Acta Aquatica Turcica

E-ISSN: 2651-5474

Home Page: https://dergipark.org.tr/actaquatr 18(2): 236-246, 2022 DOI: 10.22392/actaquatr.942961

Research Article **Araştırma Makalesi**

Monitoring of Enzymatic Activity in the Gastrointestinal Tract of Black Sea Salmon (*Salmo labrax* Pallas, 1814)

Karadeniz Alabalığının (*Salmo labrax* Pallas, 1814) Gastrointestinal Sistemindeki Enzimatik Aktivitenin İzlenmesi

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

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

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

*Corresponding author: osmantolgaozel@gmail.com

Received: 08.11.2021 **Accepted:** 27.01.2022 **Published:** 01.06.2022

How to Cite: Özel, O. T. & Ertürk Gürkan, S. (2022). Monitoring of enzymatic activity in the gastrointestinal tract of Black Sea salmon (*Salmo labrax* Pallas, 1814). *Acta Aquatica Turcica, 18*(2), 236-246. https://doi.org/10.22392/actaquatr.1020183

Abstract: In this study, it was aimed to determine the activity of digestive enzymes at different time intervals after-feeding throughout the gastrointestinal tract of Black Sea salmon (*Salmo labrax*). The study was conducted at freshwater recirculating aquaculture systems (RAS). The fish were fed by hand up to satiation for 60 days with diets containing 46.25% protein and 14.90% lipid. The fish gastrointestinal tract including stomach, anterior (with pyloric caeca), middle and posterior sections were taken together at 45th minute, 3rd, 6th, 12th, 24th, 48th, 72nd, and 96th hours post-feeding. The tissues were were stored at -80°C until analyzed. In terms of digestive enzyme activity, at the end of the study, anterior and middle intestine sections had the highest level at 3rd-hour post-feeding. Similarly, pepsin, trypsin, and lipase in the stomach had the highest activity at 3rd-hour post-feeding. However, amylase in the stomach was the highest level at 45th minute and 3rd-hour post-feeding. At 3rd-hours post-feeding, the pepsin in the stomach, the trypsin, and lipase in the anterior section, and the amylase in the middle intestine had the highest levels. Moreover, the correlations between these enzymes were strong in the positive direction. Also, the difference in pepsin, trypsin, amylase, and lipase levels in the samples dissected at different time intervals after feeding was statistically significant. Our results revealed that stomach, anterior, middle, and posterior sections in the gastrointestinal tract of Black Sea salmon had enzyme activity in the different levels, and enzyme activity of these sections changed depending on time post-feeding.

Keywords

- Aquaculture
- Digestive system
- Enzyme
- Nutrition
- Trout

Özet: Bu çalışmada, Karadeniz alabalığının (*Salmo labrax*) sindirim sistemi boyunca beslenme sonrası farklı zaman aralıklarında sindirim enzimlerinin aktivitesinin belirlenmesi amaçlanmıştır. Çalışma tatlısu ile çalışan kapalı devre araştırma ünitesinde gerçekleştirilmiştir. Balıklar deneme boyunca %46,25 protein ve %14,90 lipit içeren yemlerle 60 gün süresince doyana kadar elle beslenmiştir. Balıkların mide, ön (pilorik kese ile birlikte), orta ve arka bağırsakları içeren sindirim kanalı, yemlemeden sonra 45. dakika, 3., 6., 12., 24., 48., 72. ve 96. saatlerde alınmıştır. Dokular analiz edilene kadar -80°C'de saklanmıştır. Sindirim enzim aktivitesi beslenme sonrası 3. saatte ön ve orta bağırsak bölümlerinde en yüksek seviyeye ulaşmıştır. Benzer şekilde midedeki pepsin, tripsin ve lipaz enzimleri de beslenmeden sonraki 3. saatte en yüksek aktiviteye ulaşmıştır. Bununla birlikte, midede amilaz, beslenme sonrası 45. dakika ve 3. saatte en yüksek düzeyde bulunmuştur. Beslenme sonrası 3. saatte midede pepsin, ön bağırsakta tripsin ve lipaz ve orta kısımda amilaz en yüksek seviyelere ulaşmıştır. Bununla birlikte, bu enzimler arasındaki korelasyonlar pozitif yönde güçlü bulunmuştur. Ayrıca beslenme sonrası farklı zaman aralıklarında alınan örneklerde pepsin, tripsin, amilaz ve lipaz düzeylerindeki fark istatistiksel olarak anlamlı bulunmuştur ($P<0.05$). Sonuçlar, Karadeniz alabalığının sindirim kanalındaki mide, ön, orta ve arka bölümlerinin farklı seviyelerde enzim aktivitesine sahip olduğunu ve bu bölümlerin enzim aktivitesinin besleme sonrası zamana bağlı olarak değiştiğini göstermiştir.

