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ORIGINAL ARTICLE

Biochar Conditioned Feeds Related to Enzyme Activity in an Economically Important Carp *Labeo Bata* of Tripura, India

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Abstract:
Labeo bata is considered an important fish due to its preference as delicious protein food to the peoples. For promoting fast growth of this species under commercial farm culture knowledge of digestive enzyme activities related to effective feed is important. As efficient supplementary diet biochar conditioned feed is used. Quality of supplementary diet is identified with proximate composition of biochar feed showing occurrence of rich nutrients in the feeds.
Amylase and trypsin activities showing significant difference of value between the experiments done with biochar conditioned feeds with a comparison to other. Enzyme activities during starvation period have significant variations from that of a period after 8 hrs of feeding to *Labeo bata*. Regression analysis between the metabolic faecal nitrogen (g N/100 g dried biochar feed) and biochar feed nitrogen (%) exhibiting a significant correlation (R^2= 0.9088).
Key words: Biochar feed, enzyme, *Labeo bata*, nutrients, digestibility coefficient,

Introduction

Labeo bata plays significant role in the ecological food chain. In freshwater lentic habitat this species acts as an important nekton fauna. To the human population this fish species is also important because of its consideration as largely digestible animal protein. Knowledge of digestive enzyme activities and selection of nutritious feed are important to support the growth acceleration of this fish species. Literature reveals that the information of enhancing growth rate of *Labeo bata* is prerequisite for farm culture to meet its market demand for human population (Andaloro 1982, Einarsson *et al.* 1996, Chou *et al.* 2001).

So, for efficient growth rate of a fish species understanding of digestive enzyme activities is important and further selection of feed quality is also important. On the other efficient quality of diet is most important for optimum growth of a fish species (Reimer 1982, Ugolev and Kuz'mina 1994, Hidalgo *et al.* 1999, Lundstedt *et al.* 2002). Thus, biochemical information of digestive enzymes of the carps are significant the knowledge of which are poor on the indigenous carps (Onishi *et al.* 1976, Jonas *et al.* 1983). Literature explicitly reveals that biochemical analysis of freshwater *Labeo gonius* is poor (Alabaster and Lloyd 1980, Appel *et al.* 1974, Ash 1988, Alarcón *et al.* 1998, Takii *et al.* 1985, Munilla-Morán *et al.* 1990, Munilla-Morán and Saborido-Rey 1996a, b, Caruso *et al.* 1992, 1993a, b, 1995, 1996, 1999, 2003, 2004, 2005, 2006, 2008, 2009, Caruso and Genovese 2000).

Digestive enzyme activities of any fish species varies due to the variations of both food and feeding habit (Kitamikado and Tachino 1960a, b. c, Shimeno and Ikeda 1967, Shimeno *et al.* 1979, Chakrabarti *et al.* 1995 and Furné *et al.* 2005). A look into the existing literature explicitly reveals that knowledge of digestive enzyme activity of freshwater carp is poor (Berger and Broida 1977, Buddington *et al.* 1997, Baglole *et al.* 1998, Cahill 1990, Faranda *et al.* 1983, Fernández *et al.* 2001, Fal'ge and Fountoulaki *et al.* 2005, Deguara

et al. 2003, Einarsson *et al.* 1996, Govoni *et al.* 1986, Greco *et al.* 1989, Hofer 1979 a, b, Halver 1989, Hofer and Schiemer 1981, Hofer and Köck 1989, LS 1980, Kunitz 1947, Kolkovski 2001, Kofuji *et al.* 2005, MacDonald *et al.* 1986, Moriarty 1990, Micale and Genovese 1998, Micale *et al.* 2006, Phillips 1969, Papandroulakis *et al.* 2001, Rosch *et al.* 1989, Ribeiro *et al.* 1999, Ribeiro *et al.* 2008, Sachar *et al.* 1955, 1998, Smith 1980, Segner *et al.* 1989, Sarasquete *et al.* 1993, Seixa *et al.* 1999, Sera *et al.* 1974, Shimeno and Takeda 1985, Spedicato *et al.* 1998, Spannhof 1976, Stevens and Hume 1998, Tengjaroenkul *et al.* 2000, Tietz and Fiereck 1966, Tramati 2005, Tortonese 1975, Uys and Hecht 1987, Uys *et al.* 1987, Walter 1984, Zambonino and Cahu 2007, Zhou *et al.* 2011).

Present paper communicates analysis of digestive enzyme activities of *Labeo bata* related to biochar conditioned feeds.

Materials and Methods

Fish Sampling

Assay on the digestive enzyme activity and digestibility coefficient (%) analysis are done with *Labeo bata* for which the fish sample is being chosen during adult stage. So, the adult fish samples have been sampled in live with drag net and cast net sampler during a very early morning period at about 04.00-05.00 hrs from the littoral zone of Feni river ecosystem of South Tripura district, India (Latitude $23^0$00'02" N and Longitude $91^0$51'11"E). The studied fauna has been identified with meristic analysis (Jayaram 1999) (fig.1).

After collections from Feni river the live fish samples are being carried through a dissolved oxygenated perforated container with river water. After brought into the laboratory the fish samples of *Labeo bata* have been reared into a pre-stocking culture medium for acclimatisation for about 06-08 days period.

To know the ambient physico-chemical characters of the littoral zone of the studied river the water samples of Feni river ecosystem of South Tripura are also being collected during early morning period. The water samples are being analysed in the laboratory following the literature of APHA (2000). Among different water quality parameters of the studied river some particular parameters such as water velocity, water temperature, pH, CO_2 and DO_2 have been analysed in the field while other parameters like DOM, CO_3 and HCO_3 have been analysed in the laboratory (table 1).

Biochar Controlled Feeds

To perform the analysis of digestive enzyme activities with a carp species *Labeo bata* biochar controlled feeds are being used as major supplementary diets which are fed to the studied fish species in the present experiments. For this purpose the feeds are being collected from a village of Gomoti district of Tripura.

