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

4.1 Abstract

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

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

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

4.2 Introduction

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

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

In fish, changes in global DNA methylation have been implicated in the CHO metabolic responses in zebrafish, Chinese perch (*Siniperca chuatsi*), rainbow trout, and gibel carp (*Carassius gibelio*) (Song et al. 2019; Callet et al. 2021; Lu et al. 2022; Xiao et al. 2020; Geng et al. 2023). Global DNA methylation analysis in Nile tilapia showed that glucose injection during the alevin stage induces epigenetic changes, such as hypomethylation, that persist into adulthood (Kumkhong et al. 2020a). Epigenetic changes, including DNA methylation and histone modification, are reversible processes mediated by enzymes involved in DNA/histone methylation, demethylation, acetylation, and deacetylation. In rainbow trout, NP using HC stimulation in the broodstock causes DNA hypomethylation in the offspring through the modulation of DNA-related enzymes (Callet et al. 2021).

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

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

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

4.3 Materials and methods

4.3.1 Ethics statement

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

4.3.2 Experiment diet, plan and fish management

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

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

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

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

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

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

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

4.3.3 Fish sampling

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

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

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

4.3.4 Blood chemistry analysis

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

4.3.5 Chemical composition, glycogen, and triglyceride analysis

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

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

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

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

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

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

Table 4.9 details primers for epigenetic modification-related genes

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

4.3.7 Statistical analysis

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

4.4 Results

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

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

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

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

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

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

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

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

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

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

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

4.5 Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In this study, the epigenetic modulation of parental HC feeding history was also assessed by examining the expression of histone modification enzymes at the molecular level. In mammals, NP effects associated with high-glucose conditions in parents have been shown to modulate histone modification writers and erasers in their offspring. For example, in mice, maternal diabetes mellitus or high glucose exposure in vitro decreased the expression of *sir*t2 and *sirt6* in embryonic or neural stem cells (Yu et al., 2016). Similarly, 16 weeks of sucralose and stevia supplementation in the diets of mouse parents increased the mRNA levels of histone deacetylase 3 (*Hdac3*) in the gut of their offspring (Celume et al., 2024). In our study, NP effects of dietary HC in broodstock also induced the expression of histone modification writers and erasers in adult offspring challenged with an HC diet. Parental HC stimulation modulated: 1) H3K4me3 writers (induction: *kmt2*, *kmt2ba*, *kmt2bb*; suppression: *setd1a*) and erasers (induction: *kdm5ba*, *kdm5c*, *riox1*), 2) H3K9me3 writers (suppression: *suv39h1b*) and erasers (induction: *kdm4aa*, *kdm4ab*, *kdm4c*), 3) H3K9ac writers (induction: *kat2a*; suppression: *gtf3c4*) and erasers (induction: *sirt5*, *sirt6*) in the liver. In muscle tissue, parental HC feeding suppressed the expression of *setd1a*, *kmt2a*, *kdm5ba*, *suv39h1b*, *kat2a*, *kat2b*, and *gtf3c4*, while inducing *riox1, kdm4c*, and *sirt5*. These findings demonstrate that the modulation of histone modifications is a component of the NP effects induced by dietary CHO in broodstock. Overall, these results suggest that epigenetic mechanisms, including histone modifications, play a key role in the NP effects on CHO metabolic responses in offspring. Furthermore, these effects can persist long-term into adulthood, emphasizing the enduring impact of parental HC dietary interventions.

