MOLECULAR MECHANISMS MEDIATING ENHANCED FEED ACCEPTANCE OF PLANT-BASED DIET IN RAINBOW TROUT FOLLOWING EARLY EXPOSURE AT FIRST FEEDING

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SUMMARY

Sustainable aquaculture, which entails proportional replacement of fish-based feed sources by plant-based feed sources, is impeded by the poor growth response frequently seen in fish fed high levels of plant ingredients. In this regard, we explored the possibility of tailoring fish to adapt to plant-based feeds. Early nutritional imprinting of fish with such feed is a potential strategy, in tune with the widely accepted paradigm in mammals that early nutritional events may permanently alter the organism's physiology and metabolism in preparation to the predicted environment (Gluckman et al., 2008). In fish, studies testing the effect of early nutritional challenges employing diets with high carbohydrate levels (Geurden et al., 2007) or with different fatty acid profiles (Vagner et al., 2007) triggered persistent digestive and metabolic adaptations at the level of gene expression.

Rainbow trout (*Oncorhynchus mykiss*) swim-up fry were exposed for the first 3 weeks of exogenous feeding either i) to a plant-based diet (diet V; *V-fish*) containing a blend of plant oils (palm, linseed, rapeseed) and plant-protein sources (wheat gluten, corn gluten, soybean meal, dehulled peas, white lupin) or, ii) to an isoproteic diet containing fishmeal and fish oil as sole protein and fat source (diet M; *M-fish*). After 7-months of common rearing on diet M, *V-* and *M-fish* were then challenged to feed the plant-based diet V (*V-challenge*, see Fig. 1). The experiment was performed in three isogenic rainbow trout families (F1-F3). Voluntary feed intake (FI), growth and nutrient utilization were monitored. At the end of the early feeding period and of the V-challenge, the expression of selected marker genes from fish of both V- and M-groups was analyzed.

The results of the V-challenge (averaged over the 3 families) showed a 42% higher growth in trout juveniles of nutritional history V compared to M (Fig. 2A). This can be ascribed to the higher voluntary FI in V- compared to M-trout which had never been confronted before with diet V, an effect which was more pronounced at the beginning of the V-challenge (data not shown). Cumulated over the V-challenge and depending on the family, V-exposed fish consumed 20 to 70% more than M-fish (Fig. 2B). Besides the effects on FI, V-fish also utilized diet V more efficiently than M-fish, as reflected by the on average 18% higher feed efficiency (Fig. 2C). The positive nutritional V-history effect on FI implies that the V-fish were able to 'recognize' diet V, and this almost 7 months after the initial early exposure.

Additionally, following the early exposure to diet V, we observed changes in expression of genes whose products may mediate xenobiotic-metabolism of anti-nutritional factors (plant secondary metabolites) including Phase I, cytochrome P450 family members; Phase II, glutathione-S transferase and sulfotransferases; and Phase III drug transporters, multidrug resistance protein family members. Also, differential expression of genes encoding neuropeptides, known to play a role in regulating feed intake, was observed. Furthermore, some of these changes in gene expression were sustained following the late V-challenge, suggesting that these responses are consistent, even 7-months after the initial short-term exposure to diet V.

Our study shows that an early short-term exposure of trout fry to a plant-based diet improves both acceptance and utilization of such a diet when given later in life, irrespective of the genetic background. The phenotypic

changes were accompanied by changes in expression of genes mediating detoxification pathways and those that control feed intake, both during the initial early exposure, and 7-months later, following the *V-challenge*. In mammals, epigenetic changes at genes triggered by nutritional stress at gestational and neonatal growth have been shown to accompany the transference of persistent phenotypes to the adult organism and to future generations (Burdge et al., 2011). It is of interest to further explore these adaptive mechanisms in fish, from the perspective of employing early nutritional challenges to improve the utilization of plant ingredients for sustainable aqualculture.

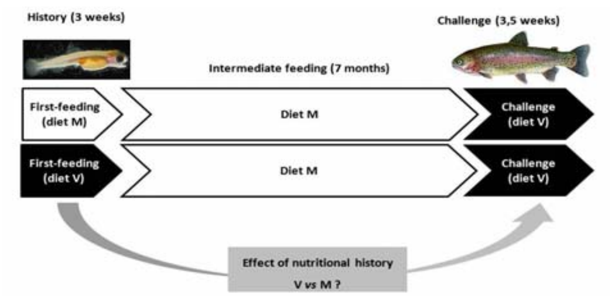

Figure 1. Experimental design. An illustration of the early nutritional exposure (history) and the subsequent challenge with an all- plant based feed (diet V) in rainbow trout.

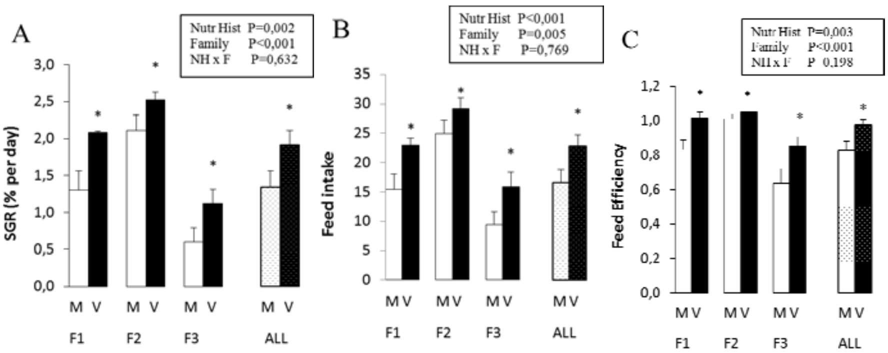

Figure 2. **A.** Specific growth rate (SGR, %BW d^{-1}), **B.** Feed intake (mg $kgBW^{-0.8}$ d^{-1}) and **C.** Feed efficiency (BW gain/dry matter intake) of the trout during the 25-day V-challenge according to early nutritional history (M or V) and family (F1, F2, F3). Values are means ±SE (n=4). ALL represent the effect of nutritional history (M or V) averaged over the 3 families. The significance of the main effects and interaction (2-way ANOVA) is shown in the rectangle, * indicates a significant effect of nutritional history (V>M, $p<0.05$).

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