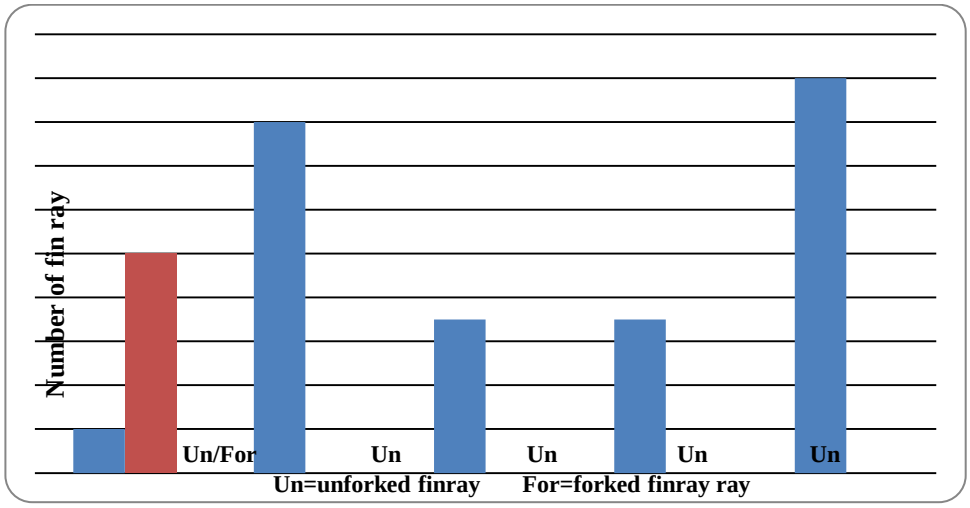

Fig. 1: Meristic character (fin formula) of *Labeo bata*

Table 1: Physico-chemical characters of Feni river ecosystem of Tripura

Parameters	Range	
water velocity	1.16 -3.12	m/sec,
water temperature	26.00 -32.00	^{0}C
pH	7.1 -7.3	
CO_2	3.23 -6.46	ppm
DO_2	4.08 -5.1	ppm
DOM	1.6 -6.62	ppm
CO_3	28.00 -38.00	ppm
HCO_3	156.00 -164.00	ppm

Analysis of Diets and the Digestive Enzymes

Proximate composition of biochar conditioned feeds is being analysed consulting the literature of Halver (1989). For assay of the activities of amylase, pepsin, and trypsin the literature of Hepher (1988) is being consulted.

Results and Discussion

To assay the digestive enzyme activities of adult stage of *Labeo bata* biochar conditioned feeds have been used. To know the effectiveness of biochar conditioned feeds proximate analysis of their nutrient composition (figs 2-4) are determined and compared to that of usual feeds variety (figs. 2a-4a). Proximate composition of biochar conditioned feeds showing relatively superior to that of usual feeds variety (figs. 2a-4a). Among biochar conditioned feeds 'Biochar conditioned Rice powder' has been a non-protein rich feed consisting of relatively greater amount of carbohydrate (r=0.9986, P<0.01) (fig. 2). Whereas 'Biochar conditioned chlorella' and 'Mustard oil cake' (figs. 3a-4a) respectively have been rich with protein (r=0.9844, P< 0.01).

Experiments during starvation period of *Labeo bata* (tables 2 and 4) showing variations in the activities of different digestive enzymes in the area of stomach, pyloric caeca and intestine as well. Values of enzyme related to feeding biochar conditioned feeds have

been compared between starvation period and a period after 8 hrs of feeding to the fish sample (tables 4-5). Examination of the digestive tract of *Labeo bata* depicting a significant difference of values of amylase, pepsin and trypsin (r=0.9882, P<0.001) among the experiments (tables 2-5). Several workers during their studies (Bondi and Spandorf 1953, Banik 2020, 2021a, 2021b) also noticed similar observations.

