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Stress mitigating and growth enhancing effect of dietary tryptophan in rohu (*Labeo rohita*, Hamilton, 1822) fingerlings

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Abstract An experiment was conducted to study the stress mitigation and growth enhancing role of dietary L-tryptophan (TRP) under thermal stress in rohu, *Labeo rohita* fingerlings for 45 days. Seven hundred and twenty fishes were distributed in three major groups that are ambient temperature (26 °C), 34 and 38 °C in triplicate following a complete randomized design. Acclimation of fishes to 34 and 38 °C over average ambient temperatures were carried out at 1 °C/day. Each group was fed with a diet supplemented with 0, 0.36, 0.72 or 1.42 % L-TRP. Results showed that blood glucose and serum cortisol level were found to be significantly higher ($p < 0.05$) in the higher temperature groups than the ambient temperature group. Similarly, aminotransferase, lactate dehydrogenase, malate dehydrogenase, CAT, superoxide dismutase activities were found to be significantly higher ($p < 0.05$) in the control groups (0 % L-TRP) and decreasing activities of these enzymes were observed with the increasing level of dietary L-TRP. In different temperature groups, L-TRP-supplemented groups were found to have higher ($p < 0.05$) growth, RGR and PER. The results obtained in the present study indicate that dietary L-TRP mitigates thermal stress and enhances growth. From the present study, we can conclude that dietary supplementation of L-TRP at the 0.72 % level in the diet is found to be optimum to reduce thermal stress even up to 38 °C in rohu, *L. rohita*. The baseline data obtained here could be useful for the farmers to formulate feeds to culture the fish in different agro-climatic zones.

Keywords *Labeo rohita* · L-Tryptophan · Thermal stress · Growth · Temperature

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Introduction

Carp culture, the mainstay of Indian freshwater aquaculture, has witnessed a sea change in technological adoption over the years in order to enhance the production levels per unit area (Saurabh et al. 2011). Rohu (*Labeo rohita*) is the main economically important aquaculture species in India as well as in southeast Asian countries with more than 11.67 lakh tonnes total production in 2010 (FAO 2012). The culture period of this species usually encounters seasonal fluctuation in water temperature. The water temperature generally goes up to 34–37 °C during summer month that is

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beyond the optimum temperature for growth of this species (Das et al. 2005). Fish physiology and behavior is significantly influenced by change in temperature, and it is known to be a pervasive factor affecting structures and functions at all levels of biological organization (Beitinger and Bennett 2000; Akhtar et al. 2011). It is well established that temperature beyond optimum limits of a particular species adversely affect the health of aquatic animal due to metabolic stress and increases oxygen demand and susceptibility to various harmful pathogens. Hence, thermal tolerance studies have generated significant attention of researchers around the world to understand the impact of global warming on animals, including fish. Lots of studies have shown that immunomodulators can trigger immune system of fish, even in stressful conditions, and therefore ameliorate the deleterious effects mediated by stress (Sahoo 2007; Saurabh and Sahoo 2008; Akhtar et al. 2010). It is, therefore, imperative to enhance the tolerance against the stress of cultured fish by modulating its immune system through dietary interventions, which has become a priority area of research.

Tryptophan (TRP) an essential amino acid is a precursor for serotonin (5-hydroxytryptamine, 5-HT), an indoleamine neurotransmitter implicated in several behavioral patterns including fear, stress, aggression, appetite regulation, social dominance and sex behavior in human and various animals including fishes (Winberg et al. 2001; Lepage et al. 2002; Hseu et al. 2003; Grimmett and Sillence, 2005; Fernandez and Strathe 2009; Laranjajr et al. 2010). Furthermore, dietary TRP has been associated with immune response and health maintenance (Le Floc'h and Seve 2007). Recent studies have shown that dietary TRP can augment growth, modulate aggressive behavior or reduce cannibalism and stress-induced anorexia and cortisol rise in different fish species (Winberg et al. 2001; Hseu et al. 2003; Tejpal et al. 2009; Costas et al. 2012). TRP has been shown to have a calmative or therapeutic effect on terrestrial farm animals and fish when supplied in excess to improve the welfare of farmed animals (Conceicao et al. 2012). Herrero et al. (2006) reported that dietary supplementation of TRP could be useful tool for reducing European sea bass stress associated with farming practices. Similarly, Hoglund et al. (2007) reported that pretreatment with dietary L-TRP attenuated stress-induced anorexia in brown trout (*Salmo trutta*). Tejpal et al. (2009) have showed mitigation of crowding stress response in *Cirrhinus mrigala* due to L-TRP. Hoseini and Hosseini (2010) have shown that TRP supplementation enhanced salt water tolerance of carp (*Cyprinus carpio*) due to anti stress effect of L-TRP and possibly increase in serotonergic activity. However, no such attempt has been undertaken to find out the growth enhancing role of dietary L-TRP in mitigation of thermal stress in rohu, *L. rohita*.

With this backdrop, the present study was conducted to delineate the role of dietary L-TRP in mitigating thermal stress in *L. rohita* fingerlings.

Materials and methods

Experimental fish

Rohu, *L. rohita* fingerlings having mean weight of 4.5 ± 0.05 were procured from the Government Fish Farm, Khopoli, Maharashtra and transported in a circular container (500 L) with sufficient aeration to the wet laboratory. During acclimation, fish were fed with control diet. After acclimatization, fish were transferred to 36 uniform size experimental tanks (80 × 57 × 42 cm) of 150 L capacity and reared for 45 days. Continuous aeration was provided throughout the experimental period.

Experimental design

Four iso-caloric (423.49–425.85 kcal 100 g^{-1}) and isonitrogenous [34.33–35.81 % crude protein (CP)] purified diets were prepared with graded levels (0, 0.36, 0.72 and 1.42 %) of L-TRP (HiMedia Laboratories, Mumbai, India). Seven hundred and twenty fishes were randomly distributed in three major groups that are ambient temperature (26 °C), 34 and 38 °C following a completely randomized design. Acclimation of fishes (20/tub) to 34 and 38 °C over average ambient temperatures were carried out at 1 °C/day to reach the experimental temperatures (34 and 38 °C). After that each group was fed with a diet supplemented with 0, 0.36, 0.72 or 1.42 % L-TRP for 45 days. Hence, total twelve experimental groups, viz. amb./0 % TRP, amb./0.36 % TRP, amb./0.72 % TRP, amb./1.42 % TRP, 34 °C/0 % TRP, 34 °C/0.36 % TRP, 34 °C/0.72 % TRP, 34 °C/1.42 % TRP, 38 °C/0 % TRP, 38 °C/0.36 % TRP, 38 °C/0.72 % TRP and 38 °C/1.42 % TRP were arranged in triplicates. The physicochemical parameters were within the optimum

