Digestive enzyme activities of *Cirrhinus mrigala* fingerlings fed with probiotic *Spirulina fusiformis* supplemented diets.

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DOI: https://doi.org/10.56293/IJASR.2023.5611

IJASR 2023
VOLUME 6
ISSUE 6 NOVEMBER – DECEMBER **ISSN: 2581-7876**

Abstract: Aquaculture is one of the fastest-growing systems in the world and has emerged as an industry that can supply protein-rich food throughout the world. The quality and quantity of fish feed consumed have a pronounced effect on the growth rate, efficiency of feed conversion, and chemical composition of fish. The use of well-balanced artificial feed is the primary basis for the success of intensive aquaculture. In recent years, fish nutrition has balanced probiotic diets that promote optimal fish growth and health. Probiotics may also be functional foods, which are defined as foods that contain some health-promoting components beyond traditional nutrients. Thus, the utilization of multifunctional probiotic-incorporated feed has emerged as a solution with huge application in aquaculture. Spirulina acts as a growth promoter and probiotic food booster for the immune system in fish. Digestive enzymes are one of the most important factors that influence the growth, efficiency of feed utilization, and feed conversion in fish. The objective of this present study is to prepare artificial probiotic pelleted feed using locally available ingredients and study the digestive enzymes of guts fed with probiotic Spirulina-incorporated feed. The present study described the development of digestive enzymes (lipase, protease, cellulose, and amylase) in the carp *Cirrhinus mrigala* fed on *Spirulina fusiformis*. The specific activity of amylase increased by 176.17 + 2.71 U/µmol in experimental fish fed on the SF-5 diet (5% inclusion). The increased cellulase activity in the gut (56.19 + 0.06 U/mg) was influenced by the higher (5%) inclusion level of *Spirulina fusiformis* (SF5). The lipase activity was found to be significantly higher in the treatment groups than in the control group. The highest lipase activity (5.85 + 0.31 U/µmol) was observed in the gut of fingerlings fed with the SF5 diet (5% Spirulina inclusion), whereas the lowest lipase activity (4.02 + 0.05, 4.18 + 0.03 U/µmol) was recorded in fish fed with the control and SF1 (1% inclusion) diets. Significantly higher levels of protease (53.97+ 0.68 U U/µmol) were observed in *Cirrhinus mrigala* fingerlings when fed on the SF5 diet.

Keywords: Spirulina, Probiotic feed, Digestive enzymes, Supplementary feed

INTRODUCTION

The growth, efficiency of feed utilization and feed conversion in fish are greatly influenced by digestive enzymes. The characterization of digestive enzymes provides information regarding the digestive capacity of fish to hydrolase carbohydrates, protein, and lipids of feed ingredients. Probiotics improves the digestion of protein, starch, and fat which could be due to higher level of enzyme activities. Enzymes are naturally occurring proteins that function as catalysts for many chemical reactions taking place in the living organism.

The assay of enzymes in the gut of a fish provides information about its nutritional physiology, it is determined by the ability of the fish to digest and absorb it (Odedeyi and Fagbenro, 2010). Protease is a class of enzymes and is associated naturally with the gastrointestinal tracts of fish. The most important proteolytic enzymes in the viscera of fish are serine proteases, chymotrypsin, collagenase, and acid proteases. Proteases catalyze the hydrolysis of proteins into smaller fragments called peptides. Lipases are responsible for the chemical breakdown of lipids, which cleaves ester bonds that attach fatty acids to glycerol within triglyceride molecules.

The pancreas secret lipases and phospholipase enzymes, which are responsible for the hydrolysis of dietary phospholipids such as cholesterol. Cellulase catalyzes cellulose, Cellulases break down the cellulose molecule into monosaccharides ("simple sugars").

The study of digestive enzymes is an essential step toward understanding the mechanism of digestion and how fish adapt to changes in the nutritional environment (Rafael Lazzari *et al.*, 2012). The enzymes that act in the intestinal lumen are generally secreted from the pancreas, α-amylase, lipase, trypsin, chymotrypsin, endo, and exopeptidases. The digestive enzyme activity mainly depends on the age, type of feeding, and diet composition in fish feed. The major source of enzymes viz; Amylase, lipase, and proteases is the hepato pancreas of fish.

