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EFFECT OF SALINITY CHANGES ON METHIONINE CONTENT IN TIGER GROUPER JUVENILE (*Epinephelus fuscoguttatus*)

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ABSTRACT

This study was conducted to determine the effect of water salinity changes on methionine content in tiger grouper juvenile (*Epinephelus fuscoguttatus*). A total of 2,560 tiger grouper juveniles were used and divided into five groups consist of 1 control group (without exposed to salinity changes) and 4 treatment groups. The salinity was changed every 2, 3, 4, and 6 hours in treatment A, B, C, and D, respectively. Salinity levels were changed during 24 hours by lowering salinity level from 32 psu to 22 psu. Twenty five of fish were collected from each treatment for methionine content analysis. Data were analysed using one way analysis of variance (ANOVA). The results showed that there was a decrease in methionine content in all treatments. A significant decrease (P<0.05) of methionine content in treatment A, B, and C were observed after 20 hours (1.15%), 18 hours (1.27%), and 16 hours (1.24%), respectively. While at 0 hours (control), the methionine content was 2.02%. Methionine level in treatment D was not significantly different (P>0.05) compared to control group. As conclusion, rearing the tiger grouper juvenile with salinity fluctuations every 6 hours did not lead to methionine deficiency.

Key words: *Epinephelus fuscoguttatus*, methionine, salinity change

ABSTRAK

Penelitian ini dilakukan untuk mengetahui pengaruh salinitas air terhadap perubahan kandungan metionin pada juvenil kerapu macan (Epinephelus fuscoguttatus). Sebanyak 2.560 juvenil kerapu macan digunakan dan dibagi ke dalam 5 kelompok yang terdiri dari 1 kelompok kontrol (tanpa perubahan salinitas) dan 4 kelompok perlakuan. Perubahan salinitas dilakukan setiap 2, 3, 4, dan 6 jam pada masing masing kelompok perlakuan A, B, C, dan D. Tingkat salinitas diubah selama 24 jam dengan menurunkan kadar salinitas dari 32 psu ke 22 psu. Sebanyak 25 ekor ikan diambil dari setiap perlakuan untuk diperiksa kandungan metionin. Data dianalisis dengan menggunakan analisis varian (ANAVA) pola searah. Hasil penelitian menunjukkan bahwa ada penurunan kandungan metionin untuk semua perlakuan. Penurunan kandungan metionin dalam perlakuan A sampai jam ke-20 (1,15%), B sampai jam ke-18 (1,27%), dan C sampai jam ke-16 (1,24%), sedangkan pada 0 jam (kontrol) kandungan metionin adalah 2,02%. Uji statistik menunjukkan bahwa kadar metionin dalam perlakuan D tidak berbeda nyata (P>0.05) dibandingkan dengan kelompok kontrol. Disimpulkan bahwa pemeliharaan juvenil kerapu macan dengan perubahan fluktuasi salinitas setiap 6 jam tidak memiliki risiko kekurangan metionin.

Kata kunci: Epinephelus fuscoguttatus, metionin, perubahan salinitas

INTRODUCTION

The coastal region is the most frequently occurring extreme environmental changes by abiotic factors that cause fluctuations in salinity both on a global scale and local scale (Nichols *et al*., 1986; Scavia *et al*., 2002; Bianchi, 2006). Changes in salinity on a global scale caused by physical factors such as tide, rain and flooding. While salinity changes at a local scale may occur due to social factors such as the increase of population in coastal environments that disturbing the state of waters salinity. Salinity changes have occurred both on a small scale and large scale (Schulte, 2007). The changes are influenced by the levels of mixing of fresh water with sea water.

Salinity changes in aquaculture or fish farming sites will give impact to the growth and development of the fish, both in the brackish water and in floating net cages that placed in rivers and estuaries (Hoar and Randall, 1988). High fluctuations in water salinitywill increase osmoregulation followed by an increase in metabolic processes. To overcome the problems, it is necessary to provide adequate nutrition (Jobling, 1994; Zhou *et al*., 2012). Proteins and amino acids are nutrition components that play important role in the preparation of the structure and metabolism in all aquatic organisms, which is required in an sufficient amount to build their growth (Lovell, 1989; Luo *et al*., 2005).

