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Effect of Feed Additives on the Development of Proteolytic Enzymes of the Tropical Sport Fish Malaysian Mahseer (*Tor tambroides* - Bleeker) Fry

[1]Jalal, K.C.A., [2]Ambak, M.A., [2]Abol, M.A.B., [1]Torla Haji. Hassan and [3]M. Zahangir Alam
[1]Faculty of Science, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur Malaysia
[2]AQUATROP, University College of Science and Technology, Malaysia, Mengabang Telipot 21030 Kuala Terengganu Malaysia
[3]Faculty of Engineering, International Islamic University Malaysia Jalan Gombak, Malaysia 53100, Kuala Lumpur Malaysia

Abstract: *Tor tambroides* fry with a mean of 8.0±0.72 mm standard length (SL) and weighing (W) 0.06±0.01g were stocked at the rate of fifty (50) individuals in each of the fifteen 150*l* rectangular fibre glass tanks for a period of 5 weeks. The development of proteolytic enzymes (Trypsin and Chymotrypsin) was studied during these feeding treatments. 45% protein diet without additives was treated as control diet. The gut of fish fry fed on control diet incorporated with 0.10% *Spirulina*, 0.10% enzyme and 0.10% vitamin additives were examined. Fish fry fed on diet with 0.10% *Spirulina* showed significantly higher enzymatic activity ($P<0.05$) compared to enzyme, vitamin and control diet throughout the experiment. Control diet without any additive showed lower activity than those of diets with additives. This study indicates that the incorporation of 0.10% *Spirulina* to a diet could be a vital factor to activate the proteolytic enzymes such as trypsin and chymotrypsin of the *Tor tambroides* fry. This suggests a new approach in the use of feed additive *Spirulina* in fish feed.

Key words: *Tor tambroides*, S*pirulina*, trypsin, chymotrypsin, feed additives

INTRODUCTION

Malaysian Mahseer *Tor tambroides* is well renowned as a splendid tropical sport fish in Southeast Asian countries. The recent catch statistics indicated that this highly valued species have declined and can be considered as endangered. In order to facilitate the restocking programme a study on nutrition was conducted.

Fish fry are primitive vertebrates in which most organs are still simple. This is true for the digestive system, one of the most important parts of a young and rapidly growing organism[1]. The development period of several species of fish fry is characterized by a more or less drastic increase of enzyme activities and some times also by changes of enzyme patterns[2,3]. The ability of an organism to digest a given substance is predominantly dependent on the presence of appropriate enzymes. It has already been widely accepted that fish can be reared on additives on enzymes, *Spirulina* and vitamin. However, different scientists documented maximum growth and survival of fish fed with these additives during their study in different fishes[4].

A mixed diet of microalgae and zooplankton, either together or sequentially are used during the production of juvenile shrimp, carp, milkfish. *Spirulina* is used as feed throughout the entire life cycle of the juvenile, even adult oysters and other bivalves. The study of growth of shrimp was faster with higher survival rate fed with additives diets (enzyme and vitamin) than that of non-additive diet in controlled conditions[5]. Addition of proteolytic enzymes in their food resulted in only small positive effects in common carp[6, 7]. The efficacy of the fish additives especially *Spirulina*, enzyme and vitamin needs to investigate in the fry of *Tor tambroides* which are expensive fish and new-fangled in aquaculture as well.

MATERIALS AND METHODS

The gut of fish fry fed on 0.10% *Spirulina*, enzyme and vitamin additives were examined. The developments of proteolytic enzymes (trypsin and chymotrypsin) were studied during these feeding treatments. In this study enzyme analysis trypsin and chymotrypsin was assayed quantitatively from the gut region.

Six fry from the original sample were fixed before the start of the experiment to establish their nutritional state before and after feeding with different diets. Intestinal segments were slit open longitudinally and

Corresponding Author: Dr. Ahmed Jalal Khan Chowdhury, Faculty of Science, International Islamic University Malaysia Jalan Gombak, 53100, Kuala Lumpur Malaysia

washed with cold saline to remove the contents. The samples were collected 3 h after the previous feeding to be certain that there was no food in the digestive tracts of fish. The sampled fish were placed in salt solution, quick frozen in dried ice and methanol and stored at -75º C until extracted for essay of enzymes.

