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RESEARCH ARTICLE

Time-dependent change of the digestive enzyme activity of Black Sea salmon (*Salmo labrax* Pallas, 1814) fed at suboptimal temperature

Osman Tolga Özel[1]* • Selin Ertürk Gürkan[2]

[1] *Central Fisheries Research Institute, Department of Aquaculture, Trabzon, Turkey*

[2] *Çanakkale Onsekiz Mart University, Faculty of Art and Science, Department of Biology, Çanakkale, Turkey*

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ABSTRACT

The present study conducted at 10.21±0.27°C water temperature in freshwater recirculating aquaculture systems (RAS) to observe the temporal variations in the digestive enzyme activities including pepsin, trypsin, amylase and lipase of Black Sea salmon (*Salmo labrax*). Seventh filial generation (F7) of Black Sea salmon (*Salmo labrax*) with average initial weights of 69.85±10.08 g were by hand fed three times daily until apparent satiation. At the end of the 75-day trial the samples were dissected that reached a weight of 179.17±31.08 g at 45th minute, 3rd, 6th, 12th, 24th, 36th, 48th, 72nd and 96nd hours post feeding. In all enzyme groups, the third hour after feeding was recorded as the time when the highest levels were observed. However, enzyme activities decreased gradually as the time after feeding was prolonged. In the nutrition studies to be conducted at a suboptimal temperature in RAS, gut sampling of Black Sea salmon can be taken at 3rd hour after feeding. For a better understanding of digestive enzyme activity for this species, however, different sections of the digestive system should be comprehensively monitored including different temperature conditions.

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Introduction

The digestive system of fish covers the region from mouth to anus. This anatomical structure is usually divided into headgut, foregut, midgut and hindgut (Floris, 2010). Chemical digestion of feeds starts in the foregut and continues in the midgut, even the midgut is where absorption takes place (Banan Khojasteh, 2012). Fish increase feed efficiency by digesting nutrients in the feed with the help of digestive enzymes (Shabana et al., 2019). The degradation of nutrients in the alimentary canal of fish is mostly dependent on the enzymes present (Hani et al., 2018). Digestive enzymes have a vital role

* Corresponding author
E-mail address: osmantolgaozel@gmail.com (O. T. Özel)

in the digestion of proteins, lipids, and carbohydrates. The absorption of digested substances from the intestinal wall for the growth and reproduction of the fish is facilitated by digestive enzymes (Amhamed et al., 2018). Most fish have seven major digestive enzymes like trypsin, carboxypeptidase a, carboxypeptidase b, maltase, amylase, lipase, and alkaline phosphatases (Hani et al., 2018). Among these enzyme groups, amylase, lipase and trypsin enzymes are the three main digestive enzymes that operate in the intestines to regulate digestion in animals. Therefore, the activities of digestive enzymes in the intestine represent the digestive status of the fish (Xu et al., 2019). The digestive enzyme activities of fish are influenced by various factors such as diet and feeding habits, fish age, growth stage, pH, and temperature, fish species, and digestive system structure (Amhamed et al., 2018). Among these factors, the temperature is one of the most important critical factors that directly affect the reproduction, survival, growth, nutrient intake, nutrient efficiency, and oxygen consumption of fish (Sun et al., 2015). Since the effectiveness of digestive enzymes highly reflects changes in diet, knowing the nutritional habits of different fish species associated with digestive enzyme activities in the gastrointestinal system is important to provide an appropriate diet for each species (Gioda et al., 2017). Therefore, determining digestive enzyme activity contributes to understanding the digestive physiology of fish (Hani et al., 2018).

