tool to achieve nutritional programing (NP) in fish. This study therefore aimed to investigate the effects of high CHO (HC) feeding stimulus in broodstock on intermediary CHO metabolic and epigenetics regulation in juvenile offspring. Two different dietary CHO diet including high carbohydrate/low protein (HC/LP) and low carbohydrate/high protein (LC/HP) were fed to mature female and male Nile tilapia. Offspring were obtained from female mouth and growth with commercial diet through juvenile stage. Our results showed that, dietary HC stimulus in broodstock could modulate CHO and its related metabolic in their offspring at juvenile stage, including increased hepatosomatic index (HSI) and hepatic triglyceride as well as induction of muscular glycolysis (*pfkma* and *pfkmb*) and suppression of hepatic gluconeogenesis (*g6pca1*) and amino acid catabolism *(alat*). When juvenile offspring were challenged with HC diet, more pronounced intermediary CHO metabolism were demonstrated in 1) increased plasma glucose and triglyceride and decrease in plasma protein, 2) increased hepatic and muscular fat, glycogen and triglyceride contents, 3) induction of hepatic glycolysis (*gck* and *pklr*) and lipogenesis (*fasn* and *g6pd*) and muscular glucose transport (*glut4*) and glycolysis (*hk1* and *hk2*), 4) suppression of hepatic gluconeogenesis (*g6pca2* and *pck2*) and amino acid catabolism (*asat*). In addition, HC feeding in broodstock regulated the mRNA level of enzymes associated with hepatic and muscular DNA (de)methylation, histone (de)methylation and (de)acetylation in juvenile offspring. Combined, hyperglucidic stimuli by dietary HC in broodstock induced NP effects in juvenile offspring, and the epigenetics were involved NP concept.

Keywords: Glycolysis; Gluconeogenesis; Amino acid catabolism; Lipogenesis; Epigenetic.

3.2 Introduction

With the rising demand for global animal-source food consumption, including products from aquaculture and terrestrial animals, an increase in global feed supply has been required. In addition, livestock and aquaculture feeds compete for feedstuff resources to develop and improve low-cost, high-quality feed, leading to explore alternative feed ingredients and/or nutrients (Sandström et al., 2022), and testing their efficient utilization with minimal negative effects. Particularly, carbohydrates (CHO) have been considered as the cheapest source of nutrients for energy that are commonly incorporated in commercially practical diets to produce low-cost feed. Therefore, several research play attentions and intensively investigate the maximum incorporation of carbohydrates and their efficient utilization for protein retention for growth improvement (Wilson, 1994; Jia et al., 2022; Yang et al., 2023). The efficient use of CHO was examined in terms of zootechnical performance and intermediary CHO metabolism (Azaza et al., 2015; Boonanuntanasarn et al., 2018b, 2018a). Recently, nutritional programming (NP) of CHO has been considered as a tool to modulate CHO metabolism and consequently improve the efficient utilization of CHO (Kamalam et al., 2017).

The concept of NP refers to the impact of nutritional environment stimuli during critical developmental windows such as gametogenesis, fetal growth and early life on long-term metabolic responses and health status (Langley-Evans 2009; Koletzko et al., 2017). Particularly, nutritional intervention of CHO at early developmental stage could modulate CHO metabolism at later life in mammals (Srinivasan et al., 2008). Application of NP of CHO was demonstrated in fish by nutrient intervention or stimulus at early life to improve efficient uptake and utilization when fish were fed a similar dietary nutrient again at later in life (Lucas 1998; Panserat et al., 2019). The effects of NP of CHO varied among fish species as well as methods of nutritional stimuli. In zebrafish, glucose injection into late embryonic life or early HC feeding in larvae could induce hepatic gluconeogenesis and lipogenesis, muscular gluconeogenesis (juvenile) and hepatic glycolysis (adult) at later juvenile and adult stage when challenged with HC diet again

(Fang et al., 2014; Rocha et al., 2015). Early HC feeding in rainbow trout showed suppression of muscular glucose transport and glycolysis in juvenile which were fed with HC diet (Geurden et al., 2014). However, early HC feeding in European sea bass larval had no effect on CHO metabolism in juvenile when they were exposed to HC diet (Zambonino-Infante et al., 2019). Overall, NP of CHO could modulate CHO metabolism in fish, and the modulatory varied among the fish species.

For world aquaculture, tilapia has been the second most economically farmed fish, after carp. Among tilapia farming, Nile tilapia (Oreochromis niloticus) dominates, and their feed cost generally accounts for 50-70% of total cost. Increasing the CHO and decreased protein content in Nile tilapia feed could be a method to reduce the cost. Intensive research on optimal CHO level (32% CHO) and CHO metabolism in Nile tilapia were demonstrated (Azaza et al., 2015; Boonanuntanasarn et al., 2018a, b). As omnivorous fish, Nile tilapia exhibited efficient tolerance and metabolic capacity to high CHO intake; therefore, NP of CHO in Nile tilapia could be an effective way to improve the efficient utilization of dietary HC (up to ~ 66 %). For example, glucose injection into yolk reserve larvae improved the growth performance in juvenile but not adult stage when they were challenged with HC diet, while intermediary CHO metabolism pathway was modulated including induction of hepatic and muscular glycolysis in both of juvenile and adult (Kumkhong et al., 2020a, 2021). Early HC feeding in Nile tilapia fry for 1 or 3 weeks and 4 weeks could enhance the growth performance and suppression of hepatic amino acid catabolism and induction of muscular glucose transport and glycolysis in both of juvenile and adult stage when they were fed HC feed (Kumkhong et al., 2020b; Srisakultiew et al., 2022). Effects of nutritional intervention of CHO in Nile tilapia varied due to the method of NP and the development stages of fish. The nutrient stimulus could be performed during broodstock. In rainbow trout, female broodstock fed with HC diet for 1 year decreased gonadosomatic index (GSI) while increased their relative fecundity. Although rainbow trout which is carnivorous showed poor utilization of dietary CHO, HC feeding in female broodstock induced several intermediary CHO and its related metabolism in offspring in long-term through juvenile stage (Callet et al., 2020; 2021; 2022a, b). The effects of NP of HC feeding in broodstock in Nile tilapia, a good user of CHO as energy source, might persisted and influenced CHO metabolism in offspring for long-term. In this study,

we therefore aimed to investigate the effects of HC dietary in broodstock on intermediary CHO metabolic responses in their juvenile offspring. To explore the effects of dietary HC in parental on the capacity of CHO utilization in offspring, juvenile fish were subjected to challenge a HC diet. In addition, the enzymes related to DNA methylation and histone modifications were examined at molecular level to investigate whether epigenetic regulation is effective in NP mechanism.

3.3 Materials and methods

3.3.1 Ethics statement

All experimental procedures involving fish cultures and sampling were approved by the Ethics Committee of the Suranaree University of Technology Animal Care and Use Committee (Approval No. SUT-IACUC-001/2023).

3.3.2 Experimental diet, design, and fish culture

Table 3.1 showed experimental diets including LC/HP (low-carbohydrate and high-protein) and HC/LP (high-carbohydrate and low-protein) diets. In addition, the composition of commercial diets used for acclimatizing fish and nursing fry was shown in Table 3.1. The proximate compositions including moisture, CP, CF, crude fiber, and ash, which were analyzed according to the standard method of the Association of Official Analytical Chemists (AOAC, 1990), are demonstrated in Table 3.1.

To avoid stressful conditions according to artificial breeding, natural breeding which is general practiced for Nile tilapia farming was applied for production of fertilized eggs. Suitable stocking rates for Nile tilapia broodstock are recommended that are ranging from 0.2 - 0.3 kg/m2 in ponds (FAO, 2009). Indeed, female to male ratio also play an important role which influenced natural reproduction rate. For instance, the sex ratio of 2:1 (female: male) in Nile tilapia broodstock showed highest number of newly hatched larvae than the sex ratio of 3:1, 4:1 and 5:1 (female: male) (Salama, 1996). Therefore, completely randomized design with two different diet including HC/LP and LC/HP with 6 females and 3 males was employed in this study. The scheme of experimental plan for feeding broodstock and offspring and their sampling was shown in Figure 3.1.

In this study, experimental male and female broodstock Nile tilapia (O. niloticus) were obtained from Suranaree University of Technology Farm (SUT Farm), Nakhon Ratchasima, Thailand. The breeding pond was the cement pond (5m * 10 m;

water depth 0.8 m) which was divided into 2 partitions (5m * 5 m; water depth 0.8 m) for HC/LP and LC/HP groups. To achieve good condition of natural breeding, six male (BW; 920 ± 16.7g) and twelve female (BW; 609.6 ± 28.4g) were transferred to each partition (3 males and 6 females) of pond according to the optimal stocking density and female to male ratio. Before the experimental trial, fish were acclimated to the experimental conditions for 4 days with commercial diet. Subsequently, the broodstock were fed with either HC/LP or LC/HP diet for 38 days. For feeding, fish were fed twice time daily (9.00 and 15.00) at 1.5% of their body weight. We did not continue culturing long-term since we observed the impact of dietary HC/LP on the number of fertilized eggs. A flow-through water change system was implemented by replacing one-third of the water in each tank twice time per week with dechlorinated water.

Since the egg size from broodstock fed HC/LP decreased with feeding time increased (Luo et al., 2025, unpublished date), this study used the fertilized eggs obtained from broodstock at 14 days after feeding to grow through juvenile stage. The healthy fertilized eggs from 6 females which were ovulated with the same egg stage (6 replications) were collected and transferred into hatching tray with circulating water. After hatching, the larvae at 7 days post-hatching (dph), were transferred to grow in cage (0.4m x 0.4m x 0.6m) which were located in cement pond (2m x 2m x 0.8 m; water depth 0.45 m) and fed with commercial diets (Table 3.1). To avoid confounding effects according to sex dimorphism in growth, fish fry was fed with commercial diet supplemented with 17 α-methyltestosterone (17-MT) at 60 mg/kg for five times daily (09.00; 11.00; 13.00; 15.00; 17.00) for 28 days (Boonanuntanasarn et al., 2018b). Subsequently, during weeks 5-21, fish were transferred to cement ponds (2 x 2 x 0.8 m^3) and fed with commercial feed (32% CP 4% CF) ad libitum twice daily (09.00 and 16.30) until week 17 (Figure 3.1). Fish mortality was daily monitored. To assess growth performance, fish were weighed, and feed intake was recorded every 4 weeks throughout the experimental period.

To explore how the offspring of HC-stimulated Nile tilapia broodstock respond to a HC diet in juvenile stage, two groups of experimental fish were challenged with HC/LP diet during weeks 18-21 (Figure 3.1). For challenging, one cage (0.6 m x 0.6 m x 0.9 m) was placed in each cement pond, twelve fish (BW: 52.08 ± 2.32) from each replicate were randomly selected and cultured in 12 cages (six cages

(replication)/treatment), then fed with HC/LP diet (3% body weight daily) for 4 weeks at 09:00 and 16:30 daily.

Throughout the experimental period, fish were grown in dechlorinated tap water under continuous aeration. A flow-through water exchange system replaced one-third of the water twice a week. Air and water temperatures were measured daily during the experimental period and ranged from 25-28°C and 28-32°C, respectively. Dissolved oxygen content and pH were measured weekly using a dissolved oxygen meter and pH meter. The values were found to be within acceptable ranges of 7.10 - 8.68 and 4.93 - 6.85 mg L−1, respectively.

3.3.3 Fish sampling

For juvenile offspring, at week 17 (before challenged with HC/LP diet) and week 21 (after challenged with HC/LP diet), twelve juvenile fish per condition (3 fish/ replication) were sampled at 5h after last meal and fish were euthanized using 0.2% clove oil. Blood samples were collected from caudal vein using hypodermic syringe, mixed with K2EDTA (at 1.5 mg mL−1 blood) as an anticoagulant, and then blood was centrifuged at 10 000xg for 5 min at 4°C to obtain plasma. The plasma was stored at −80°C until used for blood chemistry analysis. After fish bleeding, the liver was collected and weighed for calculation of hepatosomatic index (HSI). Then, the liver and epaxial muscle samples were collected and rapid frozen in liquid nitrogen and kept at −80°C for further used for nutritive composition determination and total RNA extraction for CHO and its related metabolism analysis.

3.3.4 Blood chemistry analysis

Determination of blood metabolites juvenile offspring (2 fish/ replication, n = 6 replication/broodstock diet history), including glucose, triglyceride (TAG), cholesterol, total protein, and blood urea nitrogen (BUN). Plasma glucose was quantitatively analyzed using Trinder's method (Barham and Trinder, 1972). Plasma TAG was determined using glycerol-3-phosphate oxidase-sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (GPO-ESPAS) method described by Bucolo and David (1973). Cholesterol was evaluated with using cholesterol oxidase phenol + aminophenazone (CHOD-PAP) technique described by Flegg (1973). Protein was analyzed with Biuret method (Gornall et al., 1949). BUN content was determined using a modified indophenol colorimetric method (Weatherburn, 1967).

3.3.5 Chemical composition, glycogen and triglycerides

Juvenile offspring liver and muscle (n = 6 fish/broodstock diet history) samples were determined the chemical composition including crude protein and crude fat according to AOAC (1990), glycogen and TAG.

The glycogen content was analyzed according to the method described by Kirchner et al., (2003) with modifications. Briefly, samples (200 mg) were homogenized in 1ml of 1M HCl, then an aliquot of homogenate was transferred to new tube. The homogenate was added 5M KOH to neutralization and subsequently centrifuged at 10 000xg at 4°C for10 minutes. The free glucose in supernatant was measured using a plasma glucose kit (Catalogue number: BLT00026, Erba Lachema s.r.o., Karásek Brno, Czechia), according to the manufacturer's instructions. Another aliquot of homogenate solution was then boiled at 100°C for 2.30 hours to hydrolyze glycogen. After boiling, the homogenates were cooled to room temperature and neutralized with 5M KOH. After centrifugation (10 000xg at 4°C for 10 minutes), total glucose content (free glucose and glucose that obtained from glycogen hydrolysis) in the supernatant was analyzed. The glycogen content was calculated as the amount of glucose after subtracting the total glucose with free glucose levels.

For the TAG content, samples (100 mg) were homogenized with 1ml of 5% IGEPAL in deionized water containing 2.8-mm glass beads using Bioprep-24 homogenizer. Samples were heated in water bath at 90°C for 10 minutes and then cooled down to room temperature. Then, samples were centrifuged at 10 000xg at 4°C for10 minutes, and the supernatant was collected to new tube and diluted with deionized water. TAG was measure by using triglyceride kit (Catalogue number: BLT00059, Erba Lachema s.r.o., Karásek Brno, Czechia), according to the manufacturer's instructions. Determination of blood metabolites juvenile offspring (2 fish/ replication, n = 6 replication/broodstock diet history), including glucose, triglyceride (TAG), cholesterol, total protein, and blood urea nitrogen (BUN). Plasma glucose was quantitatively analyzed using Trinder's method (Barham and Trinder, 1972). Plasma TAG was determined using glycerol-3-phosphate oxidase-sodium N-ethyl-N-(3-sulfopropyl) m-anisidine (GPO-ESPAS) method described by Bucolo and David (1973). Cholesterol was evaluated with using cholesterol oxidase phenol + aminophenazone (CHOD-PAP) technique described by Flegg (1973). Protein was analyzed with Biuret method (Gornall

et al., 1949). BUN content was determined using a modified indophenol colorimetric method (Weatherburn, 1967).

3.3.6 Total RNA extraction, cDNA synthesis, and real-time RT-PCR analysis of gene involved in glucose metabolism

Quantitative real-time reverse-transcription polymerase chain reaction (real-time RT-qPCR) was performed to determine the expression of genes related to CHO and its related metabolism. Total RNA was extracted from the liver (50 mg) and muscle (100 mg) of juvenile offspring samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The quantity of total RNA was measured using a NanoDrop spectrophotometer (Thermo Fisher, Madison, WI, USA). The total RNA was also verified using 1% agarose gel electrophoresis. Using the SuperScript III RNAseH-Reverse transcriptase kit (Invitrogen) with random primers (Promega, Charbonniéres, France), cDNA synthesis (duplicate for each sample) was performed from 1 µg of total RNA following the manufacturer's protocol.

For glucose and its related metabolism, at molecular level, we analyzed modulation of hepatic glycolysis (glucokinase (*gck*), phosphofructokinase (*pfklr*), pyruvate kinase (*pklr*)), hepatic gluconeogenesis (glucose-6-phosphatase (*g6pca1* and *g6pca2*), phosphoenolpyruvate carboxykinase cytosolic (*pck1*) and phosphoenolpyruvate carboxykinase (*pck2*), lipogenesis (fatty acid synthase (*fasn*) and glucose-6-phosphate dehydrogenase (*g6pd*)) amino acid catabolism in liver (glutamate dehydrogenase (*gdh*), alanine aminotransferase (*alat*) and aspartate amino transferase (*asat*), glucose utilization (glucose transporter; *glut4*), and muscular glycolysis (hexokinase I/II, (*hk1* and *hk2*); phosphofructokinase, (*pfk*ma and *pfkmb*); pyruvate kinase (*pkma*).

For epigenetic modification-related genes expressed in liver and muscle tissues. These include DNA methyltransferases (*dnmt1a*, *dnmt3aa*, *dnmt3ab*, *dnmt3ba*, *dnmt3bb*), TET methyl cytosine dioxygenases (*tet1*, *tet2*, *tet3*), histone (H) 3 lysine (K) 4 trimethylation (H3K4me3) writers (SET domain-containing 1A/1B [*setd1a*, *setd1ba*]; lysine methyltransferase 2A [*kmt2a*]; histone-lysine N-methyltransferase 2B [*kmt2ba*, *kmt2bb*]), H3K4me3 erasers (lysine demethylase 5A/5BA/5BB/5C [*kdm5a*, *kdm5ba*, *kdm5bb*, *kdm5c*]; bifunctional lysine-specific demethylase and histidyl hydroxylase [*riox1*]), H3K9me3 writers (histone lysine N-methyltransferase [*suv39h1b*]) and erasers

(lysine demethylase 4AA/4AB/4B/4C [*kdm4aa*, *kdm4ab*, *kdm4b*, *kdm4c*]), H3K36me3 writers (SET domain-containing 2 [*setd2*]), H3K9ac writers (lysine acetyltransferase 2A/2B/6A [*kat2a*, *kat2b*, *kat6a*]; general transcription factor IIIC subunit 4 [*gtf3c4*]), and erasers (sirtuin 2/5/6 [*sirt2*, *sirt5*, *sirt6*]).

The primer sequences used for real-time RT-qPCR are listed in Table 3.7 and Table 3.8. To analysis mRNA levels, RT-qPCR of target gene expression was performed using the Roche Applied Science E-Method according to Pfaffl (2001). Since the mRNA of ef1α of all conditions and tissues did not differ, its transcript level was used for the normalization of measured mRNA of each gene (data not shown). In all cases, PCR efficiency was measured from the slope of a standard curve using serial dilutions of cDNA and PCR efficiency values ranged between 1.8 and 2.0.

3.3.7 Statistical analysis

The statistical model utilized was was $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where y_{ij} was the response, μ was the general means, α_i was dietary (LC/HP and HC/LP) effects and ε_{ij} was the random error. All data were analyzed using SPSS for Windows version 22 (SPSS Inc., Chicago, IL, USA). An independent sample t-test was performed to analyze the differences between the LC/HP and HC/LP groups. The effects and differences were considered significant at $p<0.05$.

3.4 Results

3.4.1 Effects of dietary HC in broodstock growth performance in juvenile offspring

The growth performance of offspring from broodstock stimulated with LC/HP and HC/LP diets showed in Table 3.2. Through juvenile stage (week 17), there were no significant differences in growth performances in offspring obtained from HC/LP and LC/HP broodstock ($p>0.05$). In addition, when juvenile offspring were challenged with HC, the growth performances of offspring from HC/LP and LC/HP broodstock appeared to be similar ($p>0.05$) (Table 3.2).

3.4.2 Effects of dietary HC in broodstock on intermediary glucose and its related metabolism in juvenile offspring

The plasma metabolites and nutrients composition of liver, muscle and

whole-body in 17-weeks offspring were showed in Table 3.3. There was observed that no significant difference in plasma metabolites between offspring from LC/HP and HC/LP broodstock (p >0.05). In addition, dietary HC in broodstock showed significantly increased the hepatosomatic index (HSI) and hepatic triglyceride in juvenile offspring (p <0.05) (Table 3.3).

The responses of genes expression related to intermediary carbohydrate metabolism in liver and muscle of juvenile offspring from LC/HP and HC/LP broodstock showed in Table 3.4. Although the juvenile offspring of the two groups were fed the same diet to 17 weeks, broodstock stimulated by HC/LP diet was showed down-regulation of hepatic gluconeogenesis (*g6pca1*) and amino acid catabolism (*alat*) in their offspring (P <0.05), meanwhile, the mRNA expression of muscular glycolysis (*pfkma* and *pfkmb*) were up-regulation (P <0.05).

3.4.3 Effects of dietary HC in broodstock on intermediary glucose and its related metabolism in juvenile offspring when they were challenged with HC diet

After juvenile offspring fish was challenged with HC/LP diet for 4 weeks (week 18-21), the plasma metabolites and proximate compositions of liver, muscle and whole body in offspring were detected. Table 3.3 showed that plasma glucose and triglyceride of juvenile offspring from HC/LP-broodstock were significantly higher than offspring of LC/HP-broodstock (p <0.05), while plasma total protein was decreased in offspring of HC/LP-broodstock (p <0.05). No significant differences in cholesterol and BUN levels were found between the two experimental groups (P >0.05). In addition, the proximate composition of hepatosomatic index (HSI) and hepatic fat, glycogen and triglyceride were significantly increased in juvenile offspring of HC/LP-broodstock (p <0.05) (Table 3.3). Also, muscular fat, glycogen and triglyceride were higher in juvenile offspring of HC/LP-broodstock than that offspring of LC/HP-broodstock (p <0.05) (Table 3.3).

At molecular level, parental dietary HC diet also impact on responds of intermediary glucose and its related metabolism in juvenile offspring when they were challenged with HC diet. Table 3.4 showed that upregulation of genes related to hepatic glycolysis (*gck* and *pklr*) and lipogenesis (*fasn* and *g6pd*) while downregulation of genes related to hepatic gluconeogenesis (*g6pca1* and *pck2*) and amino acid catabolism (*asat* and *alat*) were detected in offspring derived from HC/LP-broodstock

when compared with offspring of LC/HP-broodstock (P <0.05). In addition, there were showed significant upregulation of gene related to muscular glucose transport (*glut4*) and glycolysis (*hk1*, *hk2*, *pfkma* and *pfkmb*) in offspring of HC/LP-broodstock when compared with offspring of LC/HP-broodstock (P <0.05) (Table 3.4).

3.4.4 Effects of the HC diet in broodstock on epigenetic modifications in juvenile offspring after the HC/LP diet challenge

Enzymes related to epigenetic modifications, including DNA methylation writers and erasers, were determined at the molecular level in the liver and muscle of juvenile offspring after the HC/LP diet challenge (Table 3.5). Compared with offspring from the LC/HP-fed broodstock, the parental HC/LP feeding history led to the upregulation of hepatic DNA methylation writers (*dnmt1a, dnmt3aa*) and erasers (*tet1, tet3*) (P < 0.05; Table 3.5). Additionally, the downregulation of muscular *dnmt3ab, dnmt3ba* and *tet3* were observed in fish derived from the HC/LP-fed broodstock (P < 0.05; Table 3.5). However, hepatic *dnmt3bb* and *tet2*, muscular *dnmt1a*, *dnmt3aa*, *dnmt3bb*, *tet1* and *tet2* levels were not significantly different between the two experimental groups (P > 0.05; Table 3.5).

Table 3.6 demonstrates the mRNA expression levels of genes encoding enzymes related to histone (H)3 lysine (K) 4 trimethylation (H3K4me3), H3K9me3, H3K36me3, and histone 3 lysine 9 acetylation (H3K9ac) in the livers and muscles of juvenile offspring after HC/LP-diet challenge. Compared with offspring from LC/HP-fed broodstock, parental HC/LP feeding history modulated the expression of the following genes in the liver: 1) H3K4me3 writers: induction of *setd1a* and *kmt2bb*; 2) H3K4me3 erasers: induction of *kdm5a*, *kdm5ba* and *riox1*; 3) H3K9me3 writer: suppression of *suv39h1b*; 4) H3K9me3 erasers: induction of *kdm4aa*, *kdm4ab*, and *kdm4c*; 5) H3K9ac writers: induction of *kat2a*; and 6) H3K9ac erasers: induction of *sirt5* and *sirt6* (P < 0.05; Table 3.6). In muscle tissue, parental HC/LP feeding history resulted in: 1) downregulation of *kmt2ba*, *kmt2bb*, *kdm5bb*, *kdm4aa*, *kdm4bb*, *kat6a*, *sirt5* and *sirt6*; and 2) upregulation of *kdm5a* (P < 0.05; Table 3.6). Notably, expression of the hepatic and muscular H3K36me3 writer (setd2) was not significantly different between the two experimental groups (P > 0.05; Table 3.6).

3.5 Discussion

Nile tilapia is known as a good user of dietary CHO, and dietary CHO could provide protein sparing effects (Boonanuntanasarn et al., 2018a, b). In addition, NP concept was applied in Nile tilapia to improve the efficient utilization of dietary CHO using various stimulus methods. For example, not only glucose injection into yolk sac of larvae but also early feeding with HC diet in fry stage were able to improve growth performance and modulate the intermediary CHO metabolism at later in juvenile and adult fish when they were challenged with HC feed (Kumkhong et al., 2021, 2020a, Kumkhong et al., 2020b; Srisakultiew et al., 2022). In mammals, nutritional intervention in parents could be a tool for achieving NP for tailoring metabolism in their offspring, and epigenetics including DNA methylation and histone modification were involved in metabolic changes (Riddle and Hu, 2021). In previous study, Nile tilapia broodstock exhibited several responses to HC diet such as induction of glycolysis and suppression of gluconeogenesis, and these responses were also detected in their offspring during the larvae and fry stages (Luo et al., submitted). In this study, we demonstrated the persisting effects of parental HC stimulus diet on intermediary CHO metabolism in juvenile fish. Particularly, modulation of intermediary CHO metabolism was more pronounced when fish were subjected to a challenging HC diet although no significant effects on growth performance. These indicated that dietary CHO stimulation in broodstock may serve as an effective approach for NP concept in Nile tilapia. In addition, we presented that enzymes related epigenetics modification at molecular level according to HC stimulus in broodstock would be involved with modulation of CHO metabolism in offspring in long-term, demonstrating their regulation of NP of hyperglucidic stimulus in broodstock.

3.5.1 Effects of dietary HC stimulus in broodstock on growth performance in juvenile offspring

In Nile tilapia, the NP of CHO increases CHO utilization during the juvenile stages. Early feeding of fry with an HC diet for 1–4 weeks improved the growth performance of juvenile fish when they were later challenged with an HC diet (Kumkhong et al. 2020b; Srisakultiew et al. 2022). In addition, glucose injection into yolk reserve larvae improved the growth performance in juvenile stage when challenged HC diet (Kumkhong et al., 2020a). However, in this study, with the present of

compensatory growth at week 17, the growth performance of offspring from HC/LP and LC/HP broodstock were not significant differences when fish were subjected to challenged HC/LP feeding. Previous studies showed that NP of dietary CHO stimulus in rainbow trout broodstock for long period influenced growth performance. Comparing with non-CHO fed broodstock, the male broodstock fed HC diet for 6 months resulted in higher body weight in 24-weeks juvenile offspring fed with commercial diet while juvenile offspring of broodstock fed HC diet for 1 year showed no difference growth performance when challenged with HC diet (Callet et al., 2021; 2022). Combined, the programming benefits of CHO intervention for Nile tilapia would depend on the methods of stimulus (glucose injection in larvae, early feeding in fry and broodstock feeding).