Anahtar kelimeler

- Yetiştiricilik
- Sindirim sistemi
- Enzim
- Besleme
- Alabalık

1. INTRODUCTION

Digestion is a necessary process using mechanical, chemical, and due to enzymatic methods with digestive enzymes released from multiple locations along the gastrointestinal tract for the catabolism and hydrolysis of ingested macronutrients into smaller molecules suitable for transport (Weinrauch et al. 2019). This process starts in the stomach and continues along the intestine. Ingested feeds are hydrolyzed into smaller size molecules, such as amino acids, simple sugars, and fatty acids produced by the hydrolysis of proteins, sugars, or lipids, respectively (Caruso et al. 2009). Understanding the digestive physiology of fish is quite important in the generate nutritional protocols that respond to the metabolic capabilities of feed utilization of fish species (Caruso et al. 2008). This is an important step for optimal nutritional values and cost-effectiveness (Barlaya et al. 2016). The utilization of nutrients in fish depends on the activities of digestive enzymes with different properties in the digestive organs (Tian et al. 2019). The activity of digestive enzymes in fish may be changed depending on the fish age, feeding type, season, temperature (Munilla-Moran and Saborido-Rey, 1996), fish size (Murtaza et al. 2016), feeding habits (Almeida et al. 2018), time of day, infestation with parasites (Solovyev et al. 2015) and intestinal morphology (Barlaya et al. 2016).

Digestive enzymes are secreted from the exocrine pancreas into the anterior section of the gastrointestinal tract (Tian et al. 2019), and their concentration and activity are more conspicuous in this section and decrease towards the posterior sections of the gastrointestinal tract (Gonzalez-Felix et al. 2018). In fish, activities of the digestive enzymes may change among the organs (Duarte et al. 2015). Different parts of the intestine do not have the same capacity for digestion and absorption of nutrients. Many digestive enzymes are limited to specific parts of the gastrointestinal tract (Barlaya et al. 2016). The relative activities of digestive enzymes between sections of the intestine differ from one enzyme to another (Deguara et al. 2003). The determination of digestive enzyme activities including proteases, carbohydrases, and lipases may provide information about the digestive capacity and the efficiency of species (Caruso et al. 2009). The digestive enzymes and their activities present in various sections of the intestine is quite important for understanding digestion mechanism and adaptation to nutrition in fish species (Iqbal et al. 2018). Therefore, we aimed to monitor the activities of digestive enzymes in various sections of the gastrointestinal tract of Black Sea salmon.

2. MATERIAL and METHODS

2.1. Fish and Maintenance

This study was performed 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) was used in the study. Fish were fed with commercial feeds before the experiment. Afterward, the fish were fed with a trial diet for 60 days (Table 1). Trial fish were placed randomly in 400 L (100x100 cm square with depths 40 cm) fiberglass tanks. The study was performed as triplicates containing 35 fish per trial tank. The fish were fed by hand three times a day at 08:00, 12:00, and 16:00 for 60 days up to apparent satiation. The diet used in the study was shown in Table 1. Water temperature (mean, 15.40±0.73°C), pH (mean, 7.32±0.25) and oxygen (mean, 8.48±0.57 mg/L) were recorded three times a day. Ammonia (mean, 0.04±0.02 mg/L) was measured weekly. Water change in tanks was 22 times in a day. The water supplied to the trial tanks was passed through 100, 25, 10, and 5 µ filters from the system, respectively. Trial tanks were cleaned by siphoning daily. 12 hours of darkness and 12 hours of light were applied during the experimental period.

Table 1. Formulation and proximate composition of the base 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	46.25
Crude lipid	14.90
Crude ash	9.28
Moisture	6.10

[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.

2.2. Enzyme activity

The gastrointestinal tract samples from the fish (250.67±44.09 g) were taken together at the 45th minute, 3rd, 6th, 12th, 24th, 48th, 72nd, and 96th hours 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 (in the ice cube). The stomach, anterior (with pyloric ceace), middle, and posterior intestines were examined separately. It was necessary to prepare homogenate from the digestive tract to be used and to obtain cytosolic fractions to analyse the digestive enzymes. The tissues taken were weighed and homogenized with liquid nitrogen and then taken into homogenization buffer (0.05 phosphate buffer pH 7.4). The measurements were settled following the preparation of homogenates. The specific activity of each enzyme evaluated in the study was measured spectrophotometrically. Obtained values were proportioned to the protein value in homogenate, and Bradford (1976) method was used to calculate the amount of protein.

