The Effect of Ferulic Acid on Muscular Growth in Zebrafish (Danio rerio)

その他のタイトル	フェルラ酸によるゼブラフィッシュ骨格筋増強効果に関する研究
学位授与年月日	2018-03-22
URL	http://hdl.handle.net/2261/00078170

論文の内容の要旨

水圏生物科学専攻

平成 25 年度博士課程入学

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論文題目 The effect of ferulic acid on muscular growth in zebrafish (*Danio rerio*)

（フェルラ酸によるゼブラフィッシュ骨格筋増強効果に関する研究）

According to the report of Food and Agriculture Organization (FAO) in 2016, the global fisheries and aquaculture continue to expand for meeting the needs of global protein consumptions. For rapid growth with large economic benefits, beta-agonists were used in aquaculture, which would bring potential health hazards after consumption of products with residues. Therefore, it is urgent to find a natural and safe alternative to beta-agonists with similar functions but without side-effects.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) is one ubiquitous hydroxycinnamic acid derivative in the catalog of polyphenols. It is widely distributed in the plant kingdom in both free and bound forms including woods, vegetables, fruits, grains, cereals, legumes, spices and Chinese herbs. In the primary cell wall of many plants, mono-ferulic acid and dihydroferulic would be covalently conjugated or esterified with polysaccharides and lignins for making crosslink between them and confer rigidity to the cell wall. As a naturally existing antioxidant, ferulic acid has been a hot research topic for recent years, especially in the field of human disease and health. It was reported to possess anti-inflammation, anti-hyperglycemia and cholesterol-lowering ability; it was used as the treatment for cancer, diabetes, cardiovascular disease, and Alzheimer's disease. There were several applications of ferulic acid in food industries such as food preservatives and antioxidant supplements and in cosmetics such as active ingredient being added in skin lotion and sunscreen. However, there was barely no scientific research reporting that ferulic acid would have effect on muscle growth.

Hypertrophic growth of skeletal muscle, referring to muscular hypertrophy, is characterized as increased muscle mass and enlarged cross-section area due to the growth of myofiber. Myofiber, or a skeletal muscle cell contains bundles of myofibrils, each of which is composed of sarcomeric units. In adult fast skeletal muscle, the growth process of myofibers involves the coordinate expression of numerous muscle-specific genes, especially these encoding sarcomeric unit proteins, under the regulation of series of transcription factors. As members of myogenic regulatory factors (MRFs) responsible for myogenesis, MyoD and myogenin were reported to positively regulate fast myosin heavy chain gene expression and troponin I gene expression in mice. As a ubiquitous transcription factor, serum response factor (SRF) also contributes to hypertrophic growth of fast skeletal muscle by enhancing skeletal alpha-actin gene expression as reported in chicken. On the other hand, mTOR/p70S6K/4E-BP1 signaling pathway is a crucial regulator of muscular hypertrophy. Along with the activation of this pathway, mRNA translation and protein synthesis are initiated.

Nowadays, zebrafish is becoming one of the most acceptable animal research models because of its easiness in maintaining and breeding, short culturing period due to high reproductive capacity, and the comprehensive genomic background. It has been used as the research model in aquaculture due to the similar nutritional pathways and synteny of genomes shared with those aquaculture species with economic importance such as carp, tilapia and rainbow trout.

In **Chapter 2**, zebrafish was used as the experimental subject for exploring potential the effect of ferulic acid. Male zebrafish were grouped into 3 populations with different initial body weight and length and allocated into two dietary groups for each population: normal feeding group as a negative control (NC) and ferulic acid feeding group as an experimental group (FA). Compared to the NC group, a higher body weight and BMI of zebrafish in the FA group were detected in all 3 populations after ferulic acid administration for 30 days. Differences of body weight and BMI showed up around day 10^{th}, while significances showed up around day 20^{th}. This increasing trend was maintained throughout the study. The changes in body indices suggested the growth-promoting effect of ferulic acid and provided the first clue of our study, which inspired us to found the source of weight and BMI gaining.

In the followed **Chapter 3**, body composition analysis was carried by CT scan on the whole body. The amount of total muscle mass of zebrafish in the FA group was significantly increased compared to the NC group. Furthermore, the relative increased amount of total muscle mass accounted for 88.98% of the relative increased amount of body weight, which suggested the increased muscle mass was the major source of weight gaining. For better understanding the increase in the muscle mass, frozen section of fast skeletal muscle was performed and fiber cross section area (FCSA) was measured. The enlarged size of fast skeletal myofibers as demonstrated in morphometric analyses evidenced the hypertrophic phenotype of fast skeletal muscle in adult zebrafish after ferulic acid administration for 30 days (Figure 1). In details, there were lower percentage of small fibers (FCSA<4,000 μm^2) but higher percentage of medium (with area between 4,000 μm^2 and 6,000 μm^2) and large fibers (FCSA>6,000 μm^2) showing in zebrafish of the FA group than in zebrafish of the NC group. Above findings suggested ferulic acid enlarged the size of fast myofibers, as the major symbol of muscular hypertrophy, and finally led to the increase in total amount of muscle mass.