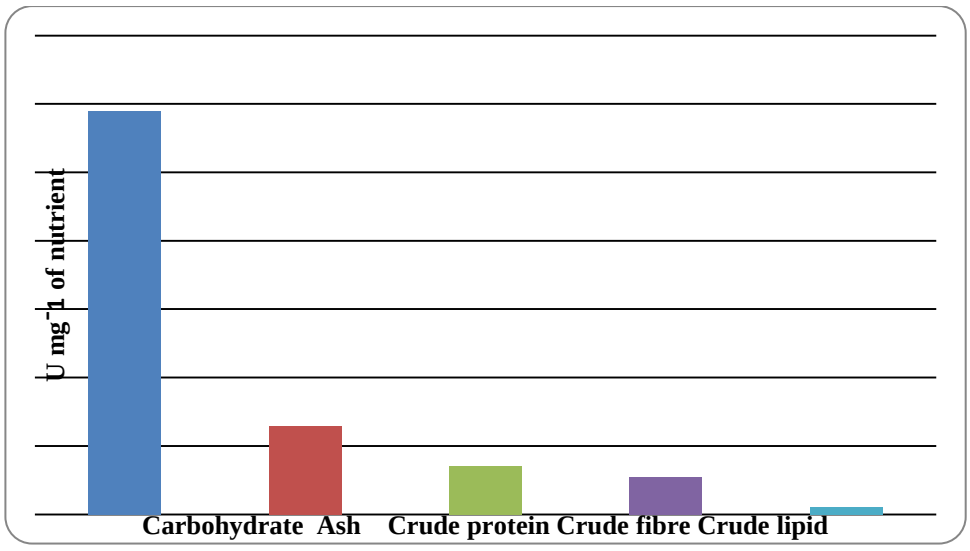

Figure 2: Proximate composition (%) of Biochar conditioned rice powder

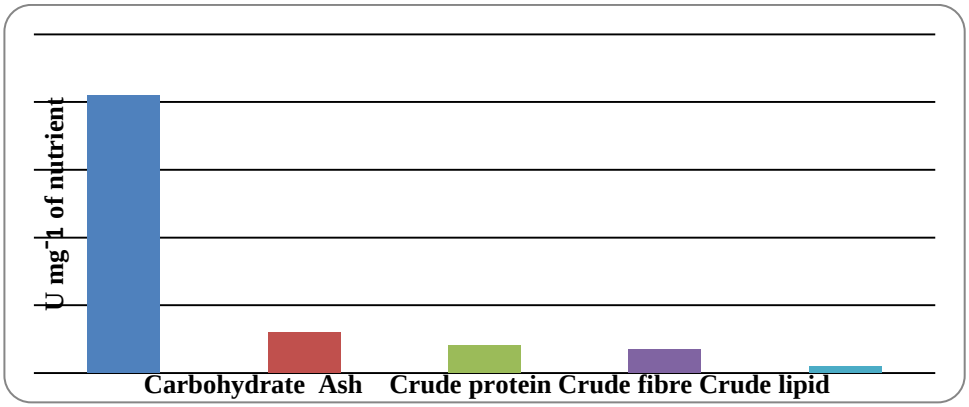

Figure 2a: Proximate composition (%) of usual rice powder

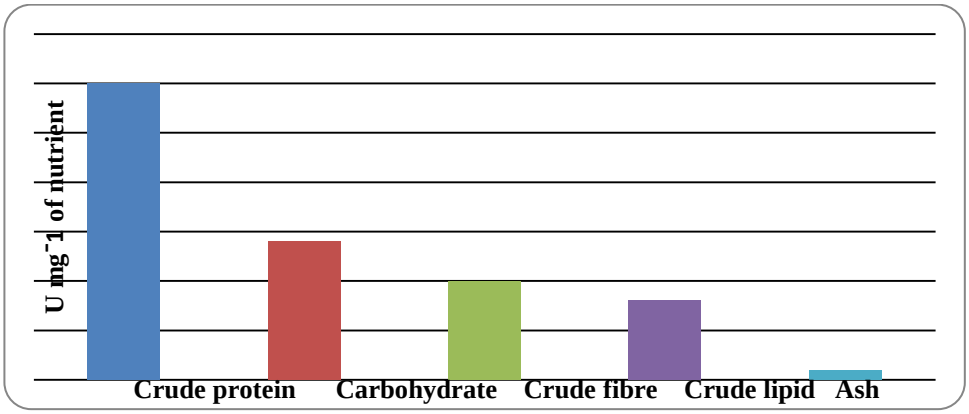

Figure 3: Proximate composition (%) of Biochar conditioned Mustard oil cake

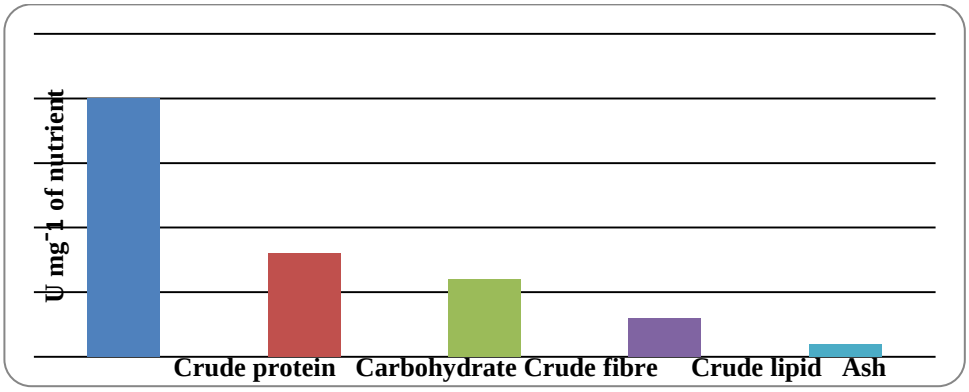

Figure 3a: Proximate composition (%) of usual Mustard oil cake

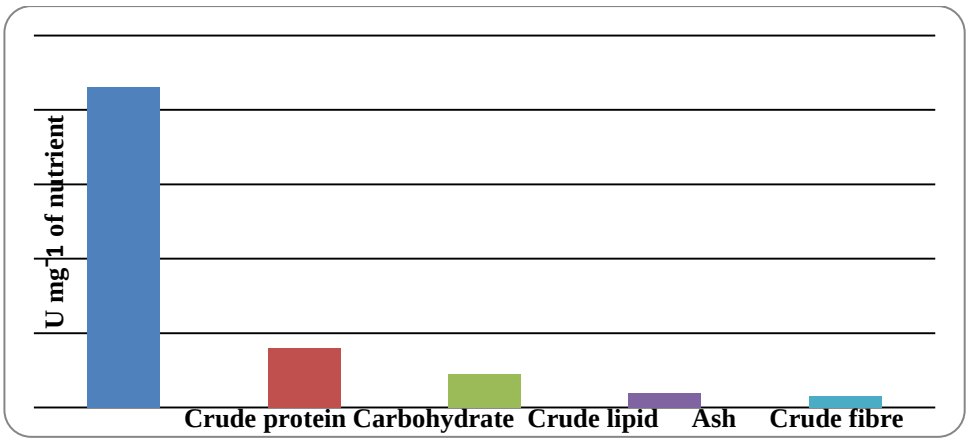

Figure 4: Proximate composition (%) of Biochar conditioned Chlorella

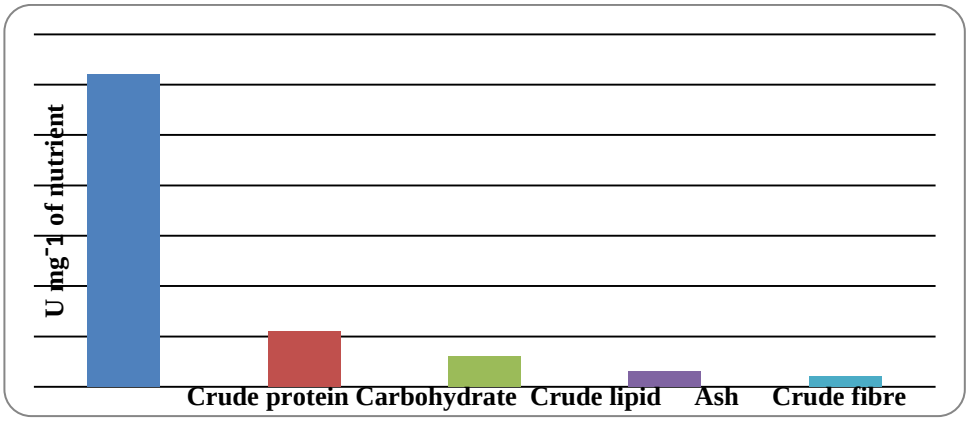

Figure 4a: Proximate composition (%) of usual Chlorella

Table 2: Digestive enzyme activity (in activity unit/ 100 g fish body weight) during adult stage of *Labeo bata* when fed usual feeds.

.....during starvation period....				
Name of the feed	**Enzyme**	**Stomach**	**Pyloric caeca**	**Intestine**
Usual Rice powder	Amylase	0.30±0.04	0.35±0.03	0.41±0.02
	Pepsin	0.01±0.02	0.0	0.0
	Trypsin	0.04±0.01	0.0	0.0
Usual Chlorella	Amylase	0.34±0.04	0.44±0.04	0.31±0.04
	Pepsin	0.14±0.04	0.0	0.0
	Trypsin	0.04±0.02	0.01±0.01	0.01±0.01
Usual Mustard Oil cake	Amylase	0.04±0.02	0.11±0.04	0.11±0.04
	Pepsin	0.11±0.04	0.0	0.0
	Trypsin	0.08±0.04	0.01±0.01	0.0
Control	Amylase	0.04±0.02	0.09±0.02	0.11±0.04
	Pepsin	0.08±0.01	0.04±0.04	0.04±0.04
	Trypsin	0.02.00	0.0	0.0

Table 3: Digestive enzyme activity (in activity unit/ 100 g fish body weight) during adult stage of *Labeo bata* when fed usual feeds.

