

## Antioxidant status and oxidative stress markers of white faeces syndrome-infected Pacific white shrimp (*Litopenaeus vannamei* Boone)

<sup>1</sup>Ardiansyah, <sup>1</sup>Andi A. Jaya, <sup>1</sup>Amrullah, <sup>1</sup>Dahlia, <sup>1</sup>Mohamad A. Baiduri, <sup>1</sup>Hartinah, <sup>1</sup>Wahidah, <sup>2</sup>Indrayani

<sup>1</sup> Aquaculture Department, Agricultural Polytechnic State of Pangkep, Makassar-ParePare Km. 83, Sulawesi Selatan 90655, Indonesia; <sup>2</sup> Faculty of Fisheries and Marine, Haluoleo University, Postal Code 93132, South East Sulawesi, Indonesia. Corresponding author: Ardiansyah, ardi\_kimsan@yahoo.com

**Abstract.** Shrimp farming industry experiences massive losses due to white faeces syndrome (WFS) infection. The virus causes oxidative damage in shrimp tissues, subsequently resulting in shrimp mortality within a relatively short time. This study aimed to evaluate the changes in antioxidant defence mechanisms and the level of protein oxidation and lipid peroxidation in the hepatopancreas, gills and flesh of WFS-infected *Litopenaeus vannamei* under laboratory conditions. The experimental animals were injected with WFS, and hepatopancreas, gill and flesh samples were collected at intervals of 0, 24, 48, and 72 hours post-infection. Lipid peroxidation, protein carbonyl contents, and antioxidant enzyme activities, including glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD), were analysed in WFS-infected shrimps. Interestingly, protein carbonyl and lipid peroxidation as oxidative markers in the shrimp's tissue were higher in infected organs than in uninfected controls. Additionally, a significant drop in GSH-Px activity was observed over the 72-hour period post-infection in all of the infected tissues analysed, with different trends observed for CAT-SOD activities. Thus, the results demonstrated that the endogenous antioxidant defences in WFS-infected shrimp failed to counteract the presence of excessive free radicals during the 72-hour period post-infection, leading to inactivation of enzymes in infected shrimp.

**Key Words:** antioxidant enzymes, SOD, CAT, GSH-Px, lipid peroxidation, white faeces syndrome, shrimp.

**Introduction.** Over the last two decades, the shrimp farming industry has emerged as a major player in the aquaculture industry. This industry offers significant employment opportunities, which may help alleviate the impoverished conditions of local coastal populations in many Asian countries (FAO 2008, 2016). In 2009, shrimp aquaculture contributed 42.2% of the total global shrimp production of 6.67 million tonnes (Pradeep et al 2012). Approximately 75% of the global farmed shrimp production occurred in Asian countries (FAO 2009). Furthermore, Walker & Winton (2010) reported that the total value of commercial shrimp species showed increasing trends and represented 17% of internationally traded seafood products. Farmed shrimps consist mainly of two species: black tiger shrimp (*Penaeus monodon*, Fabricius) and Pacific white shrimp (*Litopenaeus vannamei*, Boone).

However, the high susceptibility of these two species to WFS results in enormous economic losses of commercially farmed shrimp. The virus has been recognized since 1992 and has been shown to be the cause of massive die-offs of farmed shrimp in Asia (Durand et al 2000; Walker & Mohan 2009; Sanchez-Paz 2010). Several attempts have been made to control WFS infections, including the use of recombinant subunit vaccines (Witteveldt et al 2004) or DNA vaccines (Rout et al 2007; Johnson et al 2008), manipulating the water temperature (Rahman et al 2006), and treating shrimps with antiviral plant extracts (Peraza-Gomez et al 2009). However, these efforts have not provided encouraging results for the revival of black tiger shrimp farming. Currently,

there are no effective preventive treatments for WFS, even though various types of disinfectants are commonly applied in shrimp hatcheries and farms for the prevention of outbreaks (Chen et al 2011; FAO 2013; Byadgi et al 2014).

