Current Science
CS 5 (6), 5583-5588 (2025)
Received 07 November 2025 | **Accepted 10 December** 2025 | **Online Available 25 December** 2025
https://doi.org/10.52845/CS/2025-5-6-23

Current Science
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OPEN ACCESS
ISSN (O) 2795-8639

Original Article

Digestive Enzymatic Activities of Amylase, Pepsin and Trypsin in an Economically Significant Carp *Labeo Calbasu* of Tripura, India

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Abstract:
To meet our daily need of highly digestible animal protein freshwater fish having greater demand in the market. So, *Labeo calbasu* is considered as one of such important fish species which is preferred to human population as delicious protein food. However, knowledge of growth of such fish species in commercial view point is poor. Hence, enhancement of growth rate of this fish species with regard to supplementary feed is important. For knowledge on digestive enzyme activities of this fish species different diets are being used. Assay on activities of amylase, pepsin and trypsin (determined in activity unit per 100 g fish body weight) is being done with fingerling stage of *Labeo calbasu* both during a period of starvation and at a period after about 08 hrs of feeding to the fish samples. During a period after about 08 hrs of feeding with non-protein rich feed (e.g, Swarno musuri rice powder) significant results are being noticed with amylase (0.43±0.04 in the stomach, 0.46±0.02 in pyloric caeca and 0.76±0.02 in the intestine), pepsin (0.03±0.02 in the stomach) and trypsin (0.05±0.04 in the stomach) as well. While protein rich-feeds (e.g., chlorella and mustard oil cake) could not show any significant effect.
Key words: *Labeo calbasu;* amylase; trypsin; pepsin; supplementary diet;

1. Introduction

Labeo calbasu is considered to be preferred fish to human population due to providing larger quantity of greatly digestible protein food and delicious taste. In view of promoting fish growth in aqua-farm culture system knowledge of digestive enzyme activities with regard to supplementary diet is important. So, in view of quality of fish diet understanding of digestive enzyme activities is important. To achieve optimum growth rate in a fish species suggestions of digestive enzyme activities are indeed important which in other way can identify a diet quality (Anson 1938, Ash 1988, Caruso *et al.* 1993, 1996, 1999). With regard to the growth of freshwater carps biochemistry of digestive enzyme activities related to the diet characters are significant (Caruso *et al.* 2001, 2009, Caruso and Genovese 2000).

The present work communicates analysis on the activities of amylase, pepsin, and trypsin during fingerling stage of the studied fish species *Labeo calbasu* with regard to providing supplementary diets.

Materials and Methods

Sampling fish Fauna

During the present observations certain fish population of a carp *Labeo calbasu* during fingerling stage has been chosen to assay the digestive enzyme activities. Fingerling stage of the live fish samples have been carried out with water filled plastic container saturated with 4.64-5.28 ppm of dissolved oxygen, 0.88-2.26 ppm of carbon-di-oxide and 0.64-1.82 ppm of dissolved

organic matter etc. After collections into the laboratory those fish samples of *Labeo calbasu* have been reared into a pre-stocking artificial aquatic tank for acclimatisation for a period of 3-5 days. To get a clear knowledge about the physico-chemical characters of the studied Feni river ecosystem of Tripura, India specific quantity of water samples have been collected for analysis and the analysis have been made following some standard literature (APHA 2000).

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

Parameters	**Range**	
Water temperature	29.00 -31.00	^{0}C
Water velocity	01.86 – 2.42	m/sec
Turbidity	02.28 -05.48	NTU
pH	06.6 -07.3	
CO_2	8.36 -12.66	ppm
DO_2	4.32 – 4.78	ppm
DOM	7.34 -14.36	ppm
CO_3	24.62 -28.64	ppm
HCO_3	132.0 -146.00	ppm
PO_4P	0.002 -0.026	ppm
NO_3N	0.001 -0.024	ppm

Analysis of Diets and the Digestive Enzymes

To assay the activities of enzyme such as amylase, pepsin, trypsin, and chymotrypsin the literature of Hepher (1979) is being followed. Analysis of proximate composition of the diets used in the present studies have been done following the literature of Hepher (1988).

