Journal of Research in Biology

ISSN No: Print: 2231 –6280; Online: 2231- 6299

An International Scientific Research Journal

Journal of Research in Biology

Original Research

Effect of vitamins on digestive enzyme activities and growth performance of striped murrel *Channa striatus*

Authors:
Jeya Sheela P,
Haniffa MA,
Vigneshwaran R and
Kavitha K

Institution:
Centre for Aquaculture Research and Extension (CARE), St. Xavier's College, Palayamkottai, Tamilnadu– 627002, India.

Corresponding author:
Jeya Sheela P

ABSTRACT:

Five diets (diets incorporated with folic acid, vitamin B, vitamin C, vitamin/ mineral mix and control) were used to determine their effects on survival, digestive enzyme activity, protein profiling and growth of striped snakehead *Channa striatus* fingerlings. All vitamin diets showed significant difference in enzyme activity like protease, amylase and lipase, when compared to that of control. The fingerlings fed with vitamin C diet showed significantly (P<0.05) better performance in terms of survival rate, weight gain and Specific Growth Rate (SGR) when compared to the rest of the four diets. The electrophoretic studies revealed that high molecular weight fractions were added up in muscle tissue of *C. striatus* fed with vitamin and mineral supplemented diets.

Keywords:

Channa striatus, Vitamins, Growth, Survival, Enzyme activity.

Email Id:

sheela.care2011@gmail.com

Article Citation:

Jeya Sheela P, Haniffa MA, Vigneshwaran R and Kavitha K

Effect of vitamins on digestive enzyme activities and growth performance of striped murrel *Channa striatus*

Journal of Research in Biology (2017) 7(8): 2377-2385

Dates:

Received: 02 Oct 2017 **Accepted:** 28 Oct 2017 **Published:** 26 Nov 2017

Web Address:

http://jresearchbiology.com/documents/RA0602.pdf

INTRODUCTION

Vitamins are complex organic substances usually of low molecular weight, which are essential for a wide variety of metabolic processes. They are required in negligible quantities and are basic for fish growth, multiplication and general health. Fish can't produce vitamins and must obtain from diets. Fish raised in cages and other intensive culture systems must be supplemented with rich feeds containing vitamin supplements for better survival and development. Minimum requirement of about 15 essential vitamins have been noted for channel catfish (Andrews and Murai, 1974), common carp (Hwang and Lin, 2002) and juvenile tilapia (Shiau and Jan, 1992). The vitamin levels prescribed for pellet diets for fish are around 25% to 100% above least levels to avoid deficiency symptoms. Fish nutritionists have demonstrated that in order to promote growth, any diet must include an energy source, essential amino acids, fatty acids, vitamins and minerals.

Detailed knowledge of digestive physiology of fish is essential to promote maximum growth in fish contributed by tissue protein synthesis (Blier *et al.,* 1997). Presence and activity of certain enzymes may be used as a comparative indicator of survival rate and development (Chan *et al*., 2004). Moreover, enzyme activity depends on food content of the corresponding substrate to a greater extent. Information on the intestinal enzyme activities is essential to know the digestive efficiency of the cultured species at the particular stage of development besides that, it is equally important to characterize and to know the classes of the digestive enzyme for a better understanding for the course of action of feed in the digestive physiology. The overarching worldview is that digestive enzyme activities in fishes are characteristic of feeding environment, associating admirably with diet (Kapoor *et al*., 1975; Fernandez *et al*., 2001). Animals are believed to be flexible in their digestive enzyme synthesis in light of eating regimen, due to the fact that the metabolic cost of delivering a lot of digestive enzymes would be wasted by animals ingesting low levels of the substrates for those enzymes (Karasov, 1992; Caviedes-Vidal *et al*., 2000).

The striped murrel, commonly called snakehead, *Channa striatus*, is a freshwater fish which is a carnivorous, obligatory air breather, well known for its taste, high nutritive value, recuperative and medicinal qualities (Khanna, 1978) and is a valuable food fish in Asia (Haniffa *et al*., 2006). This study was designed to evaluate the application of different vitamins as a feed additive in the diet of *Channa striatus* fingerlings.