Following WFS infection, the formation of oxygen free radicals and reactive oxygen species (ROS) rapidly increases (Mohankumar & Ramasamy 2006; Pacheco et al 2011). ROS reacts with all main cellular components, thereby damaging tissues and causing oxidative stress-related damages, including oxidation of proteins and DNA, as well as peroxidation of unsaturated fatty acids in cell membrane structure (El-Beltagi et al 2011; Kobeasy et al 2011). However, shrimps have innate tissue defence systems, primarily made up of antioxidant enzymes. These enzymes are responsible for neutralising the effects of ROS by transforming the ROS into more stable compounds. The tissue antioxidant defence system, which consists of enzymatic and non-enzymatic components ( $\beta$ -carotene, ascorbic acid, glutathione and  $\alpha$ -tocopherol) that neutralise the ROS produced during aerobic metabolism, is indispensable for the maintenance of redox homeostasis in the organism. The enzymatic components of this system include glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione reductase (GR) (Ozturk et al 2008).

SOD is an oxidoreductase involved in the catalysis of simultaneous reduction and oxidation of superoxide anion into oxygen molecules ( $O_2$ ) and hydrogen peroxide ( $H_2O_2$ ) (Siddique et al 2013). Several isotypes of this enzyme are expressed, and are characterised by their redox-active metals in the catalytic site. The main role of CAT is eliminating excess hydroperoxide and sustaining cellular redox state. Interestingly, CAT does not require an electron donor, and it is only found in the peroxisome (Martins & English 2014). GSH-Px has been reported to be involved in fatty acid and hydroperoxide detoxification by catalysing the reduction of various hydroperoxides, thereby protecting biomembranes and many cellular components from the harmful effects of oxidative damage. In this reaction, glutathione acts as a reducing substrate to maintain proper physiological function and to prevent oxidative stress during phagocytosis (Arthur 2000; Liu et al 2004).

Enzymatic antioxidants have the capacity to prevent the effects of oxygen free radicals and ROS in aerobic organisms, thus protecting cells from oxidative stress. However, infection with a pathogen induces inhibition of antioxidant enzyme activities (Narayan et al 2017). Hence, an understanding of the relationship between antioxidant enzyme activity and WFS infection is useful in developing comprehensive methods for controlling WFS in shrimp (Mohankumar & Ramasamy 2006). Therefore, this study aims to investigate the activities of SOD, CAT, GSH-Px, and lipid peroxidation in the hepatopancreas, flesh and gills of WFS-infected shrimps.

## Material and Method

**Experimental animals and rearing conditions.** A total of 240 healthy adult *L. vannamei* (mean weight:  $29.91 \pm 1.62$  g each) were collected in April 2018 from commercial brackish water shrimp farms located in Barru Regency, Indonesia. The shrimp were then transported to the Fish Health Laboratory of Pangkep State Polytechnic of Agriculture, less than 1 hour after capture. Animal handling and sample collection were conducted in line with international guidelines and regulations. Shrimps were reared in separate 200 L fibre tanks containing 30% clean seawater ( $30 \text{ shrimp tank}^{-1}$ ) and allowed to acclimate for a period of 7 days. 120 shrimps were used for experimental infection, and the remaining shrimps were kept as controls. Continuous aerations were supplied using air pumps and air stones, and the animals were fed commercial white shrimp diets (Gold Supreme, PT Gold Coin Indonesia). Water quality parameters such as temperature, dissolved oxygen level, pH,  $NH_3$ -N,  $NO_2$ -N, and  $NO_3$ -N were recorded daily following standard protocols (APHA 2012). Uneaten feed and faecal material were removed daily, and 20% of the water volume was replaced every other day. A mechanical filter was set up to maintain the proper levels of water quality. The physiochemical properties of the water during the experimental period are shown in Table 1. After acclimation, 24 shrimps were randomly sampled to examine the health status of the animals. Diagnostic PCR

Primers for WFS were constructed based on Takahashi et al (1996). To ensure that the animals were free from WFS infection, confirmatory tests were conducted according to Lo et al (1996).

