論 文 の 内 容 の 要 旨

水圏生物科学専攻
平成 22 年度博士課程　入学
氏名　韓　ユナ
指導教員名　潮　秀樹

Studies on mechanisms for physiological modulation of energy metabolism in rainbow trout *Oncorhynchus mykiss*
(ニジマスのエネルギー代謝制御機構に関する研究)

Fish culture is conducted over fifty years in marine, brackish or freshwater. One of advantages of fish culture is the partial controllability of physiological conditions such as growth, lipid metabolism, body composition, stress responses and disease resistance. However, culture fish generally have low quality compared with wild fish. Actually lipid contents of culture fish are usually higher than those of wild fish. Lipids in fish muscle are one of the important factors for meat acceptability by human, but excessive lipid accumulation often deteriorates meat quality.

Fish, in special carnivorous fish, have the characteristics similar to mammals with type 2 diabetes in energy metabolisms. The metabolic syndrome including type 2 diabetes is a cluster of the most dangerous metabolic risk factors, including obesity, insulin resistance, cardiovascular diseases and hyperglycemia, etc. Hypertrophies of the adipocyte and insulin resistance are the major cause of being metabolic syndrome. Adipocyte is one of the most highly insulin-responsive cell. Adipocyte function not only as an energy reservoir but as an endocrine tissue. It produces and secretes adiponectin, one of the adipocytokines, which regulates energy metabolisms. The effects of adiponectin are mediated by adiponectin receptor, adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) and the signaling pathways via these receptors lead to

the controlling systems of insulin sensitivity.

γ-Oryzanol (ORZ) is one of major bioactive components in rice bran oil. ORZ has been suggested to possess the effects of lowering serum cholesterol levels, the effects enhancing lipid and carbohydrate catabolisms, the anti-inflammatory effects, the anti-cancer effects and the antioxidative effects. Although mammals hardly accumulate ORZ in their skeletal muscles, it has been recently reported that fish accumulate the compounds in their skeletal muscle. However, there is very little information about the effects of ORZ administration of energy metabolism regulation in fish.

Previous studies verified that rainbow trout adiponectin transcripts were abundant in muscle and marginally detected in adipose tissue, in consistent with mammalian cases. The recent EST analysis for rainbow trout has suggested that heart fatty acid binding protein (H-FABP), a member of intracellular fatty acid binding proteins, is useful as a adipocyte marker together with adiponectin.

The aim of this study is to disclose the mechanisms for the physiological modulation of energy metabolism in rainbow trout. This research assessed the effects of ORZ administration on fish energy metabolism regulators, including lipid metabolism, glyconeogenesis and glycolysis. This study also pursued to reveal the distribution of the key cells, adipocytes, in rainbow trout skeletal muscle and liver in order to discuss about the modulation mechanisms for energy metabolisms.

1. The effect of γ-oryzanol administration on fish energy metabolism regulation systems

In this chapter, it was analyzed the effects of ORZ administration on energy metabolism of rainbow trout based on energy metabolism-related gene expressions. Rainbow trout (*Oncorhynchus mykiss*) were divided into three groups: group 1 was fed with control diet (CD), group 2 was fed with a low-concentration of ORZ diet (8 μg/kg body weight/day, LOD) and group 3 was fed with a high-concentration of ORZ diet (40 μg/kg body weight/day, HOD). After 4-week pre-feeding and 8-week ORZ-feeding, the greatest body weight gains were achieved in the HOD groups and the administration of ORZ improved feed conversion efficiency. Gene expressions related to lipid metabolism, gluconeogenesis and glycolysis were measured in skeletal muscle and liver of rainbow trout by semi quantitative real-time PCR. mRNA expression levels of adiponectin and adiponectin receptors were also analyzed. Gluconeogenesis was suppressed by low-concentration ORZ administration for 8 weeks in rainbow trout skeletal muscle. Adiponectin expression levels increased in both concentrations of ORZ for 4 weeks.

AdipoR1 and adipoR2 gene expressions showed no significant difference in rainbow trout skeletal muscle. In liver, HOD administration would also enhance lipid catabolism pathways. Glyconeogenesis-related gene expressions were induced in the high ORZ administered group. Adiponectin and its receptor mRNA expression levels were also enhanced in the HOD group. From these resuls, ORZ administration would drive the lipid and carbohydrate metabolism equilibrium into the catabolism in rainbow trout.

2. Distribution of adipocyte-related cells in skeletal muscle and liver of rainbow trout

In mammals, adipose tissues, a bulk of adipocytes, have an important role in modulating energy metabolisms in whole body as stated above. However, there is no information about the physiologically active adipocytes corresponding to the mammalian counterpart in fish. In this chapter, anti-adiponectin and H-FABP antibodies were produced to investigate the distribution of physiologically active adipocytes in rainbow trout skeletal muscle and liver.

Antigenic peptides were designed through a prediction program (http://emboss.dbcls.jp/) and constructed 3D models using the SWISS-MODEL program (http://swissmodel.expasy.org/) and sybyl 8.1 software (Tripos, MO, USA) for the estimation of the distributions of the antigenic peptide sequences in the molecules. KLH-conjugated peptides were injected on the rabbit. After the third immunization, the rabbit was sacrificed and the sera were collected. Saturated ammonium sulfate is used to purification of antibodies. PD-10 column and HiTrap affinity column were used for desalted and purified, subsequently. Prior to investigation of the distribution of adiponectin and H-FABP proteins, the reactivity of each polyclonal antibody was confirmed by Western blot analysis.

Western blotting analysis with the anti-adiponectin antibody successfully detected the adiponectin signal around 75 kDa in a putative trimer in skeletal muscle proteins of rainbow trout. A protein signal of around 15 kDa in molecular mass was also successfully detected by the anti-H-FABP antibody. Subsequently, these antibodies were conducted immunohistochemistry in skeletal muscle and liver sections obtained with a vibratome (~500 micrometer in thickness). Adiponectin signals were detected in the small cells outside of skeletal muscle cells. H-FABP signals also showed the similar distributions. Higher magnification revealed that adiponectin and H-FABP signals were observed in adipocyte-like cells in rainbow trout skeletal muscle tissues, quite different from mammalian cases. Adiponectin and H-FABP signals were also spread in whole

liver. These results suggest that the adiponectin gene expressions in skeletal muscle and liver of rainbow trout in the previous chapter are due to the adipocyte-related cells and that the adipocyte-related cells might have important roles in controlling energy metabolisms as mammalian adipose tissues. Taken together with the results of the previous chapter that the catabolizing compound ORZ enhanced the adiponectin expressions in skeletal muscle and liver, the adipocyte-related cells might physiologically function through not only endocrine but autocrine systems for individual organs.

The present study has provided that the administration of ORZ affects fish energy metabolisms, especially lipid metabolism, gluconeogenesis and glycolysis. Adiponectin and H-FABP-positive cells were present in skeletal muscle and liver of rainbow trout and these cells might have important roles for the modulation of energy metabolisms in rainbow trout. From the results of the previous studies that ORZ in diet is accumulated in rainbow trout skeletal muscle and liver, ORZ would directly and/or indirectly work on the adipocyte-related cells and consequently energy metabolism-related gene expressions in skeletal muscle and liver. Future investigations about the isolated and cultured adipocyte-related cells discovered in the present study will disclose the mechanisms controlling energy metabolisms of fish. The present study shows that ORZ is one promising tool for improving fish energy metabolisms like the case of human type 2 diabetes, poor in the catabolizing ability of lipid and carbohydrate and for improving culture fish meat quality.