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Kinetic of Alkaline Phosphatase in Liver, Kidney, Intestine and Muscle tissue of Red Tilapia Cultured Under Mid-Hill Altitudes of Meghalaya: North Eastern India

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Abstract: The aim of the present research was to provide information on the activities of Alkaline Phosphatase (ALP) in various organs of Red Tilapia cultured under mid-hill condition of Meghalaya in captive condition, where within a year the water temperature ranges from 15-22°C. From the present study, it has been observed that, under mid-hill conditions of Meghalaya, the ALP activity was more in kidney (3983.6609±24.6838 mg L^{-1}) and the decreasing order of activity was recorded in liver (3761.9639±18.6786 mg L^{-1}), intestine (3420.1118±443.3330 mg L^{-1}) and muscle tissue (1969.1100±22.5985 mg L^{-1}). The higher distribution of ALP in kidney and other organs implies that the fish can adapt to cold temperature of mid-hill altitude and can be taken up as a candidate species for mass scale culture in mid-hill condition to meet the nutritional requirements of the people.

Key words: Kinetics, ALP, Red Tilapia

INTRODUCTION

The Meghalaya represents unique topographical conditions (Table 1). As a result, all the major water bodies of the state pose low temperature throughout the year. The maximum (22°C) and minimum (12°C) water temperature coupled with other physico-chemical parameters recorded in the year 2005 is represented in Table 2. In the present study, Red Tilapia was chosen as test organism because it is an emerging and attractive species for aquaculture sector, especially in North Eastern Hill Region of India where fish production deficit is above 48%. Beside, it grows quickly, is large when it reproduces, has a low feeding trophic level and is cheap to produce.

The alkaline phosphatase (EC 3.1.3.1) are widely distributed in nature and are characterised by a high pH optima and broad substrate specificity (Mc Comb *et al*., 1979). Non-bacterial alkaline phosphatases are membrane bound, zinc-containing glycoproteins. The enzyme catalyzes the transfer of phosphate group to water (hydrolysis) or alcohols (Tran phosphorylation) using a wide variety of phosphomonoesters. The physiological role of alkaline phosphatase remains uncertain except for a role in bone mineralization (Harris, 1989) and a possible involvement in various transport processes, such as intestinal phosphate and calcium transfer (Dupuis *et al*., 1991; Harris, 1989) and placental immunoglobulin mineralization (Makiya and Stigbrand, 1992).

The Red Tilapia used in the present study was produced in the Philippines through process of selective breeding and genetic engineering by the combination of three different species of tilapia viz. *Oreochromis niloticus, Oreochromis urolepis hornorum* and *Oreochromis mossambicus* (Majhi *et al*., 2005). In general, alkaline phosphatase from fish and poikilotherms have not been studied much, but such species like Red Tilapia from cold environments offers an opportunity for kinetic studies of the enzyme in various parts of fish body. Never the less, some comparative studies on impure preparations have been performed with alkaline phosphatase from rainbow trout, eel, carps and catfish (Sorimachi *et al*., 1983; Yora and Sakagishi, 1986) and the thermal properties of crude enzyme fractions from three deep-water fish have been studied (Gelman *et al*., 1992; 1989). Recently, the purification and properties of alkaline phosphatase from shrimp were also reported (Lee and Chuang, 1991; Olsen *et al*., 1991).

Under this backdrop, the present paper describes the distribution of alkaline phosphatase in the liver, kidney,

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Table 1: Characteristics of Mid-Hill situation of Meghalaya

Geographic location	Altitudes	Climatic condition	Annual rainfall	Air temperature	Water temperature
21.50°-29.50°N latitudes and 85.5°-97.5°E longitudes	1000-1500 m above MSL	Cold to warm pre-humid	2000 mm and above	11-26°C	10-24°C

Source: Bujarbaruah *et al.* (1996). International Conference on Organic Food, Shillong, Meghalaya

Table 2: Water quality parameters of Red Tilapia culture pond

Year	Water temp. (°C)	pH	Dissolved oxygen (ppm)	Carbon di-oxide (ppm)	Total alkalinity (ppm)	Hardness (ppm)
2005	15.0±0.5 - 22.0±0.5	7.0±0.5 - 8.0±0.5	5.0±0.4 - 9.0±0.5	5.0±0.1 - 11.5±0.5	4.5±0.5 - 14.5±0.5	15.5±0.3 - 41.5±0.5

Data show the mean value with standard deviation

muscle and intestine tissue of Red Tilapia grown in captive condition under mid-hills micro situation and its significance in counteracting stress due to cold environment.

