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Original Article

Study of digestive enzymes in marine fish, *Terapon jarbua*, from Pakistan

Estudo de enzimas digestivas em peixes marinhos *Terapon jarbua*, do Paquistão

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Abstract

Current analysis were performed to investigate the activity of various digestive enzymes, such as lipases, proteases and amylases in gut and their relationship to the other morphometric variables in a wild marine fish, *Terapon jarbua.* The descriptive data of the studied traits included fish weight, fish total length, gut weight, gut length, relative gut length, relative gut mass, Fulton's condition factor, standard length and Zihler's index. Gut length showed positive correlation with fish total length and gut weight, relative gut length (RGL) showed positive correlation with gut length. Relative gut mass (RGM) also showed positive correlation with total length (TL), gut weight (GW) and gut length (GL). Fulton's condition factor showed positive correlation with fish weight, while negative correlation with fish total length and relative gut mass. Standard length displayed positive correlation with gut weight and gut length while, it showed negative correlation with Fulton's factor. Zihler's Index displayed positive correlation with gut length, RGL and Zihler's RGM while, while showed negative correlation with Fulton's factor and fish weight. Lipase showed negative correlation with gut weight. Amylase and protease activity have no correlation with other studied traits. Lipase activity displayed negative significant correlation with gut weight. Lipase activity showed significantly negative effect on gut-weight. Amylase activity on y-axis (PC2) contributed 13% in variation but not significantly correlated with first two principal components. It showed non-significant negative correlation with fish weight, fish length and Fulton's factor while positive but not-significant correlation with other traits. Protease has positive and non-significant correlation with fish weight, RGL, Fulton's factor, lipase and amylase while non-significant negative correlation with all other traits.

Keywords: amylase, protease, lipase, *Terapon jarbua*, gut enzymes.

Resumo

No presente estudo, as análises atuais foram realizadas para investigar a atividade de várias enzimas digestivas, como lipases, proteases e amilases no intestino e sua relação com as outras variáveis morfométricas em um peixe marinho selvagem denominado "*Terapon jarbua*". Os dados descritivos das características estudadas incluíram o peso e o comprimento total do peixe, o peso e o comprimento do intestino, o comprimento e a massa relativos do intestino, o fator de condição de Fulton, o comprimento padrão e o índice de Zihler. O comprimento do intestino apresentou correlação positiva com o comprimento total e o peso do intestino dos peixes, o comprimento relativo do intestino (RGL) mostrou correlação positiva com o comprimento do intestino. A massa relativa do intestino (RGM) também apresentou correlação positiva com comprimento total (TL), peso do intestino (GW) e comprimento do intestino (CG). O fator de condição de Fulton apresentou correlação positiva com o peso do peixe, enquanto correlação negativa com o comprimento total do peixe e a massa relativa do intestino. O comprimento padrão apresentou correlação positiva com o peso e o comprimento do intestino, enquanto apresentou correlação negativa com o fator de Fulton. O índice de Zihler apresentou correlação positiva com o comprimento do intestino, RGL e RGM de Zihler, enquanto apresentou correlação negativa com o fator de Fulton e o peso do peixe. A lipase mostrou correlação negativa com o peso do intestino. A atividade de amilase e protease não tem correlação com outras características estudadas. Já a atividade da lipase apresentou correlação negativa em relação ao peso do intestino. A atividade da lipase mostrou um efeito significativamente negativo no peso do intestino. A atividade da amilase no eixo *y* (PC2) contribuiu com 13% na variação, mas não se correlacionou significativamente com os dois primeiros componentes principais, demonstrando correlação negativa e não significativa em relação ao peso e comprimento e fator de Fulton, enquanto correlação positiva, mas não significativa com outras características. A protease tem correlação positiva e não significativa com o peso do peixe, RGL, fator de Fulton, lipase e amilase, enquanto correlação negativa não significativa com todas as outras características.

Palavras-chave: amilase, protease, lipase, *Terapon jarbua*, enzimas intestinais.

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1. Introduction

An extensive type of enzymes has been described in fishes, its released and quantity mainly dependent on feeding practices, gut structures and ecological circumstances (Banerjee and Ray, 2018).