Understanding the nature of nutrients and how they are supplied in a supplemental diet would be greatly aided by the research of digestive enzymes. Since the kind and activities of digestive enzymes mostly determine the net effectiveness of the entire digestive process, the study of digestive enzymes is a very significant topic. When examining the nutritional benefits of artificial feeding and the fish's ability to adjust to dietary changes, the analysis of digestive enzymes is a crucial technique (Govind Pandey, 2013; Gisbert et al., 2009).

In the aquaculture industry, microbial (probiotic) enzymes are very much essential for the preparation of high-quality functional probiotic feeds. The gut bacterial enzymes (Probiotic enzymes) especially proteases in the intestine of fish can help in digesting the protein contained in the diet and also regulate the compound diets (Fu and Lei,2001). Probiotics may produce or stimulate the digestive enzymes of fish; they enhance feed utilization (Maurilio Lara-Flores and Olvera-Novoa, 2013). There is only limited research on the effects of probiotics on fish digestive enzymes such as amylase, lipase, cellulase, and protease. There are few publications available related to the effects of probiotics on mucosal digestive enzymes in fish.

Poor information on different digestive processes in fish alimentary canals is another hindrance to proper feed formulation for fish species. The measurement of specific digestive enzyme activities, such as proteases, amylase, cellulose, and lipase provide information about the digestive capability of fish. Several comparative studies of the digestive enzymes in different fish species have been reported earlier but very rare in our locally available cultivable carp species. Hence this study is designed to estimate the digestive enzyme activity of lipase, amylase, cellulase, and protease in the intestine of Cirrhinus mrigala fed with different types and concentrations of probiotic *Spirulina fusiformis* diets.

MATERIALS AND METHODS

Feed Ingredients

The experimental probiotic feed could be prepared using ingredients such as corn flour, wheat flour, rice bran, groundnut oil cake, tapioca, Agar agar, dried spent silk moth powder, cod liver oil, vitamins, and minerals. Flours of corn, wheat, and rice bran are used as a carbohydrate source. Silk moth powder and ground nut oil cake are excellent sources of protein, these are used as the main protein ingredient in experimental fish feeds.

Probiotic feed Preparation (Seenivasan *et al.*, 2012)

The experimental diets were formulated with selected ingredients as per "Pearson's square method" using a predetermined value of 40% protein content. The ingredients were sun-dried under hygienic conditions for 5 hrs and subsequently dried in a hot air oven at 55°C. The ingredients were made into a fine powder using an electrical mixer. The experimental feeds were prepared using known quantities of ingredients, and then the ingredients were weighed according to the formulation and kneaded by adding a sufficient quantity of distilled water and finally made into dough. The dough was autoclaved for 15 minutes and subsequently cooled for the preparation of probiotic experimental feeds. The dough was placed in the hand pelletizer and rolled out through 2mm die holes into a flat tray. The pellets were dried in a thermostatic oven at 40°C until they reached constant weight, and stored in airtight jars at room temperature (28°C). The pellets were broken manually to small sizes before being stored.

Control and experimental feed was prepared using the ingredients. viz. Corn flour, wheat flour, rice bran, tapioca powder, groundnut oil cake, silk moth (Bombyx mori) powder, cod liver oil, agar agar, vitamins and minerals, Diet without incorporation of probiotics product was served as control. *Spirulina fusiformis* was mixed in definite proportions of 1% (SF1), 2% (SF2), 3% (SF3), 4% (SF4), and 5% (SF5) to formulate experimental Spirulina feeds (Table – 1).

The proximate composition of probiotic feeds

Protein estimation

The crude protein content was determined following the micro kjeldahl method Percentage of nitrogen (N) was calculated using the following equation.

Nitrogen (%) = {(S-B) × N × 0.014 × D× 100} / (weight of sample × V) Where

D = Dilution factor,
T = Titration value = (S-B), W = weight of the sample, 0.014 = Constant value. Crude protein was obtained by multiplying the corresponding total nitrogen content by a conventional factor of 6.25. Thus, crude protein (%) = % of N × 6.25.