3.5.2 Effects of dietary HC stimulus in broodstock on intermediary glucose and its related metabolism in offspring persisted to juvenile stage

Nutritional stimulus of CHO at early stage in Nile tilapia via direct glucose injection in larvae and early feeding in fry stages revealed modulation of intermediary CHO metabolism at later life up to juvenile and adulthood including elevation of hepatic fat, triglyceride and glycogen as well as glycogen in muscle (Kumkhong et al., 2020b, 2020a; Srisakultiew et al., 2022). Our previous studies showed that dietary stimulus of Nile tilapia broodstock with HC for 2 weeks exhibited intermediary responses of CHO in offspring, i.e., increase in crude fat in 7-dph larvae and elevation of glycogen and triglyceride contents in both 7-dph larvae and 7-daf fry (Luo et al., submitted). In this study, we also found the persisting of impact of dietary CHO stimulus during broodstock in juvenile fish. Higher HSI and triglyceride in liver was observed in juvenile offspring of HC/LP broodstock when compared with offspring from LC/HP broodstock although there were no significant differences in blood metabolites in the experimental fish. In rainbow trout, broodstock fed with HC diet for 1 year resulted in lower plasma glucose and increased plasma cholesterol in juvenile offspring as well as decreased glycogen and glucose in the liver (Callet et al., 2021). Combined, nutritional stimulus of HC in broodstock could impact intermediary CHO metabolism in offspring, and the impact persisted long-term through juvenile stage although different effects in offspring might be related to fish species/habits and method of nutrient stimulation.

The effects of NP of early hyperglucidic stimulus on intermediary CHO metabolism, particularly, for glucose and its related metabolism at molecular level

were demonstrated at later development in juvenile and adult in Nile tilapia (Kumkhong et al., 2020b, 2020a; Srisakultiew et al., 2022). In previous study, NP by HC/LP feeding in broodstock demonstrated induction of glycolysis and glucose transport, while suppression of glucogenic pathway and amino acid catabolism in larvae and fry (Luo et al., submited). This study presented that the effects of parental HC feeding on glucose and its related metabolism persisted through juvenile stage. Upregulation of genes related to muscular glycolytic pathway (*pfkma* and *pfkmb*) and downregulation of genes related to hepatic gluconeogenic pathway (g6pca1) and amino acid catabolic pathway (*alat*) were observed in juvenile offspring from HC/LP broodstock, compared to that of LC/HP. Similarly, long-term effects of CHO stimulus in broodstock were presented in rainbow trout. High-CHO feeding stimulus in broodstock for 1 year led to downregulate genes related to glucose transport (*glut6*) in juvenile offspring when compared with offspring of non-CHO broodstock (Callet et al., 2021). Therefore, these findings suggested that feeding broodstock with HC could be provided as a tool for nutritional stimulus and programming, and their effects persisted up to juvenile stage.

3.5.3 Effects of dietary HC stimulus in broodstock on intermediary glucose and its related metabolism in juvenile offspring after challenged HC/LP diet

For NP concept, nutritional intervention of HC in early life was proposed to persistently influence intermediary glucose and its related metabolism, and particularly, its effects were expected to be greater when fish uptake HC containing diet later in development. In our study, with the persisting effects (increased HSI and hepatic triglyceride) of HC stimulus in broodstock, greater modulatory effects were observed when the fish were challenged with HC/LP, i.e. 1) increased the levels of blood glucose and triglyceride while decreased total protein, 2) elevated HSI in liver, and 3) increased hepatic and muscular fat, glycogen and triglyceride contents. Indeed, NP concept of CHO was revealed in Nile tilapia via hyperglucidic stimulus in larvae and fry stage. For instance, the effect of glucose injection into yolk reserve in larvae stage persisted up to juvenile (increased hepatic fat and muscular glycogen) and adult (elevated muscular glycogen) fish. When fish were challenged with HC diet in juvenile and adult stages, blood glucose (only juvenile) and HSI (only adult) were also increased (Kumkhong et al., 2021, 2020a). Early HC feeding in fry for 1 or 3 weeks led to increased hepatic fat and triglyceride in juvenile, and challenging HC diet induced elevation of blood glucose and triglyceride, glycogen in liver

and muscular fat, glycogen and triglyceride (Srisakultiew et al., 2022). Longer HC feeding in fry for 4 weeks increased hepatic fat and glycogen in both liver and muscle of adult fish. Similar effects were observed although fish were challenged with HC diet (Kumkhong et al., 2020b). Therefore, nutritional intervention of HC feeding in broodstock revealed similar NP effects although not totally the same to that during larvae and fry stages. However, the NP effects on CHO intervention in other fish varied. In rainbow trout, the effects of HC feeding in broodstock for 1 year led to decrease plasma and hepatic glucose and glycogen in liver but increase cholesterol in plasma in juvenile offspring. Nonetheless, when juvenile fish were challenged with HC/LP diet for 3 months, only whole-body lipid content increased (Callet et al., 2021). HC stimulus in zebrafish broodstock decreased blood glucose in adult offspring when they were challenged with HC diet for 1 week (Lu et al., 2022). Overall, NP of dietary CHO stimulus in broodstock could be achieved in Nile tilapia, and the impact were in similar trend of hyperglucidic stimulus during larvae and fry stages. Note that the impact of NP of hyperglucidic stimulus varied among fish species.

Coincidentally, at molecular level, the effects of nutritional intervention of HC in broodstock on CHO and its related metabolism in juvenile offspring were strengthened when fish were challenged with HC diet. In zebrafish, the offspring of broodstock fed HC diet showed suppression hepatic gluconeogenic (*fbp1a*, *fbp1b* and *pck1*) when adult fish were challenged HC feed (Lu et al., 2022). In addition, yellow catfish broodstock fed with HC diet resulted in upregulation of glycolytic genes (*gk*, *pk* and *pfk*) and sglt1 (glucose transport) in whole-body of offspring after challenging with HC diet (Xu et al., 2024). Juvenile offspring of high CHO stimulus in rainbow trout parents reinforced the hepatic cholesterol biosynthesis (*Mvdaa* and *dhcr7a*) while decelerated lipid metabolism (*acsf2a*) when fish were subjected to challenge with HC diet (Callet et al., 2022). Our results demonstrated that the persisted effects of modulation of CHO and its related metabolisms according to their parental stimulus by hyperglucidic condition were strengthened when offspring fish were challenged including 1) induction of hepatic and muscular glycolysis (liver; *gck* and *pfklr*, muscle; *hk1*, *hk2*, *pfkma* and *pfkmb*), 2) induction of muscular glucose transport (glut4), 3) induction of hepatic lipogenesis (*fasn* and *g6pdh*), 4) suppression of hepatic gluconeogenesis (*g6pca2* and *pck2*), and 5) suppression of amino acid catabolism (*asat* and *alat*). These modulatory effects according to NP at later developmental stage,

particularly, when fish were challenged with HC diet again. For example, glucose injection into yolk reserve during larval stage leading to induction of hepatic and muscular glycolysis (*gck*, *hk1* and *hk2*) in juvenile and muscular glycolysis (*pfkma*) in adults were found. Subsequently, when these fish were challenged with HC diet, there were more modulation of glucose metabolism including induction hepatic glycolysis (juvenile; *pkfr*, adult; *gck*), elevation of muscular glucose transport (only juvenile *glut4*) and glycolysis (only adult *hk2*), suppression of hepatic gluconeogenesis (juvenile; *pck1*, adult; *g6*pca1) and reduction of amino acid catabolism (only adult *asat*) (Kumkhong et al., 2021, 2020a). Similar trend of NP effects by early HC feeding in Nile tilapia fry were demonstrated with challenged with HC diet in juvenile and adult Nile tilapia (Kumkhong et al., 2020b; Srisakultiew et al., 2022). Therefore, nutritional intervention of HC in broodstock could be a method of effective stimulus which showed long-term impacts of NP of CHO in offspring, particularly, when fish intake HC diet. Future investigation was suggested to determine whether this effect would sustain in adult Nile tilapia.

3.5.4 Effects of dietary HC stimulus in broodstock on epigenetic modifications in juvenile offspring

Nutrition intervention by dietary CHO in broodstock could have impact on metabolic programming in their offspring along larvae to juvenile stage, and epigenetics modification might be involved. It was demonstrated that NP of parents during critical windows such as gametogenesis, fetal growth, and the postpartum period are related in epigenetic modifications including DNA methylation and histone modification (Gabory, 2011). Two main enzyme families including DNMT and TET enzymes were essential for the dynamic regulation of DNA methylation (Horii and Hatada, 2016). The expressions of *dnmt1*, *dnmt3a*, *dnmt3b* and *dnmt31* in oocytes of diabetic mice were significantly lower than those of the control group (Ge et al., 2013). The expression of *tet3* gene in oocytes of hyperglycemic mice was significantly lower compared to normal mice (Wu et al., 2022). In this study, HC stimulus in broodstock induced upregulation of hepatic DNA methylation writers (*dnmt1a* and *dnmt3aa*) and erasers (*tet1* and *tet3*) in juvenile offspring when they were challenged with HC/LP diet. In addition, dietary HC in broodstock downregulated *dnmt3ab*, *dnmt3ba* and *tet3* in muscle of juvenile offspring. Again, the epigenetic mechanism might involve in NP effects of dietary CHO stimulus in broodstock. Similarly, dietary HC in rainbow trout broodstock for one year resulted in overall DNA

hypomethylation in the offspring at fry stage and significantly lower expression of *dnmt3bba2* and *dnmt3bbb* (Callet et al., 2021). Overall, modified broodstock diet could impact modification of enzymes related to DNA methylation in offspring.

This study also investigated the enzymes at molecular level that are involved in histone modifications. Our results showed that HC stimulus in Nile tilapia broodstock modulated the expression of enzymes related to histone (de)methylation and (de)acetylation in juvenile offspring after challenged with dietary HC. Parental HC stimulation modulated: 1) H3K4me3 writers (induction: *setd1a* and *kmt2bb*) and erasers (induction: *kdm5a*, kdm*5ba*, and *riox1*); 2) H3K9me3 writers (suppression: *suv39h1b*) and erasers (induction: *kdm4aa*, *kdm4ab*, and *kdm4c*); and 3) H3K9ac writers (induction: *kat2a*) and erasers (induction: *sirt5* and *sirt*6) in the liver. In the muscle tissue, parental HC feeding suppressed the expression of *kmt2ba*, *kmt2bb*, *kdm5bb*, *kdm4aa*, *kdm4bb*, *kat6a*, *sirt5* and *sirt6*, while inducing *kdm5a*. These findings suggested that histone modifications could modulate by NP effects of dietary HC in broodstock. Similarly, in mammals, parental hyperglucidic condition also induced changes in enzymes associated with histone modification in their offspring. Sixteen weeks of sucralose and stevia supplementation to mouse parental diet led to increase mRNA levels of histone deacetylase 3 (*Hdac3*) in the gut of their offspring (Concha Celume et al., 2024). Overall, epigenetic modifications could be a factor regulating NP existing effects in offspring.

3.6 Conclusions

In conclusion, HC stimulus in broodstock had long-term effect on glucose and its related metabolism including increased HSI and triglyceride content in liver, induced hepatic gluconeogenesis and muscular glycolysis in juvenile offspring. In addition, parental HC stimulus history showed greater effects when offspring fish were challenging the HC/LP diet such as induction hepatic glycolysis and lipogenesis while suppression gluconeogenesis and amino acid catabolism, as well as induction muscular glucose transport and glycolysis, demonstrating NP effects of parental HC diet. The modulation of epigenetic modifications including mRNA level of writer and erase enzymes related to DNA methylation and histone modifications, indicating that epigenetics were involved in NP of dietary CHO in parental Nile tilapia.

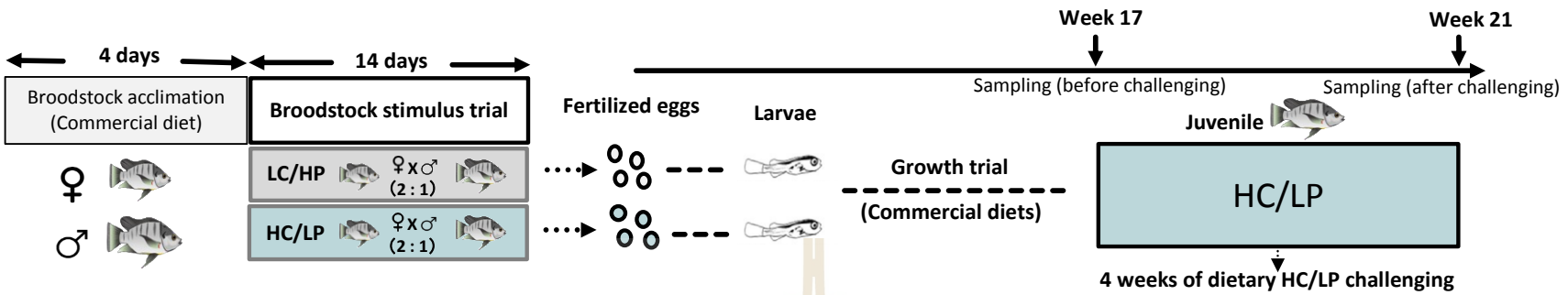

Figure 3.1 Experimental plan for nutritional programming involving dietary high-carbohydrate (HC) stimuli in broodstock and their long-term impacts on carbohydrate (CHO) metabolism in offspring. Mature male and female broodstock were acclimated to a communal breeding pond and fed a commercial diet (36% crude protein [CP], 3.4% crude fat [CF]) for 4 days. Subsequently, broodstock were fed low-carbohydrate/high-protein (LC/HP) or high-carbohydrate/low-protein (HC/LP) diets for 14 days. Fertilized eggs were collected from the mouths of females after the feeding period and cultured. The offspring were fed commercial diets (weeks 1–7: 40% CP, 8% CF; weeks 8–17: 32% CP, 4% CF) until juvenile stage (week 17). During weeks 18–21, juvenile fish were challenged with HC/LP diets and sampled to assess intermediary CHO metabolism and the epigenetic modifications.

Table 3.1 Ingredients and chemical composition (g kg−1) of the commercial diets (diets during nursing) and for stimulus diets.

Ingredients	Commercial diet			Stimulus diets		Challenge diets
	Broodstock acclimation)	Offspring nursing	Offspring growth trial	LC/HP	HC/LP	HC/LP
Fish meal	-	-	-	88	18	18
Rice flour	-	-	-	0	70	70
Fish oil	-	-	-	0	7	7
Soybean oil	-	-	-	2	0	0
Gelatin	-	-	-	8	0	0
Di-calcium phosphate	-	-	-	0	3	3
Fish premix[a]	-	-	-	2	2	2
Proximate composition (% from wet weight)						
Dry matter	91	89	89	94.2	90.2	90.2
Protein	36	40	32	57.3	15.3	15.3
Fat	3.4	8	3.9	9.2	9	9
Fiber	7.1	4.2	7.3	0.5	0.4	0.4
Ash	12.6	12.2	12.2	22.8	8.6	8.6
NFE[b]	32.5	24.3	34	4.5	56.8	56.8
Gross energy (kJ g^{-1})	13.13	14.42	12.82	14.40	15.6	15.6

[a]Vitamin and trace mineral mix provided the following (IU kg^{-1} or g kg^{-1} diet): biotin, 0.25 g; folic acid, 0.003 g; inositol, 0.25 mg; niacin, 0.0215 g; pantothenic acid, 0.03 g; vitamin A, 5,000 IU; vitamin B1, 0.0025 g; vitamin B2, 0.0012 g; vitamin B6, 0.0075 g; vitamin B12, 0.00005 mg; vitamin C, 1 g; vitamin D3, 1,000 IU; vitamin E, 100 IU; vitamin K, 0.008 g; copper, 0.02 g; iron, 0.2 g; selenium, 0.3 mg; zinc, 0.32 g.

[b]Nitrogen-free extract = dry matter − (CP + crude lipid + crude fiber + ash).

Table 3.2 Growth performances of juvenile offspring from Nile tilapia broodstock fed with low-carbohydrate/high-protein (LC/HP) diet and high-carbohydrate/low-protein (HC/LP) diet (mean ± SD, n = 6)[1].

Offspring period	Broodstock stimulus diets	Final weight (g)	Weight gain (g)	ADG[2] (g day^{-1})	SGR[3] (% day^{-1})	FCR[4]
Week 17 (before challenge)	LC/HP	51.25 ± 2.80	42.81 ± 5.33	0.36 ± 0.04	7.17 ± 0.05	0.98 ± 0.11
	HC/LP	52.92 ± 1.54	42.73 ± 1.43	0.36 ± 0.01	7.19 ± 0.02	0.97 ± 0.03
	P value	0.230	0.972	0.934	0.397	0.838
Week 21 (after challenge HC/LP diet)	LC/HP	83.54 ± 10.92	75.10 ± 13.96	0.51 ± 0.09	6.13 ± 0.09	1.38 ± 0.25
	HC/LP	85.72 ± 8.97	75.54 ± 8.98	0.51 ± 0.06	6.15 ± 0.07	1.35 ± 0.15
	P value	0.713	0.950	0.944	0.783	0.819

[1] An independent t-test analysis was used to analyse the effects of different stimulus between HP/LC and LP/HC diets.

[2] Average daily gain (ADG) = (final body weight – initial body weight)/experimental days.

[3] Specific growth rates (SGR) = 100 × [(ln final body weight – ln initial body weight) / experimental days].

[4] Feed conversion ratio (FCR) = dry feed fed / wet weight gain

Table 3.3 Plasma metabolites and proximate composition of liver, muscle and whole body in juvenile offspring before (week 17) and after (week 21) challenging with HC/LP diet for 4weeks. Juvenile offspring were obtained from broodstock fed with low-carbohydrate/high-protein (LC/HP) diet and high-carbohydrate/low-protein (HC/LP) diets (mean ± SD, n = 6)[1].

	Week 17 before challenge			Week 21 after challenge		
Broodstock diets	**LC/HP**	**HC/LP**	**P value**	**LC/HP**	**HC/LP**	**P**
Plasma metabolites						
Glucose (mM)	4.04 ± 0.45	4.12 ± 0.31	0.659	4.82 ± 0.32	5.35 ± 0.37	<0.001
Triglyceride (mM)	1.04 ± 0.33	1.09 ± 0.23	0.620	1.30 ± 0.26	2.20 ± 0.36	<0.001
BUN (mM)	0.53 ± 0.26	0.41 ± 0.28	0.265	1.18 ± 0.69	0.93 ± 0.54	0.435
Cholesterol (mM)	0.94 ± 0.17	0.99 ± 0.08	0.513	1.53 ± 0.25	1.65 ± 0.29	0.281
Total protein (g/L)	29.93 ± 2.02	28.42 ± 2.5	0.231	35.42 ± 2.07	32.67 ± 1.58	0.008
Liver (%)						
Protein	8.98 ± 0.73	8.64 ± 0.53	0.379	7.39 ± 0.42	6.86 ± 0.46	0.065
Fat	2.82 ± 0.33	3.01 ± 0.26	0.283	4.08 ± 0.39	5.18 ± 0.37	<0.001
Ash	0.82 ± 0.06	0.88 ± 0.05	0.124	0.86 ± 0.11	0.92 ± 0.07	0.331
Glycogen (mg/g)	31.45 ± 3.83	33.92 ± 2.54	0.257	43.03 ± 6.01	54.18 ± 7.58	0.028
Triglyceride (mg/g)	11.32 ± 2.32	17.68 ± 2.88	0.003	23.87 ± 1.85	27.47 ± 1.28	0.005
HSI[2]	1.33 ± 0.12	1.75 ± 0.17	0.001	3.18 ± 0.31	3.91 ± 0.33	0.003
Muscle (%)						
Protein	16.98 ± 0.45	17.38 ± 0.63	0.241	18.1 ± 0.67	17.61 ± 0.41	0.153
Fat	1.14 ± 0.25	1.19 ± 0.21	0.724	1.62 ± 0.14	1.92 ± 0.21	0.015
Ash	1.17 ± 0.10	1.11 ± 0.10	0.267	1.28 ± 0.07	1.28 ± 0.06	0.964
Glycogen (mg/g)	3.98 ± 0.27	4.06 ± 0.16	0.551	4.97 ± 0.49	6.55 ± 0.45	<0.001
Triglyceride (mg/g)	3.02 ± 0.28	3.12 ± 0.46	0.695	4.68 ± 0.23	5.31 ± 0.37	0.009
Whole body (%)						
Protein	11.17 ± 0.36	11.16 ± 0.43	0.951	12.2 ± 1.41	12.88 ± 0.47	0.300
Fat	3.13 ± 0.25	3.28 ± 0.19	0.288	6.68 ± 0.65	6.95 ± 0.58	0.467
Ash	3.16 ± 0.34	3.08 ± 0.35	0.678	3.68 ± 0.83	3.56 ± 0.54	0.776

[1] An independent t-test analysis was used to analyse the effects of different stimulus between HP/LC and LP/HC diets.

[2] Hepatosomatic index (HSI) = liver weight / fish body weight.

Table 3.4 mRNA levels of genes related to carbohydrate and its related metabolism in the liver and muscle of juvenile offspring before (week 17) and after (week 21) challenging with HC/LP diet for 4weeks. Juvenile offspring were obtained from broodstock fed with low-carbohydrate/high-protein (LC/HP) diet and high-carbohydrate/low-protein (HC/LP) diets (mean ± SD, n = 6)[1].

	Week 17 before challenge			Week 21 after challenge		
Broodstock	**LC/HP**	**HC/LP**	***P* value**	**LC/HP**	**HC/LP**	***P* value**
Liver						
Glycolysis						
gck	1.47 ± 0.33	1.61 ± 0.41	0.512	1.53 ± 0.13	1.95 ± 0.29	0.009
Pfklr	0.86 ± 0.20	1.05 ± 0.22	0.156	0.89 ± 0.12	1.08 ± 0.18	0.051
pklr	0.74 ± 0.34	0.89 ± 0.05	0.312	0.48 ± 0.19	1.27 ± 0.27	<0.001
Gluconeogenesis						
g6pca1	0.61 ± 0.05	0.22 ± 0.08	<0.001	0.58 ± 0.06	0.63 ± 0.07	0.175
g6pca2	1.05 ± 0.17	0.89 ± 0.19	0.143	0.60 ± 0.07	0.43 ± 0.04	0.001
pck1	1.14 ± 0.50	0.87 ± 0.20	0.266	0.55 ± 0.12	0.46 ± 0.03	0.135
pck2	0.61 ± 0.17	0.48 ± 0.14	0.188	0.66 ± 0.17	0.45 ± 0.11	0.032
Lipogenesis						
fasn	0.72 ± 0.39	1.05 ± 0.28	0.129	0.55 ± 0.21	0.93 ± 0.13	0.004
g6pd	0.52 ± 0.07	0.71 ± 0.25	0.126	0.52 ± 0.15	1.15 ± 0.27	0.001
Amino acid catabolism						
asat	1.09 ± 0.41	0.78 ± 0.26	0.151	0.83 ± 0.07	0.65 ± 0.11	0.005
alat	1.14 ± 0.27	0.74 ± 0.31	0.041	1.30 ± 0.29	0.53 ± 0.14	<0.001
gdh	0.96 ± 0.23	0.88 ± 0.12	0.154	0.75 ± 0.28	0.53 ± 0.11	0.126
Muscle						
Glucose transport and glycolysis						
glut4	1.01 ± 0.11	1.11 ± 0.18	0.254	0.85 ± 0.12	1.00 ± 0.07	0.029
hk1	1.07 ± 0.10	1.01 ± 0.37	0.732	0.87 ± 0.15	1.05 ± 0.10	0.031
hk2	0.91 ± 0.19	0.94 ± 0.25	0.839	0.65 ± 0.06	0.99 ± 0.15	0.001
pfkma	0.88 ± 0.15	1.27 ± 0.26	0.009	0.80 ± 0.18	1.57 ± 0.23	<0.001
pfkmb	0.70 ± 0.13	1.25 ± 0.15	<0.001	0.87 ± 0.10	1.04 ± 0.12	0.022
pkma	1.50 ± 0.16	1.97 ± 0.69	0.159	1.16 ± 0.28	1.36 ± 0.27	0.231

[1]An independent t-test analysis was used to analyse the effects of different stimulus between HP/LC and LP/HC diets.

Table 3.5 mRNA levels of genes related to DNA methylation in the liver and muscle of juvenile offspring before (week 17) and after (week 21) challenging with HC/LP diet for 4weeks. Juvenile offspring were obtained from broodstock fed with low-carbohydrate/high-protein (LC/HP) diet and high-carbohydrate/low-protein (HC/LP) diets (mean ± SD, n = 6)[1].

	Liver			Muscle		
Genes	**LC/HP**	**HC/LP**	**P value**	**LC/HP**	**HC/LP**	**P value**
DNA methylation writers						
dnmt1a	0.94 ± 0.28	1.66 ± 0.44	0.007	1.52 ± 0.44	1.44 ± 0.27	0.702
dnmt3aa	0.60 ± 0.11	1.65 ± 0.59	0.007	1.28 ± 0.11	1.21 ± 0.09	0.281
dnmt3ab	1.17 ± 0.49	1.36 ± 0.48	0.522	2.99 ± 0.98	1.12 ± 0.16	0.005
dnmt3ba	-	-	-	3.15 ± 1.23	0.73 ± 0.14	0.005
dnmt3bb	1.17 ± 0.35	1.51 ± 0.45	0.168	1.54 ± 0.65	1.16 ± 0.32	0.233
DNA methylation eraser						
tet1	0.93 ± 0.21	1.69 ± 0.21	<0.001	1.37 ± 0.17	1.22 ± 0.18	0.171
tet2	1.40 ± 0.70	2.01 ± 0.86	0.204	1.41 ± 0.28	1.21 ± 0.15	0.148
tet3	0.81 ± 0.34	1.60 ± 0.35	0.003	1.61 ± 0.31	1.27 ± 0.18	0.043

[1]An independent t-test analysis was used to analyse the effects of different stimulus between HP/LC and LP/HC diets.

Table 3.6 mRNA levels of genes related to histone modifications in the liver and muscle of juvenile offspring before (week 17) and after (week 21) challenging with HC/LP diet for 4weeks. Juvenile offspring were obtained from broodstock fed with low-carbohydrate/high-protein (LC/HP) diet and high-carbohydrate/low-protein (HC/LP) diets (mean ± SD, n = 6)[1].

