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Research Article

INTESTINAL ENZYME ACTIVITY IN RESPONSE TO DIFFERENTIALLY HEAT PROCESSED SOYBEAN ON *CIRRHINUS MRIGALA* FRY

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ABSTRACT
A two months experiment was conducted to evaluate and compare the effects of the diet containing differentially heat processed soybean on intestinal enzyme activity of *Cirrhinus mrigala* fry. To achieve this aim, six iso-nitrogenous (40% protein) and iso-energetic (20 Kjg^{-1}) experimental diets with differentially autoclaved soybean were fed to *Cirrhinus mrigala* fry and their proteolytic, amylolytic, cellulolytic and lipolytic activities were measured. Results showed that specific protease, amylase, cellulase activity increased with increase in the processing time of soybean in comparison with the fish fed on the diet containing the soybean which was not autoclaved. Specific lipase activity was observed highest in the control, fishmeal based diet. Further, both the values are not significantly different. It is concluded that autoclaved soybean for 40 min can be used as a replacer of fish meal in the diets of *Cirrhinus mrigala* fish at fry stage without interfering the overall digestive enzyme quality of fish.

***Keywords**: Fish Meal, Differentially Autoclaved, Soybean, Specific Enzyme Activity*

INTRODUCTION
Fish meal is important, but the costly source of protein in aquaculture with restricted availability. From the viewpoints of price, availability and nutritional value, soybean can be the most effective alternative to fish meal (FM) as it is easily available, palatable, has balanced amino acid profile and high nutritional value (Samocha *et al.*, 2004).

However, some deleterious effects on fish growth were also reported when fish meal was replaced in higher percentage by soybean (Lim *et al.*, 2004; Raky *et al.*, 2005; Wang *et al.*, 2006; Hernandez *et al.*, 2007; Macgoogan and Gatlin, 2007; Monzer *et al.*, 2017). It was reported that raw soybean contains some heat labile anti-nutritional factors(ANFs) (Yu *et al.*, 2013) which require pre-treatments like extrusion, pelleting (Vielma *et al.*, 2000) boiling, roasting, autoclaving, micronization and fermentation (Tiamiyu *et al.*, 2015b) of soybean before usage. Feeding on inadequately processed or raw soybean can differentially affect enzymatic activity.

For understanding the mechanism of digestion and to know the adaptive changes of an animal towards the dynamic nutritional environment, the study of digestive enzymes is quite essential (Sunde *et al.*, 2004). Further, the knowledge of digestive enzymes helps to discover digestive capabilities and efficiency of fish species under culture, which becomes the basis for selection of ingredients to be included in its diet (Lee *et al.*, 1980; Divakaran and Ostrowsky, 1990; Le Moullac *et al.*, 1994, 1996).

Fish digestive enzyme activities are associated with its innate feeding habit and diet composition (Ray, 1988). To metabolize any diet, fish requires the availability of appropriate digestive enzymes (Phillips, 1969).

Many previous comparative studies of the digestive enzymes in different fish species have been reported (Hofer and Schiemer, 1981; Hofer, 1982; Jonas *et al.*, 1983; Kuz'mina and Kuz'mina, 1990; Kuzmina and Smirnova, 1992) but very rare on our commonly available fish species. Therefore, to understand the nutritive physiology of fishes it is required to study the digestive enzymes, their secretion, and activity. A comparative study of the effect of differently heat treated soybean diet will help to show the activity pattern of different enzymes.

To achieve this target, the present experiment has been conducted on *Cirrhinus mrigala* fry fed on diets with autoclaved soybean for various time periods.

MATERIALS AND METHODS

Experiment Animal

The fish fry were purchased from Sultan Aqua and Research Foundation, Nilokheri, Karnal, Haryana, India.

Diets

Ingredients like sesame oilcake, soybean, wheat flour, fish meal, rice bran, chromic oxide, mineral premix, cod liver oil were taken. Six iso-nitrogenous (40% protein) and iso-energetic (20 Kjg^{-1}) experimental diets were formed with differentially autoclaved soybean (0, 5, 10, 20 and 40 min) following the procedure of Peres *et al.*, (2003). Mrigal fry Fish fry were the fed on these formulated diet pellets. Control diet here was prepared by using fishmeal as main protein source. Hence, total six diets were formed.