For the measurement of trypsin enzyme activity, Bieth and Metais (1968), the analysis method used in their study, and Na-Benzoyl-DL-arginine-p-nitroanilide (BAPNA) were used as substrate. Enzyme activities of the samples were measured in a spectrophotometer at 253 nm wavelength for 5 minutes. Measurement of pepsin enzyme activity was performed using a revised version of the analysis method used by Worthington (1982) by Infante and Cahu (1994). Besides, bovine haemoglobin was used as a substrate. Samples were measured at a wavelength of 280 nm for 5 minutes. Monitoring the amylase enzyme activity depended on the study conducted by Tseng et al. (1982) which they used soluble starch as a substrate. Samples were measured at 540 nm wavelength for 5 minutes. To measure 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.

2.3. Data analyses

The statistical analyses were carried out SPSS 21.0 software. Normality of data and homogeneity of variances was tested using Kolmogorov Smirnov and Levene tests, respectively. The results were compared using parametric tests (One-Way ANOVA and Tukey's sub-test) and/or non-parametric tests (Kruskal-Wallis test). Duncan's multiple range test was performed for the significance of differences of means between groups. The correlation between enzyme levels was calculated by Pearson correlation. The significant differences among concentrations were presented with different letters or figures. Two-way analysis of variance (ANOVA) was used to analyse whether there was a difference between the activities of digestive enzymes in different sections of the digestive tract and different periods. A significance level α was set at 0.05 for all analyses except correlation analysis.

3. RESULTS

The enzymatic activity in the gastrointestinal tract of Black Sea salmon fed at optimal temperature was shown in Table 2 and Table 3. The digestive enzyme activities of fish changed depending on time post-feeding and sections of the gastrointestinal tract. The difference in amylase enzyme activity in samples dissected at different time intervals after feeding was statistically significant ($F=23.65$; $df=7$; $p<0.05$) and intestinal amylase activity decreased after 6 hours post-feeding, except for the posterior part of the intestine (Table 3). The difference in amylase enzyme levels measured in different parts of the gastrointestinal tract of the samples was also statistically significant ($F=28.1$; $df=3$; $p<0.05$) (Table 2). When both time and different gastrointestinal tract sections were evaluated together, the statistical difference was significant in terms of amylase enzyme level ($F=3.19$; $df=21$; $p<0.05$).

According to the trypsin enzyme activity results, the highest trypsin activity was seen in the anterior. This was followed by the middle, posterior, and stomach, respectively ($F=160.54$; $df=3$; $p<0.05$) (Table 2). Although time-dependent changes between trypsin levels were statistically significant, ($F=184.8$; $df=7$; $p<0.05$) There were minor differences in trypsin activity in all sections at 72nd and 96th hours after feeding and were not statistically significant (Table 3). When the temporal and regional changes in trypsin enzyme level were evaluated together, the differences were statistically significant ($F=11.5$; $df=21$; $p<0.05$).

Pepsin enzyme in the stomach, front and middle reached the highest activity at the 3rd and 6th hours after feeding, followed by feeding at the 45th-minute and 12th-hour, respectively. The highest pepsin activity in the gastrointestinal tract was obtained in the stomach (Table 2). Differences in pepsin enzyme levels in different parts of the gastrointestinal tract ($F=302$; $df=3$; $p<0.05$) and differences in pepsin values measured at different times after feeding ($F=335.4$; $df=3$; $p<0.05$) were also statistically significant.

The highest lipase activity in the stomach and anterior sections was observed at the 3rd hour after feeding with very small differences. Lipase activity in the foregut was higher at the 3rd hour after feeding than in other sections. Lipase enzyme levels were also observed in different digestion sections ($F=40.1$; $df=3$; $p<0.05$) and at different times ($F=65.84$; $df=7$; $p<0.05$) were statistically different.

Table 1. The enzymatic course of the gastrointestinal tract of Black Sea salmon, U mg^{-1}

