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Digestive enzyme activities in Nile tilapia, *Oreochromis niloticus* fed diet supplemented with guar sprout meal

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Abstract

The present study was carried out to examine the specific digestive enzyme activity of Nile tilapia (*Oreochromis niloticus*) fingerlings, fed with different percentages of Guar sprout meal (GSM). The experiment was set with five isonitrogenous and isolipidic diets as T₁, T₂, T₃, T₄ and T₅ containing guar sprout meal at an inclusion rate of 0, 25, 50, 75 and 100%, respectively. The result showed a higher protease activity in the fish nourished with 0%, 25% and 50%. GSM beyond 50% showed poor protease activities especially in all parts of the gut (gastrointestinal tract). There was significant acceleration in amylase and lipase activities in the digestive tract (liver, stomach and intestine) with increased GSM levels. The results accomplish that 25-50% inclusion of GSM can be a favorable and economically sustainable protein source in the diet of Nile tilapia fingerlings.

Keywords: Tilapia, guar sprout meal, liver, amylase, protease, lipase

Introduction

Fish generally meets the requirements for the food regulation and commercial specification as it provides about 26% of the daily easily digestible high-quality protein^[1] and a wide variety of vitamins and minerals^[2]. The increasing price of fishmeal for feed preparation causes the need to find an alternative for the preparation of fish feed. Guar is the most easily available product in various parts of India having higher protein value^[3]. Tilapia is one of the widely cultured omnivorous fish species and is commercially important in tropical and subtropical areas of the world^[4]. Tilapia culture has gradually shifted from extensive farming to intensive farming systems with exclusive dependence on artificial feeds which in turn linked to the quality of protein used in the diet. For this reason, tilapia feed industry faces a momentous challenge for formulation and production of suitable, nutritionally balanced and cost-effective feeds. Fish nutrition is an important part of the aquaculture

Fish nutrition is a major part in aquaculture, which is directly and indirectly involved in the fish growth and production^[5]. Feed formulation is the most crucial part in nutrition. Fish growth is greatly influenced by its feeding habits and it can improve by consuming good quality of feed which is necessary for better enzymatic activities^[6]. The digestive enzyme study is a reliable tool to understand the digestion mechanism, its activities in different parts of the gastrointestinal tract and its adaptation to the change in nutritional environment in fish^[7-12]. The present study was aimed to understand the effect of GSM in feed, on digestive enzymes activity of liver and intestine in Nile tilapia fingerlings (*Oreochromis niloticus*).

Materials and Methods

Experimental design

The present study was conducted in the Department of Aquaculture, KUFOS, Kerala, India. A total 15 number of uniform sized FRP tanks (100:1 Volume) were stocked with 20 number of monosex Nile tilapia fingerlings with an average weight of 4.26 ± 0.02 g. The experiment was set in triplicates with five isonitrogenous and isolipidic diets as T₁, T₂, T₃, T₄ and T₅ containing GSM at an inclusion rate of 0, 25, 50, 75 and 100% respectively and other feed ingredients viz., fish meal (FM), soybean meal (SBM), groundnut oil cake (GNOC), rice polish (RP) and corn. The experimental diet with 0% guar sprout meal was considered as the control. Fish were regularly fed with the practical diets in three replicates tanks each, @ 5-7% of their body

weight. During the experimental test period of 60 days, the fish were fed twice a day.

Proximate analysis

Feed ingredients and the experimental diets were analyzed for fat, moisture, protein, fiber, ash using standard protocols (AOAC, 1990) [13]. The powdered feeds and ingredients were used for determination of the moisture after drying in a hot air oven at 100°C for 24 hours. Crude protein content was analyzed by kjeldahl method (nitrogen x 6.25) using a kjeltec system (Borosil). Crude fat was examined with low boiling organic solvent (petroleum ether/ diethyl ether, xylene) by soxhlet extraction system. The ash content of diets and fish tissues was determined by dry ashing. The Fibrotech system (Tecator 1017 hot extractor) was used to analyze fiber content.

