MOHAMAD SOFI BIN ABU HASSAN MASTER OF SCIENCE 2020

OXIDATIVE STRESS REGULATION IN *Lates calcarifer* FROM LOCAL AQUACULTURE FARMS IN THE EAST COAST OF MALAYSIA

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MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2020

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Thesis Submitted in Fulfilment of the Requirement for the Master's Degree of Science in the Institute of Oceanography and Environment Universiti Malaysia Terengganu

AUGUST 2020

DEDICATION

I dedicate this thesis to someone who has seen failures all her life. Someone who has endured struggles more than she could bear. Someone who has always believed in me with all her heart. Someone who has strength when she lost so much.

My mom

I hope it craves a smile.

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the master degree

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This study comprises of three main objectives to understand the organ-specific oxidative stress responses in caged *Lates calcarifer*. The first objective emphasised on the responses of the first line-defense of antioxidant system, superoxidase dismutase (SOD) and catalase (CAT). The second objective focussed on the responses of Glutathione dependent enzymes, glutathione-s-transferase (GST) and glutathione reductase (GR). The third objective quantified the protein damages that had occurred in body organs of *L.calcarifer* through the response of thiolation (-SH-) and carbonylation (-CH-). The fish (n=20) were sampled from fish cages in Setiu Wetland, Semerak and Tumpat. The responses of SOD, CAT, GST and GR were measured in muscle, gill and liver using enzymatic assays while protein damages were measured using 1-Dimensional electrophoresis approach through fluorescent tagging of -SH- and -CH-. The responses of SOD and CAT were consistent with the most responses in liver ($p<0.05$) followed by gill and muscle. High SOD responses were followed with lower CAT responses observed in muscle with opposite responses exhibited by both gill and liver. The responses of GST and GR in all organs agreed with the responses observed in SOD and CAT. The correlation indexes

for GST and GR in Tumpat were significant in muscle (0.89, $p < 0.01$), gill (0.95, $p < 0.01$), and liver (0.54, $p < 0.05$). Muscle remained as the least thiolated organ followed by gill and liver. Carbonylation responses were inconsistent for both sites Semerak and Tumpat with a significant level of responses ($p < 0.05$) in muscle compared to both gill and liver. Overall data were used for Integrated Biomarker Responses (IBR) computation to achieve the fourth objective and classify each study site with IBR score. Tumpat overall showed the highest score (muscle; 31, gill; 24, and liver; 29) for each studied organs and Setiu Wetland showed the lowest score overall (muscle; 18, gill; 23 and liver; 18). Semerak overall showed intermediate scores (muscle; 23, gill; 23 and liver; 26). The responses of enzymatic biomarkers and protein damages responses indicated oxidative stress and oxidative damages experienced by the fish in the study locations.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah sarjana

REGULASI TEKANAN OKSIDATIF DI DALAM *Lates calcarifer* DARIPADA SANGKAR IKAN TEMPATAN DI PANTAI TIMUR MALAYSIA

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Kajian ini mempunyai tiga objektif utama yang memfokuskan terhadap tindak balas penanda biokimia dan penanda kerosakan protein di dalam organ *L.calcarifer*. Objektif utama memfokuskan tindak balas pertahanan barisan depan sistem antioksidan dengan menggunakan enzim 'superoxidase dismutase' (SOD) dan 'catalase' (CAT). Objektif kedua memfokuskan enzim yang bergantung kepada 'glutathione', 'glutathione-s-transferase' (GST) dan 'glutathione reductase'. Objektif ketiga mengukur kerosakan protein yang telah berlaku di dalam organ dalaman ikan melalui tindak balas 'thiolation' (-SH-) dan 'carbonylation (-CH-). Ikan (n=20) disampel daripada sangkar ikan di Setiu Wetland, Semerak dan Tumpat. Tindak balas SOD, CAT, GST dan GR diukur di dalam otot, insang dan hati menggunakan kaedah ujian enzimatik manakala kerosakan protein diukur menggunakan kaedah elektroforesis 1 dimensi melauli penandaan fluorescent berdasarkann tindak balas 'thiolation' (-SH-) dan 'carbonylation' (-CH-). Tindak balas SOD dan CAT didalam hati ($p < 0.05$) menunjukkan tindak balas yang konsisten dan paling tinggi diikuti dengan insang dan otot. Tindak balas SOD yang tinggi diikuti dengan tindak balas CAT yang rendah di dalam otot manakala tindak balas yang berlawanan pula ditujukkan di dalam insang dan hati. Tindak balas GST dan GR menunjukkan

persamaan dengan tindak balas yang ditunjukkan oleh SOD dan CAT. Indeks korelasi GST dan GR adalah signifikan di dalam otot (0.89, $p < 0.01$), insang (0.95, $p < 0.01$), dan hati (0.54, $p < 0.05$). Otot adalah organ yang menujukkan tindak balas 'thiolation' terendah diikuti dengan insang dan hati. Tindak balas 'carbonylation' adalah tidak konsisten untuk kedua dua tempat Semerak dan Tumpat dengan kadar tindak balas yang signifikan dilihat di dalam otot ($p < 0.05$) berbanding insang dan hati. Kesulurahan data dikomputasi kepada 'Integrated Biomarker Index' (IBR) untuk mencapai objektif ke empat dan mengklasifikasi setiap tapak kajian megikut skor IBR. Secara kesuluruhan, Tumpat menujukkan skor tertinggi (otot; 31, insang; 24, dan hati; 29) untuk setiap organ dalaman yang dikaji dan Setiu Wetland menujukkan skor terendah secara keseluruhan (otot; 18, insang; 23 dan hati; 18). Semerak menunjukkan skor IBR pertengahan (otot; 23, insang; 23 dan hati; 26). Tindak balas enzim dan kerosakan protein menunjukkan tekanan oksidatif dan kerosakan oksidatif yang telah dialami oleh ikan di tapak kajian.