The study of fish digestive biochemistry is essential to understand factors that affect the net efficiency of food transformation and growth, and therefore aquaculture profitability (Navarro-Guillén et al., 2022)

Information of exact enzyme activities, habit of animal as well as digestive process have significance in the formulation of suitable feed of any type of organism (Candiotto et al., 2018).

Gut morphometric indices like RGL and ZI have been investigated as potential indices of fish dietary strategy based on gut length. RGM (also called digestive somatic index) has been used to determine the feeding states of fish and can give an estimation of the amount of tissue dedicated by fish to their digestive tract (German and Horn, 2006).

The performance of the enzyme amylase has been detected in the many of teleost species (comprising herbivores to austerely carnivore fishes) at the different areas of alimentary canal, even though principally in the area of pyloric caecum, as well as in the pancreas and liver of fishes (Deng et al., 2010). Endogenous enzyme performance has been described for several fish species comprising *Perca fluviatilis*, *Gadhus morhura*, *Salmo salar* and *Salvelinus alpinus* species (Torrissen and Shearer, 1992; Lemieux et al., 1999; Langeland et al., 2013).

In fact, fishes such as *Terapon jarbua* do not possesses any of prominent gut enzyme that is indicative of its generalized voracious habit. These devoured a great variety of food items, that generalized its implausible achievement in the maximal consumption of estuarine territory (Chaudhuri et al., 2012). Fish from marine habitat are thought to be as a source of great quality fatty acids and proteins (Cardoso et al., 2019). Fish is an important part of the healthy diets (Adams and Standridge, 2006; Mozaffarian and Rimm, 2006). It is vital source of many nutrients, mainly retinols, vitamin-D, proteins, selenium, vitamin-E and the essential long-chains Polyunsaturated FA, that is eicosapentaenoic acid and docosahexaenoic acid (Welch et al., 2002).

T. jarbua is a broadly dispersed fishes in Pakistani coastal water bodies. It is all around consumed by local population of coastal areas. Locally terapons are called as "Ginghra" and "Kablosh". These are captured fundamentally as shrimp by catch and in Hella fishery. It is observed that the boats conducting fishing in inshore waters with rocky bottom area get large quantities of this species than those fishing in offshore waters (Khan and Imad, 2000).

1.1. Aims and objectives of this study

The aim of this investigation is to know the gut enzyme activity in *Terapon jarbua* and its relation with measured morphometric variables.

2. Materials and Methods

A total of 25 fish samples were collected from the Fisheries Organization Karachi (Sindh), Pakistan which were captured from the Arabian Sea, Karachi Fish Harbor (Karachi, Pakistan) in September 2018. For the isolation of gut and taking measurement of the different parameters, the fresh samples were immediately transported to Laboratory, in Institute of Pure and Applied Biology, BahauddinZakariya University, Multan, Pakistan, by wrapping the fish in protected plastic bags and keeping the samples in insulation boxes with ice. On arrival at the laboratory on very next day, fish samples were removed from boxes and dried by using paper towels.

Various morphometric parameters; fish weight, fish total length, gut weight, gut length, relative gut length, relative gut mass, Fulton's factor, standard length and Zihler's index were measured to show further its relation to gut enzyme activity, for this purpose, fish were weighed and measured. These measurements were done by using an electronic digital balance (MP-3000 Chyo, Japan) to the nearest 0.01 gram. Different body lengths parameters were measured by a wooden measuring tray to the nearest of 0.1 cm. In order to remove the gut of fish, the belly of samples was excised using aluminum dissecting tray.

The removed gut was washed with chilled TrisHCl buffer (pH 7.8- 8) to clean debris and fats, after that the gut was enveloped in aluminum foil to freeze at 1°C for the safe withdrawal of digestive enzymes.

2.1. Enzyme extraction and quantitative measurement

The stored samples were transported in sample tubes to laboratory for extraction of digestive enzymes (amylases, lipases and proteases). The gut of each fish was collected in micro-centrifuge tube and homogenized manually by mean of mortar and pestle with chilled trisHCl (50 mM), after that the homogenate was centrifuged at 6000g for exact 15 minutes at lower temperature at 4°C; supernatant was collected and stored at 0°C for enzyme estimation. Various indices were noted according to given formulae by following German and Horn (2006).

