CHAPTER II
LITERATURE REVIEW

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

Figure 2.1 Tilapia (*Oreochromis niloticus*).

2.1 Carbohydrate and protein

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

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

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

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

2.2 Carbohydrate metabolism pathways

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

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

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

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

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

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

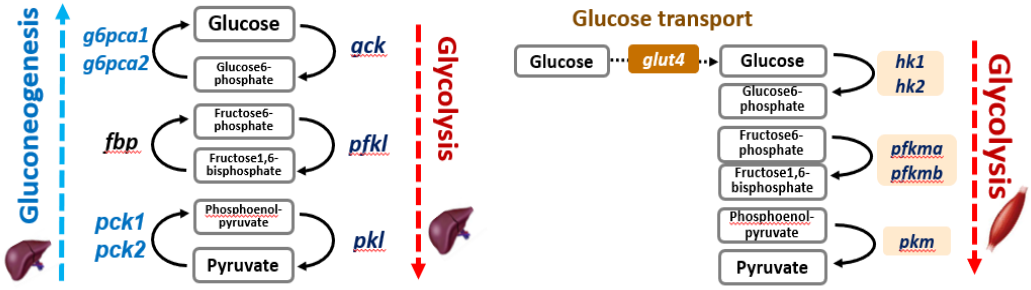

Figure 2.2 The process of glycolysis and gluconeogenesis (Tongchaitriwat et al., 2024). Liver, *g6pca*: glucose-6-phosphatase 1; *fbp*: phosphofructokinase1; *pck*: phosphoenolpyruvate carboxykinase 1; *gck*: glucokinase; *pfkl*: phosphofructokinase1; *pkl*: pyruvate kinase; muscle, *glut4*: glucose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

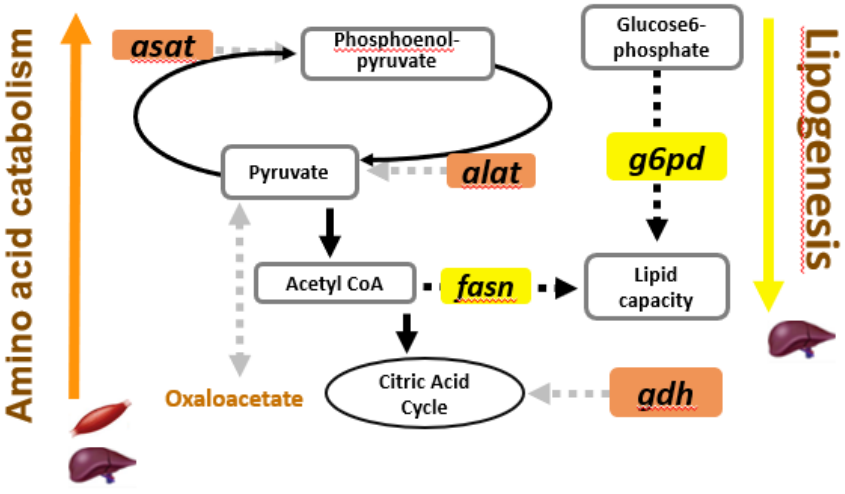

Figure 2.3 The process of lipogenesis and amino acid catabolism (Tongchaitriwat et al., 2024). *asat*: aspartate transaminase; *alat*: alanine aminotransferase; *gdh*: glutamate dehydrogenase; *fasn*: fatty acid synthase; *g6pd*: glucose-6-phosphate dehydrogenase

