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FACULTY OF FISHERIES AND PROTECTION OF WATERS
UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE

TEMPORAL AND SPATIAL EXPRESSION PATTERNS OF PREGNANE X RECEPTOR AND VITAMIN K EPOXIDE REDUCTASE GENES, TWO CORE MOLECULAR PLAYERS ON FISH VITAMIN K HOMEOSTASIS AND SKELETAL DEVELOPMENT

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Vitamin K (VK) is a liposoluble vitamin known to be essential for bone metabolism by two different pathways: (i) by its role as a coenzyme in the gamma-carboxylation of some skeletal proteins (e.g. osteocalcin (OC) and matrix Gla protein (MGP); Price et al., 1998); and (ii) through its role in skeletal gene transcription via binding to the pregnane X receptor (PXR; Azuma et al., 2010).

Carboxylation is accomplished by the enzyme gamma glutamyl carboxylase (GGCX) which requires the propeptide-containing substrate, reduced VK, CO₂, and O₂. As a result, it is produced VK epoxide, which can be recycled to reduced VK by the enzyme VK epoxide reductase (VKOR). Therefore, the rate of carboxylation is mainly controlled by the level of reduced VK available for the reactions, and indirectly by the reduced VK uptake or by the recycling activity of VKOR (reviewed in Stafford, 2005). In fish, supplementation of VK in diets has a positive effect on the incidence of skeletal deformities during fish juvenile growth (Roy and Lall, 2007); whereas nutritional imbalance (deficiency or excess) affects bone mineralization, bone mass, and increases vertebral deformities (Udagawa 2001). However, the mechanisms associated with the osteogenic and skeletogenic activity of VK in fish are still poorly understood and the optimal nutritional requirements remain to be determined.

The present work aimed to describe the temporal and spatial expression patterns of zebrafish PXR and VKOR genes by means of qPCR and *in situ* hybridizations. In addition, OC, MGP, PXR and VKOR gene expression levels, bone mineral content and incidence of skeletal deformities from fish fed different dietary VK doses (0, 250 and 1250 mg VK/kg), were analysed in triplicate. Our results highlight the effects of nutritional VK supplementation in fish skeletogenesis and bring new knowledge to the functioning of both molecular pathways (gene transcription and gamma carboxylation) involved in these effects. This knowledge represents an important contribute for future research works aiming to point out the underlying mechanisms and the nutritional requirements for better bone development and quality of fish produced in aquaculture.

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FISH SKELETAL DEFORMITIES: A MAJOR IMPACT ON AQUACULTURE WITH DIFFERENT ETIOLOGIES.

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Skeletal deformities still represent a major bottleneck for fish farmers as they are responsible for a loss of several millions of euros. Losses due to deformities occur mainly at two levels: At hatcheries, by reducing larval survival rate and growth efficiency in malformed fish; and at on-growing farms, where malformed market size fish have to be discarded or sold at lower values. Therefore, skeletal deformities hamper fish production and increase production costs (Michie, 2001; Koumoundouros et al., 2002). Moreover, skeletal deformities negatively affect fish welfare, representing a major issue in current aquaculture in relation to the consumer's perception of the industry as a healthy, environmentally and bioethical sector producing animal protein. Thus, reducing the incidence of larval deformities would reduce the production costs, and improve the quality of aquaculture products and its perception for the consumer.

Most skeletal deformities appear during the larval and juvenile stages, where many biological processes take place for organogenesis, morphogenesis and metamorphosis in a very short time. Different causative factors of skeletal deformities have been identified in recent years; however, since the aetiology of particular skeletal deformities could be related to different factors and underlying pathways, a consistent decrease on the incidence of osteological deformities has not been achieved so far.

An overview of the already identified causative factors and their related skeletal deformities will be detailed and discussed. Special emphasis will be given to describing the underlying pathways of abnormal skeletogenesis, and the knowledge needed to develop new strategies to face it. As an example, knowing how fat soluble vitamins (already known as causative factors of skeletal deformities) compete for the same lipoprotein transporters to be up taken from the intestinal lumen. Nutritional balanced diets should be developed using multifactorial approaches in fat soluble vitamins, and not by unifactorial approaches, as currently used since fish skeletogenesis depends on growth, which relies on the coordinated development of different tissues/organs such as muscle and digestive system. Multidisciplinary studies should therefore be performed, providing a full integrated view of metabolic pathways affected in fish larvae. Ongoing and future research work will be described, indicating potential converging points at underlying molecular pathways between different causative factors.

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