# THE POTENTIAL EFFECTS OF COMMERCIAL FEEDING PROTOCOL ON PROTEASE ACTIVITIES AND CORTISOL STRESS RESPONSES OF MEAGRE (*ARGYROSOMUS REGIUS*)

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**Abstract**

*The present study is designed to report the potential effects of commercial feeding protocol on protease activities and cortisol stress responses of meagre (Argyrosomus regius) from fertilized eggs to 45 days after hatching (DAH). The inhibition effects of six commercial diets (Gemma Micro 150 (100-200µ), Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) and protease contributions of live foods (enriched rotifer, Artemia nauplii and Artemia metanauplii) were tested in the study. Samples of meagre were collected from fertilized eggs to 45 DAH. All analyses were carried out in triplicates.The differences observed in the total length and weight values were statistically significant ($p< 0.05$). The lowest and highest total length values were 3.06±0.23 mm (3 DAH) mm and 33.28±0.68 mm (45 DAH), respectively. The lowest and highest weight values were 0.53±0.01 mg (3 DAH) and 467.67±1.47 mg (45 DAH), respectively. The differences in protease activities of meagre larvae were statistically significant ($p< 0.05$). The highest and lowest protease activities of meagre larvae were 477.13±39.30 U/mg protein (3 DAH) and 10.05±1.16 U/mg protein (20 DAH), respectively. The digestive proteases of meagre larvae showed the greatest sensitivity to protease inhibitors present in Gemma Micro 150 (100-200µ). Fertilized eggs of meagre had low cortisol level (0.68 ng/g). Cortisol values increased rapidly up to hatching (2.43±0.06 ng/g) and followed by a decrease in prelarval stage (1.80±0.01 ng/g) and then remained relatively until before mouth opening (1.76±0.03 ng/g). From 3 to 45 DAH, the highest and lowest cortisol values of meagre larvae were 6.13±0.18 ng/g (15 DAH) and 1.98±0.17 ng/g (30 DAH) respectively.*

*In conclusion, the presence of cortisol in fertilized eggs of Argyrosomus regius confirms that they are maternal origin. Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) are suggested due to good performances from 3 to 45 DAH but not Gemma Micro 150 (100-200µ). Caviar (200-300µ) and Caviar (300-500µ) are the moderately advisable as commercial diets in the feeding protocol of meagre larvae except for more inhibitions than 50%.*

**Key words:** *Argyrosomus regius, protease, protease inhibitions, commercial diets, cortisol*

## 1. INTRODUCTION

In present, production is based on the most important marine fish species for Mediterranean aquaculture such as gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*). Aquaculture sector need to focus on the cultured of the potential candidate species for diversification due to the decrease observed in their prices based on over productions of seabass and seabream (El-Shebly et al. 2007; Monfort 2010). There is a growing interest in the commercial culture of, *Argyrosomus regius* as a potential candidate due to rapid growth rates (Quemener 2002). Therefore, the meagre (*Argyrosomus regius*) is a potentially important aquaculture species in the Mediterranean Basin.

The larval stage is defined by a rapid change in biomass, morphology and physiology. Larval fish growth and survival are dependent on effective digestion and absorption of nutrients. Aquaculture techniques including the use of adequate diets as nutritional would solve this problem. Therefore, researchers should be focused on microdiet formulations that are efficiently ingested and meet essential nutrients needed by the larvae.

Until now, researchers have focused on growth, survival and larval rearing of meagre (Pastor et al. 2013; Vallés&Estévez 2013; Vallés&Estévez 2015), the ontogeny of digestive system of meagre (Papadakis et al. 2013) and the effects of different levels of vegetable proteins on juvenile meagre (Estévez et al. 2011). In addition, digestive enzymes of marine fish larvae such as *Dicentrarchus. labrax*, *Sparus aurata*, *Solea senegalensis*, *Diplodus sargus*, *Pagrus auriga*, *Argyrosomus regius* were investigated by some authors (Zambonino Infante&Cahu, 1994; Moyano et al. 1996; Ribeiro et al. 1999; Cara et al. 2003; Moyano et al. 2005; Süzer et al. 2013). We could only found study digestive enzymes (Süzer et al. 2013) none study on the effects of commercial feeding protocol on the cortisol stress response and protease activities of meagre larvae. In this point, cortisol stress response and inhibitory effects of the commercial diets in the larvae rearing must be investigated to solve the nutritional problem.

Cortisol is the main steroid produced and released by interrenal tissue in teleost fish, and is known to possess both glucocorticoid (affects metabolism and growth) and mineralocorticoid activities (regulates the transport of ions and water). Plasma cortisol concentrations are often used as stress indicators in teleost fishes. Wendelaar Bonga (1997) revealed that cortisol is an excellent indicator of acute stress responses. Cortisol is also known to be involved in hatching and metamorphosis during the early development of teleosts (De Jesus et al. 1991). Cortisol ontogeny keys to success in the mass production of high quality fry. In this regard, it is important to examine the development of the endocrine system during early ontogeny of marine fish.The function and ontogenesis of the endocrine system in the embryonic and larval stages of teleosts have been the subject of much study. However, little is known about cortisol in the early developmental stages of teleosts. Due to lack of knowledge about ontogeny of cortisol stress responses and intestinal proteases during commercial culture of *Argyrosomus regius*, our study were focused on the ontogeny of the cortisol stress response in meagre from fertilized eggs to 45 days after hatching(DAH), and determine the inhibition effects of six commercial diets (Gemma Micro 150 (100-200µ), Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) on protease activities of meagre larvae from 3 to 45 DAH. In addition, protease contributions of live foods (enriched rotifer, *Artemia nauplii* and *Artemia metanauplii)* were determined in the study.