Sections	Pepsin	Trypsin	Amylase	Lipase
45th minute				
Stomach	0.65±0.01^{a}	0.21±0.02^{c}	0.52±0.05^{a}	0.08±0.01^{ab}
Anterior	0.50±0.02^{b}	0.57±0.03^{a}	0.60±0.03^{a}	0.10±0.01^{a}
Middle	0.35±0.03^{c}	0.47±0.03^{b}	0.61±0.02^{a}	0.09±0.01^{ab}
Posterior	0.19±0.03^{d}	0.23±0.03^{c}	0.12±0.02^{b}	0.05±0.01^{b}
3rd hour				
Stomach	0.79±0.02^{a}	0.25±0.02^{d}	0.47±0.03^{c}	0.10±0.01^{b}
Anterior	0.61±0.03^{b}	0.68±0.05^{a}	0.61±0.02^{b}	0.14±0.01^{a}
Middle	0.44±0.02^{c}	0.54±0.04^{b}	0.73±0.02^{a}	0.10±0.01^{b}
Posterior	0.23±0.02^{d}	0.36±0.03^{c}	0.16±0.01^{d}	0.04±0.01^{c}
6th hour				
Stomach	0.73±0.01^{a}	0.25±0.01^{d}	0.41±0.03^{c}	0.08±0.01^{a}
Anterior	0.56±0.02^{b}	0.63±0.03^{a}	0.54±0.03^{b}	0.11±0.01^{a}
Middle	0.41±0.03^{c}	0.52±0.03^{b}	0.67±0.02^{a}	0.09±0.01^{a}
Posterior	0.24±0.03^{d}	0.33±0.02^{c}	0.14±0.02^{d}	0.04±0.01^{b}
12th hour				
Stomach	0.57±0.01^{a}	0.20±0.02^{c}	0.37±0.04^{a}	0.06±0.01^{ab}
Anterior	0.47±0.02^{b}	0.53±0.03^{a}	0.42±0.04^{a}	0.08±0.01^{a}
Middle	0.29±0.03^{c}	0.44±0.04^{b}	0.36±0.05^{a}	0.07±0.01^{a}
Posterior	0.15±0.02^{d}	0.26±0.02^{c}	0.09±0.01^{b}	0.04±0.01^{b}
24th hour				
Stomach	0.32±0.02^{a}	0.12±0.01^{c}	0.23±0.02^{a}	0.05±0.01
Anterior	0.28±0.02^{a}	0.35±0.03^{a}	0.27±0.03^{a}	0.06±0.01
Middle	0.20±0.01^{b}	0.28±0.03^{b}	0.29±0.03^{a}	0.04±0.01
Posterior	0.12±0.02^{c}	0.18±0.01^{c}	0.06±0.01^{b}	0.03±0.00
48th hour				
Stomach	0.18±0.02^{ab}	0.09±0.00^{c}	0.17±0.01^{a}	0.03±0.00
Anterior	0.21±0.03^{a}	0.21±0.02^{a}	0.18±0.02^{a}	0.04±0.01
Middle	0.14±0.02^{bc}	0.18±0.02^{ab}	0.19±0.02^{a}	0.03±0.01
Posterior	0.09±0.01^{c}	0.15±0.02^{b}	0.05±0.01^{b}	0.02±0.00
72nd hour				
Stomach	0.07±0.02^{b}	0.08±0.01	0.06±0.02	0.02±0.00
Anterior	0.12±0.02^{a}	0.07±0.02	0.10±0.02	0.03±0.00
Middle	0.09±0.00^{ab}	0.11±0.02	0.11±0.01	0.03±0.00
Posterior	0.07±0.01^{b}	0.06±0.01	0.09±0.02	0.02±0.00
96th hour				
Stomach	0.05±0.01^{b}	0.05±0.00	0.04±0.01	0.02±0.00
Anterior	0.07±0.00^{a}	0.03±0.01	0.06±0.01	0.03±0.00
Middle	0.06±0.01^{ab}	0.05±0.01	0.06±0.01	0.02±0.00
Posterior	0.05±0.01^{b}	0.03±0.01	0.05±0.01	0.02±0.00

Mean values in a column with different superscripts were significantly different at $p<0.05$. Values are given as means with standard errors (n=5). Each time was evaluated separately.

Table 2. Temporal variation of the enzymatic activity in the gastrointestinal tract of Black Sea salmon, U mg^{-1}

