

**EVALUATING EFFECTS OF NUTRITION ON FISH GROWTH AND
HEALTH WITH THE USE MOLECULAR PROBES.**

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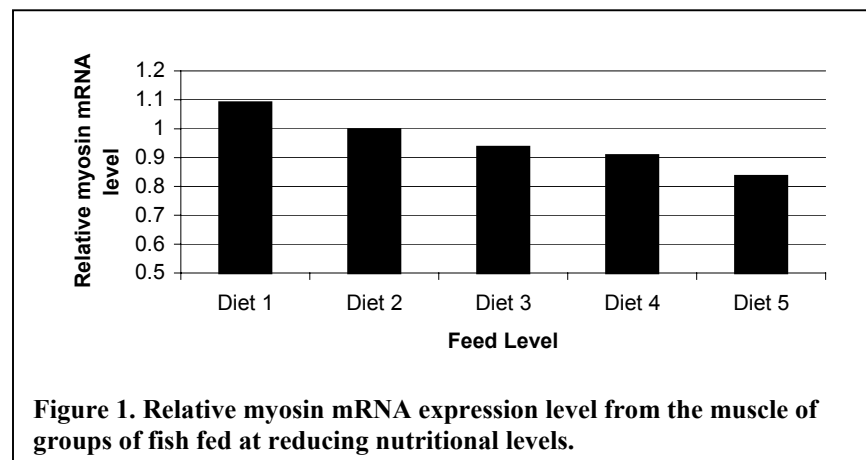
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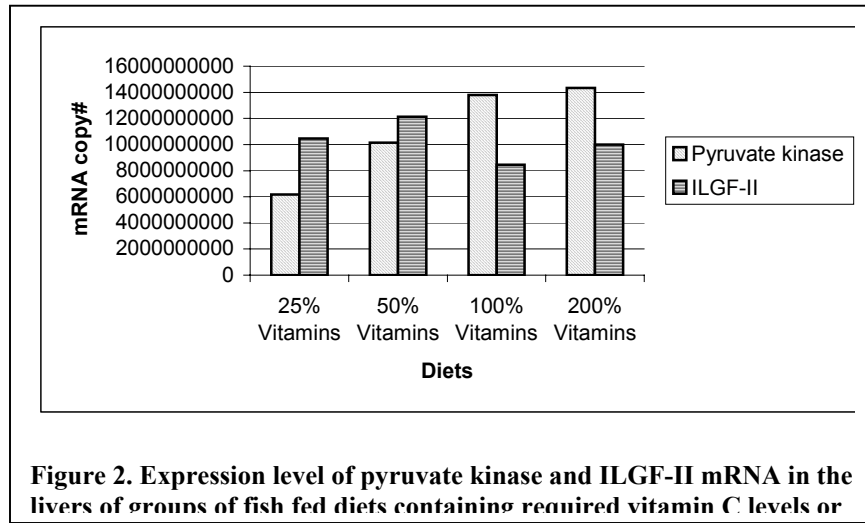
The continued selection and genetic improvement of fish stocks requires novel techniques that allow a more precise understanding of the cellular and physiological aspects that underlie traits of interest. While sequence mapping and cellular studies have greatly improved our understanding of cellular mechanisms and transcriptional changes involved with certain physical characteristics in humans and some importantly cultured domesticated animals, this type of knowledge in aquaculture is woefully lacking. To gain a more precise understanding of the specific genes that are involved with useful quantitative traits, studies have begun that analyze gene expression levels in the tissues of animals under different experimental conditions. Some studies have shown gene expression to correlate reasonably well with protein and enzymatic levels for some proteins (Rocha et al., 1996). In order to gain a better understanding of specific genes that could be involved in selectable traits of interest, specific genes were selected to monitor their expression level changes under varied experimental conditions. The genetic traits of interest that are currently under study at the Hagerman Fish Culture Experiment Station are growth, on formulated fish diets using alternative protein sources to fish meal, disease resistance, and health issues related to different diets and environmental conditions. Utilizing knowledge gained from previous work in fish and other animals, and the availability of sequence, certain transcribed genes were chosen for analysis using real-time PCR. Probes and primer pairs, such as myosin and ILGF-II, were designed and tested for growth analysis, pyruvate kinase and lactate dehydrogenase, for analysis of metabolic enzymes, and tumor necrosis factor (TNF), CD-8, interferon (MX-1), lysozyme, complement factor C3, interleukin-8 (IL-8), natural resistance associated macrophage protein-alpha (Nramp-alpha) and natural killer cell enhancement factor (NKEF), as immunological and health related indicators.

Using the myosin probe, myosin expression levels in fish on varied planes of nutrition was studied (Overturf & Hardy, 2001). Groups of fish were reared for 2 months on the following diet regimes; diet 1-100% (fed to satiation), diet-2 75% of diet 1, diet-3 50% of diet 1, diet-4 25% of diet 1, and diet-5 was held off feed during the trial. After only two weeks it was noted that myosin expression levels correlated with the nutritional plane of the groups of fish and this correlation became stronger as the trial continued ($r=0.98$)(Figure 1). This



suggests that myosin expression level, and hence muscle deposition, are directly related to the nutritional intake of the animal and that this method would be effective in monitoring nutrient quality of diets. For studies looking at metabolic enzyme mRNA expression levels, individual groups of fish were reared on complete and serial limiting vitamin levels, as these reduced levels have demonstrated adverse effects for fish growth and health (Halver, 2002). Examination of the expression levels for ILGF-II and pyruvate kinase found that after only 3 sampling periods (every 3 weeks) there were significant differences in the level of pyruvate kinase expression correlating with the vitamin level for each diet ($r=0.98$) (Figure 2). There seemed to be no correlative response of ILGF-II to varying vitamin level at this point in the study.

Another problem that has become associated with diet changes in fish, especially the use of high level replacement of fish meal with plant material as an alternative protein source, is irritation of the intestinal tract that seems to be related to immune function (Rumsey et al., 1995). As an initial assessment of probes and primers generated for analysis of aspects related to the natural and adaptive immune system, the expression level of specific immune factors was



studied in fish that were exposed to bacterial and viral pathogens. Individual groups of fish received three different dosage levels of either *Aeromonas salmonicida*, *Flavobacterium psychrophilum*, or *infectious haematopoietic necrosis virus* (IHNV) and the levels of the immune factors were determined at 1 and 5 days post-infection in the liver, spleen and head kidney of infected fish. It was found that *F. psychrophilum* did not demonstrate any major effects on the level of most of the immune factors involved in this study. *A. salmonicida* showed changes typically in the spleen and liver for factors such as tumor necrosis factor, IL-8, and CD-8. IHNV showed highly correlative expression levels for MX-1, C-3, CD-8, and TNF in the liver and somewhat in the spleen.

With these and other probe and primer sets that are being developed it is anticipated that a better understanding of the nutritional aspects related to fish health can be assessed and ultimately utilized for selection of improved stocks for aquaculture.

References

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