2.2. Study of amylase

Starch acts as a substrate in the analysis of amylase activity (Bernfeld, 1955). 5ml starch solution was mixed with 1 ml of homogenate gut product and incubated in water bath at 37°C for 30 min. 5ml blank was prepared with distilled water.1ml homogenate of enzyme was added in each tube. 1N HCl and 0.1 ml iodine solution was added in 0.5 ml aliquot taken from blank and experimental samples as well and then it was diluted to about 10.0 ml with distilled H_2O, appropriately mixed. The absorbance of each solution is then measured in a 10mm cuvette at 540nm by using Spectrophotometer (Model Hitachi-U-2900) and standard curve was plotted with maltose.

2.3. Study of lipase activity

The lipase activity was determined by using the method of Method of Tietz and Fiereck (1966). 2 ml homogenate related to gut was taken in both the test tube as well as in blank tube. These blank tubes were put in water bath (boiling) after that these were cooled down to the room temperature. 2ml Olive oil and 0.5 ml of phosphate buffer (pH = 7.4) were taken in both test and blank tubes. Tubes were then shaken and mixed by hand at the temperature of 37°C for the period of 24 hours. After the period of incubation, acetic acid (1ml) and phenolphthalein indicator (2 to 3 drops) were added in conical flask. Titrated it with standard 2N sodium hydroxide solution while waiting for the color changed in pink. Calculated lipase activities as summarized below (Equations 1-3):

$$Units\,/\,ml\ of\ enzyme\ =\ Volume\ of\ NaOH\ x \\ Normality\ of\ NaOH\ x\ 40\,/\ Volume\ of\ enzyme\ sample\ used \tag{1}$$

$$=\ Y\ \mu M\ ethanol\ released\,/\,min \tag{2}$$

$$Enzyme\ activity\ =\ Y\ x1000\,/\,254 \\ \left(Oleic\ acid\ molecular\ weight\right)\ x\ 30\,\mathrm{min}\ \ U\,/\,mL.\mathrm{min}^{-1} \tag{3}$$

2.4. Study of protease activity

Total protease activity of digestive homogenates was carried out by using Folin and Ciocalteau (1929). Casein (0.65%) was utilized for protease activity for the homogenate gut extract. Both the blank and test vials were taken with 5 ml casein solution and equilibrated at 37°C. 1ml gut homogenate was mixed in tube; it was incubated for 10 min at 37°C. Afterwards the process of incubating the tubes, TCA (5ml of 110 mM) to each vials to pause the chemical reaction, and at that moment mixed 1ml enzyme homogenate in the blank vials also. Stir each vial and incubated it at the temperature of 37°C for the period of about 30min. Whatman filter paper# 50 was used to filter the mixture. The standard curve was plotted by utilizing L-Tyrosine with help of Sodium carbonate (Na_2CO_3) and FolinCiocalteu reagent (FCR) for color development. This filtrate absorbance was noted at 660 nm and enzyme activity was calculated by comparing sample values with standard curve.

2.5. Statistical analysis

Descriptive statistics was applied on the univariate data; mean, maximum, minimum and standard deviation was calculated to check the central tendency of the data. Multivariate statistical analysis such as principal component analysis (PCA) was performed using the data obtained from studied variables with software XLSTATS-2016.

3. Results

Descriptive statistics of the studied traits observed in samples of *T. jarbua* are provided in Table 1. Gut length showed positive correlation with fish total length and gut wt., RGL showed positive correlation with gut length. RGM showed positive correlation with fish TL, GW and GL. Fulton's factor showed positive correlation with fish weight, fish total length, Standard length showed positive correlation with fish TL, GW, GL, RGM and Fulton's factor. ZI showed positive correlation with fish weight, gut length, RGL, RGM and Fulton's factor. Lipase showed negative correlation with gut length, RGM and standard length as shown in Table 2.

