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Dietary supplementation of L-tryptophan mitigates crowding stress and augments the growth in *Cirrhinus mrigala* fingerlings

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ABSTRACT

A 60 days feeding trial was conducted to study the stress mitigation and hence growth augmenting effect of dietary L-tryptophan during high density group stress in *Cirrhinus mrigala* fingerlings. Four hundred eighty fingerlings were distributed into eight experimental groups. Each group either of low density group (10 fishes/75 L water) or higher density group (30 fishes/75 L water) was fed with a diet containing either 0, 0.68, 1.36 or 2.72% L-tryptophan in the diet, thus eight experimental groups *viz.* low density control (LC) (basal feed + 0%L-tryptophan); LT1 (basalfeed + 0.68% L-tryptophan); LT2 (basalfeed + 1.36% L-tryptophan); LT3 (basalfeed + 2.72% L-tryptophan) high density control (HC) (basal feed + 0%L-tryptophan); HT1 (basalfeed + 0.68% L-tryptophan); HT2 (basalfeed + 1.36% L-tryptophan); and HT3 (basalfeed + 2.72% L-tryptophan) were fed at 3% of the body weight with isonitrogenous (34.33 ± 0.23 to 35.81 ± 0.18 CP%) and isocaloric (423.49 ± 1.76 to 425.85 ± 0.31 K.Cal/100 g) purified diets. The possible role of dietary L-tryptophan on stress mitigation was assessed in terms of blood glucose, plasma cortisol, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), alanine amino transferase (ALT), aspartate amino transferase (AST), acetyl choline esterase (AChE) assays, whereas growth was evaluated in terms of weight gain %, specific growth (SGR) and protein efficiency ratio (PER). The LDH, MDH, ALT, AST activities were found to be significantly higher ($p<0.05$) in the control groups and decreasing trend of LDH, MDH, ALT and AST activities were observed with the increasing level of dietary L-tryptophan. However, high density group exhibited significantly higher value ($p<0.05$) for the above parameters than their low density counterpart at similar level of L-tryptophan in the diet. In contrast to above parameters, AChE activity exhibited a reverse trend. In both the stocking densities, L-tryptophan supplemented groups found to have higher ($p<0.05$) growth, SGR and PER. The Plasma cortisol level was found to be significantly higher in the high density group than its low density counterpart. However, gradual supplementation of L-tryptophan in diet significantly reduced the cortisol level both in high density ($Y=-26.33x+219.53$, $r^2=0.95$) and low density group ($Y=-27.57x+169.28$, $r^2=0.92$). Similar trend was also found in blood glucose. Hence, dietary supplementation of L-tryptophan at a minimum level of 1.36% concomitantly reduced the stress in *C. mrigala* fingerlings. Though 2.72% dietary tryptophan also reduces the stress but 1.36% level appears to be cost effective.

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1. Introduction

The present growth of aquaculture production at 6.0% is highest amongst the other agriculture and livestock production sector. Still higher production is required to achieve the set target of 12 mmt during 2020 to meet the protein requirement of huge human population. Hence, shifting from traditional culture practice to semi intensive or intensive culture practice is just inevitable to achieve that target. During the culture period, fish are predisposed to stress due to environmental changes, the high stocking density, etc resulting in detrimental consequences in aquatic organisms (Reubush and Heath, 1996).

The higher stocking density causes detrimental effect like poor growth and increased incidence of diseases in fish (Barton and Iwama, 1991a) due to stress. Much research effort has been focused on evaluating the effects of rearing density on variables such as fish growth (Holm et al., 1990; Bjornsson, 1994), survival (Sodeberg and Meade, 1987), feed intake (Jorgensen et al., 1993) and hormonal related changes (Leatherland and Cho, 1985; Kebus et al., 1992). Some authors have studied the effect of rearing densities on alteration of fish behaviour (Alanara and Brannas, 1996), metabolism (Vijayan et al., 1990; Lefrançois et al., 2001) and immunity due to the stress response often associated with high stocking density (Tort et al., 1996).

High stocking density has also been considered as an aquaculture-related chronic stressor (Barton and Iwama, 1991b) causing elevation of plasma cortisol (Barton et al., 1988; Pickering and Pottinger, 1989),

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the main corticosteroid in fish (Wendelaar Bonga, 1997; Mommsen et al., 1999). Plasma cortisol level is widely used as a general indicator of stressful situations in vertebrates and particularly in fish (Pickering and Pottinger, 1989).

