Research Article

Digestive Enzymes Activity with Gut Morphometric Parameter of Carnivorous Fish *Wallago attu* (Siluridae, Siluriformes)

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Abstract | Present study attempted to determine the presence, distribution and levels of various digestive enzymes, such as lipases, proteases and amylases in the digestive tract of a wild, freshwater, carnivorous *Wallago attu*. *Wallago attu* is a fast growing catfish that belongs to family siluridae under the order siluriformes. It has good market demand as a food fish having high nutritional value, and high protein content in its flesh, Carnivorous in nature. Descriptive data of the studied traits included total body weight, total body length, gut weight, gut length, condition factor, standard length in *Wallago attu*. Fish body weight showed positive Pearson's correlation with total length, gut weight, standard length and amylase enzyme. Fulton's factor showed positive correlation with fish ZI, lipase and protease. ZI showed positive correlation with amylase and protease. Regression analysis of Protease has highly significant correlation with the entire factor except Total length. With the help of principle component analysis, fish weight was found another important trait would be investigated as dependent variable. These variables were selected on the basis of multicollinearity and principle component analysis interpretations. Present study mainly reported on overall activity of lipase, amylase and protease of *Wallago attu*. To the best of our knowledge, this is the first study on *Wallago attu* related to gut morphometrics that evaluated digestive enzymes with reported literature behalf of their species feeding habits. Gut morphometric parameters included first time in relation to *Wallago attu* morphology as Relative gut mass, length and Zihler's Index are basically explored as potential indices to identify habits.

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Introduction

The aquaculture industry is searching for feed ingredients that can be used to formulate cheap fish feed, because supply and feed costs are amongst the extreme tasks for the development of supportable fish farming (Stone, 2003). Aquaculture is the fastest growing food production part in many countries and impact in terms of economic growth and modification. Due to the high cost of feed fish nutrition is an important discipline in aquaculture. On the basis of feed preference, fish can be divided into three wide groups *viz.* Carnivorous, herbivorous and omnivorous, which is directly related to their digestive enzymes (Jhingran, 1997). Several studies have been shown about fish feed and the efficacy of growth (Roy et al., 2016). These varying types of alternative feed sources can possibly be created from plants, animals or microbes (Slawski et al., 2013; Yun et al., 2013).

Vertebrates including fishes have various type of

digestive enzymes that is responsible to digest the food which they consume, but variation in activity exists among species of various enzymes individually (Alarco´n et al., 1998). Digestive enzymes are one of the keystones of digestion process for the meant efficiency of whole process of digestion mainly depends upon the functional character and activities of parameters (Pujante et al., 2017). Nutrient degradation in the fish digestive tract largely depends upon enzymes. Therefore, determining the activity of digestive enzyme has possible interest to get complete extensive information about fish digestive physiology. Digestive enzymes are widely studied in field of aquaculture. Thus digestive secretion study have helped to define the dietary limit of utilization of carbohydrates, proteins and nutritional strategy development for fish feed and formulation of diet (Debnath et al., 2007). In all aquatic species, as in fish activity of digestive enzyme is indicative of their feeding ecology in natural conditions and are correlated with their diet (German et al., 2004).

Activity of digestive enzyme varied within the species. It is influenced by biotic factor age, size, and abiotic as food, temperature and season parameters (Hofer, 1979), that can alter the enzymatic profile or its activity level. Based upon their food preference, fish can be conventionally classified into herbivorous, omnivorous and carnivorous (Pujante et al., 2017). They can also classified according to their gut morphometric and their various parameters as relative gut mass, its length, and Zihler's Index, relation with gut length. Relative gut length and Zihler's index have been investigated as potential indices of fish dietary strategy based on length of gut (Zihler, 1981).

Wallago attu is a fast growing catfish belongs to family siluridae under the order siluriformes. It has good market demand as a food fish having high nutritional value (Lilabati and Viswanath, 1996) and high protein content in its flesh (Jafri et al., 1964). Carnivorous and predatory in nature throughout the life (Hora, 1939).

The current understanding of digestive enzyme activity in fishes indicates a strong correlation with diet: herbivores possess higher levels of carbohydrases (e.g. amylase) than are found in carnivores, and carnivorous fishes show higher protease activities (e.g. pepsin and trypsin) than are found in herbivores (Hidalgo et al., 1999; Ferna´ndez et al., 2001).

