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Effects of tetraploidy induction on rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) proteome at early stages of development

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ABSTRACT

The aim of the present study was to examine the effects of tetraploidy induction on proteome of rainbow trout during the early stages of development. After insemination, the eggs were incubated at 10 °C for 350 min. Thereafter, half of the eggs were exposed to a heat-shock of 28 °C for 10 min. The remainder were incubated normally and used as diploid controls. Fertilized egg specimens were selected 390 min post-fertilization. Samples corresponding respectively to eyed embryos and fry stages were also taken on days 18 and 76 post-fertilization. Based on two-dimensional electrophoresis, all spots that were found to differ significantly in abundance between the untreated and heat-shock treated groups were selected for identification using MALDI-TOF/TOF mass spectrometry. Out of 19 protein spots showing altered abundance in the present study, 13 spots were successfully identified. Of the spots that were shown to change in abundance in the fertilized eggs with heat-shock treatment, three were identified as vitellogenin (spots 1, 2 and 3); while the others were creatine kinase (spot 5) and nucleoside diphosphate kinase (spot 6). All of the proteins identified in the embryos were related to vitellogenin (spots 8, 12 and 13). Among the identified spots from the fry muscle extracts, two were identified as beta-globin (spots 14 and 17); while the others were parvalbumin (spots 15 and 16) and creatine kinase (spot 19). The results obtained in our study may now set the ground for investigations on gene regulation and proteome modifications in polyploid fish.

1. Introduction

Early sexual maturation is a major challenge in salmonid farming as metabolic energy is diverted from somatic growth to reproduction resulting in lower growth rate, increased incidence of diseases and deterioration of the organoleptic properties (Piferrer et al., 2009). These problems could be solved by the use of sterile fish. Manipulation of ploidy is used in aquaculture industry to induce sterility. Fish with even sets of chromosomes are generally fertile whereas those with odd sets of chromosomes are generally sterile. Artificial induction of triploidy is the most common ploidy manipulation in fish. Triploids are produced by preventing the extrusion of the second polar body during the second meiotic division. Suppression of cell division can be achieved through thermal or hydrostatic pressure treatment of eggs shortly after fertilization (Benfey, 1999).

Triploid fish can also be generated by interploid crossing, where normal haploid eggs are fertilized with the diploid sperm from a tetraploid male (Piferrer et al., 2009). Tetraploid fish can generally be produced by suppression of the first cell division in the zygote once the chromosomes have been duplicated (Chourrout, 1984).

While the basic approaches to induction of tetraploidy in rainbow trout have been reported by several authors (Thorgaard et al., 1981; Chourrout, 1984; Foisil and Chourrout, 1992; Palti et al., 1997; Zhang and Onozato, 2004; Zou et al., 2004; Hershberger and Hostuttler, 2007), few have investigated the effects of induction shocks on molecular biology of fish. Technologies such as proteomics, genomics and metabolomics are novel approaches that offer a comprehensive method to study biological systems. These technologies expand the level of research from single biomolecules to a broad range of molecules present in a cell or a tissue at once, in terms of their existence and relative abundance, without a priori knowledge (Alves et al., 2010). Recently, Babaheydari et al. (2016) showed that triploidization heat-shock treatment leads to changes in the protein contents of fertilized eggs. They related decreased abundance of vitellogenin in heat-shock treated eggs with reduced early survival rates and lowered growth performance of triploid fish. However, effects of tetraploidy induction shocks on

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rainbow trout proteome have not been studied to date. Thus, this study was designed to study the effects of tetraploidization heat-shock treatment on proteome of rainbow trout at three developmental stages including fertilized eggs, developing embryos (eyed embryos) and fry.

2. Materials and methods

2.1. Fish, experimental conditions, survival, growth and ploidy verification

This study has been performed in accordance with the ethical standards of the American Fisheries Society, American Society of Ichthyologists and Herpetologists, and the American Institute of Fishery Research Biologists. Male and female rainbow trout (approximately 4 year old) used in this study were obtained from a commercial farm in Aligoudarz, Lorestan Province, Iran. Ovulated eggs were collected from eight females and milt was stripped from six males. The gametes were checked for quality of egg and semen as described previously (Labbe and Maisse, 2001; Aegerter and Jalabert, 2004). The gametes were pooled to minimize the individual differences that may affect tetraploid yields. The eggs were then inseminated, rinsed and incubated at 10 °C. Three subsamples of eggs from fertilized egg mass were randomly sampled and subjected to heat-shock for 10 min submerged in a 28 °C uniformly heated, aerated waterbath, 350 min after fertilization (Horstgen-Schwark, 1993). Three fertilized egg subsamples were left untreated as diploid controls. Eggs were incubated in hatchery troughs (about 1650 eggs per trough) supplied by well water at 10–11 °C. At the eyed stage (18 days post-fertilization (dpf)), hatching (25 dpf), the swim-up (38 dpf), and the fry stage (76 dpf) dead embryos or fish were removed and survival rates were obtained. At the swim-up stage, fish were transferred to six fiber-glass tanks (1200 fish per 100 L tank) and reared until the fry stage (76 dpf) under the same environmental conditions (mean water temperature of 14 °C, pH of 7.5 and dissolved oxygen of 8.1 mg/L). At the beginning of exogenous feeding, fish were fed on a rainbow trout commercial diet 12 times a day at the rate of 7% of their body weight for six weeks.