Table 1

The mean temperatures, dissolved oxygen concentrations, pH values, and ammonia, nitrite, and nitrate concentrations in the water during the experimental period

<i>Parameters</i>	<i>Mean concentration</i>
Temperature (°C)	26.20±0.50
Dissolved oxygen (mg L <sup>-1</sup> )	7.24±0.16
pH	6.98±0.06
Ammonia (mg L <sup>-1</sup> )	0.13±0.01
Nitrate nitrogen (mg L <sup>-1</sup> )	1.60±0.02
Nitrite nitrogen (mg L <sup>-1</sup> )	0.20±0.04

**Preparation of viral extracts.** Preparation of the WFS extracts was performed according to the methods of Huang et al (2001), using cephalothorax tissue as a positive control for WFS PCR detection. The infected tissue was homogenised in TN (Tris-HCl, NaCl) buffer and centrifuged at 5000×g for 30 minutes at 4°C. The resulting supernatant was filtered through a 0.45 µm pore-size filter (Merck Millipore, Billerica, MA, USA) and then filtered through a 0.2 µm pore-size syringe filter (Sigma Aldrich, St. Louis, MO, USA). Aliquots were kept frozen at -80°C until use. Prior to storage, an estimation of total protein concentration of the filtrate was performed using the methods of Lowry et al (1951). The results of the previous study conducted by Pacheco et al (2011) demonstrated that intramuscular injection of WFS inoculum (20 µL total protein per shrimp) induced mortality up to 100% within 72 hours in brown shrimp (*Farfantepenaeus californiensis*). Thus, the 20 µL injection volume per shrimp was used to measure the activity of the enzyme until 72 hours post-injection. Five shrimps from each group were randomly sacrificed at different intervals (0, 24, 48, and 72 hours) post-injection to measure enzyme activity, lipid peroxidation and protein oxidation. The hepatopancreas, gills and flesh were collected from each shrimp. Measurements were performed in triplicate.

**Preparation of organ extracts for enzyme analysis.** Organ extracts were prepared by following the methods of Ahmad et al (2000) with some modifications. The hepatopancreas, gills and flesh were dissected out, and the organs were weighed and homogenised separately in a chilled phosphate buffer (pH 7.0) containing KCl. The homogenate was passed through Miracloth (Calbiochem) filter paper and subjected to centrifugation (10,000×g) for 15 minutes at 4°C. The resulting supernatant was then re-centrifuged (13,000×g) for 20 minutes at room temperature. The supernatants obtained from this centrifugation step were used for the enzyme assays.

**GSH-Px activity.** Measurement of GSH-Px activity was based on the methods of Watanabe et al (1996), and Athar & Ikbali (1998). Analyses were performed separately for each organ extract. The reaction solution consisted of 1 mL of stock solution II (100 mL of stock solution I, 0.1875 g GSH, and 0.011 g NADPH), 80 µL organ extract, 1 unit glutathione reductase, and 50 µL cumene hydroperoxide in a total volume of 1.1334 mL. Stock solution I consisted of 3 mM EDTA, 50 mM potassium phosphate buffer (pH 7.0), and 2 mM NaN<sub>3</sub>. GSH-Px activity was determined spectrophotometrically by measuring absorbance at 340 nm (Beckman DU 650 spectrophotometer).

**CAT activity.** CAT activity measurement was performed based on the methods of Ardiansyah & Indrayani (2007). The enzyme activities in organ extracts were measured separately. As much as 100µL of each organ extract was added into a quartz cuvette containing a chilled phosphate buffer (pH 7.0) and 1.2 mL of 40 mM H<sub>2</sub>O<sub>2</sub> in a total

volume of 3 mL. Decomposition of H<sub>2</sub>O<sub>2</sub> was analysed using a spectrophotometer by measuring absorbance at 240 nm.

**SOD activity.** SOD activity was determined spectrophotometrically according to the methods of Bannister & Calabrese (2006). The enzyme activities in the organ extracts were measured separately. One hundred microliters of organ extract were added into a quartz cuvette containing 0.3 mM NaCN (sodium cyanide), 1.5 mM NBT (nitro blue tetrazolium), 0.1 M EDTA, 50 mM potassium phosphate buffer (pH 7.8), 45 mM methionine, and 0.1 mM riboflavin in a total volume of 3.45 mL. The ability of the extract to inhibit SOD-dependent NBT reduction was estimated by measuring absorbance at 280 nm.