Carbohydrate estimation

The carbohydrate content was estimated by the difference method. It was calculated by subtracting the sum of the percentage of moisture, fat, protein, and ash contents from 100%.
Carbohydrate (%) = 100 – (moisture% + Fat % + Protein % + Ash %)

Fat estimation

Crude fat was determined by the Soxhlet extraction technique. The fat content of the dried samples can easily be extracted into organic solvent (petroleum ether) at 40-60 0C and followed by reflux for 6 hr. The percentage of fat content was calculated using the following formula.

Crude Fat (%) = Weight of fat in sample × 100/ Weight of dry sample.

Preparation of digestive enzyme source

The fish gut crude enzymes were extracted after the experimental period (60 days), and three fish fingerlings from each treatment were captured and sacrificed. Fish fasted for 24 hours before collection and were sacrificed by spinal cord puncture. The whole alimentary tract was dissected out in an ice-cold fish ringer solution and washed thoroughly. The digestive tract was measured and weighed. Later, the digestive tract was dissected in a petri dish, split open, and washed thoroughly in fish ringer solution. The tissue was then rinsed in cold distilled water. Then the gut tissues were homogenized separately for 2 minutes in a van potter elvehgem tissue homogenizer with buffer solution pH 7.0. The samples were then centrifuged at 12000 rpm for 5 minutes (4o C) and the supernatant was used as an enzyme source. The clean supernatant was used as the enzyme source for subsequent assay.

Estimation of crude digestive enzymes

Protease activity

Total protease activity was determined by the casein-hydrolysis method (Furne *et al.*,2005). Buffers for each pH assay were: 0.1 M glycine - NaOH pH 10.0. The reaction mixture contained casein at 1% (w/v) (0.25 ml), buffer (0.25 ml), and supernatant from the homogenates (0.1 ml) was incubated for 1 h at 37ºC. The reaction was stopped by the addition of 0.6 ml 8% (w/v) tri chloro acetic acid solution; kept for 1 h at 2ºC; centrifuged at 1800 g for 10 min and the absorbance of the supernatant was measured at 280 nm against blank. For the blank preparation, the supernatant from the homogenates was added at the end of the incubation period, just before the addition of trichloroacetic acid. Tyrosine solution was used as standard. One unit of enzyme was defined as the amount of enzyme required to catalyze the formation of 1.0 µmol of tyrosine per minute.

Amylase activity

Amylase activity was determined by the starch-hydrolysis method of Hidalgo *et al.*, (2006). A reaction mixture containing 2% (w/v) starch solution (0.125 ml), 0.1 M citrate-phosphate buffer at pH 7.5 (0.125 ml), and supernatant from the homogenates (0.05 ml) were incubated for 1 h at 37ºC. After the incubation period, the Somogyi-Nelson colorimetric method was followed and the absorbance was measured at 600 nm against reagent blank. The reagent blank was made by the addition of supernatant from the homogenate immediately after the incubation period. Maltose solution was used as standard. One unit of amylase was defined as the amount of enzyme required to produce 1.0 µ mol of maltose per minute.

Lipase activity

Lipase activity was determined following the method described by Furne *et al.*, (2005), consisting of degrading triacylglycerol to free fatty acids. A solution of 1% polyvinyl alcohol (PVA) and 5 ml of 0.1 N HCl in 1 L of distilled water was heated to 75-85ºC, cooled, filtered, and adjusted to pH 8.0 with 0.1 N NaOH. Virgin olive oil was added to an aliquot of the previous solution to obtain a substrate concentration of 0.1 M. This mixture was emulsified for 5 min. In addition, Mcllvaine buffer was prepared in 0.1 M citric acid and 0.2 M disodium phosphate. A reaction mixture containing PVA solution emulsified substrate (1 ml), Mcllvaine buffer at pH 8.0 (0.5 ml), and supernatant from the homogenates (0.5 ml) was incubated for 4 h at 37ºC. Afterward, 3 ml of 1:1 ethanol acetone solution was added to stop the reaction and break the emulsion. Phenolphthalein in ethanol 1% (w/v) was added to the reaction mixture and titrated with 0.01 M NaOH. For the blanks, the same procedure was followed but a boiled enzyme was used. Porcine type II pancreatic lipase was used as the standard. One unit of lipase was defined as the amount of enzyme required to hydrolyze 1.0 micro equivalent of fatty acids from triacylglycerols in 1 h at pH 8 and 37ºC.