Time	Pepsin	Trypsin	Amylase	Lipase
		Stomach		
45th minute	0.65±0.01^{c}	0.21±0.02^{b}	0.52±0.05^{a}	0.08±0.01^{b}
3rd hour	0.79±0.02^{a}	0.25±0.02^{a}	0.47±0.03^{ab}	0.10±0.01^{a}
6th hour	0.73±0.01^{b}	0.25±0.01^{a}	0.41±0.03^{bc}	0.08±0.01^{b}
12th hour	0.57±0.01^{d}	0.20±0.02^{b}	0.37±0.04^{c}	0.06±0.01^{c}
24th hour	0.32±0.02^{e}	0.12±0.01^{c}	0.23±0.02^{d}	0.05±0.01^{cd}
48th hour	0.18±0.02^{f}	0.09±0.00^{c}	0.17±0.01^{d}	0.03±0.00^{de}
72nd hour	0.07±0.02^{g}	0.08±0.01^{cd}	0.06±0.02^{e}	0.02±0.00^{e}
96th hour	0.05±0.01^{g}	0.05±0.00^{d}	0.04±0.01^{e}	0.02±0.00^{e}
		Anterior		
45th minute	0.50±0.02^{bc}	0.57±0.03^{bc}	0.60±0.03^{a}	0.10±0.01^{bc}
3rd hour	0.61±0.03^{a}	0.68±0.05^{a}	0.61±0.02^{a}	0.14±0.01^{a}
6th hour	0.56±0.02^{ab}	0.63±0.03^{ab}	0.54±0.03^{a}	0.11±0.01^{b}
12th hour	0.47±0.02^{c}	0.53±0.03^{c}	0.42±0.04^{b}	0.08±0.01^{c}
24th hour	0.28±0.02^{d}	0.35±0.03^{d}	0.27±0.03^{c}	0.06±0.01^{d}
48th hour	0.21±0.03^{e}	0.21±0.02^{e}	0.18±0.02^{d}	0.04±0.01^{de}
72nd hour	0.12±0.02^{f}	0.07±0.02^{f}	0.10±0.02^{e}	0.03±0.01^{e}
96th hour	0.07±0.00^{f}	0.03±0.01^{f}	0.06±0.01^{e}	0.03±0.01^{e}
		Middle		
45th minute	0.35±0.03^{b}	0.47±0.03^{ab}	0.61±0.02^{b}	0.09±0.01^{a}
3rd hour	0.44±0.02^{a}	0.54±0.04^{a}	0.73±0.02^{a}	0.10±0.01^{a}
6th hour	0.41±0.03^{a}	0.52±0.03^{ab}	0.67±0.02^{ab}	0.09±0.01^{a}
12th hour	0.29±0.03^{b}	0.44±0.04^{b}	0.36±0.05^{c}	0.07±0.01^{a}
24th hour	0.20±0.01^{c}	0.28±0.03^{c}	0.29±0.03^{c}	0.04±0.01^{b}
48th hour	0.14±0.02^{d}	0.18±0.02^{d}	0.19±0.02^{d}	0.03±0.01^{b}
72nd hour	0.09±0.00^{de}	0.11±0.02^{de}	0.11±0.01^{e}	0.03±0.00^{b}
96th hour	0.06±0.01^{e}	0.04±0.01^{e}	0.06±0.01^{e}	0.02±0.00^{b}
		Posterior		
45th minute	0.19±0.03^{ab}	0.23±0.03^{bc}	0.12±0.02^{ab}	0.05±0.01^{a}
3rd hour	0.23±0.02^{a}	0.36±0.03^{a}	0.16±0.01^{a}	0.04±0.01^{ab}
6th hour	0.24±0.03^{a}	0.33±0.02^{a}	0.14±0.02^{ab}	0.04±0.01^{ab}
12th hour	0.15±0.02^{bc}	0.26±0.02^{b}	0.09±0.01^{bc}	0.04±0.01^{abc}
24th hour	0.12±0.02^{cd}	0.18±0.01^{cd}	0.06±0.01^{bc}	0.03±0.00^{bcd}
48th hour	0.09±0.01^{cde}	0.15±0.02^{d}	0.05±0.01^{c}	0.02±0.00^{cd}
72nd hour	0.07±0.01^{de}	0.06±0.01^{e}	0.09±0.02^{c}	0.02±0.00^{cd}
96th hour	0.05±0.01^{e}	0.03±0.01^{e}	0.05±0.01^{c}	0.02±0.00^{d}

Mean values in a column with different superscripts were significantly different at $p<0.05$. Values are given as means with standard errors (n=5). Each section was evaluated separately.

It was determined that the correlation between the time-dependent changes of digestive enzyme levels in different parts of the gastrointestinal tract was significant and strong in a positive direction (Table 3).

Table 3. The correlation between time-dependent change of digestive enzyme levels in digestive tract section

Enzymes	Pepsin	Trypsin	Amylase	Lipase
Pepsin		0.6**	0.64**	0.8**
Trypsin	0.6**		0.63**	0.82**
Amylase	0.64**	0.63**		0.67**
Lipase	0.8**	0.82**	0.67**	

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

4. DISCUSSION

We preferred to use the anterior, middle, and posterior sections of the fish intestine when evaluating the studies conducted by the researchers (Albrecht et al., 2001; Deguara et al., 2003; Khojasteh et al., 2009; Deshmukh et al., 2015; Bocina et al., 2017; Gioda et al., 2017; Tian et al., 2019).