Results and Discussion

Proximate composition of three different diets have been analysed to know their qualitative characters (figs 1-3) that helped to understand the results on the assay of the digestive enzyme activities using some fish population during fingerling stage of *Labeo calbasu*. Amongst different diets chosen 'Swarno musur Rice powder' has been a non-protein rich feed containing greater amount of amylase (r=0.99, P<0.01) (fig. 1). While protein-rich diet 'Chlorella' and 'Mohua oil cake' respectively have been rich with pepsin (r=0.98, P<0.01) (fig. 2) and trypsin (r=0.98, P< 0.01) (fig. 3) as well. Data of digestive enzyme activities observed in different areas of digestive tract of the fingerling stage of *Labeo calbasu* shows variation of values of amylase, pepsin and trypsin as well. Similar observations have also been noticed by various researchers with different fish species (Appel 1974, Baglole *et al.* 1998). Some workers (Caruso *et al.*1999) however, did not see any significant difference of values of the digestive enzymes with a comparison of starvation period from that of a period after 04 hrs of feeding. Contrary to the studies of some workers (Caruso *et al.*1999) it may be pointed out that the difference of time period during their experiment had only been 04 hours and presumably due to which it could not be possible to notice a significant difference of results (Banik 2022, 2023, 2024).

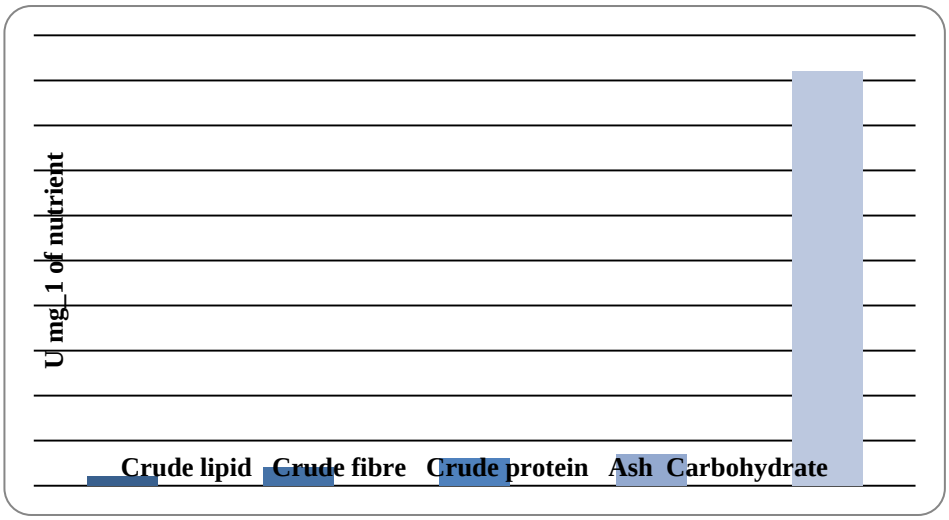

Fig. 1: Proximate composition (%) of Swarnomusuri rice powder

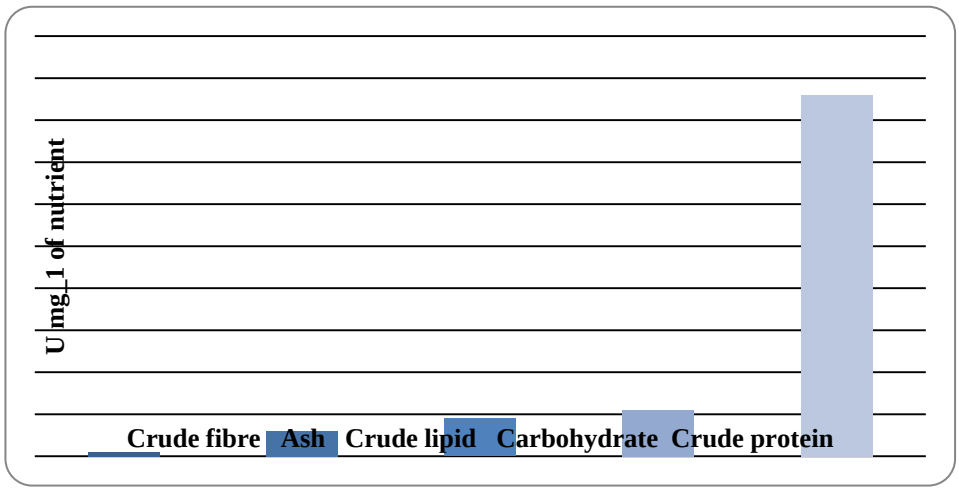

Fig. 2: Proximate composition (%) of Chlorella

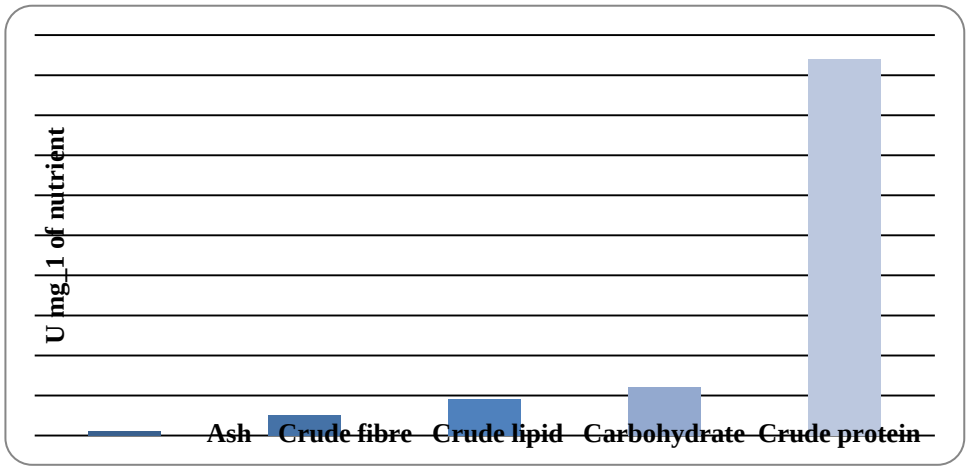

Fig. 3: Proximate composition (%) of Mohua oil cake

An analysis on the results of enzymes during 08 hrs after feeding to the fingerling stage of the studies fish samples showing variations in the values of amylase, pepsin and trypsin in the digestive tract of *Labeo calbasu*. The non-protein

diet (e.g., Swarno musur rice powder) depicting relatively greater values of amylase in the intestine and less in the pyloric caeca. While during starvation period in the digestive tract of the fish samples the non-protein diet depicting relatively low values of amylase activity in the intestine and less in the pyloric caeca as well.

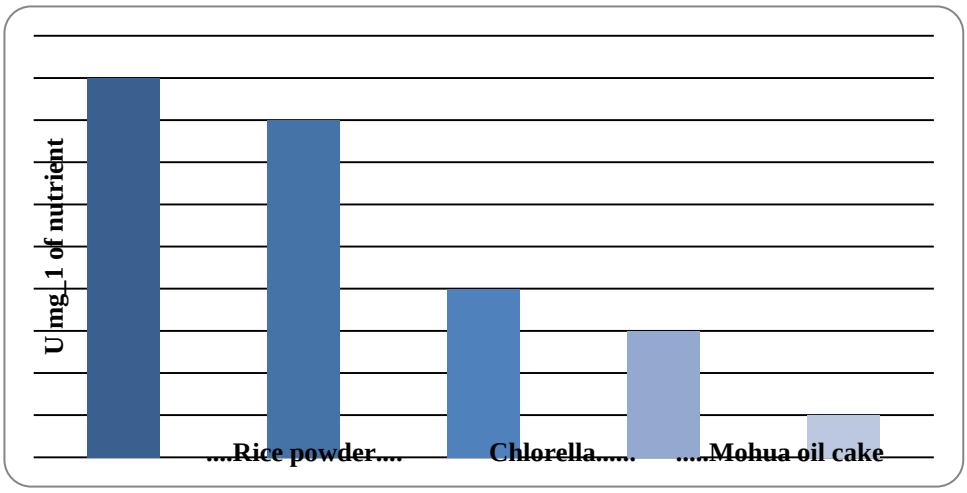

Fig. 4: Activities of amylase in intestine after 08 hrs of feeding to fingerling of *Labeo calbasu*