Though cortisol secretion is a defensive strategy of body against stress, the continuous secretion of cortisol during chronic stress is harmful as it leads to immunosuppression. Hence blocking of cortisol secretion appears to be an ideal approach to combant stress. L-tryptophan plays a vital role in synthesis of neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). The first rate-limiting step in the 5-HT biosynthesis is the hydroxylation of TRP to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase (TPH) (Boadle Biber, 1993). Serotonin in turns directly blocks cortisol in rainbow trout, *Oncorhynchus mykiss* (Lepage et al., 2002). But the effect of dietary L-tryptophan on cortisol, selectively in carp is not known.

Stress response in all vertebrate including fish results in the activation of the neuro-endocrine system, which brings about changes in metabolism, osmoregulation and blood parameters (Chatterjee et al., 2006 and Wendelaar Bonga, 1997). The metabolism shifts from anabolism to catabolism to supply the extra energy needed to combat stress (Pickering, 1992). Much of the stress studies on fish have been focused on the primary response, i.e. cortisol and catecholamine levels (Barton and Iwama, 1991a, b). Of the secondary stress response parameters, blood glucose and lactate levels are most commonly tested (Chatterjee et al., 2006). Besides, glycogen level is another important indicator of the secondary stress response. Measurement of metabolic enzyme activity can also serve as a valuable stress indicator in organisms like marine invertebrate and fishes, where the accurate determination of field's metabolic rate is difficult (Dahlhoff, 2004).

Therefore, the present study was designed to mitigate the stress response occurring due to high stocking density of *Cirrhinus mrigala* fingerlings during experimental period by supplementing L-tryptophan in the feed.

2. Materials and methods

2.1. Experimental animals

C. mrigala (cyprinid or mrigal crap) fingerlings with an average weight of 2.8 to 3.0 g were procured from Government fish farm Khopoly, Maharashtra during the month of September (2006). The fishes were transported in a big circular container (500 L) with sufficient aeration to the wet laboratory. They were carefully transferred to another circular tank (1000 L) and were left undisturbed the whole night. In order to ameliorate the handling stress the fishes were given a mild salt treatment, the next day. The stock was acclimatized under aerated conditions for 35 days.

2.2. Experimental design

Four hundred eighty fishes were randomly distributed in two major groups like low density (10 fish/75 L) and high density group (30 fish/75 L). Each group was fed with a diet supplemented with either 0, 0.68, 1.36 or 2.72% L-tryptophan. Hence total eight experimental groups *viz.* low density control (LC) (basal feed + 0% L-tryptophan); LT1 (basal-feed + 0.68% L-tryptophan); LT2 (basalfeed + 1.36% L-tryptophan); and LT3 (basalfeed + 2.72% L-tryptophan), high density control (HC) (basalfeed + 0% L-tryptophan); HT1 (basalfeed + 0.68% L-tryptophan); HT2 (basalfeed + 1.36% L-tryptophan); and HT3 (basalfeed + 2.72% L-tryptophan) were arranged in triplicates following a CRD design. The total volume of the water in each tub was maintained at 75 L throughout the experimental period. Round the clock aeration was provided. The aeration pipe in each tub was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tubs. Feed was given at 3% of body weight for 60 days twice daily at 10:00 and 18:00 h under a normal light regime (light/dark 12/12 h). Uneaten feed and faecal matter were siphoned out daily and 80% water was replaced with fresh bore well water.

2.3. Experimental diets

The composition of purified diet is given in the Table 1. Fat free casein (SD Fines Chemical Ltd) and gelatin were used as the protein source, where as cod liver oil and soybean oil were used as a lipid source, dextrin, starch, cellulose and carboxymethyl cellulose (SD Fines Chemical Ltd.) as carbohydrates source. Graded level of tryptophan was added to the respective feed as specified in Table 1. Ingredients were thoroughly mixed with water to make dough. The dough was steam cooked for 5 min in a pressure cooker. Vitamin–mineral pre-mix was mixed after cooling and the dough was pressed through a hand pelletizer with 2 mm die and then dried at 60 °C. The feeds were stored at 4 °C until use.

2.4. Growth study

Fish were weighed at the start and every 15 days interval thereafter till the termination of the experiment on the 60th day. The growth performance of fingerling was evaluated (Biswas et al., 2007) in terms of weight gain, SGR, FCR and PER, as given below

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

$$\text{Specific growth rate (SGR)} = 100\,(\log_e \text{average final weight} - \log_e \text{average initial weight}) / \text{number of culture days}.$$

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

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

2.5. Sampling procedure

Six fish per treatment (two/replicate) were sampled at the end of the experimental period in order to evaluate the haematological

Table 1
Composition of purified diets (%DM basis).

