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Physiological effects of dietary vitamin E on kelp grouper, *Epinephelus bruneus* under low water temperature stress

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저수온 스트레스시 자바리, *Epinephelus bruneus*에서의 vitamin E의 생리적 효과

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Abstract

The physiological response of the longtooth grouper, *Epinephelus bruneus* to low water temperature (LWT) stress while on three different concentrations of dietary vitamin E (1 mg/diet g, 5 mg/diet g, and 10 mg /diet g) were investigated. Plasma cortisol, glucose, and aspartate aminotransferase (AST) levels were higher in the control and sham control compared with the vitamin E supplemented groups. The most addition with vitamin E in the diet (10 mg/diet g) showed the lowest levels of cortisol, glucose, and AST. However, plasma alanine aminotransferase (ALT) showed no significant differences across all experimental conditions (P>0.05). Also the more disposing vitamin E into feeding the better resistance against stress on the LWT was shown through cortisol, glucose, ALT, and AST.

Key words : Vitamin E; Cortisol; Glucose; Alanin aminotransferase; Longtooth grouper

Ⅰ. Introduction

In breeding fish, it is inevitable for the fish to get stressed. The conditions under which the fish are suffered stress in a variety ways, including increased density, inadequate nutrition, poor sanitation, injury during handling, or low water temperature (LWT) (Gamperl et al., 1994). Stress responses can include physiological changes such as oxygen uptake and metabolic and hematological changes, redistribution of energy from growth performance and reproduction behavior, and negative effects on immune functions (Pickering and Pottinger, 1989; Barton & Iwama, 1991; Wendelaar Bonga, 1997). These changes can increase disease susceptibility leading to increased mortality and subsequent economic losses.

The physiological stresses of fish are mainly divided into three factors (Mazeaud et al., 1977). The first response is to increase internal secretion activities by promoting the secretion of catecholamine and glucocorticoid, thus inducing the second response

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where the fish then undergoes metabolic and hematological changes which subsequently induce the final and third response by which time the fish starts to exhibit obvious signs of stress and discomfort (Thompson et al., 1993).

Vitamin E is an important nutrient required to maintain flesh quality, immunity, normal resistance of red blood corpuscles to haemolysis, permeability of capillaries, and heart muscle functionality (Halver, 2002). A dietary supplement of vitamin E has been demonstrated to have positive effects in a number of species ranging from 120 mg/kg for Atlantic salmon, *Salmo salar*, to 30-50 mg/kg channel catfish, *Ictalurus punctatus* and 200 to 300 mg/kg for the common carp, *Cyprinus carpio* (Murai and Andrews, 1974; Watanabe et al., 1977; Wilson et al., 1984; NRC, 1993). Vitamin E naturally exists in many different forms. However Alpha-tocopherol α -tocopherol is traditionally recognized to have the highest vitamin E and antioxidation activity (NRC, 1993; Hamre et al., 1998).

In order to determine the physiological responses of stressed fish with added vitamin E in a dietary supplement, levels of glucose, cortisol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in their blood plasma were analyzed. Blood glucose among stressed fish is easy to measure and a good indicator of the second stress response (Wednmeyer et al., 1990). Cortisol is a species of tip hormone which is also used to gauge the level of stress in fish (Yeo and Joung, 2004). Levels of ALT and AST enzymes, both of which are associated with liver function, can also be used for the validation of fish diseases (Yeo and Joung, 2004).

Longtooth grouper, *Epinephelus bruneus* (order Perciformes, family Serranidae), is a sedentary marine fish that lives in rocky areas of shallow coastal regions and ranging to southern seas including Jeju, Korea the southern part of Japan, China, the Philippines and India (Park et al., 2008; Park and Park, 2009).

In a recent study, Park et al. (2008) has been able to show that the longtooth grouper does exhibit metabolic and physiological changes when putting under anesthetic stress. Until now, no study has investigated the effects of dietary vitamin E under low temperature stress on this species. The results of this study are expected to contribute most appropriate dietary vitamin E concentrations on the physiological responses to the stressed fish.