	Liver			Muscle		
Genes	LC/HP	HC/LP	P value	LC/HP	HC/LP	P value
H3K4me3 writer						
setd1a	1.01 ± 0.38	1.63 ± 0.44	0.025	3.18 ± 0.85	1.22 ± 0.43	<0.001
setd1ba	1.00 ± 0.71	1.46 ± 0.71	0.285	1.38 ± 0.18	1.33 ± 0.22	0.677
kmt2a	1.24 ± 0.61	1.39 ± 0.41	0.621	1.44 ± 0.21	1.30 ± 0.13	0.203
kmt2ba	1.04 ± 0.54	1.26 ± 0.37	0.440	3.48 ± 1.81	1.18 ± 0.26	0.011
kmt2bb	0.89 ± 0.13	1.31 ± 0.24	0.004	1.33 ± 0.10	1.06 ± 0.21	0.017
H3K4me3 eraser						
kdm5a	0.93 ± 0.18	1.29 ± 0.17	0.005	1.60 ± 0.22	2.33 ± 0.39	0.002
kdm5ba	1.00 ± 0.24	1.35 ± 0.25	0.029	1.40 ± 0.27	1.33 ± 0.17	0.582
kdm5bb	2.13 ± 0.46	2.00 ± 0.26	0.537	1.49 ± 0.35	1.06 ± 0.14	0.028
kdm5c	1.67 ± 0.38	1.64 ± 0.20	0.890	1.78 ± 0.23	1.61 ± 0.16	0.155
riox1	1.36 ± 0.53	2.45 ± 0.68	0.011	1.52 ± 0.18	1.67 ± 0.30	0.298
H3K9me3 specific writer						
suv39h1b	2.57 ± 0.77	1.30 ± 0.33	0.004	1.76 ± 0.31	1.66 ± 0.20	0.513
H3K9me3 specific eraser						
kdm4aa	0.84 ± 0.08	1.29 ± 0.14	<0.001	1.88 ± 0.22	1.51 ± 0.26	0.025
kdm4ab	0.77 ± 0.35	1.47 ± 0.51	0.019	2.81 ± 1.15	0.57 ± 0.29	0.004
kdm4b	1.72 ± 0.50	1.27 ± 0.16	0.087	2.00 ± 0.28	1.84 ± 0.33	0.410
kdm4c	1.44 ± 0.32	1.86 ± 0.28	0.036	1.85 ± 0.18	1.67 ± 0.24	0.167
H3K36me3 specific writer						
setd2	1.63 ± 0.18	1.97 ± 0.37	0.085	1.82 ± 0.30	1.77 ± 0.13	0.716
H3K9ac specific writer						
kat2a	1.07 ± 0.25	1.90 ± 0.78	0.049	1.77 ± 0.54	1.56 ± 0.22	0.394
kat2b	1.07 ± 0.58	1.31 ± 0.64	0.506	1.44 ± 0.67	1.27 ± 0.35	0.589
kat6a	0.87 ± 0.20	0.89 ± 0.14	0.820	1.87 ± 0.27	1.49 ± 0.14	0.013
gtf3c4	1.00 ± 0.22	0.90 ± 0.23	0.419	1.74 ± 0.31	1.63 ± 0.26	0.490
H3K9ac specific eraser						
sirt2	0.97 ± 0.41	1.01 ± 0.34	0.871	1.74 ± 0.25	1.72± 0.39	0.939
sirt5	0.70 ± 0.14	1.38 ± 0.38	0.005	1.68 ± 0.14	1.34 ± 0.15	0.003
sirt6	0.84 ± 0.27	1.63 ± 0.40	0.002	2.62 ± 1.38	0.30 ± 0.10	0.009

[1]An independent t-test analysis was used to analyse the effects of different stimulus between HP/LC and LP/HC diets.

Table 3.7 List of primers used for qRT-PCR of genes related to carbohydrate and intermediary metabolism in the liver and muscle

Genes	5'/3' Forward primer	5'/3' Reverse primer	SIZE (bps)	Access numbers
References gene				
ef1α	GCACGCTCTGCTGGCCTTT	GCGCTCAATCTTCCATCCC	250	AB075952
Hepatic glycolysis				
gck	GGGTGGTAGGATTTGGTGTG	TGCTGACACAAGGCATCTTC	186	XM003451020
pfklr	GACGAGCGAGTGGAGAAAAC	TGTCTTGATCCGAGGGAATC	162	XM003447353
pklr	AGGTACAGGTCACCCGTCAG	CATGTCGCCAGACTTGAAGA	164	XM005472622
Hepatic gluconeogenesis				
g6pca1	AGCGTTAAGGCAACTGGAGA	AAAAGCTAACAAGGCCAGCA	195	XM003448671
g6pca2	CTTCTTCCCCCTTTGGTTTC	AGACTCCTGCAGCTCCCATA	245	XM013273429
pck1	AAGCTTTTGACTGGCAGCAT	TGCTCAGCCAGTGAGAGAGA	162	XM003448375
pck2	TACGTCTTGAGCTCCCGTCT	CCTCCTGGATGATGCAAGTT	202	XM019354843
Hepatic lipogenesis				
fasn	AACCTGCTTCTCAAGCCAAA	CGTCACCCCTTGTTCTTTGT	222	XM013276809
g6pd	GTCACCTCAACCGGGAAGTA	TGGCTGAGGACACCTCTCTT	187	XM013275693
Hepatic amino acid catabolism				
alat	CACGGTGAAGAAGGTGGAGT	GCAGTTCAGGGTAGGAGCAG	200	XM005476466
asat	GCTTCCTTGGTGACTTGGAA	CCAGGCATCTTTCTCCAGAC	200	XM003451918
gdh	CGAGCGAGACTCCAACTACC	TGGCTGTTCTCATGATTTGC	203	XM003457465
Muscular glucose transport				
glut4	GAGGATGGACATGGAGAGGA	CAGGAAAAGCGAGACTACCG	235	JN900493
Muscular glycolysis				
hk1	CGTCGCTTAGTCCCAGACTC	TGACTGTAGCGTCCTTGTGG	235	XM019360229
hk2	CAGAGGGGAATTCGATTTGA	CCCACTCGACATTGACACAC	200	XM003448615
pfkma	AGGACCTCCAACCAACTGTG	TTTTCTCCTCCATCCACCAG	190	XM019349871
pfkmb	TTTGTGCATGAGGGTTACCA	CACCTCCAATCACACACAGG	208	XM003441476
pkma	TGACTGCTTCCTGGTCTGTG	CAGTGAAAGCTGGCAAATGA	249	XM005447626

*: from Yang et al. 2013

Table 3.8 List of primers used for qRT-PCR of genes related to epigenetic modification-related enzymes expressed in liver and muscle tissues

Genes		5'/3' Forward primer	5'/3' Reverse primer	SIZE (bps)	Accession numbers
Reference gene	*ef1*	GCACGCTCTGCTGGCCTTT	GCGCTCAATCTTCCATCCC	250	AB075952
DNA methylation writers	*dnmt1*	CTCACACTGCGCTGTCTTGT	ACAACGCTGAGAGAGCAAGC	188	XM_025906327.1
	dnmt3aa	CCAACAACCACGAGCAGGAA	TGCCGACAGTGATGGAGTCT	192	XM_005475084.4
	dnmt3ab	GCCGCAGCTTAGAGGACATC	CACACATGAGCACCTCTCGTC	189	XM_005477258.3
	dnmt3ba	GCTGCTGCAGATGCTACTGT	TTGCGCTGTTGTTGGCAAAG	186	XM_025901732.1
	dnmt3bb	TGCAGGAGTTCTTCGCCAAC	TGCCACATACTGACCCACCT	173	XM_025901790.1
DNA methylation eraser	*tet1*	CATCCAGTCCCAGCACAACC	CTCTATTTGGCGTGCGCTGA	194	XM_025897345.1
	tet2	GCAGCTGCCAACAAGAATGC	TGTTGCTGCTGCTGATGGAC	191	XM_005457001.3
	tet3	GCAAGCCAACCAACCAAACC	GATGTGTTGGCTCCGACCTG	177	XM_019365521.2
H3K4me3 writer (Histone Lysine methyltransferase)	*setd1a*	GGAACTCCGGTCTGGATGGT	CGAAGCTGCCCATCTGTGTT	172	XM_005468973.4
	setd1ba	AAGACAGGGAGGCAGCAGAA	CCTCAGGACTGGGAGGTCTG	198	XM_005470275.4
	kmt2a	AGAGCAGGAAAGCCAACAGC	CACTGGGCGTAGTTGTGGTC	178	XM_013274782.3
	kmt2ba	ACTCTGAGGGACCTGGAGGA	AGAGGAGGTGAAGCCGATCC	191	XM_013275905.3
	kmt2bb	GCTCCCGTCAGTGTGTCTTC	TCTGGCTCCAACCCAGTCAA	172	XM_013277028.3
H3K4me3 eraser (Histone lysine demethylases)	*kdm5a*	TCTGGCCACAGAGGAGTTGT	GTGACGTGGCTCTGCTGAAA	191	XM_005451728.4
	kdm5ba	TCTCAGAGCAGAGGGCATCC	GACCCGATGTCACACCTTGG	165	XM_003441348.2
	kdm5bb	CATCCCTGCCTACCTCCCAA	AAGGCTCCAGGTGGACTTGA	170	XM_003439103.5
	kdm5c	CTCTCCACCCTGGAGGCAAT	AGCTACCAGGCCCTCCAAAT	174	XM_005448517.4
	riox1	CCACCTGGCACACAAGGATT	TCCGGCTTCTACCACCACAT	192	XM_005475002.4
H3K9me3 specific writer	*suv39h1b*	TCCAACGCATGGCCTACAAC	CTTGATGTGCTGCAGTGTGC	197	XM_003459875.5
H3K9me3 specific eraser	*kdm4aa*	CGGATGCGAACCAAACCTCT	GGCTGGATCGACACCGTAAC	180	XM_005457300.3
	kdm4ab	TCTGTTCAGGGAGGCACACA	GCCTGTTGGCCCATCTGTTT	162	XM_005476068.4
	kdm4b	TGCTCGCTCTTCTGTCCGTA	AGCAGATCAGGAGGCTGGTT	196	XM_005453970.4
	kdm4c	CCTGCAGAGGAATGCAGTGG	GCACAGGTGCAATCTGGTGA	176	XM_005456806.2
H3K36me3 specific writer	*setd2*	AGGCAGCGATGACTTCAAGC	ATCTTGTGGCGTCCCACTCT	182	XM_019364854.2
H3K4me3 specific writer	*kat2a*	CACTGACCCTGCTGCTATGC	GTAGGCCAACCAGCCACATC	173	XM_025906390.1
	kat2b	GGCCTTTCATGGAGCCTGTG	CTCGCTCTCTGGAGGGTTGT	188	XM_003444058.3
	kat6a	CATCCCGTCCACTGCTTTCC	CCTGTTCACGCTACCACCAC	173	XM_005472980.3
	gtf3c4	CTTGTGGCGGTTCAAGCTCT	GGCTCGCCTTCCTCTTTCAC	174	XM_003440231.5
H3K9ac specific eraser	*sirt2*	GCGAGTCTAGTCAGCAGGGT	CCCAGAAGATCAGCTAGAGCCA	197	XM_003449264.5
	sirt5	ATTTGCCCAGGTGTGAGCAG	GAGCAAACATGGCTGCAGGA	177	XM_003457306.5
	sirt6	GTCAACCTGCAGTCGACCAA	TAACACCAGGCGGTGGTTTG	190	XM_003437978.5

*: from Yang et al. 2013

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CHAPTER IV

HIGH CARBOHYDRATE FEEDING IN NILE TILAPIA BROODSTCOK INDUCEDS LONG-TERM NUTRITIONAL PROGRAMMING IN ADULT NILE TILAPIA OFFSPRING

4.1 Abstract

Nutritional programming (NP) with carbohydrates (CHOs) is the exposure to a nutritional stimulus involving CHO early in life, which modulates nutrient metabolism later in life, thereby promoting the efficient CHO use. In Nile tilapia, dietary CHO stimulation in broodstock effectively transmit the effects of NP on CHO metabolic responses, although it did not influence the growth performance of offspring during the juvenile stage. The effects of NP vary depending on their growth stage. This study investigated the long-term effects of a high-CHO (HC) dietary stimulus in broodstock on the growth performance, CHO metabolic responses, and epigenetic modulation of adult offspring. To achieve early CHO stimulation, male and female Nile tilapia broodstocks were fed either an HC/low-protein (LP) diet or a low-carbohydrate/high-protein diet. The offspring were reared and cultured until the adult stage (week 25). Our results demonstrated that parental HC stimulation increased hepatopancreas size, induced muscular glycolysis, and decreased hepatic amino acid catabolism in adult fish, demonstrating that NP effects persisted into adulthood. Furthermore, during the challenging diet phase (weeks 26–29), parental HC stimulation improved growth performance, demonstrating beneficial protein-sparing effects. The NP effects of parental HC stimulation also modulated several CHO metabolic responses in adult offspring, including induction of hepatic glycolysis, lipogenesis, and muscular glucose transport induction, and suppression of gluconeogenesis and amino acid catabolism in the liver. At the molecular level, parental HC stimulation modulated several enzymes related to DNA and histone (de)methylation and histone de(acetylation) in the liver and muscle, suggesting that epigenetic modifications are involved in the long-term NP effects of CHO in Nile tilapia. Overall, dietary CHO stimulation in broodstock has long-

lasting effects, modulating CHO-related metabolic responses and growth of Nile tilapia into adulthood.

Keywords: Nutritional programming; Metabolic; Long-term effects; Effective utilization; Epigenetics

4.2 Introduction

Nutritional programming (NP) refers to the manipulation of nutrient quality or quantity during critical developmental periods (e.g. prenatal and postnatal) to program developing organisms, resulting in long-term effects throughout life (Lucas 1998). In fish, NP strategies involve exposure to specific nutrients or environmental stimuli during early life, which have long-lasting modulatory effects on the metabolic and physiological responses later in life (Hou and Fuiman 2020). Consequently, NP has been used to modulate nutrient metabolism in aquatic animals, promoting the efficient use of specific nutrients (Rocha et al. 2015; Fang et al. 2014; Vagner et al. 2007, 2009). Carbohydrates (CHOs) are among the most cost-effective resources for aquatic feed and have been intensively studied for their efficient use, particularly for their protein-sparing effects (Kamalam et al. 2017; Leung and Woo 2012; Suárez et al. n.d.). Several studies on NP using high-carbohydrate (HC) diets in fish have demonstrated that NP modulates CHO metabolism and improves CHO use later in life (Fang et al. 2014; Geurden et al. 2007, 2014; Rocha et al. 2016a, b; Gong et al. 2015).

The molecular mechanisms underlying NP-induced long-term effects are proposed to involve epigenetic modifications (Skjærven et al. 2023). Epigenetics refers to heritable changes in gene expression that do not involve alterations in DNA sequences (Hamilton 2011; Jablonka and Lamb 2002). Epigenetic analyses of the response to nutritional stimuli have primarily focused on DNA methylation and histone modifications (Marandel et al. 2016; Callet et al. 2021). Current methods to assess epigenetic modifications include global methyl cytosine level analysis, DNA bisulfite modification, histone methylation, acetylation analyses, and the detection of related modifying enzymes (Fraga and Esteller 2002; Ma et al. 2019). Persistent metabolic alterations caused by dietary glucose and lipids are associated with specific chromatin modifications (Keating and El-Osta 2015).

In fish, changes in global DNA methylation have been implicated in the CHO metabolic responses 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). Global DNA methylation analysis in Nile tilapia showed that glucose injection during the alevin stage induces epigenetic changes, such as hypomethylation, that persist into adulthood (Kumkhong et al. 2020a). Epigenetic changes, including DNA methylation and histone modification, are reversible processes mediated by enzymes involved in DNA/histone methylation, demethylation, acetylation, and deacetylation. In rainbow trout, NP using HC stimulation in the broodstock causes DNA hypomethylation in the offspring through the modulation of DNA-related enzymes (Callet et al. 2021).

Nile tilapia is an economically important omnivorous fish and its production is expected to increase to meet global protein demands. The optimal CHO level in the diet of Nile tilapia is approximately 33% (Azaza et al. 2015; Kamalam et al. 2017; Wang et al. 2005). A dietary CHO level of 50%, despite having a LP content, does not induce postprandial hyperglycaemia, demonstrating that Nile tilapia efficiently uses CHO as an energy source (Boonanuntanasarn et al. 2018a, b). Furthermore, the NP of CHO has been identified as an effective approach to enhance CHO utilisation in Nile tilapia during both the juvenile and adult stages. Gucose injection into the yolk sacs or HC feeding during the fry stage significantly improved growth performance in juvenile and adult fish fed HC diets (Kumkhong et al. 2020b, 2020a; Srisakultiew et al. 2022).

Additionally, NP of CHO in Nile tilapia has several modulatory effects on CHO metabolism, including 1) increased plasma glucose and lipid levels; 2) elevated fat, glycogen, and triglyceride content in the liver and muscle; 3) induction of hepatic glycolysis and lipogenesis; 4) suppression of hepatic gluconeogenesis and amino acid catabolism; and 5) induction of muscular glucose transport and glycolysis in juvenile and adult fish fed HC diets (Kumkhong et al. 2020a, b, 2021; Srisakultiew et al. 2022). The NP effects of HC dietary stimuli on broodstock persist in the juvenile stage of offspring (Luo et al. 2025, submitted). To further extend the scientific understanding of this concept, the present study investigated whether the effects of HC dietary stimuli on broodstock persist in the adult stage of the offspring. This study demonstrated that NP induced by parental HC feeding improved growth performance and modulated CHO

metabolic responses in adult offspring under HC diet challenge. Additionally, we examined the modulation of DNA methylation, histone methylation, and histone acetylation-related enzymes in the liver and muscle, providing evidence for the epigenetic mechanisms underlying the long-term NP effects of CHO.

4.3 Materials and methods

4.3.1 Ethics statement

All experimental procedures involving fish culture and sampling were approved by the Ethics Committee of the Suranaree University of Technology Animal Care and Use Committee (approval no. SUT-IACUC-001/2023).

4.3.2 Experiment diet, plan and fish management

Table 4.1 presents the ingredients and chemical compositions of the commercial and experimental diets, which included HC/LP and low-carbohydrate/high-protein (LC/HP) diets. Nutrient composition, including moisture, crude protein (CP), crude fat (CF), crude fibre, and ash, was determined following standard Association of Official Analytical Chemists (AOAC) methods (1990).

Figure 4.1 illustrates the experimental design, encompassing the broodstock dietary stimulus and offspring challenge periods. During the broodstock experimental phase, mature female (mean body weight [BW]: 609.6 ± 28.4 g) and male (mean BW: 920 ± 16.7 g) Nile tilapia were obtained from an earthen pond at the University Farm, Suranaree University of Technology, Nakhon Ratchasima, Thailand, and transferred to a cement pond for natural breeding. The cement pond (5 × 10 m; water depth, 0.8 m) was partitioned into two sections (5 × 5 m; water depth, 0.8 m) for the HC/LP and LC/HP diet groups, with each group containing six females (n = 6 replications) and three males. To acclimate to the broodstock, they were fed a commercial diet (36% CP, 3.4% CF; Table 4.1) for four days. Subsequently, the broodstock were fed either the HC/LP or LC/HP diet for 14 days. Fish were fed twice daily at 9:00 a.m. and 3:00 p.m. at 1.5% of their body weight.

After 14 days of feeding, fertilised eggs were collected from the female broodstock (two treatments, n = 6 replications) and transferred to hatching trays with circulating water. At seven days post-hatching, larvae were gently transferred to individual cages (one cage per replication; dimensions: 0.4 × 0.4 × 0.6 m) placed in a

cement pond (2 × 2 × 0.8 m; water depth 0.45 m) and fed a commercial diet (Table 4.1). To control for potential confounding effects of sex differences on fry growth, the fry diet (40% CP, 8% CF) was supplemented with 60 mg/kg of 17α-methyltestosterone (17-MT), administered five times daily (at 9:00, 11:00, 13:00, 15:00, and 17:00) for 28 days (Boonanuntanasarn et al. 2018b).

From weeks 5 to 25, fish were transferred to cement ponds (1 pond per replication; dimensions: 2 × 2 × 0.8 m) and fed a commercial diet ad libitum twice daily at 9:00 am and 4:00 pm. The feed composition during this period was as follows: 40% CP and 8% CF from weeks 5–7, and 32% CP and 4% CF from weeks 8 –25 (Fig. 4.1). During the growth phase, fish were weighed every four weeks to assess their growth performance, and feed intake was recorded.

To evaluate the effects of parental HC/LP dietary history on CHO metabolism in adult offspring, the offspring of HC/LP- and LC/HP-fed broodstock were challenged with the HC/LP diet (Table 4.1) for four weeks (weeks 26–29; Fig. 4.1). For this challenge phase, 10 fish per replication (mean BW: 168.04 ± 16.90 g) were randomly selected and housed in individual cages (1 cage per replication; dimensions: 0.6 × 0.6 × 0.9 m) placed in cement ponds. The fish were then fed the HC/LP diet at 3% body weight daily at 9:00 am and 4:30 pm, for four weeks.

During the experimental period, the fish were reared in dechlorinated tap water with continuous aeration. A flow-through water exchange system was employed to replace one-third of the water twice a week. Air and water temperatures were recorded daily, ranging from 25 to 28°C and 28 to 32°C, respectively. Dissolved oxygen and pH levels were measured weekly using dissolved oxygen and pH meters, respectively, ensuring that values remained within acceptable ranges of 7.10–8.68 mg/L and 4.93–6.85, respectively.

4.3.3 Fish sampling

To determine the effects of the dietary HC/LP stimulus in broodstock on adult offspring, three adult fish from each replicate were sampled at week 25 (before the challenge with the HC/LP diet) week 29 (after challenge with the HC/LP diet), and 5 h after the last meal. The fish were euthanised with 1.0% clove oil and blood was collected from the caudal vein using a hypodermic syringe containing K_2EDTA (1.5

mg/mL of blood) as an anticoagulant. The blood was centrifuged at 10,000 × g for 5 min at 4°C to obtain plasma, which was stored at −80°C for subsequent plasma metabolite analysis.

After blood collection, the livers of two fish from each replicate were dissected and weighed to calculate the hepatosomatic index (HSI). Liver and muscle samples were then collected, snap-frozen in liquid nitrogen, and stored at −80°C for later analysis of nutritive composition and extraction of total RNA for CHO metabolic response and epigenetic modification analyses. Additionally, one whole fish from each replicate was sampled for the analysis of CP, CF, and ash content.

4.3.4 Blood chemistry analysis

Two fish from each replicate (n = 6) were used to determine plasma metabolites, including glucose, triglycerides, cholesterol, protein, and blood urea nitrogen (BUN). Plasma glucose levels were determined using the GOD-PAP method (Barham and Trinder, 1972). Plasma triglyceride content was measured with 3-sulfopropyl-m-anisidine (Bucolo and David, 1973), and plasma cholesterol was quantitatively analyzed using the cholesterol oxidase phenol-aminophenazone method (Flegg, 1973). Plasma protein concentration was evaluated using the Biuret method (Gornall et al., 1949), while BUN levels were detected using a modified indophenol colorimetric method (Weatherburn, 1967).

4.3.5 Chemical composition, glycogen, and triglyceride analysis

Fish liver, muscle, and whole-body samples (two fish per replicate, n = 6), collected at weeks 25 and 29, were analyzed for their chemical composition, including protein, fat, and ash, following the methods of the Association of Official Analytical Chemists (AOAC, 1990). Glycogen content in the liver (100 mg) and muscle (200 mg) was measured using the hydrolysis technique described by Good et al. (1933) and Kumkhong et al. (2020a).

For triglyceride determination, 100 mg of liver and muscle tissue was homogenized with 1 mL of 5% IGEPAL and 2.8-mm glass beads, then heated at 90°C for 10 minutes. After cooling to room temperature, the samples were centrifuged at 10,000 × g at 4°C for 10 minutes, and the supernatant was collected. Triglyceride levels were determined using a triglyceride kit (catalog number: BLT00059, Erba Lachema s.r.o., Karasek Brno, Czechia), following the manufacturer's instructions (Luo et al., 2025, submitted).

4.3.6 Total RNA extraction, cDNA synthesis, and qRT-PCR analysis of genes involved in glucose metabolism

Liver (50 mg) and muscle (100 mg) tissue samples were collected from twelve fish per experimental group (two fish per replicate) for total RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's recommendations. The quantity and quality of total RNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher, USA) and 1% agarose gel electrophoresis, respectively. Complementary DNA (cDNA) was synthesized from 1 μg of RNA via reverse transcription using the SuperScript III RNAseH-reverse transcriptase kit (Invitrogen) and random primers (Promega, Madison, WI, USA), according to the manufacturer's protocol. Each sample was reverse transcribed in duplicate.

Relative mRNA expression levels from liver and muscle tissue were analyzed via quantitative real-time PCR on a Roche LightCycler 480 system (Roche Diagnostics, Neuilly-sur-Seine, France). Each PCR assay included duplicate samples (reverse-transcribed and PCR-amplified copies) as well as negative controls (samples without reverse transcriptase and cDNA templates). Relative quantification of target gene expression was performed using the Roche Applied Science E-Method (Pfaffl, 2001). mRNA expression levels in each tissue were normalized to the expression of ef1α. PCR efficiency was determined using serial dilutions of cDNA, with efficiency values ranging from 1.8 to 2.0, based on the slope of the standard curve.

Tables 4.8 and 4.3 list the primer sequences used for real-time RT-PCR. Table 4.2 includes primers for carbohydrate intermediate metabolism-related genes expressed in the liver and muscle. These include liver glycolysis genes (glucokinase [*gck*]; phosphofructokinase [*pfklr*]; pyruvate kinase [*pklr*]), gluconeogenesis genes (glucose-6-phosphatase [*g6pca1*, *g6pca2*]; phosphoenolpyruvate carboxykinase, cytosolic [*pck1*], and mitochondrial [*pck2*]), lipogenesis genes (fatty acid synthase [*fasn*]; glucose-6-phosphate dehydrogenase [*g6pd*]), and amino acid catabolism genes (glutamate dehydrogenase [*gdh*]; alanine aminotransferase [*alat*]; aspartate aminotransferase [*asat*]). In muscle tissue, detected genes include those for glucose transport (*glut4*) and glycolysis (hexokinase I/II [*hk1*, *hk2*]; phosphofructokinase [*pfkma*]; pyruvate kinase [*pkma*]).

Table 4.9 details primers for epigenetic modification-related genes

expressed in liver and muscle tissues. These include DNA methyltransferases (*dnmt1a*, *dnmt3aa*, *dnmt3ab*, *dnmt3ba*, *dnmt3bb*), TET methyl cytosine dioxygenases (*tet1*, *tet2*, *tet3*), histone (H) 3 lysine (K) 4 trimethylation (H3K4me3) writers (SET domain-containing 1A/1B [*setd1a*, *setd1ba*]; lysine methyltransferase 2A [*kmt2a*]; histone-lysine N-methyltransferase 2B [*kmt2ba*, *kmt2bb*]), H3K4me3 erasers (lysine demethylase 5A/5BA/5BB/5C [*kdm5a*, *kdm5ba*, *kdm5bb*, *kdm5c*]; bifunctional lysine-specific demethylase and histidyl hydroxylase [*riox1*]), H3K9me3 writers (histone lysine N-methyltransferase [*suv39h1b*]) and erasers (lysine demethylase 4AA/4AB/4B/4C [*kdm4aa*, *kdm4ab*, *kdm4b*, *kdm4c*]), H3K36me3 writers (SET domain-containing 2 [*setd2*]), H3K9ac writers (lysine acetyltransferase 2A/2B/6A [*kat2a*, *kat2b*, *kat6a*]; general transcription factor IIIC subunit 4 [*gtf3c4*]), and erasers (sirtuin 2/5/6 [*sirt2*, *sirt5*, *sirt6*]).

4.3.7 Statistical analysis

All data were analyzed using SPSS for Windows, version 22 (SPSS Inc., Chicago, IL, USA). An independent samples t-test was conducted to evaluate the differences between the LC/HP and HC/LP groups. Statistical significance was determined at $p < 0.05$.

4.4 Results

4.4.1 Effects of the HC Diet in Broodstock on Growth Performance in Adult Offspring Before and After the HC/LP Diet Challenge

Experimental fry, including those obtained from broodstock fed HC/LP and LC/HP diets, were reared on commercial diets through adulthood (week 25). Figure 4.2 shows the body weight of the experimental fish, with no significant differences observed between the groups by week 25. Similarly, there were no significant differences in growth performance between the two experimental groups ($P > 0.05$; Table 4.2).

However, when the fish were challenged with the HC/LP diet, those from parental HC/LP-fed broodstock exhibited significantly higher growth performance compared to the LC/HP group. This included increased body weight, weight gain, average daily gain (ADG), and specific growth rates (SGR) ($P < 0.05$; Table 4.2). Additionally, dietary HC in broodstock history significantly reduced the feed conversion ratio (FCR) in the offspring ($P < 0.05$; Table 4.2).