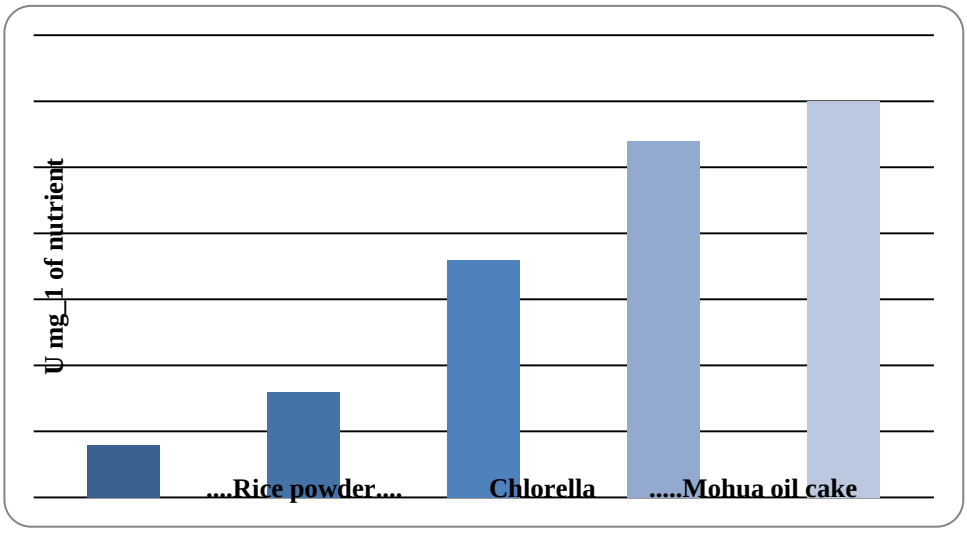

Fig. 5: Activities of pepsin in the stomach after 08 hrs of feeding to fingerling of *Labeo calbasu*

Observations on the availability of different digestive enzymes after about 08 hrs of feeding in the digestive tract of the fish samples of *Labeo calbasu* showing activities of amylase (fig. 4) with variations of values at different areas of digestive tract. Further, the results (fig. 4) reveal relatively larger activity of amylase in the intestine when carbohydrate rich non-protein diet is fed to the fingerlings. Similarly, the activity of pepsin (fig. 5) although could not showing significant value depicting appearance of certain data in the stomach area probably because of feeding protein-rich diet chlorella to the fish samples. Simultaneously, the activity of trypsin which also could not reflect significant data showing some values in the stomach area of digestive tract presumably due to feeding protein rich diet mohua oil cake (fig. 6) to the studied fish samples.

While observing the activity of pepsin and trypsin with some fish samples some workers (Banik 2022) also noticed similar results. Feeding with non-protein rich diet depicts significant effect ($r=0.99$, $P<0.01$) on the activity of amylase in the intestine area of digestive tract (fig. 4). Some researchers (Banik 2023, 2024, Hepher 1988) also pointed out similar observations during their studies with teleost fish samples. Therefore, the diet fed to the fingerling stage of the fish samples of *Labeo calbasu* depicting significant results with regard to the digestive enzyme activities. Some workers (Bernfeld 1955, Bondi and Spandorf 1953, 1954) also noticed similar results with various fish samples of different species. In this context, some workers (Bondi *et al.* 1957)

opined that in the fish population the activity of chymotrypsin varies due to variations of species, length and weight values and also because of variations in their life cycle stages as well. However, possibly due to providing protein-rich diet mohua oil cake to the studied fish samples the activities of chymotrypsin have been traced in the stomach area of digestive tract (Fernández *et al.* 2001).

Ethical Aspect

Occurrence and abundance of natural food biota exclusively preferred by *Labeo calbasu* is poor in the freshwater ecosystem. So, it is prerequisite to know which particular supplementary feeds are helpful for promoting growth rate of the freshwater fish species in general and the presently observed fish species in particular. Once such feeds can be identified they may be chosen to experiment the growth parameter under aqua-farming technology. On the other, for the need of the studies and also to understand the activities of different digestive enzymes such as amylase, trypsin, pepsin etc the studied fish samples have been observed under a period of starvation. Hence, the present work has no conflict with the ethical issue.

Conclusion

Labeo calbasu serves as preferred fish food to meet the demand of animal protein to human population. However, sufficient knowledge on the growth rate of this fish species under commercial aqua-farming system is not available. Hence, it is important to identify potential feeds which can be applied in aqua-farm. In the present studies using some feeds assay on the activities of amylase, pepsin and trypsin have been done with *Labeo calbasu* during its fingerling stage. The enzyme activities are determined in activity unit per 100 g fish body weight. The study reveals that during a period after about 08 hrs of feeding with non-protein rich feed (e.g, Swarno musuri rice powder) significant results have been noticed with amylase (in the stomach, pyloric caeca and in the intestine), pepsin (in the stomach) and trypsin (in the stomach) as well.

Acknowledgement

The author gratefully acknowledges Prof S Banik, Former Founder-Head, Department of Zoology, Former Dean, Faculty of Science and Former Director of IQAC, Tripura University; Head of Dept of Molecular Biology & Bioinformatics, Tripura University for inspiration.

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