Sample preparation for intestinal enzymes

Six fish samples from each experimental tank (three for the whole intestine, liver, stomach and three each for anterior, middle and posterior portions of the intestine) were collected. These samples were weighed and then washed with saline solution. The pre-weighed intestines, liver and stomach were homogenised in a homogeniser by adding 0.5N phosphate buffer solution. The homogenate was centrifuged at 6000 g at 4 °C for 15 min. The supernatant was collected and stored in refrigerator at -18 °C to -20 °C [14] for further analysis of enzymes activity (amylase, protease and lipase).

Amylase

The amylase activity was assayed with 1% (w/v) starch solution as substrate in 0.05 M phosphate buffer (pH 6.9). A total of 0.5 ml of substrate solution was added to 0.25 ml of enzyme preparation in the blank test tube. Both sample and reaction mixture were incubated at room temperature (37 °C) for 15 min. One ml of glucose solution (0.1%) was taken as standard in a test tube. Subsequently, 1 ml DNS (3,5-dinitrosalicylic acid) reagent was added to the sample, blank and standard test tubes respectively. All the test tubes were kept in a boiling water bath for 10 minutes. Sodium-potassium tartrate (40%) was added to stop the reaction and these test tubes were then cooled at room temperature. Further, the reaction mixture was diluted with 2 ml distilled water and absorbance of blank, standard and samples were in a spectrophotometer at 540 nm. Amylase activity was determined from the glucose standard curve and expressed as µg reducing sugars released per min. per ml [14].

Protease

The protease enzyme activity was evaluated by casein

digestion method [15]. The final reaction mixture contains 1% casein as substrate (0.25 mL), phosphate buffer (pH 7.0) (1 ml) and tissue homogenate (0.25 ml). The reaction mixture was incubated at 37 °C for 2 hrs. Further, the reaction was stopped by addition of 10% trichloroacetic acid (TCA) and then kept for overnight at 4 °C. The mixture was centrifuged for 10 min and the supernatant was collected. The protein concentration of supernatant was measured at 600 nm following Lowery *et al.* (1951) [16]. The activity of protease enzyme was determined as µg hydrolysed proteins released per min per ml sample.

Lipase

One milliliter of sample was stirred in the presence of 3.5 ml phosphate buffer (pH 7.5) and 0.5 ml olive oil for 30 min at 37° C. The enzyme activity was stopped by adding 1 ml of acetic acid followed by the addition of 3-4 drops of phenolphthalein indicator to the mixture. The mixture was titrated against NaOH (10 mm) solution until the pink colour appeared.

Lipase activity (units ml⁻¹ min⁻¹) = Vol. of NaOH × Normality of NaOH × 1000 × 40 × 1000 / Vol. of sample homogenate used × 1000 × mol. wt. of oleic acid × time (min) of incubation

Physico-chemical parameters of water

Daily siphoning was carried out with 50% of water exchange to maintain the optimum water quality parameters throughout the course of experiment trial. Water quality parameters such as water temperature, dissolved oxygen and pH were conducted on a daily basis while total alkalinity, ammonia, nitrite and nitrate were monitored on a weekly period. All the values were recorded during the morning between 06:00 hrs. and 07:00 hrs. before exchanging the water by following the American Public Health Association protocols (APHA, 1998) [17] and were found to be in the ranges of 27.1-28.85 °C, 4.7-5.5 mg/L, 7.6- 8.14, 81.95-92 mg/L, 0-0.1 mg/L, 0-0.2 mg/L and 0-4 mg/L respectively.

Statistical analysis

The data obtained were subjected to statistical analyses using the software package SPSS Version 22. The significance level was set at $P < 0.05$ and one- way analysis of variance (ANOVA) was applied to compare means.

