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Effects of Thermal Stress and Dietary Zinc on Growth Performance, Superoxide Dismutase–1 and Catalase Enzyme Activity in *Pangasianodon hypophthalmus*

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A B S T R A C T

A 60-day feeding trail was conducted to determine the effect of dietary zinc level and temperature on growth and antioxidant enzyme in *Pangasianodon hypophthalmus*. The six distinct treatment groups were fed with diets prepared with different zinc levels (16, 32 and 48 mg/kg respectively) in two temperature like 34˚C and ambient temperature. After 60 days experimental trial, the growth parameters like percent weight gain, FCR, PER, SGR and enzymes Superoxide Dismutase–I and Catalase were studied. In T3 group inclusion level of 48 mg/kg of zinc with 34˚C showed maximum SGR compared to other groups. The feed conversion ratios of different experimental groups were showed significant difference ($p<0.05$). The lowest (1.45±0.11) FCR was recorded in T3 group. The highest FCR was found in T5 (2.20±0.11) group. The highest PER value was recorded in T3 (2.32±0.04) group. The lowest PER was recorded in T5 (1.48±0.13). The value of the PER and SGR was found in same trend as specific growth and the correlation for each other. In the present study the SOD activity was significantly higher in group T3 ($p<0.05$) compared to the other groups in muscle. From the present work it can be said that dietary inclusion of zinc had better impact on growth at the two studied temperature, so the 48 mg/Kg zinc can be recommended for the inclusion in diet of *Pangasianodon hypophthalmus* at both ambient and 34˚C temperature.

Introduction

Catfishes are the favourite candidate species for aquaculture in India owing to their consumer preference, commercial and medicinal value (Auddy *et al.*, 1994). Culture practices of *Clarias batrachus* and *Heteropneustes fossilis* have been popularized widely. Studies on thermal tolerance of catfishes native to India are reported for *H. fossilis* (Vasal and Sundararaj, 1978) and *Pangasius pangasius* (Debnath*et al.*, 2006). However, expansion of aquaculture, by introducing new fish species is gaining incentive due to the wide agro-climatic conditions of India and to keep leap with the mounting demand for fish protein. *Pangasianodon hypophthalmus* (Sauvage, 1878) commonly known as Pangasius, has achieved impressive success as a commercial aquaculture species. Zinc is one of the most important trace elements involved in animal growth, because it is the most widely used metal co-factor of enzymes involved in