The fish in the different experimental groups were weighed at the onset of first feeding and at the end of rearing period for estimation of growth. Growth performance parameters were calculated according to the following formulae: average daily growth rate (ADG) = (final body weight − initial body weight) ÷ duration of the culture, and WG = final body weight − initial body weight.

Fish ploidy level was determined by erythrocyte size measurement (Benfey et al., 1984). Briefly at the end of rearing period, when the size of the fish reached approximately 2 g, blood samples were collected by cutting the caudal fin of thirty fish from each experimental group. Blood smears fixed in 95% methanol for 3 min, left to air-dry and stained with 10% Giemsa solution for 15 min. Twenty erythrocytes per slide were studied at 400× magnification to measure the size of erythrocytes using following formula. The letter "S" was used to designate the nuclear or cell area, "V" the nuclear or cell volume, "a" and "b" the small and large axis of the nucleus or cell. The formulae used for calculations, therefore, were: $S = a \times b \times \pi / 4$; and $V = [a / 2] \times [b / 2]^2 \times \pi \times 4 / 3$.

2.2. Sample collection

Fertilized egg samples from each trough of experimental groups (control and heat-shock treatment) were selected 390 min post-fertilization. Samples of eyed embryos were taken on 18 dpf. Based on ploidy determination experiment, nine diploid and nine tetraploid fry (76 dpf) were chosen for proteomic analysis (Fig. 1). Six other fish (two fish per tank) were also selected from the remaining fish in each ploidy group for physiological (thyroid hormones and cortisol) study. Egg, embryo and fry samples were then quickly frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Proteomic analysis

2.3.1. Protein extraction

To attain the necessary concentration of protein in egg or eyed embryo extracts, for each experimental group three replicates composed of five eggs or eyed embryos were prepared. In order to reduce the influence of inter-individual variation, the muscle tissues of (each) three individuals were mixed before proteome extraction so that three replicates were prepared for each experimental group (Fig. 1). Muscle tissue was swiftly dissected from behind the head and above the lateral line of fish using a clean scalpel. The surface of egg or muscle tissue samples were washed in 40 mM Tris-HCl buffer (pH = 7), before being mechanically homogenized in cold lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 50 mM dithiothreitol (DTT), 50 mM Tris (pH =

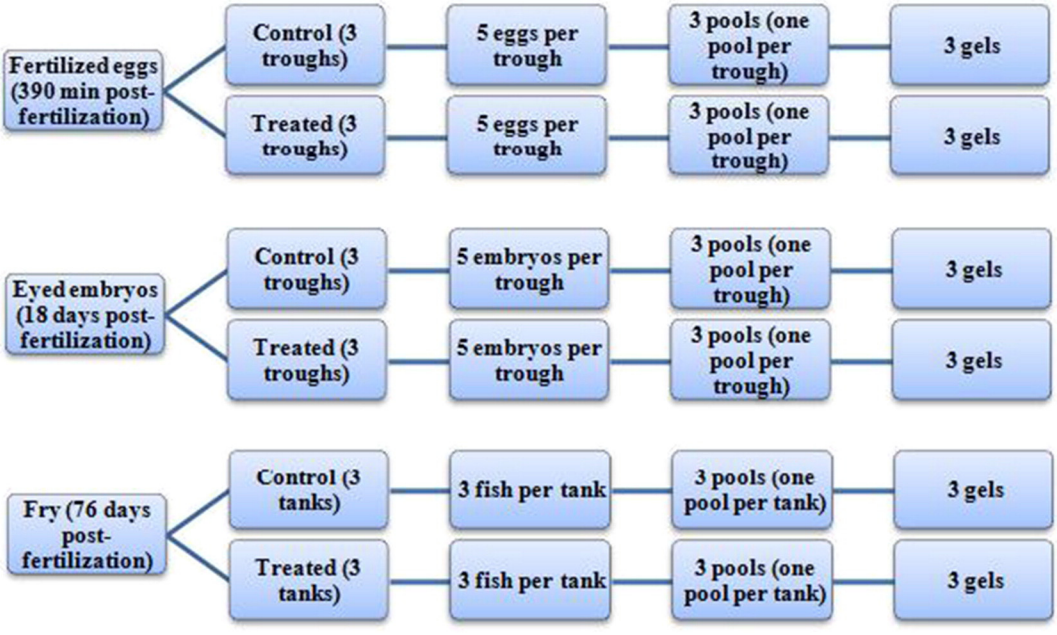

Fig. 1. Summary of proteomics experiments conducted using proteins from heat-shock treated and untreated (control) samples of rainbow trout in different developmental stages.