MATERIALS AND METHODS

C. striatus fingerlings (length: 6.5 ± 1.1cm; weight 10 ± 0.49g) reared from CARE (Centre for Aquaculture Research and Extension) Aquafarm were acclimatized in cement tanks (3.2m x 1m x 1.2m) for seven days. During acclimatization, the fishes were fed with control feed (Table 1) twice a day at 5% of its body weight. The acclimatized fingerlings were randomly selected and distributed into (3.2m x 1m x 1.2m) cement tanks filled with well water at a stocking rate of 30 fingerlings per tank. Triplicates were maintained for each of the five experimental feed (Table 1). Diet 1 served as control diet D_1 was without vitamins and the remaining four diets were incorporated with vitamins and minerals *viz.,* D_2 with folic acid, D_3 with vitamin B, D_4 with vitamin C and D_5 with vitamin - mineral mix (Table 1). Proximate composition of the feed ingredients was measured according to AOAC (1990). The fingerlings were fed 5% of their body weight twice per day for 45 days. Once in a fortnight, water change was done partially in each tank. Survival was recorded and fingerlings were weighed at 15-days interim to predict feeding rate, weight gain, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR).

To analyze the digestive enzyme activity, the intestine of the fishes homogenized (1:2w/v) with 50

Table 1. Feed formulation of different diets

S. No	Ingredients (%)	D_1	D_2	D_3	D_4	D_5
1	Fish meal	40	40	40	40	40
2	Clam meal	10	10	10	10	10
3	Soyabean flour	10	10	10	10	10
4	Tapioca flour	10	10	10	10	10
5	Wheat flour	10	10	10	10	10
6	Rice flour	10	10	10	10	10
7	Rice bran	7.5	7.5	7.5	7.5	7.5
8	Groundnut oil	2.5	2.5	2.5	2.5	2.5
9	Folic acid	−	0.02	−	−	−
10	Vitamin – B	−	−	0.02	−	−
11	Vitamin – C	−	−	−	0.02	−
12	Vitamin / Mineral mix*	−	−	−	−	0.02

*Vitamin - Mineral premix (mg kg^{-1} diet): riboflavin(80mg), biotin (6.0mg), vitamin A (6000 IU), vitamin D_3(2000 IU), vitamin E (6000 IU), nicotinic acid (0.1 mg), thiamin hydrochloride (0.6 mg), pyridoxine (15 mg), inostol (40 mg), astaxanthin (400 mg), choline chloride (60 mg), Zinc (as sulphate) (-72 mg), iron (as sulphate) (-36 mg), manganese (as sulphate) (-12 mg), selenium (as selenate) (-0.2 mg), chromium (trivalent, as chloride) (-0.8 mg), iodine (as iodide) (-1.2 mg), copper (as sulphate) (-24 mg), cobalt (as chloride) (-0.6 mg) and molybdenum (as molybdate) (-0.2 mg).

mM Tris – HCl buffer (pH 7.5) in a water bath, using a tissue homogenizer. The preparation was centrifuged at 10,000 rpm for 15 minutes at 4°C. The floating lipid fraction was removed and the aqueous supernatant was recovered and kept at -20°C until use. Soluble protein content was evaluated using Lowry *et al*. (1951), protease activity was assayed according to Anson (1938) and Folin (1929), amylase activity following Jiang (1982) and Worthington and WBC (1993) and expressed as specific activity (Umg^{-1} protein) and lipase activity based on methods of Borlongan (1990) and expressed as Umg^{-1}. Statistical analysis was done using one-way analysis of variance.

For SDS-PAGE analysis, the muscle tissue of the fish was sliced into smaller pieces and kept at -20°C prior to freeze-drying. Freeze dried tissue was homogenized to powder form. Extraction of protein from snakehead fish muscle tissue was carried out following Gam *et al*. (2006). Briefly, 1.0 mg of powdered fish muscle was extracted using 1 ml of 40 mM Tris (pH 8.8), vortexed and centrifuged at 12,000 rpm for 30 minutes at room temperature and the supernatant was recovered. SDS-PAGE was performed as described by Laemmli (1970) in 12% polyacrlyamide gel. Protein samples were then loaded and electrophoresis was performed at a constant voltage of 200 - 245 V. The run was stopped when the dye front was 2 to 3 mm away from the bottom edge of the gel. At the completion of electrophoresis, the glass sandwich was disassembled. The stacking gel was discarded and