.....after 08 hrs of feeding in the digestive tract.....				
Name of the feed	**Enzyme**	**Stomach**	**Pyloric caeca**	**Intestine**
Usual Rice powder	Amylase	0.33±0.02	0.37±0.04	0.43±0.04
	Pepsin	0.02±0.04	0.0	0.00
	Trypsin	0.08±0.01	0.04±0.02	0.08±0.04
Usual Chlorella	Amylase	0.36±0.02	0.48±0.02	0.33±0.02
	Pepsin	0.18±0.02	0.0	0.0
	Trypsin	0.07±0.02	0.04±0.02	0.14±0.02
Usual Mustard Oil cake	Amylase	0.06±0.04	0.16±0.02	0.09±0.02
	Pepsin	0.12±0.02	0.0	0.0
	Trypsin	0.1±0.02	0.06±0.02	0.14±0.02
Control	Amylase	0.03±0.02	0.1±0.04	0.14±0.02
	Pepsin	0.1±0.02	0.1±0.02	0.6±0.02
	Trypsin	0.02.00	0.01±0.02	0.0

Table 4: Digestive enzyme activity (in activity unit/ 100 g fish body weight) during adult stage of *Labeo bata* when fed Biochar conditioned feeds.

.....during starvation period...				
Name of the feed	**Enzyme**	**Stomach**	**Pyloric caeca**	**Intestine**
Usual Rice powder	Amylase	0.30±0.04	0.44±0.02	0.68±0.03
	Pepsin	0.09±0.02	0.0	0.0
	Trypsin	0.14±0.02	0.1±0.04	0.1±0.04
Usual Chlorella	Amylase	0.39±0.04	0.52±0.04	0.41±0.04
	Pepsin	0.12±0.04	0.0	0.0
	Trypsin	0.09±0.04	0.04±0.04	0.16±0.04
Usual Mustard Oil cake	Amylase	0.09±0.02	0.11±0.04	0.06±0.02
	Pepsin	0.14±0.04	0.0	0.0
	Trypsin	0.17±0.04	0.11±0.04	0.16±0.04
Control	Amylase	0.08±0.02	0.14±0.04	0.18±0.04
	Pepsin	0.11±0.02	0.10±0.02	0.9±0.04
	Trypsin	0.00	0.02±0.02	0.00

Table 5: Digestive enzyme activity (in activity unit/ 100 g fish body weight) during adult stage of *Labeo bata* when fed Biochar conditioned feeds.

.....after 08 hrs of feeding in the digestive tract.....				
Name of the feed	**Enzyme**	**Stomach**	**Pyloric caeca**	**Intestine**
Usual Rice powder	Amylase	0.32±0.02	0.53±0.03	0.72±0.04
	Pepsin	0.1±0.02	0.0	0.0
	Trypsin	0.16±0.04	0.11±0.02	0.11±0.02
Usual Chlorella	Amylase	0.42±0.03	0.54±0.02	0.46±0.02
	Pepsin	0.14±0.02	0.0	0.0
	Trypsin	0.11±0.02	0.01±0.02	0.19±0.02
Usual Mustard Oil cake	Amylase	0.12±0.04	0.15±0.02	0.08±0.03
	Pepsin	0.16±0.06	0.0	0.0
	Trypsin	0.22±0.02	0.21±0.02	0.18±0.02
Control	Amylase	0.09±0.04	0.15±0.02	0.19±0.02
	Pepsin	0.12±0.04	0.09±0.04	0.76±0.02
	Trypsin	0.0	0.04±0.02	0.0

An analysis on the values of enzymes after 08 hrs of feeding to the studied fish samples exhibiting variations in the values of amylase, pepsin and trypsin in the digestive tract of *Labeo bata* (tables 3 and 5). The non-protein diet (viz., biochar conditioned rice powder) showing relatively greater value of amylase in the intestine and somewhat less in the pyloric caeca. Protein-rich diet reflecting relatively low value of amylase activity in the intestine (tables 2-5).

Adult stage of *Labeo bata* exhibits relatively large value of pepsin and trypsin in the stomach area (tables 2-5). Hepher (1988) and Caruso *et al.* (2008, 2009) also noticed similar findings in their experiments while working with the activity of pepsin on some other fish species. While feeding non-protein feed the value of amylase has usually been increased significantly ($r=0.98$, $P<0.001$) from the pyloric caeca to the intestine during the assay of digestive enzyme activity. Similar studies have also been revealed during the experiments with teleost fish species (Hepher 1979, Lovell 1988, Banik 2020, Banik 2021a, b).

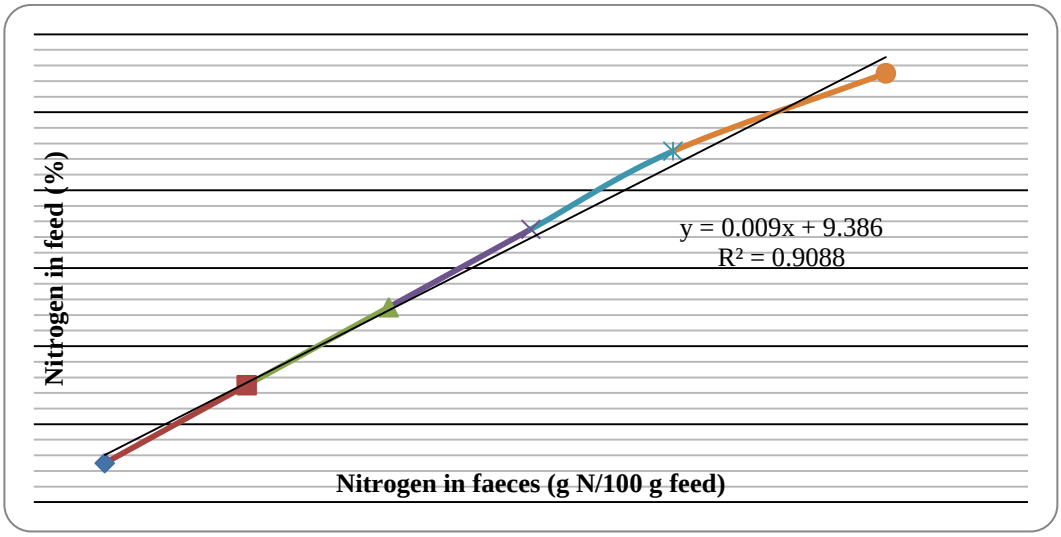

Fig. 5: Regression analysis between dietary nitrogen and faecal nitrogen in *Labeo bata*