The following methods were used for the determination of enzymatic activity. The tissues were homogenized in an adequate quantity of distilled water by a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 4000 rpm for 10 minutes. The gastric or intestinal juice was diluted with deionized water in the proportion suitable for the individual method. The supernatants were immediately used for deep-frozen in small aliquots until required[8]. The rates of enzymes activity were measured in quartz cuvettes (1 cm light and 1.5 ml capacity) in a temperature controlled (30º C) recording spectrophotometer. Protein determinations of the enzyme extracts were made according to the method with the Bio-Rad protein assay kit (Bio-Rad) Laboratories, Richmond, California[9]. The standard curve was used for the determination of protein from the enzyme extracts.

Trypsin: Trypsin activity was measured with p-tosyl-L-arginine methyl ester hydrochloride as the substrate[10]. The reaction mixture contained 0.50 ml of $1.04x10^{-3}$ M substrate and 0.40 ml of 0.04 M tris buffer containing 0.01 M $CaCl_2$, pH 8.1; 0.10 ml of diluted gut fluid was added to 1 ml of this solution and the rate of increase in absorbance of the reaction mixture was recorded at a wavelength 247 nm. Specific activity of the enzyme was expressed as micromoles of a substrate hydrolyzed per minute per milligram of protein in the extract[11].

Chymotrypsin: Chymotrypsin activity was measured with N-benzoyl-tyrosine ethyl ester as the substrate[10]. The reaction mixture contained 0.5 ml of 0.001 M substrate and 0.4 ml of 0.1 M tris-HCl buffer containing 0.01 M $CaCl_2$, pH 7.8; 0.10 ml of enzyme extract was added to 1 ml of this solution and the rate of increase in absorbance of the reaction mixture was recorded at a wavelength 254 nm. Specific activity of the enzyme was expressed as micromoles of the substrate hydrolysed per minute per milligram of protein in the extract[11].

RESULTS

Before Feeding the test diets specific activities of trypsin and chymotrypsin did not show any significant difference (P>0.05) among themselves at the size of fry 8-10 mm before feeding on test diets (Fig. 1).

Trypsin: After Feeding the test diets significant difference (P<0.05) was observed between *Spirulina* and enzyme diet up to 3rd week. The presence of trypsin enzyme in the fish fed with *Spirulina* and enzyme diet did not show any significance differences (P>0.05) between 4th and 5th week. Besides, higher enzymatic activity observed when fry reached 25-32 mm in 4th week and onwards. Enzyme and *Spirulina* diet exhibited significantly higher than those of vitamin and control diet. However, vitamin diet showed significantly (P<0.05) higher activity than that of control diet (Fig. 2).