Materials and Methods

30 Samples of *Wallago attu* were collected from various Rivers, such as Ravi, Chenab, Indus and Jhelum and transported to Fisheries Lab, IP and AB, BZU Multan, Pakistan. Then samples were weighed and the belly of samples were excised. Using dissecting tray complete digestive gut was dissected from fish sample for analysis. Digestive guts were homogenized in Tris- hydrochloride acid and kept it at 4°C for the purpose of safe withdrawal of required enzymes. Afterward, the homogenate samples were centrifuged at 6000g for 15 minute at 4°C. The supernatant was collected carefully then it was stored in freezer for further examinations.

Belly excision and gut morphometric

Whole gut was removed and washed with child Tris-HCl buffer (pH 7.5±1) to clean debris and fats. Gut was weighed, measured and enveloped in aluminum foil to freeze at 1°C for safe withdrawal of gastric enzymes. Various indices were noted according to given formulae:

1. RGM= Total gut mass (g)x[Total body mass (g)]$^{-1}$
2. RGL= Total gut length (cm) x [Standard length of fish (cm)]$^{-1}$
3. RLG = Total gut length (cm) x [Total length of fish (cm)]$^{-1}$
4. DSI= Total gut mass (g)x[Total body mass (g)]$^{-1}$ x 100
5. ZI= Total gut length (cm) x [Fish body mass (g) 1/3]$^{-1}$
6. K = Fish body mass (g) x [Total length (cm)^{3}]$^{-1}$x100

Amylase activity

For the analysis of amylase activity, soluble starch was used as a substrate. 5ml starch solution was put in falcon tubes. One ml of homogenate gut product was taken in tube. After that mixture was incubated in water bath at 37°C for 30 min. One N HCl was added then. After taking out tubes from water bath, these were properly mixed. Then 1 ml enzyme's homogenate was added in each tube. Five ml blank was prepared by distilled H_2O.

Starch solution (5 ml) was taken in falcon tubes, then 1.0 ml homogenate mixture of enzyme was added, incubated mixture inside water bath at 37°C for 30 minutes. Then, added 1 ml of 1N HCL solution. By taking out the tubes from water bath, it was properly

mixed, after that 1ml homogenate of enzyme was added in each control tube. Five ml blank solution was prepared with distilled H_2O; 0.2 ml of normal solution of HCl and iodine solution of about 0.1 ml was added in 0.5 ml aliquot taken from control and experimental group as well and then it was diluted to about 10.0 ml with distilled H_2O, appropriately mixed after that analyzed the absorbance at the wave length of 540nm.

Lipase activity

Two ml homogenate related to gut was put 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. Olive oil (2.0 ml) 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 two normal (2N) sodium hydroxide solution while waiting for the color changed in pink. Calculated lipase activities as summarized below:

Units per milli-litter of the digestive enzyme = Sodium hydroxide (NaOH) Volume x Sodium hydroxide

(NaOH) Normality x 40/ Vol. of specimen utilized = Y µM ethanol discharge per minute.

Activity of the enzyme = Y x1000/254 ($C_{18}H_{34}O_2$-Oleic acid,mol. Wt) x Half an hour (30 minutes) = (Z) U/ml/Min

Proteases Activity

Casein (0.65%) was used for protease activity for the homogenation of gut extract. Both the blank and test vials were taken with 5 ml casein solution and equilibrated at 37°C. One ml homogenate of enzyme 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 1m 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. Whitman filter paper (50) was used to filter the mixture. The standard curve was arranged by utilizing L-Tyrosine with help of Sodium carbonate (Na_2CO_3) and Folin Ciocalteu reagent (FCR) for color improvement. This filtrate absorbance was noted at 660 nm.

Results and Discussion

Descriptive data of the studied traits included total body weight, total body length, gut weight, gut length, condition factor, standard length in *Wallago attu* are given in Table 1.

Fish body weight showed positive Pearson's correlation with total length, gut weight, standard length and amylase enzyme. Fish body weight showed negative correlation with gut weight, RGL, RGM, ZI, condition factor, lipase and protease enzyme. Total length showed positive correlation with GW, GL, SL and amylase enzyme. Fish body weight showed negative correlation with gut weight, RGL, RGM, ZI, Condition factor (K), lipase and protease enzyme. Gut weight only show positive correlation with RGM, RGL, ZI and lipase enzyme while other factor showed negative correlation. Gut length showed positive correlation with RGM, K, ZI and amylase enzyme while other showed negative correlation.