Site of Experiment

The experiment was conducted in the Central Animal House, Maharshi Dayanand University, Rohtak, Haryana (India) for two months.

Experimental Design

The experiment was performed in duplicates; hence experimental system consisted of 12 glass aquaria (with water holding the capacity of 35L), 2 for each treatment. 15 fry of *Cirrhinus mrigala* fish were randomly selected among 180 fish and distributed into each aquarium. At the end of the experiment, fish were starved for one day to clear their intestine and dissected on ice tray after being pithed. Samples of intestine were taken.

For protease, amylase and cellulase activity, intestines were put in chilled distilled water and for lipase activity intestines were preserved in chilled acetone. After then samples of these intestine were homogenized by using tissue homogenizer (in 5 volumes v/w), in the same media in which these were conserved.

Homogenate was centrifuged (using cold centrifuge, Remi C-24 plus) at 10,000 rpm for 1h, at 4°C, and the supernatant was removed for determination of specific enzyme activity of protease (Walter, 1984) (using bovine serum albumin as a substrate), amylase (Tietz, 1970) (using maltose as a substrate), cellulase (Pettersson and Porath, 1966) (using carboxymethylcellulase as a substrate) and lipase (Worthington, 1991 and Zamani *et al.*, 2009) using titration method.

Statistical Analysis

The one-way analysis of variance (ANOVA) followed by Turkey HSD test was used to determine the significance of enzyme activities among various treatments.

RESULTS AND DISCUSSION

The results of digestive enzyme activity of mrigal fry are presented in table 1 and figure 1, 2, 3, 4.

Table 1: Effect of Diets with Soybean Autoclaved for the Different Time Period (0min, 5min, 10min, 20min, and 40min) on Specific Enzyme Activities (in µ/mg Protein) of Fish *Cirrhinus Mrigala* Fry

Parameters	Initial	Control (FM)	D1 (0min)	D2 (5min)	D3 (10min)	D4 (20min)	D5 (40min)
Specific Protease Activity	0.627 ±0.011	1.520 ±0.060	1.055 ±0.005	1.110 ±0.030	1.195 ±0.015	1.335 ±0.035	1.790 ±0.060
Specific Cellulase Activity	0.402 ±0.004	0.065 ±0.009	0.270 ± 0.006	0.352 ±0.039	0.362 ±0.007	0.470 ±0.020	0.649 ±0.003
Specific Amylase Activity	0.105 ±0.005	0.285 ±0.015	0.094 ±0.006	0.168 ±0.008	0.231 ±0.001	0.251 ±0.009	0.274 ±0.004
Specific Lipase Activity	0.090 ±0.001	0.617 ±0.001	0.116 ±0.001	0.098 ±0.006	0.079 ±0.001	0.067 ± 0.002	0.058 ±0.001

All the values are mean ± S.E. of mean

Digestive Enzyme Activity

Specific Protease Activity: Protease activity was recorded to be increasing order from diet D1 to diet D5. Hence, maximum value for this activity was recorded in D5 (1.790±0.060) followed by control diet (1.520±0.060).

