

Protocols supporting "Metabolomics Investigation of Dietary Effects on Flesh Quality in Grass Carp (*Ctenopharyngodon idellus*)"

Honghao Zhao,Jasmine Chong,Rong Tang,Li Li,Jianguo Xia,Dapeng Li

Abstract

The ultra-high density intensive farming model of grass carp (*Ctenopharyngodon idellus*) may elicit growth inhibition, decline flesh quality and increase disease susceptibility of fish. The quality degradation and excessive fat accumulation in cultured *C. idellus* have long been attributed to possible alterations in the lipid metabolism of fish muscle tissues as a result of over-nutrition from artificial diets. To investigate the effects of different diets on fish muscle quality, a large-scale metabolomics study was performed on 250 tails of *C. idellus*.

The experimental fish were divided into four groups based on sex and diets - female artificial feed (FAF), female grass feed (FGF), male artificial feed (MAF) and male grass feed (MGF). After 113 days rearing period, the AF group showed significantly higher total mass of muscle fat ($P < 0.01$), with the FAF group being the highest. Metabolomics profiling based on liquid chromatography-mass spectrometry (LC-MS) revealed distinctive patterns of clustering according to the four groups. Overall, artificial feeding was associated with higher concentrations of docosapentaenoic acid (DPA), dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (ARA); whereas grass-feeding was associated with elevated n-3 unsaturated fatty acids (n-3 UFAs), such as eicosapentaenoic acid (EPA), alpha-linolenic acid (ALA) and gamma-linolenic acid (GLA). Artificial feeding also resulted in significant increased docosahexaenoic acid (DHA) in MAF muscle than MGF fish, whereas no significance in the comparison of female samples. Metabolic pathway analyses using both targeted and untargeted approaches consistently revealed that the arachidonic acid metabolism and steroid hormone biosynthesis pathways were significantly different between AF and GF groups.

Our results suggested that grass is a better source of diet fatty acid and protein when compared to artificial feed. Grass feeding could effectively lower triglycerides in serum, reduce fat accumulation and alter lipid compositions in fish muscle by increasing the concentrations of n-3 UFAs, leading to better nutrition and health.

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