2.3 Carbohydrate utilization in fish

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

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

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

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

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

2.4 Nutritional programming

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

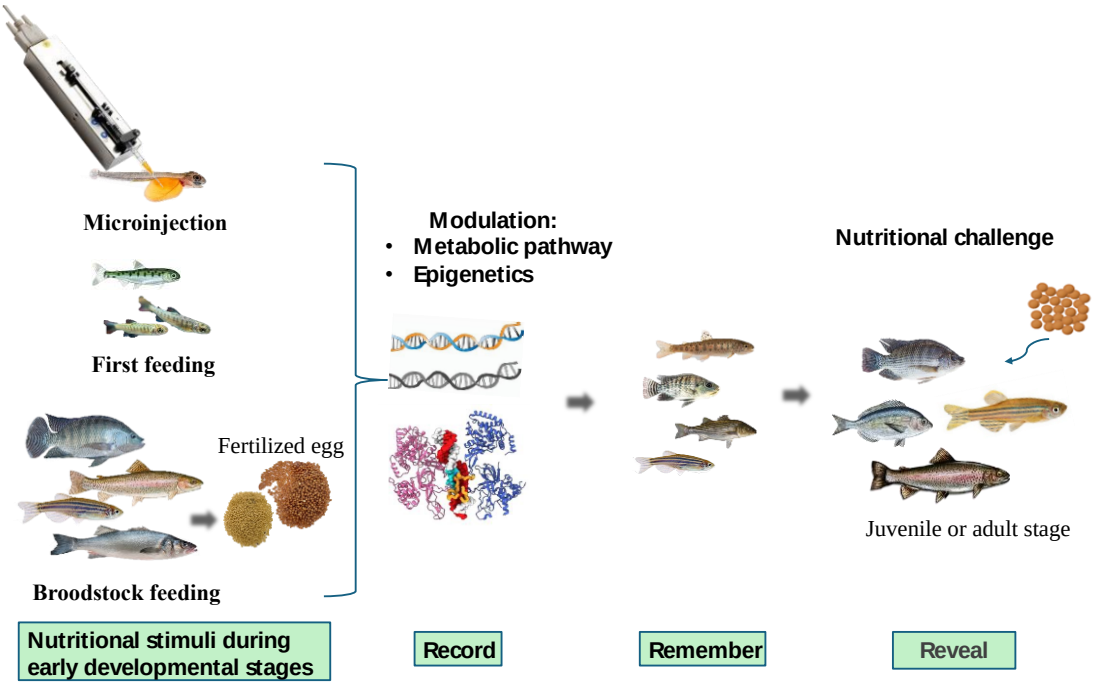

Figure 2.4 The principle of nutritional programming.

2.5 Nutritional programming of dietary carbohydrate on fish

2.5.1 Nutritional programming of dietary carbohydrate on fish early stage

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

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

2.5.2 Nutritional programming of dietary carbohydrate on broodstock zebrafish

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

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

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

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

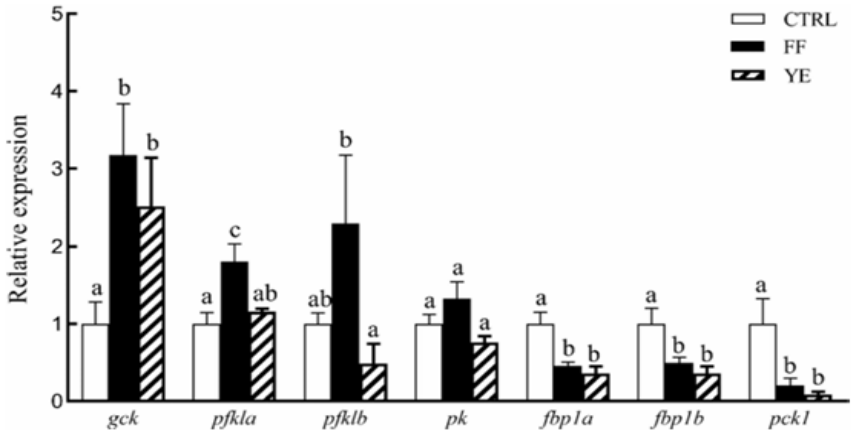

Figure 2.5 The long-term effect of early high-carbohydrate treatment on hepatic gene expression in adult zebrafish F0 (1-week HC challenge) (Lu et al 2022). CTRL, fish fed with control diet; FF, fish fed with HS from the first-feeding stage to the end of the yolk-sac larval stage; YE, fish fed with HS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