The increase in digestive enzyme activities post-feeding indicated that enzyme secretion was stimulated by the presence of nutrients in the gastrointestinal tract (Caruso et al. 2008). In our study, the activities of digestive enzymes in the gastrointestinal tract of Black Sea salmon were found at different levels at all gastrointestinal sections including the stomach, and significantly increased post-feeding. Pepsin enzyme is active in fish with stomach, but not in stomachless fish and Mediterranean fish (Susilo et al. 2018). Deguara et al. (2003) reported that the activity of pepsin in the digestive tract of gilthead sea bream (*Sparus aurata*) was only present in the stomach. Whereas, in our study, the activity of pepsin enzymes was observed even in the anterior, middle, and posterior sections of the intestine besides the stomach. Additionally, the pepsin activity in the gastrointestinal tract of the *Rhamdia quelen* and *Pimelodus maculatus*, which are omnivorous species, was highest in the stomach (Almeida et al. 2018). A similar result was obtained in our study. This condition started to change at the 24th-hour post-feeding, and it changed in favor of the anterior part at 72nd and 96th hours post-feeding. This can be explained by the fact that the digestive activity in the stomach decreases depending on time and the enzymatic activity continues in this section for a while as the digestive contents are pushed into the anterior intestine over time. The trypsin activity in the intestine of *Pimelodus maculatus* was higher compared to the stomach (Duarte et al. 2015). Similarly, the activity of the trypsin enzyme of the gilthead sea bream was significantly lower in the stomach than in other sections of the intestine (anterior, middle, and posterior) (Deguara et al. 2003). A similar result was obtained in our study. In a previous study, Barlaya et al. (2016) reported that the activity of trypsin enzyme in the anterior intestine was higher than middle and posterior intestine (Coccia et al. 2011). In an additional study, Gioda et al. (2017) reported that the trypsin activity was highest in the anterior for *Ctenopharyngodon idella* (Herbivore), in the middle intestine for *Leporinus obtusidens* (Omnivore), in the posterior intestine for *Rhamdia quelen* (Omnivore). However, trypsin was highest in all intestine portions for *Hoplias malabaricus* (Carnivore). In our study, the highest trypsin activity was obtained in the anterior intestine until the 48th hour post-feeding, and this course of activity remained the same in all sections at the 72nd and 96th hours. Tian et al. (2019) found that the trypsin activity in anterior and middle sections of the intestine in carp (*Gymnocypris przewalskii*) were maximum levels at 2nd-hour post-feeding, and then to the basic level at 8 hour after feeding. Caruso et al. (2008) reported that the activity of trypsin enzyme in the intestine of European eel (*Anguilla anguilla*) reached its highest level 8th hour post-feeding, and the activity level remained unchanged until 24th hours post-feeding. Amylase enzyme in fish is localized throughout the gastrointestinal tract (Barlaya et al. 2016). The amylase enzyme activity was higher in the anterior and middle intestines for *Ctenopharyngodon idella* (Herbivore), in the anterior intestine for *Leporinus obtusidens* (Omnivore), but very low in all intestine portions for *Hoplias malabaricus* (Carnivore) (Gioda et al. 2017). In our study, while the amylase activity was highest in the middle intestine at 3th and 6th hours post-feeding, there was no difference in

the stomach, anterior and middle sections as the time passed post-feeding was prolonged. Fountoulaki et al. (2005) reported that amylase activity in the digestive tract of gilthead sea bream (*Sparus aurata*) increased significantly at 5 h post-feeding. Tian et al. (2019) found that the amylase activity in the anterior section of the intestine in carp (*Gymnocypris przewalskii*) were maximum levels at the 2nd hour post-feeding, and then decreased to the basic level at 6th hour post-feeding. In a previous study, Barlaya et al. (2016) reported that the activity of amylase enzyme in the anterior intestine of Indian major carp (*Labeo rohita*) was higher than in the middle and posterior intestine. In an additional study, Coccia et al. (2011) reported that amylase activity, the highest among carbohydrates, was the highest in the intestine as compared to the stomach in the Crayfish (*Cherax albidus*). The activity of the amylase enzyme was similar to each other in the anterior, middle, and posterior sections of the intestine, whereas had significantly lower activity in the stomach (Deguara et al. 2003). In our study, the posterior section of the intestine of Black Sea salmon had the lowest amylase activity until 48th hour post-feeding. Also, our results with the activity course of the amylase in the stomach were consistent with the results of Caruso et al. (2008) found that the amylase activity in the stomach of European eel (*Anguilla anguilla*) was progressively decreased until 24th hours post-feeding. Similar to our study, Weinrauch et al. (2019) reported that lipase activity was determined at all gastrointestinal sections and was significantly higher in the anterior section when compared to the posterior section. Lipase activity of *Cherax albidus* was higher in the intestine compared to the gastric juice (Coccia et al. 2011). Moreover, lipase enzyme activity in the posterior intestine of *Labeo rohita* was higher than in the anterior section (Iqbal et al. 2018). However, the activity of lipase enzyme in Nile tilapia (*Oreochromis niloticus*.) was higher in the anterior section as compared to pseudostomach and posterior intestine (Klahan et al. 2009). The lipase enzyme in the anterior intestine and pyloric caeca had more activity in the sections of the gastrointestinal tract of *Totoaba macdonaldi* (Gonzalez-Felix et al. 2018). In our study, while lipase activity was the highest level in the anterior section at the 3rd-hour post-feeding, there were no differences in the stomach, anterior and middle sections as the time passed post-feeding was prolonged. Tian et al. (2019) reported that the activity lipase enzyme in the anterior section of the intestine in carp (*Gymnocypris przewalskii*) were maximum levels at 2nd-hour post-feeding, and then decreased to the basic level at 6th-hour post-feeding. Moreover, Caruso et al. (2008) reported that lipase activity in the intestine of European eel (*Anguilla anguilla*) was highest at 8th-hour post-feeding, and progressively decreased over time.