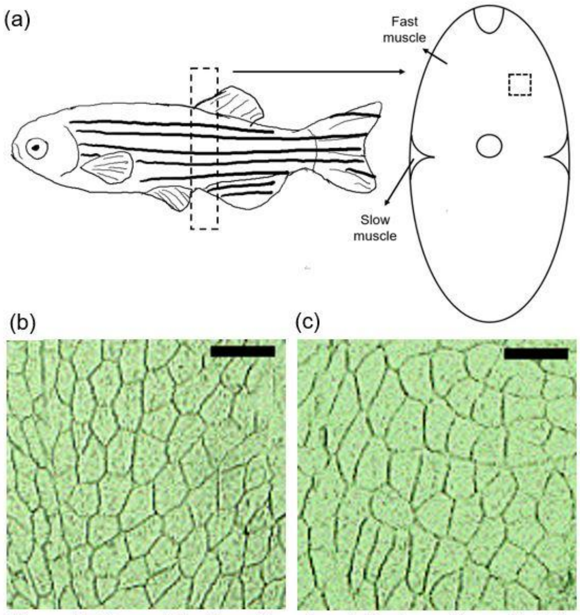

Figure 1 Effects of ferulic acid on fast skeletal myofibres of adult zebrafish. (a) Image of fast skeletal muscle tissue used for cross section. Frame with dotted line shows the position of filet dissection. (b) Cross-sectional fast skeletal muscle of zebrafish in NC group and (c) in FA group; Square with dotted line shows the area of (b) and (c).

For further confirmation of the hypertrophic phenotype and for deciphering the molecular changes occurring in fast skeletal muscle, gene expression level of transcription factors including MyoD, myogenin and SRF were examined due to their important roles in muscular hypertrophy. As described in **Chapter 4**, higher transcription levels of MyoD, myogenin and SRF genes were detected in the FA group than the NC group, which evidenced the occurring of hypertrophic growth of fast skeletal muscle after ferulic acid administration. In addition, higher transcriptional levels of genes encoding sarcomeric unit proteins were indeed observed in the FA group than the NC group, probably contributing to the enlarged skeletal myofiber cell size. The increased expressions of skeletal alpha-actin, myosin heavy chain, troponin I and tropomyosin genes were under the regulation of above transcription factors.

On the other hand, increased protein synthesis is another major symbol of muscular hypertrophy, which could be regulated by the major anabolic pathway—mTOR/p70S6K/4E-BP1 signaling pathway. As the work been done in **Chapter 5**, relatively higher levels of phosphorylated zebrafish target of rapamycin (zTOR) at residue Ser2448, phosphorylated p70S6K at residue Ser371, and phosphorylated 4E-BP at residue Thr 37/46 were observed in the FA group than the NC group, which suggest an enhanced mRNA translation efficiency and increased overall rate of protein synthesis after ferulic acid administration. Therefore, with the combination of a higher transcription level of genes encoding sarcomeric unit proteins, a higher translation efficiency and enhanced protein synthesis ability, a higher amount of proteins is expected in hypertrophic muscle cells. The higher protein level of myosin heavy chain (MyHC), one subunits of myosin, detected in fast skeletal muscle of zebrafish of the FA group, supported the above viewpoint. These changes and adaptations occurring on molecular level would finally converge and lead to the increase in muscle mass.

The present study is the first study discovering the muscular growth-promoting effect of ferulic acid in the aquaculture model—zebrafish, which could be extended to other teleost species, especially them with economic importance. Considering that skeletal muscle is the primary product of fish and dietary protein source, the increased muscle mass is closely linked with the profitability of aquaculture production. Muscular hypertrophic phenotype was identified by the larger cross-section area of myofibers and was further evidenced by the changes occurring on molecular level. The relative mRNA expression level of transcriptional factors (MyoD, myogenin and SRF) involving myogenesis and hypertrophy and their target genes encoding sarcomeric unit proteins (skeletal alpha-actin, myosin heavy chain, tropomyosin and troponin I) was elevated. The translation efficiency and protein synthesis capacity of fast muscle cells were enhanced resulting from a higher activated status of the zTOR/p70S6K/4E-BP1 pathway. Furthermore, the activation of zTOR was reported to inhibit autophagy, leading to the protein retention. Finally, all above alternations will lead to enlarged size of myofibers containing more amount of sarcomeric unit proteins such as myosin, confirmed by the higher protein level of myosin heavy chain (MyHC). Given to its natural, ubiquitous and low toxic property, ferulic acid could be therefore a candidate for feedstuff additives in aquaculture for muscular growth-promoting purpose.

KEY WORDS Zebrafish, Ferulic acid, Skeletal muscle mass, Muscular hypertrophy, Protein synthesis