4.6 Conclusions

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

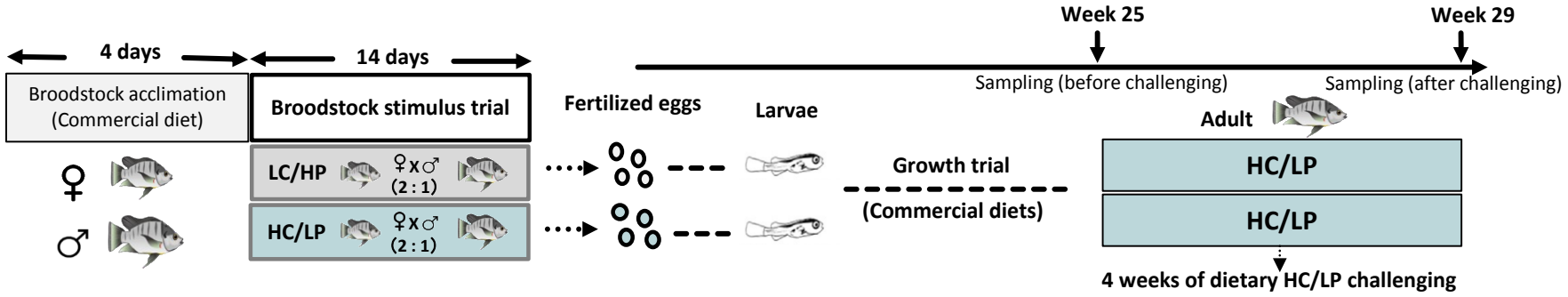

Figure 4.1 Experimental plan for nutritional programming involving dietary high-carbohydrate (HC) stimuli in broodstock and their long-term impacts on carbohydrate (CHO) metabolism in offspring. Mature male and female broodstock were acclimated to a communal breeding pond and fed a commercial diet (36%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

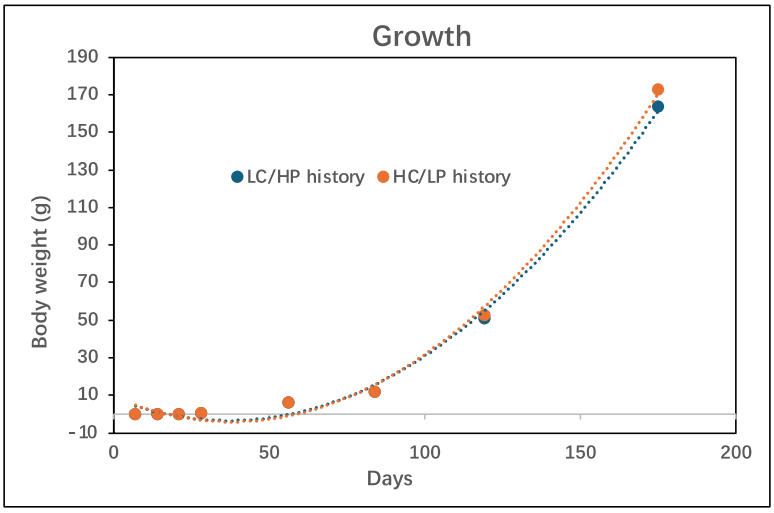

Figure 4.2 Growth of experimental offspring fish from Nile tilapia broodstock fed LC/HP and HC/LP diets for 14 days (mean ± SD, n = 6). During the growth trial, all experimental fish were fed commercial diets (days 1–49: 40% crude protein [CP], 8% crude fat [CF]; days 50–175: 32% CP, 4% CF). Abbreviations: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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

*: from Yang et al. 2013

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

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

*: from Yang et al. 2013

4.7 References

AOAC, 1990. Official methods of analysis. **Association of Official Analytical Chemists**, 62, 2742-2744.

Azaza, M. S., Khiari, N., Dhraief, M. N., Aloui, N., Kraïem, M. M., & Elfeki, A. (2015). Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus L*., in response to dietary starch to protein ratios. **Aquaculture research**. 46(1), 14-27.

Boonanuntanasarn, S., Jangprai, A., Kumkhong, S., Plagnes-Juan, E., Veron, V., Burel, C., . . . Panserat, S. (2018b). Adaptation of Nile tilapia (*Oreochromis niloticus*) to different levels of dietary carbohydrates: New insights from a long term nutritional study. **Aquaculture**. 496, 58-65.