Table 2. Proximate composition of feed ingredients

S. No	Ingredients	Protein (%)	Carbohydrate (%)	Crude fat (%)
1	Fish meal	76.24	1.7	10.3
2	Clam meal	30.24	1.9	12.2
3	Soyabean flour	26.58	29.5	0.3
4	Tapioca flour	13.9	38.9	0.4
5	Wheat flour	19.7	32	11.9
6	Rice flour	6.88	15.63	0.18
7	Rice bran	2.72	5.04	0.06

Table 3. Body composition of *C. striatus* fed different diets

S. No	Diets	Crude protein (%)	Crude carbohydrate (%)	Crude fat (%)
1	Control (D_1)	30.58±2.19	17.57±1.29	11.65±0.72
2	Folic acid diet (D_2)	33.75±1.60	15.57±1.19	10.82±1.01
3	Vitamin B diet (D_3)	34.80±1.07	15.14±1.06	10.20±0.93
4	Vitamin C diet (D_4)	36.91±1.64	13.14±1.32	9.75±0.97
5	Vitamin - Mineral mix diet (D_5)	35.52±2.10	14.00±1.09	9.68±0.84

the resolving gel was stained using coomassie brilliant Blue. Molecular weights of the proteins were determined by comparing relative mobility of protein bands to the standard protein markers. The presence or absence of the protein bands were scored for similarity index studies. The dendrogram was constructed based on the similarity index values using UPGMA algorithm in NTSYS-pc, version 2.01 and the molecular weight of the protein bands were calculated using Total Lab 100 software.

$$\text{Similarity index } (S) = \frac{\text{Number of common bands}}{\text{Total number of bands}} \times 2$$

The similarity index was calculated according to Lamont *et al*. (1986).

RESULTS

Mean temperature (28 ± 1.5ºC), dissolved oxygen (5.9 ± 0.6 mg/l), and total ammonia (0.5 ± 0.2 mg/l) were recorded during the feeding trial. Proximate composition of feed ingredients was recorded in Table 2 and the body composition of *C. striatus* fed on different diets was given in Table 3. The highest survival rate (93%) was observed in (93.5±2.56%) vitamin C and (93.7±1.81%) vitamin - mineral mix incorporated diets. Significant differences (P<0.05) in the survival rate were noticed as a function of vitamin C and vitamin - mineral mix incorporated diets when compared to folic acid (92.1±1.56%), vitamin B (91.6±1.81%) incorporated diets and control diet (82.3± 2.36%) as illustrated in Table 4. After 45 days, there was a significant difference between the mean weights as a function of different experimental diets (D_1 – D_5), when compared to control diet.

The highest weight gain (11.46±0.52g) was noticed in *Channa striatus* fed on diet incorporated with vitamin C. The mean weight gain of *C. striatus* fingerlings for each vitamin diet (8.61 to 9.13 g) was significantly higher than that of the control (7.03±0.11g)**.** Among the experimental fishes, the highest protein content was noticed in vitamin C incorporated diet (36.91%), followed by vitamin - mineral mix incorporated diet (35.52%), vitamin B incorporated diet (34.80%), folic acid incorporated diet (33.75%) and control (30.58%). The highest carbohydrate content was found in control diet (17.57%) whereas the lowest carbohydrate content was found in vitamin C (13.14%). Fat was found as the highest in control (13.65%) and the lowest in vitamin C incorporated diet (10.75%).

After 45days of culture, the activity of digestive enzymes observed as a function of Vitamin diets showed significant difference when compared to that of the control. The protease activity was significantly higher in vitamin C diet (110.58±2.49 Umg^{-1}), followed by