4.4.2 Effects of the HC Diet in Broodstock on Intermediary Carbohydrate (CHO) Metabolism in Adult Offspring Before and After the HC/LP Diet Challenge

To investigate the effects of dietary HC/LP in broodstock history on intermediary metabolism in adult offspring, plasma metabolites and the proximate composition of the liver, muscle, and whole body were analyzed (Tables 4.3 and 4.4). No significant differences were observed in plasma metabolites, including glucose, triglycerides, blood urea nitrogen (BUN), cholesterol, and total protein levels (Table 4.3), nor in the proximate composition of the liver, muscle, or whole body of offspring derived from HC/LP- and LC/HP-fed broodstock ($P > 0.05$; Tables 4.3 and 4.4). However, the hepatosomatic index (HSI) was significantly higher in adult offspring of HC/LP-fed broodstock compared to offspring of LC/HP-fed broodstock ($P < 0.05$; Table 4.4).

After adult offspring were challenged with the HC/LP diet for 4 weeks (weeks 26–29), plasma glucose and triglyceride levels were significantly increased, while total protein levels were decreased in offspring of HC/LP-fed broodstock compared to those of LC/HP-fed broodstock ($P < 0.05$; Table 4.3). Notably, there were no significant differences in BUN and cholesterol levels between the two experimental groups ($P > 0.05$; Table 4.3). Additionally, dietary HC/LP in broodstock history significantly increased the fat, glycogen, and triglyceride contents in the liver and muscle of their adult offspring ($P < 0.05$; Table 4.4). HSI remained significantly elevated in offspring from HC/LP-fed broodstock ($P < 0.05$; Table 4.4). Furthermore, whole-body fat content was significantly increased in adult offspring of HC/LP-fed broodstock ($P < 0.05$; Table 4.4). However, there were no significant differences in protein or ash content in the liver, muscle, or whole body ($P > 0.05$; Table 4.4).

The modulatory effects on CHO metabolism and related pathways were further examined at the molecular level in liver and muscle tissues, as shown in Table 4.5. When offspring were reared on commercial diets until week 25, the parental HC/LP stimulus history caused downregulation of hepatic amino acid catabolism genes (*alat*) and upregulation of muscular glycolysis genes (*pfkma* and *pfkmb*) in adult offspring compared to those of LC/HP-fed broodstock ($P < 0.05$; Table 4.5). After the HC/LP diet challenge, the HC/LP broodstock history induced 1) induction of hepatic glycolysis (*gck*, *pfklr*, *pklr*) and lipogenesis (*fasn*, *g6pd*), 2) Induction of muscular glucose transport

(*glut4*) and muscular glycolysis (*hk1*, *hk2*, *pfkma*, *pkma*), and 3) Suppression of hepatic gluconeogenesis (*g6pca1*, *pck1*, *pck2*) and hepatic amino acid catabolism (*asat*, *alat*, *gdh*), when compared to offspring of LC/HP-fed broodstock ($P < 0.05$; Table 4.5).

4.4.3 Effects of the HC Diet in Broodstock on Epigenetic Modifications in Adult Offspring After the HC/LP Diet Challenge

The enzymes related to epigenetic modifications, including DNA methylation writers and erasers, were measured at the molecular level in the liver and muscle of adult offspring after the HC/LP diet challenge (Table 4.6). Compared to offspring from LC/HP-fed broodstock, parental HC/LP feeding history led to the upregulation of hepatic DNA methylation writers (*dnmt1a*, *dnmt3aa*, *dnmt3ab*, *dnmt3bb*) and erasers (*tet2*, *tet3*) ($P < 0.05$; Table 4.6). Additionally, the upregulation of dnmt3aa and downregulation of dnmt1a were observed in fish derived from HC/LP-fed broodstock ($P < 0.05$; Table 4.6). However, hepatic *tet1* and muscular *dnmt3ab*, *dnmt3bb*, *te*t1, *tet2*, and *tet3* levels were not significantly different between the two experimental groups ($P > 0.05$; Table 4.6).

Table 4.7 presents the mRNA expression levels of genes encoding enzymes related to histone (H)3 lysine (K) 4 trimethylation (H3K4me3), H3K9me3, H3K36me3, and histone 3 lysine 9 acetylation (H3K9ac) in the liver and muscle of adult offspring after the HC/LP diet challenge. Compared to offspring from LC/HP-fed broodstock, parental HC/LP feeding history modulated the expression of genes in the liver as follows: 1) H3K4me3 writers: Induction of *kmt2a*, *kmt2ba*, *kmt2bb* and suppression of *setd1a*, 2) H3K4me3 erasers: Induction of *kdm5ba*, *kdm5c*, *riox1*, 3) H3K9me3 writer: Suppression of *suv39h1b*, 4) H3K9me3 erasers: Induction of *kdm4aa*, *kdm4ab*, *kdm4c*, 5) H3K9ac writers: Induction of *kat2a* and suppression of *gtf3c4*, 6) H3K9ac erasers: Induction of *sirt5*, *sirt6* ($P < 0.05$; Table 4.7). In muscle tissue, parental HC/LP feeding history resulted in 1) Downregulation of *setd1a*, *kmt2a*, *kdm5ba*, *suv39h1b*, *kat2a*, *kat2b*, *gtf3c4* and 2) Upregulation of *riox1*, kdm4c, *sirt5* ($P < 0.05$; Table 4.7). Notably, the expression of the H3K36me3 writer (*setd2*) was not significantly different between the two experimental groups ($P > 0.05$; Table 4.7).

4.5 Discussion

Nile tilapia is known for its high capacity to utilize carbohydrates (CHO) as an energy source. Nutritional stimuli involving high-carbohydrate (HC) intake, such as glucose injection into the yolk reserves during the alevin stage and early feeding with an HC diet, have been shown to generate nutritional programming (NP) effects that modulate CHO metabolism and related pathways (Boonanuntanasarn et al., 2018a, b; Kumkhong et al., 2020b, 2021; Srisakultiew et al., 2022). These studies also demonstrated that applying the NP concept via direct HC stimuli during early life could enhance protein-sparing effects, improving growth performance during juvenile and adult stages.

In a previous study, dietary HC stimuli in broodstock were found to induce NP effects, which were transmitted to their offspring and modulated CHO-related metabolic responses. However, these NP effects did not enhance growth performance in juvenile offspring fed an HC diet (Luo et al., 2025, submitted). To expand the understanding of NP effects across the entire life cycle of Nile tilapia, this study introduced a nutritional stimulus via broodstock diets. This study demonstrated the successful application of NP of CHO and highlighted the long-term impacts of dietary HC in broodstock on their adult offspring. The parental HC stimulus effects persisted into adulthood, resulting in significant improvements in growth performance and CHO-related metabolic responses when the offspring were challenged with an HC diet. Additionally, this study identified dynamic modulations of hepatic and muscular enzymes involved in epigenetic modifications at the molecular level. These findings suggest that epigenetic mechanisms were involved in the long-term effects of NP of CHO.

4.5.1 Dietary HC in broodstock improved the growth performance of adult offspring when challenged with an HC diet

The concept of nutritional programming (NP) of carbohydrates (CHO) has been proposed as a tool to enhance the dietary utilization of CHO, thereby improving protein-sparing effects. Protein-sparing effects refer to the provision of sufficient non-protein energy sources, such as carbohydrates (the most economical option) and/or lipids, in feed. This reduces the use of protein as an energy source, maximizing the efficiency of protein utilization in feed (Mohanta et al., 2007). In Nile tilapia, the NP of CHO has been shown to increase the utilization of CHO during juvenile and adult stages.

For example, early feeding of fry with an HC diet for 1–4 weeks improved growth performance in both juvenile and adult fish when they were later challenged with an HC diet (Kumkhong et al., 2020b; Srisakultiew et al., 2022). Similarly, in our study, dietary HC stimulus in broodstock improved growth performance in adult offspring when challenged with an HC diet, suggesting that broodstock HC feeding enhances CHO utilization for protein-sparing effects in adult offspring. However, the effects of NP vary depending on the growth stage and the methods of nutritional stimulus.

In a previous study, HC feeding in broodstock did not improve the efficient utilization of dietary CHO in juvenile offspring (Luo et al., 2025, submitted). Additionally, glucose injection into yolk reserves in larvae improved growth performance in juveniles but not in adults when fed an HC diet (Kumkhong et al., 2020a, 2021). These findings suggest that the effects of NP of CHO on the efficient use of CHO for growth are particularly evident in Nile tilapia, a species well-adapted to using CHO as an energy source. However, the effects of NP of CHO on growth performance differ in carnivorous and omnivorous fish. For instance, while CHO utilization and protein-sparing effects are enhanced in Nile tilapia, studies have shown varying results in other species, including carnivorous fish (Callet et al., 2022; Song et al., 2019; Xiao et al., 2020; Zambonino-Infante et al., 2019; Rocha et al., 2015; Lu et al., 2022; Geng et al., 2023). These differences highlight the species-specific nature of NP responses. In summary, the NP of CHO can improve protein-sparing effects in fish species with a high capacity for CHO utilization, such as Nile tilapia.

4.5.2 Dietary HC in broodstock influenced intermediary carbohydrate (CHO) metabolism in offspring throughout the adult stage, with stronger effects observed when the offspring were challenged with a dietary HC regimen

Different nutritional stimulus histories, including glucose injection into yolk reserves during the alevin stage and early feeding of high-carbohydrate (HC) diets in fry, directly modulated intermediary carbohydrate (CHO) metabolic responses in fish. These responses persisted into juvenile and adult stages and included increased glycogen, fat, and triglyceride levels in the liver and muscle. These modulatory effects were more pronounced when accompanied by increases in the hepatosomatic index (HSI), plasma glucose, and triglyceride levels after fish were re-exposed to HC diets

(Kumkhong et al., 2020b; Srisakultiew et al., 2022; Kumkhong et al., 2021, 2020a). Applying a similar NP concept, although not identical, different nutritional stimulus methods in broodstock have also been shown to modulate intermediary metabolic responses in their offspring. These modulatory effects, transmitted from broodstock to offspring, persisted through the juvenile stage, leading to increased HSI and triglyceride content in the liver. Moreover, when juvenile offspring were challenged with an HC diet, parental dietary CHO stimulus history resulted in elevated plasma glucose and triglyceride levels, as well as increased fat, glycogen, and triglyceride levels in the liver and muscle (Luo et al., 2025, submitted). In this study, dietary HC in broodstock led to increased HSI in adult offspring. Furthermore, when adult offspring were challenged with an HC diet, parental HC stimulation increased plasma glucose and triglyceride levels, hepatic and muscular fat, glycogen, and triglyceride levels, as well as whole-body fat content. These findings suggest that the modulatory effects of parental HC stimulation persist long-term, extending into adulthood. Therefore, in Nile tilapia, within the framework of the NP of CHO concept, different CHO stimulus histories-whether direct CHO stimuli during early life stages or CHO stimuli applied to the broodstock-can modulate similar long-term intermediary metabolic responses.

This study demonstrated that at the molecular level, the modulatory effects of nutritional programming (NP) on intermediary carbohydrate (CHO) metabolism in broodstock were sustained in their offspring through adulthood. Dietary HC in broodstock resulted in the downregulation of hepatic amino acid catabolism (*alat*) and upregulation of muscular glycolysis (*pfkma* and *pfkmb*) in adult offspring. These effects were further amplified when offspring were challenged with an HC diet, resulting in: 1) induction of hepatic glycolysis and lipogenesis, 2) suppression of hepatic gluconeogenesis and amino acid catabolism, and 3) induction of muscular glucose transport and glycolysis. The modulatory effects observed in adult offspring were consistent with findings during the juvenile and fry stages (Luo et al., 2025, submitted). These results indicate that HC stimuli in broodstock can be effectively transmitted to their offspring, with impacts persisting long-term into adulthood. The NP effects of CHO via HC stimuli in broodstock were comparable to the outcomes observed with direct CHO stimuli, such as glucose injection during the alevin stage and early HC feeding in fry (Kumkhong et al., 2020a, b, 2021; Srisakultiew et al., 2022). However, the modulatory

effects of NP of CHO can vary across fish species. For instance, in zebrafish, although dietary HC in broodstock suppressed gluconeogenesis (*fbp1a*, *fbp1b*, *pck1*), it did not affect hepatic glycolytic genes (*gck*, *pfkla*, *pfklb*, *pk*) in adult offspring after a 1-week HC diet challenge (Lu et al., 2022). Overall, as with other early-life nutritional stimuli in fish, HC stimuli in broodstock can be transmitted to offspring, generating NP effects on CHO metabolism. The modulation of CHO and related metabolic responses in offspring appears to be consistent and sustained through adulthood.

4.5.3 Dietary HC in broodstock influenced epigenetic modifications in adult offspring, particularly when challenged with a dietary HC diet

Nutrigenomics refers to the analysis of the interactions between diet, gene expression, and epigenetics, which are proposed to play a key role in gene-diet interactions (Pal, 2022). In fish, dietary interventions with nutrients such as protein and carbohydrates during early life can modulate metabolic pathways over the long term. Epigenetic chromatin changes, including DNA methylation and histone modifications, have been shown to regulate metabolic responses, with effects that can persist throughout life (Skjærven et al., 2023). In other words, epigenetic mechanisms are implicated in the long-term modulatory effects of nutritional programming (NP) on metabolic responses. Heritable epigenetic modifications associated with NP are reversible and can be assessed through global DNA methylation analysis, DNA bisulfite sequencing, and the expression of genes related to DNA methylation processes (Song et al., 2019; Xiao et al., 2020; Horii and Hatada, 2016). For example, in diabetic mice, the downregulation of *dnmt1*, *dnmt3a*, *dnmt3b*, and *dnmt31* was observed in oocytes (Ge et al., 2013). Similarly, downregulation of *tet3* was detected in the oocytes of hyperglycemic mice (Wu et al., 2022). In this study, we demonstrated the effects of NP through parental HC feeding on the expression of genes related to DNA methylation in adult offspring challenged with an HC diet. Dietary HC in broodstock modulated epigenetic stability, including the upregulation of dnmt*1a*, *dnmt3aa* (also observed in muscle), *dnmt3ab*, *dnmt3bb, tet1*, and *tet2* in the liver, alongside the downregulation of dnmt1a in muscle. These findings suggest that epigenetics plays a role in the long-term impacts of nutritional interventions in broodstock that are transmitted to their offspring. Notably, the history of NP through CHO feeding in broodstock induced the upregulation of both DNA methylation writers and erasers in adult offspring, highlighting

the dynamic and reversible nature of epigenetic mechanisms. However, NP effects of dietary HC in broodstock vary across fish species. For instance, in rainbow trout, maternal HC dietary stimulation suppressed *dnmtabba2* and *dnmt3bbb* expression in fry, which was associated with global DNA hypomethylation (Callet et al., 2021). Differences in epigenetic modulation at the molecular level may be attributed to species-specific habits and the developmental stage of the offspring. In summary, nutritional stimuli in broodstock have long-term impacts on the metabolic responses of offspring, and epigenetic modifications likely play a significant role in mediating the effects of NP of CHO.

In this study, the epigenetic modulation of parental HC feeding history was also assessed by examining the expression of histone modification enzymes at the molecular level. In mammals, NP effects associated with high-glucose conditions in parents have been shown to modulate histone modification writers and erasers in their offspring. For example, in mice, maternal diabetes mellitus or high glucose exposure in vitro decreased the expression of *sirt2* and *sirt6* in embryonic or neural stem cells (Yu et al., 2016). Similarly, 16 weeks of sucralose and stevia supplementation in the diets of mouse parents increased the mRNA levels of histone deacetylase 3 (*Hdac3*) in the gut of their offspring (Celume et al., 2024). In our study, NP effects of dietary HC in broodstock also induced the expression of histone modification writers and erasers in adult offspring challenged with an HC diet. Parental HC stimulation modulated: 1) H3K4me3 writers (induction: *kmt2*, *kmt2ba*, *kmt2bb*; suppression: *setd1a*) and erasers (induction: *kdm5ba*, *kdm5c*, *riox1*), 2) H3K9me3 writers (suppression: *suv39h1b*) and erasers (induction: *kdm4aa*, *kdm4ab*, *kdm4c*), 3) H3K9ac writers (induction: *kat2a*; suppression: *gtf3c4*) and erasers (induction: *sirt5*, *sirt6*) in the liver. In muscle tissue, parental HC feeding suppressed the expression of *setd1a*, *kmt2a*, *kdm5ba*, *suv39h1b*, *kat2a*, *kat2b*, and *gtf3c4*, while inducing *riox1, kdm4c*, and *sirt5*. These findings demonstrate that the modulation of histone modifications is a component of the NP effects induced by dietary CHO in broodstock. Overall, these results suggest that epigenetic mechanisms, including histone modifications, play a key role in the NP effects on CHO metabolic responses in offspring. Furthermore, these effects can persist long-term into adulthood, emphasizing the enduring impact of parental HC dietary interventions.

4.6 Conclusions

Dietary high-carbohydrate (HC) intake in Nile tilapia broodstock induced nutritional programming (NP) that was transmitted to their offspring, persisted through adulthood, and modulated CHO metabolism. The NP of CHO improved growth performance in offspring and elicited stronger CHO metabolic responses when they were challenged with an HC diet. The modulation of gene expression related to DNA methylation and histone modification in offspring suggests that epigenetic mechanisms are involved in the NP benefits of dietary CHO in Nile tilapia broodstock.

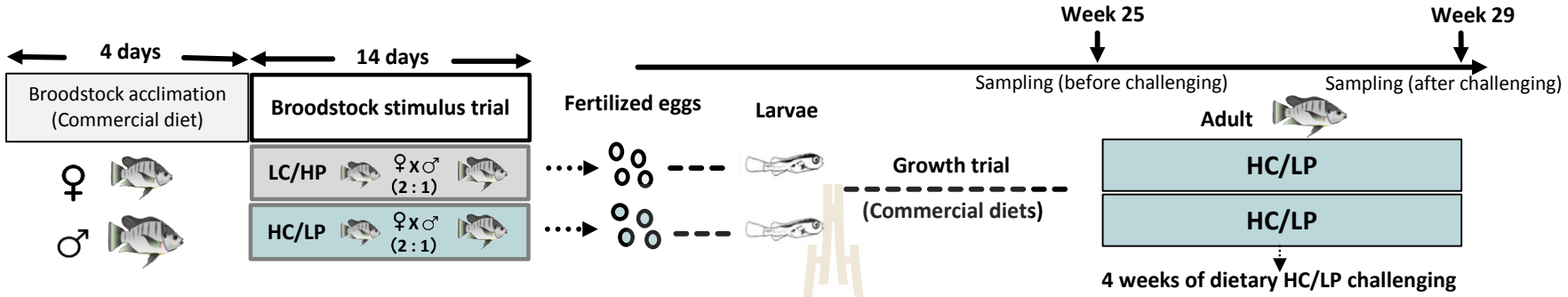

Figure 4.1 Experimental plan for nutritional programming involving dietary high-carbohydrate (HC) stimuli in broodstock and their long-term impacts on carbohydrate (CHO) metabolism in offspring. Mature male and female broodstock were acclimated to a communal breeding pond and fed a commercial diet (36% crude protein [CP], 3.4% crude fat [CF]) for 4 days. Subsequently, broodstock were fed low-carbohydrate/high-protein (LC/HP) or high-carbohydrate/low-protein (HC/LP) diets for 14 days. Fertilized eggs were collected from the mouths of females after the feeding period and cultured. The offspring were fed commercial diets (weeks 1–7: 40% CP, 8% CF; weeks 8–25: 32% CP, 4% CF) until adulthood (week 25). During weeks 26–29, adult fish were challenged with HC/LP diets and sampled to assess intermediary CHO metabolism and the epigenetic modifications

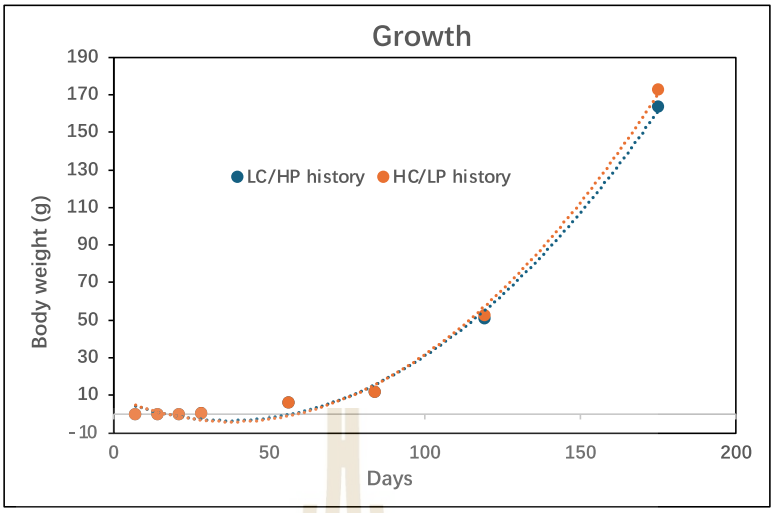

Figure 4.2 Growth of experimental offspring fish from Nile tilapia broodstock fed LC/HP and HC/LP diets for 14 days (mean ± SD, n = 6). During the growth trial, all experimental fish were fed commercial diets (days 1–49: 40% crude protein [CP], 8% crude fat [CF]; days 50–175: 32% CP, 4% CF). Abbreviations: HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

Table 4.1 Ingredients and chemical compositions (%) of the challenge diets,

Ingredients	Commercial diet			Challenge diets	
	Broodstock acclimation (36% protein)	Offspring nursing (40% protein)	Offspring growth trial (32% protein)	LC/HP	HC/LP
Fish meal	-	-	-	88	18
Rice flour	-	-	-	-	70
Fish oil	-	-	-	-	7
Soybean oil	-	-	-	2	-
Gelatin	-	-	-	8	-
Di-calcium phosphate	-	-	-	0	3
Fish premixa	-	-	-	2	2
Proximate composition (% from wet weight)					
Dry matter	91	89	89	94.2	90.2
Protein	36	40	32	57.3	15.3
Fat	3.4	8.0	3.9	9.2	9.0
Fiber	7.1	4.2	7.3	0.5	0.4
Ash	12.6	12.2	12.2	22.8	8.6
NFEb	32.5	24.3	34	4.5	56.8
Gross energy (kJ g−1)	13.13	14.42	12.82	14.40	15.6

[a] Vitamin and trace mineral mix provided the following (IU kg−1 or g kg−1 diet): biotin, 0.25 g; folic acid, 0.003 g; inositol, 0.25 mg; niacin, 0.0215 g; pantothenic acid, 0.03 g; vitamin A, 5,000 IU; vitamin B1, 0.0025 g; vitamin B2, 0.0012 g; vitamin B6, 0.0075 g; vitamin B12, 0.00005 mg; vitamin C, 1 g; vitamin D3, 1,000 IU; vitamin E, 100 IU; vitamin K, 0.008 g; copper, 0.02 g; iron, 0.2 g; selenium, 0.3 mg; zinc, 0.32 g.

[b] Nitrogen-free extract = dry matter − (CP + crude lipid + crude fibre + ash). HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

Table 4.2 Growth performances of adult offspring before (week 25) and after (week 29) fed with HC/LP diet for 4 weeks (mean ± SD, n = 6)[1].

Experimental periods	Broodstock diets	Final weight (g)	Weight gain (g)	ADG[2] (g day $^{-1}$)	SGR[3] (% day $^{-1}$)	FCR[4]
Week 25 before challenge	LC/HP	163.50 ± 16.38	155.06 ± 16.14	0.89 ± 0.09	5.54 ± 0.05	1.21 ± 0.13
	HC/LP	172.58 ± 17.63	162.40 ± 17.52	0.93 ± 0.10	5.56 ± 0.06	1.16 ± 0.14
	P value	0.377	0.468	0.463	0.355	0.516
Week 29 after challenge	LC/HP	278.75 ± 18.23	270.31 ± 18.05	1.33 ± 0.09	5.04 ± 0.03	1.32 ± 0.09
	HC/LP	310.75 ± 12.71	300.57 ± 12.80	1.48 ± 0.06	5.09 ± 0.03	1.18 ± 0.05
	P value	0.005	0.007	0.006	0.020	0.012

[1] An independent t-test was used to analyse the effects of different stimuli (HP/LC and LP/HC diets)

[2] Average daily gain (ADG) = (final body weight – initial body weight)/experimental days

[3] Specific growth rates (SGR) = 100 × ([in final body weight – in initial body weight]/ experimental days)

[4] Feed conversion ratio (FCR) = dry feed fed/wet weight gain

HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

Table 4.3 Plasma metabolites in adult offspring before (week 25) and after (week 29) fed with HC/LP diet for 4 weeks (mean ± SD, n = 6)[1].

	Week 25 (before challenge)			Week 29 (after challenge)		
Broodstock diets	LC/HP	HC/LP	P value	LC/HP	HC/LP	P value
Glucose (mM)	4.45 ± 0.44	4.53 ± 0.46	0.608	6.05 ± 0.49	6.69 ± 0.54	0.034
Triglyceride (mM)	1.46 ± 0.13	1.52 ± 0.13	0.399	2.02 ± 0.23	2.68 ± 0.23	<0.001
BUN (mM)	1.06 ± 0.33	0.92 ± 0.23	0.276	1.52 ± 0.54	1.24 ± 0.40	0.156
Cholesterol (mM)	1.21 ± 0.24	1.24 ± 0.23	0.805	2.53 ± 0.37	2.88 ± 0.43	0.093
Total protein (g/L)	37.35 ± 2.6	36.74 ± 2.84	0.634	41.94 ± 1.60	39.16 ± 1.96	0.002

[1]An independent t-test was used to analyse the effects of different stimuli (HP/LC and LP/HC diets). HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

Table 4.4 Proximate composition of liver, muscle and whole body in adult offspring before (week 25) and after (week 29) fed with HC/LP diet for 4 weeks (mean ± SD, n = 6)[1].

	Wee 25 (before challenge)			Week 29 (after challenge)		
Broodstock diets	LC/HP	HC/LP	*P* value	LC/HP	HC/LP	*P* value
Liver (%)						
Protein	10.67 ± 0.65	11.40 ± 0.49	0.055	8.06 ± 0.72	7.43 ± 0.53	0.111
Fat	5.49 ± 0.63	5.67 ± 0.46	0.600	6.21 ± 0.24	7.13 ± 0.19	<0.001
Ash	1.19 ± 0.10	1.21 ± 0.10	0.679	1.11 ± 0.10	1.11 ± 0.09	0.880
Glycogen (mg/g)	36.06 ± 2.16	37.15 ± 2.21	0.447	53.65 ± 7.06	69.39 ± 4.56	0.002
Triglyceride (mg/g)	12.52 ± 2.82	15.55 ± 1.91	0.078	30.77 ± 2.32	36.79 ± 2.03	0.001
HSI[2]	1.61 ± 0.12	1.92 ± 0.23	0.015	3.18 ± 0.31	4.02 ± 0.67	0.021
Muscle (%)						
Protein	18.75 ± 0.56	18.44 ± 0.41	0.203	18.38 ± 0.69	18.51 ± 0.4	0.691
Fat	1.38 ± 0.12	1.46 ± 0.26	0.513	1.76 ± 0.15	2.15 ± 0.13	<0.001
Ash	1.05 ± 0.05	1.12 ± 0.08	0.100	1.23 ± 0.11	1.30 ± 0.11	0.238
Glycogen (mg/g)	5.11 ± 1.02	5.22 ± 1.23	0.884	6.36 ± 0.72	7.88 ± 0.35	0.002
Triglyceride (mg/g)	4.05 ± 0.38	4.09 ± 0.65	0.889	5.64 ± 0.51	6.74 ± 0.87	0.035
Whole body (%)						
Protein	13.53 ± 0.97	13.82 ± 0.53	0.546	13.75 ± 0.83	13.52 ± 0.96	0.664
Fat	5.77 ± 0.49	5.92 ± 0.45	0.593	8.23 ± 0.59	9.47 ± 0.48	0.002
Ash	3.32 ± 0.59	3.29 ± 0.81	0.946	3.45 ± 0.62	3.33 ± 0.53	0.717

[1] An independent t-test was used to analyse the effects of different stimuli (HP/LC and LP/HC diets). HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

[2] Hepatosomatic index (HSI) = liver weight / fish body weight

Table 4.5 mRNA levels of genes related to intermediary CHO metabolism in the liver and muscle of adult offspring before (week 25) and after (week 29) fed with HC/LP diet for 4 weeks (mean ± SD, n = 6)[1].