Cellulase activity

Cellulase activity was determined by the method of Maria del Carmen González-Peña *et al.*, 2002. The gastric fluid from individual prawns was transferred to 1.5ml centrifuge tubes, homogenized in 1 volume of 10 mM ice-cold sodium citrate at pH 7.0 with a motorized homogenizer at maximum speed for 1 min, and centrifuged at 4ºC at 10,000 g for 3 min. The supernatant was then transferred to a new tube and stored at -20ºC until analysis. The supernatant was then treated as for gastric fluid. Take 1 ml of 1% microcrystalline cellulose, 1 ml of 0.1 M phosphate buffer, and 1 ml of enzyme extract solution in a test tube. Incubate the test tubes for 1 h at 37°C. After 1 h, stop the reaction by the addition of 0.5 ml of dinitro salicylic acid reagent. Note the absorbance at 540 nm. Deduce the value from the standard curve prepared using glucose. One unit of cellulase is defined as the amount of enzyme per ml which releases one µg of glucose per minute.

Statistical analysis

The results presented are mean ± SD (n = 3). The data are analyzed using one-way ANOVA and the differences among mean were compared by Duncan's multiple range test. All tests were conducted with a significance level of $P < 0.05$.

Results and Discussion

Biochemical constituents of experimental feeds

Analyzing the results of biochemical constituents of different probiotic *Spirulina fusiformis feed* types (SF), the protein percentage varied considerably among 1,2,3,4 and 5% inclusion. The crude protein was highest (43.47%) in the SF5 feed type (5% inclusion of *Spirulina)* and lowest (40.17%) in the SF1 (1% inclusion of *Spirulina*) feed type. From the data presented in Table 1, it could be observed that the higher percentage values of carbohydrate (41.8. & 41.63%) and fat (3.90% & 3.86%) were recorded in SF4 and SF5 feed types. On the other hand, the lower percentage of carbohydrates (38.54%), and lower percentage of fat (3.26%) were studied in SF2 and SF3 feed types respectively (Table 2). These results agree with the findings of Abdulrahman (2014), who reported that the percentage of Spirulina diet was 34-40%.

Table 1: The ingredients used in the probiotic *Spirulina fusiformis* contain experimental fish feed.

Sl.No	Ingredients	Feed types and quantity of each ingredient (g/100g)					
		Control (0%)	SF1(1%)	SF2 (2%)	SF3 (3%)	SF4 (4%)	SF5 (5%)
1	Dried silk moth powder	20.13	20.89	20.89	20.89	20.89	20.89
2	Groundnut oil cake	17.15	16.51	15.51	15.51	15.51	15.51
3	Rice bran	12.75	12.63	12.63	11.63	11.63	11.63
4	Corn flour	18.35	17.17	17.17	17.17	16.17	15.17
5	Wheat flour	17.35	17.17	17.17	17.17	17.17	17.17
6	Tapioca powder	10.27	10.63	10.63	10.63	10.63	10.63
7	Cod liver oil	2.00	2.00	2.00	2.00	2.00	2.00
8	Vitamins & Minerals	1.00	1.00	1.00	1.00	1.00	1.00
9	Agar	1.00	1.00	1.00	1.00	1.00	1.00
10	*Spirulina fusiformis* powder	-	1.00	2.00	3 .00	4.00	5.00
	Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Table 2: Experimental diets included biochemical components of the probiotic *Spirulina fusiformis.*

S. No	Probiotic feed types	Crude Protein (%)	Crude carbohydrates (%)	Crude Lipids (%)
1	CONTROL	39.78 ± 0.09	37.18 ± 1.79	3.18 ± 0.03
2	SF1	40.17 ± 0.14	39.16 ± 0.42	3.84 ± 0.33
3	SF2	41.18 ± 0.33	38.54 ± 0.33	3.54 ± 0.01
4	SF3	41.00 ± 0.06	40.00 ± 2.20	3.26 ± 0.16
5	SF4	41.86 ± 0.57	41.80 ± 0.66	3.90 ± 0.05
6	SF5	43.47 ± 0.33	41.63 ± 0.89	3.86 ± 0.02

Digestive enzyme activities of fish fed with *Spirulina fusiformis* diet (SF-feeds).