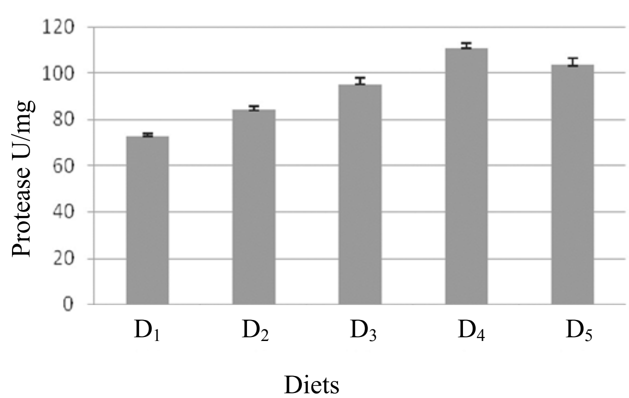

Figure 1. Protease activity of *Channa striatus* fingerlings fed on test diets

Table 4. Growth performances of *Channa striatus* fed with experimental diets

S. No	Growth parameters	Control (D_1)	Folic acid Diet (D_2)	Vitamin B Diet (D_3)	Vitamin C Diet (D_4)	Vitamin - Mineral mixed Diet (D_5)
1	Initial weight (g)	10.8±0.09	10.6±0.12	10.7±0.45	10.6±0.67	10.8±0.36
2	Final weight (g)	17.83±0.11	19.11±0.34	20.15±0.49	22.06±0.52	20.93±0.48
3	Survival rate (%)*	82.3±2.36	92.1±1.56	91.6±1.81	93.5±2.56	93.7±1.81
4	Weight gain (g)**	7.03±0.11	8.61±0.31	9.45±0.45	11.46±0.52	9.13±0.39
5	SGR***	1.11±0.28	1.33±0.09	1.56±0.14	1.62±0.23	1.49±0.04
6	FCR ****	3.42±0.01	2.75±0.02	2.51±0.04	2.06±0.01	2.43±0.03

vitamin mineral mix diet(103.41±2.92 Umg^{-1}), vitamin B diet (95.21±2.77 Umg^{-1}), and folic acid diet (84.12±1.58 Umg^{-1}) (Figure 1). The average value of amylase activity was significantly higher in vitamin C diet (53.50±2.49 Umg^{-1}) when compared to all other diets (Figure 2). The average value of lipase activity in the intestine of *C. striatus* was higher in vitamin C diet (93.80±1.42 Umg^{1}) (Figure 3). All Vitamin diets showed difference in enzyme activity as a function of nature of vitamin incorporated and the difference was statistically significant when compared to that of the control. With regard to growth parameters, control diet produced least growth which showed variation when compared to vitamin diets. From the SDS-PAGE analysis, more number of protein bands indicating increase in qualitative protein in fish fed with vitamin and mineral supplemented diets were observed. A total of 63 bands were observed and the similarity coefficients ranged from 0.64 – 0.95. More number of bands (16) occurred in vitamin C diet fed fish while the least (9) in control diet fed fish. Molecular weight of the fish muscle tissues varied from 1.9 kDa to 153 kDa (Table 5). High molecular weight protein bands were observed in the four diets supplemented with vitamin and mineral diets.

DISCUSSION

Vitamin requirements of fish have been reported to vary with species, size or age, protein quality, level of energy, water quality, presence of natural food, feeding and culture management. In the present study, vitamin supplemented diets resulted in an increase in weight gain and specific growth rate of *C. striatus.* The results showed that survival and growth rate of fingerlings increased ($P < 0.05$) with 200 mg of vitamin C / Kg feed. The present finding correlated with those of Adham *et al*. (2000), who stated about the dietary requirement of ascorbyl sulphate as vitamin C sources in *Clarias gariepinus.* Gupta (2006) investigated the relative efficiency of various level of dietary vitamin E on SGR and FCR of *Labeo rohita* fingerlings. The

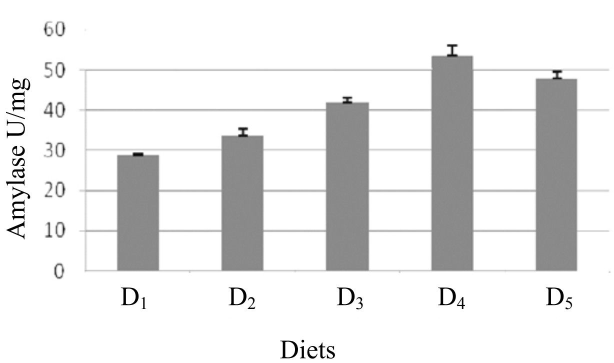

Figure 2. Amylase activity of *Channa striatus* fingerlings fed on test diets

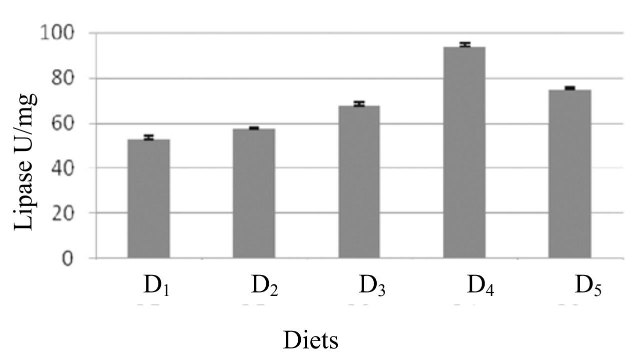

Figure 3. Lipase activity of *Channa striatus* fingerlings fed on test diets

Table 5. Molecular weight and Rf values of protein isolated from *C. striatus* fingerlings fed on different experimental diets