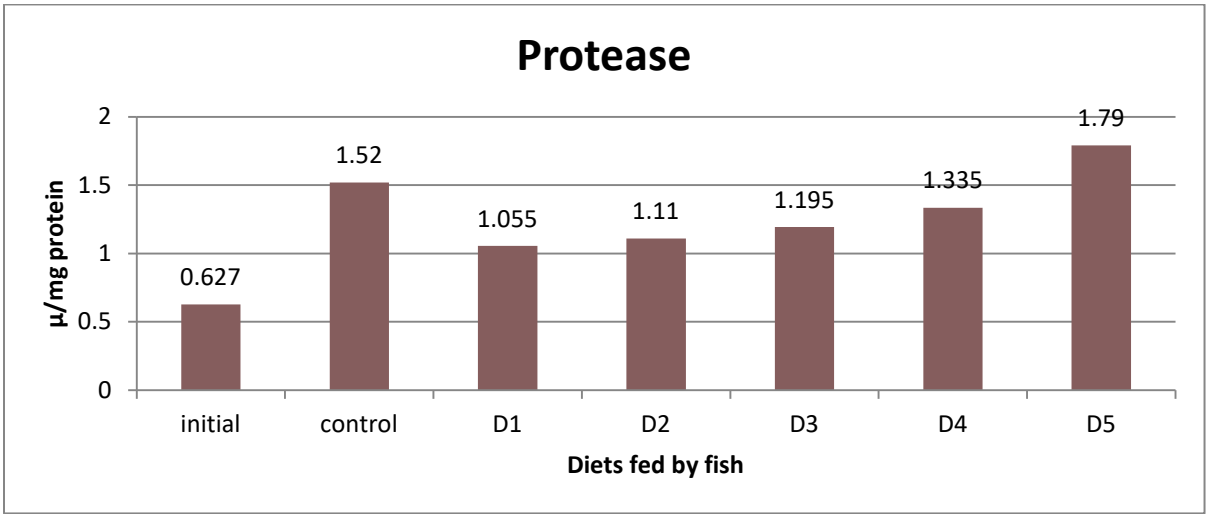

Figure 1: Protease Activity (in µ/mg Protein) of *Cirrhinus Mrigala* Fry after Feeding Differently Autoclaved Soybean after 60 Days

Specific Cellulase Activity: Some increasing trends were recorded for Cellulase activity. Here also maximum activity was recorded in diet D5. However, the fish which were fed on reference diet had comparatively low Cellulase activity (0.065±0.009). Hence, here both values were significantly different.

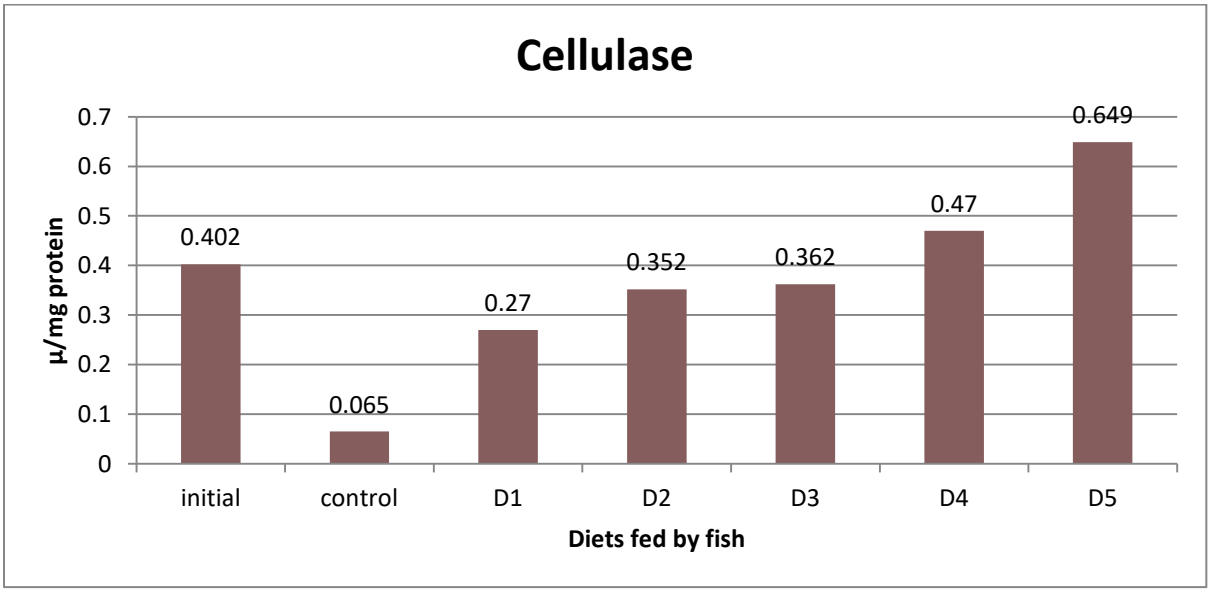

Figure 2: Cellulose Activity (in µ/mg Protein) of *Cirrhinus Mrigala* Fry after Feeding Differently Autoclaved Soybean after 60 Days