Table 1 Feed composition and proximate composition of the experimental diets (% dry matter basis) fed to *L. rohita* fingerlings

	Diets			
	0 % TRP	0.36 % TRP	0.72 % TRP	1.44 % TRP
Ingredients (%)				
Soybean meal	40	40	40	40
Fish meal	20.50	20.50	20.50	20.50
Corn flour	15	15	15	15
Wheat flour	14	13.64	13.28	12.44
Fish oil	8	8	8	8
Carboxy methyl cellulose	1	1	1	1
L-Tryptophan	0	0.36	0.72	1.44
Vitamin–mineral mix[a]	0.5	0.5	0.5	0.5
Vitamin B complex[b]	0.05	0.05	0.05	0.05
Vitamin C	0.95	0.95	0.95	0.95
Total	100	100	100	100
Proximate composition of diets				
Organic matter	96.44 ± 0.31	96.53 ± 0.07	96.83 ± 0.02	96.70 ± 0.01
Crude protein	34.33 ± 0.23	34.73 ± 0.16	35.06 ± 0.06	35.81 ± 0.18
Total carbohydrate	54.56 ± 0.02	54.07 ± 0.17	54.19 ± 0.21	53.08 ± 0.10
Ether extract	7.55 ± 0.10	7.73 ± 0.08	7.59 ± 0.29	7.81 ± 0.07
Ash	3.57 ± 0.32	3.48 ± 0.08	3.17 ± 0.02	3.30 ± 0.01
Digestible energy[c]	423.49 ± 1.76	424.75 ± 0.70	425.27 ± 1.53	425.85 ± 0.31

[a] Composition of vitamin–mineral mix (agrimin) (quantity/kg): vitamin A—6,25,000 IU, vitamin D_3—62,500 IU, vitamin E—250 mg, nicotinamide—1 g, Cu—312 mg, Co—45 mg, Mg—6 g, Fe—1.5 g, Zn—2.13 g, I—156 mg, Se—10 mg, Mn—1.2 g, Ca—247.34 g, P—114.68 g, S—12.2 g, Na—5.8 mg, K—48.05 mg

[b] Composition of vitamin B complex (quantity g^{-1}): thiamine mononitrate—20 mg, riboflavin—20 mg, pyridoxine hydrochloride—6 mg, vitamin B_{12}—30 mcg, niacinamide—200 mg, Ca pantothenate—100 mg, folic acid—3 mg, biotin—200 mcg, vitamin C: (Hoffman La Roche, Nutley, NJ, USA), 15 % ascorbic acid activity, L-tryptophan: (HiMedia)

[c] Digestible energy (kcal/100 g) = (%CP × 4) + (%EE × 9) + (%TC × 4)

range (dissolved oxygen: 6.6–7.6 mg/L; pH 7.6–8.1; ammonia nitrogen: 0.15–0.28 mg/L; nitrite nitrogen: 0.001–0.005 mg/L; nitrate nitrogen: 0.02–0.05 mg/L) throughout the experimental period. All the groups were fed their respective diets throughout the experimental period. Feed was given at 3 % of body weight twice daily at 10:00 and 18:00 h under a normal light regime (light/dark 12/12). Uneaten feed and fecal matter were siphoned out daily and 40 % water was replaced with clean bore well water, maintained at the same temperature. Round the clock aeration was provided.

Proximate analysis of feed

The proximate composition of the experimental diets was determined following the standard methods of AOAC (AOAC 1995) and is presented in Table 1. Dry weight of feed was estimated after drying at 105 °C for 12 h and ashing (6 h at 600 °C). Crude fat (CF) content of the samples was estimated using Soxtec system (Model HT2, 1045 extraction unit Foss Tecator, Sweden) using diethyl ether as a solvent. Nitrogen in the feed and carcass was estimated by a micro Kjeldahl method (Foss Tecator 2200 Kjeltec), and CP was calculated as $N \times 6.25$. Total carbohydrate was calculated by difference, i.e., total carbohydrate (%) = 100 − (CP% + CF% + ash%). The digestible energy (DE) of the feed and of the carcass was calculated following Halver (1976).

Growth study

Fish were weighed at the start and every 15 days interval thereafter till the end of the experiment on the

45 days. The growth performance of fingerlings was evaluated in terms of weight gain, RGR, FCR and PER as given below:

$$\text{Weight gain}\% = (\text{final weight} - \text{initial weight})/\text{initial weight} \times 100.$$

$$\text{RGR}\,(\%/\text{day}) = (e^{g} - 1) \times 100$$

where e is the Nepper number and $g = [\ln(w2) - \ln(w1))/(t2 - t1)$, $w2$ is the final average weight, $w1$ is the initial average weight and t is the time.

$$\text{Feed conversion ratio (FCR)} = \text{total feed given (dry weight) (g)/weight gain (wet weight) (g).}$$

$$\text{Protein efficiency ratio (PER)} = \text{total wet weight gain (g)/crude protein fed (g).}$$

Sample preparation

At the end of the experiment, three fish from each replicate with a total of nine fish from each treatment were anesthetized with clove oil at 50 µL/L. For enzyme assay, separate homogenates were prepared for each tissue like muscle, liver, gill and brain. Tissues were homogenized with chilled 0.25 M sucrose solution using a mechanical tissue homogenizer. The homogenized samples were centrifuged (5,000 × *g* for 10 min at 4 °C), and supernatants were collected and stored at −20 °C for subsequent enzyme assay. For serum, again two fish from each replicate with a total of six fish from each treatment were anesthetized; the blood was collected without anticoagulant and allowed to clot for 2 h, centrifuged (3,000 × *g* for 5 min at 4 °C) and then kept at −80 °C until use.

Blood glucose and serum cortisol assays

Blood glucose was estimated at 540 nm by the method of Nelson and Somogyi (1945). Serum cortisol was estimated by using a validated radioimmunoassay (RIA) modified by Olsen et al. (1992) as described by Winberg and Lepage (1998) and expressed as ng/mL.