MATERIALS AND METHODS

Experimental animals: A red tilapia of 1+ age with a weight of 200 g was randomly selected and taken for the study. The fish was collected from ICAR Complex fish farm, Meghalaya. The maintenance of fish and feeding conditions were those of the fish farm. The present study was conducted during November-December 2005 at Division of Fisheries, ICAR Research Complex for NEH Region, Meghalaya, India.

After 48 h without food, the fish was killed and various organs like liver, kidney, intestine and muscle were removed. The samples were immediately placed in deep freeze at 0°C until analysed. Table 3 presents the parameters and biometric indices of the animal used in the assay.

Treatment of the sample: To the 0.3 g each of various organs removed from the fish, 5.7 mL of 0.25 M sucrose was added and homogenized in an electric homogeniser at 0°C. The homogenates were centrifuged at 5,000 rpm for 5 min in a refrigerated centrifuge (REMI C24 Model). After centrifugation, the supernatant was collected and analysed within 8 h.

Table 3: Biometrics parameters

Weight of fish (g)	Sample tissue	Weight of sample (g)	DSI
200	Liver	0.3	0.6
200	Kidney	0.3	0.6
200	Intestine	0.3	0.6
200	Muscle	0.3	0.6

DSI: Weight of sample tissue (g)/Fish weight (g) × 100

Enzymatic determination: The alkaline phosphatase activity in various organs of Red Tilapia was estimated at 25°C by using Merck Enzyme Kit (Ecoline (R) 1117675.0001) and the methodology described by Bergmeyer (1972). The test concentration was Diethanolamine HCl buffer (pH 9.8), Magnesium chloride and 4-nitrophenylphosphate as substrate. Twenty microliter of supernatant sample was taken and 1000 µL of reaction solution was added in to it. The sample and reaction solution was thoroughly mixed and kept for 1 min. Then after, the absorbance of supernatants was measured spectrophotometrically (Thermo Genesys 10) at 405 nm during a 1-3 min reaction time. The calibration of blank was done by keeping distil water in the blank. The samples were assayed in triplicates and blanks in duplicate. The principle of assay is defined as, the rate of increase in 4-nitrophenolate is determined photometrically and is directly proportional to the alkaline phosphatase activity in the sample material and is expressed as Enzyme activity (mg L^{-1}) = (ΔA/Min)×2754 (Factor)

Statistical analysis: The results are expressed as Mean ± Standard Deviation (SD). The differences among the organs for alkaline phosphatase activity were tested for significance using t-test (MSTAT-C package).

RESULTS AND DISCUSSION

Overall, the enzyme activity in kidney was high (3983.6609±24.6838 mg L^{-1}) followed by liver

Table 4: Alkaline phosphates activities in studied organs of Red Tilapia

Enzyme	No. of Observation	Studied organs	Enzyme activity (mg L^{-1})	t-value
Alkaline Phosphates	04	Liver[1]	3761.9639±18.6786[a]	1 and 2:-11.0837
				1 and 3: 1.5956
		Kidney[2]	3983.6609±24.6838[b]	1 and 4: 442.2659
		Intestine[3]	3420.1118±443.3330[a,b]	2 and 3: 2.4171
				2 and 4: 99.9308
		Muscle[4]	1969.1100±22.5985[c]	3 and 4: 6.7715

Different superscript[a, b, c] indicates significant difference at 5% level. The superscripts[1,2,3,4] indicate studied organs and are used in presenting t-values between the organs

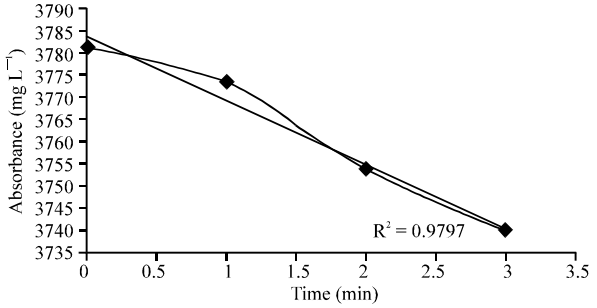

Fig. 1: Alkaline phosphatase in liver sample of Red Tilapia (Absorbance at 405 nm)