Fulton's factor showed positive correlation with fish ZI, lipase and protease. ZI showed positive correlation with amylase and protease. Lipase showed positive correlation with Amylase and protease activity have no correlation with other study traits as shown in Table 2.

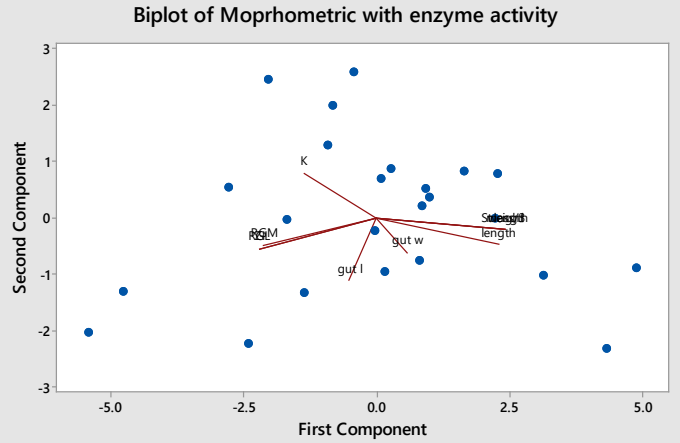

Figure 1: *Biplot of fish samples and generated through principal component analysis explained overall variation in the data with enzyme activity.*

Regression analysis of Lipase activity showed highly significant correlation with TL (0.02), ZI (0.03), RGL (0.02) while other factors showed nonsignificant correlation. Fish sample number 1,2,3 and 5, exhibited maximum lipase activity while other samples were with low lipase activity as shown in Table 3 and Figure 1.

Table 1: *Descriptive stats of the studied traits observed in 25 samples of Wallago attu.*

Statistic	Minimum	Maximum	Mean	SD	1st quartile	3rd quartile
fish wt.	223 g	915.6 g	517.11	177.93	415.7	580.8
Fish TL	30 cm	63 cm	43.16	9.09	35	48.5
Gut weight	9.20 g	17.3 g	13.30	2.59	11.93	15.9
Gut Length	22 cm	45 cm	31.25	5.79	27.75	35.5
RGL	0.032	0.187	0.071	0.037	0.050	0.083
RGM	0.013	0.051	0.028	0.010	0.022	0.033
Faulton's F	0.366	1.422	0.692	0.287	0.537	0.701
St. Length	208.5	903.18	502.619	177.939	401.2	566.3
Zihler's index	0.095	0.524	0.207	0.104	0.146	0.240
Lipase	16	25	19.14	2.522	17.925	20
Amylase	0.123	0.254	0.186	0.048	0.163	0.216
Protease	1.998	0.383	0.760	0.468	0.494	0.729

Table 2: *Pearson's correlation coefficient of the traits under study in Wallago attu.*

Variables	Fish wt.	Fish TL	Gut weight	Gut length	RGL	RGM	Fulton's F	St length	Zihler's index	Lipase	Amylase
Fish TL	0.92										
Gut weight	0.25	0.25									
Gut Length	-0.01	0.07	0.11								
RGL	-0.77	-0.62	-0.19	0.54							
RGM	-0.32	-0.66	0.25	0.31	0.83						
Fulton's F	-0.51	-0.78	-0.28	-0.15	0.23	0.23					
St Length	1	0.92	0.25	-0.01	-0.75	-0.77	-0.51				
Zihler's index	-0.75	-0.63	-0.18	0.54	0.99	0.83	0.23	-0.75			
Lipase	-0.02	-0.21	0.44	-0.10	-0.04	0.55	0.22	-0.02	-0.05		
Amylase	0.19	0.16	-0.56	0.32	0.01	0.37	-0.10	0.19	0.02	0.90	
Protease	-0.36	-0.37	-0.03	-0.27	0.07	0.25	0.30	-0.36	0.06	0.07	-0.50

Values are different from 0 with a significance level alpha=0.05

Regression analysis of Amylase activity on y axis (Principle components) contributed variation in data but showed non-significant negative correlation with fish TL, RGL, RGM, GW and ZI while factor while highly significant correlation with other traits as shown in Table 3 and Figure 1.