	Control	T1	T2	T3
Casein (fat free)[a]	35.00	35.00	35.00	35.00
Gelatin[a]	9.50	9.50	9.50	9.50
Dextrin[a]	10.00	10.00	10.00	10.00
Starch[a]	25.00	25.00	25.00	25.00
Cellulose[a]	7.30	8.00	8.00	7.98
CMC[a]	1.50	1.50	1.50	1.50
Soya bean oil + cod liver oil	8.00	8.00	8.00	8.00
L-tryptophan[a]	–	.68	1.36	2.72
Vitamin + mineral mix	2.90	2.90	2.90	2.90
Vitamin C	0.10	0.10	0.10	0.10
BHT[a]	0.02	0.02	0.02	0.02

Casein fat free: 75% CP (HImedia).
Gelatin: 96% CP (HImedia).
L-tryptophan (HImedia).
Carboxymethyl cellulose (CMC): (SD Fines Chemical Ltd).
Dextrin: (HImedia).
Vitamin C: (Hoffman La Roche, Nuetly. NJ. USA). 15% ascorbic acid activity.
Composition of vitamin mineral mix (EMIX PLUS) (quantity 2.5 kg-14): Vitamin A 55,00,000 IU; Vitamin D3 11,00,000 IU; Vitamin B2 2000 mg; Vitamin E 750 mg; Vitamin K 1000 mg; Vitamin B6 1,000 mg; Vitamin B12 6 mcg; Calcium Pantothenate 2500 mg; Nicotinamide 10 g; Choline Chloride 150 g; Mn 27,000 mg; I 1000 mg; Fe 7500 mg; Zn 5000 mg; Cu 2000 mg; Co 450 mg; Ca 500 g; P 300 g; L-lysine 10 g; DL-Methionine 10 g; Selenium 50 ppm; Selenium 50 ppm; Satwari 250 ppm; (*Lactobacillus* 120 million units and Yeast culture 3000 crore units).
Butylated hydroxyl toluene (BHT) (SD Fines Chemical Ltd).
[a] Indicate significant difference ($P<0.05$) among the control and treatment groups.

Table 2
Growth performance (mean ± SE) of *Cirrhinus mrigala* fingerlings fed with different experimental diet.

Treatment	% Wt gain	FCR	PER	SGR
Stocking density				
Low density	$356.97^{b} \pm 11.63$	$1.73^{a} \pm 0.05$	$1.65^{b} \pm 0.04$	$1.30^{b} \pm 0.03$
High density	$183.00^{a} \pm 8.55$	$3.05^{b} \pm 0.12$	$0.95^{a} \pm 0.04$	$0.79^{a} \pm 0.02$
L-tryptophan level				
0%TRP	224.20 ± 33.74	2.75 ± 0.36	1.13 ± 0.15	0.91 ± 0.11
0.68%TRP	264.12 ± 42.25	2.48 ± 0.34	1.27 ± 0.17	1.03 ± 0.13
1.36%TRP	290.50 ± 44.95	2.26 ± 0.29	1.38 ± 0.18	1.10 ± 0.13
2.72%TRP	301.12 ± 36.35	2.08 ± 0.21	1.44 ± 0.14	1.14 ± 0.10
Stocking density X L-tryptophan				
L.(control)	$298.56^{d} \pm 9.68$	$1.96^{b} \pm 0.05$	$1.45^{d} \pm 0.03$	$1.15^{d} \pm 0.02$
LT_1(0.68%TR)	$357.99^{e} \pm 10.20$	$1.73^{ab} \pm 0.02$	$1.65^{e} \pm 0.02$	$1.30^{e} \pm 0.02$
LT_2(1.36%TR)	$389.98^{f} \pm 12.55$	$1.61^{a} \pm 0.03$	$1.76^{f} \pm 0.04$	$1.38^{e} \pm 0.03$
LT_3(2.72%TR)	$381.34^{ef} \pm 8.76$	$1.62^{a} \pm 0.01$	$1.75^{f} \pm 0.01$	$1.36^{e} \pm 0.02$
H.(control)	$149.85^{a} \pm 8.47$	$3.54^{f} \pm 0.17$	$0.80^{a} \pm 0.04$	$0.67^{a} \pm 0.03$
HT_1(0.68%TR)	$170.25^{ab} \pm 3.39$	$3.23^{e} \pm 0.05$	$0.88^{a} \pm 0.04$	$0.74^{ab} \pm 0.01$
HT_2(1.36%TR)	$191.10^{b} \pm 6.95$	$2.90^{d} \pm 0.07$	$0.98^{b} \pm 0.02$	$0.82^{b} \pm 0.02$
HT_3(2.72%TR)	$220.90^{c} \pm 9.81$	$2.54^{c} \pm 0.08$	$1.12^{c} \pm 0.03$	$0.91^{c} \pm 0.03$