Black Sea salmon, *Salmo labrax*, is a subspecies of the brown trout distributed at the Eastern Black Sea, and an opportunistic ecotype. Black Sea salmon is an endemic species for Turkey. This species of is represented by three different ecotypes: (i) sea ecotype: that migrates to the sea in the Black Sea basin, (ii) stream ecotype: that does not migrate to the sea but resident in the stream in the Black Sea Region, and (iii) lake ecotype: that residents in lakes in the Black Sea Region (Tabak et al., 2002). Under Black Sea conditions, following stripping, Black Sea salmon are grown in freshwater ponds until smolt stage (about 12 cm and 15 g). Individuals who reach the smolt are smoltified by transferring to seawater. Fish are kept during the periods (November-May) allowed by the water temperature in marine cages. In May, when the sea water reaches its critical value (18°C) of the temperature for Black Sea salmon aquaculture, the fish are harvested or transferred to freshwater ponds again for broodstock maintenance. Additionally, in the dam lakes where the water temperature, water depth and water current are suitable for aquaculture, Black Sea salmon can also be produced throughout the year or at a certain period of the year (SUMAE, 2010). The juvenile Black Sea salmon, which are grown up to 2-5 g in freshwater ponds, can be grown up to 350-400 g before the fillet size by transporting to the dam lake cages. During the months when the sea water temperature is suitable for Black Sea salmon production, fish can be reached fillet size by transporting from the dam lake cages to the marine cages. Moreover, Black Sea salmon can also be grown in freshwater ponds throughout all year without being transported to marine and dam lake cages. During these production processese, optimal and non-optimal water temperatures in both freshwater and sea water occur depending on the season. This condition can lead to the creation of different maintenance and feeding programs in aquaculture. Therefore, it is important to know the changes that occurred in the digestive physiology and metabolism of the species at optimum or non-optimum temperatures. This study aimed to determine the effect of suboptimal temperature on digestive enzyme activity in the gastrointestinal tract of Black Sea salmon.

Material and Methods

Material

The present study conducted between November 2018 and February 2019 at freshwater recirculating aquaculture systems (RAS) at Central Fisheries Research Institute, Trabzon, Turkey. Seventh filial generation (F7) of Black Sea salmon *Salmo labrax* (Pallas, 1814) with average initial weights of 69.85±10.08 g were selected for the study. In November 2019, fish were placed randomly in 500 l tanks. The trial was performed as triplicates containing 110 fish per tank. The fish were hand fed three times a day at 08:00, 12:00 and 16:00 for 75 days until apparent satiation. The basal diet used in the study was shown in Table 1. Water temperature (10.21±0.27°C), pH (7.09±0.28) and oxygen (9.39±0.32 mg/l) were recorded three times a day. Ammonia (0.03±0.02 mg/l) was measured weekly. Water changes in the tanks were conducted 20 times a day. Cleaning of tanks was performed by siphoning daily. The sampling was carried out according to both the European Union Directive (2010/63/EU) (European Commission, 2010) and ARRIVE ethical guidelines (Kilkenny et al., 2010). The study was performed with the approval of the Ethical Committee of Animal Experiments of Central Fisheries Research Institute (coded as ETIK-2017/1).

Methods

The tissue sampling was carried out at 08:00. For this, the midgut part of the intestine tissues from the fish was dissected at 45th minute (min.), 3rd, 6th, 12th, 24th, 36th, 48th, 72nd and 96nd hours (h) post feeding. Tissue samples were stored at -80°C until analyzed. Then, they were brought to Çanakkale Onsekiz Mart University, Faculty of Arts and Science, Biology Department, Water Ecology Laboratory in the cold chain. It

was necessary to prepare homogenate from the tissues to be used and to obtain cytosolic fractions to analyze the digestive enzymes. The tissues taken were weighed and homogenized in a 1:5 ratio with homogenization buffer (0.05 phosphate buffer pH 7.4). The specific activity of each enzyme evaluated in the study was measured spectrophotometrically. Bradford (1976) method was used to calculate the amount of protein.

Table 1. Formulation and proximate composition of the experimental diet

Ingredients	**%**
Fish meal	31
Soybean meal	20
Wheat gluten	6
Pea protein	12
Sunflower seed meal	7
Wheat flour	12.5
Fish oil	11
Vitamin mix[1]	0.22
Mineral mix[2]	0.16
Vit C	0.12
Proximate Composition	
Crude protein	46.31
Crude lipid	14.91
Crude ash	9.34
Moisture	6.19

Note: [1]Supplied the following: inositol 300 mg, biotin (Vit B7) 200 mg, tocopherol (Vit E) 200 mg, calcium pantothenate (Vit B5) 50 mg, riboflavin (Vit B2) 30 mg, pyridoxine (Vit B6) 20 mg, thiamine (Vit B1) 20 mg, menadione (Vit K3) 12 mg, niacin (Vit B3) 6 mg, retinol (Vit A) 0.6 mg, folic acid (Vit B9) 0.5 mg, cholecalciferol (Vit D3) 0.05 mg, cobalamin (Vit B12) 0.05 mg. [2]Supplied the following: ferric sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) 50 mg, manganese (II) oxide (MnO) 50 mg, zinc oxide (ZnO) 50 mg, copper sulfate pentahydrate ($CuO_4S \cdot 5H_2O$) 10 mg, calcium iodate (Ca_2IO_6) 0.8 mg, cobalt carbonate hexahydrate ($CoCO_3 \cdot 6H_2O$) 0.15 mg, sodium selenite (Na_2SeO_3) 0.15 mg.