Ref Band No	D_1		D_2		D_3		D_4		D_5	
	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf
1	-	-	149.4	0.66	149.9	0.06	153.8	0.05	151.3	0.06
2	-	-	136.5	0.09	130.2	0.10	143.4	0.07	142.2	0.07
3	-	-	-	-	-	-	134.3	0.09	130.7	0.10
4	112.8	0.13	109.1	0.14	114.3	0.13	127.2	0.10	112.4	0.14
5	89.81	0.19	-	-	101.7	0.16	107.1	0.15	102.2	0.16
6	-	-	90.6	0.19	86.36	0.2	94.7	0.18	94.7	0.18
7	66.98	0.25	-	-	-	-	84.4	0.20	84.7	0.20
8	-	-	-	-	-	-	48.47	0.30	-	-
9	51.30	0.29	59.5	0.27	61.81	0.26	37.4	0.34	53.2	0.29
10	-	-	44.6	0.32	44.4	0.32	-	-	40.9	0.33
11	39.18	0.32	37.8	0.34	31.6	0.37	27.2	0.39	29.5	0.38
12	22.42	0.41	24.8	0.40	21.4	0.41	18.6	0.43	-	-
13	4.45	0.54	14.2	0.46	9.66	0.49	10.5	0.48	17.9	0.43
14	-	-	-	-	-	-	7.26	0.51	9.32	0.49
15	-	-	5.92	0.52	3.73	0.55	3.38	0.56	3.27	0.57
16	2.06	0.62	-	-	-	-	2.17	0.60	2.28	0.6
17	2.06	0.69	2.03	0.62	1.99	0.65	2.06	0.65	2.09	0.65

enhanced growth performance of *C. striatus* in the present study might be due to the increase in digestive enzyme activity as induced by the vitamins. Our findings were similar to the increase in digestibility by enzyme activities as demonstrated by Ghosh *et al*. (2002) in rohu (*Labeo rohita*). It is normal that tissue levels achieve a most extreme or saturation level past which they don't increase with additionally increase in dietary concentration; the excess intake is discharged or metabolized (Sahoo and Mukhopadhyay, 2008). The reduced growth performances in fish fed on control diet clearly indicated the vitamins had a specific effect on growth of fish as already suggested by Ram (1966).

Carnivorous fishes as often as possible show higher proteolytic enzyme activities, to process their high -protein animal dietary from foods (Fish, 1960; Cockson and Bourne, 1972; Reimer, 1982). Digestive enzyme activities in fishes, in any case, differ among species and can be influenced by age and in addition by the amount and composition of diet (Péres *et al*., 1998). The digestive enzyme activities and nutrient digestive values were affected by dietary composition, with carbohydrate and fat changes eliciting the strongest effect. Storebakken and Goswami (1996) reported the interrelationship between astaxanthin and vitamin A in Atlantic salmon. Mahajan and Agrawal (1980) reported that, the hatchlings of *Cirrhina mrigala,* fed purified diets with evaluated levels of crystalline ascorbic acid, the ideal necessity was 650-700 mg ascorbic acid/kg feed in light of weight gain, mortality and behavioral and morphological criteria. This level gives off an impression of being on the high side, potentially because of the utilization of crystalline ascorbic acid which is a unstable type of vitamin C (Sahoo and Mukhopadhyay, 2008). Mishra *et al*. (2007) reported better immunity, growth, and survival in rohu fingerlings after feeding diets containing 500 mg/kg ascorbic acid for eight weeks.

The incorporation of vitamin-C in diets enhanced digestive enzyme activities, including protease, amylase and lipase, which have subsequently brought about better growth. Vitamins could influence the enzyme activities in a different manner and vitamin C actuated a more intense stimulation than the others. This stimulation of amylase and protease activity was associated with the growth. The production of digestive enzymes can be regulated by genes, hormones, and food (Péres *et al*., 1998). The agreement is that protease, lipase, and amylase secretion and contents change relatively in light of the amount of their respective substrates (Lhoste *et al*., 1994).