Mean value in the column under each category (stocking density, L-tryptophan level and stocking density × L-tryptophan) with different superscript differ significantly ($P<0.05$), Data expressed as mean ± SE.

parameters. Fish were quickly captured and anaesthetized with clove oil at 50 µl/L. Blood samples were taken from the caudal vein using heparinised syringes, and transferred to heparinised tubes kept on ice until centrifugation. Blood samples were collected by the bleeders to ensure that blood collection was completed within 1 min as described by Cristina et al. (2006). Plasma was separated from blood by centrifuging at 5 °C for 10 min at 5000 ×g (Debnath et al., 2007).

2.6. Sample preparation for enzyme assay

Four fish per treatment were anaesthetized with clove oil at 50 µl/L and killed by decapitation. For enzyme assay, separate homogenates prepared for each tissue, where tissue was homogenized with chilled 0.25 M sucrose solution using a mechanical tissue homogenizer. The homogenized samples were centrifuged (5000 *g* 4 °C for 10 min) and supernatants were collected and stored at −20 °C for subsequent enzyme assays.

AST and ALT activities were measured by the estimation of oxaloacetate and pyruvate released, respectively, after incubation of the reaction mixture at 37 °C for 60 min (Wooten, 1964). LDH and MDH activities were measured by the change in optical density (OD) at 340 nm for 5 min using the method of Wrobleuiski and Ladue (1955) and Ochoa (1955), respectively.

AChE activity as measured by the change in optical density (OD) at 540 nm by following the method of Hestrin (1949) modified by Augustinsson (1957).

Glucose was estimated by the method of Nelson-Somogii (1945) as described by Oser (1965). Cortisol in fish plasma was estimated by using a validated radioimmunoassay (RIA) modified by Olsen et al. (1992), as described by Winberg and Lepage (1998). Plasma cortisol was expressed as ng/ml.

2.7. Statistical analysis

Main effect of L-tryptophan level or stocking density was found out by two-way ANOVA. However, the comparison of the eight treatments was done by one-way ANOVA. Comparisons were made at the 5% probability level. Regression analysis was made between level of L-tryptophan and blood glucose, plasma cortisol and weight gain %. A statistical package SPSS version 14 was used for data analysis. Duncan's multiple range test was used to determine the significant differences between the treatment means.

3. Results

3.1. Growth parameters

The weight gain %, SGR, FCR and PER values are given in Table 2. Percentage weight gain, SGR and PER of low density group was significantly higher ($p<0.05$) than the high density group. A significant interaction was found between the level of L-tryptophan and the stocking density. Though there was no significant variation within the tryptophan treated groups of either low or high density group, but supplementation of L-tryptophan in both the groups exhibited higher growth rate than their respective control. Like other growth parameter FCR was lower in low density group than its corresponding high density counterpart. Irrespective of the stocking density, tryptophan supplemented groups exhibited lower FCR.

3.2. Plasma cortisol and blood glucose

Higher plasma cortisol level was recorded in the control groups of both the stocking density (Fig. 1). Similar trend was also found for blood glucose (Fig. 2). But supplementation of L-tryptophan in the diet gradually decreased the cortisol and blood glucose level. However, it was apparently visible that supplementation of L-tryptophan at ≥1.36% was almost similar.

3.3. Enzyme assays

3.3.1. ALT and AST

ALT activity in muscle exhibited a significant difference ($p<0.05$) between the stocking densities and is presented in Table 3. Highest activity was recorded in the control group than their tryptophan treated counterpart irrespective of the stocking density. However, control of high density group exhibited higher value ($p<0.05$) than the control of low density group in both muscle and liver. A clear trend of decreasing ALT activity was observed due to gradual supplementation of dietary L-tryptophan in both the groups. Comparatively higher activity was found in muscle than the liver. Similar trend was also observed for the AST.