The present study described the development of digestive enzymes (Lipase, protease, cellulose, and amylase) in carp *Cirrhinus mrigala* fed on *Spirulina fusiformis* as a probiotic supplement. The results are presented in Table 3. When *Spirulina fusiformis* food is offered at different incorporation levels (1, 2, 3, 4, 5 %). The specific activity of amylase increased 176.17 ± 2.71 U/µmol in experimental fish fed on SF 5 diet (5% inclusion). A decrease in the activity of amylase 100.90 ±1.15, 114.18 ± 4.20 U/µmol was observed in the control and SF1 diets (1% inclusion) respectively. High amylase values obtained may be associated with better performance of the *Spirulina* as supported by different authors on some species of grouper fish (Harikrishnan *et al.*, 2011) Nile tilapia (Ngamkala *et al.*, 2010) and Faheem *et al.,2022)* in Grass Carp (*Ctenopharyngodon idella*). Similar results have been reported in other carps fed on *the Spirulina* supplement diet; *Spirulina* is highly beneficial to the fish because it improves the intestinal amylase activity

leading to the accumulation of carbohydrates and protein-sparing effect (Delhi Bai, 2016). Various workers have demonstrated that amylase activity is greater in herbivorous fish like carp. The highest amylase activity was found in SF5 diet-fed groups, this might be due to the formulated *Spirulina* feed SF 5 containing the higher carbohydrate 41.63 ± 0.89% level (Table 3)

Table 3: The digestive enzyme activity of fish that are fed with the probiotic *Spirulina fusiformis* feed.

S.No	Feed	Lipase(U/µmol)	Protease(U/µmol)	Cellulase(U/mg)	Amylase(U/µmol)
1	Control	4.02 ± 0.05	29.36 ± 0.17	31.68 ± 0.52	100.90 ± 1.15
2	SF1	4.18 ± 0.03^{c}	40.16 ± 0.99^{b}	47.09 ± 1.62^{b}	114.18 ± 4.20^{b}
3	SF2	4.45 ± 0.20^{c}	46.68 ± 0.51^{b}	40.65 ± 0.45^{b}	126.19 ± 2.50^{b}
4	SF3	4.67 ± 0.13^{c}	48.59 ± 0.61^{b}	42.45 ± 0.44^{b}	136.22 ± 1.08^{b}
5	SF4	4.94 ± 0.51^{c}	47.27 ± 0.74^{b}	47.69 ± 0.55^{b}	153.89 ± 0.56^{b}
6	SF5	585 ± 0.31^{c}	53.97 ± 0.68^{b}	56.19 ± 0.06^{b}	176.17 ± 2.71^{b}

Values are expressed as mean ± S.E.M. Mean values with different superscripts are significantly different from each other as indicated by one way ANOVA where a-P< 0.05, b- p<0.0001, c- NS

The cellulase activity was highly influenced by *Spirulina* diets. A sharp decline in cellulase activity (31.68 ± 0.52 U/mg) was observed in the fish fed with the control diet. The increased cellulase activity in the gut (56.19 ± 0.06 U/mg) was influenced by the higher (5%) inclusion level of *Spirulina fusiformis* (SF5). The exogenous cellulase was secreted in the digestive tract of carp fed with plant proteins. The cellulase enzyme secretion was positively correlated with the amount of plant as well as algal detritus in gut carp. Cellulase activity has been detected earlier in carp fingerlings (Lesel *et al*., 1986), carps showed relative cellulase enzyme activity due to the presence of natural complex cellulose-containing plant ingredients (wheat flour, corn flour, Tapioca & Rice bran) and microalgae products in the diet (table-3). The presence of cellulase enzyme reveals that *Cirrhiuns mrigala* fingerlings are a consumer of a complex diet. The potential of cellulase activity suggests that could avail all the necessities to provide more carbohydrates in the diet.