Protease has highly significant correlation with the entire factor except Total length that showed non-significant correlation as shown in Table 3 and Figure 1.

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 all the related co study factors. To test the assumption, lipase activity showed non-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 was showed non significance and only protease showed a highly signification correlation which may determine the nature of fish as shown in Table 3.

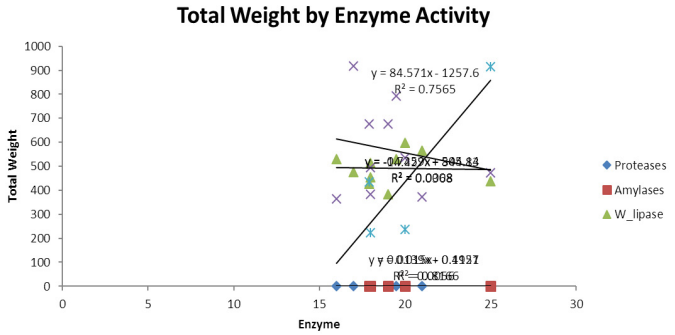

Figure 2: *Relationship of total weight with enzyme activity.*

With the help of principle component analysis, fish weight was found another important trait that would be investigated as dependent variable. Another

model was designed with multiple variability which explained effect of GW, GL, Condition factor and ZI and lipase activity on weight of fish. These variables were selected on the basis of multicollinearity and principle component analysis interpretations. Model parameters by drawing a scatter graph with body weight and total length of fish along with enzyme activity showed in Figures 2 and 3.

Table 3: *Model summary of multiple linear regression of enzyme independent variables with morphometry (g) as dependent Y-variable.*

	Lipase activity	Protease activity	Amylase activity
	P- value	p- value	p-value
Total weight	0.75 n.s	0.02***	0.002***
Total length	0.02***	0.94 n.s	0.77 n.s
Faulton's K	0.83 n.s	0.005***	0.04***
Zilhar's Index	0.03***	0.002***	0.29 n.s
Gut weight	0.50 n.s	0.001***	0.15 n.s
Gut length	0.15 n.s	0.019***	0.01***
Relative gut length	0.03***	0.002***	0.30 n.s
Relative gut mass	0.55 n.s	0.003***	0.15 n.s

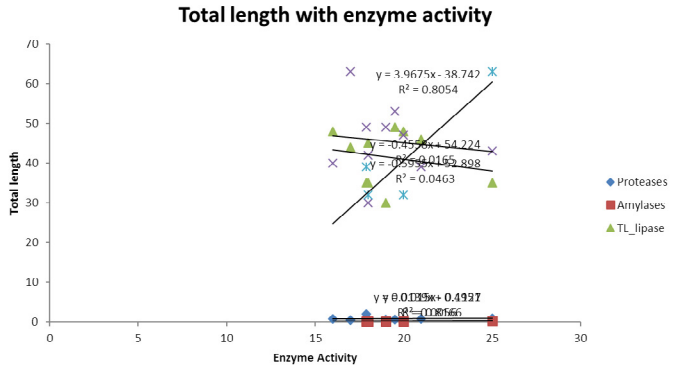

Figure 3: *Relationship of total length with enzyme activity.*

Present study is conducted to determine the activity of three digestive enzymes (i.e. amylase, protease and lipase) and their morphometric parameters (gut length, gut weight, relative gut length, relative gut mass, condition factor and Zihler's Index) of *Wallago attu.*

Carnivorous fish species show relatively higher protease activity in 1 feeding category and showed higher protease activity than others like herbivorous fishes (Chakrabarti et al., 1995; Drewe et al., 2004). Carnivorous species usually have higher proteineous requirements than others omni and herbivores (35 to 55% in case of carnivores and other less than 30 %) (Horn et al., 1995). Tilapia with thin wall of its stomach need more acidic media capable for protein digestion protein as compared to African catfish that rely more on mechanical breakdown of chime or food that results in less pepsin secretion (Uys and Hecht, 1987). Yu et al. (2002) found the maximum proteases activity in stomach of *Pseudobagrus, fulvidraco*, *Leiocassis longirostris* and *Silurus meridionalis* was at acidic media as 2.5, 2.6 and 3.0 respectively. It was generally acknowledged that carnivorous species had high protease activity than other fish species with variable nutritional diet (Chong et al., 2002). Present study also marked general agreement with study as the nature of species protease activity relatively higher than other enzymes.

