Journal of Thermal Biology 36 (2011) 84–88

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Dietary pyridoxine enhances thermal tolerance of *Labeo rohita* (Hamilton) fingerlings reared under endosulfan stress

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ARTICLE INFO

Article history:
Received 26 August 2010
Accepted 20 October 2010

Keywords:
Pyridoxine
CTmax
CTmin
LTmax
Thermal tolerance
Labeo rohita
Endosulfan

ABSTRACT

A preliminary experiment was carried out to study the effect of dietary pyridoxine (PN) on thermal tolerance of *Labeo rohita* fingerlings exposed to endosulfan (1/10th 96 h LC_{50}=0.2 ppb) stress, reared at 26.0 ± 0.5 °C to assess its culture potential in different agro-climatic zones. Two hundred seventy fingerlings were randomly distributed into six treatment groups in triplicate. Five iso-caloric and iso-nitrogenous purified diets were prepared with graded levels of pyridoxine. Six treatment groups were T_0 (10 mg PN+without endosulfan), T_1 (0 mg PN+endosulfan), T_2 (10 mg PN+endosulfan), T_3 (50 mg PN+endosulfan), T_4 (100 mg PN+endosulfan) and T_5 (200 mg PN+endosulfan). After feeding for 60 days, critical temperature maxima (CTmax), lethal temperature maxima (LTmax), critical temperature minima (CTmin) and lethal temperature minima (LTmin) were determined in each group. There was significant ($P<0.05$) effect of dietary pyridoxine on temperature tolerance (CTmax, LTmax, CTmin and LTmin) of the groups fed diets supplemented with 100 and 200 mg PN/kg diet compared to other experimental groups. Positive correlations were observed between CTmax and LTmax (R^2=0.85) as well as between CTmin and LTmin (R^2=0.97). The effect was more prominent on lower thermal tolerance limit (CTmin and LTmin). The overall results obtained in this preliminary study indicated that pyridoxine supplementation at 100 mg PN/kg diet enhances the thermal tolerance of endosulfan exposed *L. rohita* fingerlings.

1. Introduction

Labeo rohita (Hamilton), one of Indian Major Carps, is considered to be the major aquaculture candidate species in India as well as in South East Asian countries (FAO, 2001) and provides livelihood to millions of people. But, global warming along with indiscriminate use of antibiotics, synthetic growth promoters and pesticides like endosulfan is likely to potentially affect its productivity in wild fish populations as well as in aquaculture systems globally (Ficke et al., 2007). As fish is an ectotherm, any alterations in the water temperature have a marked and direct effect on many of the key physiological processes and behavioural activities (Brett and Groves, 1979; Jonassen et al., 1999). Each species has a range of temperature (thermal tolerance range) over which it survives, and a narrower range where optimum growth occurs (Katersky and Carter, 2007). However, temperature beyond optimum limits of a particular species adversely affects the health of aquatic animal due to metabolic stress and increases oxygen demand and susceptibility to diseases (Wedemeyer et al., 1999). Hence, thermal tolerance studies have gained significant attention of scientists to understand the impact of global warming on animals, including fish, as well. Recent studies have demonstrated that immunostimulants can trigger defence system, even in stressful conditions, and therefore ameliorate the harmful effects mediated by stress (Ortuno et al., 2003; Sarma et al., 2009; Akhtar et al., 2010). It is, therefore, imperative to enhance the tolerance against stress of cultured fish by modulating its immune system through dietary interventions, which has also become a priority area of research.

