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Protein Profile Study In Selected Ornamental Fishes Using Natrum Muriaticum 30c Potency

Sudha.C,

Assistant Professor, PG and Research Department of Zoology, National College, Tiruchirapalli-620001.

Abstract: One of the most diverse groups of vertebrate is teleosts. They use a wide range of reproductive methods. Vitellogenin molecules of different fish species have been widely studied for their role in fish reproduction. The protein fractions of selected ornamental fish have been analyzed in the present study. The ovary tissue samples of oviparous fish (goldfish and rosybarb) and viviparous fish (white molly and guppy) treated with natrum muriaticum exhibited different protein fractions on SDS-PAGE analysis. Results revealed that there are striking similarities between the protein fractions of ovary tissues. The analysis of polypeptides of oviparous fish by SDS-PAGE revealed the presence of nine fractions and eight fractions in viviparous fish. Three bands were more prominent in both categories. These are considered as specific bands for reproductive functions of oviparous and viviparous fish on the observations made in ornamental fish. The variation in their number and staining intensities of different fractions may reflect their different type of metabolic activity, reproductive age, and their environmental conditions. The result showed that the synthesis of proteins during natrum muriaticum exposure in ornamental fish signifies that and these proteins are female specific proteins. These proteins are involved in vitellogenesis. However, further experiments are needed to sequence the whole vitellogenin at gene level.

Key words: Protein profile, Natrum muriaticum 30c, Oviparous and Viviparous ornamental fish.

INTRODUCTION

One of the most diverse groups of vertebrate is teleosts. They use a wide range of reproductive methods [11]. The most common method is oviparity. Oviparous animals lay eggs with little or no embryonic development and the embryos are supplied with nutrition via yolk [1].Vitellogenesis is the process of sequestering yolk, the oocyte accumulates nutritional reserves needed for the developing embryo [5].Vitellogenin is a large glyco-phospho-lipo-protein. It occurs as a dimer with two equal subunits of about 200 kDa [21]. Vitellogenesis have been observed in many fish species using various analytical methods viz, ion-exchange chromatography, electrophoresis, ultra centrifugation and immunological methods[6]. Vitellogenin molecules of different fish species have been widely studied for their role in fish reproduction. Detecting the onset of puberty and progression of maturation in female fish in laboratory studies and in aquaculture and fisheries research, vitellogenin served as an ideal marker [7]. The SDS-PAGE proteins studies may reveal to certain extend the protein fractions due to homeopathy induction among the different fish groups. With this view in mind, the protein fractions of selected ornamental fish have been analyzed in the present study.

MATERIALS AND METHODS

The selected mature female fish of Goldfish (*Carassius auratus)*, Rosy barb (*Puntius conchonius)*, White molly (*Poecilia sphenops)* and Guppy (*Poecilia reticulate)* were treated with natrum muriaticum, mixed in water medium. From natrum muriaticum, 30 centesimal potency with 0.025% dilution was prepared. (0.1 ml of natrum muriaticum 30 c potency was diluted by adding 400 ml water).

The fish were autopsied at different day's interval (i.e.) 0 day and 3rd day for protein profile studies. SDS polyacrylamide gel electrophoresis [10] was done to study the protein profile in the ovary tissue.

RESULTS

The ovary tissue samples of oviparous fish (goldfish and rosybarb) and viviparous (white molly and guppy fish) treated with natrum muriaticum exhibited different protein fractions on SDS-PAGE analysis. The analysis of polypeptides of oviparous fish by SDS-PAGE revealed the presence of nine fractions with molecular weight ranging from 38 kDa to140 kDa. In the ovary nearly two polypeptides were with high molecular weight (above 100kDa) and the rest were with low molecular weight polypeptides. Similarly, in the viviparous fish the presence of eight fractions with molecular weight ranging from 45kDa to 150kDa was obvious. Among this, two of them were with high molecular weight (above 100kDa) and the remaining were

with low molecular weight of 95kDa and 45kDa. Three bands in all the fish were more prominent with molecular weight of 52 kDa, 63 kDa and 66 kDa in oviparous fish and with molecular weight of 58 kDa, 79 kDa and 95 kDa in viviparous fish.

Figure-1- A. Electrophoresis of total ovary protein extracts from previtellogenic or vitellogenic oviparous females-goldfish and rosybarb –protein profile. M-Protein ladder; Lane-1-goldfish-0-day; Lane-2-goldfish-3rd day Control and Lane-3-goldfish-3rd day Experimental. Lane-4-rosybarb-0-day; Lane-5-rosybarb-3rd day Control and Lane-6- rosybarb-3rd day Experimental.