Enzyme assays

Aspartate aminotransferase (AST) (E.C.2.6.1.1) and alanine aminotransferase (ALT) (E.C.2.6.1.2) activities were measured by the estimation of oxaloacetate and pyruvate released, respectively, after incubation of the reaction mixture at 37 °C for 60 min (Wooton 1964). Lactate dehydrogenase (LDH) (L-lactate NAD + oxidoreductase; E.C.1.1.1.27) was assayed using 0.1 M phosphate buffer (pH 7.5), 0.2 mM NADH solution in 0.1 M phosphate buffer. The reaction was initiated by adding 0.2 mM sodium pyruvate as the substrate and optical density (OD) was recorded at 340 nm (Wroblewski and Ladue 1955). A similar reaction mixture was used for the estimation of malate dehydrogenase (MDH) (L-malate: NAD+ oxidoreductase; E.C.1.1.1.37) except for the substrate (1 mg oxaloacetate/mL of chilled triple distilled water) (Ochoa 1955). Acetylcholine esterase (acetyl hydroxylase, E.C. 3.1.1.7) activity was measured by change in optical density at 540 nm by following the method of Hestrin (1949) modified by Augustinsson (1957). Superoxide dismutase (SOD) (E.C.1.15.1.1) activity was estimated by the method of Misra and Fridovich (1972). The assay is based on the oxidation of epinephrine-adrenochrome transition by the enzyme. The reaction mixture consisted of 50 µL of sample, 1.5 mL phosphate buffer and 0.5 µL epinephrine. The solution was mixed well, and the OD was immediately read at 480 nm. The protein content was analyzed from the supernatant (Lowry et al. 1951) for calculating enzyme activities. Catalase (E.C. 1.11.1.6) was assayed using 50 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 30 % H_2O_2 as the substrate, and OD was recorded at 240 nm (Claiborne 1985).

Statistical analysis

Each experimental assay was performed in triplicate, and values are presented as mean ± SE. Two-way analysis of variance was used to determine difference between different temperature and graded L-TRP and their interaction for each parameters according to methods of Zar (2009) followed by Duncan's multiple range test (Duncan, 1955) to determine the significant difference at the 5 % ($p < 0.05$) level.

Results

Growth parameters

The weight gain%, RGR, FCR and PER values at different temperature and graded level of dietary L-TRP

Table 2 Growth performance (mean ± SE) of *L. rohita* fingerlings fed with different experimental diets

Treatment	% Weight gain	FCR	PER	RGR
Temperature (°C)				
Ambient	247.38 ± 17.38[a]	1.39 ± 0.04[c]	1.31 ± 0.05[a]	1.34 ± 0.04[a]
34	226.10 ± 17.63[b]	2.39 ± 0.16[b]	1.09 ± 0.06[b]	0.83 ± 0.03[b]
38	212.40 ± 18.79[b]	2.46 ± 0.13[a]	0.96 ± 0.09[c]	0.78 ± 0.05[c]
L-Tryptophan (%)				
0	143.22 ± 5.10[d]	2.60 ± 0.25[a]	0.84 ± 0.08[d]	0.78 ± 0.09[d]
0.36	219.18 ± 11.76[c]	2.19 ± 0.22[b]	1.02 ± 0.06[c]	0.95 ± 0.09[c]
0.72	256.19 ± 10.04[b]	1.86 ± 0.14[c]	1.22 ± 0.04[b]	1.05 ± 0.08[b]
1.42	295.91 ± 4.97[a]	1.67 ± 0.09[d]	1.40 ± 0.03[a]	1.15 ± 0.09[a]

Mean values of all the assays were subjected to two-way analysis of variance. Different superscripts in the same column indicate significant difference ($p < 0.05$) amongst different treatments. Values are expressed as mean ± SE ($n = 3$)

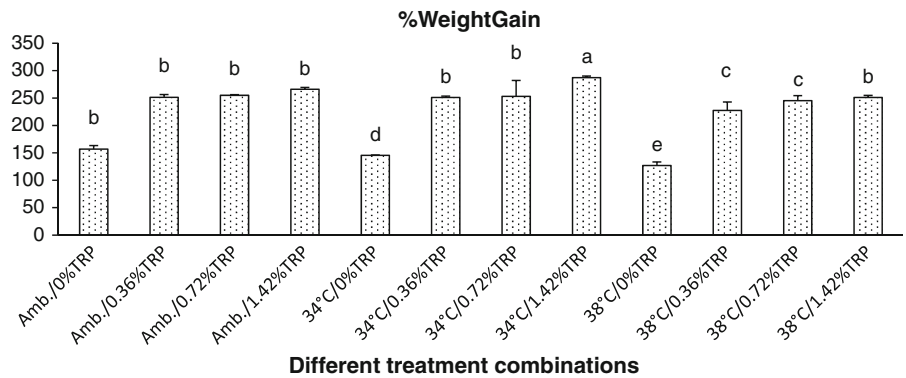

Fig. 1 Interaction between different temperature and graded dietary L-tryptophan on percent weight gain of *L. rohita* fingerlings. Mean values of the assay were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

and their interaction of the different experimental groups are shown in Table 2, Figs. 1 and 2. Percentage weight gain, RGR and PER at ambient temperature were significantly higher ($p < 0.05$) than the other group (34 and 38 °C). Irrespective of the temperature, TRP-supplemented groups exhibited higher percentage weight gain, RGR PER, and lower FCR. A significant interaction was found between the level of L-TRP and the temperature. The interaction study reveals that the percentage weight gain, FCR, PER and RGR in different experimental group varied significantly ($p < 0.05$). The highest percentage weight gain, PER and RGR were recorded in 34 °C/1.42 % TRP group and the lowest in the 38 °C/0 % TRP group. The lowest FCR was recorded in 34 °C/1.42 % TRP group and highest in 38 °C/0 % TRP group.

Blood glucose level

Effect of different temperatures and graded levels of dietary L-TRP and their interaction on blood glucose of the different experimental groups is shown in Table 3 and Fig. 3. The highest value was found in high temperature group, i.e., 38 °C, and the lower value was found in the ambient temperature group. Blood glucose level increases significantly ($p < 0.05$) with increase in temperature and decreases significantly ($p < 0.05$) with increase in dietary L-TRP supplementation. The interaction study reveals that blood glucose value in different experimental group varied significantly ($p < 0.05$). Highest value was observed in 38 °C/0 % TRP group, and lowest value was found in amb./1.42 % TRP group, which does not vary significantly with amb./0.72 % TRP group.