The lipase activity was found to be significantly higher in the treatment groups than in the control group. The highest lipase activity (5.85 ± 0.31 U/µmol) was observed in the gut of fingerlings fed with SF5 diet (5% *Spirulina* inclusion), whereas the lowest lipase activity (4.02 ± 0.05, 4.18 ± 0.03 U/µmol) was recorded in fish fed with control and SF1 (1% inclusion) diet. Lipase activity is correlated with lipid digestion and lipid absorption. The lipase activity is low in fishes fed with *Spirulina* feeds when compared to other digestive enzymes; because the lipid level of *Spirulina* feed type is lower than carbohydrate and protein (table-3). The results were in line with the findings of Chakrabarti *et al*., (1995), who found that lipase enzymes may be of particularly important because fish utilize lipids as their main nutritional source. The present observations corroborate the results of Mukhopadhyay and Rout (1996) in fry of *Catla catla*, the least lipid activity may be due to the lack of lipid in the diet. Fagbenro *et al*., (1993) reported that lipase activity was average and restricted to the gut region of the *H.bidrosalis* fed with *Spriulina* diet. *Spirulina* diet enhances lipase activity than control diet-fed fishes; similar results have also been reported for other fish species fed on *Spirulina* (Gaylord *et al*., 2008).

Significantly higher protease (53.97± 0.68 U/µmol) was observed in *Cirrhinus mrigala* fingerlings when fed on the SF5 diet. The lower level of protease (29.36 ± 0.17 and 40.16 ± 0.99) was found in the control and SF1-fed fish groups. Similarly variable levels of proteases (46.68 ± 0.51, 48.59 ± 0.61 and 47.27 ± 0.74 U/µmol) were recorded in SF2, SF3 and SF4 diets respectively. Similar to the present work, protease enzyme secretion was favorable in fish fed with *Spirulina-based* diets. *Spirulina* diet also significantly improves protein utilization and protease enzyme activity in tilapia (Hussain, 2012). In *Spirulina* feed, protease enzyme activity was increased with increasing dietary *Spirulina* level (1 to 5%). The present results also agree with Fagbenro *et al*., (2001) and Mohammadiazarm *et al., (*2020) who reported that, high protease activity in the stomach of catfish and on Oscar fish, *Astronotus ocellatus* respectively. The high protease activity recorded in this study can be attributed to *Cirrhinus mrigala* consuming protein-rich *Spirulina* diets (PS-diets). *Spirulina* was found to enhance intestinal protease activity and total body protein which ultimately results in faster growth of *Cyprinus carpio* fingerlings in a shorter period (Delhi Bai, 2016).

The experimental *Spirulina fusiformis* diets were protein-rich and hence the gut extract of the treatment group showed higher (40.16 – 53.97 U/µmol) protease activity than the control 29.36 U/µmol) fish group. This result corroborates with the prior report where increased protease enzyme activity in carp was observed with a protein basal diet. Gastrointestinal digestive enzyme activities are in close correlation with food absorption. The food absorption was high in fish fed with SF5 feed, which has higher enzyme activities.

The results of digestive enzyme activities in fish fingerlings fed with *Spirulina fusiformis* diets are also in agreement with the previous research findings that the type of food can influence enzyme activity (Caruso *et al.*, 2009). Fingerlings fed with 5% *Spirulina fusiformis* incorporated basal feed showed better digestive enzyme activities and significantly higher protease activities compared with control and other concentrations of *Spirulina* treatments, protease enzymes play a considerable role in food digestion, which might in turn contribute to the better food absorption in experimental fish *Cirrhinus mrigala* fingerlings. Results are similar to the findings of Jobling (1995) and Wang (2011).