Ⅱ. Materials and methods

Specimen seemingly healthy longtooth grouper, *Epinephelus bruneus* of 19.2±1.58 cm (Mean±SE) average body length 170.1±13.97 g and average body weight were selected from our laboratory in Busan and kept in a flow-through tank under natural conditions of temperature (25±0.5°C).

Light was provided by four 40-W (5400 K) fluorescent bulbs controlled by an electric timer, which kept the photoperiod at a 12:12 hrs light/ dark cycle. No lights were used during the dark period. The dissolved oxygen levels were maintained at more than 9.5 mg/L, ammonia at less than 0.001 ppm, nitrous acid at 0.08~0.1 ppm, nitric acid at 2~5 ppm, and pH at 7.3~8.3. Fish were divided equally and observed under five experimental conditions; control, sham control, 1 mg vitamin E/g diet, 5 mg vitamin E/g diet, and 10 mg vitamin E/g diet for a period of two weeks prior to the commencement of LWT stress treatment.

The amount of feed supplied 3 % of fish body weight equally. The control group was fed a dry

commercial feed (E-hwa Oil & Fat Ind. Co., Korea: more than 52 % crude protein, more than 10 % crude lipid, less than 3 % ash less than 16 % crude fiber, more than 1.5 % calcium, less than 2.7 % phosphor) while the sham control group was only fed the squid liver oil. The three other experimental groups were fed 100 ml of squid live oil, a dry feed commercial feed, as well as a supplemental concentration of 1 mg vitamin E/g diet, 5 mg vitamin E/g diet, and 10 mg vitamin E/g diet.

The low water temperature stress test commenced after a consistent diet for two weeks. Each sample was randomly selected and experimented in a rectangular glass tank (Dimensions W109 x L69 x H47 cm) followed by the admission of 50 longtooth groupers. Water temperature in tanks were decreased at 1 °C every hour in order to allow for acclimatization and to avoid possible sudden death from shock. Once the water temperature had reached 15 °C, it was maintained at that level for a further 10 hours.