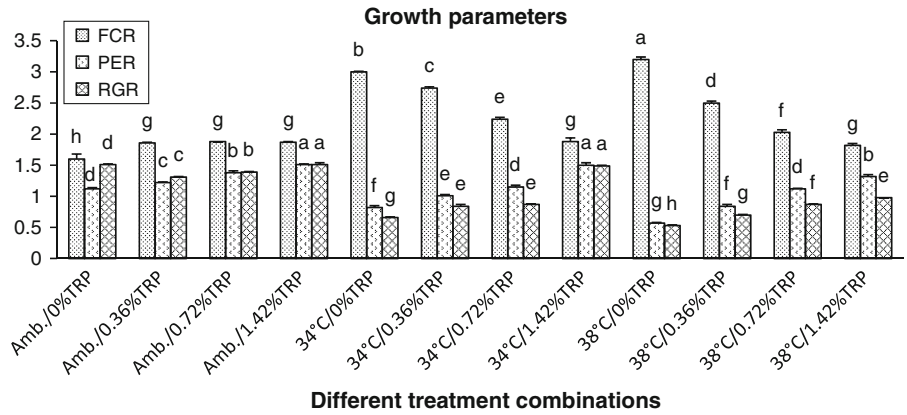

Fig. 2 Interaction between different temperature and graded dietary L-tryptophan on other growth parameters of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

Table 3 Effect of different temperature and graded dietary L-tryptophan level on the activity of blood glucose, serum cortisol, AST and ALT of *L. rohita* fingerlings

Treatment	Blood glucose	Serum cortisol	AST		ALT	
			Liver	Muscle	Liver	Muscle
Temperature (°C)						
Ambient	84.15 ± 3.88^{b}	135.51 ± 8.28^{c}	21.36 ± 1.44^{c}	26.52 ± 1.79^{c}	9.05 ± 1.20^{c}	10.33 ± 2.76^{b}
34	103.43 ± 5.20^{a}	220.38 ± 29.64^{b}	31.05 ± 6.28^{b}	29.35 ± 0.87^{b}	10.15 ± 2.87^{b}	13.42 ± 1.27^{b}
38	102.83 ± 4.66^{a}	400.92 ± 33.68^{a}	31.58 ± 0.93^{a}	35.05 ± 2.95^{a}	12.77 ± 2.25^{a}	20.90 ± 1.32^{a}
L-Tryptophan (%)						
0	117.09 ± 3.21^{a}	360.45 ± 54.27^{a}	42.59 ± 6.20^{a}	40.69 ± 2.78^{a}	20.48 ± 2.07^{a}	20.68 ± 1.82^{a}
0.36	102.59 ± 4.16^{b}	288.16 ± 46.47^{b}	27.70 ± 1.33^{b}	28.69 ± 1.28^{b}	10.61 ± 0.42^{b}	14.82 ± 2.10^{b}
0.72	90.64 ± 4.26^{c}	200.22 ± 35.59^{c}	22.42 ± 2.03^{c}	26.52 ± 0.78^{c}	6.55 ± 1.07^{c}	13.25 ± 2.48^{bc}
1.42	76.89 ± 1.18^{d}	160.24 ± 23.44^{d}	19.27 ± 2.10^{d}	25.34 ± 0.86^{d}	4.98 ± 1.29^{d}	10.78 ± 3.07^{c}

Mean values of all the assays were subjected to two-way analysis of variance. Different superscripts in the same column indicate significant difference ($p < 0.05$) amongst different treatments. Values are expressed as mean ± SE ($n = 3$). Units: blood glucose (mg/100 mL), cortisol (ng/mL of plasma), nanomoles oxaloacetate released/mg protein/min at 37 °C (AST), nanomoles of sodium pyruvate formed/mg protein/min at 37 °C (ALT)

Serum cortisol level

Effect of different temperatures and graded levels of dietary L-TRP and their interaction on serum cortisol level of the different experimental groups is given in Table 3 and Fig. 3. The higher cortisol level was found in high temperature group, i.e., 38 °C, and the lower level was found in the ambient temperature group. Serum cortisol level increases significantly ($p < 0.05$) with increase in temperature and decreases significantly ($p < 0.05$) with increase in dietary L-TRP supplementation. The interaction study reveals that serum cortisol level in different experimental group varied significantly ($p < 0.05$). Highest value was observed in 38 °C/0 % TRP group, and lowest value was found in amb./1.42 % TRP group.

Enzyme assays

AST and ALT

Data on AST and ALT activity in liver and muscle of *L. rohita* fingerlings of the different experimental groups are shown in Table 3, Figs. 4 and 5. An

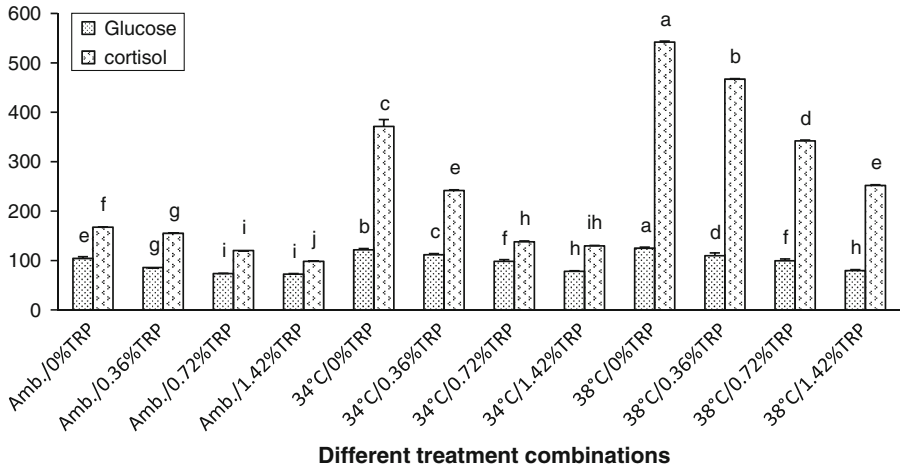

Fig. 3 Interaction between different temperature and graded dietary L-tryptophan on blood glucose and serum cortisol of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$). Unit: blood glucose (mg/100 mL), cortisol (ng/mL of plasma)