Specific Amylase Activity: The values for amylase activity of control (0.285±0.015), D3 (0.231±0.001), D4 (0.251±0.009), D5 (0.274±0.004) fish were recorded nearly same as no significant difference was observed here.

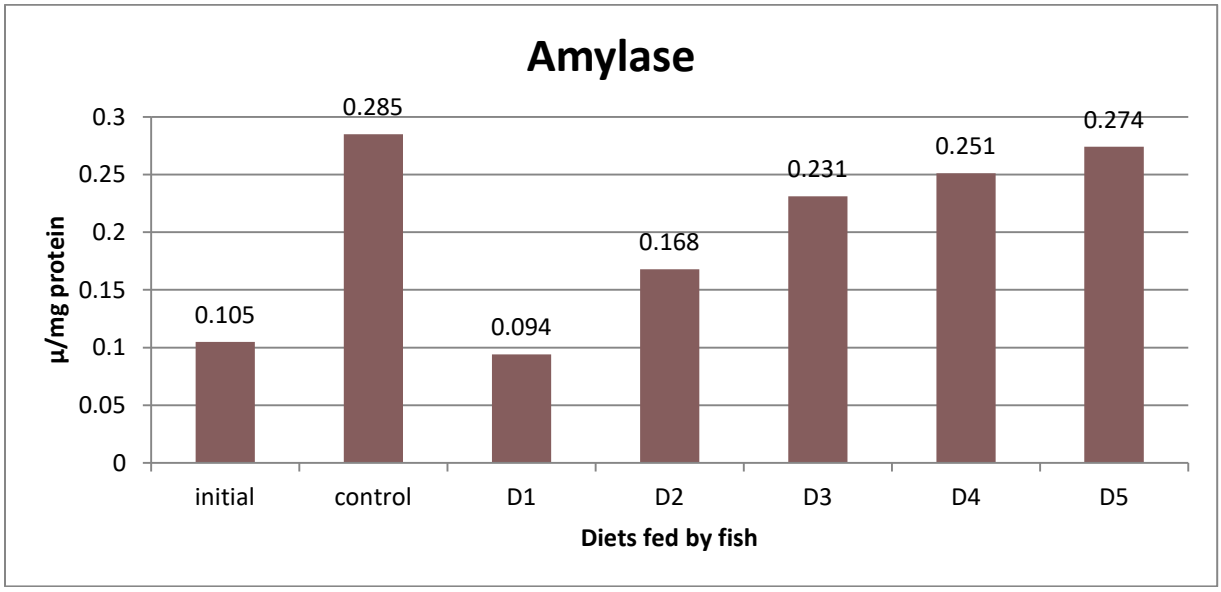

Figure 3: Amylase Activity (in µ/mg Protein) of *Cirrhinus Mrigala* Fry after Feeding Differently Autoclaved Soybean after 60 Days

Specific Lipase Activity: Maximum lipase activity was recorded in fish fed on control diet (0.617±0.001). Further, lipase activity decreases with increasing the time of the autoclaving process of soybean. Hence, minimum (0.058±0.001) lipase activity was recorded in D5.