Experiments with the controlled condition depicting a trend of increase in the values of amylase in the gut from the stomach to the intestine followed by pyloric caeca (tables 2-3). While comparing the values of table 3 it shows a relatively greater results than those of table 2. Several workers have also been noticed similar results with different fish species (Nose (1964, 1967, 1971). The biochar feeds provided to *Labeo bata* showing significant observations in the digestive enzyme activities. When similar nature of feeds used in their experiments with different fish species several workers (Bondi and Spandorf 1953, 1954 and Bondi *et al.* 1957) also observed similar findings. Zhou *et al.* (2011) pointed out that in the fish fauna the activity of chymotrypsin varies in relation to species, age and weight etc. In the present experiment activities of chymotrypsin remains in a poor or negligible concentration during 6 hrs after feeding the diet to *Labeo bata* (fig. 3).

Regression analysis (fig. 5) between the metabolic faecal nitrogen (g N/100 g dried biochar feed) and biochar feed (non-protein) nitrogen (%) showing a significant correlation (R^2= 0.9088). Ogino *et al.* (1973) and Kim (1974) also determined the metabolic faecal nitrogen in the feed and nitrogen excreted in the faecal matter per 100 g feed ingested by the studied fish sample.

Table 6: Apparent protein digestibility coefficient during adult stage of *Labeo bata.*

Protein (%) in Biochar feed	Metabolic protein (g)	Digestible protein (g)	Apparent digestibility coefficient (%)
10	3.8	6.20	6.20 x 100/10= 62.00
20	3.8	14.11	14.11 x 100/20= 70.55
40	3.8	32.21	32.21 x 100/40= 80.52

Table 7: Apparent protein digestibility coefficient during adult stage of *Labeo bata.*

Protein (%) in Biochar feed	Metabolic protein (g)	Digestible protein (g)	Apparent digestibility coefficient (%)
60	3.8	52.82	52.82 x 100/60= 88.03
80	3.8	73.64	73.64 x 100/80= 92.05
100	3.8	98.26	98.26 x 100/100= 98.26

The present results showing apparent digestibility coefficient of protein (tables 6-7) in *Labeo bata* with usual feed and biochar conditioned feed as well. The values of protein percentage are relatively greater in biochar conditioned feeds than that of usual feed. It exhibits from the study (table 7) that the apparent digestibility coefficient (%) of protein increases when protein content is accelerated in biochar conditioned feeds. Contrary to that the protein content has been relatively low

in usual feed where apparent digestibility coefficient of protein is also relatively low (table 6). Some workers (Nose 1967, Hepher 1979) also noticed similar observations.

Multivariate regression analysis (table 8) showing a significant correlation of different biochar feeds used in the experiment (P<0.01) related to the population density of *Labeo bata* (table 8). 'R' (=0.9273) value exhibiting a significant level of multiple correlation coefficient. Coefficient of multiple determination (R^2=0.8597) further indicating that the regression is significant. ANOVA depicting a significant level of 'F' (=17.39) value suggests that the total regression analysis is significant (P<0.01).

Table 8: Multivariate regression analysis on the influence of biochar feeds and physico-chemical parameters of water on the population density of *Labeo bata*

Variables	Coefficient	±SE	F	Beta coefficient
Biochar rice	-9.822	0.3921	49.78	-8.9454*
Biochar mustard oil cake	-3.951	0.8341	52.44	-1.3141*
Biochar chlorella	9.7914	0.3755	50.67	10.1268*
HCO_3	-106.021	0.9933	5.80	-0.3308
DO_2	-2.885	2.2811	1.60	-0.1323
NO_3 N	-96.011	0.5574	1.33	-0.1122

Multiple correlation coefficient (R)= 0.9273

Coefficient of multiple determination (R^2)=0.8597

Variables not in regression equation:

Variables	Partial correlation	Tolerance
Water temperature	0.017	0.650
pH	0.236	0.566
CO_2	0.108	0.638
CO_3	0.149	0.354
DOM	0.095	0.801
PO_4 P	0.078	0.696

Analysis of variance table:

Source of variation	Degree of freedom	Sum of SQ	Mean SQ	F	Conf level
Regression	6	45541.610	9090.18	17.39	100.00*
Residual	17	8888.27	522.84		
Total	23				

* (P<0.01)

Ethical Aspect

In freshwater ecosystem occurrence and abundance of natural food biota specific to *Labeo bata* is too low. So, basic aim of the present work is to know how growth of *Labeo bata* can be promoted with biochar controlled feeds. In order to effectively use these feeds for efficient growth of the studied fish species experiments with starvation are being done to know fish biology related to digestive enzyme activity. Therefore, the work has no conflict with ethical issue.

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identify the fish sample from the Feni river ecosystem of Tripura, India.