2.6 Epigenetics modification

2.6.1 Main research contents of epigenetics

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

2.6.1.1 DNA methylation

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

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

2.6.1.2 Histone modifications

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

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

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

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

2.6.1.3 Non-coding RNA

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

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

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

2.6.2 The role of epigenetic modifications in glucose metabolism

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

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

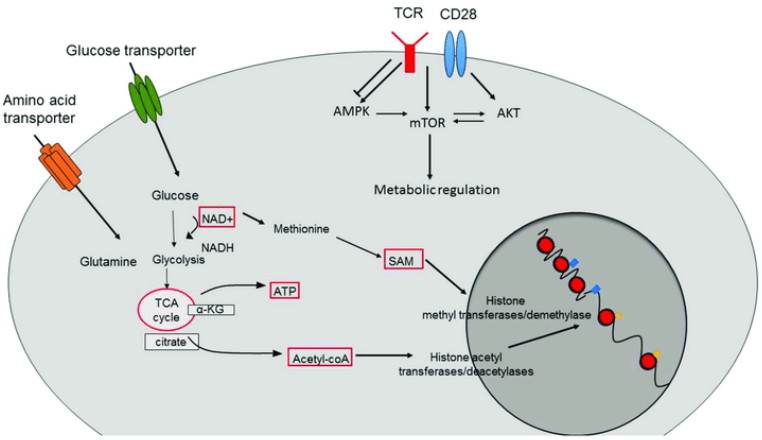

Figure 2.6 The role of epigenetic modifications in glucose metabolism (Yerinde et al.2019).

2.6.3 The role of epigenetic modifications in lipid metabolism

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

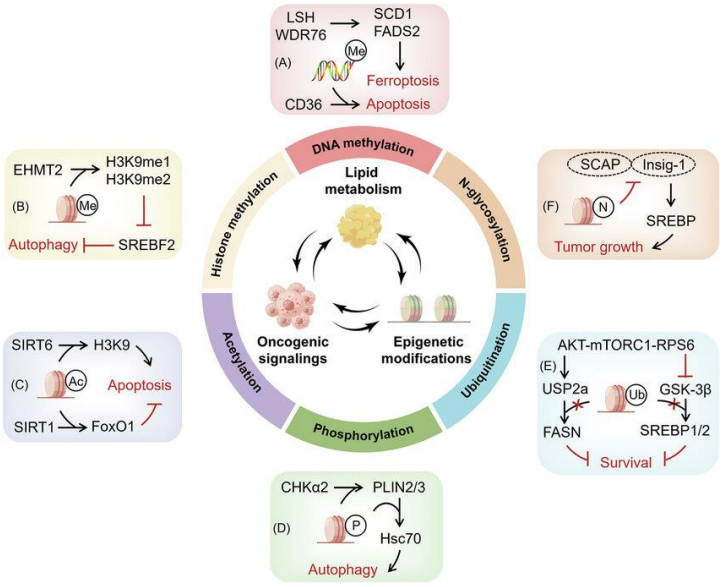

Figure 2.7 The role of epigenetic modifications in lipid metabolism (Zhang et al. 2022).

2.6.3.1 DNA methylation and lipid metabolism

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

2.6.3.2 Histone modification and lipid metabolism

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

2.6.3.3 Non-coding RNA and lipid metabolism

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

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

2.7 Effect of dietary high carbohydrate on fish epigenetics modification

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

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

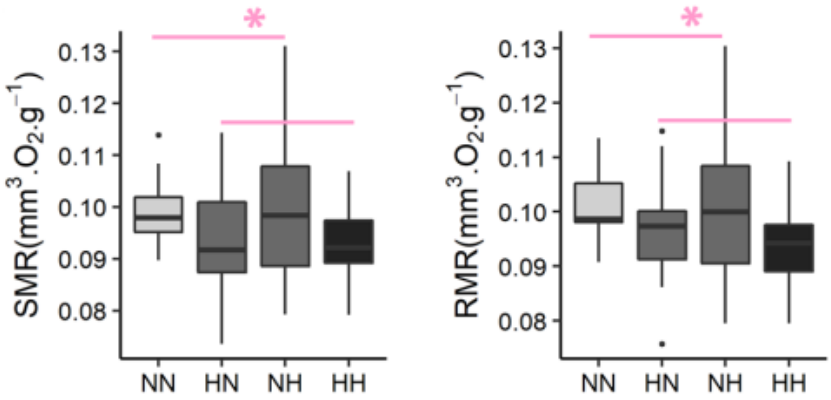

Figure 2.8 Effect of parental LP/HC diet on fry metabolic rates (Callet et al 2021).