	Wee 25 (before challenge)			Week 29 (after challenge)		
Broodstock diets	**LC/HP**	**HC/LP**	**P value**	**LC/HP**	**HC/LP**	**P value**
Hepatic glycolysis						
gck	0.57 ± 0.16	0.65 ± 0.22	0.486	1.32 ± 0.23	1.82 ± 0.43	0.030
pfklr	0.91 ± 0.25	1.00 ± 0.41	0.651	0.78 ± 0.20	1.21 ± 0.25	0.008
pklr	0.70 ± 0.20	0.94 ± 0.36	0.175	0.58 ± 0.22	1.31 ± 0.42	0.003
Hepatic gluconeogenesis						
g6pca1	0.93 ± 0.51	0.85 ± 0.23	0.723	1.23 ± 0.06	0.72 ± 0.29	0.007
g6pca2	0.80 ± 0.16	0.62 ± 0.23	0.136	1.39 ± 0.36	1.04 ± 0.08	0.060
pck1	1.15 ± 0.38	0.80 ± 0.38	0.134	1.79 ± 0.22	0.75 ± 0.18	<0.001
pck2	2.00 ± 0.33	1.81 ± 0.24	0.279	1.28 ± 0.43	0.41 ± 0.27	0.002
Hepatic lipogenesis						
fasn	0.71 ± 0.20	0.73 ± 0.14	0.896	0.52 ± 0.04	0.91 ± 0.18	0.002
g6pd	0.64 ± 0.22	0.74 ± 0.22	0.441	0.32 ± 0.08	1.27 ± 0.26	<0.001
Hepatic amino acid catabolism						
asat	1.12 ± 0.38	0.77 ± 0.13	0.087	1.82 ± 0.40	1.02 ± 0.26	0.002
alat	0.98 ± 0.10	0.74 ± 0.23	0.045	1.27 ± 0.30	0.89 ± 0.05	0.025
gdh	1.00 ± 0.20	0.85 ± 0.12	0.162	1.28 ± 0.31	0.82 ± 0.20	0.012
Muscular glucose transport and glycolysis						
glut4	0.93 ± 0.43	1.11 ± 0.36	0.458	0.75 ± 0.08	0.90 ± 0.07	0.007
hk1	1.15 ± 0.37	1.22 ± 0.48	0.769	0.76 ± 0.12	0.93 ± 0.10	0.025
hk2	0.69 ± 0.14	0.79 ± 0.10	0.189	0.72 ± 0.19	1.42 ± 0.15	<0.001
pfkma	0.67 ± 0.39	1.37 ± 0.38	0.011	0.66 ± 0.36	1.50 ± 0.44	0.005
pfkmb	0.75 ± 0.29	1.16 ± 0.14	0.012	0.69 ± 0.19	0.96 ± 0.23	0.052
pkma	1.74 ± 0.77	2.09 ± 0.43	0.353	1.06 ± 0.14	1.42 ± 0.19	0.010

[1] An independent t-test was used to analyse the effects of different stimuli (HP/LC and LP/HC diets). HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

Table 4.6 mRNA levels of genes related to DNA methylation in the liver and muscle of adult offspring fed with HC/LP diet for 4 weeks (week 26-29) (mean ± SD, n = 6)[1].

	Liver			Muscle		
Broodstock diets	**LC/HP**	**HC/LP**	**P value**	**LC/HP**	**HC/LP**	**P value**
DNA methylation writers						
dnmt1a	0.48 ± 0.40	1.31 ± 0.20	0.002	1.32 ± 0.71	0.05 ± 0.03	0.007
dnmt3aa	0.63 ± 0.30	1.26 ± 0.37	0.009	0.96 ± 0.05	1.15 ± 0.09	0.001
dnmt3ab	0.48 ± 0.26	1.43 ± 0.50	0.002	1.74 ± 0.76	0.99 ± 0.08	0.061
dnmt3bb	0.65 ± 0.42	1.25 ± 0.27	0.014	1.58 ± 0.17	1.88 ± 0.45	0.172
DNA methylation erasers						
tet1	0.92 ± 0.45	1.18 ± 0.24	0.241	1.09 ± 0.18	0.95 ± 0.09	0.115
tet2	0.82 ± 0.12	1.34 ± 0.37	0.017	1.15 ± 0.18	1.08 ± 0.18	0.524
tet3	0.76 ± 0.31	1.28 ± 0.07	0.008	1.11 ± 0.20	1.10 ± 0.12	0.878

[1] An independent t-test was used to analyse the effects of different stimuli (HP/LC and LP/HC diets). HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein.

Table 4.7 mRNA levels of genes related to histone modifications in the liver and muscle of adult offspring fed with HC/LP diet for 4 weeks (week 26-29) (mean ± SD, n = 6)[1].

Broodstock	Liver			Muscle		
diets	LC/HP	HC/LP	P value	LC/HP	HC/LP	P value
H3K4me3 writers						
setd1a	2.50 ± 0.54	1.40 ± 0.20	0.001	1.65 ± 0.34	1.04 ± 0.14	0.005
setd1ba	0.91 ± 0.28	1.14 ± 0.21	0.128	1.26 ± 0.31	1.06 ± 0.24	0.256
kmt2a	0.61 ± 0.26	1.14 ± 0.34	0.013	1.79 ± 0.49	1.19 ± 0.18	0.029
kmt2ba	0.80 ± 0.25	1.19 ± 0.13	0.007	1.49 ± 0.37	1.27 ± 0.20	0.237
kmt2bb	0.64 ± 0.34	1.27 ± 0.27	0.005	1.31 ± 0.16	1.29 ± 0.16	0.775
H3K4me3 erasers						
kdm5a	0.98 ± 0.29	0.87 ± 0.24	0.567	1.25 ± 0.17	1.28 ± 0.08	0.745
kdm5ba	0.57 ± 0.33	1.26 ± 0.26	0.002	1.99 ± 0.43	1.12 ± 0.11	0.003
kdm5bb	0.99 ± 0.18	1.22 ± 0.26	0.102	1.53 ± 0.45	1.28 ± 0.25	0.264
kdm5c	0.81 ± 0.33	1.28 ± 0.21	0.015	1.26 ± 0.11	1.28 ± 0.08	0.711
riox1	0.36 ± 0.30	1.35 ± 0.22	<0.001	1.14 ± 0.06	1.41 ± 0.10	<0.001
H3K9me3 writers						
suv39h1b	1.84 ± 0.84	0.53 ± 0.24	0.011	3.72 ± 1.01	1.17 ± 0.31	<0.001
H3K9me3 erasers						
kdm4aa	0.59 ± 0.26	1.27 ± 0.48	0.012	1.25 ± 0.11	1.32 ± 0.06	0.170
kdm4ab	0.58 ± 0.45	1.39 ± 0.38	0.007	1.01 ± 0.18	1.18 ± 0.17	0.112
kdm4b	1.22 ± 0.22	1.44 ± 0.42	0.295	1.33 ± 0.22	1.44 ± 0.13	0.299
kdm4c	0.53 ± 0.33	1.43 ± 0.38	0.001	1.19 ± 0.11	1.47 ± 0.08	<0.001
H3K36me3 writers						
setd2	1.35 ± 0.20	1.29 ± 0.18	0.597	1.37 ± 0.20	1.21 ± 0.15	0.128
H3K9ac writers						
kat2a	0.56 ± 0.39	1.34 ± 0.26	0.002	1.69 ± 0.44	1.22 ± 0.13	0.046
kat2b	0.52 ± 0.20	1.31 ± 0.77	0.056	1.90 ± 0.79	0.35 ± 0.08	0.005
kat6a	0.62 ± 0.15	0.63 ± 0.09	0.839	1.57 ± 0.21	1.37 ± 0.21	0.130
gtf3c4	1.25 ± 0.34	0.33 ± 0.06	0.001	3.21 ± 1.53	1.11 ± 0.21	0.019
H3K9ac erasers						
sirt2	0.98 ± 0.23	1.04 ± 0.38	0.775	1.54 ± 0.19	1.42 ± 0.08	0.198
sirt5	0.81 ± 0.19	1.18 ± 0.24	0.016	1.22 ± 0.04	1.40 ± 0.08	<0.001
sirt6	0.51 ± 0.22	1.19 ± 0.33	0.002	1.16 ± 0.33	1.17 ± 0.25	0.946

Table 4.8 List of primers used for qRT-PCR of genes related to carbohydrate and intermediary metabolism in the liver and muscle

Genes	5'/3' Forward primer	5'/3' Reverse primer	SIZE (bps)	Access numbers
References gene				
ef1α	GCACGCTCTGCTGGCCTTT	GCGCTCAATCTTCCATCCC	250	AB075952
Hepatic glycolysis				
gck	GGGTGGTAGGATTTGGTGTG	TGCTGACACAAGGCATCTTC	186	XM003451020
pfklr	GACGAGCGAGTGGAGAAAAC	TGTCTTGATCCGAGGGAATC	162	XM003447353
pklr	AGGTACAGGTCACCCGTCAG	CATGTCGCCAGACTTGAAGA	164	XM005472622
Hepatic gluconeogenesis				
g6pca1	AGCGTTAAGGCAACTGGAGA	AAAAGCTAACAAGGCCAGCA	195	XM003448671
g6pca2	CTTCTTCCCCCTTTGGTTTC	AGACTCCTGCAGCTCCCATA	245	XM013273429
pck1	AAGCTTTTGACTGGCAGCAT	TGCTCAGCCAGTGAGAGAGA	162	XM003448375
pck2	TACGTCTTGAGCTCCCGTCT	CCTCCTGGATGATGCAAGTT	202	XM019354843
Hepatic lipogenesis				
fasn	AACCTGCTTCTCAAGCCAAA	CGTCACCCCTTGTTCTTTGT	222	XM013276809
g6pd	GTCACCTCAACCGGGAAGTA	TGGCTGAGGACACCTCTCTT	187	XM013275693
Hepatic amino acid catabolism				
alat	CACGGTGAAGAAGGTGGAGT	GCAGTTCAGGGTAGGAGCAG	200	XM005476466
asat	GCTTCCTTGGTGACTTGGAA	CCAGGCATCTTTCTCCAGAC	200	XM003451918
gdh	CGAGCGAGACTCCAACTACC	TGGCTGTTCTCATGATTTGC	203	XM003457465
Muscular glucose transport				
glut4	GAGGATGGACATGGAGAGGA	CAGGAAAAGCGAGACTACCG	235	JN900493
Muscular glycolysis				
hk1	CGTCGCTTAGTCCCAGACTC	TGACTGTAGCGTCCTTGTGG	235	XM019360229
hk2	CAGAGGGGAATTCGATTTGA	CCCACTCGACATTGACACAC	200	XM003448615
pfkma	AGGACCTCCAACCAACTGTG	TTTTCTCCTCCATCCACCAG	190	XM019349871
pfkmb	TTTGTGCATGAGGGTTACCA	CACCTCCAATCACACACAGG	208	XM003441476
pkma	TGACTGCTTCCTGGTCTGTG	CAGTGAAAGCTGGCAAATGA	249	XM005447626

*: from Yang et al. 2013

Table 4.9 List of primers used for qRT-PCR of genes related to epigenetic modification-related enzymes expressed in liver and muscle tissues

Genes		5'/3' Forward primer	5'/3' Reverse primer	SIZE (bps)	Accession numbers
Reference gene	*ef1*	GCACGCTCTGCTGGCCTTT	GCGCTCAATCTTCCATCCC	250	AB075952
DNA methylation writers	*dnmt1*	CTCACACTGCGCTGTCTTGT	ACAACGCTGAGAGAGCAAGC	188	XM_025906327.1
	dnmt3aa	CCAACAACCACGAGCAGGAA	TGCCGACAGTGATGGAGTCT	192	XM_005475084.4
	dnmt3ab	GCCGCAGCTTAGAGGACATC	CACACATGAGCACCTCTCGTC	189	XM_005477258.3
	dnmt3ba	GCTGCTGCAGATGCTACTGT	TTGCGCTGTTGTTGGCAAAG	186	XM_025901732.1
	dnmt3bb	TGCAGGAGTTCTTCGCCAAC	TGCCACATACTGACCCACCT	173	XM_025901790.1
DNA methylation eraser	*tet1*	CATCCAGTCCCAGCACAACC	CTCTATTTGGCGTGCGCTGA	194	XM_025897345.1
	tet2	GCAGCTGCCAACAAGAATGC	TGTTGCTGCTGCTGATGGAC	191	XM_005457001.3
	tet3	GCAAGCCAACCAACCAAACC	GATGTGTTGGCTCCGACCTG	177	XM_019365521.2
H3K4me3 writer (Histone Lysine methyltransferase)	*setd1a*	GGAACTCCGGTCTGGATGGT	CGAAGCTGCCCATCTGTGTT	172	XM_005468973.4
	setd1ba	AAGACAGGGAGGCAGCAGAA	CCTCAGGACTGGGAGGTCTG	198	XM_005470275.4
	kmt2a	AGAGCAGGAAAGCCAACAGC	CACTGGGCGTAGTTGTGGTC	178	XM_013274782.3
	kmt2ba	ACTCTGAGGGACCTGGAGGA	AGAGGAGGTGAAGCCGATCC	191	XM_013275905.3
	kmt2bb	GCTCCCGTCAGTGTGTCTTC	TCTGGCTCCAACCCAGTCAA	172	XM_013277028.3
H3K4me3 eraser (Histone lysine demethylases)	*kdm5a*	TCTGGCCACAGAGGAGTTGT	GTGACGTGGCTCTGCTGAAA	191	XM_005451728.4
	kdm5ba	TCTCAGAGCAGAGGGCATCC	GACCCGATGTCACACCTTGG	165	XM_003441348.2
	kdm5bb	CATCCCTGCCTACCTCCCAA	AAGGCTCCAGGTGGACTTGA	170	XM_003439103.5
	kdm5c	CTCTCCACCCTGGAGGCAAT	AGCTACCAGGCCCTCCAAAT	174	XM_005448517.4
	riox1	CCACCTGGCACACAAGGATT	TCCGGCTTCTACCACCACAT	192	XM_005475002.4
H3K9me3 specific writer	*suv39h1b*	TCCAACGCATGGCCTACAAC	CTTGATGTGCTGCAGTGTGC	197	XM_003459875.5
H3K9me3 specific eraser	*kdm4aa*	CGGATGCGAACCAAACCTCT	GGCTGGATCGACACCGTAAC	180	XM_005457300.3
	kdm4ab	TCTGTTCAGGGAGGCACACA	GCCTGTTGGCCCATCTGTTT	162	XM_005476068.4
	kdm4b	TGCTCGCTCTTCTGTCCGTA	AGCAGATCAGGAGGCTGGTT	196	XM_005453970.4
	kdm4c	CCTGCAGAGGAATGCAGTGG	GCACAGGTGCAATCTGGTGA	176	XM_005456806.2
H3K36me3 specific writer	*setd2*	AGGCAGCGATGACTTCAAGC	ATCTTGTGGCGTCCCACTCT	182	XM_019364854.2
H3K4me3 specific writer	*kat2a*	CACTGACCCTGCTGCTATGC	GTAGGCCAACCAGCCACATC	173	XM_025906390.1
	kat2b	GGCCTTTCATGGAGCCTGTG	CTCGCTCTCTGGAGGGTTGT	188	XM_003444058.3
	kat6a	CATCCCGTCCACTGCTTTCC	CCTGTTCACGCTACCACCAC	173	XM_005472980.3
	gtf3c4	CTTGTGGCGGTTCAAGCTCT	GGCTCGCCTTCCTCTTTCAC	174	XM_003440231.5
H3K9ac specific eraser	*sirt2*	GCGAGTCTAGTCAGCAGGGT	CCCAGAAGATCAGCTAGAGCCA	197	XM_003449264.5
	sirt5	ATTTGCCCAGGTGTGAGCAG	GAGCAAACATGGCTGCAGGA	177	XM_003457306.5
	sirt6	GTCAACCTGCAGTCGACCAA	TAACACCAGGCGGTGGTTTG	190	XM_003437978.5

*: from Yang et al. 2013

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CHAPTER V

Effects of nutritional programming by dietary carbohydrate in broodstock on utilization of high plant based diets in adult offspring

5.1 Abstract

Nutritional programming (NP) approach was demonstrated to provide protein sparing effects in Nile tilapia. To optimize the NP effects of CHO and promote the development of low-cost and high-quality feed, this study 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 (week 57-60) which obtained from parental LC/HP and HC/LP stimuli history. Our results showed that, the NP effects of broodstock modulated CHO response in offspring persisted into harvestable adult offspring including suppression gluconeogenesis (*g6pca1*, *g6pca2*) and amino acid catabolism (*asat*, *alat*) in liver and induction glycolysis (*pfkma*) in muscle. After offspring challenged with high plant-based diets, irrespective of the NP effects of CHO in broodstock, plant-based HC diet inhibited the growth performance, increased hepatic and muscular fat, glycogen and triglyceride as well as whole-body fat content in fish. Also, at molecular level, plant-based HC diet modulated CHO and its related metabolism and induced dynamic genes expression of enzymes associated with DNA methylation and histone methyl/acetylation in liver and muscle. These suggested that Nile tilapia could adapt our high plant-based diets without postprandial hyperglycemia and varied CHO level could induced epigenetics modifications. In addition, when irrespective of the challenge diets in adult offspring, broodstock HC history improved growth performance, pronounced CHO metabolic and induced dynamic epigenetic modification at molecular level in offspring. These demonstrated that parental NP effects could improve the efficient utilization of high plant-based diets in harvestable offspring. Overall, the NP concept could persist impact in Nile tilapia life cycle and epigenetics were accompanied by NP effects.

Keywords: Plant-based; Intermediary carbohydrate metabolism; Epigenetics; Long-term implication; Feed optimization.

5.2 Introduction

Nile tilapia (*Oreochromis niloticus*), one of the major species in global aquaculture, is a high-quality animal protein source for humans and ensures global food security. However, the expansion of large-scale farming has also increased the dependence on feed resources, of which protein demand was a central challenge (FAO, 2022). The main protein supplier of tilapia feed was fish meal, which were highly dependent on the Marine fishing industry in the world. Overfishing has led to resource limitation and price increase (Tacon and Metian, 2015; IFFO, 2023). Improving protein retention in tilapia diets and developing low-cost and high-quality diets are the key to sustainable tilapia farming (Lopez-Elias et al., 2019).

The nutritional programming (NP) approach was demonstrated to provide protein sparing effects in Nile tilapia. NP refers to the stimulation of high nutrients (such as carbohydrates (CHO)) during the critical early stages of life development forms memory (such as metabolic memory) and could persist to affect later life for a long term (Lucas, 1998). The NP methods of CHO in Nile tilapia were demonstrated at varied developmental stage including in yolk, fry and broodstock. Glucose injection in yolk of larvae and feeding HC in fry could persist impact on CHO and its related metabolism in juvenile and adult stage. When the fish challenged with HC diet again, the CHO response were more pronounced and growth performance improved (Kumkhong et al., 2020a, b; Srisakultiew et al., 2022). In addition, these NP effects in broodstock with dietary HC stimulation also could transmit to their offspring and persisted through early adult stage. More obvious metabolic and increased growth performance were observed in early adult offspring after challenged HC diet (Luo et al., 2025a, b, submitted). Therefore, NP strategy could improve the efficient utilization of CHO in Nile tilapia, providing the protein sparing effects.

Furthermore, epigenetics was found involve in the NP effects of CHO in Nile tilapia. Epigenetics refers to the genetic information that changes the conformation of chromatin through chemical modifications (such as methylation and acetylation) without changing the DNA sequence, and makes these changes retained for a long

time (Bird, 2007). The epigenetic modifications in fish could induce by dietary CHO feeding. In rainbow trout, HC diet led to global DNA hypomethylation and histone hypermethylation (H3K9me3, histone 3 lysine trimethylation) in liver of fish (Marandel et al., 2016). Moreover, HC diet induced difference mRNA expression trend of enzymes related to DNA methylation at differ feeding trial after fasting, demonstrating dynamic epigenetic modifications (Liu et al., 2022). When applied NP of CHO in Nile tilapia, HC stimulation history induced epigenetic modifications in later life. Such as high-glucose stimuli in larvae led to global DNA hypomethylation in liver and muscle of juvenile fish after challenged with CHO-M and CHO-H diet (Kumkhong et al., 2020a). Dietary HC in broodstock modulated the dynamic genes expression of enzymes related to DNA methylation, histone methylation and acetylation in liver and muscle of adult offspring after fed HC diet (Luo et al., 2025b, submitted). The occurred dynamic epigenetic modifications in fish later life might be due to the HC stimuli in Nile tilapia early developmental stage.

Again, the CHO source in challenge diets in NP effects for provide protein saving efficient in Nile tilapia were from rice flour. Which is a quality CHO source, but its price is not dominant among many available CHO sources. In addition, the plant-based source of the challenge diets was simple (soybean meal and rice only) (Kumkhong et al., 2020a, b; Srisakultiew et al., 2022; Luo et al., 2025b, submitted), which were not suitable for the development of aquafeed with NP strategy in Nile tilapia farming. Nile tilapia is a good user of CHO, therefore, explore the optimizing formula from high-quality and low-cost CHO resources, combined with the protein retention effect provided by the NP strategy, might be optimize the NP effect and promote the development of aquafeed, promoting economic benefits and sustainable development of aquaculture.

Thereby, in present study, 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. And the genes expression of enzymes associated with DNA/histone methylation and histone acetylation were measured to

explore whether the epigenetics involved in NP mechanism for long term.

5.3 Materials and methods

5.3.1 Ethics statement

All animal experiments were approved by the Animal Care and Use Committee of the Suranaree University of Technology (Nakhon Ratchasima, Thailand) (SUT-IACUC-012/2020).

5.3.2 Experiment diet, plan and fish management

Table 5.1 presents the ingredients and chemical compositions of the commercial and experimental diets, which included HC/LP and low-carbohydrate/high-protein (LC/HP) diets. Nutrient composition, including moisture, crude protein (CP), crude fat (CF), crude fibre, and ash, was determined following standard Association of Official Analytical Chemists (AOAC) methods (1990).

Fig. 5.1 illustrates the experimental design, encompassing the broodstock dietary stimulus and offspring challenge periods. During the broodstock experimental phase, mature female (mean body weight [BW]: 609.6 ± 28.4 g) and male (mean BW: 920 ± 16.7 g) Nile tilapia were obtained from an earthen pond at the University Farm, Suranaree University of Technology, Nakhon Ratchasima, Thailand, and transferred to a cement pond for natural breeding. The cement pond (5 × 10 m; water depth, 0.8 m) was partitioned into two sections (5 × 5 m; water depth, 0.8 m) for the HC/LP and LC/HP diet groups, with each group containing six females (n = 6 replications) and three males. To acclimate to the broodstock, they were fed a commercial diet (36% CP, 3.4% CF; Table 5.1) for four days. Subsequently, the broodstock were fed either the HC/LP or LC/HP diet for 14 days. Fish were fed twice daily at 9:00 a.m. and 3:00 p.m. at 1.5% of their body weight.

After 14 days of feeding, fertilized eggs were collected from the female broodstock (two treatments, n = 6 replications) and transferred to hatching trays with circulating water. At seven days post-hatching, larvae were gently transferred to individual cages (one cage per replication; dimensions: 0.4 × 0.4 × 0.6 m) placed in a cement pond (2 × 2 × 0.8 m; water depth 0.45 m) and fed a commercial diet (Table 5.1). To control for potential confounding effects of sex differences on fry growth, the fry diet (40% CP, 8% CF) was supplemented with 60 mg/kg of 17α-methyltestosterone

(17-MT), administered five times daily (at 9:00, 11:00, 13:00, 15:00, and 17:00) for 28 days (Boonanuntanasarn et al., 2018b).

From weeks 5 to 56, fish were transferred to cement ponds (1 pond per replication; dimensions: 2 × 2 × 0.8 m) and fed a commercial diet ad libitum twice daily at 9:00 am and 4:00 pm. The feed composition during this period was as follows: 40% CP and 8% CF from weeks 5-7, 32% CP and 4% CF from weeks 8-29 and 30% CP and 4% CF from week 30-56 (Fig. 5.1). During the growth phase, fish were weighed every four weeks to assess their growth performance, and feed intake was recorded.

To evaluate the effects of parental HC/LP dietary history on CHO metabolism in adult offspring, the offspring of HC/LP- and LC/HP-fed broodstock were challenged with the plant-based medium carbohydrate (PBHC) and plant-based high carbohydrate (PBHC) diets (Table 5.1) for four weeks (weeks 57–60; Fig. 5.1). For this challenge phase, 6 fish per replication (mean BW: 1485.5 g) were randomly selected and placed in cement ponds (2 × 2 × 0.8 m). The fish were then fed the HC/LP diet at 1% body weight daily at 9:00 am and 4:30 pm, for four weeks.

During the experimental period, the fish were reared in dechlorinated tap water with continuous aeration. A flow-through water exchange system was employed to replace one-third of the water twice a week. Air and water temperatures were recorded daily, ranging from 25 to 28°C and 28 to 32°C, respectively. Dissolved oxygen and pH levels were measured weekly using dissolved oxygen and pH meters, respectively, ensuring that values remained within acceptable ranges of 7.10–8.68 mg/L and 4.93–6.85, respectively.

5.3.3 Fish sampling

To determine the effects of the broodstock NP history and high plant-based diets on adult offspring, three adult offspring fish from each replicate were sampled at week 56 (before challenge) and week 60 (after challenge with PBMC and PBHC diets), and 5 h after the last meal. The fish were euthanised with 1.0% clove oil and blood was collected from the caudal vein using a hypodermic syringe containing K_2EDTA (1.5 mg/mL of blood) as an anticoagulant. The blood was centrifuged at 10,000 × g for 5 min at 4°C to obtain plasma, which was stored at −80°C for subsequent plasma metabolite analysis.

After blood collection, the livers of two fish from each replicate were

dissected and weighed to calculate the hepatosomatic index (HSI). Liver and muscle samples were then collected, snap-frozen in liquid nitrogen, and stored at −80°C for later analysis of nutritive composition and extraction of total RNA for CHO metabolic response and epigenetic modification analyses. Additionally, one whole fish from each replicate was sampled for the analysis of CP, CF, and ash content.

5.3.4 Blood chemistry analysis

Two fish from each replicate (n = 6) were used to determine the plasma metabolites, including glucose, triglycerides, cholesterol, protein, and blood urea nitrogen (BUN). Plasma glucose levels were determined using the GOD-PAP method (Barham and Trinder, 1972). Plasma triglyceride content was measured using 3-sulfopropyl-m-anisidine (Bucolo and David, 1973) and plasma cholesterol was quantitatively analysed using the cholesterol oxidase phenol-aminophenazone method (Flegg, 1973). Plasma protein concentrations were evaluated using the biuret method (Gornall et al., 1949), whereas BUN levels were detected using a modified indophenol colorimetric method (Weatherburn, 1967).

5.3.5 Chemical composition, glycogen, and triglyceride analysis

Fish liver, muscle, and whole-body samples (two fish per replicate, n = 6), collected at weeks 56 and 60, were analysed for their chemical composition, including protein, fat, and ash, following the methods of the AOAC (1990). The glycogen content in the liver (100 mg) and muscle (200 mg) was measured using the hydrolysis technique described by Good et al., (1933) and Kumkhong et al., (2020a).