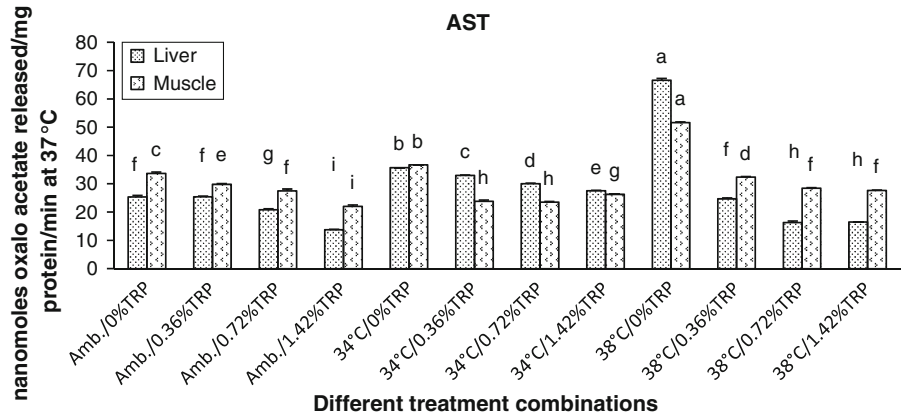

Fig. 4 Interaction between different temperature and graded dietary L-tryptophan on the activity of AST in liver and muscle of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

increase in AST and ALT activity was evident in the both liver and muscle with increasing temperature ($p < 0.05$). Gradual decrease in their activity is evident in both liver and muscle with the increasing level of dietary L-TRP. The interaction studied reveals that the AST and ALT activity in both liver and muscle vary significantly ($p < 0.05$) among different experimental groups. In both liver and muscle, highest AST activity was observed in 38 °C/0 % TRP, whereas lowest in amb./1.42 % TRP group, whereas the ALT activity in liver and muscle was highest in 38 °C/0 % TRP group, whereas lowest in 38 °C/1.42 % TRP group.

LDH and MDH

Data pertaining to LDH and MDH activity in liver and muscle of *L. rohita* fingerlings at different temperature and graded levels of dietary L-TRP and their interaction of the different experimental groups are shown in Table 4, Figs. 6 and 7. Activity of LDH and MDH in the liver and muscle at ambient temperature was significantly lower ($p < 0.05$) than the other groups (34 and 38 °C). Similarly, TRP-supplemented groups exhibited significantly lower ($p < 0.05$) activity of LDH and MDH. The interaction study reveals that LDH and MDH activity in different experimental group varied significantly ($p < 0.05$). The highest activity was recorded in 38 °C/0 % TRP group and the lowest in the 38 °C/0.72 % TRP group, which is similar to 38 °C/1.42 % TRP.

AChE

Acetylcholine esterase (AChE) activity of brain tissue is given in Table 5 and Fig. 8. AChE activity significantly decreases with increase in temperature, whereas dietary L-TRP supplementation increases

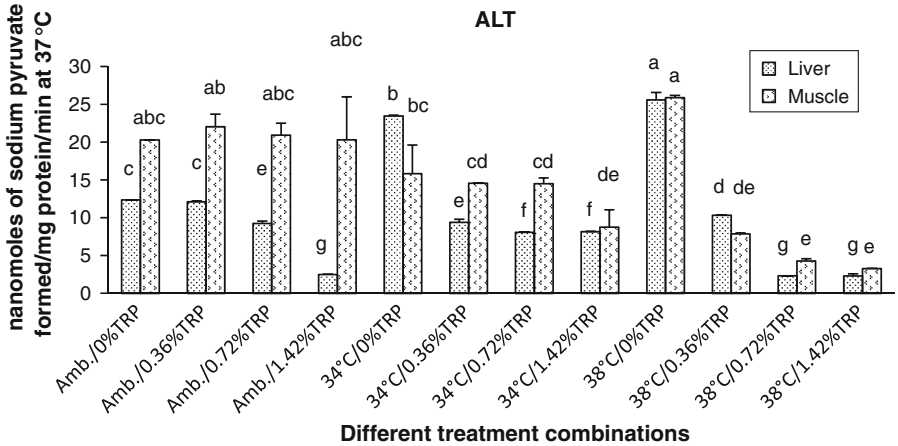

Fig. 5 Interaction between different temperature and graded dietary L-tryptophan on the activity of ALT in liver and muscle of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

Table 4 Effect of different temperature and graded dietary L-tryptophan level on the activity of LDH and MDH in liver and muscle of *L. rohita* fingerlings

Treatment	LDH		MDH	
	Liver	Muscle	Liver	Muscle
Temperature (°C)				
Ambient	3.12 ± 0.32^{b}	3.47 ± 0.48^{a}	1.17 ± 0.10^{c}	2.43 ± 0.15^{b}
34	3.51 ± 0.60^{b}	4.49 ± 0.54^{b}	4.22 ± 0.38^{b}	2.63 ± 0.29^{c}
38	4.38 ± 0.18^{a}	4.97 ± 0.68^{c}	5.04 ± 0.25^{a}	2.96 ± 0.16^{a}
L-Tryptophan (%)				
0	4.83 ± 0.56^{a}	5.72 ± 0.85^{b}	4.58 ± 0.90^{a}	3.33 ± 0.06^{a}
0.36	2.72 ± 0.14^{b}	2.70 ± 0.16^{c}	3.14 ± 0.55^{bc}	3.17 ± 0.24^{b}
0.72	2.71 ± 0.25^{b}	2.83 ± 0.17^{c}	3.17 ± 0.40^{b}	2.31 ± 0.08^{c}
1.42	2.71 ± 0.47^{b}	2.75 ± 0.18^{c}	3.01 ± 0.54^{c}	1.89 ± 0.15^{d}

Mean values of all the assays were subjected to two-way analysis of variance. Different superscripts in the same column indicate significant difference ($p < 0.05$) amongst different treatments. Values are expressed as mean ± SE ($n = 3$). Units: nanomoles of pyruvate utilized/mg protein/min at 37 °C (LDH), nanomoles of oxaloacetate utilized/mg protein/min at 37 °C (MDH)

the AChE activity ($p < 0.05$). The interaction studied reveals that the AChE activity varies significantly ($p < 0.05$) among different experimental groups. The highest AChE activity was observed in amb./1.42 % TRP group and lowest in 38 °C/0 % TRP group.