3.3.2. LDH and MDH

A significant difference ($p<0.05$) in LDH activity was observed in the liver, gill and muscle between low and high density groups and is

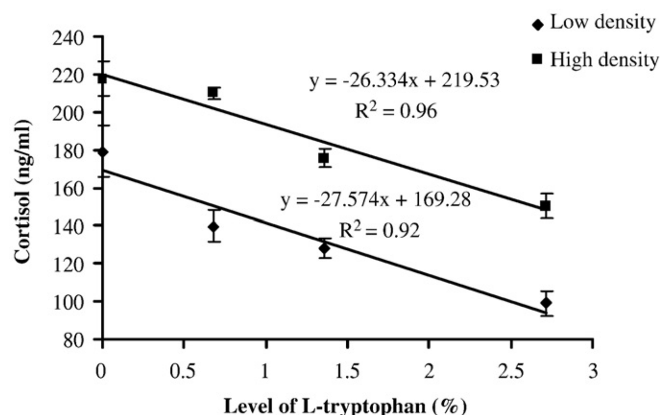

Fig. 1. Impact of dietary L-tryptophan in feed on plasma cortisol in *Cirrhinus mrigala* fingerlings exposed to both low density group and high density group (Mean of six values).

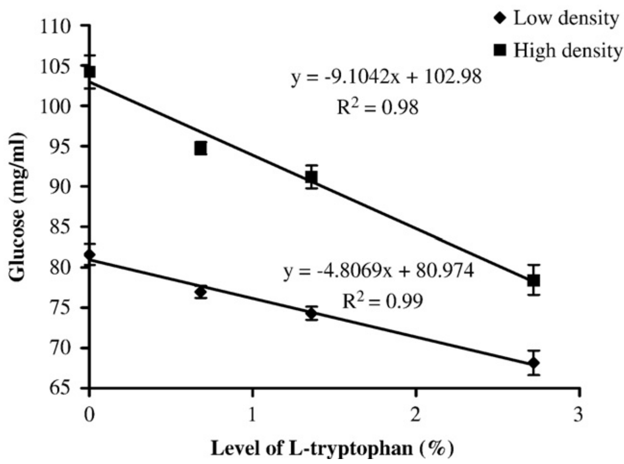

Fig. 2. Impact of dietary L-tryptophan in feed on Blood glucose in *Cirrhinus mrigala* fingerlings exposed to both low density group and high density group (Mean of six values).

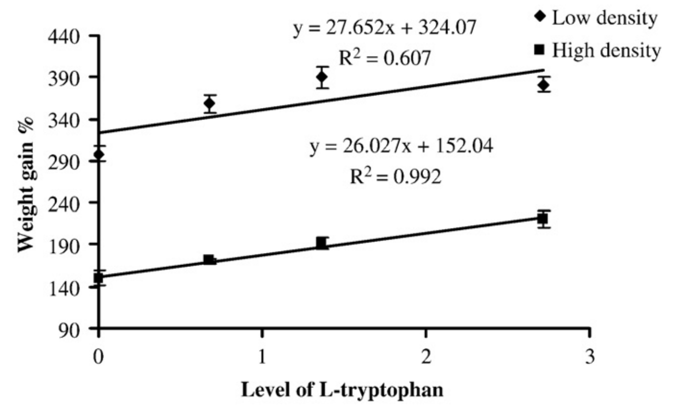

Fig. 3. Impact of dietary L-tryptophan in feed on weight gain% in *Cirrhinus mrigala* fingerlings exposed to both low density group and high density group (Mean of six values).

presented in Table 3. Like AST and ALT activity, LDH and MDH activities of control groups in both the stocking densities also exhibited higher activity, which gradually decreased with the increasing level of dietary L-tryptophan. Similarly, LDH activity of muscle was comparatively higher than the liver and gill. Low density group showed higher values of LDH and MDH than their high density counterpart at each level of tryptophan supplementation. The trend of MDH activity was similar as that of LDH.

3.3.3. AchE

AChE activity showed significant difference ($P<0.05$) amongst different treatment groups and is presented in Table 3. Unlike above enzymes, the lowest AChE activity was observed in the control groups of both the stocking densities, which increased gradually with the increasing level of L-tryptophan in the diet.