**Lipid peroxidation assay.** Lipid peroxidation was analysed by measuring thiobarbituric acid-reactive substances (TBARS), using malondialdehyde (MDA) equivalents (Siddique et al 2012). Determination of MDA concentrations was performed according to the methods of Eze et al (2008) using a standard curve based on the coloured product resulting from the condensation of thiobarbituric acid (TBA) with MDA. The homogenates were centrifuged (10,000×g) for 10 minutes at 4°C. The resulting supernatant from each sample was used for the lipid peroxidation assay. The supernatant and standard were evaluated simultaneously. To this end, 1400 µL stock solution (0.375% [w/v] TBA, 15% [w/v] trichloroacetic acid [TCA], and 0.25 N HCl) was added to the samples and standards, and 5% (w/v) butylated hydroxytoluene (BHT) was added. The mixture was then vortexed, heated at 100°C for 20 minutes, and cooled at room temperature for 30 minutes. Once cool, samples and standards were centrifuged at 13,000×g for 15 minutes. Subsequently, the resulting supernatants were analysed spectrophotometrically at 532 nm.

**Protein carbonyl assay.** Measurement of protein oxidation in shrimp tissue was based on the modified methods of Weber et al (2015) with some modifications. The tissue samples were mixed with 100 µL of cell lysis reagent and incubated for 10 minutes at room temperature. The sample was vortexed and centrifuged at 17,000 rpm for 15 minutes. The supernatants (cell lysate) in a 50 mM potassium phosphate buffer (pH 7.2) were mixed with 4 µL of 2.5 M HCl DNPH. The blank sample was mixed with 2.5 M HCl and incubated for 1 hour in the dark. Protein was precipitated with 6 µL of 20% trichloroacetic acid (TCA) and washed with 5 µL of ethanol: ethyl acetate mixture (1:1). The protein pellet was dissolved in 2 µL of 6 M guanidine hydrochloride.

**Statistical analysis.** The results are expressed as the mean±standard error (SE). Data was statistically analysed with SPSS 22 (IBM SPSS Advanced Statistics 22). The differences between group means were analysed by one-way ANOVA, followed by Tukey's honestly significant difference (HSD). Differences were reported as statistically significant when p values were less than 0.05 (p < 0.05).

**Results and Discussion.** All experimental shrimps injected with the viral extract were susceptible to WFS infection. Observations indicated that there was a sudden reduction in food consumption, and the shrimp became lethargic, exhibited disorientation during swimming, and showed reddish discoloration of the cuticle. At 72 hours post-infection, 82% of infected shrimps had died. Clinical signs of WFS in shrimp include dramatically decreased appetite, lethargy, collapse of the cuticle, discoloration of the shrimp body, and the appearance of white spots, ranging from 0.5 to 2 mm in diameter, on the carapace, appendages, and cuticle (Sriurairatana et al 2014). Pathogenic infection induces membrane lipid peroxidation, which is closely associated with some specific functions, such as digestion, absorption, and detoxification (Pan et al 2003). Thus, accumulation of these insults causes damage that eventually leads to death within a relatively short time after infection.

Variations in the activities of SOD in the hepatopancreas, gills and flesh of WFS-infected *L. vannamei* and control shrimp at different stages of infection are shown in

Figure 1. Injection of the viral extract into *L. vannamei* elevated SOD activities in the hepatopancreas, gills and flesh to 0.53, 0.46, and 0.44 U·mg<sup>-1</sup> of protein respectively, at 24 hours post-infection. The rate of elevation was significantly lower at 48 hours and fell to baseline levels below that of the control group at 72 hours post-infection. No significant changes ( $p > 0.05$ ) in SOD activity were observed in the control shrimp. Elevations in CAT activities were observed in infected shrimp at 24 hours following viral infection. The rate of elevation was significantly lower ( $p < 0.05$ ) at 48 hours and then fell below that of the control group at 72 hours post-injection. No significant changes ( $p > 0.05$ ) in CAT activity were observed in the control group (Figure 2). Increased CAT-SOD activities may indicate a higher need to destroy ROS (Arun & Subramanian 1989). Campa-Cordova et al (2002) reported a similar increase in SOD activity in tissues from white shrimps infected with *Vibrio parahaemolyticus* at 18 and 24 hours post-infection, potentially indicating that oxidative stress was increased due to the presence of the superoxide anion radical. Increased SOD activity may also indicate a higher capacity to avoid cytochrome *c* reduction by O<sub>2</sub><sup>-</sup>. This suggests that the capacity of SOD to prevent cellular damage is decreased (Neves et al 2000).