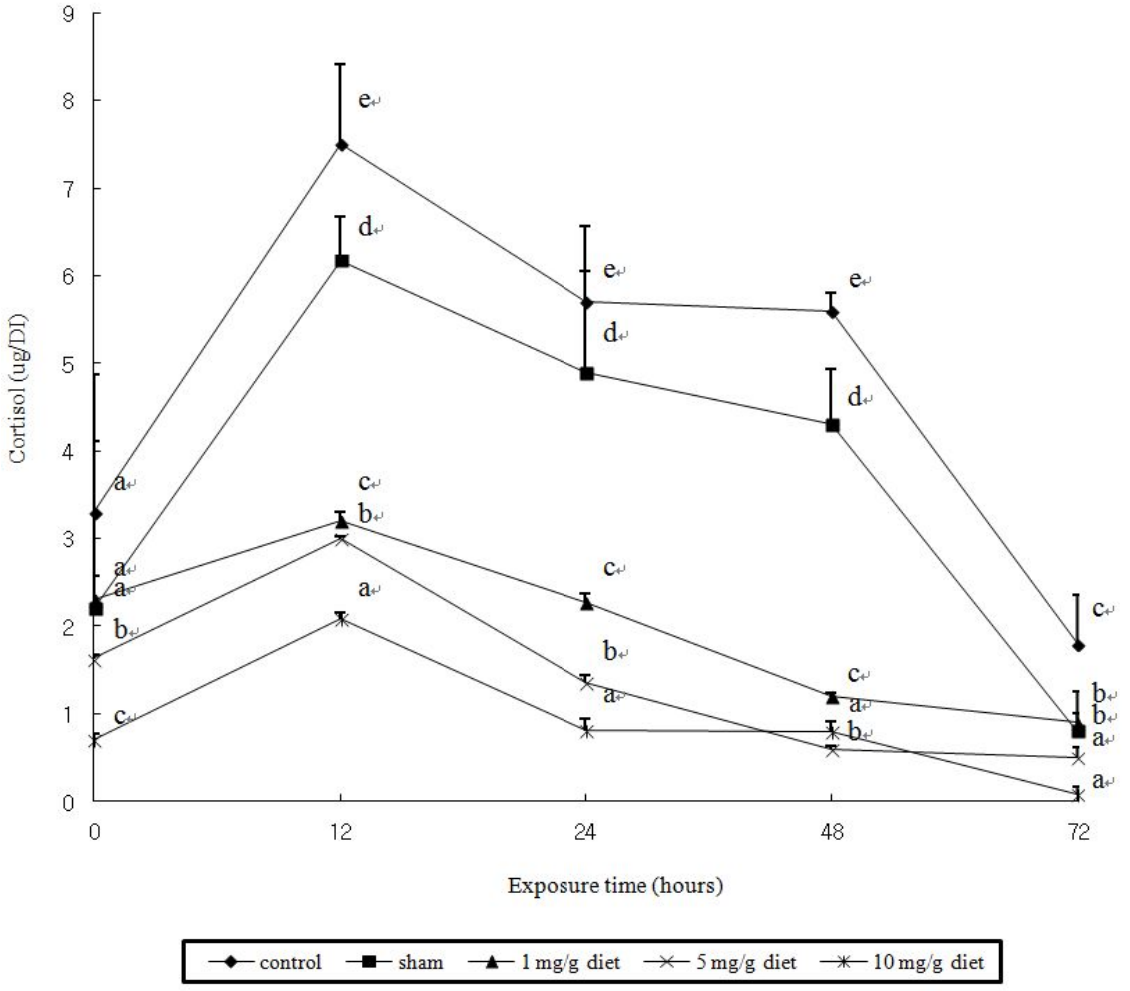
Using syringes lined with the anticoagulant heparin blood was extracted in order to assay fixed intervals of 0, 12, 24, 48, and 72 hrs and filled into capillary tubes. All samples were analyzed after centrifuging at 5,600 g for 5 minutes. The glucose, ALT, and AST concentrations were analyzed using blood automatic analysis (Boehringer Mannhein Reflotron, Germanry). The cortisol concentration was measured using the method of rabbit anti-Cortisol-3-CMO-BSA antibody (Cosmo-Bio Co. Ltd., Tokyo, NH, USA) and radiation mark 〔1,2,5,6-H3〕- cortisol (Amersham Life Science, England).

The experiments were performed in triplicate. Data were analyzed by ANOVA with the SPSS statistical package (SPSS 9.0, SPSS Inc., USA). Means were performed by using Duncan's multiple range test and significant difference were found using $P<0.05$ (Duncan, 1955).

Ⅲ. Results

The cortisol concentrations of the five different dietary experiments subjected to cold water stress in longtooth grouper, *Epinephelus bruneus* were presented in [Fig. 1]. The cortisol levels exhibited a sharp initial increase across all dietary conditions within the first 12 hrs. The highest levels were observed in the control experiment (7.5 ± 0.92 μg/DI), followed by the sham control, 1 mg/g E, 5 mg/g E, and 10 mg/g of dietary vitamin E respectively. After peaking at 12 hrs under stress, cortisol levels declined across all experiments for the duration of the experiment following the same hierarchical order as above. One way ANOVA on the 1 mg/g E group showed there to be statistically significant differences at 12 hrs of stress ($P<0.05$). The same scenario is also exhibited by the other two vitamin E supplemented experiments.