Results and Discussion

Data presented in Table 1 showed the proximate composition of locally available various feed ingredients and the composition and proximate analysis of the experimental diets is mentioned in Table 2.

Table 1: Proximate composition of feed ingredients

Parameter	Fishmeal	Soybean meal	Guar sprout meal	GNOC	Rice polish
Moisture (%)	10.08±0.20	9.98±0.69	8.13±0.35	8.50±0.50	9.55±0.25
Protein (%)	63.08±0.18	37.66±1.25	35.10±0.44	33.38±0.78	11.48±1.29
Fat (%)	9.38±0.62	8.15±0.25	3.35±0.04	8.97±0.24	13.85±0.25
Ash (%)	13.33±0.04	9.18±0.64	5.90±0.02	9.35±0.78	8.28±0.32
Fiber (%)	2.02±0.53	12.28±0.31	3.25±0.05	7.02±0.58	8.05±0.35
NFE (%)	0.30±0.20	22.29±0.98	43.72±0.06	31.82±1.28	47.35±0.85

Table 2: Formulation of experimental diets fed to fingerlings of Tilapia (g/100 g)

Ingredients	Diet ¹				
	T ₁	T ₂	T ₃	T ₄	T ₅
Fishmeal	20.0	15.0	10.0	5.0	0.0

Soybean meal	22.0	22.0	22.0	22.0	22.0
Guar sprout meal	0.0	09.0	16.0	27.0	38.0
GNOC	10.0	10.0	10.0	10.0	10.0
Rice polish	21.0	18.0	17.0	15.0	12.0
Corn	22.5	21.5	20.5	16.5	13.5
Veg oil	02.0	02.0	02.0	02.0	02.0
Vit C ²	0.5	0.5	0.5	0.5	0.5
Vit + min ³	02.0	02.0	02.0	02.0	02.0
Proximate chemical composition%					
Moisture	2.99	2.91	2.48	2.46	2.68
Crude protein	29.31	29.23	28.96	28.88	28.79
Crude lipid	7.70	7.08	8.03	7.26	7.34
Ash	14.12	13.51	13.05	12.80	12.22

¹Percentage replacement of fish meal protein by sprout guar meal protein in the diets: (F1) 0%; (F2) 25%; (F3) 50%; (F4) 75%; (F5) 100%.

²Vitamin C: Ascorbic acid I.P., 100 mg; sodium ascorbate I.P., 450 mg; eq. ascorbic acid, 400 mg.

³Vitamin- Mineral Mixture: Vitamin (IU or g kg⁻¹premix): retinol palmitate, 50000 IU; thiamine, 5; riboflavin,5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamin, 5; ascorbic acid, 10; cholecalciferol, 50000 IU; α -tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25.

Minerals (g kg⁻¹): CaCO₃, 336; KH₂PO₄, 502; MgSO₄.7H₂O, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; MnSO₄.H₂O, 3.12; ZnSO₄.7H₂O, 4.67; CuSO₄.5H₂O, 0.62; KI, 0.16; CoCl₂.6H₂O, 0.08; ammonium molybdate, 0.06; NaSeO₃, 0.02.