Boonanuntanasarn, S., Kumkhong, S., Yoohat, K., Plagnes-Juan, E., Burel, C., Marandel, L., & Panserat, S. (2018a). Molecular responses of Nile tilapia (*Oreochromis niloticus*) to different levels of dietary carbohydrates. **Aquaculture**. 482, 117-123.

Bucolo, G., & David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. **Clinical chemistry**, 19(5), 476-482.

Callet, T., Li, H., Surget, A., Terrier, F., Sandres, F., Lanuque, A., . . . Marandel, L. (2021). No adverse effect of a maternal high carbohydrate diet on their offspring, in rainbow trout (*Oncorhynchus mykiss*). **PeerJ**, 9, e12102.

Callet, T., Li, H., Heraud, C., Larroquet, L., Lanuque, A., Sandres, F., ... & Marandel, L. (2022b). Molecular programming of the hepatic lipid metabolism via a parental high carbohydrate and low protein diet in rainbow trout. **Animal**, 16(12), 100670.

Celume, F. C., Pérez-Bravo, F., & Gotteland, M. (2024). Sucralose and stevia consumption leads to intergenerational alterations in body weight and intestinal expression of histone deacetylase 3. **Nutrition**, 125, 112465.

Fang, L., Liang, X. F., Zhou, Y., Guo, X. Z., He, Y., Yi, T. L., ... & Tao, Y. X. (2014). Programming effects of high-carbohydrate feeding of larvae on adult glucose metabolism in zebrafish, *Danio rerio*. **British journal of nutrition**. 111(5), 808-818.

Flegg, H. M. (1973). Ames award lecture 1972. An investigation of the determination of serum cholesterol by an enzymatic method. **Annals of Clinical Biochemistry**.

Fraga, M. F., & Esteller, M. (2002). DNA methylation: a profile of methods and applications. **Biotechniques**, 33(3), 632-649.

Ge, Z. J., Liang, X. W., Guo, L., Liang, Q. X., Luo, S. M., Wang, Y. P., ... & Sun, Q. Y. (2013). Maternal diabetes causes alterations of DNA methylation statuses of some imprinted genes in murine oocytes. **Biology of reproduction**, 88(5), 117-1.

Geng, H., Xu, W., Wu, L., Dong, B., Han, D., Liu, H., Zhu, X., Yang, Y., Xie, S., Jin, J. (2023). Early programming effects of a hyperglucidic diet on carbohydrate and lipid metabolism in gibel carp (*Carassius gibelio*). **Aquaculture Reports**, 33, 101838.

Geurden, I., Aramendi, M., Zambonino-Infante, J., & Panserat, S. (2007). Early feeding of carnivorous rainbow trout (*Oncorhynchus mykiss*) with a hyperglucidic diet during a short period: effect on dietary glucose utilization in juveniles. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology**, *292*(6), R2275-R2283.

Geurden, I., Mennigen, J., Plagnes-Juan, E., Veron, V., Cerezo, T., Mazurais, D., Zambonino-Infante, J., Gatesoupe, J., Skiba-Cassy, S., Panserat, S. (2014). High or low dietary carbohydrate:protein ratios during first-feeding affect glucose metabolism and intestinal microbiota in juvenile rainbow trout. **Journal of Experimental Biology**, 217, 3396–3406.

Gong, G., Xue, M., Wang, J., Wu, X., Zheng, Y., Han, F., Liang, X., Su, X. (2015). The regulation of gluconeogenesis in the Siberian sturgeon (*Acipenser baerii*) affected later in life by a short-term high-glucose programming during early life. **Aquaculture**, 436, 127–136.

Good CA, Kramer H & Somogyi M. (1933). The determination of glycogen. **JOURNAL OF BIOLOGICAL CHEMISTRY**, 100, 485–491.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. **JOURNAL OF BIOLOGICAL CHEMISTRY**, 177(2), 751-766.

Hamilton, J.P. (2011). Epigenetics: Principles and Practice. **Digestive Diseases**, *29*, 130–135.

