



Research Article

Evaluation of Catalase Activity in Organs of Fresh Water Fish, *Ctenopharyngodon idella* Fed with Vitamin E

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Authors' Contributions

MS executed the research work. SA supervised the research. HN and TA helped in writing article. KA facilitated in conducting the research work in his laboratory. SM and MAH revised the article.

Keywords

CAT, Fish, Tissues, Dietary effect, Vitamin E

Abstract | This work was performed to evaluate the catalase (CAT) activity in *Ctenopharyngodon idella* fed with vitamin E (VE) supplemented diet. Four different diets viz. T₁ (VE-0 mg/Kg Control), T₂ (VE-200 mg/Kg), T₃ (VE-350 mg/Kg) and T₄ (VE-500 mg/Kg) were prepared containing different levels of VE. The *C. idella* was fed @ 3% of body weight three times in a day for 30 days. At the end of trail fish was dissected and tissues viz. liver, muscle and gills were obtained to analyze the CAT activity. The CAT level was reduced in all treatments when compared control. CAT activity was observed higher in the gills, muscle and liver tissues of T₁ diet fed *C. idella* as 14.68±0.90, 12.76±0.87 and 20.34±0.99 UmL⁻¹, respectively. Comparison among diets for CAT activity showed the trend as T₄<T₃<T₂<T₁. The maximum CAT level was noted in liver than that of gills and muscles.

Novelty Statement | This research was conducted to study the impact of Vitamin E on oxidative stress enzyme (catalase) of fish. Vitamin E has proven beneficial in protecting cellular membranes against oxidation and increases the resistance to stress.

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Introduction

Organic substances such as vitamins are very essential micronutrient for proper maintenance, growth, and normal development of animals like fish. These substances cannot be synthesized by the animals rather supplied in diet (Falathkar *et al.*, 2006). Unlike other single compound vitamins, the vitamin E (VE) is composed of four

tocopherols and four tocotrienols (Shiau and Hsu, 2002).

During normal metabolic reactions free radicals are produced known as reactive oxygen species (ROS). Some of these radicals have a key role in transduction of signals (Hancock *et al.*, 2001) but their high level can induce damage in cells of organisms (Wang *et al.*, 2007). Most reactive free radical is hydroxyl which has high ability to cause oxidative damage to any molecule upon interacting with tissues (Castro and Freeman, 2001).

Vitamin E is a fat-soluble compound and act as an antioxidant scavenges of the free radicals (Paul *et al.*, 2004)

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and save the cell's membrane lipid-proteins from oxidation (Lee and Shiau, 2004), lipid peroxidation (Paul *et al.*, 2004). These radicals also responsible for damage induce to intracellular components such as enzymes, membrane and DNA/RNA resulted in decreased growth (Paul *et al.*, 2004). Therefore, VE supposed to be a very vital organic compound in body (Blazer, 1992). Many authors have documented the dietary requirement of VE for different fish species including Channel catfish (Wilson, 1984), Carps (Takeuchi, 1993) and Bass (Kocabas, 1999).

Among fish body organs, gills are direct in contact with aquatic environment and are most susceptible to change in water quality. In addition to this, gills are also accountable for respiration and osmoregulation includes acid-base balance and excretion of nitrogenous waste (Au, 2004). Muscles are the most important tissue of fish body used as food and greatly affect the human health (Pirrone and Mahaffey, 2005). Fish liver is a metabolic organ performs the function of excretion, biotransformation and accumulation of toxicants. The physiological condition of animal can be evaluated through suitable biomarkers in fish such as organs (Figueiredo-Fernandes *et al.*, 2006). Therefore, this study was carried out to check the dietary vitamin E impact on catalase activity of *Ctenopharyngodon idella*.

Materials and Methods

This study was conducted on a 90-day old *Ctenopharyngodon idella* commonly known as Grass carp. Fingerlings were obtained from local Fish Seed Hatchery and shifted to Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. Initially, fish were acclimatized in cemented tanks for few days and then transferred to 70-L glass aquaria for dietary trial. Table 1 show the different diets contained various level of vitamin E and fish fed with these diets at 3% of their body weight. Feed ingredients includes gluten, soybean oil, fish meal, premix and rice polish were ground and mixed with soya oil and then make the paste followed by pellet formation. Fish were fed with experimental diets three times a day at 7:00-8:00AM, 1:00-2:00PM and 6:00-7:00PM for

30 days. At the end, fish were killed and tissues viz. gills, muscles and liver were obtained to check the catalase (CAT) activity. During trail, water quality characteristics such as temperature, total hardness, dissolved oxygen and pH were stabilized as 28°C, 230 mg/L, 5ppm and 7.25, respectively.

CAT extract's preparation

At the end of the trial, fish was dissected and gills, muscle and liver were isolated. In each organ phosphate buffer of pH 7.0 was mixed, separately, in ratio of 1:4 (w/v) and homogenized for 10 minutes. Homogenate was filtered and centrifuged at 10,000 rpm at 4°C for 12 minutes. Supernatant was collected for catalase study.