Fish cannot perform the synthesis of all kinds of amino acids, therefore, it is required the intake of food sources or exogenous amino acid intake (Lovell, 1989). The newly hatched Atlantic halibut larvae (*Hippoglosus hippoglosus* L.) live in low salinity (< 26 psu) and high (> 32 psu) will be stressed againts salinity changes (Lein *et al*., 1997). Rearing the fish in different water salinity affects the growth, survival, and food consumption ratio in juvenile tiger grouper (Muhammadar *et al*., 2011). The artificial feed for tiger grouper is formulated to contain high and digestible proteins and amino acids (Muhammadar *et al*., 2011; Muhammadar *et al*., 2014). Deficiency of essential amino acids (EAA) can lead to a decrease in the growth and utilization of feed. Therefore, feeds containing the appropriate amino acid or balanced indispensable amino acids was needed for juvenile fish that exposed to extreme salinity either low or high (below or above normal). The amino acids are derived from food and consumed by the fish must be balanced both number and type (Zhou *et al*., 2007). The changes in the aquatic environment that affect fish physiological conditions can be overcomes by maintaining the stability of amino acids between cells through the use of free amino acids (Willett and Burton, 2002). The amino acid balance can be obtained if fish living in extreme salinity do not affect the absorption of amino acids (Perez and Teles, 2008). Therefore it is necessary to study the effect of water salinity fluctuations on methionine content in juvenile tiger grouper.

MATERIALS AND METHODS

The research was conducted in Brackish Water Aquaculture Center, Ujong Batee, Indonesia. A total of 2,560 tiger grouper juveniles were used and divided into five treatment groups with two replications. Three hundreds twenty tiger grouper juveniles were placed in each pond containing 100 L water. Salinity levels were changed during 24 hours by lowering salinity level from 32 psu to 22 psu. The salinity level in treatment A (control) was 32 psu and the level were changed every 2, 3, 4, 6 hour in treatments B, C, D, and E, respectively.

Twenty five of fish were collected from each treatment for methionine content analysis purposes. The content of methionine were analyzed in the FST laboratory, National University of Malaysia. Samples were dried in an oven at a temperature of 105° C for 24 hours. To avoid the effect of fat from the sample, separation of coarse fat was performed by extraction using soxhletation method (Soxhlet System-Texator, Sweden) (AOAC, 1990). Then, a total of 0.2 grams of the powder sample was added with 6N hydrochloric acid (HCl) in the borosilicate test tube. The tubes were covered with parafilm and masked tape tightly. The samples were placed in a furnace at temperature of 110° C for 24 hours. The samples were then cooled at room temperature for 20 minutes. A total of 400 µL of alpha amino butyric acid (AABA) was added into sample and filtered with sartorius stedim filter paper (Biotech, 1288) into 100 mL flask. The samples were then diluted with deionized water and then filtered with aqueous syringe nylon filter 0.2 µm. A total of 2 mL filtered sample were cooled at -20° C prior to amino acid analysis using HPLC standart procedure (AOAC, 1990; Muhammadar *et al*., 2011).

Analysis Data

Data were statistically analyzed using ANOVA (analysis of variance). If the results of the analysis indicate different values at significant level of $P<0.05$, then continued with Duncan test (Dytham, 2003; Bhujel, 2008).

RESULTS AND DISCUSSION

The results showed that numerically methionine content decreased in treatments A, B, C, and D compared to control treatment (2.02%). A significant decrease of methionine content ($P<0.05$) in treatment A, B, and C were observed after 20 hours (1.15%), 18 hours (1.27%), and 16 hours (1.24 %), respectively after exposed to salinity changes. However, the levels of methionine in treatment D was not significantly different ($P<0.05$). The levels of methionine content in each treatment are presented in the Figure 1, Figure 2, Figure 3, and Figure 4.