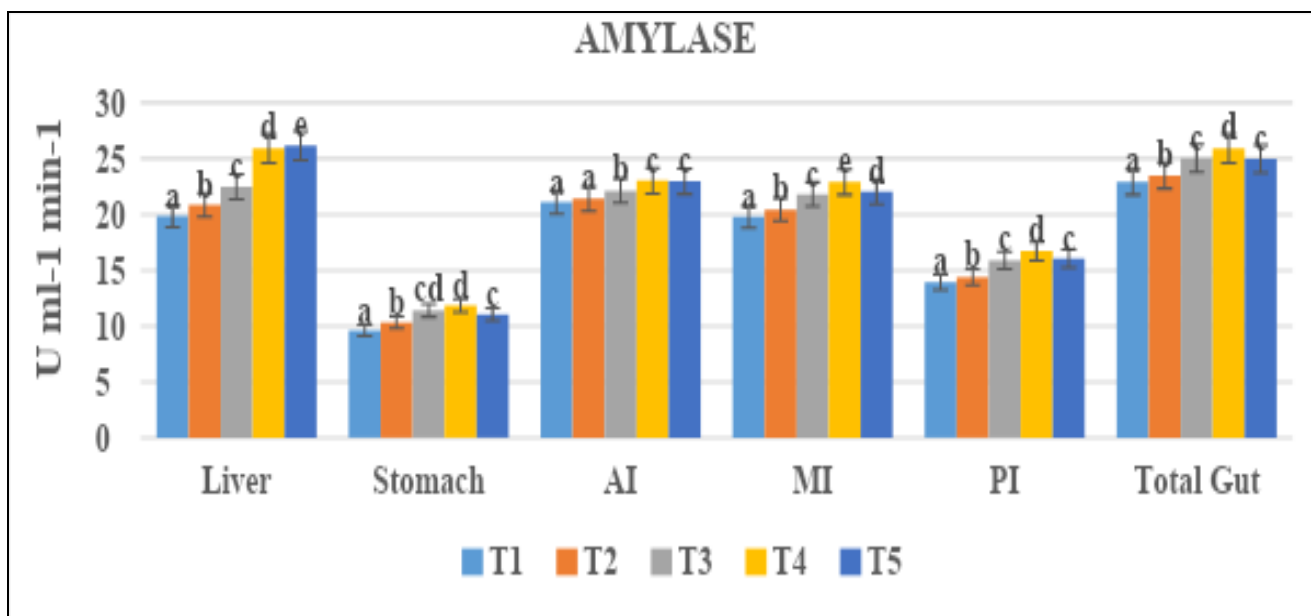


Fig 1: Effect of experimental diets on the amylase enzyme activities of different gastrointestinal parts of *Oreochromis niloticus*