References

1. Alabaster, J. S. and Lloyd, R. 1980. Water quality criteria for freshwater fish. Butterworths, London, 297 pages,
2. Alarcón FJ, Díaz M, Moyano FJ, Abellán E. 1998.Characterization and functional properties of digestive proteases in two sparids; gilthead seabream (*Sparus aurata*) and common dentex (*Dentex dentex*). Fish Physiol Biochem; 19: 257-267.
3. Andaloro F. 1982. About the catch, the diet, the reproduction, the size frequencies and distribution of *Pagellus acarne* (Risso, 1826) in the Strait of Messina area. Rapp Proc Réun Comm Int Expl Mer Médit; 23: 33-37.
4. Anson ML 1938. The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. J Gen Physiol ; 22: 79-89.
5. Ash R. 1988. Protein digestion and absorption. In: Cowey CB, Mackie AM, Bell JG, Ed. Nutrition and feeding in fish. Academic Press; 69-94.
6. APHA. 2000. Standard methods for the examination of water and waste water. American Public Health Association, Philadelphia, reprint edition,
7. Appel W. 1974. Carboxypeptidases. In: Bergmeyer HU, Ed. Methods of Enzymatic analysis. Academic Press; 996-997.
8. Baglole CJ, Goff GP, Wright GM. 1998. Distribution and ontogeny of digestive enzymes in larval yellowtail and winter flounder. J Fish Biol; 53: 767-784.
9. Banik, R. 2020. Biochemical characters of some herbivore, omnivore and carnivorous fish species of Tripura. India. Unpublished data from MSc dissertation, Department of Molecular Biology & Bioinformatics, Tripura University,
10. Banik, R. 2021a. Some aspects of biochemistry of a near threatened fish *Chitala chitala* of Tripura. India. Unpublished data from MSc dissertation, Department of Molecular Biology & Bioinformatics, Tripura University,
11. Banik, R. 2021b. Biochemistry of an endangered fish *Ompok bimaculatus* of Tripura, India related to proximate analysis of Pizum rice powder, Chlorella and Mohua oil cake. Unpublished data from MSc dissertation, Department of Molecular Biology & Bioinformatics, Tripura University,
12. Banik, R. 2023. Amylase and trypsin activities in an economically important fish *Labeo calbasu* of Tripura, India. Current Science CS 3 (4): 371-378
13. Banik, R. 2024. Biochar conditioned feeds related to digestive enzyme activities of an economically important carp *Labeo gonius* of Feni river ecosystem of Tripura, India. Current Science (accepted),
14. Berger L & Broida D. 1977. The quantitative colorimetric determination in serum and uring of leucine aminopeptidase (LAP). Sigma Technical Bulletin 251, Sigma Chemical Co., St. Louis, Missouri,
15. Bernfeld P. 1955. Amylase alpha and beta. In: Colowich SP, Ed. Methods in Enzymology. Academic Press 149-150.
16. Bondi, A. and Spandorf, A. 1953. The activity of digestive enzymes of the carp. *In Hebrew:* Bamidgeh 5 (7-8): 116-130.
17. Bondi, A. and Spandorf, A. 1954. The action of the digestive enzymes of the carp. Br. J. Nutr. 8: 240-246.
18. Bondi, A., Spandorf, A. and Calmi, R. 1957. The nutritive value of various feeds for carp. Bamidgeh 9 (1): 13-18.
19. Buddington RK, Krogdahl A, Bakke-Mckellep AM. 1997. The intestine of carnivorous fish: structure and functions and the relations with diet. Acta Physiol Scandin ; 161: 67-80.
20. Cahill MJ. 1990. Bacterial flora of fishes: a review. Microb Ecol; 19: 21-41.
21. Caruso G, Genovese L, Greco S. 1993. Effect of two diets on the enzymatic activity of *Pagellus acarne* (Brunnich 1768) in intensive rearing. Eur Aquaculture Soc Special Publication; 19: 332.
22. Caruso G, Genovese L, Greco S. 1996.Preliminary investigation on the digestive enzymes of reared *Pagellus acarne* (Risso 1826) juveniles in relation to two different diets. Oebalia; 22: 3-13.
23. Caruso G, Genovese L, Maimone G, Manganaro A, Mancuso M, Palmegiano GB. 2003. Enzimi digestivi in *Pagellus bogaraveo*: tre diete sperimentali a confronto. Biol Mar Medit: 10: 430-433.
24. Caruso G, Costanzo MT, Palmegiano GB, Gai F, Genovese L. 2005. Blackspot sea bream

(*Pagellus bogaraveo*) fed on rice protein concentrate meal: effect on digestive enzymes. Eur Aquaculture Soc Spec Publ; 35: 158-159.
25. Caruso G, Genovese L. 1993. Attivita' enzimatica nell'apparato digerente di *Seriola dumerilii* (Risso 1810) in allevamento intensivo. Biol Mar Medit; 1: 257-258.
26. Caruso G, Genovese L, Monticelli L. 1999. Observations on the enzymatic activities in the digestive tract of some *Pagellus bogaraveo* (Brunnich 1768) specimens in intensive rearing. Oebalia; XXV: 31-42.
27. Caruso G, Genovese L, Micale V, Spedicato MT, Mancuso M. 2001. Preliminary investigation of the digestive enzymes in *Pagellus erythrinus* (Linneo 1758) larvae. Mar Freshwater Behav Physiol; 34: 265-268.
28. Caruso G, Genovese L 2000. Osservazioni sulla distribuzione degli enzimi digestivi in differenti specie di Sparidi. Biol Mar Medit 2000; 7:621-623.
29. Caruso G, Denaro MG, Genovese L. 2008.Temporal changes in digestive enzyme activities in the gastrointestinal tract of European eel (*Anguilla anguilla*) (Linneo 1758) following feeding. Mar Freshwater Behav Physiol. 41: 215-228.
30. Caruso, G, Denaro MG and Genovese L. 2009. Digestive Enzymes in Some Teleost Species of Interest for Mediterranean Aquaculture. The Open Fish Science Journal, 2009, 2, 74-86
31. Chakrabarti I, Gani Md A, Chaki KK, Sur R, Misra KK. 1995. Digestive enzymes in 11 freshwater teleost fish species in relation to food habit and niche segregation. Comp Biochem Physiol; 112A: 167-177.
32. Chou R-L, Su M-S, Chen H-Y. 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture; 193: 81-89.
33. Deguara S, Jauncey K, Agius C. 2003. Enzyme activities and pH variations in the digestive tract of gilthead sea bream. J Fish Biol; 62: 1033-1043.
34. Einarsson S, Davies PS, Talbot C. 1996. The effect of feeding on the secretion of pepsin, trypsin and chymotrypsin in the Atlantic salmon, *Salmo salar* L. Fish Physiol Biochem; 15: 439-446.
35. Faranda F, Cavaliere A, Lo Paro G, Manganaro A. 1983. Accrescimento di *Pagellus acarne* alimentati con due diverse diete. Mem Biol Mar Oceanogr; XII: 55-63.
36. Fal'ge R, Spannhof L. 1976. Amylase, esterase and protease activity in the gut contents of the rainbow trout *Salmo gairdneri* after feeding. J Ichthyol; 16: 672-677.
37. Fernández I, Moyano FJ, Díaz M, Martínez T. 2001. Characterization of alpha-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J Exp Mar Biol Ecol; 262: 1-12.
38. Fountoulaki E, Alexis MN, 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.
39. Furné M, Hidalgo MC, López A, García-Gallego M, Morales AE, Domezain A, Domezainé J, Sanz A. 2005. Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquaculture ; 250: 391- 398.
40. Genovese L, Patti F, Caruso G. 1995. Studies of the digestive cycle in the amberjack (*Seriola dumerilii*) (Risso 1810) in intensive rearing. Oebalia; 21: 5-15.
41. Genovese L, Patti F, Caruso G. 1992. Intestinal microflora and native enzymes in specimens of *Seriola dumerilii* (Risso 1810) in intensive rearing: preliminary studies. In Szabò J, Ed. Proceedings of the 2nd International Colloquium Microbiology of Poikilotherms; June; 15-17, Budapest, Hungary.
42. Greco S, Cavaliere A, Lo Paro G, Manganaro A, Sturniolo G, Caridi F. 1989. Effetti di diete bilanciate nell'allevamento intensivo di *Pagellus bogaraveo* (Rovello). Oebalia; XV-2: 637-644.
43. Govoni JJ, Boehlert GW, Watanabe Y. 1986. The physiology of digestion in fish larvae. Environ Biol Fishes; 16: 59-77.
44. Halver JE. 1989. Fish nutrition, 2nd ed. Academic Press: San Diego 1989.
45. Hepher, B. 1979. Supplementary diets and related problems in fish culture. *In:* S.D. Gerking (ed.) Ecology of freshwater fish production, Blackwell Scientific Publishing, Oxford, pages 447-468,

