PRELIMINARY RESULTS ON THE EFFECT OF BROODSTOCK DIET ON CYSTEINE PROTEINASES ACTIVITY DURING EMBRYONIC DEVELOPMENT OF COMMON SOLE, *Solea solea*.

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Introduction

In many cultured fish species, particularly in those relatively new for aquaculture, such as common sole *Solea solea*, unpredictable and variable reproductive performance is an important limiting factor for the successful mass production of juveniles. An improvement in broodstock nutrition and feeding has been shown to greatly enhance both gemetes quality and seed production. To date knowledge on nutrient requirements, feed quality attributes and feeding levels to be used in rearing broodstock of common sole is scarce and the question of whether eggs quality can be influenced by different broodstock diets remains unanswered. During embryonic development, materials that accumulated in the oocyte are utilized by the embryo and several studies demonstrated the involvement of some intracellular proteases, the cathepsins, both during vitellogenesis process and yolk protein degradation during embryonic development. Considering the role of this set of enzymes, the present study was aimed at evaluating the effect of different natural supplements in common sole broodstock diets on changes in yolk protein components and in cathepsin D, L, and B activities, during embryogenesis.

Material and methods

Thirty-nine males (298.5±129.14g) and thirthy-two females (576.63±211.08g) of common sole were reared in six concrete sandy bottom *indoor* tanks (4m^2) at 0.8-1kg /m^2 stocking density, adopting a nearly balanced male:female ratio (1:1). Out of the spawning season (June to January), all fish were fed a semi-moist pellet (P) (55% Crude Protein and 16% Crude lipid on dry matter) while from February up to the end of the spawning season (May), fish were subjected to three different dietary treatments each tested in duplicate. Two fish groups were fed diet P alone (controls); PP groups were given the same semi-moist pellet supplemented with live polychaetes (*Perinereis cultrifera*,), while PM groups received diet P supplemented with fresh mussels (*Mytilus edulis*). Broodfish were fed twice a day, with a ration ranging from 0.9-1 % of total biomass. The incidence of polychaetes and mussels on total ration mass as such was about 30%. During the spawning season (April) overflow eggs collectors of the broodstock tanks were checked during the evening 8:30-9:00 p.m. and the floating fertilized eggs were collected in a plankton net. They were incubated in 60-L conical-bottom tanks at 17°C until hatching. During the following 48h embryo development were checked under the microscope and tissue were collected in order to have the same developmental stage for all treatments (half gastrula, neurula and pre-hatching). Two hundred mg of tissue were collected in duplicate, placed in three volumes of distilled water, mixture were homogenized and centrifuged at 13.000g for 10 min at 4°C. The supernatant was recovered, immediately frozen in liquid nitrogen and stored at -80°C for electrophoretic and Western blot assay previously described by Carnevali et al. (2001). Cathepsin D, B and L activity were analysed following the methods described by Takahashi and Tang (1981), Barret and Kirschke (1981) and Kamboj et al. (1993);

respectively. Enzymatic activity were subjected to two-way ANOVA (diet x stage of development) and, if appropriate, to Holmon-Sidak post-hoc test was applied for diet comparison.

Results

Gel electrophoresis and Western Blot Analysis. The electrophoretic pattern of developing embryos was similar irrespective of the broodstock dietary treatment. The sole yolk proteins, stained by Comassie blue showed at the half gastrula stage six major bands with apparent molecular weights of: 85, 70, 45, 30, 15 and 13 kDa. In the following neurula stage embryos showed an increase in 70 and 30 kDa band intensity; while at pre-hatching stage the electrophoretic pattern of yolk components showed a decreasing intensity of the 85 kDa component whereas that of the 70 and 30 kDa ones was increased. The seabream (*Sparus aurata*) VTG antibody used in the Western blot analysis recognized all components stainable by Comassie blue indicating that those products were derived from vitellogenin. *Enzymatic assay*. The Cathepsins activity in embryos showed a marked effect of the broodstock diet. Cat D activity was higher ($P<0.05$) in embryos of fish fed PP diet 1.78 ±0.34 U/mg when compared to those given diets P and PM (1.42±0.07 U/mg vs. 1.12 ±0.37 U/mg; respectively, $P>0.05$). No significant differences were observed among the different stage of development ($P>0.05$). Cathepsin B and L activities were influenced by both broodstock diet and stage of development. The highest Cat B activity was observed in embryos of fish fed diet PP (17.4±2.1 nmol/min/mg/ml) and its activity significantly increased ($P<0.05$) during embryogenesis, with the highest value recorded at the pre-hatching stage (23.4±3.1 nmol/min/mg/ml). A similar pattern was observed also for the activity of Cat L.

Discussion

Degradation of yolk proteins is essential for the early development of the embryo. Here we focused on the activity of three lysosomal enzymes which are known to affect viability of eggs as well as yolk utilization during embryonic development, in many farmed fish species. In this study cathepsin activities were found to be related to changes occurred throughout the embryonic development, confirming the role played by these enzymes in yolk reserve mobilization. A similar pattern was observed in the European seabass (Carnevali et al., 2001) where cathepsin L was found to be responsible for the second proteolytic cleavage of the lipovitellin components of higher molecular weight. For the first time, we also found that serine and cysteine proteinases activities in embryos to be affected somehow by broodstock diet manipulation. In particular, supplementing the broodstock diet with live polychaetes seems to increase the activity of cathepsin L possibly resulting in improved utilization of yolk proteins by the embryos. The activity of cathepsin D, was found to be influenced by the broodstock diet but it did not show major changes during the embryo development so that its role in yolk proteins degradation of is still questionable.

References

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