protein, nucleic acid, carbohydrate and lipid metabolism as well as control of gene transcription and other fundamental biological processes. A dietary input is vital at least in fresh water fish and the requirement levels are between 15 to 30 mg/kg. The zinc requirement has been estimated at 15-30 mg/kg feed for rainbow trout, *Oncorhynchus mykiss* (Ogino and Yang, 1978) and 37-57 mg/kg feed for Atlantic salmon, *Salmo salar* (Maage and Julshamn, 1993; Maage *et al.*, 2001). Shim and Lee (1993) reported that zinc deficient diet causes poor growth rate, low feed efficiency and high mortality in guppy. Zinc (Zn) has significant roles in the organism for growth and protein metabolism, energy production, gene regulation, maintaining the health of cell membranes and bones probably because it is a cofactor of over 200 enzymes such as alkaline phosphatase, alcohol dehydrogenase and carbonic anhydrase etc. (Watanabe *et al.,* 1997; Yamaguchi, 1998). One of the most significant functions of zinc is related to its antioxidant role and its participation in the antioxidant defence system (Powell, 2000). Zinc deficiency shows growth retardation, cataract, skin erosion, and higher mortality, oxidative damage through the effects of free radical activity (Ogino and Yang, 1978; Powell *et al.*, 1994; Salgueiro *et al.,* 2000) and changes the status of antioxidant enzymes and substances (Prasad *et al.*, 1993). The process by which Zn exerts its antioxidant activity is not well specified. Nevertheless, it has been proposed that it increases the synthesis of metallothionein, a cysteine-rich protein, which plays as an important role to act as free radical scavenger (Prasad *et al.*, 1993; Bales *et al.*, 1994). Zinc deficiency increases oxidative damage due to free radical activity (Powell *et al.,* 1994; Salgueiro *et al.,* 2000). In animals, aerobic tissues continuously generate superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) at the mitochondrial and endoplasmic reticulum membranes as by-products of the oxidative metabolism. The primary antioxidant protection against these species is provided by the enzymes superoxide dismutase (SOD) and catalase (CAT), respectively (Chance *et al.,* 1979). Consequently, these antioxidant enzymes contribute to the maintenance of a relatively low level of the reactive and harmful species hydroxyl radical ($^{\cdot}OH$), which is generated through the Haber–Weiss reaction between (O^{2-}) and H_2O_2 in the presence of Cu^{2+} and/or Fe^{3+}. Brian and co-worker (2001) reported that the variable thermal environment can induce thermal stresses to aquatic animals and potentially affects the enzyme activity and antioxidant defence system in aquatic organisms (Abele *et al.,* 1998; Portner, 2002). Higher temperature is reported to increase reactive oxygen species release and enhances the risk of oxidative damage (Abele *et al.,* 1998). Most living systems adapt to oxidative stress by increasing their antioxidant potential which seems to be the most important effective protection against oxidative stress (Hermes-Lima, 2004). Increased availability of anti-oxidative enzymes like superoxide dismutase and catalase is believed to minimize oxidative stress (Portner, 2002). They directly detoxify harmful reactive oxygen species and oxidative damage to cellular components. Akther *et al.,* (2013) reported SOD and catalase activities in liver, gill and kidney tissues of *T. putitora* were significantly higher at higher acclimation temperatures which are a clear indication of higher magnitude of oxidative stress in these groups.

Realizing the importance of dietary Zinc the present study was conducted with the objective to *examine the effects of thermal Stress* and *Dietary Zinc on* growth performance, Superoxide Dismutase–I and Catalase enzyme activity in *Pangasianodon hypophthalmus.*

Materials and Methods

The experiment was conducted at the wet laboratory of ICAR- Central Institute of Fisheries Education (CIFE), Mumbai. The fishes were procured from Kolkata (W.B). The fishes were transported in the well aerated syntax tanks. They were carefully transferred to a circular tank (1000 L).

The experiment was conducted for a period of 60 days in the wet laboratory of old campus, CIFE, Mumbai. The setup consisted of 18 plastic rectangular tubs (80 X 57 X 42 cm, 150 L capacity) covered with perforated lids. Animals used for the experiment were advanced fingerlings of *Pangasianodon hypophthalmus* (Sauvage, 1822) with an initial weight ranging from 5.32 to 5.70 g. Two hundred thirty four (234) fishes were randomly distributed in 18 distinct experimental groups in triplicates, following a completely randomized design.

Water quality parameters *viz*. temperature, pH, dissolved oxygen, free carbon dioxide, total hardness, ammonia, nitrite and nitrate were recorded during the experimental period.

Formulation and preparation of experimental diets

Purified ingredients such as casein (vitamin free), gelatin, dextrin, starch, cellulose, carboxymethylcellulose (CMC), betainehydrochloride, butylated hydroxytoluene (BHT), cod liver oil, sunflower oil and vitamin-mineral mixture (zinc free) were taken for feed formulation (Table 1). Three diets with the same composition were prepared which contained zinc acetate in different concentrations. The diets were T1 (53.7 mg Zn acetate/kg =16 mg Zn/kg), T2 (107.4 mg Zn acetate/kg =32 mg Zn/kg) and T3 (161.2 mg Zn acetate/kg = 48 mg Zn/kg).

Feeding

Feeding was initially done @ 3% of the body weight and the feeding rate was adjusted accordingly. The daily ration was divided into two equal parts and was fed at 8.00 am in the morning and 5.00 pm in the evening.