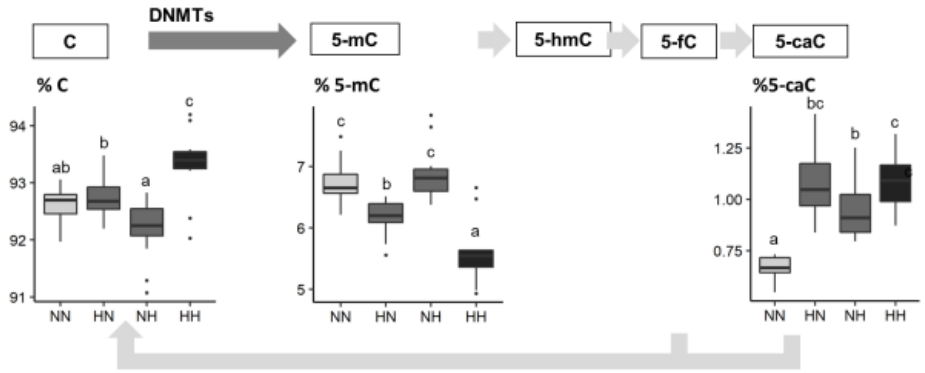

Figure 2.9 Effect of parental LP/HC diet on fry DNA methylation (Callet et al 2021).

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

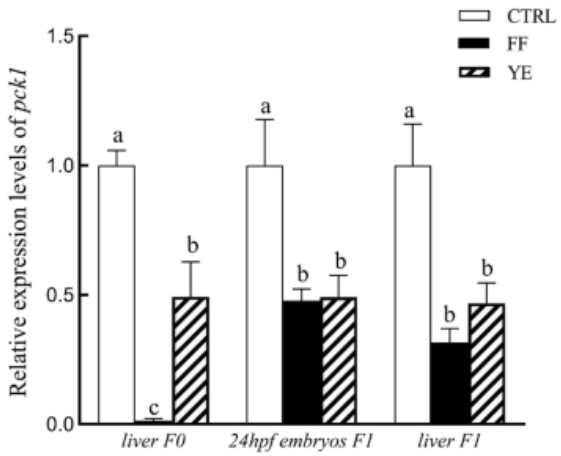

Figure 2.10 The pck1 expression levels in FF, YE, and the control group in F0 and F1. pck1, phosphoenolpyruvate carboxykinase 1 (Lu et al. 2022).

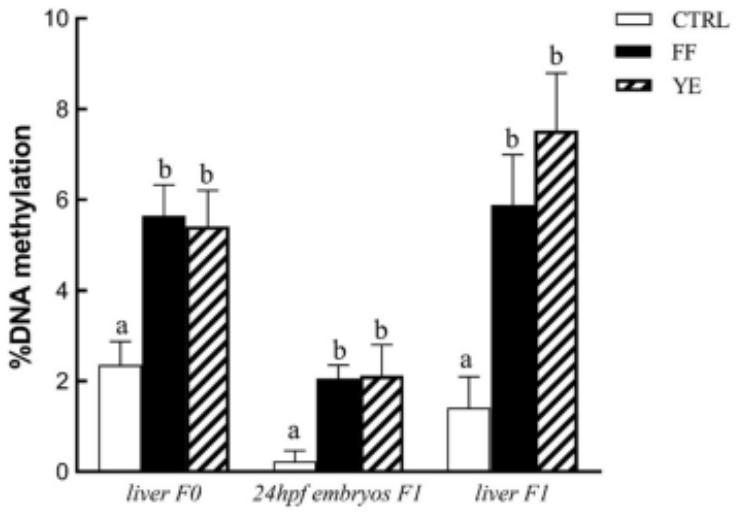

Figure 2.11 Gene-specifc DNA methylation in the promoter region of pck1 (Lu et al. 2022).

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

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

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

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

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

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