CAT assay

Activity of CAT was calculated by adopting the protocol of Chance and Mehaly (1977). The 2 ml of reaction mixture was prepared contained 0.05 mL CAT extract and 1.95 mL of buffered substrate and activity was checked at A_{240nm} .

$$\text{Activity} \left(\frac{\text{units}}{\text{mL}} \right) = \frac{\frac{\Delta A}{\text{min}} \times \text{dilution} \times 2\text{mL}}{0.04\text{mM}^{-1} \text{cm}^{-1} \times 0.05\text{mL}}$$

Statistical analysis

Each dietary trial was conducted three times and ANOVA and Tukey's tests were applied to see the statistical differences and to compare the means among different variables under study (Steel *et al.*, 1997). Statistix version 8.1 software was used for data analyses.

Results and Discussion

The results showed that *C. idella* fed with VE had lowered CAT activity in organs when compared with T₁ (without VE). The lower CAT activity is due to low production of reactive oxygen species (ROS) as VE also act as an antioxidant. It was observed that CAT activity was higher in gills, muscle and liver of fish fed with T₁ diet as 14.68±0.90, 12.76±0.87 and 20.34±0.99 UmL⁻¹, respectively (Table 2). Among diets CAT activity showed

Table 1: Composition of experimental diet.

Ingredient (g kg ⁻¹ diet)	Compositions of diets			
	T1 (Control) E-0 mg/Kg	T2 E-200 mg/Kg	T3 E-350 mg/Kg	T4 E-500 mg/Kg
Rice Polish g/Kg	330	329.96	329.93	329.9
Gluten g/Kg	320	319.96	319.93	319.9
Fish meal g/Kg	300	299.96	299.93	299.9
Soyabean oil g/Kg	20	19.96	19.93	19.9
Premix g/Kg	30	29.96	29.93	29.9
α-Tocopherol (mg/kg diet)	0	0.20	0.35	0.50

Table 2: Effect of Vitamin E on CAT activity of *C. idella*.

Diets	Muscles	Gills	Liver	*Overall mean
T ₁ VE-0	12.76±0.87a	14.68±0.90a	20.34±0.99a	15.93±3.94a
T ₂ VE-200	9.55±0.67b	10.22±0.73b	15.65±0.94b	11.81±3.35b
T ₃ VE-350	7.46±0.53c	8.10±0.60c	12.32±0.81c	9.29±2.64c
T ₄ VE-500	4.28±0.37d	6.63±0.45d	8.23±0.68d	6.38±1.99d
Overall mean	8.51±3.57c	9.91±3.51b	14.14±5.13a	

Means with similar letters in a single row are statistically non-significant at $p < 0.05$.

Table 3: Regression analyses on CAT activity in *C. idella*

Organs	Regression equation	SE	R	R ²
Muscle	Activity = 12.9 - 0.0166** (Diets)	0.0009671	0.994	0.99
Gills	Activity = 14.1 - 0.0161* (Diets)	0.002126	0.975	0.95
Liver	Activity = 20.4 - 0.0240 ** (Diets)	0.0006957	0.998	0.997

SE= Standard Error; r=Multiple Regression Coefficient; R²; Coefficient of Determination; ** Highly significant; * Significant at $P < 0.01$.

the following trend: $T_4 < T_3 < T_2$ (Figure 1). The maximum CAT level was noted in liver of fish than that of gills and muscles. Regression analyses showed the negative relation between activity and VE (Table 3). Similarly, Fatima et al. (2019) also studied the reduced CAT level in *Labeo rohita* fed with high doses of vitamin E. CAT level significantly decreased in large yellow croaker fed with VE supplemented diet (Wang et al., 2016). Grass carp showed reduced CAT level when fed with VE supplemented diet (Li et al., 2014). Tocher et al. (2002) also studied the lowered CAT level in sea bream fed with diet contained VE1000 as compared to VE0. Vitamin E supplemented diet decreased the CAT activity in gills of *C. mrigala* (Iqbal et al., 2018). Some authors also observed high level of CAT in liver when fish fed with VE supplemented diet (Zhang et al., 2016). There is little information on fish regarding to VE effect but researcher have done work with other mammals such as Hsu et al. (2001) reported the lowered CAT level in rabbits fed with VE. Vitamin E is also an antioxidant have defensive role against harmful impact of ROS (NRC, 2011), but high level of α -Toc may promote the lipid peroxidation in fish (Kim et al., 2007). It is concluded that vitamin E act as antioxidant and plays role to minimize the oxidative stress in fish.

Conflict of interest

The authors have declared no conflict of interest.

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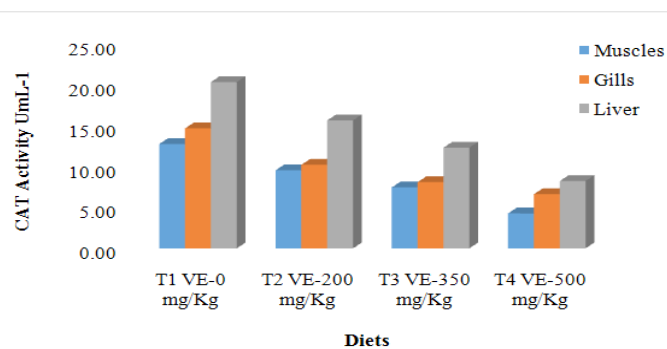


Figure 1: CAT activity in organs of *C. idella* fed with vitamin E supplemented diet

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