Growth parameters

Sampling for growth was done at every 15 days to assess the body weight of the fishes. Fishes were starved overnight before taking the weight. The growth performance was assessed by using the following formula:

Percentage weight gain

The percentage weight gain was calculated using the following formula

$$\text{Weight gain}(\%) = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Specific growth rate (SGR)

The specific growth rate was calculated by the following formula

$$\text{SGR}\ (\%) = \frac{\text{Log}_e\ \text{Final weight} - \text{Log}_e\ \text{Initial weight}}{\text{Number of days}} \times 100$$

Feed conversion ratio (FCR)

The feed conversion ratio was calculated by the following formula

$$\text{FCR} = \frac{\text{Feed given(Dry weight)}}{\text{Body weight gain (wet)}}$$

Protein efficiency ratio (PER)

Protein efficiency ratio was calculated by the following formula

$$PER = \frac{\text{Net wet gain (Wet weight)}}{\text{Protein fed}}$$

Survival rate

At the end of the experiment, all the experimental tubs were dewatered and the number of the experimental animals in each tub was counted and the survival rate (%) was calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{Total number of harvested animal}}{\text{Total number of stocked}} \times 100$$

Enzyme assays

At the end of the experiment fishes were collected from each tank ((T1, T2, T3, T4, T5 and T6) and anaesthetized with clove oil (50 $\mu l.L^{-1}$). Fishes were then dissected and the tissues *viz.*, liver, gills, and muscle, were immediately removed. A 5% tissue homogenate was prepared in chilled 0.25 M sucrose solution by Teflon coated mechanical homogenizer (REMI Equipment, Mumbai, India). The whole procedure was followed in ice cold condition. Homogenized samples were centrifuged at 8000 rpm for 10 min at 4˚C. The supernatant was collected in glass vials and stored in deep freezer (-20^0C) for enzyme assay. Suitable dilution of the samples was done as and when required.

Quantification of protein of the different tissues was carried out by using Bradford method (Bradford, 1976). The Bradford assay relies on the binding of the dye Coomassie blue G250 to protein.

Tissue homogenate (20 µl) was taken along with 180 µl distilled water and 250 µl 1N NaOH added. After that 5 ml Bradford reagent added and kept for 5 mins. Reading was taken at 595 nm against the blank. Protein content was expressed in mg/g wet tissue.

Superoxide dismutase was assayed according to the method described by Mishra and Fridovich (1972) based on the oxidation of epinepherine-adrenochrome transition by the enzyme. Fifty microlitre of the sample was taken in the cuvette and 1.5 ml 0.1M carbonate–bicarbonate buffer containing 57 mg/dl EDTA (pH 10.2) and 0.5 ml epinephrine (0.3 mM) was added and mixed well. Change in optical density at 480 nm was read immediately for 3 min in a Shimadzu–UV spectrophotometer. One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto-oxidation. SOD expressed as unit activity (amount of protein required to give 50% inhibition of epinephrine auto-oxidation).

Catalase was assayed according to the method described by Takahara *et al.*, (1960). To a reaction mixture of 2.45 ml phosphate buffer (50 mM, pH 7.0), enzyme source was added and the reaction was started by the addition of 1.0 ml of H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 15 sec intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml distilled water instead of H_2O_2 solution. Enzyme activity was expressed as nano moles H_2O_2 decomposed /min /mg protein

Proximate analysis of the experimental diets and carcass tissue

Proximate analysis of the diets and carcass tissue was done by standard methods (AOAC, 1995) at Fish Nutrition Laboratory, CIFE. The moisture content of the experimental diets and carcass tissue was determined by taking a known weight of the sample in the petri dish and drying it in a hot air oven at 105^0C till a constant weight was achieved. Nitrogen content of the experimental diets and carcass tissue dried samples were estimated quantitatively by Kjeltec semi-automated system (2200 Kjeltec Auto

Distillation, Foss Tecator, and Sweden) using titration as the means for determining nitrogen percentage. The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.Ether extract of dried experimental diets and carcass tissue samples were estimated by Soxhlet apparatus using petroleum ether (boiling point 40-60 ^{0}C) as the solvent. Ash content of the experimental diets and carcass tissue was estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 550˚C for 6 hours. Digestible energy of the experimental diets was calculated following Halver (1976) formula:

Digestible energy (kcal/100 g) =protein (%) x 4 + lipid (%) x 9 + carbohydrate (%) x4

Statistical analysis

Statistical significance of different enzyme activities was analysed using two-way analysis of variance (ANOVA) via SPSS 22.0 for Windows. Duncan's multiple range test was used for post hoc comparison of mean (P<0.05) between different acclimation temperatures. All data presented in the text, figures and tables are means ± standard error and statistical significance for all statistical tests was set at P<0.05.