46. Hepher, B. (ed). 1988. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, 388 pages,
47. Hidalgo MC, Urea E, Sanz A. 1999. Comparative study of digestive enzymes in fish with different nutritional habitus. Proteolytic and amylase activities. Aquaculture 1999; 170: 267-83.
48. Hofer R. 1979a. The adaptation of digestive enzymes to temperature, season and diet in roach, *Rutilus rutilus* L., and rudd, *Scardinius erythrophthalmus* L. 1. Amylase. J Fish Biol; 14: 565-572.
49. Hofer R. 1979b. The adaptation of digestive enzymes to temperature, season and diet in roach, *Rutilus rutilus* L., and rudd, *Scardinius erythrophthalmus* L. Proteases. J Fish Biol; 15: 373-379.
50. Hofer R, Schiemer F. 1981. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia (Berl.); 48: 342-345.
51. Hofer R, Köck G. 1989. Method for quantitative determination of digestive enzymes in fish larvae. Polskie Arch Hydrobiol; 36: 439- 441.
52. Hummel BCW. 1959. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Can J Biochem Physiol; 37: 1393-1399.
53. Jayaram, K. C. 1999. The freshwater fishes of Indian Region. Narendra Publishing House, Delhi, 551 pages,
54. Jonas E, Ragyansszki M, Olah J, Boross L. 1983. Proteolytic digestive enzymes of carnivorous (*Silurus glanis* L.), herbivorous (*Hypophthalmiichthys molitrix* Val.) and omnivorous (*Cyprinus carpio* L.) fishes. Aquaculture; 30: 145-154.
55. Kim, Y. K. 1974. Determination of true digestibility of dietary proteins in carp with chromic oxide containing diet. Bull. Jap. Soc. Sci. Fish 40:651-653
56. Kitamikado, M. and Tachino, S. 1960a. Studies on the digestive enzymes of rainbow trout. I. Carbohydrates. Bulletin of Japanese Society of Science Fish 26: 679-684.
57. Kitamikado, M. and Tachino, S. 1960b. Studies on the digestive enzymes of rainbow trout. II. Proteases. Bulletin of Japanese Society of Science Fish 26: 685-690.
58. Kitamikado, M. and Tachino, S. 1960c. Studies on the digestive enzymes of rainbow trout. III. Esterases. Bulletin of Japanese Society of Science Fish 26: 691-694.
59. Kofuji PYM, Akimoto A, Hosokawa H, Masumoto T. 2005. Seasonal changes in proteolytic enzymes of yellowtail *Seriola quinqueradiata* (Temminck & Schlegel; Carangidae) fed extruded diets containing different protein and energy levels. Aquaculture Res; 36: 696-703.
60. Kolkovski S. 2001. Digestive enzymes in fish larvae and juveniles- implications and applications to formulated diets. Aquaculture; 200: 181-201.
61. Kunitz M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. J Gen Physiol; 320: 291-310.
62. Lauff M, Hofer R. 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture; 37: 335-346.
63. Lovell T. (ed.) 1988. Nutrition and feeding of Fish. Chapman & Hall: New York
64. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem; 193: 265-275.
65. Lundstedt LM, Melo JFB, Santos Neto C, Moraes G. 2002. Diet influences proteolytic enzyme profile of the South American catfish *Rhamdia quelen.* In: International Congress on the Biology of Fish, Biochemical and Physiological Advances in Finfish Aquaculture. Vancouver, Canada,
66. MacDonald NL, Stark JR, Austin B. 1986. Bacterial microflora in the gastro-intestinal tract of Dover sole (*Solea solea* L.), with emphasis on the possible role of bacteria in the nutrition of the host. FEMS Microbiol Lett; 35: 107-111.
67. Moriarty DJW 1990. Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. In: Lésel R, Ed. Microbiology in Poecilotherms. Elsevier Science Publishers 1990; 217-222.
68. Micale V & Genovese L. 1998. Supporto della ricerca allo sviluppo di tecniche di acquacoltura per specie innovative: *Pagellus erythrinus,Pagellus bogaraveo, Seriola dumerilii*. Biol Mar Medit; 5: 1192-1199.
69. Micale V, Caruso G, Garaffo M, Genovese L, Spedicato MT, Muglia U. 2006.