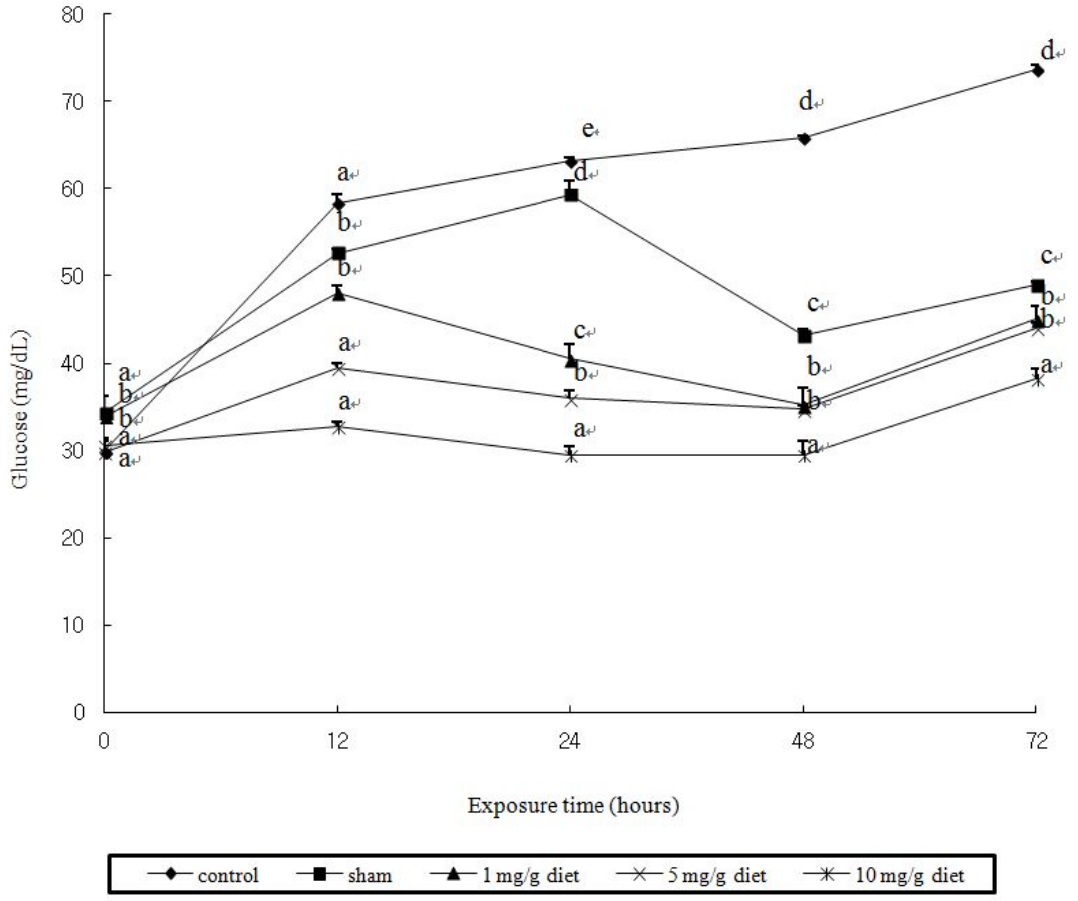
The glucose concentrations of the five different dietary experiments subjected to cold water stress were presented in [Fig. 2]. As cortisol levels, glucose concentration increased across all experimental conditions within the first 12 hrs. However, this is where the similarity ends. The control experiment continued to increase in glucose levels for the duration of the experiment while the sham experiment peaked at 24 hrs declining rapidly thereafter before recovering again after 48 hrs. Glucose levels for the vitamin E experimental groups were more uniform with all three exhibiting an initial increase peaking at 12 hrs, but subsequently declining during the next 24 hrs before recovering again. The 1 mg/g dietary vitamin E group showed the sharpest incline to peak at 12 hrs (3.2 ± 0.11 mg/dL).



[Fig. 1] Cortisol concentration variations in blood plasma of longtooth grouper (*Epinephilus bruneus*) during 72 hrs of stress period. Values represent means±SE (n=50). Different letters on the bars indicate statistically significant difference (P<0.05).

Analysis of variance with the control experiment showed there to be found significant differences in concentrations (P<0.05). These were followed by the 5 mg/g and 10 mg/g dietary vitamin E groups, respectively. The 10 mg/g dietary vitamin E groups exhibited the lowest change in glucose concentration increasing from 30.6 ± 0.57 mg/dL at 0 hr to 38.2 ± 1.24 mg/dL at 72 hrs.