Results and Discussion

Proximate composition of feed

In the present study, dietary zinc supplemented based diets were maintained with a specific range of moisture content, dry matter, crude protein and ether extract were found to be in 10.51±0.19 to 11.15±0.06%, 88.85±0.06 to 89.49±0.19%, 34.35±0.68 to 35.7±0.10% and 7.02±0.20 to 8.01±0.40% respectively. The calculated average digestible energy was 406.41 kcal/100 gm feed. Above range of nutrients were as described by several authors. Phumee *et al.*, (2009) suggested that optimum protein and lipid requirement of *Pangasianodon hypophthalmus* ranges between 30-35% and 8-12% respectively. The digestible energy content of experimental diets was found to be within the range of 368.68-375.09 kcal/100 g in a study by Rostagno *et al.*, (2000).

Physico-chemical parameters of water

The physico-chemical parameters of water such as temperature (˚C), pH, dissolved oxygen (mg.L^{-1}), free carbon dioxide (mg.L^{-1}), total hardness (mg.L^{-1}), ammonia (mg.L^{-1}), Nitrite-N (mg.L-1), Nitrate-N (mg.L^{-1}), Zinc level (mg.L^{-1}) are presented in table 2. All the physico-chemical parameters of water such as temperature, pH, dissolved oxygen, free carbon dioxide, carbonate hardness, ammonia, nitrite- N, nitrate-N were observed to be within the optimum range of requirements for fish. Temperature plays an important role in regulating the metabolism of animals, so an optimum range of temperature is required for optimum metabolic activity, which in turn gives maximum yield so we designed one ambient temperature and 34˚C. Since *Pangasianodon hypophthalmus* can thrive well at temperature range of 20-35˚C (Choudhury, 2000). It supported the range of temperature from 34.05˚C to 34.22˚C during the experimental period. The pH of water in all the experimental groups were ranged from 7.5-8.4, which is within the acceptable range (6.7-8.6) as suggested by Andrew *et al.*, (1972) and 6.5-9.0 as suggested by Swingle (1967). The dissolved oxygen level in water was varied with a large number of factors such as water temperature, metabolic rate, biomass density, aeration *etc*. The dissolved oxygen level in different experimental tubs was recorded to be within the range of 4.8-7.3 mg.L^{-1} which is within the optimum range of 5.0-8.0 mg.L^{-1} for Thai pangus as suggested

by Sarker (2000). It is assumed that dissolved oxygen was optimum throughout the experimental period, which is due to continue aeration. In the present study, the carbon dioxide concentration was found to be negligible, and hence did not have any adverse effect on the survival and performance of the experimental animals. This may be due to low biomass and daily water exchange during the experimental period. The carbonate hardness was found to be 230-253 $mg.L^{-1}$ during the experimental period**.**