A decrease in the methionine content in treatment A, B, and C showed that the use of methionine is necessary in the process of tiger grouper metabolism. Methionine is an essential amino acid needed by the fish. Mechanism of the use of methionine in the fish body is highly dependent on its requirements in maintaining the osmoregulation balance (Wilson,

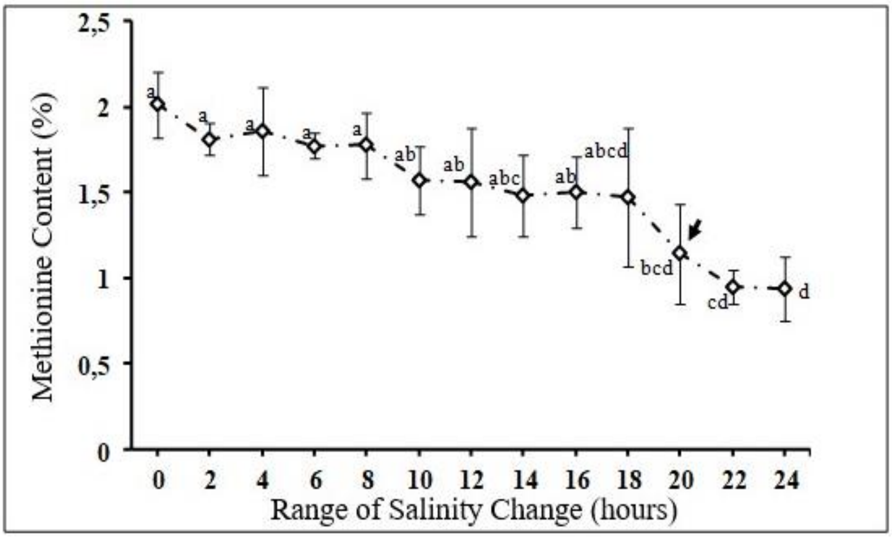

Figure 1. The methionine content of treatment A with a range of 2 hours salinity change. Arrow indicates a significant decrease of methionine levels ($P<0.05$)

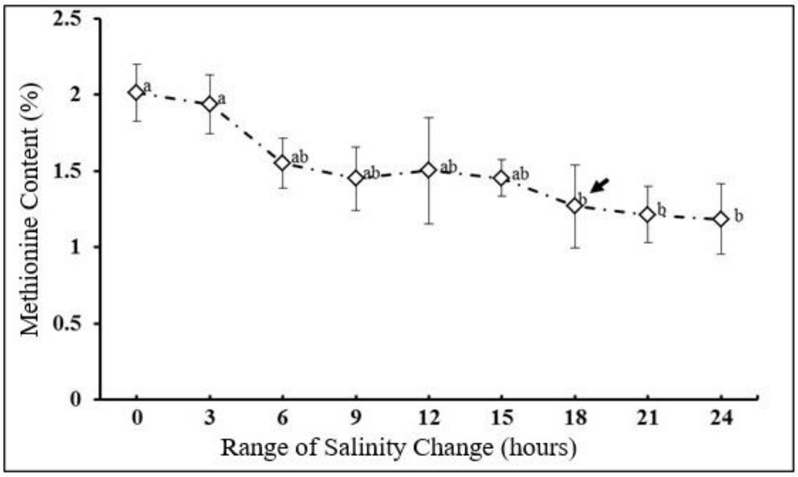

Figure 2. The methionine content of treatment B with a range of 3 hours salinity change. Arrow indicates a significant decrease of methionine levels ($P<0.05$)