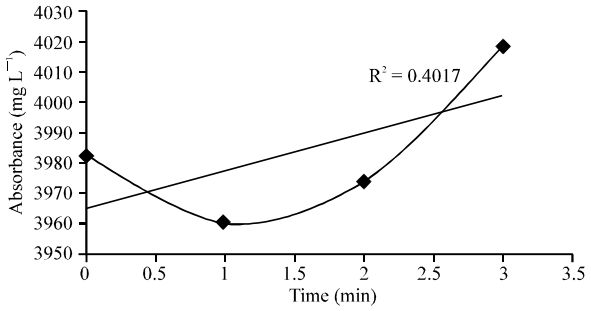

Fig. 2: Alkaline phosphatase in kidney sample of Red Tilapia (Absorbance at 405 nm)

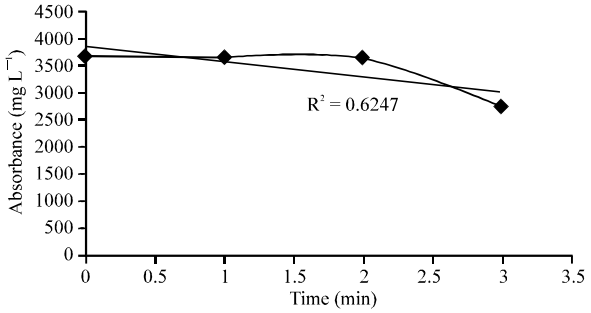

Fig. 3: Alkaline phosphatase in intestine sample of Red Tilapia (Absorbance at 405 nm)

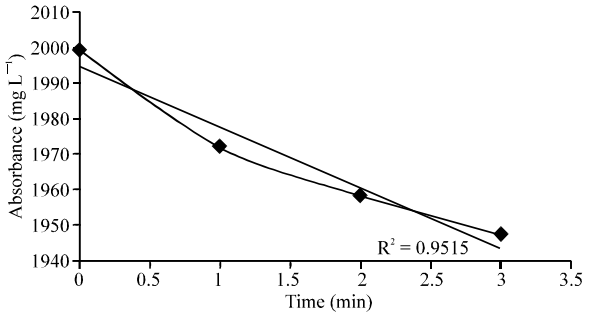

Fig. 4: Alkaline phosphatase in muscle sample of Red Tilapia (Absorbance at 405 nm)

(3761.9639±18.6786 mg L^{-1}), intestine (3420.1118±443.3330 mg L^{-1}) and muscle tissue (1969.1100±22.5985 mg L^{-1}), (Table 4 and Fig. 1-4).

The present study on activities of alkaline phosphatase in various organs of Red Tilapia revealed that, the activity of the alkaline phosphatase was more in kidney. The increase in alkaline phosphatase activity in kidney may be due to the fact that, the phosphatase are very important for regulation of various metabolic processes that occurs by phosphorylation and dephosphorylation with kinase, especially in temperate condition to meet the energy requirement during stress condition, which is also reported by Sparks and Brautigan (1986). Furthermore, Molina *et al*. (2005) have also reported that, the alkaline phosphatase activity increases significantly in liver of tilapia exposed to physiological stress and the changes are more pronounced in kidney. The physiological stress to Red Tilapia cultured under mid-hill situation of Meghalaya is due to cold water temperature. The Red Tilapia is basically a sub-tropical fish and perform better in a temperature range of 25-28°C, thus adaptation to cold temperature might have put the fish under stress by virtue of which the ALP activities in different tissues have increased to compensate the physiological stress. The alkaline phosphatase is basically a membrane bound enzyme and any perturbation in the membrane properties caused by the interaction with environment could alterate ALP activities (Molina *et al*., 2005). The functions of alkaline phosphatase are numerous and are widely distributed in the nature. In higher animals, ALP activities is involved in bone formation and in membrane transport activities. In blue crab *Callinectes sapidus*, ALP modulates the osmoregulatory response (Lovett *et al*., 1994).

In addition to above, the tissue pH values of most red-flesh fish and white-flesh fish are 5.6-5.8 and 6.2-6.3, respectively (Asgeirsson *et al*., 1995). In the present study, white-flesh fish was taken for the investigation. Therefore, the largely distribution of ALP in all the tested organs may be due to alkaline properties of fish flesh tissue. Kuda *et al*. (2002) reported that, the ALP activities in the organs might also be due to leaching from internal organs, apart from stress factor. Furthermore, he revealed that, heavy microbial load in the aquatic system having ALP activity can also lead to accumulation of ALP in different organs. The fish sample used in the present study was collected from a well-maintained culture pond having optimum organic load. Thus, probability of ALP accumulation due to heavy microbial load is rejected. Thus, the ALP distribution in major organs of Red Tilapia is only to compensate the physiological stress arises due to cold water temperature. Karuppasamy (2002) also reports the similar finding, who has studied the ALP activities changes due to environmental stress in fish.