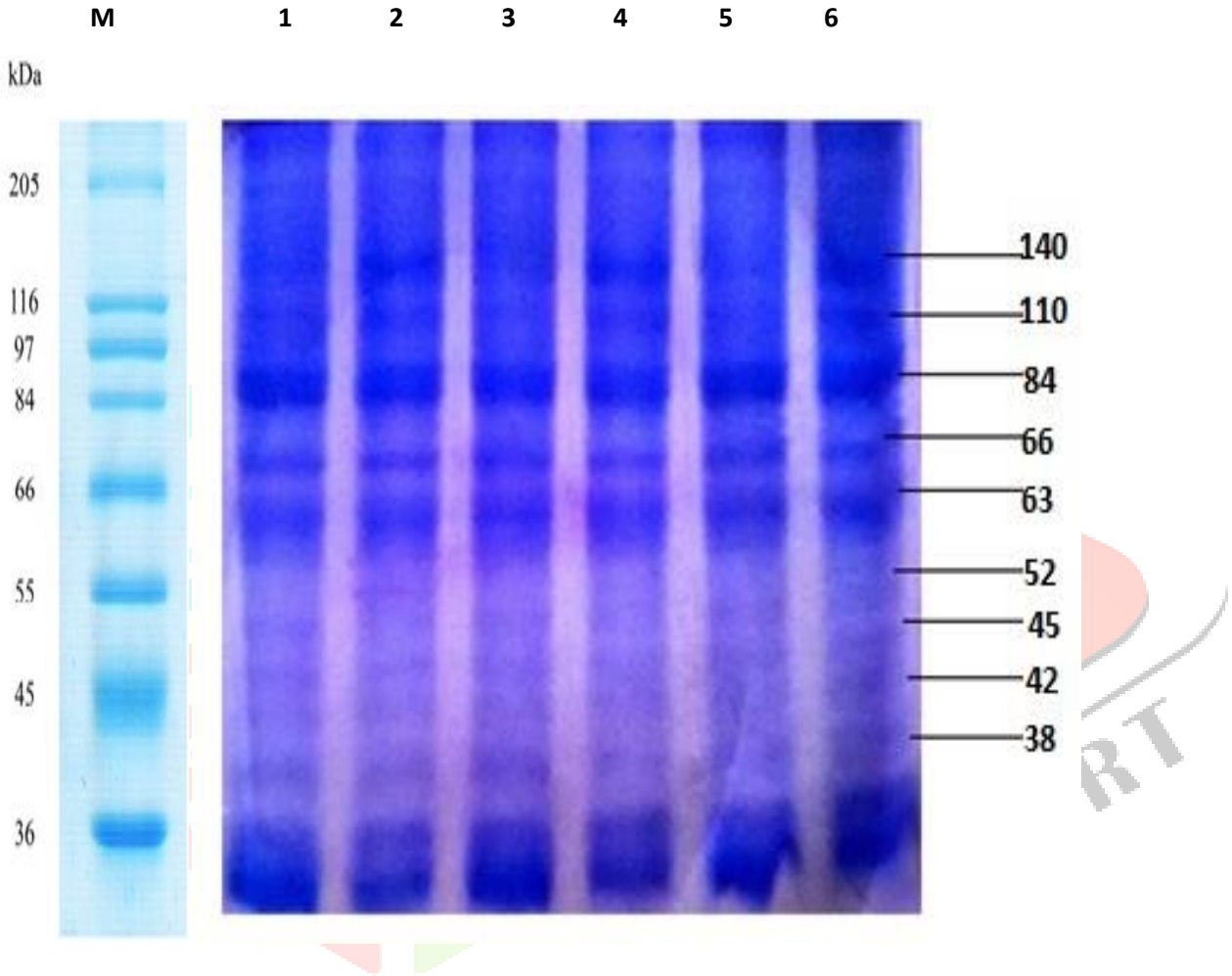
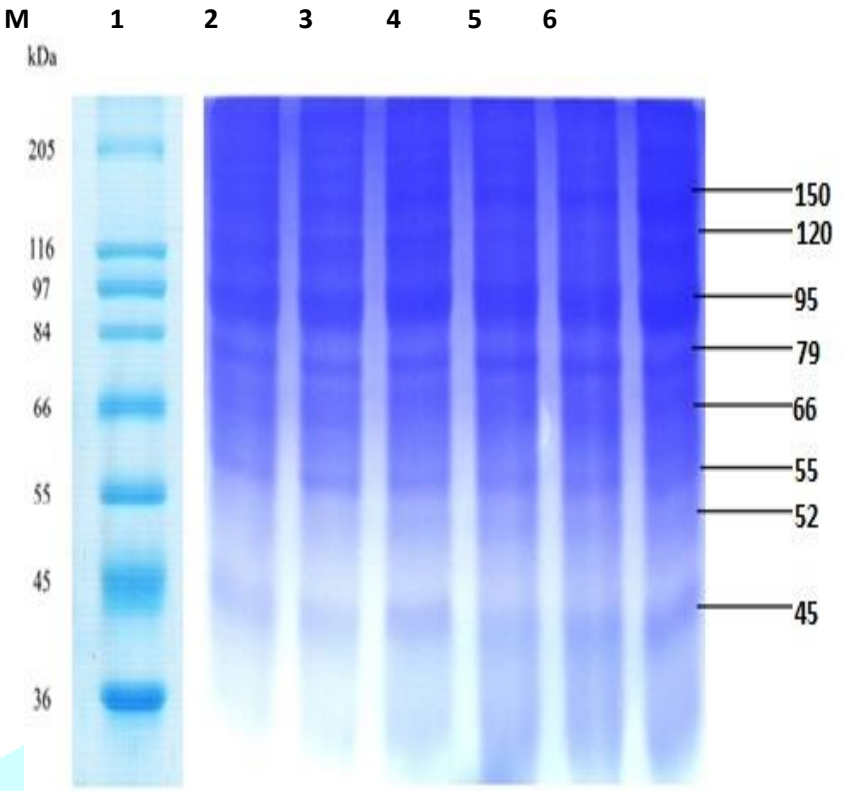