For triglyceride determination, 100 mg of liver and muscle tissue was homogenised with 1 mL of 5% IGEPAL and 2.8-mm glass beads, then heated at 90°C for 10 min. After cooling to room temperature (24-26 °C), the samples were centrifuged at 10,000 × g at 4°C for 10 min, and the supernatant was collected. Triglyceride levels were determined using a triglyceride kit (catalogue number: BLT00059, Erba Lachema s.r.o., Karasek Brno, Czechia), following the manufacturer's instructions (Luo et al., 2025, submitted).

5.3.6 Total RNA extraction, complementary DNA (cDNA) synthesis, and qRT-PCR of glucose metabolism and enzymes related to epigenetics genes

Liver (50 mg) and muscle (100 mg) tissue samples were collected from 12 fish per experimental group (two fish per replicate) for total RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's

recommendations. The quantity and quality of the total RNA were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA) and 1% agarose gel electrophoresis, respectively. cDNA was synthesised from 1 μg of RNA via reverse transcription using the SuperScript III RNAseH-reverse transcriptase kit (Invitrogen) and random primers (Promega, Madison, WI, USA), according to the manufacturer's protocol. Each sample was reverse transcribed in duplicate.

Relative mRNA expression levels in the liver and muscle tissues were analysed via quantitative real-time PCR using a Roche LightCycler 480 system (Roche Diagnostics, Neuilly-sur-Seine, France). Each PCR assay included duplicate samples (reverse transcribed and PCR-amplified copies) and negative controls (samples without the reverse transcriptase or cDNA templates). Relative quantification of target gene expression was performed using Roche Applied Science E-Method (Pfaffl, 2001). mRNA expression levels in each tissue were normalised to the expression of ef1α. PCR efficiency was determined using serial dilutions of cDNA, with efficiency values ranging from 1.8 to 2.0, based on the slope of the standard curve.

Tables 5.10 and 5.11 list the primer sequences used for the real-time RT-PCR. Table 5.10 includes the primers for carbohydrate intermediate metabolism-related genes expressed in the liver and muscle. These include liver glycolysis genes (glucokinase [gck]; phosphofructokinase [pfklr]; pyruvate kinase [pklr]), gluconeogenesis genes (glucose-6-phosphatase [g6pca1, g6pca2]; phosphoenolpyruvate carboxykinase, cytosolic [pck1], and mitochondrial [pck2]), lipogenesis genes (fatty acid synthase [fasn]; glucose-6-phosphate dehydrogenase [g6pd]), and amino acid catabolism genes (glutamate dehydrogenase [gdh]; alanine aminotransferase [alat]; aspartate aminotransferase [asat]). In the muscle tissue, the detected genes included those for glucose transport (glut4) and glycolysis (hexokinase I/II [hk1, hk2], phosphofructokinase [pfkma], and pyruvate kinase [pkma]).

Table 5.11 shows the primers for epigenetic modification-related genes expressed in liver and muscle tissues. These include DNA methyltransferases (dnmt1a, dnmt3aa, dnmt3ab, dnmt3ba, and dnmt3bb), TET methyl cytosine dioxygenases (tet1, tet2, and tet3), histone (H) 3 lysine (K) 4 trimethylation (H3K4me3) writers (SET domain-containing 1A/1B [setd1a and setd1ba]; lysine methyltransferase 2A [kmt2a]; histone-lysine N-methyltransferase 2B [kmt2ba andkmt2bb]), H3K4me3 erasers (lysine

demethylase 5A/5BA/5BB/5C [kdm5a, kdm5ba, kdm5bb, and kdm5c]; bifunctional lysine-specific demethylase and histidyl hydroxylase [riox1]), H3K9me3 writers (histone lysine N-methyltransferase [suv39h1b]), and erasers (lysine demethylase 4AA/4AB/4B/4C [kdm4aa, kdm4ab, kdm4b, and kdm4c]), H3K36me3 writers (SET domain-containing 2 [setd2]), H3K9ac writers (lysine acetyltransferase 2A/2B/6A [kat2a, kat2b, and kat6a]; general transcription factor IIIC subunit 4 [gtf3c4]), and erasers (sirtuin 2/5/6 [sirt2, sirt5, and sirt6]).

5.3.7 Statistical analysis

All data were analysed using SPSS for Windows version 22 (SPSS Inc., Chicago, IL, USA). An independent samples t-test was conducted to evaluate differences between the LC/HP and HC/LP groups. Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups. Statistical significance was set at $P < 0.05$.

5.4 Results

5.4.1 Growth performance of harvestable adult offspring fed with commercial diets and after challenged with high plant-based diets

Hatchlings from broodstock fed with LC/HP and HC/LP diets were cultured with commercial diets until harvestable adulthood (week 56). Fig.5.1 demonstrated the body weight of two groups experimental fish, there were no significant difference between two groups at week 56. However, after fish challenged with plant-based MC and HC diets, the growth performance was significantly changed (Table 5.2). When irrespective of broodstock stimulation history, comparing plant-based MC diet, dietary plant-based HC significantly inhibited final body weight (FW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR) and increased feed conversion ratio (FCR) in fish ($P < 0.05$) (Table 5.2). When irrespective of challenge diets in harvestable adult offspring, compared to the broodstock-LC/HP history, dietary HC/LP in broodstock improved the final body weight, WG, ADG, SGR and decreased FCR in offspring fish ($P < 0.05$) (Table 5.2).

5.4.2 Dietary HC in broodstock effect on intermediary CHO metabolism in harvestable adult offspring fed with commercial diets

To determine the effects of broodstock NP history on intermediary CHO metabolism in harvestable adult offspring, the plasma metabolites and proximate compositions of liver, muscle and whole body were measured in offspring at week 56 (before challenge). The plasma metabolites were showed on Table 5.3, when compared with broodstock LC/HP diet, dietary HC in broodstock no effect on plasma glucose, triglyceride, blood urea nitrogen (BUN), cholesterol and total protein in offspring fish (P >0.05) (Table 5.3). Table 5.4 demonstrated the proximate composition of offspring fish, there were no significant difference of hepatic and muscular protein, fat, ash, glycogen and triglyceride content between two experimental groups (P >0.05) (Table 5.4). The protein, fat and ash content in whole body also no significant difference between two experimental groups (P >0.05) (Table 5.4).

In addition, at molecular level, comparing broodstock LC/HP diet, dietary HC in broodstock significantly induced suppression gluconeogenesis (*g6pca1, g6pca2*) and amino acid catabolism (*asat, alat*) in liver and induction glycolysis (*pfkma*) in muscle of offspring (P <0.05) (Table 5.5). There were not significantly observed in hepatic glycolysis (*gck, pfklr, pklr*) and lipogenesis (*fasn, g6pd*) between two groups (P >0.05) (Table 5.5).

5.4.3 Intermediary CHO metabolism of harvestable adult offspring after challenged with high plant-based diets

To examine the effects of broodstock NP history and challenge diets on CHO and its related metabolism in harvestable adult offspring, the plasm metabolites, proximate compositions and related metabolic pathway were analyzed after offspring fish challenged with plant-based MC and HC diets. The plasma metabolites demonstrated in Table 5.3, when irrespective of broodstock stimulation history, compared with plant-based MC diet, dietary plant-based HC significantly decreased plasma total protein in fish (P <0.05) (Table 5.3). The plasma glucose, triglyceride, BUN and cholesterol in fish were no significant difference between experimental groups (P >0.05) (Table 5.3). Moreover, when irrespective of challenge diets in offspring fish, dietary HC in broodstock increased plasma glucose and triglyceride in fish, comparing fish of LC-fed broodstock (P <0.05) (Table 5.3). When analyzed the interaction of

broodstock history and challenge diets in offspring, broodstock HC stimulation history and plant-based HC challenge feed significantly decreased plasma protein in offspring fish ($P < 0.05$) (Table 5.3).

Table 5.4 showed the proximate composition of liver, muscle and whole body in fish. Our results showed that, dietary plant-based HC significantly decreased hepatic protein while increased hepatic and muscular fat, glycogen and triglyceride as well as whole body fat content in offspring fish irrespective of the broodstock simulation history ($P < 0.05$) (Table 5.4). When irrespective of challenge diets in offspring, broodstock HC stimulation history significantly increased hepatic and muscular fat, glycogen and triglyceride as well as whole body fat content in offspring fish ($P < 0.05$) (Table 5.4). In addition, broodstock HC stimulation history and plant-based HC challenge diet enhanced triglyceride in liver while reduced protein in muscle in offspring fish ($P < 0.05$) (Table 5.4). Also, broodstock LC stimulation history and plant-based HC challenge diet lower the protein in liver of offspring fish ($P < 0.05$) (Table 5.4).

The related CHO metabolic pathway in fish were exhibited in Table 5.5. The results demonstrated that, dietary plant-based HC significantly induced 1) induction hepatic glycolysis (*gck*) and lipogenesis (*fasn*, *g6*pd), 2) suppression hepatic gluconeogenesis (*g6pca1, g6pca2, pck1*) and amino acid catabolism (*asat, alat*), 3) induction muscular glucose transport (*glut4*) and glycolysis (*hk1, pfkmb, pkma*) in offspring fish irrespective of the broodstock simulation history ($P < 0.05$) (Table 5.5). Moreover, broodstock HC stimulation history significantly induction hepatic *g6pd* and muscular *hk1* and *pfkma*, but suppression hepatic *g6pca1, g6pca2, pck1, asat, alat* and *gdh* in offspring fish irrespective of the challenge diets ($P < 0.05$) (Table 5.5). Finally, broodstock HC stimulation history and plant-based HC challenge diet downregulation *g6pca2*, *pck1* and *asat* while upregulation *fasn* in liver of offspring fish ($P < 0.05$) (Table 5.5). Additionally, broodstock LC stimulation history and plant-based HC challenge diet downregulation *g6pca1* and *pck2* in liver of offspring fish ($P < 0.05$) (Table 5.5).

5.4.4 mRNA levels of enzymes related to DNA methylation, histone methylation and acetylation of harvestable adult offspring fed with commercial diets and after challenged with high plant-based diets

The effects of broodstock NP history and difference level CHO challenge diets on genes expression of enzymes related to epigenetic modifications in harvestable

adult offspring were demonstrated at before and after challenged experiment feeds. Our results revealed that, before offspring fish challenged with experimental diets (week 56), when compared with broodstock LC stimulation history, dietary HC in broodstock no effect on the mRNA level of DNA methylation, histone methylation and acetylation writers and their erasers in liver and muscle of 56-weeks offspring fed with commercial diets (P >0.05) (Table 5.6, Table 5.7, Table 5.8 and Table 5.9).

After harvestable adult offspring challenged with plant-based MC and HC diets, we found that, when irrespective of the broodstock simulation history, dietary plant-based HC significantly induction DNA methylation writer (*dnmt1a, dnmt3aa, dnmt3ab, dnmt3ba, dnmt3bb*) and its erasers (*tet1, tet2, tet3*) in muscle, however, suppression *dnmt3aa, tet1, tet2* and *tet3* in liver of adult fish (P <0.05) (Table 5.6 and Table 5.7). Additionally, irrespective of the challenge diets, broodstock HC stimulation history significantly induced induction *dnmt3aa, dnmt3ab and dnmt3ba* in liver while suppression *dnmt3aa* and *tet1* in muscle of adult offspring (P <0.05) (Table 5.6 and Table 5.7). Comparing fish of LC-fed broodstock, dietary plant-based HC significantly downregulated *tet1* in liver of fish from HC-fed broodstock (P <0.05) (Table 5.6). Furthermore, when analyzed the interaction of broodstock NP history and plant-based challenge diets in offspring fish, we found that 1) broodstock LC stimulation history and plant-based HC challenge diet downregulation the *dnmt3aa, dnmt3ba* and *tet2* in liver, 2) broodstock HC stimulation history and plant-based MC challenge diet downregulation *dnmt3bb* in liver, 3) broodstock HC stimulation history and plant-based HC challenge diet downregulation *tet3* in liver, 4) broodstock LC stimulation history and plant-based HC challenge diet upregulation *dnmt3ab* and *tet3* in muscle (P <0.05) (Table 5.6 and Table 5.7).

In addition, Table 5.8 and Table 5.9 showed the mRNA level of enzymes related to histone methylation and acetylation in liver and muscle of offspring fish. When irrespective of the broodstock simulation history, dietary plant-based HC significantly induction 1) H3K4me3 writer (*setd1a, setd1ba, kmt2a, kmt2ba, kmt2bb*) and its erasers (*kdm5a, kdm5ba, kdm5bb, kdm5c, riox1*), 2) H3K9me3 writer (*suv39h1b*) and its eraser (*kdm4aa, kdm4ab, kdm4b, kdm4c*), 3) H3K36me3 writer (*setd2*) and 4) H3K9ac writers (*kat2a, kat2b, kat6a, gtf3c4*) and its erasers (*sirt6*) in muscle of fish (P <0.05) (Table 5.9). In liver, dietary plant-based HC significantly induced suppression

setd1akdm5a, kdm5ba, kdm5bb, kdm5c, sirt5 and induction *kdm4aa, setd2, kat2b, gtf3c4* in fish (P <0.05) (Table 5.8). Moreover, When irrespective of the challenge diets in offspring fish, broodstock simulation history induction *kmt2a, kmt2ba, kmt2bb, kdm5a, kdm5bb, riox1, kdm4aa, kdm4b, kdm4c, kat2a, kat2b, gtf3c4, sirt6* in liver and *kat2a* in muscle, while suppression muscular sirt5 of fish (P <0.05) (Table 5.8 and Table 5.9). Besides, broodstock LC stimulation history and plant-based MC challenge diet upregulation hepatic *setd1a* and downregulation *kdm4c, kat2b, setd1a, kmt2ba, kdm5a, kdm5ba, kdm5c* and *kat6a* in liver and muscle of offspring fish (P <0.05) (Table 5.8 and Table 5.9).

5.5 Discussion

The present study continued to deduce that the long-term effects of parental HC stimulation on Nile tilapia offspring could persist into harvestable adulthood. Although harvestable adult offspring challenges were different from HC diets in previous studies (Luo et al., 2025b), the NP strategy also promoted growth performance and modulated CHO-related metabolism in offspring fed the high plant-based diets, providing a protein sparing effects. In addition, the dynamic epigenetic modifications including DNA/histone methylation and histone acetylation at molecular level were observed in the long-term NP effects of CHO.

5.5.1 mRNA levels of enzymes related to DNA methylation, histone methylation and acetylation of harvestable adult offspring fed with commercial diets and after challenged with high plant-based diets

Nile tilapia is a good user of dietary CHO. Increasing dietary CHO to reduce protein content is the superior solution to reduce feed cost and then improve economic efficiency (Watanabe, 2002; Kamalam et al., 2017). However, intensive studies have shown that low-protein and high-CHO in diet decreased the growth performance of fish. For instance, in juvenile Nile tilapia, fish fed 40% starch (STA, from maize meal and corn starch meal)/ 21% protein (CP) diet for 45 days appeared lower growth performance than that fish fed with 16% STA/34% CP, 24% STA/31% CP and 32% STA/26% CP diet (Azaza et al., 2015). In adult Nile tilapia, compared with 14%CHO (from dextrin)/63%CP and 32%CHO/45%CP diet, fish fed 50%CHO /27%CP diet for 90days or form fist feeding to week 40 showed lowest growth performance

(Boonanuntanasarn et al., 2018a,b). Therefore, if the efficient utilization of CHO for energy source in the diet is improved, protein sparing effects can be provided, and nutritional programming strategy could achieve these (Hou and Fuiman, 2020). NP effects of CHO in Nile tilapia early developmental stage demonstrated that the utilization of CHO was improved in fish later life at juvenile and adult stage. For example, glucose injection into yolk of larvae and HC feeding in fry enhanced the growth performance in juvenile and adult fish when received HC diet again (Kumkhong et al., 2020a,b; Srisakultiew et al., 2022). In addition, parental HC stimulation also appeared the NP effects in their offspring. Although dietary HC in Nile tilapia broodstock no effect on body weight in their juvenile offspring but significantly improved the growth performance of early adult offspring (weeks 26-29) when they were fed an HC diet (Luo et al., 2025a, b, submitted). In this study, our results showed that, irrespective of the NP effects of CHO in broodstock, offspring fish fed with plant-based MC diet for 4 weeks (week 57-60) showed better growth than that fish fed plant-based HC diet. These results were consistent with previous data on the effects of different levels of CHO/CP diet on the growth in adult tilapia (Boonanuntanasarn et al., 2018a,b; Kumkhong et al., 2020b; 2021). Noteworthy, dietary HC in broodstock significantly improved growth performance in adult offspring, irrespective of whether they were challenged with a plant-based MC or HC diet. These findings exhibited that the beneficial effects of parental NP approach on efficient utilization of CHO in offspring persisted through harvestable adult stage. Overall, the NP of CHO strategy demonstrated positive influenced throughout the life cycle of Nile tilapia. Furthermore, the use of high plant-based sources in this study highlights the potential for developing low-cost, high-quality feeds through NP approaches.

5.5.2 Dietary HC in broodstock persisted long-term effect on intermediary CHO metabolism in harvestable adult offspring

The effective NP effects were reflected in the enhancement of intermediary CHO metabolism pathways and sustained for a long term. The NP effects of CHO in Nile tilapia parents demonstrated a long-term modulation of CHO metabolism in their offspring and persisted through early adult stage. The sustained parental NP effects on CHO response in offspring including 1) increased fat (larvae), glycogen and triglyceride content in hatched-larvae and 7days-fry, 2) increased HSI and

hepatic triglyceride (juvenile only) in juvenile and adult offspring fish. Also, these persist NP effects were observed at molecular level, dietary HC stimuli in broodstock induced 1) upregulation of glycolysis (*pklr, hk2, pfkma, pfkmb*) and glucose transport (*glut4*), downregulation of amino acid catabolism (*alat, gdh*) in hatched-larvae and 7days-fry, 2) downregulation of hepatic gluconeogenesis (*g6pca1*, juvenile only) and *alat*, upregulation of muscular *pfkma* and *pfkmb* in juvenile and adult offspring (Luo et al., 2025a,b, submitted). Sequentially, this study revealed that parental NP effects of CHO influenced on CHO metabolic pathway in offspring persisted until harvestable adult stage. The results presented suppression of hepatic gluconeogenesis (*g6pca1* and *g6pca2*) and amino acid catabolism (*asat* and *alat*), and induction of muscular glycolysis (*pfkma*) in 56-weeks offspring. These findings align with the CHO response observed in juvenile and adult Nile tilapia that had a history of hyperglucidic stimulation during the larval (via glucose injection into the yolk) and fry (via HC feeding) stages (Kumkhong et al., 2020a,b;2021; Srisakultiew et al., 2022). These suggested that the sustained NP effects in Nile tilapia are independent of the stimulation method. Also, in other fish species, such as rainbow trout, Chinese perch (Siniperca Chuatsi), zebrafish, European sea bass and yellow catfish (Tachysurus fulvidraco), sustained NP effects of CHO were observed later in life, despite varying stimulation methods and stimuli at varied developmental stages. (Callet et al., 2021a,b; Fang et al., 2014; Lu et al., 2022; Rocha et al., 2015; Xiao et al., 2020; Xu et al., 2024; Zambonino-Infante et al., 2019). Combined, these findings highlight the robustness and universality of the sustained regulatory effects of NP on CHO metabolism across a variety of fish species.

5.5.3 Broodstock NP history modulated the intermediary CHO metabolism in harvestable adult offspring after challenged with plant-based MC and HC diet

Feeding Nile tilapia with a high carbohydrate diet could induce CHO-related metabolic responses in fish organs, albeit with varied CHO sources and stage of fish. For example, in juvenile fish, fed with 40%/21% starch/protein (starch from maize meal and corn starch meal) diet for 45 days enhanced HSI and hepatic enzyme activities (G6PD, 6PG-DH, PFK-1 and PK), when compared with fish fed with 16%/34%, 24% /31% and 32% /26% starch/protein diet (Azaza et al., 2015). Feeding adult fish with CHO-H (from dextrin) diet for 45 and 90 days increased plasma glucose, triglyceride

and cholesterol, upregulation hepatic glycolysis (45 day; *gck*) and downregulation hepatic amino acid metabolism (45 days; *alat, gdh*, 90 days; *asat*), comparing with fish fed with CHO-M and CHO-L diet (Boonanuntanasarn et al., 2018b). In addition, when compared with CHO-M (form rice flour) diet, CHO-H diet increased plasma triglyceride, HSI, hepatic and muscular fat content as well as upregulation hepatic lipogenesis (*fasn, g6pdh*) and downregulation hepatic acid catabolism (*asat, alat*) in juvenile and adult fish (Kumkhong et al., 2020a,b; 2021). In this study, irrespective of broodstock stimuli history, plant-based HC (from broken rice, casava) diet modulated CHO and its related metabolism in harvestable adult fish. Our results showed that, compared with dietary plant-based MC, dietary plant-based HC decreased plasma and hepatic protein while increased HIS, hepatic and muscular fat, glycogen and triglyceride as well as whole body fat content in harvestable adult fish. Meanwhile, at molecular level, plant-based HC induced 1) induction hepatic glycolysis (*gck, pfklr*) and lipogenesis (*fasn, g6pd*), 2) suppression hepatic gluconeogenesis (*g6pca1, g6pca2, pck1*) and amino acid metabolism (*asat, alat*), and 3) induction muscular glucose transport (*glut4*) and glycolysis (*hk1, pfkma, pkma*) in adult fish. These finding suggested that Nile tilapia could adapt well to different HC diets with varied CHO sources without postprandial hyperglycemia. Overall, the adaptability of Nile tilapia to various CHO resources may serve as a scientific information for search of more low-cost CHO resources to design cost-effective and nutritionally optimized aquafeed formulations for Nile tilapia aquaculture.

A complete NP mechanism, the history of NP effects in addition to persist for a long term, the associated CHO metabolism would be more pronounced when challenged with the CHO diet at a later stage. For example, in pervious study, NP effects of glucose injection into yolk of larvae and HC feeding in fry histories also induced more obvious metabolic in later at juvenile and adult stage when they were received CHO diets (CHO-M and CHO-H) again. The results were present as NP histories increased plasma glucose level, increased hepatic and muscular glycogen content, upregulation hepatic glycolysis (*gck, pklr*) and muscular glut4, hk2 and downregulation hepatic *g6pca1, asat* in juvenile and adult fish with challenged CHO diet (Kumkhong et al., 2020a, 2021; Srisakultiew et al., 2022). Additionally, NP of CHO in broodstock also strengthen the CHO response in their juvenile and adult offspring when they were

challenged with an HC diet. The CHO response in juvenile and adult offspring were observed including 1) increased plasma glucose and triglyceride, 2) increased hepatic and muscular fat, glycogen and triglyceride content, 3) induction *gck, pklr, fasn, g6pd* and suppression *pkc2* in liver, 4) induction muscular *glut4, hk1and hk2* (Luo et al., 2025a, b submitted). In this study, the NP effects of HC in broodstock also could strengthen the CHO and its related response in harvestable adult offspring when challenged with high plant-based diets. Our results showed that, irrespective of adult offspring challenged with plant-based MC or HC diet, dietary HC stimuli broodstock led to 1) increased plasma glucose, triglyceride and decreased plasma protein level, 2) increased hepatic fat, glycogen and triglyceride content, 3) increased whole-body fat content, 4) suppression hepatic gluconeogenesis (*g6pac1, g6pca2, pck1*) and amino acid catabolism (*asat, alat, gdh*), 5) induction hepatic lipogenesis (*g6pd*) and 6) induction muscular glycolysis (*hk1, pfkma*) in offspring fish, comparing with fish of LC-fed broodstock. Taken together, the improvement of the CHO metabolic capacity of Nile tilapia by the NP effect on the use of the CHO diet is not limited to the NP methods and CHO source, these could be suggested to Nile tilapia are good NPs strategy implementers. However, parental NP effects of CHO varied among fish species. In rainbow trout, feeding HC in male broodstock increased whole-body and digestive tract lipid content and induction hepatic cholesterol biosynthesis (*hmgcs1, mvdaa, mvdab*) in juvenile offspring when challenged with HC diet (Callet et al., 2022). In zebrafish, HS (53% CHO/25% CP) diet stimulus in broodstock decreased plasma glucose and suppression hepatic *pck1* in adult offspring feeding with HC (35%CHO/43%CP) diet (Lu et al., 2022). These suggested that under the influence of NP effects, carnivorous fish respond to relevant lipid metabolism and glucose regulation with HC feed challenging. Combined, the regulatory pathways of NP effects in fish species are complex. Further research to understand the relationship between epigenetic mechanisms and NP effects is conducive to optimizing the application of NP strategies in aquaculture.

Deeply, selection of appropriate interactions of historical factors and challenge diets on intermediary CHO metabolism in later life is one way to optimize NP effects. In pervious study, with 0.85% NaCl / 2M glucose injection history and CHO-M / CHO-H challenge diets in juvenile and adult Nile tilapia fish, glucose injection

history significantly increased fat, induction lipogenesis (*fasn*) and suppression amino acid catabolism (*asat*) in liver and induction muscular glycolysis (*pkma*) of fish fed with CHO-H diet (Kumkhong et al., 2020a, 2021). In addition, with the histories of early feeding dietary LC and HC in fry and the challenge diets of CHO-M and CHO-H in adult stage, HC feeding history significantly increased hepatic fat and induction muscular glucose transport (*glut4*) in adult fish challenged with CHO-H diet (Kumkhong et al., 2020b). In this study, when we analyze the interaction between parental LC and HC stimulus history and adult offspring challenge diets (plant-based MC and HC), our results showed that, parental HC history significantly increased triglyceride content, induction lipogenesis (*fasn)* and suppression gluconeogenesis (*g6pca2, pck1*) and amino acid catabolism (*as*at) in liver of harvestable adult offspring when fed with plant-based HC diet. Taken together, comparing with the findings of HC stimuli history (including glucose injection in larvae, HC feeding in fry and dietary HC in broodstock) induced more pronounce CHO response in fish at juvenile and adult stage after challenged CHO-M/CHO-H and plant-based MC/HC diets, the response of CHO related metabolic of the interaction of HC history and HC challenge diet in fish were not same obvious. These findings suggested that the suitable CHO level of challenge feed in fish later life could optimize the benefits of the NP of CHO approach. And then combine with the excellent and cheap CHO resources, the application advantage of NP effects in aquatic animals will be magnified.

5.5.4 Broodstock NP history regulated the mRNA level of enzymes related to DNA methylation and histone modifications in harvestable adult offspring after challenged with plant-based MC and HC diet

Dietary CHO with varied level could regulate the mRNA level of enzymes related to epigenetic modifications such as DNA methylation and histone modifications in fish. For instance, in carnivorous Mandarin Fish, feeding HC diet (8%CHO) led to upregulation H3K4 histone methyltransferase (*setd1b*) in liver when compared with fish fed artificial diet (You et al., 2020). In addition, in rainbow trout, different level of CHO could induce dynamic DNA methylation at molecular level. After fasting, dietary HP-NC and MP-HC induction DNA methyltransferases (*dnmt1b, dnmt3ab2*; MP-HC only, *dnmt3ab1, dnmt3ba1, dnmt3bbb*) and DNA demethylases (*tdgaa, tdgab*) in liver at first feeding trial 1. However, at feeding trial 2, after feed deprived, dietary MP-HC

suppression dnmt3aa and DNA demethylases (*tet1a, tet1b, tet2a/b, tdgbb*) in liver (Liu et al., 2022). In this study, our results demonstrated that, irrespective of broodstock stimulation history, different level of dietary CHO (plant-based MC and HC) modulated the genes expression of enzymes related to DNA/histone methylation and histone acetylation in harvestable adult offspring. There results presented that, compared with plant-based MC diet, dietary plant-based HC induction muscular *dnmt1a, dnmt3aa, dnmt3ab, dnmt3ba, dnmt3bb, tet1, tet2 and tet3* while suppression hepatic *dnmt3aa, tet1, tet2 and tet3* in adult fish. Furthermore, dietary plant-based HC upregulation 1) H3K4me3 writers (*setd1a, setd1ba, kmt2a, kmt2ba, kmt2bb*) and its erasers (*kdm5a, kdm5ba, kdm5bb, kdm5c, riox1*), 2) H3K9me3 writer (*suv39h1b*) and its erasers (*kdm4aa, kdm4ab, kdm4c*), 3) H3K36me3 writer (*se*td2), 4) H3K9ac writers (*kat2a, kat2b, kat6a, gtf3c4*) and its eraser (*sirt6*) in muscle, downregulation *setd1a, kdm5a, kdm5ba, kdm5bb, kdm5c, setd2, sirt5* and upregulation *kdm4aa, kat2b, gtf3c4* in liver. These findings suggested that dietary CHO could induce dynamic epigenetic modifications at molecular level in adult Nile tilapia and the varied expression level were among the organs. Combined, the heritable epigenetic modifications induced by dietary CHO stimulation seem to provide the basis for the long-term persistence of NP effects of CHO in fish.