## 2. MATERIALS AND METHODS

### *2.1. Larvae rearing and sampling*

Sampling stage of the present study was carried out at the EGEMAR Aquaculture Food Industry and Commercial Incorporated Company. Eggs were obtained with hormone injection from meagre broodstocks (GnRH; 20 µg/kg ♀ and 10 µg/kg ♂). Fertilized eggs of meagre were collected from the broodstock tanks and incubated in conical fiberglass tanks at a temperature of 22.0 ±0.2 ºC. Newly hatched larvae were transferred from the incubators to fiber glass 7 $m^3$ ellipsoidal fiberglass tanks with black walls until 15 days after hatching (DAH; stocking density; at 0-15 DAH 75-80 larvae/L). From 15 to 45 DAH, larvae were stocked to concrete raceway 15 $m^3$ tanks (stocking density; at 16-32 DAH 10-12 larvae/L and at 33-45 DAH 6-8 larvae/L). The rearing tanks supplied with running sea water that had been filtered through a sand, bag and UV filters. Temperature, salinity, oxygen levels, and pH were 20.8-22.2 ºC, 27.0-37.0 g/L, 7.9-12.7 mg/L, and 7.8-8.0, respectively. Air and fresh sea water were introduced into the surface of the tanks to prevent water stratification until 15 DAH. Rearing tanks were exposed to a photoperiod (16:8).

*Nannochloropsis occulata* were used for green water technique from 3 to 15 DAH. Rotifer (*Brachionus plicatilis*) was cultured with Algamac Protein Plus (Aquafaune Bio-Marine Inc. Hawthorne USA) and *Nannochloropsis occulata*. The average water temperature and salinity during the culture were 25 ºC and 28 g/L, respectively. Rotifer was enriched with Spresso (INVE Aquaculture) prior transfer to the larval feeding tanks. The average water temperature and salinity during the enrichment were 26 ºC and 28 g/L, respectively. *Artemia* nauplii (***Artemia* Cysts;** Vinh Chau-Bac Lieu Artemia Co.O) were cultured at 29 ºC and 28 g/L. *Artemia* metanauplii (*Artemia* **EG;** *Artemia* SepArt EG >250.000 npl/g INVE Aquaculture Salt Lake City Utah/USA) were cultured at 29

ºC and 28 g/L. *Artemia* metanauplii were enriched with enrichments (Spresso-INVE Aquaculture) for 24 h at 26ºC and 28 g/L.

The feeding regime consisted of *B. plicatilis* from 3 to 8 DAH, reaching a maximum concentration of 10-15 prey/mL, *Artemia* nauplii from 7 to 11 DAH, with a maximum density of 4-6 prey/mL, *Artemia* metanauplii from 10 to 15 DAH, with a maximum density of 2-4 prey/mL, *Artemia metanauplii* from 16 to 32 DAH with a maximum density of 2-5 prey/mL. Commercial diets such as **Gemma Micro 150** (100-200µ; **Skretting AS**) from 16 to 22 DAH, **Caviar** (200-300µ; **BernAqua**) from 21 to 24 DAH, **Caviar** (300-500µ; **BernAqua**) from 24 to 29 DAH, Perla Larva Proactive 4.0 (300-500µ; **Skretting AS**) from 28 to 44 DAH and Perla MP-S(700 µ; **Skretting AS**) from 34 to 45 DAH and Perla Plus 3.0(400-800 µ; **Skretting AS**) at 45 DAH were used in the commercial feeding procedure of meagre larvae. Also, *Nannochloropsis occulata* was added into the growth tanks from 16 to 26 DAH. Proximate compositions of commercial diets used in the present study were given in **Table 1**. Samples of meagre larvae fed on commercial feeding procedure were collected in triplicates from 3 to 45 DAH. Larvae were taken before the morning feeding and immediately stored in liquid nitrogen (-196 ºC) to prevent protein autolysis.

**Table 1.** Proximate compositions of commercial diets used in the present study.

| Microdiets | Size (µm) | Proteins (%) | Lipids (%) | Ash (%) | Fibre (%) |
|---|---|---|---|---|---|
| SKRETTING | | | | | |
| Gemma Micro 150 | 100-200 | 59,0 | 14,0 | 15,0 | 0,2 |
| Perla LP 4.0 | 300-500 | 62,0 | 11,0 | 8,0 | 1,2 |
| Perla MP-S | 700 | 56 | 15 | 10 | 0,25 |
| Perla Plus 3.0 | 400-800 | 57 | 15 | 8 | 0,6 |
| BERNAQUA | | | | | |
| Caviar 200-300 | 200-300 | 55,0 | 15,0 | 15,0 | 2,0 |
| Caviar 300-500 | 300-500 | 55,0 | 15,0 | 15,0 | 2,0 |
| Σn-3 HUFA 25,0 mg/g | DHA 1,0 mg/g | EPA 10,0 mg/g | | | |

### *2.2. Extracts of larvae and live foods*

The samples of meagre larvae and live foods (enriched rotifer, *Artemia nauplii* and *Artemia metanauplii*) were rinsed in distilled water after thawing and then the extracts of whole larvae and live foods were homogenizationed and centrifuged (16,000 g, 30 minute 4ºC).