4. Discussion

The main objectives of the present experiment was to study the efficacy of dietary L-tryptophan for mitigating stress caused due to higher stocking density and augmentation of growth in *C. mrigala* fingerlings. Biochemical parameters serve as reliable indicators of physical status of organism (Ferry-Graham and Gibb, 2001). Stress response in fish includes a cascade of reactions such as primary, secondary and tertiary responses. It is initiated after the perception of stress in hypothalamus, which in turn activates the pituitary-inter-renal axis, resulting in the release of the hormone cortisol and catecholamine. The secondary stress response includes metabolic, haematological and immunological changes due to of the action of

Table 3
Impact of dietary L-tryptophan in feed on AST, ALT, LDH, MDH, and AChE activity of *Cirrhinus mrigala* fingerlings during low density group and high density group.

Parameters	Tissue	Stocking density	Treatments			
			Control (0%)	T_1 (0.68%)	T_2 (1.36%)	T_3 (2.72%)
AST	Liver	Low density	$82.00^{d}\pm6.07$	$66.40^{c}\pm5.37$	$44.57^{b}\pm4.23^{A}$	$29.55^{a}\pm2.32^{A}$
		High density	$94.35^{c}\pm3.69$	$76.83^{b}\pm4.63$	$61.42^{a}\pm2.25^{B}$	$51.97^{a}\pm1.74^{B}$
	Muscle	Low density	$94.90^{c}\pm6.43^{A}$	$73.96^{b}\pm1.85^{A}$	$63.58^{b}\pm2.73^{A}$	$34.92^{a}\pm0.93^{A}$
		High density	$120.78^{d}\pm6.05^{B}$	$92.53^{c}\pm3.19^{B}$	$79.93^{b}\pm1.93^{B}$	$50.60^{a}\pm2.63^{B}$
ALT	Liver	Low density	$14.58^{d}\pm0.59^{A}$	$11.48^{c}\pm0.58$	$8.40^{b}\pm0.25^{A}$	$4.83^{a}\pm0.32$
		High density	$21.87^{d}\pm0.89^{B}$	$17.22^{c}\pm0.86$	$12.60^{b}\pm0.38^{B}$	$7.25^{a}\pm0.48$
	Muscle	Low density	$26.30^{d}\pm0.97^{A}$	$21.44^{c}\pm0.48^{A}$	$15.96^{b}\pm0.97^{A}$	$10.35^{a}\pm0.61$
		High density	$36.55^{d}\pm1.70^{B}$	$31.50^{c}\pm0.39^{B}$	$25.48^{b}\pm0.89^{B}$	$7.91^{a}\pm1.11$
LDH	Liver	Low density	$1.10^{c}\pm0.02^{A}$	$0.77^{b}\pm0.07^{A}$	$0.68^{b}\pm0.04^{A}$	$0.48^{a}\pm0.05^{A}$
		High density	$3.29^{d}\pm0.06^{B}$	$2.88^{c}\pm0.07^{B}$	$2.16^{b}\pm0.07^{B}$	$1.69^{a}\pm0.08^{B}$
	Gill	Low density	$0.88^{d}\pm0.03^{A}$	$0.62^{c}\pm0.01^{A}$	$0.55^{b}\pm0.01^{A}$	$0.37^{a}\pm0.01^{A}$
		High density	$1.50^{d}\pm0.07^{B}$	$0.97^{c}\pm0.03^{B}$	$0.51^{b}\pm0.01^{B}$	$0.28^{a}\pm0.02^{B}$
	Muscle	Low density	$4.65^{d}\pm0.12^{A}$	$3.94^{c}\pm0.07^{A}$	$3.09^{b}\pm0.04^{A}$	$2.72^{a}\pm0.06^{A}$
		High density	$6.32^{d}\pm0.12^{B}$	$4.88^{c}\pm0.16^{B}$	$3.90^{b}\pm0.15^{B}$	$3.01^{a}\pm0.05^{B}$
MDH	Liver	Low density	$3.47^{c}\pm0.13^{A}$	$2.41^{b}\pm0.09^{A}$	$1.52^{a}\pm0.08^{A}$	$1.33^{a}\pm0.13^{A}$
		High density	$3.98^{d}\pm0.09^{B}$	$3.23^{c}\pm0.17^{B}$	$2.47^{b}\pm0.22^{B}$	$1.74^{a}\pm0.06^{B}$
	Gill	Low density	$3.74^{d}\pm0.02$	$3.18^{c}\pm0.08$	$2.37^{b}\pm0.10^{B}$	$1.63^{a}\pm0.13^{A}$
		High density	$3.98^{c}\pm0.21$	$2.96^{b}\pm0.13$	$2.05^{a}\pm0.10^{A}$	$1.71^{a}\pm0.12^{B}$
	Muscle	Low density	$3.28^{d}\pm0.08^{A}$	$2.74^{c}\pm0.06^{A}$	$2.24^{b}\pm0.06^{A}$	$1.55^{a}\pm0.09^{A}$
		High density	$4.34^{d}\pm0.15^{B}$	$3.47^{c}\pm0.08^{B}$	$2.53^{b}\pm0.12^{B}$	$1.66^{a}\pm0.12^{B}$
AchE	Brain	Low density	$0.18^{a}\pm0.003^{B}$	$0.23^{b}\pm0.003^{B}$	$0.25^{c}\pm0.004^{B}$	$0.28^{d}\pm0.001^{B}$
		High density	$0.14^{a}\pm0.009^{A}$	$0.18^{b}\pm0.002^{A}$	$0.19^{c}\pm0.003^{A}$	$0.23^{d}\pm0.004^{A}$