The current study shows that higher protease activity in the gut, it has also showed that pepsin in stomach was significantly higher than the complete gut protease results in correspondingly higher than other carnivorous fish species with developed muscular stomach as *Boleophthalmus pectinirostris* and *Scophalmus maximus* (Fu et al., 2005; Wu et al., 2007). Along the gut of the carnivorous *G. maculatum*, the level of amylase was significantly in inner of gut than other parts. As relative tendency is also found in other fish species this is due to digestion of starch and absorption of glucose occurs mainly in this portion (Uys and Hecht, 1987; Lundstedt et al., 2004). Lower activity of amylase was in the stomach as by Uys and Hecht (1987) thought that amylase is in lower concentration because of contamination exogenous in intestinal portion. Munilla-Mora´n and Saborido-Rey (1996) studied that possibility of digestion of carbohydrate begin from stomach side of marine turbot due to amylase presence and activity starts there. In addition, moderate or lower amylase activity have been reported in many carnivorous species (Sabapathy and Teo, 1993; Munilla-Mora´n and Saborido-Rey, 1996). Present study justifies that the protease showed a signification relation with gut morphometrics and also found that in some cases amylase show signification because of digestion of starch and absorption of glucose in gut.

Several fish species despite of their diets showed ontogenetic rise in gut's length relation with its total length (Kramer and Bryant, 1995). Ontogenetic rises in fish gut length documented in the freshwater and marine fish species (Drewe et al., 2004). Carnivore species enhance their gut length with increased in fish

standard length (Kramer and Bryant, 1995). Present study can justify as in general agreement with this study.

Some previously studies report that feeding habits of various fishes, such as *G. aculeatus* could be deduced by studying their contents of stomach (Wotton, 1994). To the best of our knowledge, this is the first study on *Wallago attu* related to gut morphometrics that evaluated digestive enzymes with reported literature behalf of their species feeding habits. Gut morphometric parameters included first time in relation to *Wallago attu* morphology as Relative gut mass, length and Zihler's Index are basically explored as potential indices to identify habits (German and Horn, 2006).

Relative gut mass is also known as digestive somatic index, that indicates the fish feeding condition; species which utilize active diet have heavier guts as compared to starved (Lloret and Planes, 2003). Relative gut mass is characteristically utilizing to evaluate the tissue quantity (German and Horn, 2006). Relative gut mass in *Wallago attu* showed positive correlation with gut weight and length and values ranged between 0.25 to 0.31, because relative gust mass was also found to be increased with feed relation (Toloza and Diamond, 1990). Relative gut length and Zihler's Index are basic sightsee indices fish feeding natures rely on their gut length (German and Horn, 2006). As feeding nature categorized fishes as carni, omni or herbivorous species (Al-Hussaini, 1949). Current study on *Wllago attu* suggested that increasing in gut mass is technique used to enhance the energy intake from food components.

Kramer and Bryant (1995) investigated that Zihler's index value range characterized fishes with various masses as low body mass of body varies from 0.3 to 3.0g referred to as a carnivore. In the present study, Zihler's index was founded in range related to relative gut length range of carnivore fishes, and Zihler's index values of *Wallago attu* was between that which might be according to the feeding habits in wild due to which it is not palpable similar findings have been reported by Hani et al. (2018).

Conclusions and Recommendations

Present study mainly reported on overall activity of lipase, amylase and protease of *Wallago attu*. This study reveals the presence of major digestive enzymes and typical patterns of varying activity that predicted the primary study of digestive physiology of *Wallago attu*. Further research would be conceded to study more detail about their nutrition and digestion.

Novelty Statement

Present result will be helpful to predict the nature by mean of various enzymatic activities as confirmed in present study. Moreover to the best of our knowledge, this study is the first to characterize the digestive activity of *Wallago attu* by focusing on three digestive enzymes and gut morphometric parameters.

Author's Contribution

Parsa Riaz conducted the experiment and lab work, collected and analyzed data and wrote the manuscript. Muhammad Naeem supervised the research work, made available the necessary circumstances for the completion of experiment and assisted in the manuscript writing.

Conflict of interest

The authors have declared no conflict of interest.

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