### *2.3. Extracts of commercial diets*

Six commercial diets (Gemma Micro 150 (100-200µ), Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) were tested with *in vitro* techniques. Extracts of commercial diets prepared by homogenization (100 mg/mL in distilled water) followed by centrifugation (15,000 g, 10 minute) were used in protease inhibition analyses.

### *2.4. Determination of protease activities of larvae and live foods*

Total protease activities of meagre larvae and live foods were measured as described by Walter (1984), using casein (10 mg/mL) in 50 mM Tris-HCl buffer at pH 8.5 as the substrate. The mixtures including extracts of larvae and substrate were incubated and then the reaction was stopped by addition of 500 µL trichloroaceticacid (TCA) (120 g/L). One unit of enzyme activity was defined as 1 µg of tyrosine release per minute. The soluble protein concentrations of meagre larvae were determined according to Bradford (1976).

### *2.5. Effects of commercial diets on protease activities of larvae*

The inhibitory effects of commercial diets on protease activities of meagre larvae were determined by measuring the reduction in protease activity of extracts using a modification of the method described by García-Carreno (1996). The method is based on the measurement of residual protease activity remaining after preincubation with different commercial diets such as Gemma Micro 150 (100-200µ), Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ) .

### *2.6. Cortisol Extraction and ELISA*

Whole body cortisols of meagre were determined according to Alderman and Bernier (2009). Samples obtained from fertilized egg to 45 DAH were collected and frozen at -80 °C up to cortisol assays. Larvae were thawed and then homogenized in 500 µL of ice cold Phosphate buffered saline buffer. 5 mL of diethyl ether were added to each sample. Then, the samples were vortexed for 1 min and centrifuged at 3500 rpm for 5 min. Following centrifugation, the organic layer containing cortisol was removed from each sample and placed in a separate test tube. The process was repeated 3 times to provide maximal cortisol extraction. Samples were kept overnight to allow for evaporation of ether. Cortisol was reconstituted in 1 mL of Phosphate buffered saline after ether evaporation and incubated overnight at 4 °C. Cortisol values of meagre larvae was determined according to Fish Cortisol ELISA Kit (CUSABIO BIOTECH CO.,Ltd) procedures.

### *2.7. Statistical methods*

All measurements were carried out in triplicates. The experimental data were subjected to one-way (ANOVA) and mean±standard error (SE) differences were made by Duncan test at *P*=0.05 content level by using SPSS 15.0 statistical package (SPSS 2006).

## 3. RESULTS

Growth of meagre larvae are summarised in Figure 1 and Figure 2. The differences determined in the total length and weight values from 3 to 45 DAH were statistically significant ($p< 0.05$). The lowest and highest weight values were 0.53±0.01 mg (3 DAH) and 467.67±1.47 mg (45 DAH), respectively. Larval weight remained relatively constant until 10 DAH and followed by a sharp increase in larval weight continued until 45 DAH ($p < 0.05$). The lowest and highest total length values were 3.06±0.23 mm (3 DAH) mm and 33.28±0.68 mm (45 DAH). The total length value of meagre larvae remained relatively up to 5 DAH. After 5 DAH, the total length value tended to increase until 45 DAH ($p < 0.05$).