Trypsin enzyme activity: The measurement was done due to Tseng et al. (1982) analysis method and Na-Benzoyl-DL-arginine-p-nitroanilide (BAPNA) was used as substrate. Enzyme activities of the samples were measured in a spectrophotometer at 253 nm wavelength for 5 minutes.

Pepsin enzyme activity: The measurement was performed using a revised version of the analysis method used by Worthington (1982) by Infante & Cahu (1994). Besides, bovine hemoglobin was used as a substrate. Samples were measured at a wavelength of 280 nm for 5 minutes.

Amylase enzyme activity: The enzyme levels were obtained depending on the study conducted by Bieth & Metais (1968) they used soluble starch as a substrate. Samples were measured at 540 nm wavelength for 5 minutes.

Lipase enzyme activity: α -naphthyl caprylate was used as the substrate, and the analysis method was used in the study conducted by Versaw et al. (1989). The measurements were done at 490 nm wavelength for 10 minutes.

Statistical Analyses

The descriptive statistics were presented as mean±Sx. Data were statistically analyzed by the one-way analysis of variance (ANOVA) procedure of SPSS 21.0 (Table 1). Duncan's multiple range test was performed for the significance of differences of means between groups. Probability levels of $p<0.05$ were chosen for statistical significance.

Results

Table 2 presents the results obtained from the digestion enzyme activities at suboptimal temperature. Significant differences were observed in the digestive enzyme activities of Black Sea salmon (One way ANOVA, $p<0.001$). The highest enzyme activity was obtained in pepsin (144.57±9.64 U mg^{-1}). This was followed by trypsin (60.64±11.24 U mg^{-1}) and amylase (3.69±0.42 U mg^{-1}). The lowest enzyme activity was obtained in lipase (0.04±0.00 U mg^{-1}). According to the results, the highest activity for all enzymes was observed at 3rd-hour post feeding. Digestive enzyme activities increased rapidly until 3 hours post feeding. After 3rd-hour post feeding, all enzyme activities tended to decrease over time. The decrease in amylase enzyme was faster than other enzymes. But, the increase in amylase enzyme was faster than other enzymes until 3rd-hour post feeding (Figure 1). From the 3rd hour to the 96th hour, the activity of pepsin, trypsin, amylase and lipase enzymes decreased in 95.45, 93.50, 95.12, and 95 % levels, respectively. In addition to, from the 45th minute to the 3rd hour, the activity of pepsin, trypsin, amylase and lipase enzymes increased in 56.07%, 64.10%, 78.86%, and 50% level, respectively.

When the correlations between digestive enzyme activities were examined, it was determined that the correlation between all measured digestive enzymes in specimens was found to be significant (Table 3), and the correlations between these enzymes were found to be strong in the positive direction.

Discussion

The activity of digestive enzymes in fish species can be influenced by factors including fish age, type of feeding, temperature (Munilla-Moran & Saborido-Rey, 1996), fish size, season, and origin (Hani et al., 2018). Temperature is one of the

Table 2. Time-dependent change of digestive enzyme activities of *S. labrax*, U mg^{-1}

Time	Pepsin	Trypsin	Amylase	Lipase
45min.	50.32±3.40^{c}	21.77±0.95^{b}	0.78±0.12^{b}	0.02±0.00^{b}
3h.	114.57±9.54^{a}	60.64±11.24^{a}	3.69±0.42^{a}	0.04±0.00^{a}
6h.	82.42±3.77^{b}	26.40±1.56^{b}	0.66±0.04^{bc}	0.02±0.00^{b}
12h.	45.96±2.93^{c}	21.32±1.61^{b}	0.35±0.01^{cd}	0.02±0.00^{b}
24h.	28.17±3.03^{d}	17.73±1.29^{bc}	0.24±0.01^{d}	0.01±0.00^{c}
36h.	19.54±3.05^{de}	9.96±1.07^{cd}	0.21±0.01^{d}	0.01±0.00^{c}
72h.	15.50±2.02^{ef}	6.45±0.82^{d}	0.18±0.01^{d}	0.01±0.00^{d}
96h.	5.29±0.88^{f}	3.94±0.46^{d}	0.18±0.01^{d}	0.002±0.00^{d}
P values	0.000	0.000	0.000	0.000

Note: Mean values in row with different superscripts were significantly different (P<0.001). Values are given as mean±Sx (n=5).