Digestive enzyme activity can be prompted as a response to growth or attempting to drive more supplements from a deficient meal during digestion (Wormhoudt *et al*., 1980). The outcomes for *Octopus maya* (Aguila *et al*., 2007) recommended that the enzyme capacity of octopuses and, thus, food digestibility could be enhanced utilizing supplements that fortify enzyme secretion. In this sense, it was discovered that enzyme secretion can be directed by visual, chemical and endocrinological stimuli. The increase in the protein content of the *C. striatus* was illustrated by the protein banding pattern of the muscle tissue by SDS-PAGE.

CONCLUSION

In short, the results of the present study showed the effect of vitamins on the growth performance of *C. striatus*, and also on digestive enzymes. It is concluded that, use of 200 mg kg^{-1} supplement of vitamin C in *C. striatus* diet could be recommended based on results of this study. The electrophoretic studies revealed that high molecular weight fractions are added up in muscle tissue of *C. striatus* fed with vitamin and mineral supplemented diets. This clearly suggests that both qualitative and quantitative increase in the protein content of the fish.

ACKNOWLEDGEMENT

We acknowledge the financial assistance received from Indian Council of Agricultural Research – National Agricultural Innovation Project (ICAR-NAIP F.No. 1(5)/2007-NAIP dt. 22 August 2008) to carry out this study. We are grateful to Rev. Dr. A. Joseph, S.J., Principal and Consortium Leader, St. Xavier's College, for providing necessary facilities for our research work.

REFERENCES

Adham KG, Hashem HO, Abu-Shabana MB and Kamel AH. (2000). Vitamin C deficiency in the catfish *Clarias gariepinus*. *Aquaculture Nutrition*, 6(2): 129-139.

Alain Van Wormhoudt, Hubert Ceccaldi J and Bernard Martin. (1980). Adaptation de la teneur en enzymes digestives de l'hepatopancreas de Palaeomon serratus (Crustacea, Decapoda), a la compostition alimentes experimentaux. *Aquaculture,* 21(1): 63-78.

Andrews JW and Murai T. (1974). Studies on the vitamin C requirements of channel catfish (*Ictalurus punctatus*). *Journal of Nutrition*, 105: 557–561

Anson ML. (1938). The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. *Journal of General Physiology,* 22: 79-89

AOAC. (1990). Official methods of analysis. association of official analytical chemists. 15th ed., Inc., Arlington, Virginia, USA.

Ashok Gupta K. (2006). Effect of dietary vitamin E in relation to growth, feed conversion and deficiency symptoms for the fingerlings of *Labeo rohita* (Hamilton). *Fish Management and Aquatic Environment,* Chapter 5, 38 p.

Blier P, Pelletier D and Dutil JD. (1997). Does aerobic capacity set a limit on fish growth rate? *Reviews in Fisheries Science,* 5(4): 323–340.

Borlongan IG. (1990). Studies on the digestive lipases of milkfish, *Chanos chanos. Aquaculture,* 89(3): 315-325.

Caviedes-Vidal E, Afik D, Martı ´nez del Rio C and Karasov WH. (2000). Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. *Comparative Biochemistry and Physiology,* 125(1): 11–24.

Chan AS, Horn MH, Dickson KA and Gawlicka A. (2004). Digestive enzyme activities in carnivores and herbivores: comparisons among four closely related prickleback fishes (Teleostei: Stichaeidae) from a California rocky intertidal habitat. *Journal of Fish Biology*, 65(3): 848-858.

Cockson A and Bourne D. (1972). Enzymes in the digestive tract of two species of euryhaline fish. *Comparative Biochemistry and Physiology,* 41(4): 715-718.

Fernandez I, Moyano FJ, Dıaz M and Martınez T. (2001). Characterization of a-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). *Journal of Experimental Marine Biology and Ecology,* 262(1): 1–12.

Fish GR. (1960). The comparative activity of some digestive enzymes in the alimentary canal of tilapia and perch. *Hydrobiologia*, 15(1-2): 161–178.

Folin O and Ciocalteu V. (1929). On tyrosine and tryptophan determinations in proteins. *The Journal of Biological Chemistry,* 73: 627- 650.