Different superscript (a, b, c, d) in the row indicate significant difference ($p<0.05$) among the control and treatment groups (control, T_1, T_2 and T_3) (Duncan's multiple range test, $\alpha=0.05$). Different superscripts (A, B) in the column indicate significant difference ($p<0.05$) among the stocking groups (low density group and high density group). Values are expressed as mean ± SE ($n=6$).

Units: nano moles oxalo acetate released/mg protein/min at 37 °C (AST); nano moles of sodium pyruvate formed/mg protein/min at 37 °C (ALT); nanomoles of Oxaloacetate utilized/mg protein/min (MDH); nanomoles of pyruvate utilized/mg protein/min (LDH); Acetyl choline hydrolysed/mg protein/min (AChE).

cortisol and catecholamine. The tertiary response is the final stage, which leads to disease or exhaustion, growth retardation and finally death (Chatterjee et al., 2006).

4.1. Growth

In the present study, dietary supplementation of L-tryptophan showed a significant effect on the weight gain % and specific growth rate in both the stocking densities. Control groups of both the groups showed lowest growth indices compared to the tryptophan treatment groups. In general the weight gain and specific growth rate of low density group were significantly higher ($p<0.05$) than the high density group (Fig. 3), which appears to be due to the high density stress. Similar type of observation was also reported by (Hoglund et al., 2007). In high density group dietary L-tryptophan 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 tryptophan than the control group.

4.2. Plasma cortisol

Elevations of plasma cortisol and glucose levels are often used as indicators of stress (Barton and Iwama, 1991a). Several studies have demonstrated the effects of stressors on concentrations of the corticosteroid cortisol in fish. Plasma concentration of cortisol is dependent on the duration and strength of the stressor (Barton and Iwama, 1991a) for which this parameter has been used as an indicator of stress.

In the present study the highest plasma cortisol was found in high density group control (HC) group fed without dietary L-tryptophan, indicates secretion of cortisol due to the stress caused by high density group. Gradual decrease in plasma cortisol level of both low density ($Y=-27.57x+169.28$, $r^2=0.92$) and high density group ($Y=-26.33x+219.53$, $r^2=0.95$) with the increasing level of dietary L-tryptophan confers that dietary L-tryptophan mitigates the high density group stress in *C. mrigala* fingerlings. The trend line of low and high density group appears to be parallel, suggesting a constant difference in cortisol level was maintained due to L-tryptophan supplementation in both the groups. This difference was similar to the difference between the cortisol levels of both the control groups (Fig. 1). But it was observed that the difference in cortisol level in the high density group in comparison to its low density counterpart was very close at 1.36% and 2.72%. Hence it appears to be logical to use 1.36% considering the cost involve at higher inclusion level of (2.72%). Similarly, Lepage et al., (2002) reported that feeding rainbow trout with L-tryptophan supplemented diets for 7 days resulted in a dose-dependent suppression of the stress-induced elevation of plasma cortisol.

4.3. Blood glucose level

The measurement of blood glucose level is still considered as an effective method to evaluate the stress effect of variety of stressors due to its simple methodology. The elevation of blood sugar levels in fish by both glucocorticoid, corticosteroids and catecholamines makes it the ideal parameter to study the secondary stress response, on activation of direct sympathetic (Chromaffin tissue) as well as humoral (internal tissue) pathways (Wedemeyer and Mcleay, 1981). Ample literature exists on the rise of glucose level on application of various stressors like handling (Wedemeyer, 1972; Carey and McCormick, 1998), transportation (Barton and Schreck, 1987), claw ablation of prawn (Manush et al., 2005), packing density (Chatterjee et al., 2006). In the present study the highest blood glucose level was found in both the control groups (Fig. 2) fed without dietary L-tryptophan, but supplementation of L-tryptophan caused decrease in blood glucose level in high density ($Y=-9.107x+102.98$, $r^2=0.98$) and low density group ($Y=-4.8069x+80.97$, $r^2=0.99$) as well. Decreasing levels of blood glucose content in both control and treatment groups with feeding increasing level of dietary L-tryptophan shows effectiveness in mitigating stress in *C. mrigala* fingerlings.