Pyridoxine (vitamin B_6) including pyridoxal and pyridoxamine, is essential for absorption and metabolism of amino acids and is also involved in development of red blood cells (David et al., 2004). These act as coenzymes for transaminases (Rogers and Mohan, 1994) and control the biosynthesis of neurotransmitters like GABA, dopamine and serotonin (5-HT), important for development and function of the central nervous system (CNS) and thereby have an anti-stress effect (Ernahrungswiss, 1996). High tissue levels of pyridoxal phosphate may work both centrally and peripherally to mitigate the physiological consequences of stress (McCarty, 2000). Studies have shown that high dose of pyridoxine mitigates stress-mediated immune suppression (Lettko and Meuer, 1990). Chen et al. (2005) has reported that dietary pyridoxine plays an important role in stimulating the immune responses by increasing phagocytic activity and respiratory burst activity of abalone, *Haliotis discus hannai*. However, this vitamin has a protective effect on Chinook salmon, *Oncorhynchus tshawytscha*

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doi:10.1016/j.jtherbio.2010.10.003

(Walbaum) fingerlings challenged with a virulent strain of *Vibrio anguillarum*, (Hardy et al., 1979). In our previous study (Akhtar et al., 2010), we observed that dietary pyridoxine mitigates endosulfan stress and modulates immunity in *L. rohita* fingerlings.

Apart from all these advantages, the present experiment was conducted to elucidate the role of dietary pyridoxine in thermal tolerance of endosulfan exposed *L. rohita* fingerlings to assess its culture potential in different agro-climatic zones across the countries.

2. Material and methods

2.1. Experimental animal

Fingerlings of *L. rohita* (6.5 ± 0.26 g, average weight $\pm$ SE) were procured from Prem Fisheries Consultancy, Gujarat, India and transported in a circular container (500 L) with sufficient aeration to the experimental facilities at Central Institute of Fisheries Education, Mumbai, India. The fish were given a salt treatment (1% NaCl w/v) for 5 min and $KMnO_4$ (5 ppm) dip treatment to ameliorate the handling stress and then acclimatized to the laboratory conditions for 15 days. During acclimation fish were fed with control diet containing normal pyridoxine requirement. After acclimatization, fishes were transferred to 18 uniform size experimental plastic tanks ($80 \times 57 \times 42$ cm^3) of 150 L capacity and reared for 60 days at 26.0 ± 0.5 °C. Continuous aeration was provided throughout the experimental period.

2.2. Experimental design and feeding

Five iso-caloric (404.85–409.99 kcal/100 g) and iso-nitrogenous (35.45–35.75% crude protein) purified diets were prepared with graded levels (0, 10, 50, 100, and 200 mg PN/kg) of pyridoxine (Himedia Laboratories, Mumbai, India). Two hundred and seventy fish were randomly distributed in six experimental groups in triplicate following a Completely Randomized Design. The six treatment groups were T_0 (10 mg PN+without endosulfan), T_1 (0 mg PN+endosulfan), T_2 (10 mg PN+endosulfan), T_3 (50 mg PN+endosulfan), T_4 (100 mg PN+endosulfan) and T_5 (200 mg PN+endosulfan). All the experimental groups except T_0 {10 mg PN/kg feed, which is normal requirement (Murthy, 2002)}, were exposed to sub-lethal concentration of endosulfan (1/10th 96 h LC_{50}=0.2 ppb) throughout the experimental period. Endosulfan treatment was given in a pulsed manner. The physicochemical parameters of water were within the optimum range (dissolved oxygen: 6.4–7.2 mg/L; pH: 7.5–8.1; temperature: 26.0 ± 0.5 °C; ammonia nitrogen: 0.14–0.27 mg/L; nitrite nitrogen: 0.001–0.005 mg/L; nitrate nitrogen: 0.02–0.07 mg/L) throughout the experimental period. All the groups were fed their respective diets. Feeding was done at 2.5% of the body weight. Daily ration was divided into two split doses: about 2/3rd of the total ration was given at 09:00 h and the rest at 18:00 h. The uneaten feed and faecal matters were removed by siphoning and about 50% water of the tank was exchanged with endosulfan treated water daily.

2.3. Proximate analysis of diet

The proximate composition of the experimental diets was determined following the standard methods of AOAC (AOAC, 1995) and is presented in Table 1. The moisture content was determined by drying at 105 °C to a constant weight. Nitrogen content was estimated by Kjeldahl (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) method and crude protein was estimated by

Table 1
Diet composition and proximate analysis of the experimental diets (% dry matter (DM) basis) fed to *L. rohita* fingerlings during the experimental period.