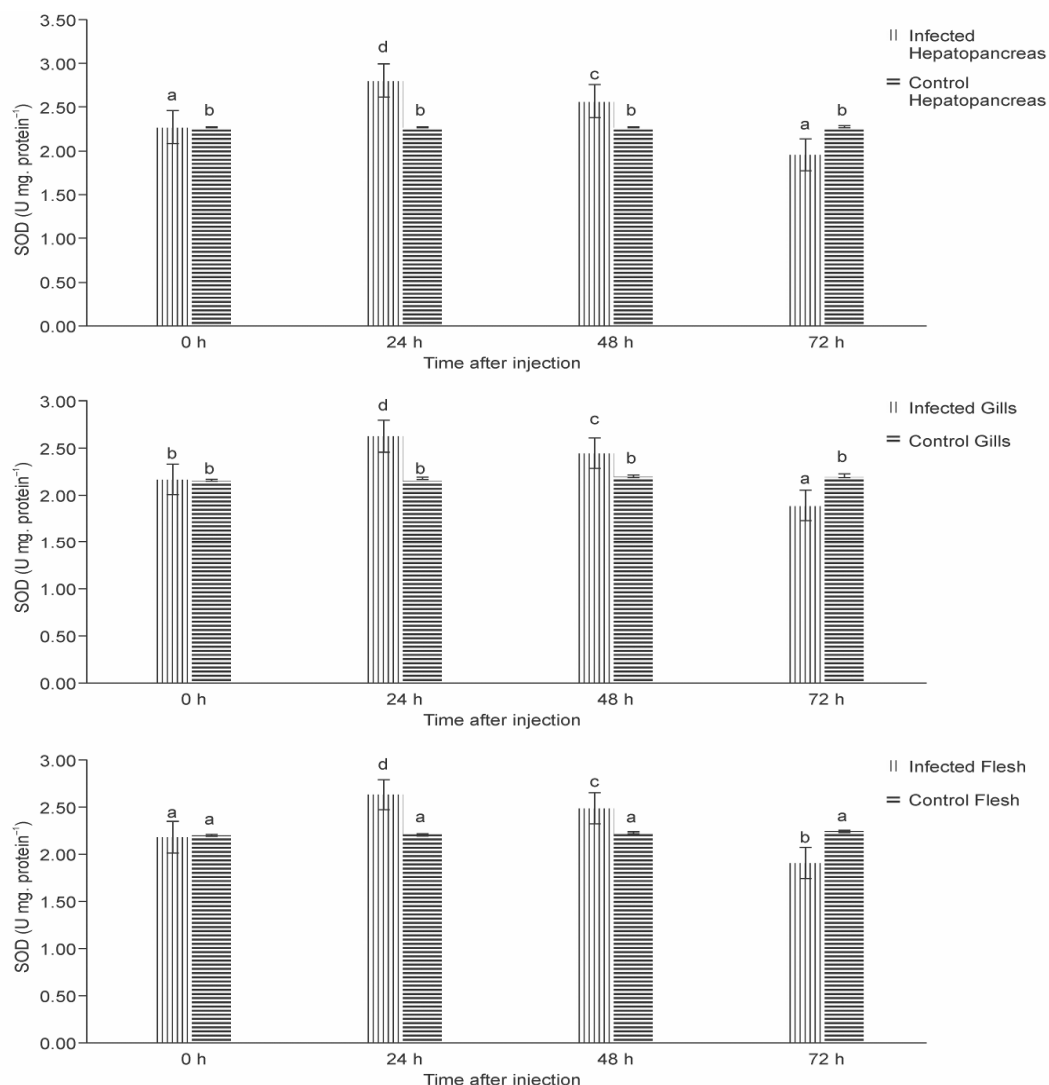


Figure 1. Variations in SOD activity in the hepatopancreas, gills, and flesh of WFS-infected *L. vannamei* and control shrimp. Same superscripts mean no significant differences ( $p > 0.05$ ).