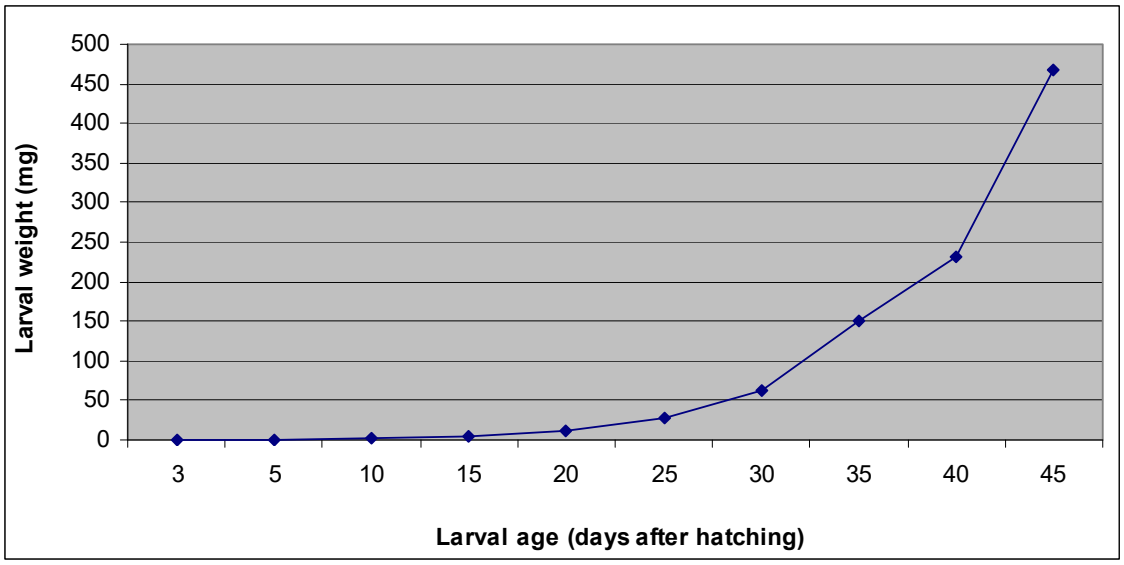


**Figure 1.** The weight values of meagre (*Argyrosomus regius*) larvae (mg) observed during the study. Results are expressed as mean± standard error (a pool of 30 larvae).

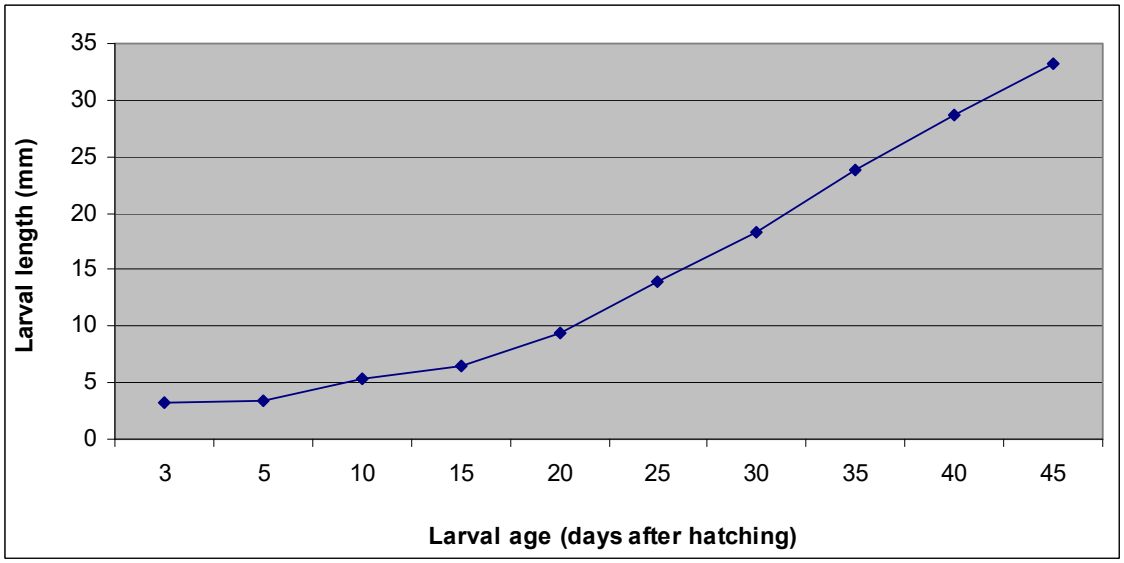


**Figure 2.** The total length values of meagre (*Argyrosomus regius*) larvae (mm) observed during the study. Results are expressed as mean± standard error (a pool of 30 larvae).

The changes measured in protease activities of meagre larvae are given in Figure 3. The differences observed in protease activities from 3 to 45 DAH were statistically significant ($p< 0.05$). The highest and lowest protease activities of meagre larvae were 477.13±39.30 U/mg protein (3 DAH) and 10.05±1.16 U/mg protein (20 DAH), respectively. Protease activities of larvae showed a sharply decrease from 3 to 10 DAH. After 10 DAH, an increase until 15 DAH and then, a decrease up to 20 DAH was observed. Protease activities of larvae tended to increase from 20 to 25 DAH and then, followed by a decrease up to 30 DAH. From 30 to 40 DAH, protease activities of meagre larvae increased and followed by a decrease at 45 DAH. Protease activities of live foods used in commercial feeding protocol are given in Figure 4. Protease activities of live foods such as enriched rotifer, *Artemia nauplii* and *Artemia metanauplii* were determined as 11.49±3.96, 24.30±3.31 and 569.67±15.65, respectively. *Artemia metanauplii* had the highest protease contribution between live foods used in the feeding protocol of meagre larvae.