Growth parameters

In the present study, the different zinc level supplemented diets were showed significant effect on weight gain percentage. In T3 group Inclusion level of 48 mg/kg of zinc with 34˚C showed maximum SGR compared to T5 (Zn 48 mg/kg with ambient temperature) as well as the other group. This may be correlated with the fact that 48 mg/kg supplemented zinc with 34˚C were better utilized by *Pangasianodon hypophthalmus* while the lower inclusion level reflected the reduced growth. The lowest weight gain percentage and SGR were found in T4 group and growth improvements observed in the dietary zinc supplemented at levels of 32 mg/kg, and maximum in 48 mg/kg supplemented. However, growth retardation has been encountered in T4 (16 mg/kg) group. Which is comparatively high temperature exposed group and it clearly reflects that at the elevated level of temperature there is reduced growth which may be either due to elevated dietary requirement of zinc or high rate of metabolism at higher temperate while the substantial increase of the growth at the group T6 Indicates positive effect of the dietary zinc at high temperature as well determining the dietary requirement is optimum at higher level of inclusion. It also clearly indicates that decreasing level of zinc negatively effect on growth at ambient temperature and elevated temperature. Dietary zinc supplemented diet (48 mg/kg) can be considered as adequate level of zinc, which had significant effect on growth at ambient temperature and elevated temperature. Since the highest growth has been found in highest inclusion of dietary zinc so the further study is required in this area for exploring the maximum inclusion level at ambient and elevated temperature. Similarly, Ogino and Yong (1978) reported that zinc deficiency induced retarded growth and high mortality in common carp at ambient temperature. The feed conversion ratios of different experimental groups were showed significant difference. The FCR of different experimental groups were varied significantly ($p<0.05$). The lowest ($1.45^{a}\pm0.11$) FCR was recorded in T3 group. The highest FCR was found in T5 ($2.20^{bc}\pm0.11$) group. While the T4 group, lowest level of supplementation at 16 mg/kg level and ambient temperature has no significant difference with T5 group i.e. 32 mg/kg with ambient temperature. The mean per value was significantly different ($p<0.05$) among the different treatment groups. The highest PER value was recorded in T3 ($2.32^{d}\pm0.04$) group. The lowest PER was recorded in T5 ($1.48^{a}\pm0.13$). The value of SGR were varied significantly ($p<0.05$) among different treatment groups. The lowest SGR value was found in T4 ($1.55^{a}\pm0.02$) group and higher SGR was found in T3 group (2.00 ± 0.04). The value of the PER and SGR was found in same trend as specific growth and the correlation holds the support for each other. This result is supported by Eid *et al.*, (1993) reported that zinc deficient diet showed higher FCR and lower growth rate in *Oreochromis niloticus*. So, the present study indicated that dietary zinc supplementation up to 48 mg/kg diet with 34˚C has direct influence on FCR of *Pangasianodon hypophthalmus*. There was no mortality observed during the experimental period (Table 3).

Table.1 Composition of purified experimental diets

Ingredients	T1 (Low level Zinc)	T2 (Medium level Zinc)	T3 (High level zinc)
Casein purified	35.00	35.00	35.00
Starch	24.00	24.00	24.00
Dextrin	7.00	7.00	7.00
Cellulose	14.87	14.87	14.87
CMC	4.00	4.00	4.00
Gelatine	3.00	3.00	3.00
Oil	10.10	10.10	10.10
BHT	0.02	0.02	0.02
Zn-acetate g/100g	0.0054	0.0107	0.00161
VM+MM(Zn deficient)	2	2	2
Total	100	100	100

Composition of vitamin mineral mix (quantity/250g starch powder) : Vitamin A-55,00,00 IU; Vitamin D3-11,00,00 IU; Vitamin B1-20mg; VitaminB2-200mg; Vitamin E-75mg; VitaminK-100mg; VitaminB12-0.6mcg; Calcium pantothenate-250mg; Nicotinamide-1000mg; Pyridoxine-100mg; Mn-2700mg; I-100mg; Fe-750mg; Choline chloride-500mg; Cu-200mg; Co- 45mg; Ca-50g; P-30g; Se-0.5ppm

Table.2 Physico-chemical parameters of water during the experimental period of 60 days for different experimental groups

Parameter	T1	T2	T3	T4	T5	T6
Dissolved oxygen(mg.L^{-1})	4.8-7.4	4.8-7.3	4.8-7.3	6.2-7.3	6.2-7.3	6.2-7.3
Temperature (˚C)	33.8-34.3	33.9-34.1	33.6-34.4	27.3-28.7	27.6-28.2	26.8-28.8
pH	7.6-8.2	7.8-8.6	7.7-8.3	7.5-8.1	7.6-8.4	7.7-8.2
Hardness (mg.L^{-1})	227-236	238-240	238-240	238-242	239-246	229-243
Ammonia (mg.L^{-1})	0.21-0.24	0.21-0.23	0.22-0.26	0.13-0.20	0.14-0.20	0.13-0.20
Nitrite(mg.L^{-1})	0.001-0.002	0.001-0.002	0.001-0.002	0.001-0.002	0.001-0.002	0.001-0.002