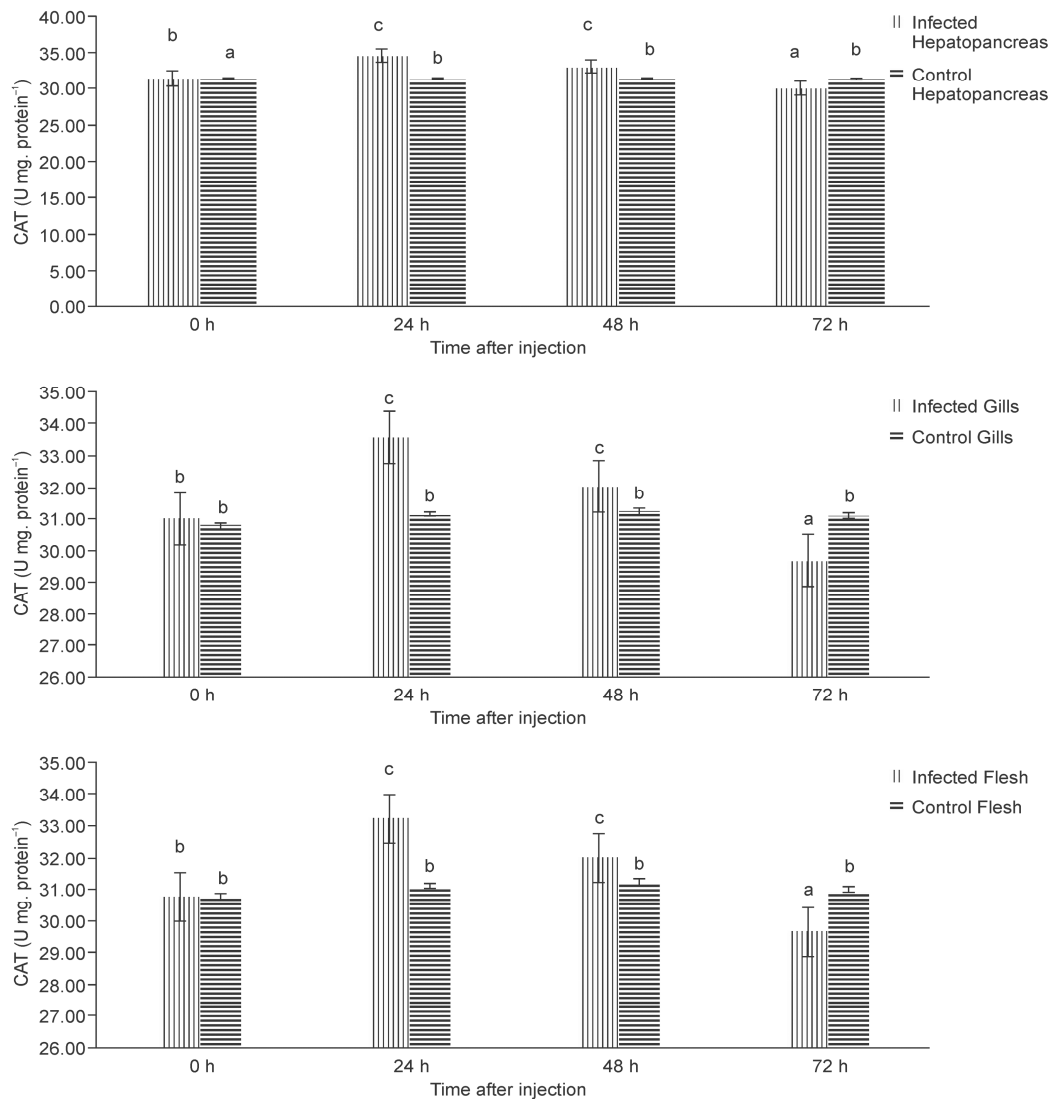


Figure 2. Variations in CAT activity in the hepatopancreas, gills, and flesh if WFS-infected *L. vannamei* and control shrimp. Same superscripts mean no significant differences ( $p > 0.05$ ).