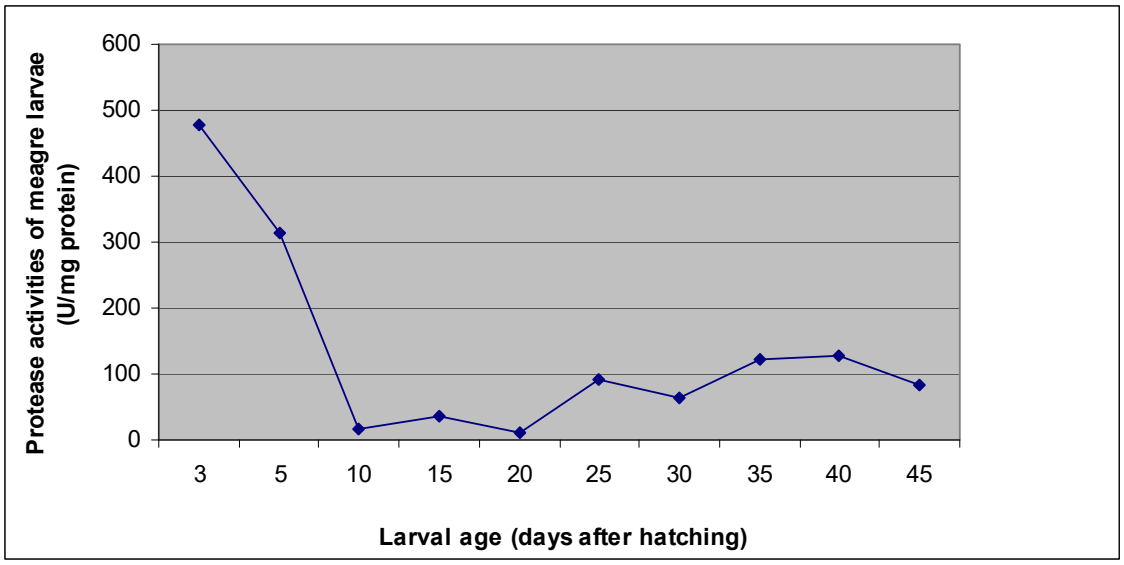


**Figure 3.** The changes determined in protease activities of meagre (*Argyrosomus regius*) larvae during the study (U/mg protein). Results are expressed as mean± standard error

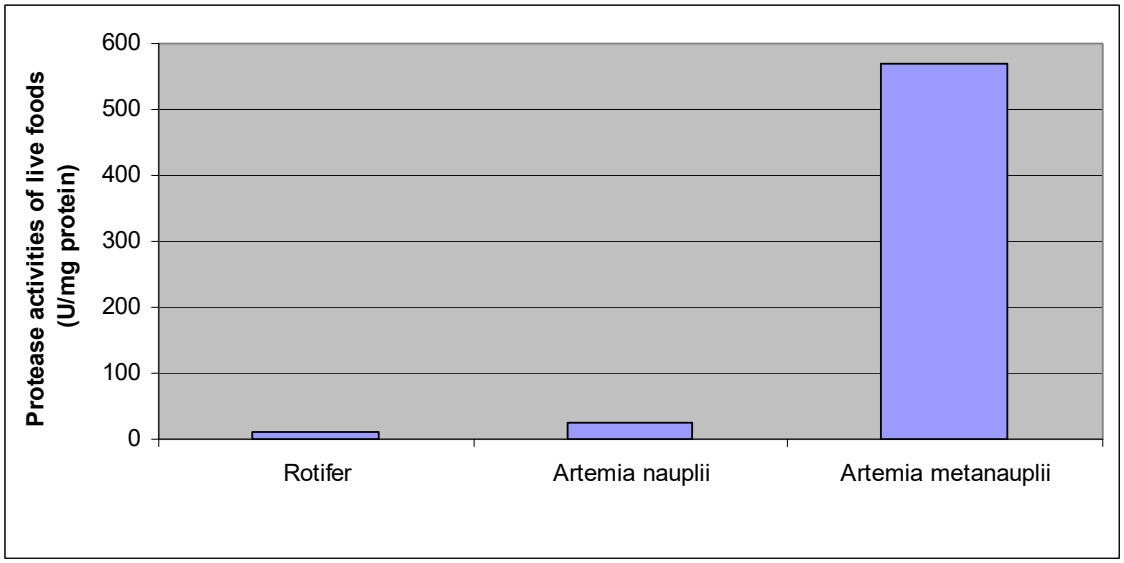


**Figure 4.** The changes determined in protease activities of live foods used during the study (U/mg protein). Results are expressed as mean± standard error

The inhibitory effects of commercial diets on protease activities of meagre larvae are given in Figure 5. The high inhibitions of protease activities of meagre larvae were obtained when extracts were incubated in the presence of solutions prepared with commercial diets used in the present study. The highest and lowest inhibitions of commercial diets on protease activities of meagre larvae were observed in Gemma Micro 150 (100-200µ) at 12 DAH (90.68%) and Caviar (200-300µ) at 12 DAH (11.45%), respectively.

The inhibitory effects of Gemma Micro 150 (100-200µ) on protease activities of meagre larvae were higher than 50% except for 7, 30, 32 and 42 DAH. Caviar (200-300µ) exhibited more inhibitions than 50% at 25 and 27 DAH while Caviar (300-500µ) showed more inhibitions than 50% at 20, 25, 27, 30, 32, 35 and 37 DAH. Perla Larva Proactive 4.0 (300-500µ) had more inhibitions than 50% at 5, 35, 40 and 45 DAH. Perla MP-S(700 µ) showed more inhibitions than 50% at 5, 10, 35, 37 and 45 DAH while Perla Plus 3.0(400-800 µ)) exhibited lower inhibitions than 50% except for 35 DAH.

Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) had good performance in the critical larval stage (from 3 to 15 DAH) but not Gemma Micro 150 (100-200µ). From 15 to 45 DAH, Caviar (200-300µ) showed better performance than that of Caviar (300-500µ) except for 25, 40 and 45 DAH. On the other hand, Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) had good performance from 15 to 45 DAH.