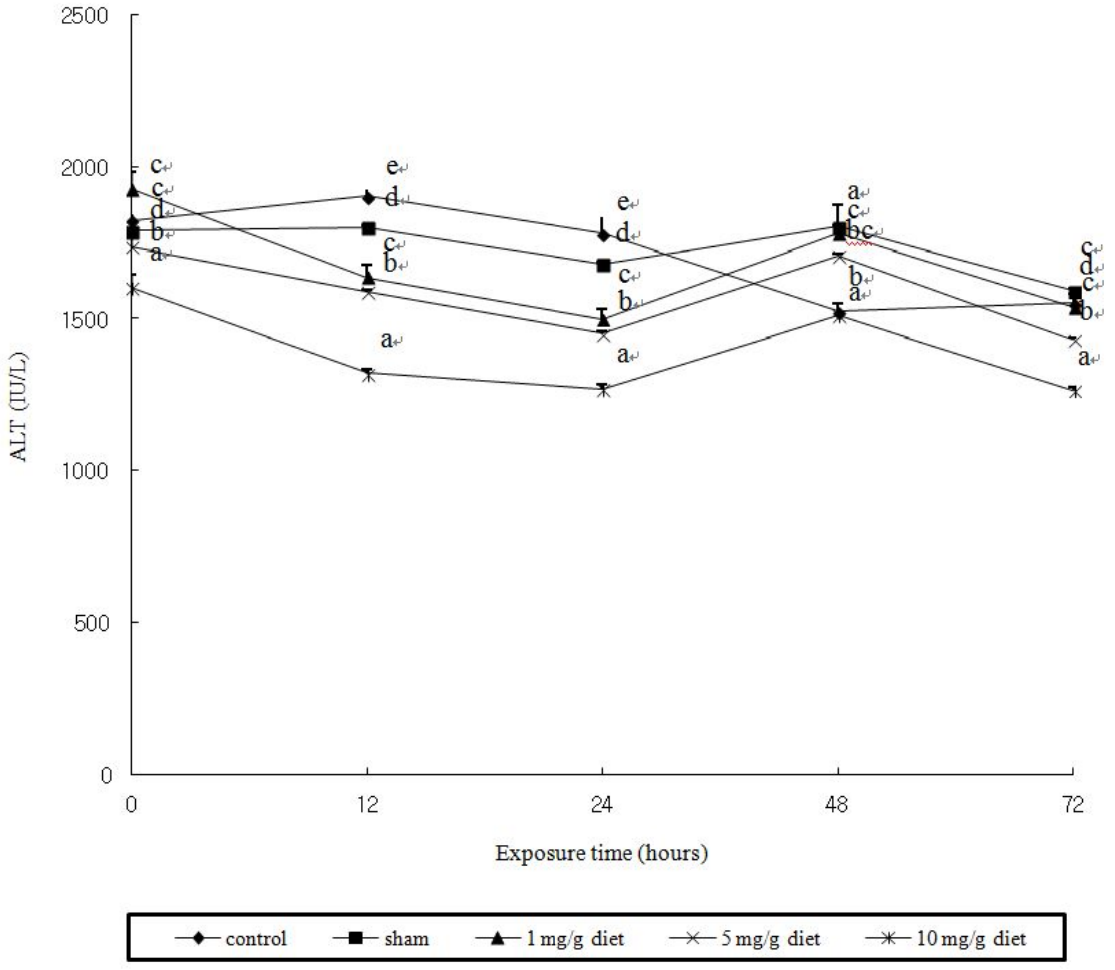
ALT concentrations of the five different dietary experiments subjected to cold water stress were presented in Fig. 3. ALT concentration trends exhibited marked variations between the experimental groups. The 1 mg/g dietary E group showed the highest concentration at start of the experiment but then exhibited a varied trend declining for the next 24 hrs before recovering during the next 24 hrs and declining again after that.



[Fig. 2] Glucose concentration variations in blood plasma of longtooth grouper (*Epinephilus bruneus*) during 72 hrs of stress period. Values represent means±SE (n=50). Different letters on the bars indicate statistically significant difference (P<0.05).

The sham control and control showed slight increases in ALT concentrations for the first 12 hrs after which levels declined further for the control experiment while the sham control underwent an up and down trend for the next 48 hrs. In the two remaining dietary vitamin E groups, the fishes were treated with both 5 mg/g and 10 mg/g vitamin E and initially showed decreasing ALT concentrations during the first 24 hrs before recovering briefly during the next 24 hrs and again declining during the last 24 hrs period.