Because in previous study, we found that epigenetic modifications were involved in the NP effects of CHO. There were demonstrated that the history of glucose injection into yolk reserve larvae induced DNA hypomethylation in liver and muscle of juvenile stage fish when fed with CHO-M and CHO-H diets (Kumkhong et al., 2020a). In addition, NP of CHO in broodstock also modulated epigenetic modifications at molecular level in early adult offspring. Dietary HC stimuli in parents induced 1) induction DNA methylation writers and its erasers in liver, 2) suppression *dnmt1a* and induction *dnmt3aa* in muscle, 3) induction H3K4me3, H3K9me3 and H3K9ac writers and their erasers in liver and 4) suppression *kmt2a, kdm5ba, suv39h1b, kat2a, kat2b*, and induction *kdm4, sirt5* in muscle of adult offspring after challenged HC feed for 4 weeks (week 26-29) (Luo et al., 2025b). In this study, NP effects on the modulatory mRNA level of enzymes associated with DNA methylation and histone modifications were also observed in harvestable adult offspring, irrespective of they challenged with plant-based MC or HC diet for 4 weeks (week 57-60). Our results showed that, parental HC stimulation history led to 1) upregulation *dnmt3aa, dnmt3ab, dnmt3bb* and

downregulation *tet1* in liver, 2) downregulation *dnmt3aa* and upregulation *tet1* in muscle, 3) upregulation *kmt2a, kmt2ba, kmt2bb, kdm5a, kdm5bb, riox1, kdm4aa, kdm4b, kdm4c, setd2, kat2a, kat2b, gtf3c4* and downregulation *sirt5* in liver, 4) upregulation *kat2b* in muscle of adult offspring after fed high plant-based diets. These findings demonstrated that the epigenetic modifications associated with the long-term NP effects of CHO in offspring persist in early adult stage through to the harvestable adulthood, suggesting that epigenetics plays a role in the NP mechanism throughout the Nile tilapia life cycle. Moreover, epigenetics exist in parental NP effects has also been found in carnivorous fish. In rainbow trout, maternal dietary HC feeding induced hepatic global DNA hypomethylation in juvenile offspring after challenged with HC feed (Callet et al., 2022). In zebrafish, parental HC stimuli history increased the level of gene-specific DNA methylation in the promoter region of *pck1* in liver of adult offspring after fed HC diet (Lu et al., 2022). These results revealed that the epigenetic regulation induced by parental nutritional intervention was stable in offspring, either omnivorous or carnivorous fish, thus might facilitate the long-term effects of NP. Further exploration of editing epigenetic modifications such as DNA methylation, histone methylation and acetylation in combination with NP strategy may further promote the utilization of low-cost and high-quality nutrients in aquaculture and increase economic benefits.

5.6 Conclusions

In conclusion, feeding HC in broodstock induced CHO response in their offspring and persisted through harvestable adulthood. These NP effects improved growth performance and pronounced intermediary CHO metabolism in offspring after challenged with plan-based CHO diets. We found that varied CHO level could induce genes expression of enzymes related to DNA/histone methylation and histone acetylation in liver and muscle of fish. In addition, parental NP history significantly effect on mRNA level of enzymes associated with epigenetic modification in early adult offspring into harvestable adult stage. Our findings suggested that the NP effects could throughout Nile tilapia life cycle and could provide protein effects with varied CHO sources, optimizing the CHO level and selecting the excellent and inexpensive source of CHO might amplify the NP effects in aquaculture. Editing the epigenetic

modifications present in NP effects may be a way to facilitate the development of NP strategies.

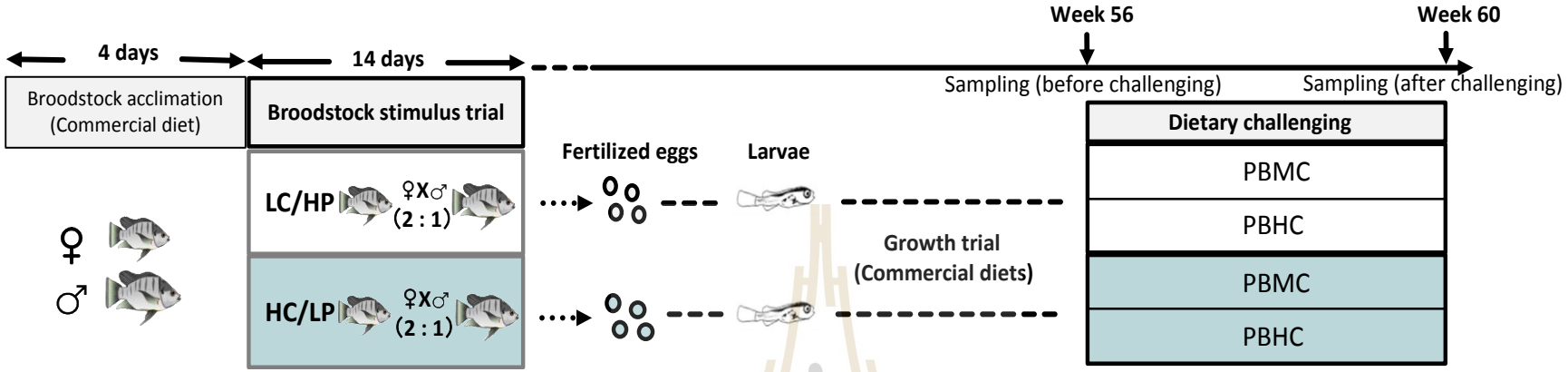

Figure 5.1 Experimental plan for nutritional programming involving dietary high-carbohydrate (HC) stimuli in broodstock and their long-term impacts on carbohydrate (CHO) metabolism in offspring. Mature male and female broodstock were acclimated to a communal breeding pond and fed a commercial diet (36% crude protein [CP], 3.4% crude fat [CF]) for 4 days. Subsequently, broodstock were fed low-carbohydrate/high-protein (LC/HP) or high-carbohydrate/low-protein (HC/LP) diets for 14 days. Fertilized eggs were collected from the mouths of females after the feeding period and cultured. The offspring were fed commercial diets (weeks 1–7: 40% CP, 8% CF; weeks 8–29: 32% CP, 4% CF; weeks 30-56: 30% CP, 4% CF) until adulthood (week 56). During weeks 57–60, adult offspring fish were challenged with different level of dietary carbohydrate diets which mainly derived from plant resources (PBMC, plant-based medium carbohydrate; PBHC, plant-based high carbohydrate). At week 56 (before challenge) and week 60 (after challenge), the fish were sampled to determine the growth performance, intermediary CHO metabolism and epigenetic modifications.

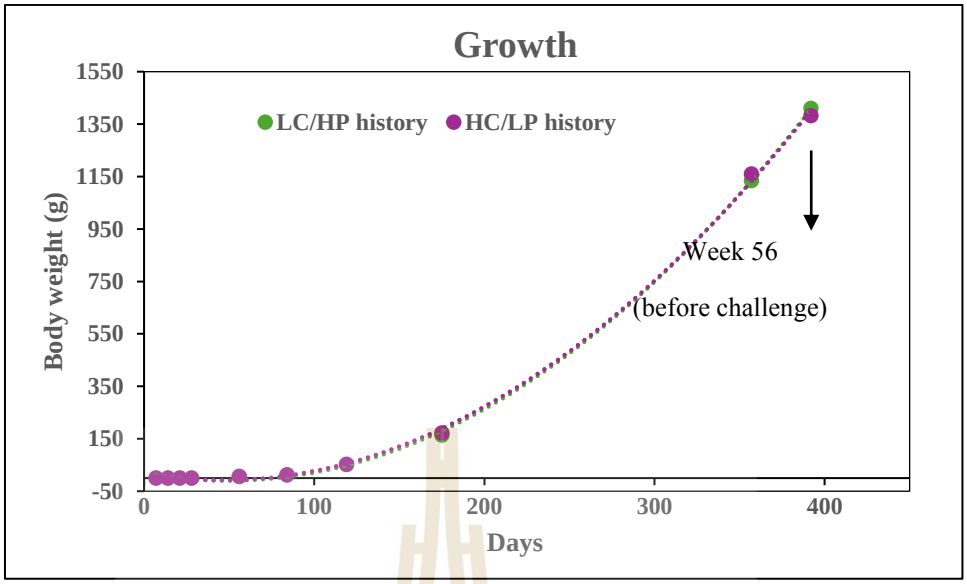

Figure 5.2 Growth of experimental offspring fish from Nile tilapia broodstock fed LC/HP and HC/LP diets for 14 days (mean ± SD, n = 6). During the growth trial, all experimental fish were fed commercial diets (days 1–49: 40% crude protein [CP], 8% crude fat [CF]; days 50–203: 32% CP, 4% CF; days 204–392: 30% CP, 4% CF). HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein

Table 5.1 Ingredients and chemical compositions (%) of the experiment diets.

Ingredients	Broodstock diets		Challenge diets	
	LC/HP	HC/LP	PBMC	PBHC
Fish meal	88	18	19	8
Rice flour	0	70	-	-
Soybean meal	-	-	30	8
Broken rice	-	-	20.5	30
Rice bran	-	-	12	22
Casava	-	-	17	30
Vitamin C	-	-	0.5	0.5
Glycine			0.25	0.25
Methionine			0.25	0.25
Fish oil	0	7	-	-
Soybean oil	2	0	-	-
Gelation	8	0	-	-
Di-calcium phosphate	0	3	-	-
Fish premix[a]	2	2	1	1
Proximate composition (%)				
Dry matter	94.2	90.2	90.42	89.79
Protein	57.3	15.3	28.65	14.52
Fat	9.2	9.0	5.64	5.74
Fiber	0.5	0.4	4.49	4.37
Ash	22.8	8.6	7.09	4.46
NFE[b]	4.5	58.9	44.55	60.69
Gross energy(kJ/g)	14.40	15.6	11.84	11.82

[a]Vitamin and trace mineral mix provided the following (IU kg^{-1} or g kg^{-1} diet): biotin, 0.25 g; folic acid, 0.003 g; inositol, 0.25 mg; niacin, 0.0215 g; pantothenic acid, 0.03 g; vitamin A, 5,000 IU; vitamin B1, 0.0025 g; vitamin B2, 0.0012 g; vitamin B6, 0.0075 g; vitamin B12, 0.00005 mg; vitamin C, 1 g; vitamin D3, 1,000 IU; vitamin E, 100 IU; vitamin K, 0.008 g; copper, 0.02 g; iron, 0.2 g; selenium, 0.3 mg; zinc, 0.32 g.

[b]Nitrogen-free extract = dry matter – (CP + crude lipid + crude fibre + ash).

HC, high-carbohydrate; HP, high-protein; LC, low-carbohydrate; LP, low-protein; PBMC, plant-based medium carbohydrate; PBHC, plant-based high carbohydrate.

Table 5.2 Growth performances of adult offspring fed with PBMC and PBHC diets for 4 weeks (week 57-60) (mean ± SD, n = 6)[1].

Broodstock	LC/HP history		HC/ LP history		*P* value		
Challenge diets	PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
IW[2](g)	1488.89 ± 40.37	1485 ± 51.37	1483.33 ± 34.96	1486.11 ± 49.91	0.904	0.976	0.857
FW[3] (g)	1752.78 ± 26.70	1701.67 ± 27.79	1811.11 ± 34.43	1740.56 ± 31.30	0.001	<0.001	0.440
WG[4] (g)	263.89 ± 49.91	216.67 ± 69.12	327.78 ± 13.61	254.44 ± 28.02	0.013	0.004	0.489
ADG[5] (g/day)	9.42 ± 1.78	7.74 ± 2.47	11.71 ± 0.49	9.09 ± 1.00	0.013	0.004	0.489
SGR[6] (%)	0.58 ± 0.11	0.49 ± 0.16	0.71 ± 0.03	0.57 ± 0.07	0.027	0.011	0.556
FCR[7]	1.55 ± 0.40	1.96 ± 0.61	1.20 ± 0.05	1.56 ± 0.16	0.022	0.020	0.849
HSI[8]	1.73 ± 0.26	2.43 ± 0.05	1.75 ± 0.22	2.46 ± 0.24	0.769	<0.001	0.986

[1] Two-way ANOVA was used to analyse the effects of broodstock stimulus (history), challenging diet (diet) and their interaction (history × diet).

[2] Initial body weight (IW).

[3] Final body weight (FW).

[4] Weight gain (WG) = Final body weight – Initial body weight.

[5] Average daily gain (ADG) = (Final body weight – Initial body weight)/Experimental days.

[6] Specific growth rate (SGR) = 100 × ((Final body weight – Initial body weight)/Experimental days).

[7] Feed conversion ratio (FCR) = Dry feed fed/Wet weight gain.

[8] Hepatosomatic index (HSI) = 100 × (liver weight/body weight).

Table 5.3 Plasma metabolites in adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock diets	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interactions
Glucose	4.20 ± 0.66	4.02 ± 0.84	0.685	5.45 ± 0.99	5.27 ± 0.88	5.89 ± 0.65	6.27 ± 0.94	0.008	0.684	0.253
Triglyceride	0.72 ± 0.29	0.64 ± 0.25	0.623	1.28 ± 0.31	1.37 ± 0.12	1.56 ± 0.38	1.78 ± 0.41	0.003	0.146	0.545
BUN[2]	1.49 ± 0.52	1.43 ± 0.41	0.813	1.36 ± 0.35	1.25 ± 0.39	1.31 ± 0.27	1.14 ± 0.49	0.444	0.215	0.758
Cholesterol	1.12 ± 0.26	1.18 ± 0.21	0.603	1.78 ± 0.39	1.83 ± 0.55	1.87 ± 0.43	1.98 ± 0.42	0.331	0.531	0.545
Total protein	25.83 ± 2.36	26.55 ± 2.16	0.501	40.41 ± 0.21^{c}	39.55 ± 0.37^{c}	37.13 ± 0.92^{a}	34.16 ± 1.43^{b}	<0.001	<0.001	0.014

[1] An independent t-test was used to analyse the effects of different broodstock stimuli (HP/LC and LP/HC diets) at week 56 (before challenge). Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups ($P <$ 0·05).

[2] Blood urea nitrogen.

Table 5.4 Proximate composition of liver, muscle, and whole body in adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock diets	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interactions
Liver (%)										
Protein	10.94 ± 1.29	10.74 ± 0.73	0.754	9.52 ± 0.79^{b}	7.78 ± 0.42^{a}	8.61 ± 0.78^{ab}	8.26 ± 0.39^{ab}	0.406	0.001	0.013
Fat	5.92 ± 1.96	6.01 ± 1.92	0.942	9.15 ± 0.52	10.45 ± 0.59	10.62 ± 0.50	12.27 ± 0.69	<0.001	<0.001	0.467
Ash	0.94 ± 0.09	0.92 ± 0.18	0.870	0.89 ± 0.11	0.88 ± 0.12	0.80 ± 0.16	0.83 ± 0.11	0.186	0.843	0.661
Glycogen(mg/g)	68.73 ± 6.36	71.95 ± 5.35	0.407	75.13 ± 12.75	95.66 ± 11.59	78.8 ± 6.67	115.24 ± 11.19	0.026	<0.001	0.115
Triglyceride(mg/g)	24.43 ± 1.92	25.94 ± 1.81	0.231	32.25 ± 1.37^{a}	36.78 ± 2.42^{b}	35.69 ± 2.36^{b}	46.68 ± 2.19^{c}	<0.001	<0.001	0.003
Muscle (%)										
Protein	19.59 ± 0.88	19.05 ± 0.85	0.465	19.6 ± 1.04	20.1 ± 0.48	20.97 ± 0.91	19.29 ± 1.48	0.513	0.181	0.018
Fat	1.85 ± 0.62	1.92 ± 0.34	0.810	2.06 ± 0.10	2.16 ± 0.14	2.34 ± 0.13	2.57 ± 0.16	<0.001	0.006	0.250
Ash	1.24 ± 0.05	1.20 ± 0.04	0.170	1.46 ± 0.12	1.52 ± 0.04	1.56 ± 0.05	1.54 ± 0.06	0.099	0.526	0.190
Glycogen(mg/g)	5.92 ± 0.42	6.48 ± 0.88	0.230	7.48 ± 0.62	8.55 ± 0.28	8.84 ± 0.42	9.93 ± 0.43	<0.001	<0.001	0.987
Triglyceride(mg/g)	4.83 ± 0.57	4.99 ± 0.52	0.652	6.34 ± 0.32	6.82 ± 0.26	7.16 ± 0.22	7.81 ± 0.46	<0.001	0.001	0.545
Whole body (%)										
Protein	16.09 ± 0.92	15.94 ± 0.82	0.764	16.39 ± 1.06	16.27 ± 1.44	17.07 ± 1.16	16.44 ± 0.87	0.376	0.436	0.593
Fat	7.23 ± 1.48	8.45 ± 1.93	0.247	6.98 ± 0.35	7.24 ± 0.44	8.33 ± 0.37	9.18 ± 0.56	<0.001	0.005	0.110
Ash	4.31 ± 1.54	4.68 ± 1.55	0.686	3.03 ± 1.18	3.75 ± 0.89	4.36 ± 1.09	3.97 ± 1.03	0.086	0.700	0.212

[1] An independent t-test was used to analyse the effects of different broodstock stimuli (HP/LC and LP/HC diets) at week 56 (before challenge). Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups ($P < 0{\cdot}05$).

Table 5.5 mRNA levels of genes related to intermediary CHO metabolism in the liver and muscle of adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
Hepatic glycolysis										
gck	0.70 ± 0.44	1.00 ± 0.58	0.342	0.76 ± 0.10	1.39 ± 0.46	0.80 ± 0.17	1.45 ± 0.34	0.688	<0.001	0.904
pfklr	0.79 ± 0.34	1.10 ± 0.18	0.730	0.65 ± 0.14	0.98 ± 0.44	0.63 ± 0.09	0.68 ± 0.17	0.119	0.078	0.178
pklr	0.96 ± 0.23	1.05 ± 0.20	0.528	1.10 ± 0.18	0.78 ± 0.23	0.98 ± 0.15	1.01 ± 0.07	0.389	0.051	0.123
Hepatic gluconeogenesis										
g6pca1	1.41 ± 0.31	0.64 ± 0.28	0.001	1.63 ± 0.12^{b}	0.61 ± 0.06^{a}	0.92 ± 0.42^{a}	0.67 ± 0.02^{a}	0.001	<0.001	<0.001
g6pca2	1.45 ± 0.49	0.71 ± 0.37	0.014	1.89 ± 0.12^{c}	0.78 ± 0.10^{b}	0.90 ± 0.37^{b}	0.46 ± 0.10^{a}	<0.001	<0.001	0.001
pck1	1.51 ± 0.91	0.56 ± 0.48	0.054	3.36 ± 0.21^{c}	0.07 ± 0.06^{a}	0.42 ± 0.33^{b}	0.05 ± 0.01^{a}	<0.001	<0.001	<0.001
pck2	1.01 ± 0.30	0.71 ± 0.35	0.147	1.05 ± 0.18^{b}	0.67 ± 0.06^{a}	0.68 ± 0.28^{a}	0.80 ± 0.10^{a}	0.098	0.086	0.003
Hepatic lipogenesis										
fasn	0.75 ± 0.38	1.14 ± 0.56	0.186	0.85 ± 0.32^{ab}	0.84 ± 0.23^{ab}	0.50 ± 0.11^{a}	1.06 ± 0.48^{b}	0.627	0.048	0.042
g6pd	1.28 ± 0.96	1.35 ± 0.57	0.889	0.61 ± 0.16	0.96 ± 0.21	0.68 ± 0.12	1.22 ± 0.12	0.021	<0.001	0.154
Hepatic amino acid catabolism										
asat	1.14 ± 0.12	0.64 ± 0.26	0.004	1.74 ± 0.16^{c}	0.24 ± 0.03^{a}	1.04 ± 0.43^{b}	0.16 ± 0.06^{a}	0.001	<0.001	0.004
alat	1.26 ± 0.05	0.85 ± 0.18	<0.001	1.06 ± 0.04	0.83 ± 0.08	0.80 ± 0.18	0.51 ± 0.11	<0.001	<0.001	0.499
gdh	0.97 ± 0.10	0.94 ± 0.14	0.708	1.00 ± 0.20	0.94 ± 0.16	0.76 ± 0.07	0.72 ± 0.13	0.001	0.398	0.903
Muscular glucose transport and glycolysis										
glut4	0.75 ± 0.25	1.09 ± 0.32	0.067	0.66 ± 0.15	1.25 ± 0.20	1.03 ± 0.42	1.26 ± 0.37	0.148	<0.001	0.155
hk1	0.82 ± 0.31	1.08 ± 0.63	0.381	0.54 ± 0.11	0.86 ± 0.20	1.04 ± 0.17	1.36 ± 0.67	<0.001	0.008	0.680
hk2	0.81 ± 0.30	1.07 ± 0.28	0.160	0.90 ± 0.19	1.07 ± 0.49	1.10 ± 0.14	1.09 ± 0.20	0.361	0.531	0.462
pfkma	0.45 ± 0.19	1.40 ± 0.55	0.007	0.49 ± 0.22	1.06 ± 0.65	1.11 ± 0.23	1.31 ± 0.25	0.011	0.022	0.238
pfkmb	0.81 ± 0.08	1.00 ± 0.22	0.093	0.88 ± 0.10	1.10 ± 0.26	1.06 ± 0.06	1.04 ± 0.13	0.370	0.153	0.080
pkma	0.78 ± 0.24	0.96 ± 0.32	0.290	0.70 ± 0.11	0.92 ± 0.19	0.83 ± 0.15	0.96 ± 0.23	0.239	0.023	0.562

Table 5.6 mRNA levels of genes related to DNA methylation in the liver of adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
DNA methyltransferases										
dnmt1a	0.92 ± 0.27	0.92 ± 0.31	0.985	1.01 ± 0.29^{ab}	0.74 ± 0.13^{a}	0.76 ± 0.23^{a}	1.15 ± 0.16^{b}	0.402	0.525	0.009
dnmt3aa	1.07 ± 0.19	1.09 ± 0.24	0.840	1.11 ± 0.15	0.70 ± 0.19	1.20 ± 0.23	0.99 ± 0.05	0.011	<0.001	0.159
dnmt3ab	0.97 ± 0.23	1.22 ± 0.37	0.201	0.82 ± 0.07	0.93 ± 0.27	1.07 ± 0.23	1.27 ± 0.17	0.001	0.075	0.574
dnmt3ba	1.45 ± 0.39	1.32 ± 0.41	0.579	1.44 ± 0.36^{a}	1.29 ± 0.29^{a}	1.37 ± 0.52^{a}	2.03 ± 0.29^{b}	0.039	0.108	0.011
dnmt3bb	1.15 ± 0.44	1.24 ± 0.47	0.753	1.12 ± 0.20^{b}	0.89 ± 0.20^{ab}	0.67 ± 0.11^{a}	1.09 ± 0.12^{b}	0.081	0.173	<0.001
DNA demethylases										
tet1	1.15 ± 0.68	0.9 ± 0.18	0.396	1.26 ± 0.22	0.87 ± 0.17	0.96 ± 0.13	0.76 ± 0.06	0.004	<0.001	0.139
tet2	1.13 ± 0.16	1.06 ± 0.23	0.560	1.14 ± 0.10^{b}	0.81 ± 0.15^{a}	0.99 ± 0.08^{b}	1.08 ± 0.09^{b}	0.208	0.011	<0.001
tet3	1.01 ± 0.26	1.04 ± 0.15	0.810	1.05 ± 0.11^{bc}	0.98 ± 0.19^{ab}	1.21 ± 0.13^{c}	0.80 ± 0.09^{a}	0.861	<0.001	<0.001

[1] An independent t-test was used to analyse the effects of different broodstock stimuli (HP/LC and LP/HC diets) at week 56 (before challenge). Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups ($P < 0•05$).

Table 5.7 mRNA levels of genes related to DNA methylation in the muscle of adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
DNA methyltransferases										
dnmt1a	1.85 ± 0.79	1.49 ± 0.30	0.323	0.75 ± 0.22	1.89 ± 0.52	1.06 ± 0.32	1.77 ± 0.53	0.598	<0.001	0.230
dnmt3aa	1.62 ± 0.67	1.18 ± 0.25	0.170	0.85 ± 0.33	1.58 ± 0.60	0.83 ± 0.17	0.96 ± 0.22	0.048	0.010	0.057
dnmt3ab	1.48 ± 0.53	1.27 ± 0.31	0.424	0.82 ± 0.17^{a}	1.33 ± 0.16^{c}	0.95 ± 0.11^{ab}	1.13 ± 0.11^{b}	0.517	<0.001	<0.001
dnmt3ba	1.51 ± 0.83	1.42 ± 0.55	0.835	1.17 ± 0.37	1.36 ± 0.40	1.10 ± 0.21	1.53 ± 0.37	0.750	0.041	0.391
dnmt3bb	1.44 ± 0.82	1.11 ± 0.41	0.387	0.77 ± 0.33	1.31 ± 0.34	0.83 ± 0.14	0.94 ± 0.15	0.167	0.006	0.054
DNA demethylases										
tet1	1.48 ± 0.65	1.12 ± 0.20	0.223	0.85 ± 0.22	1.6 ± 0.68	0.75 ± 0.20	1.03 ± 0.16	0.043	0.003	0.123
tet2	1.22 ± 0.25	1.52 ± 0.37	0.129	0.84 ± 0.18	1.27 ± 0.27	1.06 ± 0.29	1.46 ± 0.24	0.058	<0.001	0.897
tet3	1.28 ± 0.35	1.58 ± 0.48	0.242	0.51 ± 0.17^{a}	1.4 ± 0.55^{b}	1.05 ± 0.30^{b}	1.18 ± 0.18^{b}	0.263	0.002	0.002

[1] An independent t-test was used to analyse the effects of different broodstock stimuli (HP/LC and LP/HC diets) at week 56 (before challenge). Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups ($P < 0•05$).