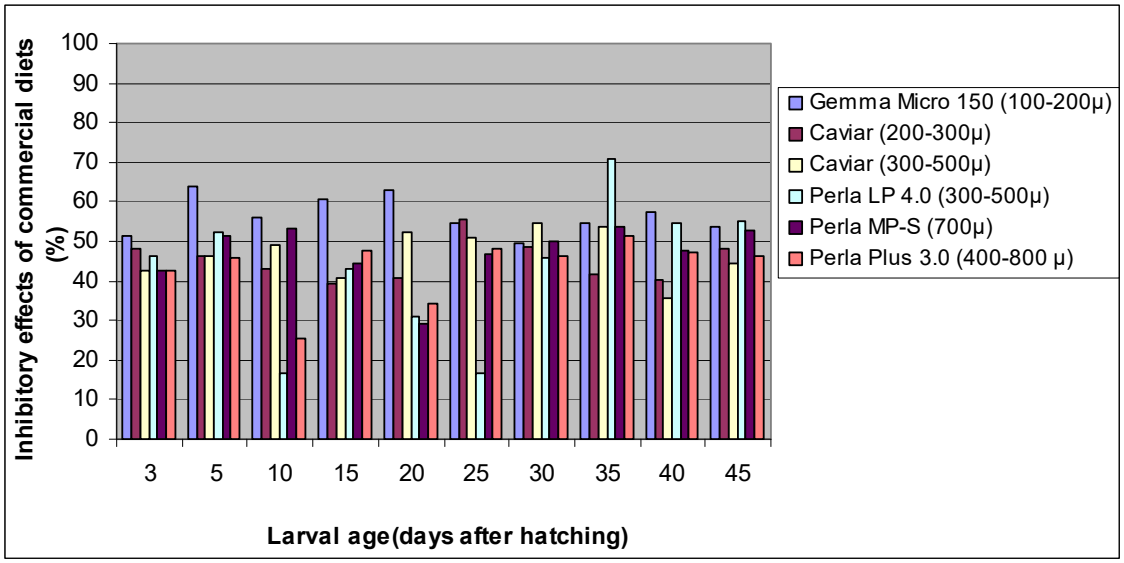


**Figure 5.** The inhibitory effects of commercial diets such as Gemma Micro 150, Caviar (200-300µ), Caviar (300-500 µ), Perla LP 4.0 (300-500 µ), Perla MP-S (700 µ) and Pearla Plus 3.0 (400-800 µ) on protease activities of meagre (*Argyrosomus regius*) larvae (%).

Cortisol was measured by ELISA technique from fertilized egg to 45 DAH. Figure 6 shows the cortisol values of meagre from fertilized egg to before mouth opening. The differences observed in cortisol values from eggs to before mouth opening were statistically significant ($p< 0.05$). Fertilized eggs of meagre contained cortisol (0.68 ng/g) that presumably originated from the mother. Cortisol values increased rapidly up to hatching (2.43±0.06 ng/g) and then, followed by a decrease in prelarval stage (1.80±0.01 ng/g). Cortisol values remained relatively until before mouth opening (1.76±0.03 ng/g).

In addition, the cortisol values of meagre larvae from 3 to 45 DAH are given in Figure 7. The differences observed in cortisol values from 3 to 45 DAH were statistically significant ($p< 0.05$). The highest and lowest cortisol values of meagre larvae were 6.13±0.18 ng/g (15 DAH) and 1.98±0.17 ng/g (30 DAH) respectively.