Ingredients	Diets				
	1 (0 mg PN)	**2** (10 mg PN)	**3** (50 mg PN)	**4** (100 mg PN)	**5** (200 mg PN)
Casein (vitamin free)[a]	30.00	30.00	30.00	30.00	30.00
Gelatin[a]	10.00	10.00	10.00	10.00	10.00
Dextrin[a]	10.00	10.00	10.00	10.00	10.00
Starch soluble[a]	30.00	30.00	30.00	30.00	30.00
Cellulose powder[a]	8.00	7.99	7.95	7.90	7.80
Cod liver oil[b]	4.00	4.00	4.00	4.00	4.00
Sunflower oil[c]	4.00	4.00	4.00	4.00	4.00
Vit.Min mix (Pyridoxine.free)[d]	1.96	1.96	1.96	1.96	1.96
Vitamin.C[e]	0.01	0.01	0.01	0.01	0.01
Carboxymethylcellulose(CMC)[a]	2.00	2.00	2.00	2.00	2.00
Betaine hydrochloride[a]	0.02	0.02	0.02	0.02	0.02
Butylated hydroxy toluene (BHT)[a]	0.01	0.01	0.01	0.01	0.01
Pyridoxine hydrochloride (mg PN/kg)[a]	0	10	50	100	200
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition of diets					
Moisture	8.38 ± 0.20	8.04 ± 0.06	8.71 ± 0.25	8.42 ± 0.65	8.48 ± 0.41
Crude protein (CP)	35.70 ± 0.24	35.45 ± 0.29	35.68 ± 0.42	35.56 ± 0.13	35.75 ± 0.28
Ether extract (EE)	8.54 ± 0.13	8.7 ± 0.37	8.69 ± 0.43	8.84 ± 0.14	8.81 ± 0.40
Ash	9.09 ± 0.40	8.44 ± 0.26	9.65 ± 0.68	9.10 ± 0.38	9.02 ± 0.58
Dry matter (DM)	91.62 ± 0.20	91.96 ± 0.06	91.29 ± 0.25	91.58 ± 0.65	91.52 ± 0.41
Total carbohydrate (TC)	46.67 ± 0.32	47.37 ± 0.7	45.98 ± 0.6	46.5 ± 0.43	46.41 ± 1.1
Digestible energy (DE[f])	406.34 ± 1.15	409.9 ± 1.9	404.8 ± 4.7	407.8 ± 2.0	407.9 ± 2.2

Data expressed as Mean $\pm$ SE, $n=3$

[a] Himedia Laboratories, India.
[b] Procured from local market.
[c] Ruchi Soya Industries Ltd., Raigad, India.
[d] Prepared manually and all components from Himedia Ltd., India. Composition of vitamin mineral premix (quantity/250 g starch powder) prepared manually: ++Vitamin A 55,00,00 IU; ++Vitamin D3 11,00,00 IU; Vitamin B_2 200 mg; Vitamin E 75 mg; Vitamin K 100 mg; +Vitamin B_{12} 30 μg; calcium pantothenate 250 mg; Nicotinamide 1000 mg; choline chloride 15 g; Mn (Mnso4) 2700 mg; I (KI) 100 mg; Fe (ferric citrate) 75 mg; Zn ($Znso_4$) 500 mg; Cu ($Cuso_4$) 200 mg; Co (CoC_{l2}) 45 mg; Ca ($CaCo_3$ and dibasic calcium phosphate) 50 g; P (dibasic calcium phosphate) 30 g; selenium (sodium selenite) 50 ppm. ++Glaxo Pharmaceuticals, Mumbai, India.
[e] Sd Fine Chemicals Ltd., India.
[f] Digestible energy (kcal 100 g^{-1})=(%CP × 4)+(%EE × 9)+(TC × 4), DM%=100 − moisture%.