4.4. Enzyme assays

Metabolism is a physiological process reflecting the energy expenditure of living organisms. In the present study, the control of high density (HC) group showed highest activity but supplementation of L-tryptophan showed the lower activity than its control which was similar also for the low density group. The increase in LDH activity in HC group is attributed to the production of preferred substrate (Lactate) for gluconeogenesis (Chatterjee et al., 2006). Generally LDH activity increases in stress *viz.* temperature starvation (Vijayaraghavan and Rao, 1986), confinement (Chatterjee et al., 2006). Lower activity in the treatment groups suggested that the supplementation of dietary L-tryptophan reduces induced cortisol effect due to stress in the *C. mrigala* fingerlings.

Besides lactate, amino acids are the preferred substrates for gluconeogenesis in fish. Fish utilize protein and lipid sources rather than carbohydrate for energy fulfillment (Demeal, 1978). Teleosts are known for their ability to convert amino acids into glucose. Therefore, the higher activity of AST and ALT indicates the mobilization of aspartate and alanine via gluconeogenesis for glucose production to cope with stress Knox and Greengard (1965) and Chatterjee et al. (2006) also reported that elevated level of transaminase activity during stress would lead to increased feeding of ketoacids into TCA cycle, there by affecting oxidative metabolism. Dietary L-tryptophan enrichment significantly lower the liver contents of saturated and monounsaturated acids and increased poly and highly-unsaturated fatty acids (Papoutsoglou et al., 2005). In the present study, the activity of both the transferase decreased with the gradual increase of L-tryptophan and higher activity in those groups not fed with dietary L-tryptophan. This is also supported by the higher growth rate of treatment groups supplemented with L-tryptophan, as amino acids were not diverted for energy production but utilized for growth. Less activity in the L-tryptophan supplemented groups can be inferred that addition of L-tryptophan reduces the stress in *C. mrigala* fingerlings. The activity of MDH was highest in muscle followed by gill and liver. The activity of the enzyme was found higher in the control groups than the treatment groups. This is supported by the findings of Das et al. (2006), who found elevated MDH activity in fishes acclimated at higher temperature. Higher activity of MDH indicates greater activity of TCA cycle due to increased energy demands. Lower activity in the treatment groups support our findings that the supplementation of dietary L-tryptophan reduced the energy demands in the *C. mrigala* fingerlings as stress was also reduced.

Acetylcholine is widely distributed in nervous tissue, stored and released from the synaptic vesicles. It helps in the depolarization in an adjacent, skeletal or smooth muscle fiber, thus passing on the neuron impulse. Acetylcholine is synthesized in nervous tissue by enzyme choline acelytase. Acetylcholine is rapidly destroyed by cholinesterase, a group of related enzymes, which are hydrolytic in action (Stowe, 1969). In the present study, acetylcholine esterase in brain of *C. mrigala* fingerlings was assayed at the end of the experiment and found to be significantly ($p<0.05$) difference in both the stocking density groups. Cholinesterase hydrolyzes the acetyl choline and hence its production decreases at higher activity of cholinesterase. Gradual increase in choline esterase activity was observed in both the density groups with increasing level of dietary L-tryptophan. Significantly lower activity was observed in control group and the lowest activity in control of high density group indicates highest stress due to accumulation of acetylcholine. Similarly Murthy et al., (1984) observed the inhibition of acetylcholine esterase in tilapia on exposure to acidic media and reduction of enzyme activity and

accumulation of acetylcholine with aberrant behavior (Murthy et al., 1984; Jones et al., 1999).

5. Conclusion

The overall results of the present investigation suggest that higher stocking density have a marked effect on the metabolism, leading to chronic stress in fish. But dietary supplementation of L-tryptophan at a minimum level of 1.36% reduced the high density group stress and improved growth performance in *C. mrigala* fingerlings as well. Though 2.72% dietary tryptophan also reduces the stress but 1.36% level appears to be cost effective. This result may be useful for the farmer for reducing stress caused due to over high density group.

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