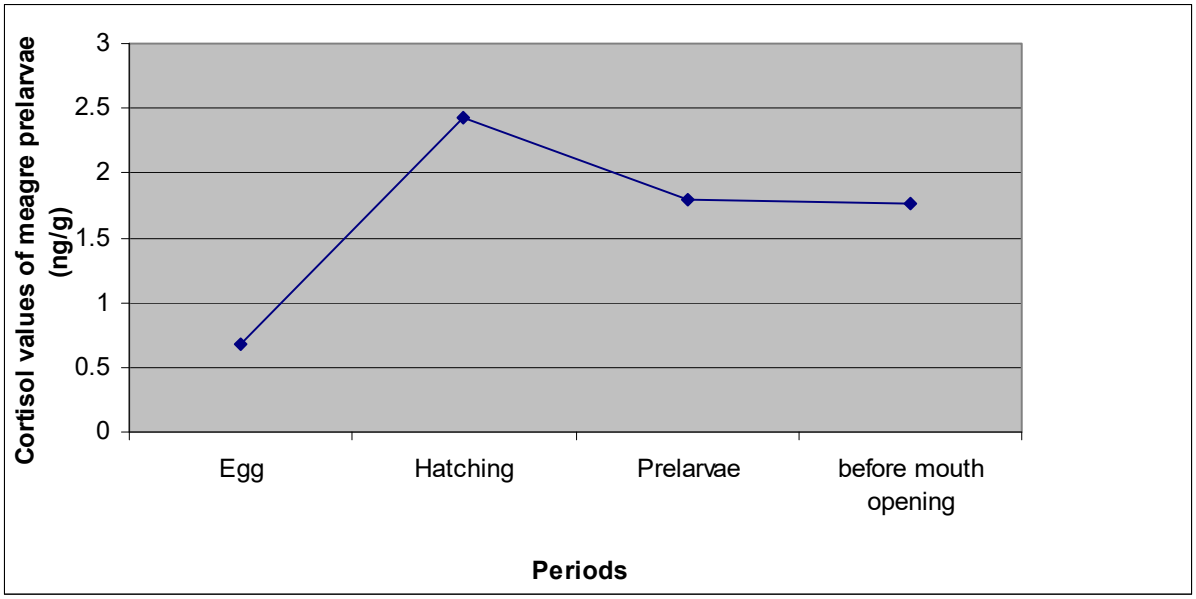


**Figure 6.** The cortisol values observed in meagre prelarvae during the study.

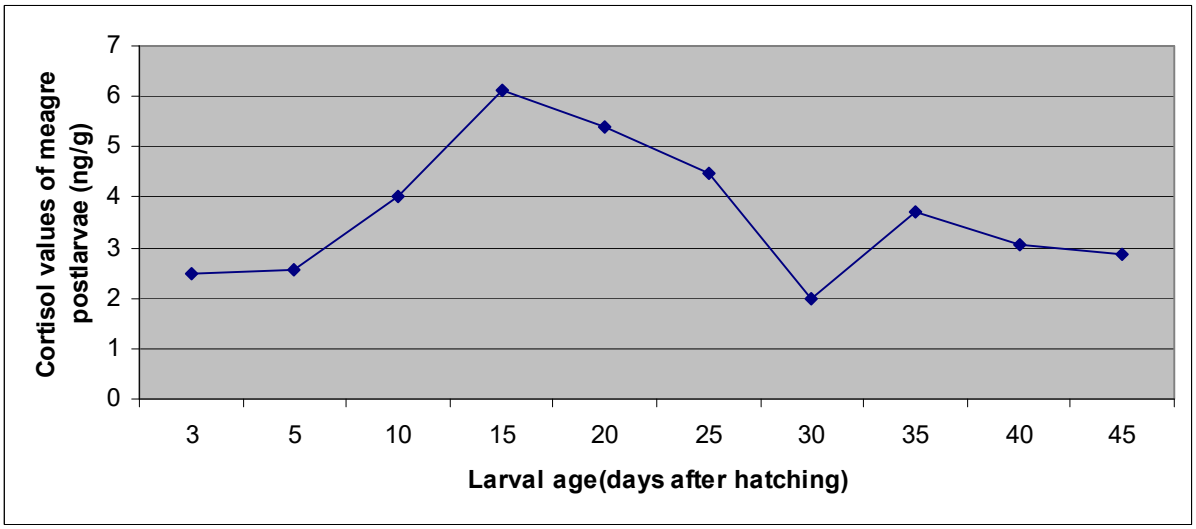


**Figure 7.** The cortisol values observed in meagre postlarvae during the study.

## 4. DISCUSSION

The present study were focused on the ontogeny of the cortisol stress response in meagre from fertilized eggs to 45 days after hatching(DAH), and determine the inhibition effects of six commercial diets (Gemma Micro 150 (100-200µ), Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) on protease activities of meagre larvae from 3 to 45 DAH. In addition, protease contributions of live foods were tested in the study.

Growth parameters (total length and weight) of meagre larvae were determined. The results of the study showed that larvae had high growth rates. Quemener (2002) supported that the most important advantage of meagre larvae was high growth rates.

Currently, we could only found study about digestive enzymes of meagre larvae (Süzer et al. 2013) and none study on the effects of commercial feeding protocol on the cortisol stress response and protease activities of meagre larvae. Ueberschär (1988) indicated that analysis of digestive enzyme activities is an easy and reliable methodology that can be used as indicator of digestive processes and

nutritional condition of larvae. In present study, the fluctuations in protease activities of meagre larvae were observed from 3 to 45 DAH. Protease activities of larvae showed a sharply decrease from 3 to 10 DAH. The lowest level of protease activities of meagre larvae was observed at 20 DAH. Protease activity values increased from 20 to 25 DAH and then remained relatively constant until 45 DAH. Zambonino Infante and Cahu (2001) indicated that the fluctuations observed in specific activities of enzymes is not due to a diminution in enzyme synthesis but is the result of an increase in tissue proteins. In addition, *Artemia metanauplii* had the highest contribution of protease between the live foods tested. Results obtained on live foods were supported by Naz (2008).

Our results showed that commercial diets used in the study caused an important inhibition on protease activities of meagre larvae. Cahu and Zambonino Infante (1994) showed that the survival and growth of marine fish larvae fed solely on microdiet through weaning period are known to be very poor, but supplementation with live foods usually results in a marked improvement. Naz (2008) revealed that the highest contribution of the digestive enzymes derived from live food commonly used in marine fish culture provide by *Artemia* metanauplii. For the reduction the inhibitions of commercial diets observed on protease activities of meagre larvae, microdiets exhibiting more inhibitions than 50% may be advised to use together with live food due to the highest enzyme contribution of *Artemia meta*nauplii as the mentioned by Naz (2008).