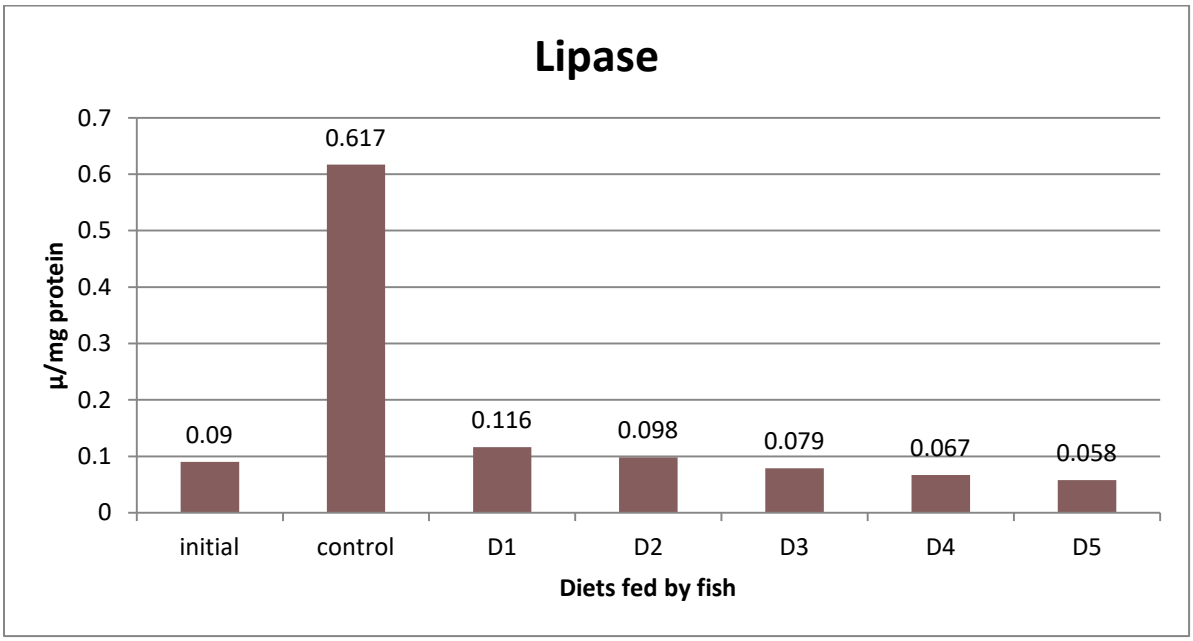

Figure 4: Lipase Activity (in µ/mg Protein) of *Cirrhinus Mrigala* Fry after Feeding Differently Autoclaved Soybean after 60 Days

Digestive enzyme activity plays an important role in feed utilization by fish which is further essential to improve diet formulation. Digestive enzymes are very important to study during digestion of a particular food. Distribution of endogenous enzymes and their activity in the digestive tract of fish very much depends on their feeding habits and with the composition of diets (Steffens, 1989); some trends were also

recorded in our study. According to Ray (1988), diet composition has a great impact on digestive enzyme activity. Therefore, changing the diet may induce changes in the enzymatic activity. Our results also prove this as maximum lipase activity was recorded in the fishmeal based diet. Also, high value of cellulase activity was observed in the diet based on soybean. Further, the digestive enzyme activity appeared to depend on both the inclusion levels of soybean in the diets as well as the time period for heat processing of soybean. High level of raw soybean is appeared to depress enzyme activity compared to diets containing the adequately processed full-fat soybean. In our study also the raw soybean based diets had low enzyme activity as compared to processed soybean based diet. ANFs of soybean could affect fish health and growth rate by reducing the activity of fish digestive enzymes (Robaina, 1997). On combining with nutrients ANFs could form indigestible compounds which will further affect absorption of nutrients, hence slower fish growth rate (Buttle *et al.*, 2001). Generally, fish require higher dietary protein levels in the feed as compare to carbohydrates (Kikuchi, 1990). High dietary unprocessed soybean meal was proved to be a potent inhibitor of intestinal protease in jundia (Lazzari *et al.*, 2010). By calculating specific enzyme activities of enzymes (protease, amylase, cellulase, and lipase), the whole digestive capability and efficiency of fish species under treatment can be understood.

Conclusion

The aim of this paper was to determine the specific activity of intestinal enzymes of *Cirrhinus mrigala* fish when fed with differently heat processed full-fat soybeans. The result from this study will be used as a basis to develop cheep food formulation with optimal nutritional values for fish farmers.

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