5. CONCLUSION

To conclude, pepsin activity was at the highest level in the stomach and gradually decreased towards the end of the gastrointestinal tract. The amylase enzyme in the stomach was the enzyme with the highest activity after pepsin among the enzymes studied, and its activity in the stomach was higher than the posterior section. Trypsin enzyme was more intensely secreted in the anterior section. This was followed by the middle intestine. A similar conclusion can also be said for the lipase enzyme.

It can be said that protein, carbohydrate and lipid digestion in the Black sea salmon begins in the stomach, and the anterior intestine contributed more to the digestion of nutrients. Also, the response of digestive enzyme activity in the gastrointestinal tract of Black Sea salmon to feeding was quite fast. Indeed, digestive enzyme activity reached the highest levels at 3rd-hour post-feeding. However, enzyme activities decreased progressively by time after 3rd-hour post-feeding.

ACKNOWLEDGEMENTS

This research was carried out within the project named “Investigation of possibilities of using some phytobiotic added feeds in feeding of Black Sea trout "

FINANCIAL SUPPORT

This research was supported by General Directorate of Agricultural Research and Policies, TAGEM/HAYSUD/2017/A11/P-01/3.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Both of the authors equally contributed to the present study.

ETHICAL STATEMENT

This study was approved by the Central Fisheries Research Institute Animal Experiments Local Ethics Committee (2017/1).

DATA AVAILABALITY STATEMENT

Research data is not shared.

REFERENCES

Albrecht, M. P., Ferreıra, M. F. N., & Caramaschi, E. P. (2001). Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; Anostomidae). *Journal of Fish Biology*. *58***,** 419–430. https://doi.org/10.1006/jfbi.2000.1462

Almeida, A.P.G., Zardo, E.L., Toni, C., Behr, E.R., da Silv, L.P., Vieira, J.P., Loro, V.L., & Baldisserotto, B. (2018). Composition of gastrointestinal content, protease and lipase activities in summer and winter of four freshwater siluriforms (*Teleostei: Actinopterygii*) with two different feeding habits. *Zoologia. 35*, e13286. https://doi.org/10.3897/zoologia.35.e13286

Barlaya, G., Sridhar, N., Kushwaha, J. P., & Gangadhar, B. (2016). Digestive enzyme activities in different size groups and segments of the digestive tract in *labeo rohita* (day, 1878). *Journal of Aquaculture & Marine Biology. 4*(5).

Bieth, J. & Metais, P. (1968). The simultaneous presence of trypsin and trypsin inhibitors in some pathological effusions. *Clinica Chimica Acta*, *22*, 639-642.

Bocina I, Santic Z, Restovic I, & Topic S. 2017. Histology of the digestive system of the garfish *Belone belone* (Teleostei: Belonidae). *The European Zoological Journal. 84*(1), 89-95. https://doi.org/10.1080/11250003.2016.1276977

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254.

Caruso, G., Denaro, M. G., & Genovese, L. (2008). Temporal changes in digestive enzyme activities in the gastrointestinal tract of European eel (*Anguilla anguilla*) (Linneo 1758) following feding. *Marine and Freshwater Behaviour and Physiology*, *41*(4), 215-228, https://doi.org/10.1080/10236240802492931

Caruso, G., Denaro, M. G., & Genovese, L. (2009). Digestive Enzymes in Some Teleost Species of Interest for Mediterranean Aquaculture. *The Open Fish Science Journal*, *2*, 74-86.

Coccia, E., Varricchio, E., & Paolucci, M. (2011). Digestive Enzymes in the Crayfish Cherax albidus: Polymorphism and Partial Characterization. *International Journal of Zoology, 2011*, 310371. https://doi.org/10.1155/2011/310371

Deguara, S., Jauncey, K., & Agius, C. (2003). Enzyme activities and pH variations in the digestive tract of gilthead sea bream. *Journal of Fish Biology. 62*, 1033–1043. https://doi.org/10.1046/j.1095-8649.2003.00094.x

Deshmukh M. R., Chirde S. G., & Gadhikar Y. A. (2015). Histological and histochemical study on the stomach and intestine of catfish *heteropneustes fossilis* (bloch 1794). *G.J.B.A.H.S. 4*(1), 16-23.

Duarte, S., Bemquerer, M., & Araújo, F. G. (2015). Enzymatic Activity in the Gastrointestinal Tract of *Pimelodus maculatus* (*Teleostei, Siluriformes*) in Two Neotropical Reservoirs with Different Trophic Conditions. *Brazilian Archives of Biology and Technology*. *58*(4) 605-612.