Conclusion

The results of this study indicate that feeding *Cirrhinus mrigala* fingerlings with probiotic *Spirulina fusiformis* supplemented diets enhanced the activity of the digestive enzymaes like protease, lipase, cellulose and amylase.Thus supplementation of *Spirulina fusiformis* along with fish diet can improve all the enzymes involved in digestive process which ultimately increase all the metabolic activities and enhances the growth.

References

1. Abdulrahman,N,M and Hamad Ameen, H,J.(2014). Replacement of fishmeal with microalgae Spirulina on common carp weight gain, meat and sensitive composition and survival. *Pakistan Journal of Nutrition.* 13 (2),93-96.
2. Caruso,G., Denaro,M.G. and Genovese, L. (2009). Digestive enzymes in some teleost species of interest for Mediterranean aquaculture. *The open fish science journal.***2**, 74-86.
3. Chakrabarti.I., Gani, Md, A.,Chaki,A and Sur.R., Mirsa.K.K , 1995, Digestive enzymes in 11 freshwater teleost fish species in relation to food habit and niche segregation. *Comp. Biochem. physiol.*112, 167-179
4. Chantal Cahu. and Jose Luis Zambonino-Infante, (2004). Substitution of live food by formulated diets in marine fish larvae. *Aquaculture.* 200(1-2), 161-180.
5. Delhi Bai, S. (2016), Effect of dietary supplementary feed of Spirulina on digestive enzyme in fingerlings of common carp (*cyprinus carpio*). *Eurepean Journal. of pharmaceutical and medical research* ,3(8), 421-426.
6. Fagbenro,O,A., Adedire, C,O., Owoseeni, E,A and Ayotunde, E.O,(1993), Studies on the biology and aquaculture potential of feral catfish *Hetorobrnachus bidorsalis.* (GeoffroySt. Hilaire 1809) (Clariidae) *Trop.Zool.***6**; 67-79.
7. Fagbenro,O,A., Adedire, C,O & Aiyegbeni,M,L., (2001), Food composition and digestive enzymes in the gut of the African Catfish, *Malapterurs electicus,* (Gmelin 1789)(Malapteru AE) *Trop.Zool.* 1, 14-16.
8. Faheem, M., Jamal,R., Nazeer ,N., Khaliq,S Hoseinifar,S.H.,Doan,H.V. and Paolucci,M.(2022), Improving Growth, Digestive and Antioxidant Enzymes and Immune Response of Juvenile Grass Carp (*Ctenopharyngodon idella*) by Using *Dietary Spirulina platensis.* Fishes, 7, 237
9. Furne, M., Hidalgo, M, C., Lopez, A., Garcia Gallego, M., Morales, A, E., Domezain, A., Domezaine, J, and Sanz, A., (2005), Digestive enzyme activities in Adriatic *sturgeon Acip- enser naccarii* and rainbow trout *Oncorhynchus mykiss*, A comparative study. *Aquaculture.* 250 (1-2), 391–398.
10. Fu,Y., Sies, H & Lei, X,G., (2001), Opposite roles of selenium-dependent glutathione peroxidase-1 in superoxide generator diquat- and peroxynitrite-induced apoptosis and signaling. *J Biol Chem,*276(46),43004–9.
11. Gaylord, T ,G., Barrows, F, T and Rawles, S, D. (2008), Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for rainbow trout, *Oncorhynchus mykiss. J. World Aquacult. Soc.*, 39 (6), 827–834.
12. Gisbert, E., Gimenéz, G., Fernández, I., Kotzamanis, Y & Estevéz, A., (2009), Development of digestive enzymes in common dentex *Dentex dentex* during early ontogeny. *Aquaculture*, 287(3-4), 381–387.
13. González-Peña, M,C., Anderson,A,J, Smith,D,M and Moreira,G,S., (2002), Effect of dietary cellulose on digestion in the prawn *Macrobrachium rosenbergii. Aquaculture*, 211 (1-4), 291-303.
14. Govind Pandey. (2013), Feed formulation and feeding technology for fishes. *Int. Res. Jr. Pharmacy*,4 (3),23-30.