Table 3. Pearson correlation coefficients between the specific activity of digestive enzymes of the *S. labrax* (n=38)

Enzymes	Pepsin	Trypsin	Amylase	Lipase
Pepsin		0.84**	0.76**	0.92**
Trypsin	0.84**		0.84**	0.81**
Amylase	0.76**	0.84**		0.79**
Lipase	0.92**	0.81**	0.79**	

Note: **Correlation is significant at the 0.01 level (2-tailed).

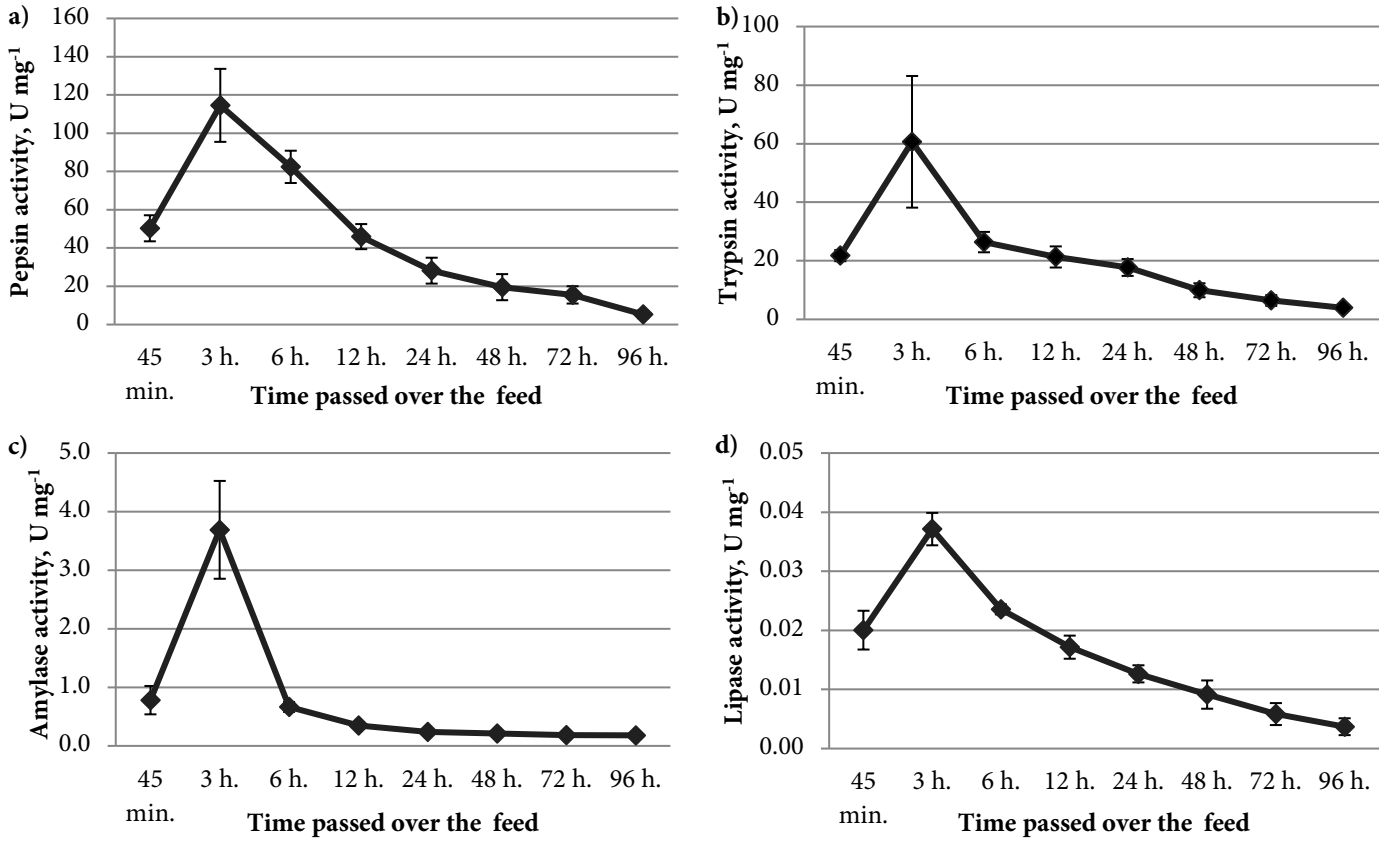

Figure 1. Time-dependent change of digestive enzymes post feeding, a: Pepsin, b: Trypsin, c: Amylase, d: Lipase.

