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 *sir*t6) 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.

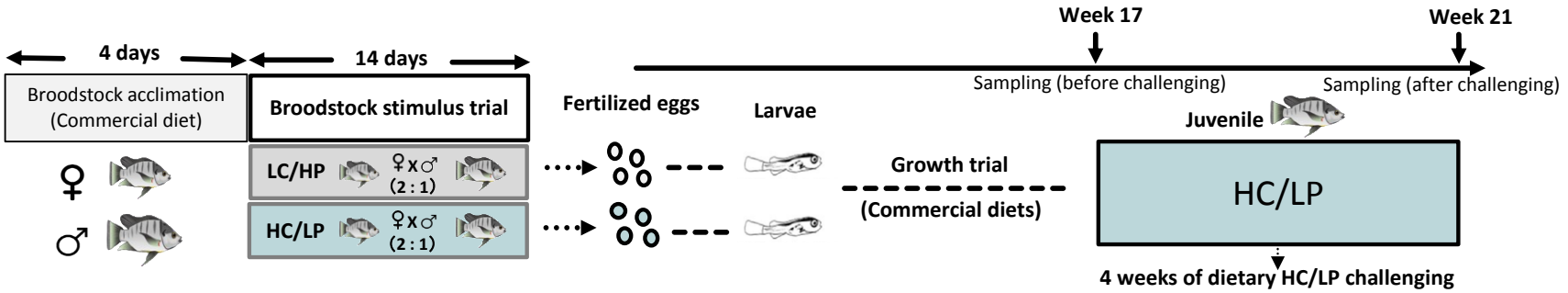

Figure 3.1 Experimental plan for nutritional programming involving dietary high-carbohydrate (HC) stimuli in broodstock and their long-term impacts on carbohydrate (CHO) metabolism in offspring. Mature male and female broodstock were acclimated to a communal breeding pond and fed a commercial diet (36% 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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