Table.3 Growth parameters of the different experimental groups fed different Experimental diets at the end of the experiment

Treatments	Weight Gain%	SGR	FCR	PER
T1	193.22^{b}±3.49	1.77^{c}±0.080	1.85^{b}±0.11	1.86^{b}±0.07
T2	173.37^{ab}±5.09	1.68^{a}±0.04	1.88^{bc}±0.14	1.74^{ab}±0.06
T3	235.69^{d}±8.48	2.00^{d}±0.04	1.45^{a}±0.11	2.32^{d}±0.04
T4	162.34^{a}±5.78	1.59^{a}±0.05	2.16^{bc}±0.09	1.58^{ab}±0.08
T5	156.21^{a}±7.47	1.55^{a}±0.02	2.20^{c}±0.06	1.48^{a}±0.13
T6	184.78^{b}±8.44	1.73^{b}±0.02	1.85^{b}±0.06	1.77^{b}±0.07

Data expressed as Mean ± SE, n=3.The different treatments were found to be significantly different ($p<0.05$) from each other

Table.4 Proximate composition of the fish carcass

Treatments	Moisture (%)	Crude Protein (%)	Ether Extract (%)	Ash (%)
T1	73.78±0.650	15.85±0.51	7.35±0.55	1.74±0.08
T2	73.46±0.16	16.61±0.38	7.11±0.11	1.71±0.01
T3	72.50±0.70	16.21±0.40	7.98±0.45	1.88±0.01
T4	73.04±0.24	16.09±0.46	7.52±0.02	1.97±0.005
T5	74.39±0.40	15.81±0.72	7.66±0.04	1.69±0.035
T6	73.86±0.23	15.89±0.55	7.22±0.42	1.79±0.03

Data expressed as Mean ± SE, n=3.The different treatments were found to be significantly different ($p<0.05$) from each other.

Fig.1 SOD-1 activity in muscle of *Pangasianodon hypophthalmus* fingerlings Fed with different experimental diets

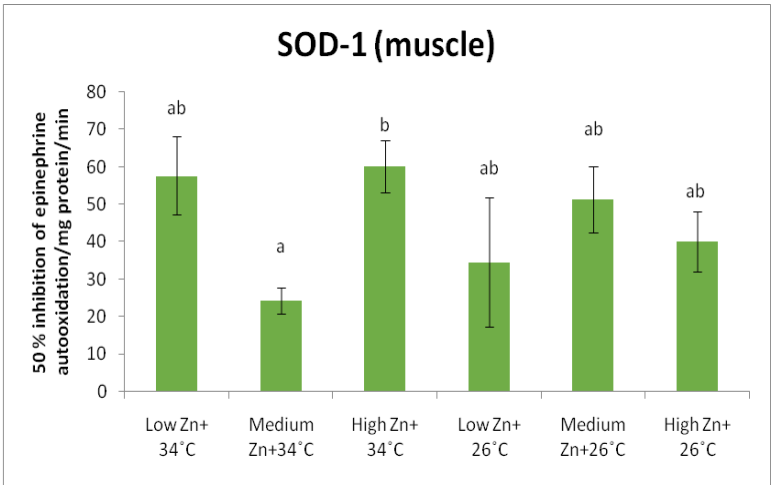


Fig.2 SOD-1 activity in gill of *Pangasianodon hypophthalmus* fingerlings Fed with different experimental diets