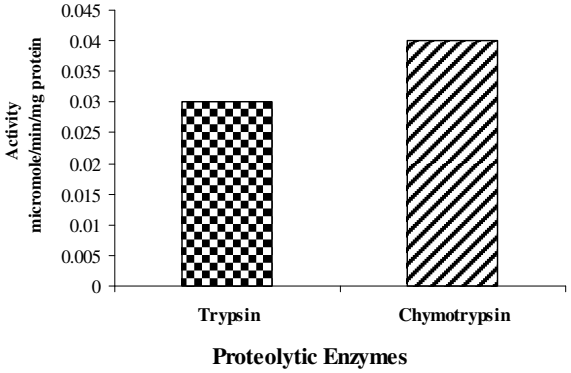

Fig. 1: Proteolytic enzymes before feeding

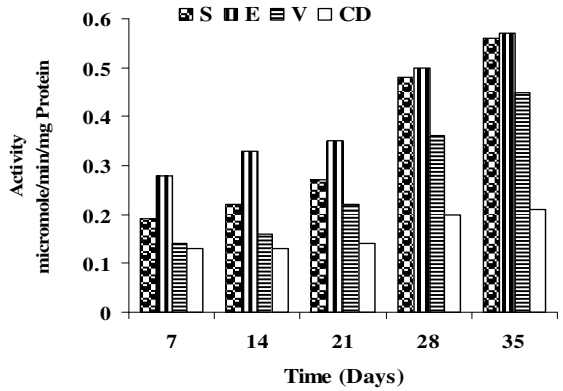

Fig. 2: Trypsin activities after feeding on different diets S- *Spirulina*, E- Enzyme, V- Vitamin, Cd ~ Control Diet

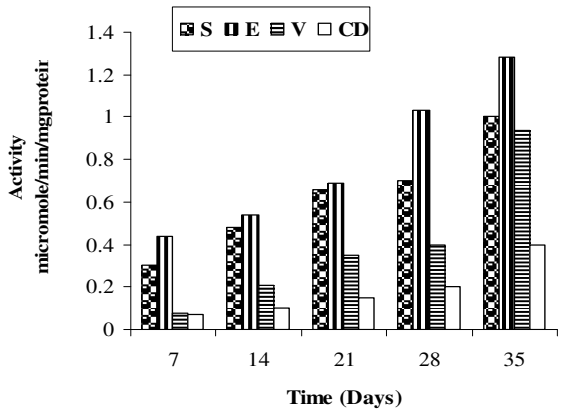

Fig. 3: Chymotrypsin activities after feeding on different diets

Chymotrypsin: Chymotrypsin activity seemed to be higher than that of trypsin throughout the experiment (Fig.3).

The activity of chymotrypsin was not similar to that of trypsin. Significantly higher activity ($P<0.05$) was also observed in diets with enzyme and *Spirulina* compared to vitamin and control diet throughout the experiment. Control diet without any additive showed lower activity than those of diets with additives (Fig. 2).

DISCUSSION

An increase in trypsin and chymotrypsin activity was also observed in the carp fry[6]. It was found that proteolytic enzyme activity increased considerably when common carp fry were provided with bovine trypsin in their diet. The activities of trypsin and chymotrypsin were also found in the digestive system of stomachless gold fish [12]. This is evident in our study in those fry fed dry diet having exogenous enzyme.

A surprisingly low tryptic activity and proteolytic action have been found in larvae fed on *Artemia* that of artificial diet for carp[13]. The increased rate of chymotrypsin indicated that this enzyme was strongly influenced by the diet which agrees with the findings of other researchers[14]. The trypsin activity in the carnivorous fish is four times higher than chymotrypsin activity[15]. The situation is reverse in the omnivorous *Cyrinus carpio* and the herbivorous *Hypophthalmichthys molitrix*, where chymotrypsin activities were found to be almost four times higher than trypsin activities. Although the vitamin diet showed good survival rate but the activity of proteolytic enzymes were lower than those of *Spirulina* and enzyme diet in *T. tambroides* fry. Likewise activities of enzymes in fish fed on artificial diet without any additive seemed to be low throughout the study period. This perhaps indicates non-optimum their digestion with the absence of exogenous enzyme or diet not enriched with quality additive *Spirulina*. *T. tambroides* seemed much less adaptive when the diets have no additive like vitamin and enzyme during their fry stages. Thus fry receiving the *Spirulina and* enzyme exhibited the highest level of protease activity whereas vitamin acted as a intermediate level. The trypsin activities in larval *Coregenous* sp. were increased from approximately 0.10 to 0.30 micromole/mg body weight between 10 to 30 days of rearing period[16]. In our previous study fish fed on diet incorporated with 0.10% *Spirulina* showed an increase in growth. Fish fry fed on 0.10% *Spirulina* diet showed an increase trend of protease activity in tissues suggesting the presence of proteolytic enzymes such as trypsin and chymotrypsin. Thus, the incorporation of *Spirulina* to artificial diet could play a vital role for the growth and survival of *T. tambroides* fry.

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