Morphological development and enzyme activities of the digestive tract in larval pandora, *Pagellus erythrinus* L. Italian J Anat Embryol; 111: 109.
70. Munilla-Morán R, Stark JR, Barbour A. 1990. The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). Aquaculture; 88: 337-350.
71. Munilla-Morán 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*). Comp Biochem Physiol; 113B: 395-402.
72. Munilla-Morán R, Saborido-Rey F. 1996. Digestive enzymes in marine species. II. Amylase activities in gut from seabream (*Sparus aurata*), turbot (*Scophthalmus maximus*) and redfish (*Sebastes mentella*). Comp Biochem Physiol; 113B: 827-834.
73. Nose, T. 1964. Protein digestibility of several test diets in cray and prawn fish. Bulletin of Freshwater Fish Res. Lab., Tokyo 14:23-28
74. Nose, T. 1967. Recent advances in the study of fish digestion in Japan. FAO/EIFAC Technical paper 3:83-94
75. Nose, T. 1971. Determination of nutritive value of food protein in fish. III. Nutrtive value of casein, whitefish meal and soybean meal in rainbow trout fingerlings. Bulletin of Freshwater Fish Res. Lab., Tokyo 21: 85-98.
76. Ogino, C., Kakino, J. and Chen, M. S. 1973. Protein nutrition in fish. II. Determination of metabolic faecal nitrogen and endogenous nitrogen excretions of carp. Bull. Jap. Soc. Sci. Fish 39: 519-523.
77. Onishi T, Murayama S, Takeuchi M. 1976. Changes in digestive enzyme levels in carp after feeding –III. Response of protease and amylase of twice-a-day feeding. Bull J Soc Sci Fish; 42: 921-929.
78. Papandroulakis N, Divanach P, Anastasiadis P, Kentouri M. 2001. The pseudo-green water technique for intensive rearing of sea bream (*Sparus aurata*) larvae. Aquaculture Int; 9: 205-216.
79. Phillips AM Jr. 1969. Nutrition, digestion and energy utilization. In: Hoar WS, Randall DJ, Ed. Fish Physiology. Academic Press: London 1969; pp. 391-432.
80. Reimer G. 1982. The influence of diet on the digestive enzymes of the amazon fish Matrincha, *Bricon* cf. *melanopterus*. J Fish Biol; 21: 637-42.
81. Ribeiro L, Zambonino-Infante JL, Cahu C, Dinis MT. 1999. Development of digestive enzymes in larvae of *Solea senegalensis* (Kaup 1858). Aquaculture 1999; 179: 465-473.
82. Ribeiro L, Couto A, Olmedo M, Alvarez-Blazquez B, Linares F, Valente LMP 2008. Digestive enzyme activity at different developmental stages of blackspot seabream, *Pagellus bogaraveo* (Brunnich 1768). Aquaculture Res; 39: 339-346.
83. Savona B. 1998. Variabilità nei processi enzimatici della digestione in *Diplodus puntazzo* (Pisces, Sparidae) a diverse condizioni di allevamento. PhD Dissertation, University of Palermo; 1-72.
84. Sachar LA, Winter KK, Sicher N, Frankel S. 1955. Photometric method for estimation of elastase activity: Proceedings of the Society for Experimental Biology and Medicine, 90: 323-326.
85. Sarasquete MC, Polo A, Gonzales De Canales ML. 1993. A histochemical and immunochemical study of digestive enzymes and hormones during larval development of the sea bream, *Sparus aurata* L. The Histochem. J; 25: 430-437.
86. Seixa Filho JT, Oliveira MGA, Donzele JL, Gomide ATM, Menin E. 1999. Atividade de amilase em quimo de três espécies de peixes Telostei de áqua doce. Rev Brasil Zootecnia; 28: 907-913.
87. Sera H, Ishida Y, Kadota M. 1974. Bacterial flora in the digestive tracts of marine fish. In: Colwell RR, Morita RY, Ed. Effect of the ocean environment on microbial activities. University Park Press; 467-490.
88. Segner H, Rosch R, Schmidt H, 1989. Von Poeppinghausen KJ. Digestive enzymes in larval *Coregonus lavaretus* L. J Fish Biol; 35: 249-263.
89. Shimeno, S. and Ikeda, S. 1967. Studies on glucose-6-phosphatase of aquatic animals. II. The enzyme activities in fish tissues. Bulletin of Japanese Society of Science Fish 33: 112-116.
90. Shimeno, S., Hosokawa, H. and Takeda, M.1979. The importance of carbohydrate in

the diet of carnivorous fish. In: J. E. Halver and K Tiews (eds). Finfish nutrition and fishfeed technology, Vol. I pp. 127-143, Heenemann Verlagsgesel publ, Berlin
91. Smith LS. 1980. Digestion in teleost fish. In: Lectures presented at the FAO/UNPD training course in fish feed technology. ADCP/REP/80/11; 3-17.
92. Spedicato MT, Contegiacomo M, Lembo G. 1998. Sistemi di produzione innovativi orientati alla maricoltura di nuove specie: *Pagellus erythrinus,Pagellus bogaraveo*. Biol Mar Medit 1998; 5: 1180-1185.
93. Stevens CE, Hume ID. 1998. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol Rev 1998; 78: 393-427.
94. Takii K, Shimeno S, Takeda M. 1985. Changes in digestive enzyme activities in eel after feeding. Bull J Soc Sci Fish ; 51: 2027-2031.
95. Tengjaroenkul B, Smith BJ, Caceci R, Smith SA. 2000. Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia *Oreochromis niloticus* L. Aquaculture; 182: 317- 27.
96. Tietz NW, Fiereck EA. 1966. A specific method for serum lipase determination. Clin Chem Acta ; 13: 352-358.
97. Tortonese E, Ed. Fauna d'Italia. Osteichthyes (Pesci ossei). 1975. Vol. II. Bologna: Calderini.
98. Tramati C, Savona B, Mazzola A. 2005. A study of the pattern of digestive enzymes in *Diplodus puntazzo* (Cetti, 1777) (*Osteichthyes, Sparidae*): evidence for the definition of nutritional protocols. Aquaculture Int; 13: 89-95.
99. Ugolev AM, Kuz'mina VV. 1994. Fish enterocyte hydrolases. Nutrition adaptations. Comp Biochem Physiol 1994; 107A: 187-93.
100. Uys W, Hecht T, Walters M. 1987. Changes in digestive enzyme activities of *Clarias gariepinus* (Pisces: *Clariidae*) after feeding. Aquaculture; 63: 243-250.
101. Uys W, Hecht T. 1987. Assays on the digestive enzymes of sharptooth catfish, *Clarias gariepinus* (Pisces: *Clariidae*). Aquaculture ; 63: 301-313.
102. Walter HE. 1984. Proteinases: methods with hemoglobin, casein and azocoll as substrates. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis. Verlag Chemie; 270-277.
103. Zambonino Infante JL, Cahu CL. 2007. Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: applications to diet formulation. Aquaculture ; 268: 98- 105.
104. Zhou Liang, Budge Suzanne M., Ghaly Abdel E., Brooks Marianne S. and Deepika Dave. 2011. Extraction, Purification and Characterization of Fish Chymotrypsin: A Review. American Journal of Biochemistry and Biotechnology 7 (3): 104-123.