SOD

Data related to SOD activity of different experimental groups exposed to different temperatures and fed with varied levels of dietary L-TRP is given in Table 5 and Fig. 9. In both liver and gill, SOD activity seems to be significantly decreasing with both increase in temperature and dietary L-TRP supplementation ($p < 0.05$). The interaction studied showed that the SOD activity in both liver and gill differ significantly ($p < 0.05$). In liver, highest activity of SOD was observed in amb./0 % group which is similar to amb./0.36 % TRP, amb./0.72 % TRP and amb./1.42 % TRP group, whereas lowest activity was observed in 38 °C/1.42 % TRP group, which is similar to 34 °C/0.72 % TRP, 34 °C/1.42 % TRP, 38 °C/0.36 % TRP, and 38 °C/0.72 % TRP groups. In gill, highest SOD activity was observed in 38 °C/0 % TRP group,

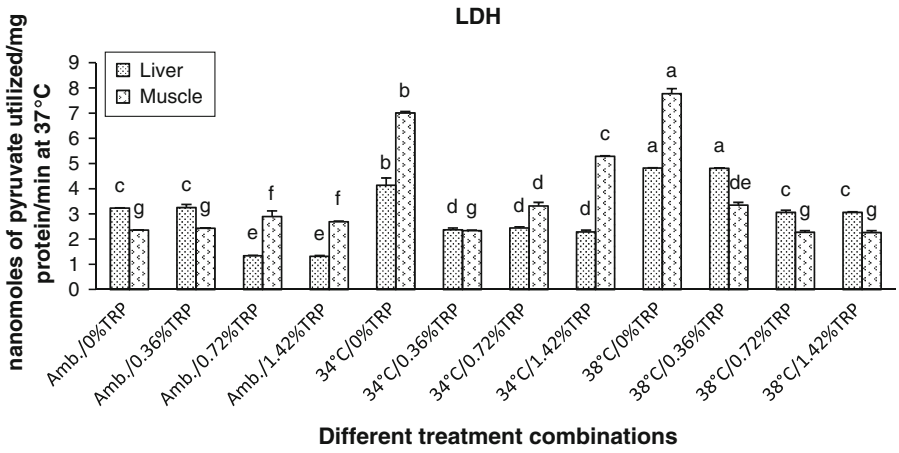

Fig. 6 Interaction between different temperature and graded dietary L-tryptophan on the activity of LDH in liver and muscle of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

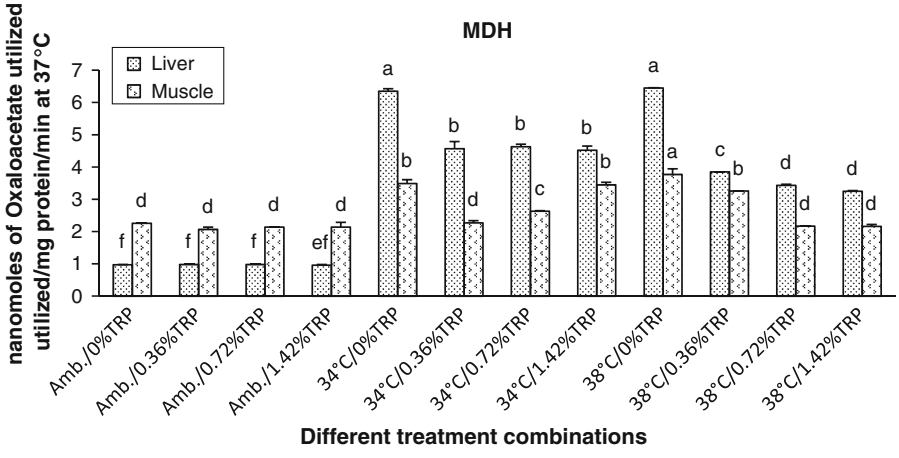

Fig. 7 Interaction between different temperature and graded dietary L-tryptophan on the activity of MDH in liver and muscle of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

whereas lowest activity was found in amb./1.42 % TRP group, which is similar to amb./0.72 % TRP and 34 °C/1.42 % TRP group.

Catalase activity

Data on catalase activity in liver and gill of *L. rohita* fingerlings of the different experimental groups is shown in Table 5 and Fig. 10. Significantly increase in catalase activity with increasing temperature and decrease in catalase activity with increasing L-TRP was observed in both liver and gill of the all experimental groups ($p < 0.05$). The interaction studied reveals that the catalase activity in both liver and gill varies significantly ($p < 0.05$) among different experimental groups. The highest catalase activity in both liver and gill was observed in 38 °C/0 % TRP group and lowest in 38 °C/1.42 % TRP group.

Discussion

Stress is a general term proposed by Selye in 1953 (Selye 1953) applying to a situation in which a person or an animal is subjected to a challenge that may result in a real or symbolic danger for its integrity (Tort 2011). Stress is an event that most animals experience and include a cascade of reactions such as primary, secondary and tertiary responses. Very few reports are available on the effect of dietary L-TRP on body metabolism and mitigation of stress in fish (Lepage et al. 2002; Tejpal et al. 2009). Thus, the current study

Table 5 Effect of different temperature and graded dietary L-tryptophan level on the activity acetylcholine esterase (AChE), catalase and SOD activity in different tissues of *L. rohita* fingerlings

Treatment	AChE	SOD		CAT	
	Brain	Liver	Gill	Liver	Gill
Temperature (°C)					
Ambient	30.89 ± 0.05[a]	13.49 ± 1.06[a]	17.33 ± 0.99[a]	50.81 ± 2.39[b]	5.68 ± 0.60[b]
34	16.59 ± 0.03[b]	3.24 ± 0.34[b]	8.93 ± 0.77[b]	52.08 ± 0.67[b]	14.27 ± 7.47[ab]
38	10.84 ± 0.01[c]	2.58 ± 0.41[b]	7.55 ± 1.10[c]	60.59 ± 8.83[a]	24.83 ± 6.84[a]
L-Tryptophan (%)					
0	10.36 ± 0.01[d]	6.75 ± 1.09[ab]	16.00 ± 1.68[a]	73.21 ± 7.69[a]	25.01 ± 9.41[a]
0.36	18.51 ± 0.03[c]	6.19 ± 1.50[b]	11.81 ± 1.37[b]	58.43 ± 3.41[b]	7.12 ± 0.75[b]
0.72	24.70 ± 0.04[b]	5.63 ± 2.88[c]	8.92 ± 1.93[c]	45.19 ± 1.61[c]	5.37 ± 0.20[c]
1.42	30.33 ± 0.05[a]	5.07 ± 1.70[c]	8.35 ± 1.22[d]	41.13 ± 3.36[c]	4.20 ± 9.58[c]

Mean values of all the assays were subjected to two-way analysis of variance. Different superscripts in the same column indicate significant difference ($p < 0.05$) amongst different treatments. Values are expressed as mean ± SE ($n = 3$). Units: unit activity amount of protein required to give 50 % inhibition of epinephrine auto oxidation at 37 °C (SOD), nano moles H_2O_2 decomposed/min/mg protein at 37 °C (CAT), micromoles acetylcholine hydrolyzed/mg protein/min at 37 °C (AChE)