Horii, T., & Hatada, I. (2016). Regulation of CpG methylation by Dnmt and Tet in pluripotent stem cells. **Journal of Reproduction and Development**, 62(4), 331-335.

Hou, Z., Fuiman, L.A. (2020). Nutritional programming in fishes: insights from mammalian

studies. **Reviews in Fish Biology and Fisheries**, 30, 67–92.

Jablonka, E., Lamb, M.J. (2002). The Changing Concept of Epigenetics. **Annals of the New York Academy of Sciences**, 981, 82–96.

Kamalam, B.S., Medale, F., Panserat, S. (2017). Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies. **Aquaculture**, 467, 3–27.

Keating, S.T., El-Osta, A. (2015). Epigenetics and Metabolism. **Circulation Research**, 116, 715–736.

Kumkhong, S., Marandel, L., Plagnes-Juan, E., Veron, V., Boonanuntanasarn, S., Panserat, S. (2020a). Glucose Injection Into Yolk Positively Modulates Intermediary Metabolism and Growth Performance in Juvenile Nile Tilapia (*Oreochromis niloticus*). **Frontiers in Physiology,** 11, 286.

Kumkhong, S., Marandel, L., Plagnes-Juan, E., Veron, V., Panserat, S., Boonanuntanasarn, S. (2020b). Early feeding with hyperglucidic diet during fry stage exerts long-term positive effects on nutrient metabolism and growth performance in adult tilapia (*Oreochromis niloticus*). **British Journal of Nutrition**, 9, e41.

Kumkhong, S., Marandel, L., Plagnes-Juan, E., Veron, V., Panserat, S., & Boonanuntanasarn, S. (2021). Glucose injection into the yolk influences intermediary metabolism in adult Nile tilapia fed with high levels of carbohydrates. **Animal**, 15(9), 100347.

Leung, L.Y., Woo, N.Y.S. (2012). Influence of dietary carbohydrate level on endocrine status and hepatic carbohydrate metabolism in the marine fish *Sparus sarba*. **Fish Physiology And Biochemistry**, 38, 543–554.

Lu, K., Liang, X.-F., Liu, T., Cai, W., Zhuang, W., Zhang, Y., Bibi, A. (2022). DNA methylation of pck1 might contribute to the programming effects of early high-carbohydrate diets feeding to the glucose metabolism across two generations in zebrafish (*Danio rerio*). **Fish Physiology And Biochemistry**, 48, 1619–1633.

Lucas, A. (1998). Programming by Early Nutrition: An Experimental Approach. **The Journal of Nutrition**, 128, 401S-406S.

Ma, F., Jiang, S., Zhang, C. (2019). Recent advances in histone modification and histone modifying enzyme assays. **Expert Review of Molecular Diagnostics**, 19, 27–36.

Marandel, L., Lepais, O., Arbenoits, E., Véron, V., Dias, K., Zion, M., Panserat, S. (2016).

Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (*Oncorhynchus mykiss*) by nutritional status and dietary carbohydrates. **Scientific Reports**, 6, 32187.

Mohanta, K.N., Mohanty, S.N., Jena, J.K. (2007). Protein-sparing effect of carbohydrate in silver barb, *Puntius gonionotus* fry. **Aquaculture Nutrition**, 13, 311–317.

Pal, A. (2022). Nutrigenomics, in: Protocols in Advanced Genomics and Allied Techniques, Springer Protocols Handbooks. **Springer US, New York, NY,** pp. 559–569.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. **Nucleic acids research**, *29*(9), e45-e45.

Rocha, F., Dias, J., Engrola, S., Gavaia, P., Geurden, I., Dinis, M.T., Panserat, S. (2015). Glucose metabolism and gene expression in juvenile zebrafish (*Danio rerio*) challenged with a high carbohydrate diet: effects of an acute glucose stimulus during late embryonic life. **British Journal Of Nutrition**, 113, 403–413.