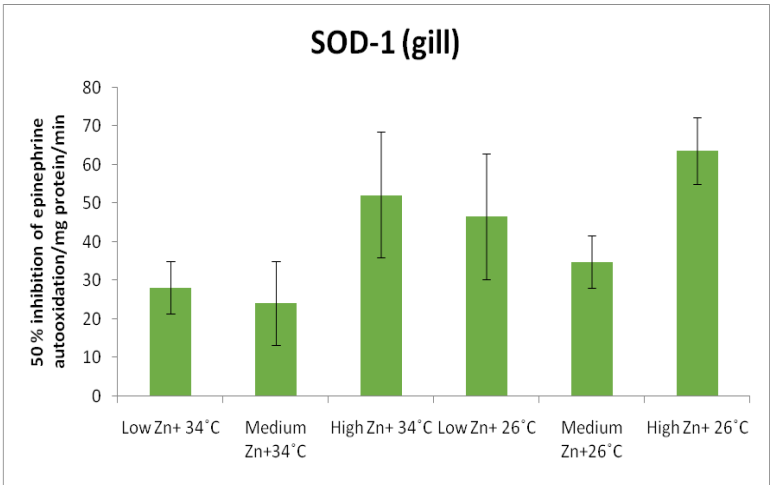


Fig.3 Catalase activity in liver of *Pangasianodon hypophthalmus* fingerlings Fed with different experimental diets

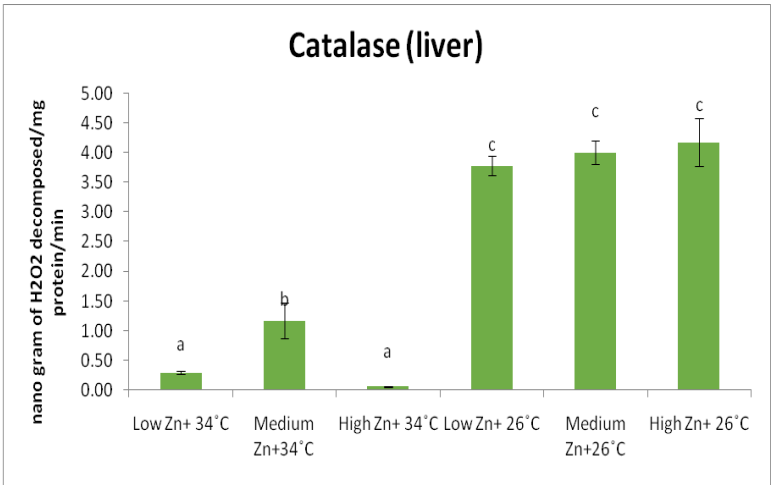
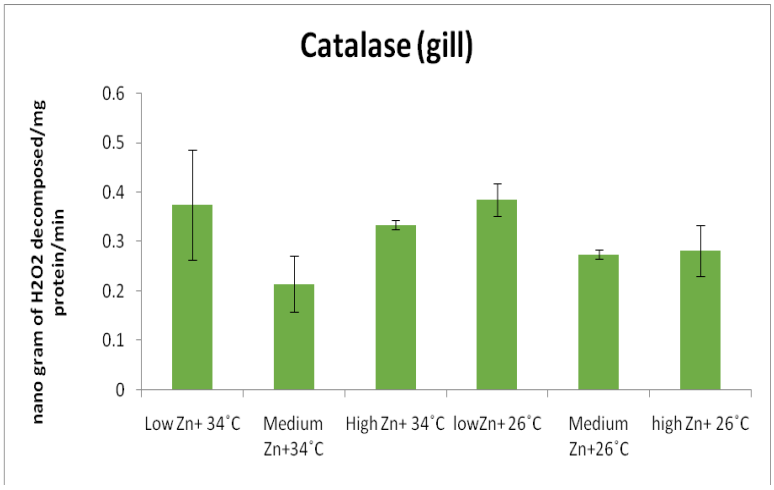


Fig.4 Catalase activity in gill of *Pangasianodon hypophthalmus* fingerlings Fed with different experimental diets