PCA explained 58.44% of the total variation expressed by twelve variables. Two principal components, PC1 and PC2 along x-axis and y-axis respectively, were calculated which showed 4.46 and 2.55 eigenvalues and explained 37.15% and 58.44 percent of total variation respectively (Figure 1) Gut weight is negatively correlated with enzyme activity. All the variables present at x- axis i.e., Fish length, RGM, gut length, standard length and gut weight are strongly showing correlation.

Lipase activity showed negative significant correlation (- 0.57) with gut weight. Fish sample number 20, 1, 14 and 5, exhibited maximum lipase activity as well as low gut weight while sample 19, 9 and 8 were with low lipase activity thus high gut weight (Figure 1).

Amylase activity on y-axis (PC2) contributed 13% in variation but not significantly correlated with first two principal components. It showed non-significant negative correlation with fish weight, fish length and Fulton's factor while positive but not-significant correlation with other traits as shown in (Figure 1)

Table 1. Descriptive stats of the studied traits observed in 25 samples of *T. jarbua*.

Statistic	Minimum	Maximum	Mean	SD	1st Quartile	3rd Quartile	CV%
Fish wt.(g)	111.70	281.40	192.64	53.53	152.60	226.90	27.23
Fish Total length(cm)	22.70	31.00	25.40	2.24	23.50	26.40	8.64
Gut weight(g)	5.20	23.62	12.39	4.88	9.32	13.70	38.58
Gut Length(cm)	23.40	47.20	32.71	5.10	29.40	34.20	15.29
RGL(cm)	1.23	1.99	1.55	0.19	1.43	1.66	12.29
RGM(g)	0.03	0.11	0.07	0.02	0.04	0.08	35.58
Fulton's F	0.48	2.22	1.24	0.50	0.84	1.51	39.56
St. Length(cm)	17.60	25.80	21.16	1.91	19.50	22.20	8.86
Zihler's index	0.32	0.92	0.55	0.19	0.40	0.76	33.80
Lipase (U/ml.min^{-1})	23.75	29.77	28.21	1.15	27.88	28.74	4.00
Amylase (U/ml.min^{-1})	0.12	0.13	0.12	0.00	0.12	0.12	3.92
Protease(U/ml.min^{-1})	0.24	0.25	0.24	0.00	0.24	0.24	1.45

SD = Standard Deviation.

Table 2. Pearson's correlation coefficient of the traits under study of *T. jarbua*.

Variables	fish wt.	Fish TL	Gut weight	Gut Length	RGL	RGM	Fulton's F	Standard Length	Zihler's index	Lipase	Amylase
Fish TL	-0.15										
Gut weight	0.38	0.39									
Gut Length	-0.09	**0.56**	**0.50**								
RGL	-0.14	0.05	0.18	**0.79**							
RGM	-0.32	**0.51**	**0.72**	**0.64**	0.33						
Fulton's F	**0.80**	**-0.69**	0.02	-0.36	-0.09	**-0.54**					
St Length	0.01	**0.88**	**0.58**	**0.58**	-0.03	**0.63**	**-0.50**				
Zihler's index	**-0.88**	0.35	-0.14	**0.49**	**0.47**	**0.55**	**-0.81**	0.21			
Lipase	-0.18	-0.04	**-0.57**	-0.10	-0.03	-0.37	-0.04	-0.14	0.16		
Amylase	-0.02	-0.01	0.12	0.14	0.13	0.12	-0.04	0.07	0.00	0.01	
Protease	0.17	-0.27	-0.15	-0.03	0.11	-0.25	0.33	-0.20	-0.13	0.07	0.02

Values in bold are different from 0 with a significance level alpha=0.05. The bold digits in the columns displayed significant correlation with the different variables in the rows of first column.