Overall, the ALP are helpful in numerous activities in fish tissues including osmoregulation, membrane transport and bone formation etc. Thus, distribution of ALP in all the studies organs indicate that, the Red Tilapia can be well domesticated in mid-hills conditions for mass-scale production and can be incorporated as a candidate species in integrated fish farming system.

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REFERENCES

Asgeirsson, B., R. Hartemink and J.F. Chlebowski, 1995. Alkaline phosphatase from Atlantic cod (*Gadus morhua*). Kinetic and structural properties, which indicate adaptation to low temperature. Comp. Biochem. Physiol., IIOB: 315-329.

Bergmeyer, H.U., 1972. Standardization of enzyme assays. Clin. Chem., 18: 1305-1311.

Bujarbaruah, K.M., A. Das and S.K. Nanda, 1996. Status of animal husbandry in Meghalaya. ICAR Research Complex for NEH Region Publication, 12: 27-29.

Dupis, Y., S. Tardival, Z. Poremska and P. Fournier, 1991. Effect of some alkaline phosphatase inhibitors on intestinal calcium transfer. Intl. J. Biochem., 23: 175-180.

Gelman, A., U. Cogan and S. Mokady, 1992. The thermal properties of fish enzymes as a possible indicator of the temperature adaptation potential of the fish. Comp. Biochem. Physiol., 101B: 205-208.

Gelman, A., S. Mokady and U. Cogan, 1989. The thermal properties of intestinal alkaline phosphatase of three kinds of deep-water fish. Comp. Biochem. Physiol., 94B: 113-116.

Harris, H., 1989. The human alkaline phosphatase: What we know and what we don't know. Clin. Chim. Acta., 186: 133-150.

Karuppasamy, R., 2000. Effects of phenyl mercuric acetate on acid and alkaline phosphatase activities in selected tissue of fish. Environ. Ecol., 18: 643-650.

Kuda, T., C. Matsumoto and T. Yano, 2002. Changes in acid and alkaline phosphatase activities during the spoilage of raw muscle from horse mackrel *Trachurus japonicus* and gurnard *Lepidotriga microptera*. J. Food Chem., 76:443-447.

Lee, A.C. and N.N. Chuang, 1991. Characterization of different molecular forms of alkaline phosphatase in the hepatopancreases from the shrimp *Penaeus monodon*. Comp. Biochem. Physiol., 99B: 845-850.

Lovett, D.L., D.W. Towle and J.E. Paris, 1994. Salinity-sensitive alkaline phosphatase activities in gills of blue crab *Callinectes sapidus* Rathbun. Comp. Biochem. Physiol., B 109: 163-173.

Majhi, S.K., B.K. Mahapatra, K. Vinod and B.K. Mandal, 2005. Growth performance of Red Tilapia through feeding different livestock excreta under mid-hill micro situation of Meghalaya, North Eastern India. Environ. Ecol., 23: 277-281.

Makiya, R. and T. Stigbrand, 1992. Placental alkaline phosphatase in related to human IgG internalisation in Hep2 cells. Biochem. Biophys. Res. Commun., 182: 624-630.

McComb, R.B., G.N. Bowers and S. Posen, 1979. Alkaline Phosphatase. Plenum Press, New York.

Molina, R., I. Moreno, S. Pichardo, A. Jos and R. Moyano, 2005. Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis* sp.) exposed sub chronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. J .Toxins., 46: 725-735.

Olsen, R.L., K. Overbo and B. Myrnes, 1991. Alkaline phosphatase from the hepatopancreases of shrimp (*Pandalus borealls*): A dimeric enzyme with catalytically active subunits. Comp. Biochem. Physiol., 99B: 755-761.

Quilliam, M.A., 1999. Phylotoxins. J. AOAC, 82: 773-781.

Sorimachi, K., H. Mizuno, R. Konno, A. Niwa, Y. Yasumura and S. Uchiyama, 1983. Alkaline phosphatase in various animal species and heat stability of the enzyme in catfish. Zool. Mag., 92: 226-230.

Sparks, J.W. and D.L. Brautigan, 1986. Molecular basis for substrate specificity of protein kinases and phosphatases. Intl. J. Biochem., 18: 497-504.

Yora, T. and Y. Sakagishi, 1986. Comparative biochemical study of alkaline phosphatase isozymes in fish, amphibians, reptiles, birds and mammals. Comp. Biochem. Physiol., 85B: 649-658.