SOD-1 and catalase enzyme activity

The enzymes of antioxidant defence viz. SOD-1 and Catalase are presented in figures 1, 2, 3 and 4 respectively. The antioxidant defences are very important in maintaining the homeostasis and overcome by pro-oxidant forces and reactive oxygen species play important role in it (Sies *et al.,* 1992). Living organisms are protected from the ROS by several defence mechanisms, including antioxidant enzymes such as SOD and catalase. In the present study, the SOD activity was significantly higher in group T3 ($p<0.05$) compared to the other groups in muscle which may be due to the interactive impact of the highest dietary inclusion of the zinc and temperature. While SOD activity was not significantly different among the different experimental groups in gill. Higher

temperature is reported to increase reactive oxygen species (Abele *et al.,* 1998). Most living systems adapt to oxidative stress by increasing their antioxidant potential which seems to be the most important effective protection against oxidative stress (Hermes-Lima, 2004). Increased availability of anti-oxidative enzymes like superoxide dismutase to minimize oxidative stress (Portner, 2002) and increase the animal competence of effect of temperature and higher bioavailability of zinc at higher temperature (Phillips, 1978), this indicates that the free radicals are effectively scavenged by the SOD-1. Metabolism is also dependent on acclimation temperature, acclimation period and species (Das *et al.*, 2004; Manush *et al.,* 2004). The role of Zn (II) in Cu-Zn SODs is structural rather than functional. Replacement of Zn (II) with Co (II), Hg (II), Cd (II) or Cu (II) does not affect activity (Cudd and Fridovich, 1982, Bordo *et al.,* 1994; Marino *et al.,* 1995). Even a complete removal of Zn (II) from the protein has little effect on activity. Zn (II) probably confers structural stability to the active site (Bordo *et al.,* 1994, Marino *et al.,* 1995). However, it was found that, in the muscle, the SOD activity in the 34˚C temperature with Zn interaction in experimental groups was significantly different ($p<0.05$).

Catalase is another major primary antioxidant defence component that works primarily to catalyse the decomposition of H_2O_2 to H_2O, sharing this function with glutathione peroxidase (GPX). Whereas the catalase activity of the liver and gill tissues in 60 day periods was determined. There was a significant difference ($p<0.05$) in the enzyme activity of the different treatment groups in the liver. In the liver, the least catalase activity was found in the T3 group whereas the highest enzyme activity was found in the T6 whereas no significant difference was followed in the gill but the least activity was in the T2 group and highest in T4. As the optimum temperature for the pangasius lies to be in 30 to 35˚ C (Debnath*et al.,* 2006). The increase in the antioxidant enzymes at higher or lower side of the optimum temperature has been shown to increase catalase activity due to oxidative stress at suboptimal temperature especially in gill. In a similar report by Madeira (2013) oxidative stress response was not directly correlated to temperature. It was lowest at the optimal temperature (24˚C) and it increased in European sea bass, *Dicentrarchus labrax* outside the upper and lower optimum thermal limits. It was concluded that, although these biomarkers have been used mostly as indicators of the effects of contamination in field studies, they are very sensitive to temperature either higher or lower side of the thermal optimal range.

Biochemical composition of the fish carcass

Data relating to the biochemical composition of all the experimental animals in terms of moisture, protein, lipid and ash at the end of the experiment reflect no significant variation ($P>0.05$). The moisture content of all the experimental fish sampled for proximate analysis varied from 72.50 to 74%. The crude protein content (wet weight basis) varied from 15.81 to 16.61%. The ether extract of all the fish was estimated within 7.11 to 7.98%.The total ash content varied from 1.69 to 1.97%. The proximate composition of the fish tissues is shown in table 4.

Catfishes are the preferred candidate species for aquaculture in India owing to their consumer preference, commercial and medicinal value. In the present study, the maximum growth in *Pangasianodon hypophthalmus* fingerlings has been achieved at dietary zinc supplementation of 48 mg/kg with 34˚C. Therefore, in conclusive way it can be said that the 48 mg/kg of dietary inclusion of zinc is required for the

Pangasianodon hypophthalmus optimum growth at elevated temperature in optimum range. Since, the highest dietary inclusion of zinc had given better result at ambient and 34˚C temperature.

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