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Abstract

Transcriptomic Analysis of Differentially Expressed Genes in Kidney and Intestine of *Dicentrarchus labrax* Fed Different Nutritional Amounts of Inorganic Phosphate †

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Abstract: Phosphorus (P), in the form of inorganic phosphate (Pi), is one of the most important macronutrients for all organisms, including fish. It is indispensable for the formation of hard tissues such as bones, but also for cell signalling and cell membrane formation, and energy transduction, among many other functions and is kept under well-controlled conditions, since its deficiency or overload may lead to skeletal malformation or ectopic calcification, disturbances of intermediary metabolism, growth and function impairment, endocrine dysfunction, and eventually death. Fish feeds used in aquaculture are therefore P-rich but excess/unused/excreted P in the effluents can lead to eutrophication and a consequent deleterious change in the aquatic ecosystem. The objective of this study was to evaluate the expression profiles and transcripts modified by dietary P, to identify pathways and mechanisms involved in P transport and regulation in the kidney and intestine. Juvenile *Dicentrarchus labrax* were fed using a commercial feed (1.1% P) or tailored-made feeds containing 0.05%, 1.1%, or 3% Pi. Fish (duplicate tanks, n = 10) were fed for 70 days and weighed periodically to evaluate growth changes. Kidney and intestine were used for RNA extraction. Next-Generation Sequencing and RNAseq library preparation were performed in an Illumina system following the manufacturer's recommendations. Annotation was performed using the available sea bass genome assembly. Bioinformatic analysis showed significant differences in expression patterns among the three conditions tested in both tissues. In the kidney, increased P led to a total of 135 differentially expressed genes (DEGs; 82 up and 53 down), while only 54 (11 up and 43 down) genes responded to P restriction. In the intestine, high P affected the expression of 50 genes (16 up and 34 down) whereas only 26 (6 up and 20 down) were modified by low P. However, DEGs between high and low P were 156 in kidney and 154 in intestine. Preliminary analysis suggests the most affected pathways were those involved in cellular metabolism and phosphorylation but also on the structure of cell membranes, either for maintaining membrane integrity or in genes related to transmembrane ion transport. We expect this research to reveal the molecular implications of dietary P imbalance looking at specific targets such as membrane transporters and regulatory factors, but also to the larger metabolic pathways affected in these two key organs for P uptake and excretion.

Keywords: inorganic phosphate; fish feeds; nutritional physiology; transcriptomics; aquaculture; environmental protection

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