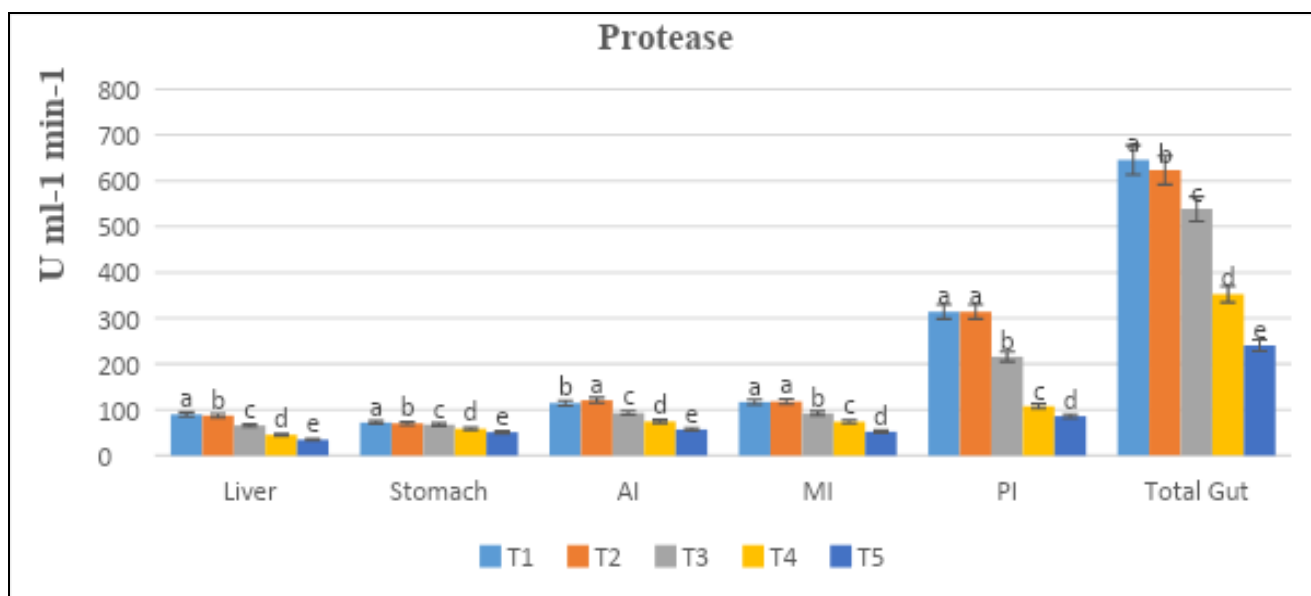


Fig 2: Effect of experimental diets on the protease enzyme activities of different gastrointestinal parts of *Oreochromis niloticus*

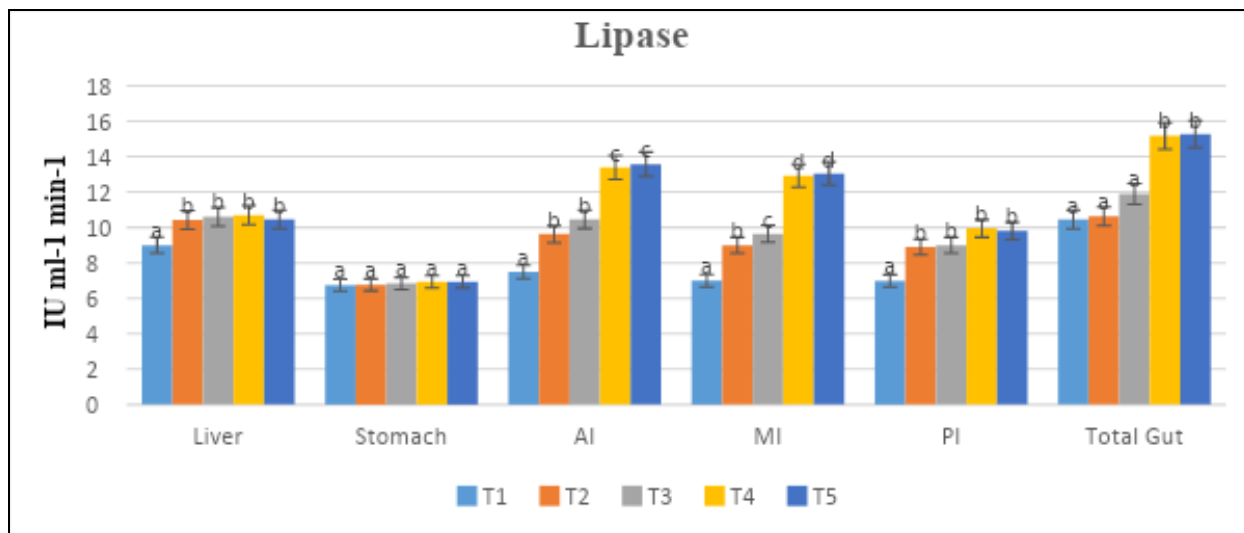


Fig 3: Effect of experimental diets on the lipase enzyme activities of different gastrointestinal parts of *Oreochromis niloticus*

Five feed ingredients were chosen for making the experimental diets with different percentage inclusion of spout guar meal. Among all ingredients the highest protein content was recorded in fishmeal, soybean meal and sprout guar meal respectively. These feed ingredients are easily available in the local market and can be used for aquaculture directly. Excellent nutritional value including high levels of crude protein, complementary amino acid profile and relatively high nutrient digestibility make the fish meal a much sought-after input in the commercial aquaculture. In recent centuries, the feed manufacturing industry has been demanding to focus more on plant protein sources and trying to reduce the fish meal usage due to its high cost and reduced availability. Being a drought tolerant crop with a short cultivation period of 30-45 days in the summer months of April- July in India (ranging between 50-450mm) and moderate protein content guar meal serves as a potential alternative to fish protein source. The plant can use its tap root system to scavenge deeper- lying nutrients left over in the soil from preceding crops. However, guar requires good crop management practices to achieve optimal yield, including field selection. Consequently, replacing fishmeal with guar meal has high ecological significance as far as the perspective of water conservation is concerned. Obviously, using guar meal in the aqua feed industry will have long term repercussions in terms of water footprint and sustainability [18].