Figure-2- B. Electrophoresis of total ovary protein extracts from previtellogenic or vitellogenic oviparous females-whitemolly and guppy –protein profile. M-Protein ladder; Lane-1- whitemolly -0-day; Lane-2-whitemolly -3rd day Control and Lane-3- whitemolly -3rd day Experimental. Lane-4- guppy -0-day; Lane-5-guppy -3rd day Control and Lane-6- guppy -3rd day Experimental. The molecular weight of the standard proteins is indicated on the left: myosin (200kDa),b**-galactosidase (116.25 kDa), phosphorylase-**b **(97.4 kDa), BSA (66.2 kDa) and ovalbumin (45 kDa).**



DISCUSSION

Fish vitellogenin displays a high variability in their protein size and subunit number in general [1]. Fish vitellogenins are quite unstable. Vitellogenin molecular weight 176 kDa was identified in female rainbow trout [16]. In goldfish, three monomeric polypeptides ranging in molecular weight from 140 to 147 kDa were reported by [3]. Vitellogenins composed of monomers or dimers in most species, but vitellogenin of goldfish and Japanese eel (*Anguilla japonica*) composed of from three to four subunits. In rainbow trout, only one dominant subunit was found but several minor bands were present. It may also be attributable to vitellogenin as suggested by [19].

Vitellogenin in teleosts are ranging from 300 to 600 kDa indifferent species [7]. Vitellogenin isolated from African catfish *Clarias gariepinus* is 520 kDa [14] and for trout *Salmo gairdneri* is 470 kDa [2], 440 kDa for sea trout *Salmo trutta* [17].Vitellogenin for sea bass *Dicentrarchus labrax* is 445 kDa [12], 500 kDa for viviparous blenny *Zoarces viviparous* [9],490 kDa for common carp *Cyprinus carpio* [4] .

Vitellogenin isolated from copper redhorse *Moxostoma hubbsi* is 425kDa and 450 kDa for shorthead redhorse *Moxostoma macrolepidotum*[13]. A similar SDS-PAGE pattern was obtained by [20] while studying grouper (*Epinepheles malabaricus*) vitellogenins. They found two major bands of 140 and 113 kDa.

Two minor bands and two major bands of 113and 140 kDa in SDS-PAGE of grouper (*Epinepheles malabaricus*)[5]. [4] reported that two bands corresponding to 156 and 190 kDa are seen in *Cyprinus carpio* under SDS-PAGE. Similar electrophoretic conditions were reported in atlantic salmon *Salmo salar* and in greenback flounder *Rhombosolea tapirina* vitellogenin dissociated into three major subunits (~ 86, 117, 159 kDa and 79, 104, 155 kDa, respectively by [24].According to [8], SDS-PAGE of the oocyte extract of Tilapia, *O.mossambicus* showed minor bands at 26 and 24 and 23kDa, a major protein band at 106 and very faint bands at 83and 175kDa. [11] identified the vitellogenin in cat fish as 200kDa through SDS-PAGE. Teleost vitellogenins are reported to be dimers consisting of identical monomers in general. Vitellogenin monomers normally vary between 85 and 220 kDa in molecular weight [18].

In the present study two subunits were with more or less same molecular weight 110kDa and 140kDa in oviparous as well as120kDa and 150kDa in viviparous fish. Hence, it is more likely that they may be involved in vitellogenesis. Similar results have been reported by [23] who has explained at least three polypeptides, ranging in molecular weight from 140kDa to 147kDa were identified by sds-gel electrophoresis of vitellogenin in native goldfish. The variation in their number and staining intensities of different fractions may reflect their different type of metabolic activity, reproductive age, and their environmental conditions. The result showed that the synthesis of proteins during natrum muriaticum exposure in ornamental fish signifies that and these proteins are female specific proteins. These proteins are involved in vitellogenesis. However, further experiments are needed to sequence the whole vitellogenin at gene level.

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