AST concentrations of the five different dietary experiments subjected to cold water stress were presented in [Fig. 4]. The concentration for all treatments was similar at the start of the experiment but follow markedly different paths thereafter. There was rapid increase in the concentrations of the control and sham control within the fist 12 hrs followed by a marked up and down trend.

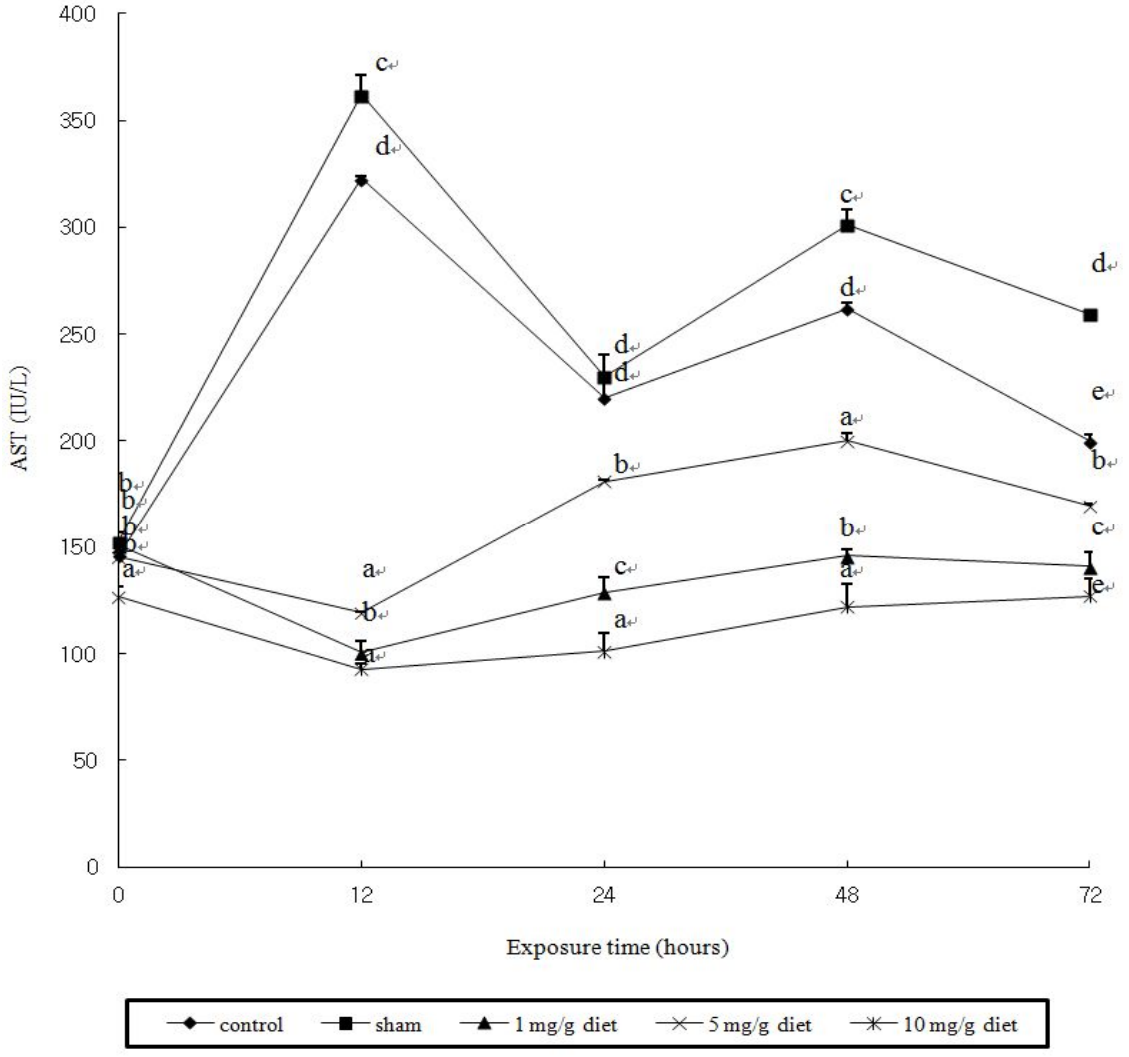


[Fig. 3] Alanine aminotransferase (ALT) concentration variations in blood plasma of longtooth grouper (*Epinephilus bruneus*) during 72 hrs of stress period. Values represent means±SE (n=50). Different letters on the bars indicate statistically significant difference (P<0.05).