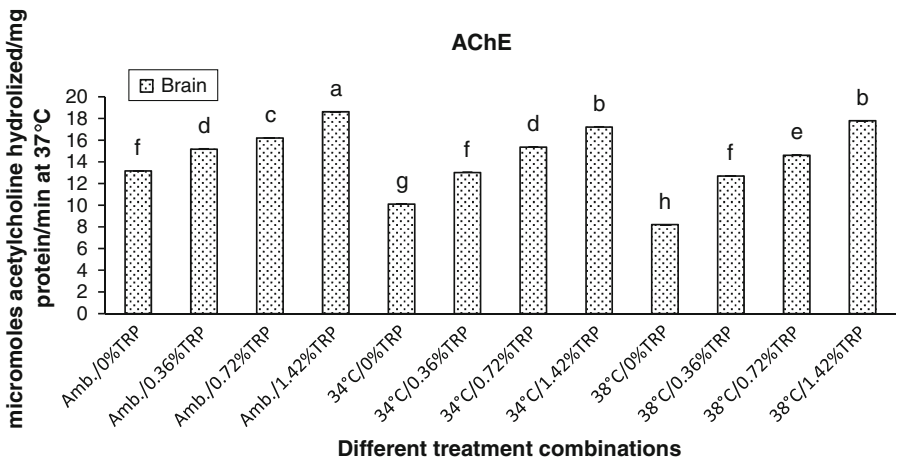

Fig. 8 Interaction between different temperature and graded dietary L-tryptophan on the activity of AChE in the brain of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

was conducted to evaluate the efficacy of dietary L-TRP for mitigating thermal stress and growth enhancing effect in rohu, *L. rohita* fingerlings.

In the present study, dietary supplementation of L-TRP showed a significant effect on the growth parameters in all the experimental groups. Control groups of different temperature showed lowest growth indices compared with their respective TRP treatment groups. In general, the percentage weight gain, protein efficiency ratio and relative growth rate at ambient temperature were significantly higher ($p < 0.05$) than the high temperature groups (34 and 38 °C) which appears to be due to the thermal stress. In higher temperature groups (34 and 38 °C), dietary L-TRP supplementation improved the growth parameters of fish, which is supported by the finding of Walton et al. (1984) who found better growth and survival in rainbow trout when fed with TRP than the control group. Similarly, Tejpal et al. (2009) reported that L-TRP supplementation in diet mitigate crowding stress and improved growth in *C. mrigala* fingerlings.

The measurement of blood glucose level is considered as an effective method to evaluate the stress effect of a variety of stressors and is the ideal parameter to study the secondary stress response (Wedemeyer and Mcleay 1981). In the present study, blood glucose

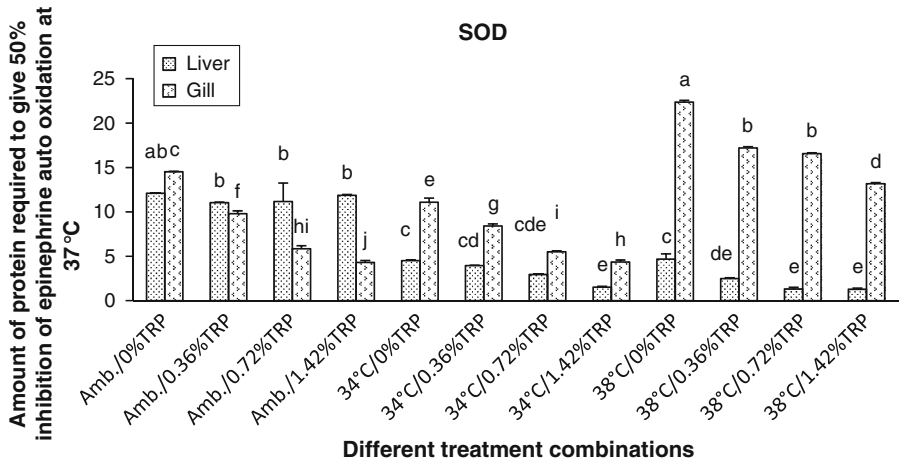

Fig. 9 Interaction between different temperature and graded dietary L-tryptophan on the activity of SOD in liver and gill of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

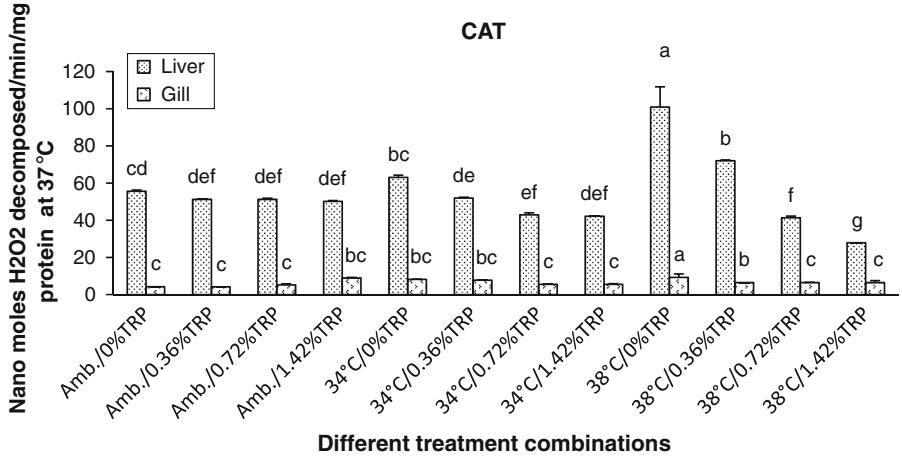

Fig. 10 Interaction between different temperature and graded dietary L-tryptophan on the activity of CAT in liver and gill of *L. rohita* fingerlings. Mean values of all the assays were subjected to two-way analysis of variance. Mean value in the column with *different superscript* differs significantly ($p < 0.05$). Values are expressed as mean ± SE ($n = 3$)

level increases significantly with increase in temperature but decreases significantly with increase in dietary L-TRP supplementation. Positive correlation between glucose level and temperature acclimation is reported by Costas et al. 2012. The reason for increase in glucose level could be increased in glycolysis is also indicated by increased LDH activity in liver and muscle due to higher production of lactate. The decrease in blood glucose level in dietary L-TRP-supplemented group could inhibit release of cortisol, hence reducing glucose levels. The lower blood glucose content in L-TRP fed group suggests that the L-TRP is an effective thermal stress mitigator for fish. Tejpal et al. (2009) and Hoseini and Hosseini (2010) also showed the crowding and osmotic stress mitigation effect of L-TRP in *C. mrigala* and *C. carpio,* respectively.