Table 5.8 mRNA levels of genes related to histone modifications in liver of adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
H3K4me3 writer (Histone lysine methyltransferases)										
setd1a	1.08 ± 0.17	1.04 ± 0.15	0.703	1.12 ± 0.17[b]	0.82 ± 0.19[a]	1.10 ± 0.09[b]	1.07 ± 0.13[b]	0.088	0.013	0.009
setd1ba	0.93 ± 0.32	1.03 ± 0.27	0.537	0.88 ± 0.05	0.85 ± 0.24	0.89 ± 0.15	1.02 ± 0.07	0.142	0.438	0.224
kmt2a	1.00 ± 0.30	1.27 ± 0.31	0.168	0.84 ± 0.13	0.91 ± 0.25	1.03 ± 0.10	1.18 ± 0.08	0.001	0.104	0.513
kmt2ba	1.00 ± 0.20	1.02 ± 0.27	0.887	0.93 ± 0.09	0.74 ± 0.23	0.99 ± 0.12	0.99 ± 0.09	0.013	0.146	0.103
kmt2bb	1.04 ± 0.27	0.99 ± 0.15	0.697	0.90 ± 0.16	0.75 ± 0.19	1.05 ± 0.13	0.96 ± 0.05	0.007	0.052	0.616
H3K4me3 eraser (Histone lysine demethylases)										
kdm5a	0.95 ± 0.29	1.07 ± 0.16	0.411	0.89 ± 0.04	0.71 ± 0.16	1.09 ± 0.17	1.04 ± 0.10	<0.001	0.044	0.197
kdm5ba	1.17 ± 0.34	1.10 ± 0.06	0.635	0.95 ± 0.13	0.74 ± 0.13	0.91 ± 0.12	0.90 ± 0.09	0.193	0.037	0.052
kdm5bb	0.95 ± 0.25	1.08 ± 0.17	0.325	0.99 ± 0.10	0.89 ± 0.14	1.23 ± 0.33	1.03 ± 0.09	0.026	0.070	0.508
kdm5c	1.07 ± 0.36	1.02 ± 0.15	0.728	1.00 ± 0.14	0.88 ± 0.14	1.11 ± 0.29	0.87 ± 0.05	0.460	0.021	0.434
riox1	1.13 ± 0.50	1.05 ± 0.27	0.722	0.94 ± 0.18	0.69 ± 0.14	1.18 ± 0.45	1.15 ± 0.13	0.004	0.199	0.328
H3K9me3 specific writer (Histone lysine methyltransferases)										
suv39h1b	0.98 ± 0.32	0.89 ± 0.23	0.562	0.79 ± 0.12	0.64 ± 0.22	0.65 ± 0.24	0.75 ± 0.14	0.890	0.742	0.124
H3K9me3 specific eraser (Histone lysine demethylases)										
kdm4aa	0.91 ± 0.35	1.16 ± 0.34	0.242	0.68 ± 0.04	0.85 ± 0.09	1.11 ± 0.19	1.30 ± 0.12	<0.001	0.002	0.895
kdm4ab	1.30 ± 0.36	1.27 ± 0.21	0.886	1.06 ± 0.13	0.97 ± 0.22	1.08 ± 0.23	1.27 ± 0.13	0.480	0.495	0.074
kdm4b	1.00 ± 0.55	1.27 ± 0.20	0.288	0.62 ± 0.14	0.70 ± 0.20	1.07 ± 0.35	0.84 ± 0.11	0.004	0.406	0.095
kdm4c	1.03 ± 0.47	1.23 ± 0.12	0.347	0.51 ± 0.03[a]	0.80 ± 0.20[b]	0.90 ± 0.19[b]	0.76 ± 0.13[b]	0.009	0.245	0.002

Table 5.8 Continued

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
H3K36me3 specific writer (Histone lysine methyltransferases)										
setd2	0.65 ± 0.56	1.36 ± 0.67	0.076	1.01 ± 0.08	0.62 ± 0.22	1.12 ± 0.22	0.82 ± 0.11	0.036	<0.001	0.495
H3K9ac specific writer (Histone lysine acetylase)										
kat2a	0.65 ± 0.56	1.36 ± 0.67	0.076	0.61 ± 0.13	0.69 ± 0.20	1.49 ± 0.24	1.78 ± 0.30	<0.001	0.059	0.261
kat2b	1.31 ± 0.47	1.89 ± 0.69	0.117	0.57 ± 0.07[a]	1.1 ± 0.40[a]	0.92 ± 0.19[a]	2.35 ± 0.81[b]	<0.001	<0.001	<0.001
kat6a	0.88 ± 0.13	1.03 ± 0.22	0.184	0.94 ± 0.18	1.02 ± 0.31	0.89 ± 0.14	0.96 ± 0.13	0.507	0.350	0.960
gtf3c4	0.86 ± 0.47	1.13 ± 0.42	0.331	0.61 ± 0.09	0.95 ± 0.25	0.94 ± 0.17	1.07 ± 0.13	0.004	0.002	0.148
H3K9ac specific eraser (Histone lysine deacetylase)										
sirt2	0.99 ± 0.31	0.88 ± 0.24	0.497	0.92 ± 0.12	0.76 ± 0.17	0.87 ± 0.21	0.88 ± 0.09	0.552	0.221	0.186
sirt5	0.97 ± 0.30	0.85 ± 0.22	0.409	0.98 ± 0.10	0.74 ± 0.12	0.77 ± 0.20	0.63 ± 0.06	0.008	0.002	0.366
sirt6	0.68 ± 0.64	1.18 ± 0.64	0.199	0.51 ± 0.09	0.75 ± 0.15	1.01 ± 0.34	1.09 ± 0.13	<0.001	0.065	0.331

[1] An independent t-test was used to analyse the effects of different broodstock stimuli (HP/LC and LP/HC diets) at week 56 (before challenge). Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups ($P < 0{\cdot}05$).

Table 5.9 mRNA levels of genes related to histone modifications in muscle of adult offspring before (week 56) and after (week 60) fed with PBMC and PBHC diets for 4 weeks (mean ± SD, n = 6)[1].

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge diets	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
H3K4me3 writer (Histone lysine methyltransferases)										
setd1a	1.29 ± 0.40	1.42 ± 0.31	0.538	0.73 ± 0.14^{a}	1.35 ± 0.22^{b}	0.88 ± 0.22^{a}	1.16 ± 0.14^{b}	0.822	<0.00	0.034
setd1ba	1.26 ± 0.21	1.49 ± 0.58	0.380	0.97 ± 0.27	1.94 ± 0.69	1.31 ± 0.49	1.53 ± 0.37	0.851	0.007	0.075
kmt2a	1.19 ± 0.20	1.32 ± 0.28	0.398	0.89 ± 0.07	1.31 ± 0.27	0.96 ± 0.21	1.24 ± 0.24	0.985	<0.00	0.405
kmt2ba	1.32 ± 0.37	1.39 ± 0.34	0.778	0.66 ± 0.09^{a}	1.43 ± 0.47^{c}	0.83 ± 0.22^{ab}	1.11 ± 0.2^{bc}	0.499	<0.00	<0.001
kmt2bb	1.68 ± 0.42	1.70 ± 0.41	0.925	0.67 ± 0.13	1.10 ± 0.25	0.77 ± 0.19	1.29 ± 0.21	0.094	<0.00	0.635
H3K4me3 eraser (Histone lysine demethylases)										
kdm5a	1.36 ± 0.34	1.30 ± 0.31	0.745	0.51 ± 0.11^{a}	1.18 ± 0.32^{b}	0.82 ± 0.23^{a}	0.83 ± 0.10^{a}	0.802	<0.00	<0.001
kdm5ba	1.06 ± 0.36	1.06 ± 0.27	0.979	0.43 ± 0.10^{a}	1.10 ± 0.30^{c}	0.61 ± 0.21^{ab}	0.85 ± 0.16^{bc}	0.673	<0.00	<0.001
kdm5bb	1.79 ± 0.57	1.61 ± 0.39	0.542	1.01 ± 0.30	1.64 ± 0.55	1.18 ± 0.32	1.27 ± 0.14	0.492	0.023	0.085
kdm5c	1.74 ± 0.52	1.70 ± 0.37	0.902	0.79 ± 0.21^{a}	1.61 ± 0.34^{c}	1.02 ± 0.23^{ab}	1.29 ± 0.16^{bc}	0.620	<0.00	<0.001
riox1	1.74 ± 0.60	1.58 ± 0.39	0.591	0.93 ± 0.32	1.52 ± 0.49	0.91 ± 0.22	1.15 ± 0.18	0.155	0.006	0.196
H3K9me3 specific writer (Histone lysine methyltransferases)										
suv39h1b	1.65 ± 0.8	1.5 ± 0.82	0.757	0.57 ± 0.11	2.30 ± 0.71	0.91 ± 0.18	1.93 ± 1.10	0.959	<0.00	0.204
H3K9me3 specific eraser (Histone lysine demethylases)										
kdm4aa	1.68 ± 0.54	1.61 ± 0.48	0.821	0.87 ± 0.20	1.58 ± 0.45	1.13 ± 0.24	1.40 ± 0.19	0.750	<0.00	0.085
kdm4ab	1.82 ± 0.6	1.60 ± 0.41	0.479	0.93 ± 0.20	1.81 ± 0.52	1.12 ± 0.20	1.49 ± 0.24	0.631	<0.00	0.076
kdm4b	1.77 ± 0.46	1.79 ± 0.24	0.939	0.90 ± 0.31	1.34 ± 0.61	1.02 ± 0.28	1.11 ± 0.33	0.739	0.123	0.286
kdm4c	1.76 ± 0.5	1.69 ± 0.36	0.812	1.00 ± 0.15	1.58 ± 0.35	1.11 ± 0.23	1.25 ± 0.25	0.341	0.002	0.470

Table 5.9 Continued.

Broodstock	LC/HP	HC/LP	P value	LC/HP History		HC/LP History		*P* value		
Challenge	Week 56 (before challenge)			PBMC	PBHC	PBMC	PBHC	History	Diet	Interaction
H3K36me3 specific writer (Histone lysine methyltransferases)										
setd2	1.75 ± 0.58	1.68 ± 0.38	0.797	0.97 ± 0.17	1.57 ± 0.35	1.13 ± 0.25	1.57 ± 0.28	0.493	<0.001	0.493
H3K9ac specific writer (Histone lysine acetylase)										
kat2a	1.73 ± 0.73	1.62 ± 0.19	0.730	0.90 ± 0.24	1.57 ± 0.50	1.05 ± 0.31	1.42 ± 0.26	0.976	0.001	0.284
kat2b	1.91 ± 0.56	2.05 ± 0.60	0.681	0.97 ± 0.23	1.43 ± 0.17	1.36 ± 0.38	1.94 ± 0.74	0.022	0.009	0.749
kat6a	2.52 ± 0.64	2.62 ± 0.94	0.836	0.71 ± 0.14^{a}	1.60 ± 0.35^{c}	1.32 ± 0.31^{bc}	1.09 ± 0.16^{b}	0.625	0.004	<0.001
gtf3c4	1.34 ± 0.36	1.48 ± 0.63	0.631	0.53 ± 0.11	1.55 ± 0.60	0.86 ± 0.13	1.43 ± 0.37	0.471	<0.001	0.134
H3K9ac specific eraser (Histone lysine deacetylase)										
sirt2	2.13 ± 0.52	1.79 ± 0.35	0.214	1.21 ± 0.41	1.48 ± 0.49	1.37 ± 0.31	1.26 ± 0.23	0.830	0.592	0.228
sirt5	1.59 ± 0.39	1.51 ± 0.38	0.703	1.10 ± 0.20	1.32 ± 0.21	1.15 ± 0.35	1.25 ± 0.22	0.916	0.142	0.568
sirt6	1.60 ± 0.58	1.49 ± 0.32	0.683	0.96 ± 0.30	1.53 ± 0.63	1.17 ± 0.29	1.66 ± 0.54	0.360	0.010	0.834

[1] An independent t-test was used to analyse the effects of different broodstock stimuli (HP/LC and LP/HC diets) at week 56 (before challenge). Two-way ANOVA was used to analyse the effects of broodstock stimulus diet (history), challenging diet (diet) and their interaction (history × diet). One-way ANOVA following Tukey's range test was used to rank the treatment combination groups when the interaction of the factors was statistically significant. Different letters indicate significant differences in the mean values for four combination groups ($P < 0•05$).

Table 5.10 List of primers used for qRT-PCR of genes related to carbohydrate and intermediary metabolism in the liver and muscle.

Genes	5'/3' Forward primer	5'/3' Reverse primer	SIZE (bps)	Access numbers
References gene				
ef1α	GCACGCTCTGCTGGCCTTT	GCGCTCAATCTTCCATCCC	250	AB075952
Hepatic glycolysis				
gck	GGGTGGTAGGATTTGGTGTG	TGCTGACACAAGGCATCTTC	186	XM003451020
pfklr	GACGAGCGAGTGGAGAAAAC	TGTCTTGATCCGAGGGAATC	162	XM003447353
pklr	AGGTACAGGTCACCCGTCAG	CATGTCGCCAGACTTGAAGA	164	XM005472622
Hepatic gluconeogenesis				
g6pca1	AGCGTTAAGGCAACTGGAGA	AAAAGCTAACAAGGCCAGCA	195	XM003448671
g6pca2	CTTCTTCCCCCTTTGGTTTC	AGACTCCTGCAGCTCCCATA	245	XM013273429
pck1	AAGCTTTTGACTGGCAGCAT	TGCTCAGCCAGTGAGAGAGA	162	XM003448375
pck2	TACGTCTTGAGCTCCCGTCT	CCTCCTGGATGATGCAAGTT	202	XM019354843
Hepatic lipogenesis				
fasn	AACCTGCTTCTCAAGCCAAA	CGTCACCCCTTGTTCTTTGT	222	XM013276809
g6pd	GTCACCTCAACCGGGAAGTA	TGGCTGAGGACACCTCTCTT	187	XM013275693
Hepatic amino acid catabolism				
alat	CACGGTGAAGAAGGTGGAGT	GCAGTTCAGGGTAGGAGCAG	200	XM005476466
asat	GCTTCCTTGGTGACTTGGAA	CCAGGCATCTTTCTCCAGAC	200	XM003451918
gdh	CGAGCGAGACTCCAACTACC	TGGCTGTTCTCATGATTTGC	203	XM003457465
Muscular glucose transport				
glut4	GAGGATGGACATGGAGAGGA	CAGGAAAAGCGAGACTACCG	235	JN900493
Muscular glycolysis				
hk1	CGTCGCTTAGTCCCAGACTC	TGACTGTAGCGTCCTTGTGG	235	XM019360229
hk2	CAGAGGGGAATTCGATTTGA	CCCACTCGACATTGACACAC	200	XM003448615
pfkma	AGGACCTCCAACCAACTGTG	TTTTCTCCTCCATCCACCAG	190	XM019349871
pfkmb	TTTGTGCATGAGGGTTACCA	CACCTCCAATCACACACAGG	208	XM003441476
pkma	TGACTGCTTCCTGGTCTGTG	CAGTGAAAGCTGGCAAATGA	249	XM005447626

*: from Yang et al. 2013

Table 5.11 List of primers used for qRT-PCR of genes related to epigenetic modification-related enzymes expressed in liver and muscle tissues

Genes		5'/3' Forward primer	5'/3' Reverse primer	SIZE (bps)	Accession numbers
Reference gene	*ef1*	GCACGCTCTGCTGGCCTTT	GCGCTCAATCTTCCATCCC	250	AB075952
DNA methylation writers	*dnmt1*	CTCACACTGCGCTGTCTTGT	ACAACGCTGAGAGAGCAAGC	188	XM_025906327.1
	dnmt3aa	CCAACAACCACGAGCAGGAA	TGCCGACAGTGATGGAGTCT	192	XM_005475084.4
	dnmt3ab	GCCGCAGCTTAGAGGACATC	CACACATGAGCACCTCTCGTC	189	XM_005477258.3
	dnmt3ba	GCTGCTGCAGATGCTACTGT	TTGCGCTGTTGTTGGCAAAG	186	XM_025901732.1
	dnmt3bb	TGCAGGAGTTCTTCGCCAAC	TGCCACATACTGACCCACCT	173	XM_025901790.1
DNA methylation eraser	*tet1*	CATCCAGTCCCAGCACAACC	CTCTATTTGGCGTGCGCTGA	194	XM_025897345.1
	tet2	GCAGCTGCCAACAAGAATGC	TGTTGCTGCTGCTGATGGAC	191	XM_005457001.3
	tet3	GCAAGCCAACCAACCAAACC	GATGTGTTGGCTCCGACCTG	177	XM_019365521.2
H3K4me3 writer (Histone Lysine methyltransferase)	*setd1a*	GGAACTCCGGTCTGGATGGT	CGAAGCTGCCCATCTGTGTT	172	XM_005468973.4
	setd1ba	AAGACAGGGAGGCAGCAGAA	CCTCAGGACTGGGAGGTCTG	198	XM_005470275.4
	kmt2a	AGAGCAGGAAAGCCAACAGC	CACTGGGCGTAGTTGTGGTC	178	XM_013274782.3
	kmt2ba	ACTCTGAGGGACCTGGAGGA	AGAGGAGGTGAAGCCGATCC	191	XM_013275905.3
	kmt2bb	GCTCCCGTCAGTGTGTCTTC	TCTGGCTCCAACCCAGTCAA	172	XM_013277028.3
H3K4me3 eraser (Histone lysine demethylases)	*kdm5a*	TCTGGCCACAGAGGAGTTGT	GTGACGTGGCTCTGCTGAAA	191	XM_005451728.4
	kdm5ba	TCTCAGAGCAGAGGGCATCC	GACCCGATGTCACACCTTGG	165	XM_003441348.2
	kdm5bb	CATCCCTGCCTACCTCCCAA	AAGGCTCCAGGTGGACTTGA	170	XM_003439103.5
	kdm5c	CTCTCCACCCTGGAGGCAAT	AGCTACCAGGCCCTCCAAAT	174	XM_005448517.4
	riox1	CCACCTGGCACACAAGGATT	TCCGGCTTCTACCACCACAT	192	XM_005475002.4
H3K9me3 specific writer	*suv39h1b*	TCCAACGCATGGCCTACAAC	CTTGATGTGCTGCAGTGTGC	197	XM_003459875.5
H3K9me3 specific eraser	*kdm4aa*	CGGATGCGAACCAAACCTCT	GGCTGGATCGACACCGTAAC	180	XM_005457300.3
	kdm4ab	TCTGTTCAGGGAGGCACACA	GCCTGTTGGCCCATCTGTTT	162	XM_005476068.4
	kdm4b	TGCTCGCTCTTCTGTCCGTA	AGCAGATCAGGAGGCTGGTT	196	XM_005453970.4
	kdm4c	CCTGCAGAGGAATGCAGTGG	GCACAGGTGCAATCTGGTGA	176	XM_005456806.2
H3K36me3 specific writer	*setd2*	AGGCAGCGATGACTTCAAGC	ATCTTGTGGCGTCCCACTCT	182	XM_019364854.2
H3K4me3 specific writer	*kat2a*	CACTGACCCTGCTGCTATGC	GTAGGCCAACCAGCCACATC	173	XM_025906390.1
	kat2b	GGCCTTTCATGGAGCCTGTG	CTCGCTCTCTGGAGGGTTGT	188	XM_003444058.3
	kat6a	CATCCCGTCCACTGCTTTCC	CCTGTTCACGCTACCACCAC	173	XM_005472980.3
	gtf3c4	CTTGTGGCGGTTCAAGCTCT	GGCTCGCCTTCCTCTTTCAC	174	XM_003440231.5
H3K9ac specific eraser	*sirt2*	GCGAGTCTAGTCAGCAGGGT	CCCAGAAGATCAGCTAGAGCCA	197	XM_003449264.5
	sirt5	ATTTGCCCAGGTGTGAGCAG	GAGCAAACATGGCTGCAGGA	177	XM_003457306.5
	sirt6	GTCAACCTGCAGTCGACCAA	TAACACCAGGCGGTGGTTTG	190	XM_003437978.5

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CHAPTER VI
OVERALL CONCLUSION AND IMPLICATION

6.1 Overall conclusion

Nutritional programming (NP) of high-carbohydrates (HC) in Nile tilapia early life (glucose injection into yolk of larvae and HC feeding in fry) could improve the efficient utilization of carbohydrate (CHO) in later life of juvenile and adult stage, which could provide protein sparing effects. To obtain scientific information on the NPs concept of CHO in the life cycle of Nile tilapia, the effects of dietary HC stimuli in broodstock on their offspring could be an approach to deep understanding the NP strategy in fish. In pervious study, parental HC stimulation modulated the intermediary CHO metabolism in their offspring at larvae and fry stage, demonstrating the NP effects. Therefore, this study continued explore whether the effects of NP of CHO in broodstock on offspring could persist on long term in juvenile and adult offspring. Moreover, the juvenile and adult offspring were challenged with a HC diet, respectively, to exam the capacity of carbohydrate utilization. In addition, numerous studies have shown that there is an epigenetic role in the effect of NP. To investigate whether epigenetics is included in the NP effects of CHO in broodstock, the present study identified enzymes associated with epigenetic modifications at the molecular level in juvenile and adult offspring fish after they were challenged the HC diet. The main results are summarized as follows (Figure 6.1):

6.1.1 HC stimulus in broodstock had long-term effect on glucose and its related metabolism including increased HSI and triglyceride content in liver, induced hepatic gluconeogenesis and muscular glycolysis in juvenile offspring. In addition, parental HC stimulus history showed greater effects when offspring fish were challenging the HC/LP diet such as induction hepatic glycolysis and lipogenesis while suppression gluconeogenesis and amino acid catabolism, as well as induction muscular glucose transport and glycolysis, demonstrating NP effects of parental HC diet. The modulation of epigenetic modifications including the writer and eraser enzymes related to DNA

methylation and histone modifications at molecular levels, indicating that epigenetics were involved in NP of dietary CHO in parental Nile tilapia.

6.1.2 Dietary HC intake in Nile tilapia broodstock induced NP that was transmitted to offspring, persisted through adulthood, and modulated CHO metabolism. CHO NPs improved the growth performance of offspring and elicited stronger CHO metabolic responses when challenged with an HC diet. The modulation of gene expression related to DNA methylation and histone modification in the adult offspring suggests that epigenetic mechanisms are involved in the NP benefits of dietary CHO in Nile tilapia broodstock. Our results suggested that NP of CHO strategy could be an application to improve the efficient utilization of CHO in later life of fish and could provide a scientific theoretical basis for the development of low cost and high-quality feed, enhance economic performance in tilapia farming.

6.1.3 Feeding HC in broodstock induced CHO response in their offspring and persisted through harvestable adulthood (week 56). These NP effects improved growth performance and pronounced intermediary CHO metabolism in offspring after challenged with plan-based CHO diets for 4 weeks (week 57-60). We found that varied CHO level could induce genes expression of enzymes related to DNA/histone methylation and histone acetylation in liver and muscle of fish. In addition, parental NP history significantly effect on mRNA level of enzymes associated with epigenetic modification in early adult offspring into harvestable adult stage. Our findings suggested that the NP effects could throughout Nile tilapia life for long term at least to week 60 and could provide protein effects with varied CHO sources, optimizing the CHO level and selecting the excellent and inexpensive source of CHO might amplify the NP effects in aquaculture. Editing the epigenetic modifications present in NP effects may be a way to facilitate the development of NP strategies.

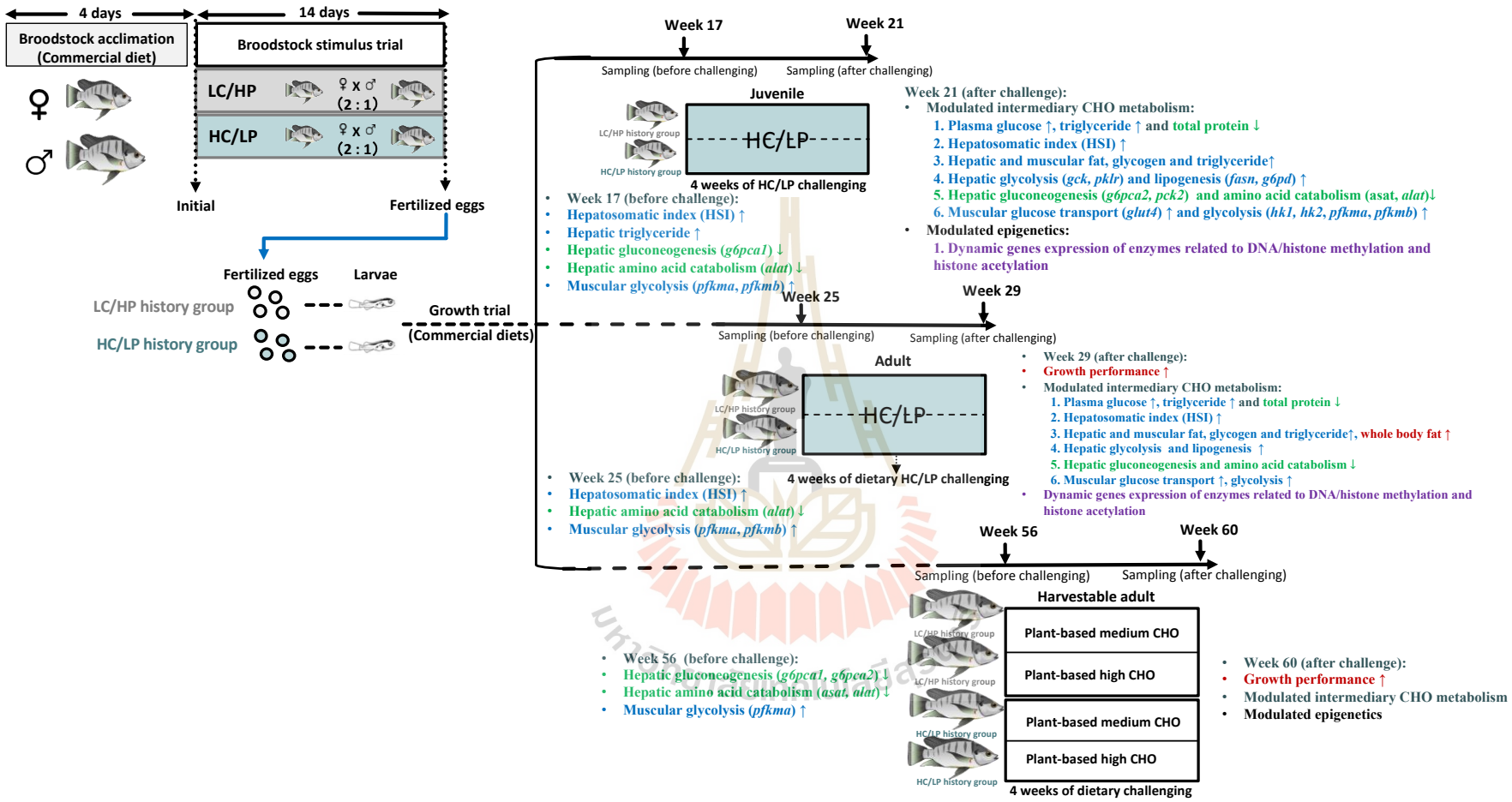

Figure 6.1 Conclusion map.

6.2 Implication

The findings from this study hold significant promise for advancing sustainable aquaculture practices by enhancing carbohydrate utilization in Nile tilapia through nutritional programming (NP) strategies. By elucidating the long-term effects of broodstock dietary carbohydrate intervention on offspring metabolism across juvenile, adult, and harvestable stages, this research could pave the way for cost-effective feed formulations that reduce reliance on expensive protein sources like fishmeal. The potential identification of epigenetic mechanisms underlying NP effects, such as DNA methylation and histone modifications, may provide novel insights into metabolic plasticity in fish, enabling targeted interventions to optimize nutrient utilization.

Future research should focus on unraveling the specific epigenetic pathways involved in mediating NP effects, including detailed analyses of DNMT, TET, and histone-modifying enzymes, to establish causal relationships between chromatin dynamics and metabolic reprogramming. Additionally, exploring the interaction between plant-based sources and NP-induced metabolic adaptations could further refine low-cost, high-efficiency feed formulations. Longitudinal studies tracking transgenerational epigenetic inheritance and metabolic resilience under varying environmental conditions would deepen understanding of NP sustainability.

The integration of NP strategies into aquaculture systems could revolutionize feed management by improving carbohydrate efficiency, reducing production costs, and minimizing ecological impacts linked to overfishing and fishmeal dependency. Ultimately, this approach may serve as a model for other economically important species, contributing to global food security and the sustainable expansion of aquaculture industries.

BIOGRAPHY

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