The AST levels in the control increased from 146 ± 1.25 IU/L to 322.7 ± 9.45 IU/L within the first 12 hrs mirrored by a similar increase in the sham control. Conversely the concentrations for the three vitamin E supplementary diets decreased initially to their lowest level at 12 hrs but began to moderately increase again thereafter analysis of variance revealed that the concentration levels at 12 hrs between the control/sham control and the vitamin E groups were markedly different (P<0.05).

Ⅳ. Discussion

The trends in cortisol, glucose, ALT, and AST concentrations measured in this experiment are indicative of stressed reactions in longtooth grouper, *Epinephelus bruneus*. As expected, plasma cortisol levels significantly increased in response to the



[Fig. 4] Aspartate aminotransferase (AST) concentration variations in blood plasma of longtooth grouper (*Epinephilus bruneus*) during 72 hrs of stress period. Values represent means±SE (n=50). Different letters on the bars indicate statistical significance (P<0.05).

beginning of a chronic stress situation but then declined back to initial values thereafter (Pickering and Pottinger, 1989; Barton and Iwama, 1991). More importantly our results revealed that the groups with the dietary vitamin E supplements showed the more moderate changes in cortisol levels compared with the unstable and marked changes in the sham control and control.

The addition of vitamin E to diet has been shown to decrease plasma corticosteroids in rats and calves (Watson and Petro, 1982; Mudron et al., 1996). The vitamin E also known as a substance that improves the disease resistance (Blaszer, 1992; Lall and Olivier, 1993). In this study the group on the higher

concentrations of vitamin E (i.e 5 and 10mg/g) showed the less unpredictable changes thus indicating better resistance against LWT stress ([Fig. 2]). Gilthead seabream, *Sparus aurata* fed added with a lower levels of vitamin E (18.5 mg/Kg diet) had caused higher plasma cortisol levels after get stressed when compared with fish fed added with a higher vitamin E (167.5 mg/kg diet) (Montero et al., 1998).

In this study, the glucose formation appeared to closely followed to change of cortisol amount. Elevated cortisol secretion under stress increases the activation of plasma glucose by activity of the gluconeogenesis enzyme which subsequently triggers the second reaction to stress (Barton and Iwama, 1991). Das et al. (2004) suggested that a occurrence of glucose for increased cell metabolism during early exposure may have overwhelmed the increase in blood glucose, even though glycogenolysis would have increased during this period. However, because of dysfunctional cell metabolism the lower use of glucose later in the exposure period (after 48 hrs) resulted in an increase in blood glucose levels.

ALT concentration showed changes from initial to final period in all experimental groups. However, Hur and Habibi. (2007) was experimented that goldfish, *Carassius auratus* gold was not showed significant differenece between control and experimental group under the water temperature shock ($P<0.05$). This was in contrast to the trends seen in our experiment. Possible reasons for this could include mechanical problems measuring stress, species-specific stress responses, or some other unknown factors.

AST concentration showed significant increases within initial 12 hrs period for the control and sham control. This trend was in agreement with AST levels of goldfish under similar LWT stress levels (Hur and Habibi, 2007). However, our results exhibited significant differences between the control and experimental groups. Ortuño et al. (2000) also observed reduced blood glucose and AST levels due to numerous stress variations (environmental changes, water stirring, density, and exposure to air during 2 min) in gilthead seabream fed with vitamin E. In conclusion, vitamin E added to fish diets has been proved to enhance the resistance of several disease in a number of species (Blaszer, 1992; Lall and Olivier, 1993). Our study confirms this expectation in this species.

Acknowledgements

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