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Research Article

USE OF ENZYMES IN DETERGENT ON INTESTINAL ENZYME ACTIVITY IN FISH, *CIRRHINUS MRIGALA*, MRIGAL

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ABSTRACT
Use of enzymes in detergent on intestinal enzyme activity (Protease) in fish fry *Cirrhinus mrigala* was investigated. The fishes of mean body weight (1.25g±0.12) were exposed to three concentrations of detergent (surf excel easy wash) i.e, 0.5mg/lit., 1mg/lit., 3mg/lit respectively. The control group was also maintained simultaneously. During exposure period, the test fish exhibited no signs of deformity. However, the fish showed significantly ($p < 0.05$) increased in protease activity with increased conc. of detergent in aquarium.

Keywords: *Enzymes, Detergent, Protease*

INTRODUCTION
Detergents are common household products used for the cleaning of domestic materials. The "after wash" of these detergents are either deliberately drained into the aquatic environment such as pond, lakes, rivers, streams etc or find their way into the aquatic environment by natural seepage. Toxicity is a function of concentration and the duration of exposure of an organism to a toxicant. The validity of a detergent as a toxicant depends on the response of the test animal, their mode of action and the toxicity of the substance in relation to their chemical and physical structures (Moss *et al.*, 1980). Fish is of one of the most important non-target aquatic organism affected by detergent pollution. Abel (1974) documented that synthetic detergents are acutely toxic to fish of concentrations between 0.4 and 40mg/L, Okwuosa and Osuala, (1993) reported that considerable amount of detergent have been found to exist in Nigerian freshwater systems where they generally affect several aquatic organisms-Communal washing, a common practice in many water bodies in Nigeria could lead to a build up of detergent level in natural waters.
Environmental regulations and consumer concerns are putting pressure on the entire detergent value chain to offer more environment friendly detergents to the market. The environmental impact of doing laundry in the U.S. is primarily affected by the raw materials used to manufacture detergents and, more importantly, the temperature used in washing machines. The development of new enzyme solutions by Novozymes allows detergent manufacturers to reduce the environmental impact of their detergents by replacing traditional chemicals such as surfactants with a multienzyme solution (Nielsen *et al.*, 2010). This can be done without compromising performance or cost, while at the same time allowing for good cleaning performance at low wash temperatures. Enzymes are natural substances that are efficient at low concentrations, readily degradable in the environment, deliver low toxicity levels, and most importantly, perform well at low wash temperatures. Thus, detergent manufacturers can reformulate their detergents with enzymes to make them more sustainable and attractive to their customers. It has already been illustrated that there are significant environmental benefits from replacing surfactants with enzymes in a powder detergent in the European market (Nielsen *et al.*, 2007) and from reformulation of detergents for the French market (Dewaele, 2006). The present study highlights the use of enzymes in detergent on intestinal enzyme (protease) activity of fish *Cirrhinus mrigala*.

MATERIALS AND METHODS
For this study Fish fry *Cirrhinus mrigala* were collected from Government Fish Seed Farm, Jhajjar. The fishes were acclimatized to the laboratory conditions in a glass aquarium for 7 days before commencement of experiment. Water was replaced daily during acclimatization. 20 fries and four aquarium were used for the study, 5 fries in each (labelled the aquarium as aquarium 1, 2, 3 and 4).The

body weight of fish ranges from 0.17-2.32gm. Experiment was conducted in glass aquarium containing 33 litres of water. The fish fry were exposed to three concentrations of detergent (surf excel easy wash) i.e. 0.5mg/lit., 1mg/lit., 3mg/lit (Table-1). The control group was also maintained simultaneously. At the end of 15 day fish were dissected and intestine was collected for analysis.

Table 1: Concentration of detergent (surf excel)

S.no.	Name of fish	Groups
1	*Cirrhinus mrigala*	Control
2		Conc.0.5mg/lit
3		Conc.1mg/lit
4		Conc.3mg/lit

Determination of Protease Enzyme Activity

Preparation of crude enzyme extract-

Firstly, the fry were dissected on a ice tray and intestine was removed carefully. Intestine was homogenized in 5 volumes of ice cold distilled water using tissue homogenizer after that the contents was centrifuged at 4˚C at 10,000 rpm and the supernatant was used for the determination of protease enzyme activity

Determination of Enzyme Activity

1ml of the substrate (1%BSA) solution, 1ml of 0.1 M phosphate buffer, 1ml of CaCl2 and 1ml of crude enzyme extract were taken in a test tube then test tubes were incubated at 37˚ C for 1 hour. After 1 hour reaction was stopped by adding 3ml of 5% TCA solution. The precipitates were removed by centrifugation at 3000rpm for 10 minutes. The supernatant was used for determination of digested protein by Lowry's method For the application on each treatment following formula was used-

$$\text{Specific protease activity} = \frac{\text{Total protease activity}}{\text{Total protein}}$$

ANOVA followed by Turkey HSD test was applied to assess the significance of the differences among treatments.

RESULTS AND DISSCUSSION

The result of present study indicates that the specific protease activity was increased as increase in concentration of detergent. The increase in specific protease activity was due to presence of enzyme in detergent, which causes the activity of protease to rise (Table 2).

Table-2 Effect of detergent (surf excel) on Protease activity of *Cirrhinus mrigala*

S.no.	Groups	Specific Protease activity (mg of tyrosine mg−1 of protein h−1)
1	Control	8.25±0.02
2	Conc.0.5mg/lit	8.32±0.06
3	Conc.1mg/lit	9.05±0.04
4	Conc.3mg/lit	10.72±0.05

Values are mean ±SE of mean.

Interaction of surfactants with proteins has been studied by many workers as this forms an important part of extraction and solubilisation of membrane proteins and enzymes (Higgins, 1987). Also interactions of surfactants with many enzymes have also been investigated by many workers. Large amount of research has been done on surfactant-protein interactions. But there are very few data is available concerning use of enzymes in detergents. Recently, intestine of common carp (*Ciprinus carpio*) and tambaqui (*Colossoma macropomum*) were proposed as sources of alkaline proteases (Espósito *et al.*, 2009) because of their compatibility with commercial laundry detergents. In the presence of Tween 20 and sodium

choleate, the enzyme extract retained about 70 % of its activity and saponin did not seem to affect its value. Surfactant SDS was the only agent capable of inactivating it. Similar results have been described for the intestine proteases from carp (*Cyprinus carpio*) and tambaqui (*C. macropomum*) including the strong effect of SDS in protease denaturation (Espósito *et al.*, 2009). The cost-neutral replacement of surfactant with enzymes in a standard laundry detergent allow for reduction of laundry wash temperatures and toxicity impact on water bodies without compromising the total wash performance. Enzyme use is small compared with surfactant savings and the environmental impact caused by the extra enzyme use is insignificant compared with the savings obtained by reducing the surfactant content and particularly reducing consumption of energy used to heat wash water (Nielsen *et al.*, 2010). Data quality assessments and sensitivity analyses show that exact results are uncertain and depend on the magnitude of the temperature reduction, the applied water heating systems and a range of other factors.

Conclusion

The enzyme-rich detergent examined in this study costs the same as the conventional detergent and substantial environmental impact improvements can be realized while saving on the consumers' electricity bill. These are significant environmental savings and Novozymes is pleased to join forces with colleagues in the detergent industry across the value chain to realize this potential in the coming years.

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