CAT activity in infected tissues was increased at 48 hours post-infection, which was in agreement with the findings of Parilla-Taylor et al (2013). Increased CAT activity may occur in an attempt to eradicate excessive free radicals generated from WFS infection, which can result from an ongoing increase in damage to ectodermal and mesodermal cells of the shrimp (Rajan et al 2000; Wang et al 2017). However, SOD and CAT activities later decreased in all infected tissues at 72 hours post-infection. Chang et al (2003) and Mohankumar & Ramasamy (2006) reported that the activity of CAT-SOD decreased significantly in the hemolymph, hepatopancreas and flesh following WFS infection in *P. monodon*. Interestingly, viral infection seems to trigger significant changes in cellular activity, leading to dysfunction of the complex antioxidant defence system. Indeed, in our study, failure of the antioxidant defence system, which was noted during the later stages of infection, clearly indicated that the tissue antioxidant defence status during WFS infection in *L. vannamei* was operating at a lower rate, despite the increased need for antioxidant defences to neutralise the increased production of free radicals. Decreased CAT-SOD levels may lead to a reduced capacity to neutralise ROS and an increased susceptibility to oxidative stress. Wang et al (2010) reported that the decrease in SOD activity may be due to the consumption of this enzyme during the conversion of  $O_2^{\cdot-}$  to  $H_2O$ . This result indicates that SOD itself was damaged because of the high levels of ROS generated in shrimp tissues. Such reduced enzyme activities would allow the accumulation of oxidative damage and ROS, promoting symptoms of the viral infection.

Variations in GSH-Px activities are shown in Figure 3. The enzyme activity was lower in infected shrimp than in control shrimp for the 72 hour experimental period. GSH-Px activity initially fell to below control values by 24 hours after viral challenge and then continued to decline throughout the remaining study period. At 72 hours, the activities of the enzyme in the hepatopancreas, gills and flesh of infected shrimp were 0.40, 0.38, and 0.44 U·mg<sup>-1</sup> protein, respectively, below the control values. No significant differences ( $p > 0.05$ ) were observed in the control group over the 72 hour study period. These results differ from a previous study on WFS-resistant *P. japonicus* (He et al 2005). A substantial decrease in GSH-Px activity 24 hours after virus challenge may indicate increased levels of H<sub>2</sub>O<sub>2</sub> and lipid hydroperoxide in shrimp cells, which may be caused by the lower scavenging abilities of SOD and CAT (Searle & Wilson 1980; Fijałkowski et al 2018). Reduction in GSH-Px activity causes the lipid environment of cellular and subcellular membranes to be more susceptible to oxidative damage, leading to the production of oxidized glutathione and other disulfides (Espinosa-Diez et al 2015). Sustained reduction of GSH-Px activity over 72 hours post-infection may also indicate higher formation of singlet oxygen ( $^1O_2$ ) and H<sub>2</sub>O<sub>2</sub>, which in turn form the hydroxyl radical (OH<sup>·</sup>) and carry a number of adverse reactions to the shrimp's cell membranes (Fridovich 2004). This data suggests that GSH-Px is essential for initial elimination of various hydroperoxides before the involvement of another endogenous antioxidant.