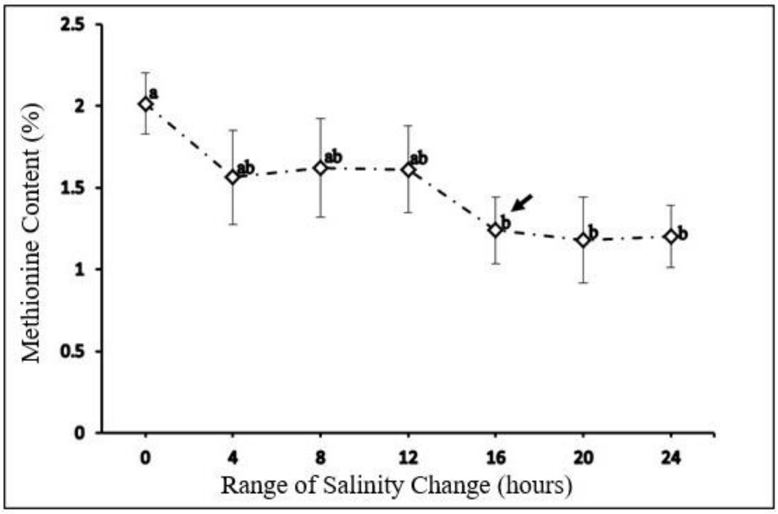

Figure 3. The methionine content of treatment C with a range of 4 hours salinity change. Arrow indicates a significant decrease of methionine levels ($P<0.05$)

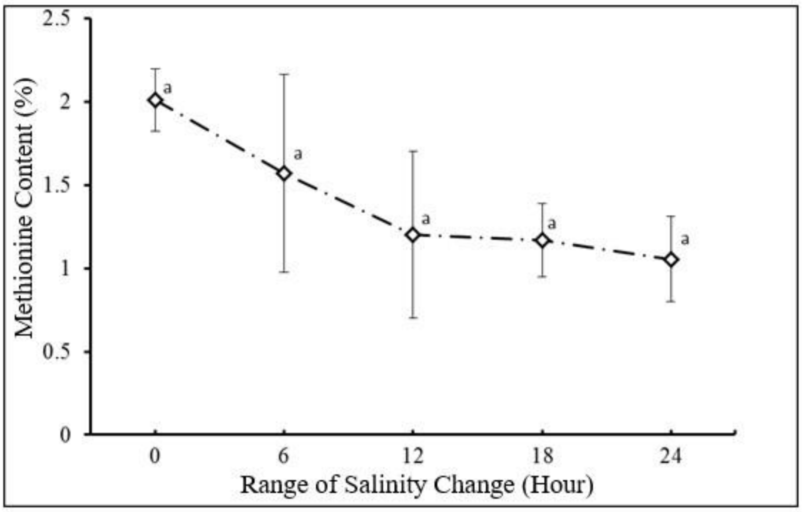

Figure 4. The methionine content of treatment D with a range of 6 hours salinity change

2002). Peres and Teles (2008) found that growth of *Dicentrachus labrax* would be good if the ratio content between essential amino acids and non-essential amino acid is 50:50. However, the content of essential amino acids such as methionine in the fish body will be less if the intake through food is also reduced. The feed formulations with high methionine content is very important, especially low protein feed (Zhou *et al*., 2006). Methionine is an EAA which is required by the fish for growth and energy metabolism (Lovell, 1989; Keembiyehetty and Gatlin, 1993; Zhou *et al*., 2006). Furthermore, methionine and cystine in food derived from animals sulfur amino acid, however cystine is not necessary if the methionine can be synthesized well (Bhagavan, 1992; Zhou *et al*., 2006).

Wilson (2002) expressed that the required methionine content for the fish were in the range of 1.8 - 3.2 %, and Luo *et al*. (2005) reported that Malabar grouper (*Epinephelus coioides*) juvenile required methionine content in the range of 1 , 31 to 2.73 %. The present results showed that methionine content in tiger grouper with salinity change every 3 hours are still within the limits specified above (2.01% at 0 hours and 1.46% at 15 hours. While the methionine content at 18 hours (1.27%) up to 24 hours (1.19%) were below optimum limit required or there has been a shortage. However, the lack of methionine in the fish muscle could be enriched through food. Methionine deficiency will hamper growth, appetite, and encourages the formation of cataracts in the eyes of juvenile fish (Keembiyehetty and Gatlin, 1993).

Results of treatment D with salinity was changed every 6 hours (Figure 4), did not show significant differences (P>0.05) during 24 hours of the treatment. This result suggests that the difference duration in salinity change does not necessarily affects the decrease in the methionine content. Therefore the rearing grouper juvenile with salinity changed every six-hour did not require the enrichment of methionine in the diet.

CONCLUSION

In conclusion, the culture of tiger grouper juvenile with salinity fluctuate every 6 hours has no risks on the decreasing of methionine content.

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