Cortisol is also widely accepted as an indicator of stress in fish, generally increases after exposure to stressors (Wendelaar Bonga 1997; Davis 2004; Tejpal et al. 2009; Hoseini and Hosseini 2010; Costas et al. 2012). In the present study, the highest serum cortisol was found in higher temperature groups (34 and 38 °C) fed without dietary L-TRP, indicates secretion of cortisol due to stress caused by high temperature. Gradual decrease in serum cortisol level in all three temperature groups fed with the increasing level of dietary L-TRP confers that dietary L-TRP mitigates the higher temperature stress in *L. rohita* fingerlings. The present findings are in agreement with observations of Lepage et al. (2002) for *Oncorhnchus mykiss*; Tejpal et al. (2009) for *C. mrigala* and Hoseini and Hosseini (2010) for *C. carpio* who reported that feeding with L-TRP-supplemented diets resulted in suppression of

stress-induces elevation of serum cortisol. Hence, it can be concluded that increased cortisol levels normally led to immunosuppression in fish and therefore, dietary TRP supplementation may result in a positive immune-modulation at high temperatures. However, the present finding is contradictory to the observation of Martins et al. (2013) who observed significant reduction in cortisol level in unstressed fish fed with TRP-supplemented diet, and there is no suppression of stress-induces elevation of serum cortisol when feeding with TRP-supplemented diets.

The higher activity of AST and ALT indicates the mobilization of aspartate and alanine via gluconeogenesis for glucose production to cope with stress. Previous studies also reported that elevated level of transaminase activity during stress would lead to increased feeding of keto acid into TCA cycle, thereby affecting oxidative metabolism (Knox and Greengard 1965; Vijayan et al. 1997; Verma et al. 2007). Costas et al. (2012) reported the augmentation of plasma aspartate and glutamate concentration in Senegalese sole at higher acclimation temperature due to increased transamination process which increased utilization of amino acids as energetic substrate at higher acclimation temperature stress. The activity of both the transferase, on the contrary, decreased with the gradual increase in L-TRP and higher activity in those groups not fed with dietary L-TRP. This is also supported by the higher growth rate of groups supplemented with L-TRP, as amino acids were not diverted for energy production but utilized for growth. Less activity in L-TRP-supplemented groups can be inferred that addition of L-TRP reduces the thermal stress in *L. rohita* fingerlings. Similar finding was observed by Tejpal et al. (2009) in *C. mrigala* due to crowding stress. Costas et al. 2012 reported the reduction in plasma TRP level at higher acclimation temperature which may be due to increased up take of plasma TRP in brain of fish to increased synthesis of serotonin (5-hydroxytrptamine), which is essential to combat the stress.

In the present study, high temperature (34 and 38 °C) groups showed highest LDH activity in comparison with ambient temperature group. The increase in LDH activity in high temperature groups is attributed to the production of preferred substrate (Lactate) for gluconeogenesis under thermal stress due to oxygen limited condition in the cell. A similar observation was made under confinement (Chatterjee et al. 2006; Tejpal et al. 2009), thermal acclimation (Grigo 1975; Verma et al. 2007) and starvation (Vijayaraghaban and Rao 1986). On the other hand, low activity in the L-TRP-supplemented groups suggested that the L-TRP supplementation reduces cortisol-induced thermal stress. The present finding is in agreement with observation of Tejpal et al. (2009) who reported dietary L-TRP reduces induced cortisol effect due to crowing stress in the *C. mrigala* fingerlings. The activity of MDH was found higher in high temperature (34 and 38 °C) groups as compared with ambient temperature group. Previous studies also reported elevated MDH activity in fishes, acclimated at higher temperature (Das et al. 2006; Verma et al. 2007). Higher MDH activity was also observed under crowding stress (Tejpal et al. 2009), confinement stress (Chatterjee et al. 2006). Higher MDH activity indicates greater activity of the TCA cycle in order to use the product (oxaloacetate) due to the higher activity of AST for production of more energy (ATP). Glucose might have therefore mobilized through non-carbohydrate source; mainly by protein, as transaminase activities increased at higher temperatures. Result also strengthens the fact that higher temperature induces amino acid mobilization (alanine, aspartate). On the other hand, lower activity in the L-TRP-supplemented group supports our findings that the supplementation of dietary L-TRP reduced the energy demands in the *L. rohita* fingerlings and hence thermal stress.

The AChE in brain of *L. rohita* fingerlings was assayed at the end of the experiment. There was a significant decrease ($p < 0.05$) in the activity of acetylcholine esterase among the treatment groups. The different temperature groups fed without L-TRP showed a decrease in the acetylcholine esterase activity which indicates stress to the animals induced by higher temperature, due to accumulation of acetylcholine. A similar observation was observed in *C. carpio* at higher acclimation temperature (Verma et al. 2007). Gradual increases in cholinesterase activity were observed in different temperature groups fed with increasing level of dietary L-TRP, and hence, L-TRP mitigates the thermal stress. Similarly, improvement in acetylcholine esterase activity was observed with dietary L-TRP in *C. mrigala* under crowding stress (Tejpal et al. 2009).

Several studies demonstrated that changes in antioxidant enzyme activities could be used as stress

indicators (Akhtar et al. 2010; Ciji et al. 2012). The exposure to various stressors in aquatic environment can enhance the intracellular formation of reactive oxygen species (ROS) capable of inducing oxidative damage (Livingstone et al. 1990). The ROS can be detoxified by an enzyme defense system, comprising SOD and catalase. The present study demonstrated that both liver and gill catalase and SOD activity was significantly higher in the group fed without L-TRP-supplemented diet. Among the L-TRP fed groups, the lowest activity was found in the group fed with either 0.72 or 1.44 % L-TRP. This indicates that oxidative stress is lower in the L-TRP fed groups than the group fed without L-TRP. Hence, it can be concluded that L-TRP might have antioxidative properties in mitigating oxidative stress caused due to increased temperature.

In conclusion, the overall results of this study suggested that dietary supplementation of L-TRP mitigates thermal stress and enhanced growth. Dietary supplementation of L-TRP at the 0.72 % level in the diet is found to be optimum to reduce thermal stress even up to 38 °C in rohu, *L. rohita.* The baseline data obtained here could be useful for the farmers to formulate feeds to culture this fish in different agro-climatic zones. However, in future, more elaborate studies should be carried out to elucidate the mode of action of dietary TRP in enhancing thermal tolerance of other major carp, for development of better management practices of the freshwater aquaculture sector of the country.

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