The highest resistances to protease inhibitors in Gemma Micro 150 (100-200µ), Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) were found at 30, 15, 40, 25, 20 and 10 DAH, respectively. Results obtained from the *in vitro* inhibition assays also revealed to negative effects of feed ingredients used in six commercial diets tested on the protease activities of meagre larvae.

Moyano et al. (1999) indicated that the negative effects of using protease inhibitor-containing diets on fish growth may be related to dietary factors such as the type of meal and the sensitivity of a given fish species to the antinutritional compounds. Alarcon et al. (1999) showed that ovalbumin significantly reduced (60%) the activity of proteases in 8 days old seabream larvae. Similar results were found when commercially produced microcapsules containing ovalbumin were tested using shrimp proteases (Alarcon et al. 1997). Reductions in nutritional value of commercial diets are the results of the presence of antinutritional compounds found in feed ingredients commonly used in the formulation of aquaculture feeds. The present study indicates a different sensitivity of meagre proteases to inhibitors present in six commercial diets, especially Gemma Micro 150 (100-200µ) exhibiting more inhibition than 50% from 3 to 45 DAH except for 7, 30, 32 and 42 DAH. For this reason, Gemma Micro 150 (100-200µ) should not be recommended as the sole diet for weaning and critical larval periods of meagre. The high inhibitions observed in commercial diets may be overcome by a careful combination of the most suitable ingredients in the formulation after the individually inhibitory effects of feed ingredients on protease activities of marine fish larvae were determined.

Caviar (200-300µ), Caviar (300-500µ), Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) had good performance in the critical larval stage (from 3 to 15 DAH) but not Gemma Micro 150 (100-200µ). From 15 to 45 DAH, Caviar (200-300µ) showed better performance than that of Caviar (300-500µ) except for 25, 40 and 45 DAH. On the other hand, Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) had good performance from 15 to 45 DAH. Result revealed that Perla Larva Proactive 4.0 (300-500µ), Perla MP-S(700 µ) and Perla Plus 3.0(400-800 µ)) are suggested due to good performances in critical larval stage (from 3 to 15 DAH) as well as weaning period (from 15 to 45 DAH). Caviar (200-300µ) and Caviar (300-500µ) are the moderately advisable as commercial diets in the feeding protocol of meagre larvae except for more inhibitions than 50%.

Pavlidis et al. (2011) indicated that cortisol is readily accumulated in the oocytes of cultured fish and significant amount is present in fertilized eggs, embryos and larvae. In present study, cortisol has been detected in all development stages examined such as fertizied eggs and larvae of *Argyrosomus regius*. The results were supported by Pottinger and Mosuwe (1994) and Auperin and Geslin (2008). Fertilized eggs of meagre contained cortisol (0.68 ng/g) that presumably originated from the mother.

Li et al. *(*2010) and Ghaedi et al (2013) determined that whole body cortisol content in fertilized eggs of rainbow trout were 3.2±0.3 ng/g and 5.09 ± 0.12 ng/g, respectively. Others investigators also determined the low or high levels of cortisol in fertilized eggs such as 2.5 ng/g in Japanese flounders (De Jesu et al. 1991) and 1.65 ng/g in *Lates calcarifer*a (Sampath-Kumar et al. 1995). Previous study results were higher than our study results.

In present study, cortisol values increased rapidly up to hatching (2.43±0.06 ng/g) and then, followed by a decrease in prelarval stage (1.80±0.01 ng/g). Cortisol values remained relatively until before mouth opening (1.76±0.03 ng/g). The fluctuations in cortisol values of meagre larvae were observed from 3 to 45 DAH. The highest and lowest cortisol values of meagre larvae were 6.13±0.18 ng/g (15 DAH) and 1.98±0.17 ng/g (30 DAH) respectively. Researchers revealed that diets used in aquaculture may play an important role in stress response of fishes such as African catfish (*Claria gariepinus*) and juvenile gilthead sea bream (*Sparus aurata*) (Merchie et al. 1997; Montero et al. 2001). Tocher et al. (2002) indicated that higher stress is observed when diets are used in feeding of larvae. Cahu et al. (2003) showed that this might be a result of the lack of essential nutrients needed to provide optimal growth.

Presence of cortisol in fertilized eggs in present study confirm that they are maternal in origin, Thereafter, egg cortisol decreased during development, and endogenous cortisol production began around hatching, as shown from the increase in cortisol concentration. Literature results showed that, de novo synthesis of cortisol starts after hatching (Sampath-Kumar et al. 1995; Szisch et al. 2005). Based on these findings it is concluded for the present study that the endocrine system becomes functional after hatching in *Argyrosomus regius*.

Results suggest that the need of a preliminary evaluation of both positive and negative effects of the feed ingredients used in commercial diets in future. Also, the present paper reveals the usefulness of using *in vitro* assays for a preliminary assessment of the effects of commercial diets used in the feeding of fish larvae. Results obtained form the study confirm the existence of protease inhibitors in feed ingredients used. In addition, the results of the study provide important contributions to determine the most suitable commercial diet for using of meagre larvae.

When such data become available, they will serve the regulation of feeding protocol of cultured marine fish larvae. For this reason, the potential effects of commercial feeding protocol on protease activities and cortisol stress responses of marine fish larvae to sustainable aquaculture should be investigated in future studies.

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