Rocha F, Dias J, Geurden I, et al. (2016a). Dietary glucose stimulus at larval stage modifies the carbohydrate metabolic pathway in gilthead seabream (*Sparus aura*ta) juveniles: an in vivo approach using 14C-starch. **Comparative Biochemistry and physiology: Molecular and Integrative Physiology**, 201, 189–199. 25.

Rocha F, Dias J, Geurden I, et al. (2016b) High-glucose feeding of gilthead seabream (*Sparus aurata*) larvae: effects on molecular and metabolic pathways. **Aquaculture**, 451, 241–253.

Skjærven, K.H., Adam, A., Saito, T., Waagbø, R., Espe, M. (2023). Epigenetics in Fish Nutritional Programming, in: Piferrer, F., Wang, H. (Eds.), Epigenetics in Aquaculture. **Wiley**, pp. 231–244.

Song, Y., Alami-Durante, H., Skiba-Cassy, S., Marandel, L., Panserat, S. (2019). Higher glycolytic capacities in muscle of carnivorous rainbow trout juveniles after high dietary carbohydrate stimulus at first feeding. **Nutrition & Metabolism**, 16, 77.

Srisakultiew, N., Kumkhong, S., Marandel, L., Plagnes-Juan, E., Panserat, S., Boonanuntanasarn, S. (2022). Short initial period of high carbohydrate feeding improves nutrient utilisation in juvenile Nile tilapia (*Oreochromis niloticus*) fed a high carbohydrate diet. **Aquaculture**, 561, 738661.

Suárez, M. D., Sanz, A., Bazoco, J., & García-Gallego, M. (2002). Metabolic effects of changes in the dietary protein: carbohydrate ratio in eel (Angilla anguilla) and trout (Oncorhynchus mykiss). **Aquaculture International**, 10, 143-156.

Barham, D., & Trinder, P., 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. **Analyst**. 97(1151), 142-145.

Vagner, M., Robin, J.H., Zambonino-Infante, J.L., Tocher, D.R., Person-Le Ruyet, J. (2009). Ontogenic effects of early feeding of sea bass (*Dicentrarchus labrax*) larvae with a range of dietary n-3 highly unsaturated fatty acid levels on the functioning of polyunsaturated fatty acid desaturation pathways. **British Journal of Nutrition**, 101, 1452.

Vagner, M., Zambonino Infante, J.L., Robin, J.H., Person-Le Ruyet, J. (2007). Is it possible to influence European sea bass (*Dicentrarchus labrax*) juvenile metabolism by a nutritional conditioning during larval stage? **Aquaculture**, 267, 165–174.

Wang, Y., Liu, Y.-J., Tian, L.-X., Du, Z.-Y., Wang, J.-T., Wang, S., Xiao, W.P. (2005). Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, *Oreochromis niloticusxO*. aureus. **Aquaculture Research**, 36, 1408–1413.

Weatherburn, M. (1967). Phenol-hypochlorite reaction for determination of ammonia. **Analytical chemistry**, 39(8), 971-974.

Wu, X., Xu, S., Weng, J. (2022). Hyperglycemia‐mediated oocyte TET3 insufficiency predisposes offspring to glucose intolerance. **Journal of Diabetes Investigation**, 13, 1649–1651.

Xiao, Q., Li, J., Liang, X.-F., Tang, S., Zhang, Y., Zhang, Z., Kuang, Y., Dou, Y., He, S. (2020). Programming of high-glucose diet acceptance in Chinese perch (*Siniperca Chuatsi*) following an early exposure. **Aquaculture Reports**, 18, 100534.

Yu, J., Wu, Y., Yang, P. (2016). High glucose‐induced oxidative stress represses sirtuin deacetylase expression and increases histone acetylation leading to neural tube defects. **Journal of Neurochemistry**, 137, 371–383.

Zambonino-Infante, J.L., Panserat, S., Servili, A., Mouchel, O., Madec, L., Mazurais, D. (2019). Nutritional programming by dietary carbohydrates in European sea bass larvae: Not always what expected at juvenile stage. **Aquaculture**, 501, 441–447.