Ghosh K, Sen SK and Ray AK. (2002). Growth and survival of rohu, *Labeo rohita* (Hamilton) spawn fed diets supplemented with fish intestinal microflora. *Acta Icthologica et Piscatoria*, 32(1): 83-92.

Haniffa MA, Dhanaraj M, Muthu Ramakrishnan C and Arthi Manju R. (2006). Simple herbal treatment for epizootic ulcerative syndrome in murrels (snakehead). *Aquaculture Asia*, 3: 18-21.

Hwang DF and Lin TK. (2002). Effect of temperature on dietary vitamin C requirement and lipid in common carp. *Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology,* 131(1): 1-7.

Josué Aguila, Gerard Cuzon, Cristina Pascual, Pedro Domingues M, Gabriela Gaxiola, Ariadna Sánchez, Teresita Maldonado and Carlos Rosasc. (2007). The effects of fish hydrolysate (CPSP) level on *Octopus maya* (Voss and Solis) diet: Digestive enzyme activity, blood metabolites, and energy balance. *Aquaculture*, 273 (4): 641-655.

Jiang CK. (1982). Activity measuring for implemental Enzyme. Science and Technology Press, Shangai.

Kapoor BG, Smit H and Verighina IA. (1975). The alimentary canal and digestion in teleosts. *Advances in Marine Biology*, 13: 109-239.

Karasov WH. (1992). Test of the adaptive modulation hypothesis for dietary control of intestinal transport. *American Journal of Physiology,* 267(3-2): R496–R502.

Khanna SS. (1978). An introduction to fishes. 2nd ed. Central Book Department. Allahabad. 428 p.

Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T_4. *Nature,* 227: 680-685.

Lamont RJ, Retire DT, Meluin WT and Postlethwaite R. (1986). An investigation of the taxonomy of *Listeria* species in comparison of electrophoretic patterns 1985–86, In Listeriose, Listeria, Listeriosis, eds. Courtiuuie AL, Espaze L and Rehaud AE. Nantes: Universite de Nantes. 41-46 p.

Lay-Harn Gam, Chiuan-Yee Leow and Saringat Baie, (2006). Proteomic analysis of snakehead fish (*Channa striata*) muscle Tissue. *Malaysian Journal of*

Biochemistry and Molecular Biology, 14: 25-32.

Lhoste EF, Rszlewicz M, Gueugneau AM and Coning T. (1994). Adaptation of exocrine pancreas to dietary proteins: effect of the nature of protein and rat strain on enzyme induction and messenger mRNA levels. *Journal of Nutritional Biochemistry,* 5: 84–94.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1): 265-277.

Mahajan CL and Agrawal NK. (1980). Nutritional requirement of ascorbic acid by Indian major carp, *Cirrhina mrigala*, during early growth. *Aquaculture*, 19(1): 37-48.

Minakshi Sahoo and Mukhopadhyay PK. (2008). Dietary ascorbic acid requirements of fingerlings of genetically improved rohu (*Labeo rohita* Ham). *The Israeli Journal of Aquaculture – Bamidgeh,* 60(2): 100-106.

Mishra CK, Das BK, Mukherjee SC and Pradhan J. (2007). Effects of dietary vitamin C on immunity, growth and survival of Indian major carp *Labeo rohita*, fingerlings. *Aquaculture Nutrition,* 13(1): 35-44.

Péres A, Zambonino Infante JL and Cahu CL. (1998). Dietary regulation of activities and mRNA levels of trypsin and amylase in sea bass *Dicentrarchus labrax* larvae. *Fish Physiology and Biochemistry,* 19(2):145–152.

Ram MM. (1966). Growth rate and protein utilization in vitamin C deficiency. *Indian Journal of Medical Research*, 54(1): 964-970.

Reimer G. (1982). The influence of diet on the digestive enzymes of the amazon fish Matrincha, *Brycon melanopterus*. *Journal of Fish Biology,* 21(6): 637–642.

Shiau S and Jan FL. (1992). Dietary ascorbic acid requirement of juvenile tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. *Nippon Suisan Gakkaishi,* 58: 671-675.

Storebakken T and Goswami UC. (1996). Plasma carotenoid concentration indicates the availability of dietary astaxanthin for Atlantic salmon, *Salino salur*. *Aquaculture*, 146: 147-153.