15. Harikrishnan,R., Balasundaram ,C and Heo, M,S.(2011), Lactobacillus Sakei B.K. 19 enriched diet enhances the immunity status and disease resistance to kelpgruper *Epinephelus bruneus*. *Fish shelfish Immunol.***29** (6) 1037-1043.
16. Hidalgo,M,C., Skalli, A., Abellan,E., Arizcun, M and Cardenete, G. (2006), Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex* L.):Effects on growth performance, survival and liver proteolytic activities. *Aquacult. Nutr.*12 (4), 256-266.
17. Hussain, M, (2012), Effect of feed manure and their Combination on the growth of cyprinus carpio fry and fingerlings. *Egypt.J. Aquat. Bio. Fisheries.* 16 (2) 153-168.
18. Jobling, M., (1995), Digestion and Absorption. In.Jobling.M.(ed); Environmental Biology of Fishes. Chapman and Hall. London, UK. 175-210.
19. Lesel, R., Fromageot, C and Lesel, M. (1986), Cellulose digestibility in grass carp, *Ctenopharyngodon idella* and in goldfish, *Carassius auratus Aquaculture* .54 (1-2),11-17.
20. Lu.J., Yoshizaki, G, Sakai,K and Takeuchi, T., (2002) ,Acceptability of raw *spirulina platensis* by larval tilapia *Oreochromis niloticus. Fish sci,* 68(1),51-58.
21. Maria del Carmen González-Peña, Gomes, S. Z. and Moreira,G.S. (2007), Effects of Dietary Fiber on Growth and Gastric Emptying Time of the Freshwater Prawn *Macrobrachiurn rosenbergii* (De Man, 1879), J.World aquac .Soc. 33(4),441-447.
22. Maurilio Lara-Flores and Olvera-Novoa,M.A, (2013), The use of lactic acid bacteria isoloated from intestinal tract of nile tilapia (*Oreochromis niloticus*) as growth promoters in fish fed low protein diets. *Lat. Am.J.Aquat. Res.*41 (3),490-497.
23. Mohammadiazarm, H., Maniat,M., Ghorbanijezeh,K. and Ghotbeddin,N.(2020),Effects of spirulina powder (Spirulina platensis) as a dietary additive on Oscar fish, Astronotus ocellatus: Assessing growth performance, body composition, digestive enzyme activity, immune-biochemical parameters, blood indices and total pigmentation. Aquaculture Nutrition 27(1):252-260
24. Mukhopadhyay,P,K and Rout,S,K.,(1996), Effects of different dietary lipids on growth and tissue fatty acid changes in fry of the carp Catla catla (Hamilton). *Aquaculture research*, 27 (8), 623-630.
25. Ngamkala, S., Futami, K., Endo, M., Maita, M and Katagiri, T. (2010), Immulotgical effect of glucan and *Lactobacillus rhamnons* a probiotic bacterium on Nile tilapia *Oreochromis niloticus* intestine with oral *Aeromonas challges*. *Fisheries Sci.***76;** 833-840.
26. Odedeyi,D,O and Fagbenro,O,A.(2010), Feeding habits and digestive enzymes in the gut of *Mormyrus rume (Osteichthyes mormyridae*). *Trophical Zoology*, 23(1):75-89.
27. Rafael Lazzari., João Radünz Neto., Fabio de Araújo Pedron., Vania Lucia Loro., Alexandra Pretto. and Carolina Rosa Gioda (2012), Protein sources and digestive enzyme activities in jundia (*Rhamdia Quelen*). *Sci. Agric.*67(3) 259-266.
28. Seenivasan,C, Radhakrishnan, S,Muralisankar, T & Saravana Bhavan,P.,*(2012),Bacillus subtilis* on survival, growth, biochemical constituents and energy utilization of the freshwater *prawn Macrobrachium rosenbergii* post larvae. *Egyptian journal of aquatic Research*, 38 (3), 195-203.
29. Wang,Y. (2011), Use of probiotics Bacillus coagulans, *Rhodopseudomonas palustris* and *Lactobacillus acidophilus* as growth promoters in grass carp (*Ctenopharyngodon idella*) fingerlings. *Aquac. Nutr.*, 17(2),e372 –e 378.