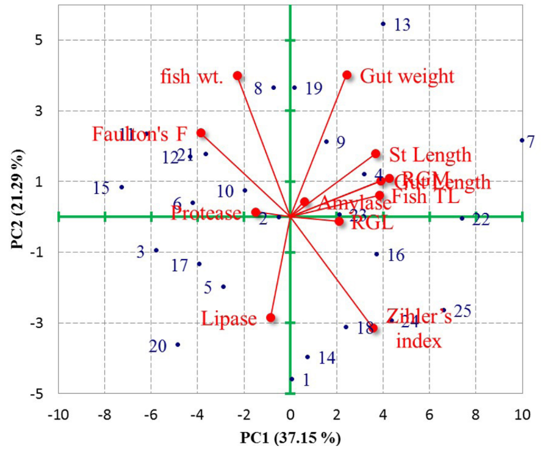

Figure 1. Biplot of 25 fish samples *T. jarbua* and 12 traits generated through principal component analysis (PCA) explained 58.44% of overall variation in the data.

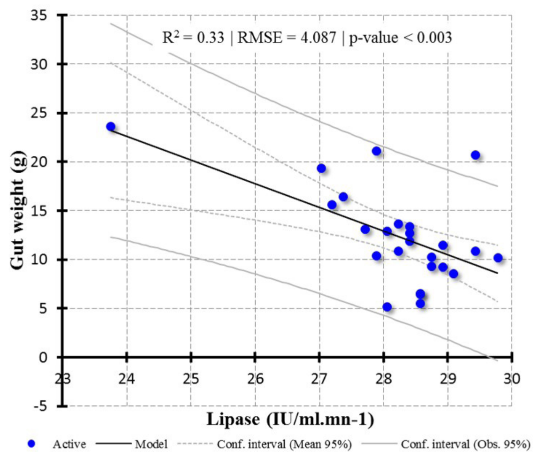

Figure 2. Simple linear regression of gut weight (g) by lipase (IU/ml.mn^{-1}).

Protease has positive and non-significant correlation with fish weight, RGL, Fulton's factor, lipase and amylase while non-significant negative correlation with all other traits. Protease showed fifteen percent variation and 15 percent contribution with PC3 but not significantly correlated with first two PCs as shown in (Figure 1).

3.1. Model summary interpret evaluation

Two separate regression models were applied by taking gut weight as dependent variable. First model was simple linear regression applied to see the effect of lipase activity on gut weight. To test the assumption, lipase activity shows significant effect on gut weight, simple linear regression was applied and this assumption was made by seeing correlation table. Analysis of variance of the model (Table 3) was significant ($p < 0.003$) with RMSE of 4.087 and coefficient of determination (R^2) was 0.33 (Figure 2). It has been observed (Table 3) that with each unit change in lipase activity, 2.426 times of gut weight was decreased.

With the help of PCA, fish weight was found another important trait would be investigated as dependent variable. Another model (multiple regression) was designed which explained effect of GW, GL, Condition factor and ZI and lipase activity on fish weight. These variables were selected on the basis of multi-collinearity statistics and PCA interpretations. Analysis of variance of the model (Table 3) was highly significant displaying the RMSE of 13.41 and coefficient of determination (R^2) was 0.95 (Figure 3). Model parameters showed that GL, Fulton's factor and ZI have significant effect in determination offish weight (Table 4).

Table 3. Model summary of simple linear regression between gut weight (g) as dependent variable (Y-variable) and lipase activity (X-variable).

Source	DF	SS	MS	F	Pr> F
Model	1	187.206	187.206	11.205	0.003
Error	23	384.272	16.707		
Corrected Total	24	571.478			

Source	Value	SE	T	Pr> |t|	Lower bound (95%)	Upper bound (95%)
Intercept	80.835	20.463	3.950	0.001	38.505	123.166
Lipase	-2.426	0.725	-3.347	0.003	-3.925	-0.927

SE = Standard Error, DF=Degree of Freedom, SS=Sum of Square, MS=Mean Square.

Table 4. Model summary of multiple linear regression of selected independent variables with fish weight (g) as dependent Y-variable.

Source	DF	Sum of squares	Mean squares	F	Pr> F
Model	5	65363.987	13072.797	72.702	< 0.0001
Error	19	3416.464	179.814		
Corrected Total	24	68780.451			

Source	Value	SE	T	Pr> |t|	Lower bound (95%)	Upper bound (95%)
Intercept	63.233	90.155	0.701	0.492	-125.463	251.928
Gut weight	1.204	0.965	1.247	0.227	-0.816	3.225
Gut Length	3.911	0.891	4.392	0.000	2.047	5.775
Fulton's F	23.745	9.768	2.431	0.025	3.300	44.189
Zihler's index	-247.286	31.230	-7.918	< 0.0001	-312.650	-181.922
Lipase	3.341	3.028	1.104	0.284	-2.996	9.679

SE = Standard Error.