Fountoulaki, E., Alexis, M. N., Nengas, I., & Venou, B., (2005). Effect of diet composition on nutrient digestibility and digestive enzyme levels of gilthead sea bream (*Sparus aurata* L.). *Aquaculture Research, 36*, 1243–1251.

Gioda, C. R., Pretto, A., Freitas, C. S., Leitemperger, J., Loro, V. L., Lazzari, R., Lissner, L. A., Baldisserotto, B. & Salbego, J. (2017). Different feeding habits influence the activity of digestive enzymes in freshwater fish. Ciência Rural, Santa Maria, 47(3), e20160113. http://dx.doi.org/10.1590/0103-8478cr20160113

Gonzalez-Felix, M. L., Santana-Bejarano, E. B., Perez-Velazquez, M., & Villalba-Villalba, A. G. (2018). Partial characterization, quantification and activity of pancreatic lipase in the gastrointestinal tract of *Totoaba macdonaldi*. *Archives of Biological Sciences., 70*(3), 489-496. https://doi.org/10.2298/ABS180202009G

Infante, J. Z. & Cahu, C. L. (1994). Influence of diet on pepsin and some pancreatic enzymes in sea bass (*Dicentrarchus labrax*) larvae. *Comparative Biochemistry and Physiology Part A: Physiology*, *109*, 209-212.

Iqbal, K.J., Ashraf, M., Javıd, A., Chaudhry, M.S., Khan, N., Majeed, H., & Abbas, F. (2018). Effect of different feed ingredients on digestive enzymes activity and on the histology of liver and intestine in *Labeo rohita* Hamilton, 1822. *Indian Journal of Fisheries, 65*(4): 93-101. https://doi.org/10.21077/ijf.2018.65.4.62647-11

Khojasteh S. M. B., Sheikhzadeh F., Mohammadnejad D., & Azami A. (2009). Histological, Histochemical and Ultrastructural Study of the Intestine of Rainbow Trout (Oncorhynchus mykiss). *World Applied Sciences Journal*, *6*(11), 1525-1531.

Klahan, R., Areechon, N., Yoonpundh, R., & Engkagul, A. (2009). Characterization and activity of digestive enzymes in different sizes of Nile tilapia (*Oreochromis niloticus* L.). *Kasetsart Journal - Natural Science*, *43*, 143 - 153.

Munilla-Moran, R., & Saborido-Rey, F. (1996). Digestive Enzymes in Marine Species. I. Proteinase Activities in Gut from Redfish (Sebastes mentella), Seabream (Sparus aurata) and Turbot (Scophthalmus maximus). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 113(2), 395-402.

Murtaza, M., Abdullah, S., Hassan, W., Abbas, K., Naz, H., & Zia, M. A. (2016). Studies on amylase and lipase activity in fishes fed with diet containing different feed ingredients. *Punjab University Journal of Zoology*, *31* (2), pp. 165-169.

Solovyev, M.M., Kashinskaya, E.N., Izvekova, G.I., & Glupov, V.V. (2015). pH values and activity of digestive enzymes in the gastrointestinal tract of fish in lake Chany (*West Siberia*). *Journal of Ichthyology*, *55*(2). 251–258.

Susilo, U., Sukardi, P., & Affandi, R. (2018). The age dependent activities of digestive enzymes in Rasbora, *Rasbora lateristriata* Blkr., (Pisces: Cyprinidae). *Molekul, 13*(1), 80 – 91. https://doi.org/10.20884/1.jm.2018.13.1.418

Tian, H., Meng, Y., Li, C., Zhang, L., Xu, G., Shi, Y., Shi, J., Qi, H., & Ma, R. (2019). A study of the digestive enzyme activities in scaleless carp (*Gymnocypris przewalskii*) on the Qinghai-Tibetan Plateau. *Aquaculture Reports 13*, 100174. https://doi.org/10.1016/j.aqrep.2018.10.002

Versaw, K. W., Cuppet, L. S., Winters, D. D., & Williams., L. E. (1989). An improved colorimetric assay for bacterial lipase in nonfat dry milk. *Journal of Food Science*, *54*, 1557-1558.

Weinrauch, A.M., Schaefer, C.M., & Goss, G.G. (2019). Activity and post-prandial regulation of digestive enzyme activity along the Pacific hagfish (*Eptatretus stoutii*) alimentary canal. *PLoS ONE 14*(4): e0215027. https://doi.org/10.1371/journal.pone.0215027

Worthington, T. M. (1982). Pepsin enzymes and related biochemicals. Freehold, NJ: Worthington Diagnostic System Inc. Retrieved from http://www.worthington-biochem.com/index/manual.html