The amylase, protease and lipase activity of liver, stomach, anterior intestine, middle intestine, posterior intestine and whole gut of the experimental animals fed with different inclusion levels of sprout guar meal over a period of 60 days are given in Figure 1, 2 and 3 respectively. The experimental feed incorporating 75% sprout guar showed significantly higher amylase activity in liver, stomach, anterior intestine, middle intestine, posterior intestine and whole gut. Maximum amylase activity was observed in total gut and liver. Similarly, more amylase secretion is recorded in anterior intestine and middle intestine however minimum amylase is recorded in the stomach and posterior part of the intestine. Protease enzyme activity was recorded maximum in intestine and minimum protease in liver and stomach. However, the posterior intestine showed higher protease than anterior and middle intestine. Fish fed with experimental diet T1, T2 and T3 significantly showed better protease enzyme activity than fish fed with T4 and T5 experimental diets. Greater lipase

amount was recorded in the intestine than the liver and stomach. While, lipase activity in anterior, posterior and whole intestine was found variable ($p < 0.5$) for all the experimental diets. Maximum and minimum lipase activity was observed in the whole intestine when fish were fed with 100% and 0% sprout guar meal diets respectively.

Feed constituents work as a driving force for digestive enzymes in fish [19]. According to Fernandez and his co-worker digestive adaptation in different species exhibits closer correlation with their diet rather than on their taxonomic category Suzer *et al.* (2007) [20] documented significant differences in amylase and protease activity in *Pagrus pagrus* when fish meal partially replaced by plant protein sources. He also explained that the specific activity of digestive enzymes highly depends on the variation in feed ingredients. Similar results were observed by Rodiles *et al.* (2012) [21] in juvenile Senegalese sole. However, Kumar *et al.* (2011) [22] predicted significant differences in amylase and protease activity in *Labeo rohita* in response to different feed ingredients. Aliyu-Paiko *et al.* (2010) [23] and Li *et al.* (2012) [24] stated that Lipases are inducible enzymes which could be stimulated by the dietary lipid content. According to Ismat *et al.* (2013) [14] highest lipase activity observed in *Catla catla* fed with SBM diets whereas poor performance detected in *Hypophthalmichthys molitrix*. Likewise, Kuzmina (1996) [15] explained the feeding behavior and biochemical composition of feed could affect the digestive enzyme activity. Khalid and his co-worker (2018) [25] recorded maximum amylase activity in *Labeo rohita* fed with canola meal while maximum protease activity was recorded in fish fed with cotton seed meal. He also explained that rohu, *Labeo rohita* fed with guar meal diets showed maximum lipase activity in the whole intestine. Similarly, Ma (2014) [26] described that fish fed with plant products showed the positive correlation between lipase activity and dietary lipid content. However, *P. pagrus* fed with diets of different feed composition showed dissimilar activity of digestive enzymes in intestine of fish [27].

Conclusion

The replacement of fishmeal with guar sprout meal is a desirable promising and sustainable result on a long-term basis under the light of fish meal shortage observed around the world. Based on the results of the present study, it can be concluded that every experimental diet performs differently in the various parts of the gut due to differences in nature of

nutrients. Fish fed with diets containing sprout guar meal incorporation of 25% and 50% by replacing fish meal showed better protease level than the feed containing 75% and 100% sprout guar meal which could be possible for partial replacement of guar meal with fish meal that ultimately enhanced fish growth, nutrient utilization and health of fish.

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Conflict of interest

There is no conflict of interest involved in this manuscript.

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