most important external factors affecting the metabolic rate in ectotherm animals and has a direct effect on fish activity. At suboptimal temperature, the activities of various enzymes may decrease, which may result in decreased growth rate, and reduced digestibility of feeds (Bowyer et al., 2014). In our study, pepsin, trypsin, amylase, and lipase activity of Black Sea salmon were significantly influenced by time passed post feeding. A similar result was seen in Özel & Gürkan (2019). In addition, Gheisvandi et al. (2014) indicated that the activities of trypsin and amylase of Caspian kutum (*Rutilus kutum*) larvae fed at

18.4°C were highest at 8-hour post feeding. Our study demonstrated that trypsin, pepsin, amylase, and lipase enzymes increased from 45th minute to 3rd-hour post feeding, but decreased after 3rd hour. Unlike our study, Özel & Ertürk Gürkan (2019) found that the highest digestive enzyme activities in Black sea trout fed at 15°C were in 45th-minute post feeding. Hani et al. (2018) stated that most aquatic organisms have the ability to adapt to different temperatures because they are ectothermic animals. Miegel et al. (2010) stated that water temperature can have a direct effect on feed intake and enzyme activity. Intestine motility decreases at low water temperatures. This condition can result in higher intestinal enzyme activity (Miegel et al., 2010). Ahmad et al. (2014) found that the lipase and trypsin activities of *Clarias batrachus* were found to be the lowest at 10°C, which is the lowest temperature. Unlike the Özel & Ertürk Gürkan (2019), pepsin enzyme activity in the digestive system was higher at suboptimal temperature in our study, but amylase was lower. Kofuji et al. (2005) found that pepsin activity in the stomach of yellowtail (*Seriola quinqueradiata*) was lower in the colder water temperatures, but trypsin activity in the intestine was higher. The difference from our result could possibly be due to the sampling section of the gastrointestinal tract, water temperature, and also fish species. Gabriel et al. (2017) stated that the distribution and activities of digestive enzymes such as pepsin, trypsin, amylase, and lipase are at different levels throughout the gastrointestinal tract. This depends generally on the nature and composition of the diet. Regarding these studies, we can also say that the effect of suboptimal temperature on the activity of digestive enzymes may be at different levels depending largely on the digestive physiology of the fish species. Indeed, Wei et al. (2010) stated that the activity of digestive enzymes is an important indicator in understanding the digestive physiology of fish species.

Conclusion

We found that activity of digestive enzymes (pepsin, trypsin, amylase, and lipase) in the midgut section of the intestine of Black Sea salmon fed at 10.21°C in culture condition reached the highest level by increasing 3rd-hour post feeding. Enzyme activities decreased gradually by time after the 3rd hour. Among digestive enzymes, the highest activity was seen in the pepsin enzyme. This was respectively followed by trypsin and amylase. The lowest activity was seen in the lipase enzyme. We suggest that to determine digestive enzyme activities in nutrition researches to be conducted at the suboptimal temperature, the midgut intestine tissues of Black Sea salmon (appr. 179.17 g) can be taken at 3rd-hour post feeding. However, in order to better understand the effect of suboptimal temperature on the digestive enzymes of the species in the gastrointestinal tract, it is necessary to study the stomach, pyloric caeca and anterior, middle and posterior intestines should be monitored separately by supported with nutrition studies.

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Compliance With Ethical Standards

Authors' Contributions

Author OTÖ did conceptualization, methodology, design of the experiments and feed formulations, experiments, feeding studies and manuscript writing, SEG determined digestion enzyme activity, OTÖ and SEG performed data analysis, validation, and review.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The authors confirm that the ethical policies of the journal have been adhered to and the appropriate ethical review committee approval has been received from the Animal Ethics Committee of Central Fisheries Research Institute, Turkey (application number ETIK-2017/1). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

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