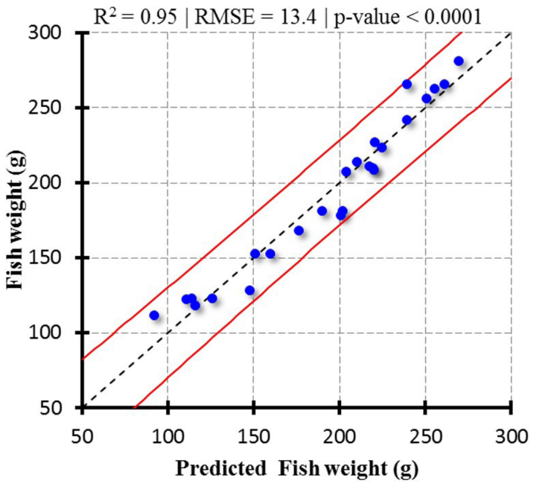

Figure 3. Observed and predicted values of dependent variable fish weight in grams as revealed by the multiple regression model.

4. Discussion

Gut length in *Terapon jarbua* showed positive correlation with total length and gut weight this is because numerous herbivore species of fish appeared to start life as carnivorous or omnivorous and change to a further herbivorous food as they grow in size (White, 2012), and its length of gut typically increase consequently (Stoner and Livingston, 1984; Drewe et al., 2004). Ontogenetic rises in the length of gut of fishes are well recognized in the marine and freshwater herbivore fish species these observations are in consistent with the findings of Ribble and Smith (1983), Montgomery (1977), Benavides et al. (1994), Gallagher et al. (2001) and Drewe et al. (2004). Even carnivore fish species could enhance their length of gut with increased in the standard length, but then herbivorous one has tendency to represent a quicker increase (Kramer and Bryant, 1995).

RGM in *Terapon jarbua* displayed a positive correlation with fish total length, gut weight and gut length and its valued ranged between 0.03 to 0.11, this is because the relative gust mass (RGM) also found to be increased in the tadpoles and herbivore feed in relation to those nourished on a carnivorous diet which is consistent with the finding of Toloza and Diamond (1990), German and Horn (2006). This current analysis on *T. jarbua* also coincide with analysis of Karasov and Douglas (2013) who suggested that, increasing in the mass of gut is one of technique utilized the by animals to enhance the intake of energy from food components. In this present study, Relative gut length (RGL) of *Terapon jarbua* was from 1.23 to 1.99 and related to relative gut length range of carnivore fishes, and Zihler's index values of *Terapon jarbua* was between

0.32 to 0.92, that did not coincide with any of three groups defined by researchers, this might be according to their feeding habits in wild due to which it is not palpable similar findings have been reported by Hani et al. (2018). Four other variables such as RGL, lipase, amylase and protease activity showed different lines of variation other than PC1 and PC2 (Figure 1). Gut weight of fish is negatively correlated with enzyme activity similar analyses were reported by Hofer and Schiemer (1981). All the variables present at x- axis i.e. Fish length, RGM, gut length, standard length and gut weight are strongly showing correlation.

The lipase activity in *Terapon jarbua* displayed negative significant correlation with gut weight. In *T. jarbua* amylase showed non-significant negative correlation with fish weight, fish length and Fulton's factor while positive but not-significant correlation with other traits as shown in Figure 1.

5. Conclusion

The results may be helpful in future work for comparison of data related to enzymatic activities and morphometric variable in different fish species, to study the aquatic environment, and for different nutrition studies and food authorities.

The results of the present study will contribute to the proper management of this species in the wild and can be used to fill a gap in the current knowledge in this area. Moreover, this study seems to be the first to portray the digestive activity of *T. jarbua* by focusing on three digestive enzymes and its relation to gut morphometric variables.

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