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.

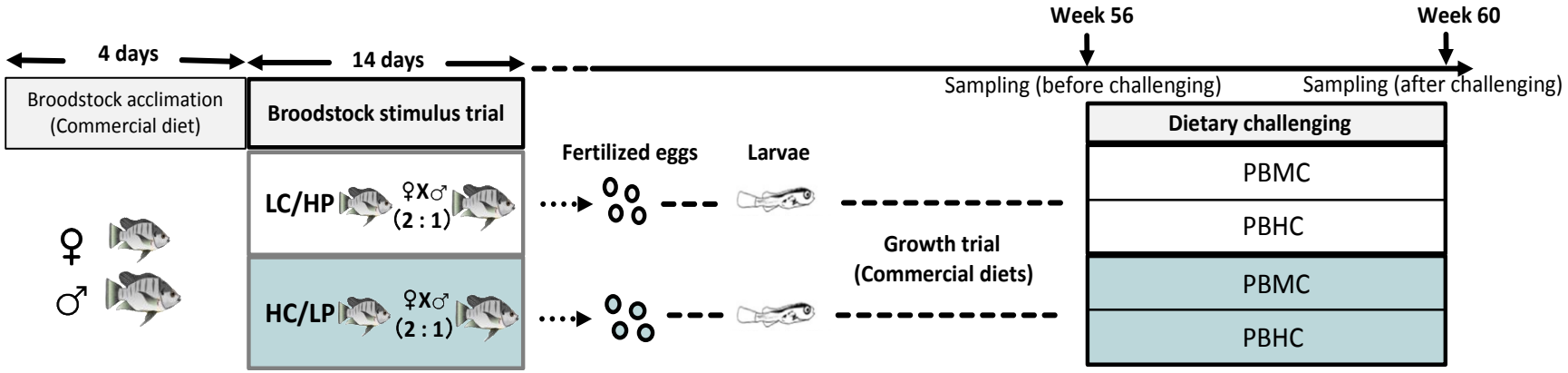

Figure 5.1 Experimental plan for nutritional programming involving dietary high-carbohydrate (HC) stimuli in broodstock and their long-term impacts on carbohydrate (CHO) metabolism in offspring. Mature male and female broodstock were acclimated to a communal breeding pond and fed a commercial diet (36% 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.

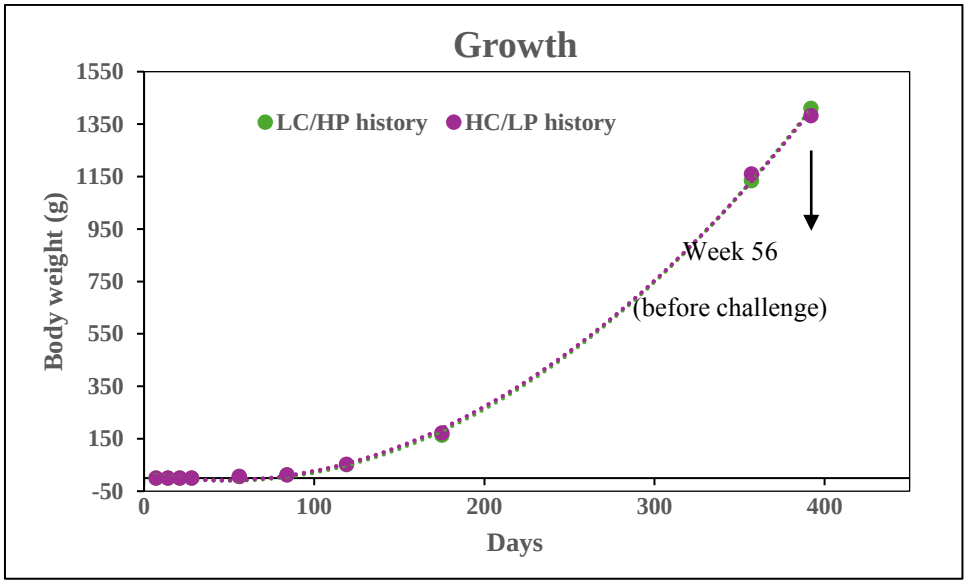

Figure 5.2 Growth of experimental offspring fish from Nile tilapia broodstock fed LC/HP and HC/LP diets for 14 days (mean ± SD, n = 6). During the growth trial, all experimental fish were fed commercial diets (days 1–49: 40% crude protein [CP], 8% crude fat [CF]; days 50–203: 32% CP, 4% CF; days 204–392: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