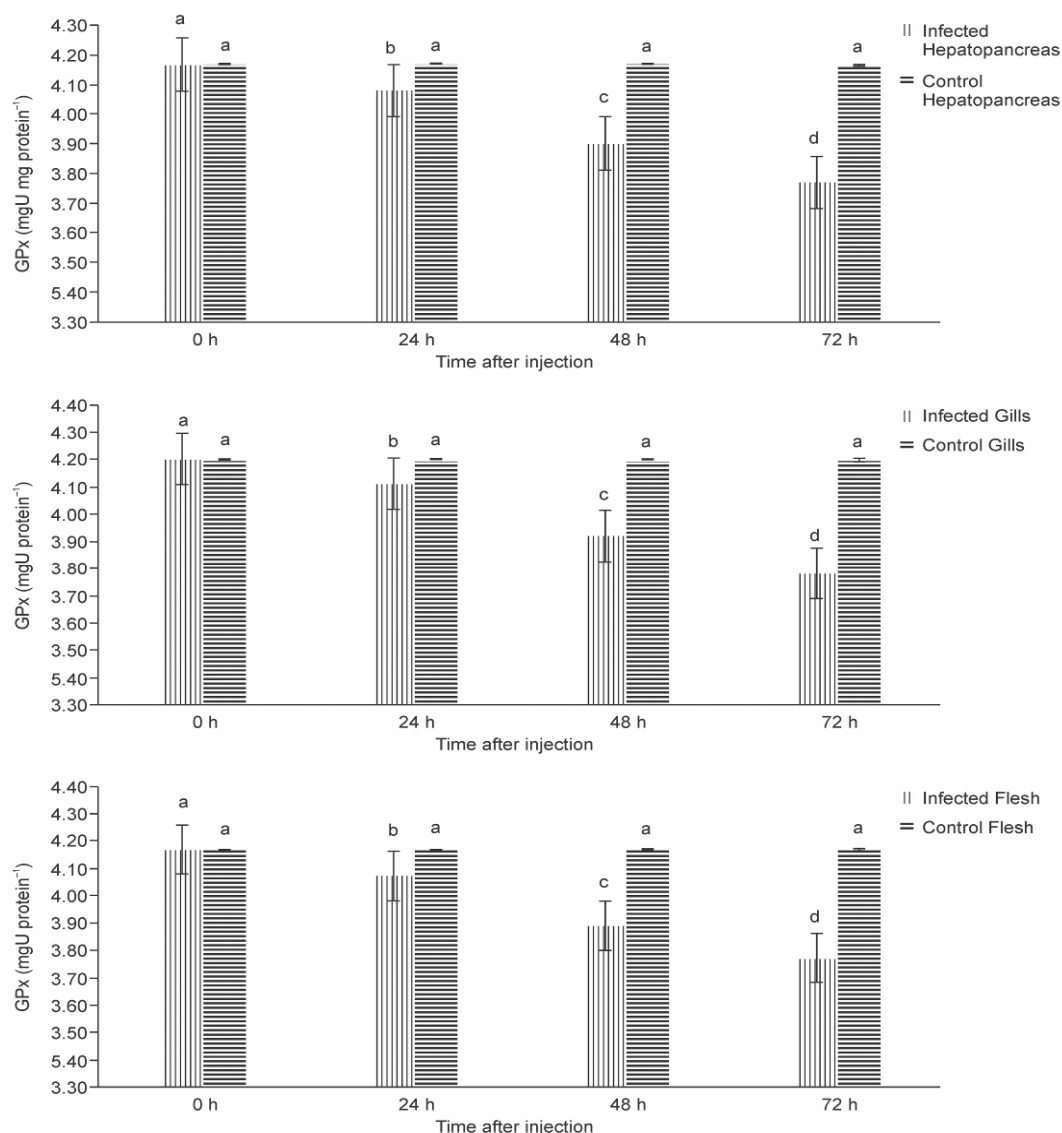


Figure 3. Variations in GSH-Px activity in the hepatopancreas, gills, and flesh of WFS-infected *L. vannamei* and control shrimp. Same superscripts mean no significant differences ( $p > 0.05$ ).

TBARS is an indicator of oxidative damage in the hepatopancreas, gills, and flesh in infected and uninfected shrimp. Formation of TBARS (nmol MDA·mg<sup>-1</sup> protein) in shrimp organs over time is shown in Figure 4. Overall, TBARS values increased over time in infected shrimp, while no significant variations ( $p > 0.05$ ) were observed in the control group. The highest TBARS values in the hepatopancreas, gills, and flesh of infected shrimp were recorded at 48 and 72 hours post-infection. At this time, the TBARS values in infected tissues were about three times higher than those in the control group.

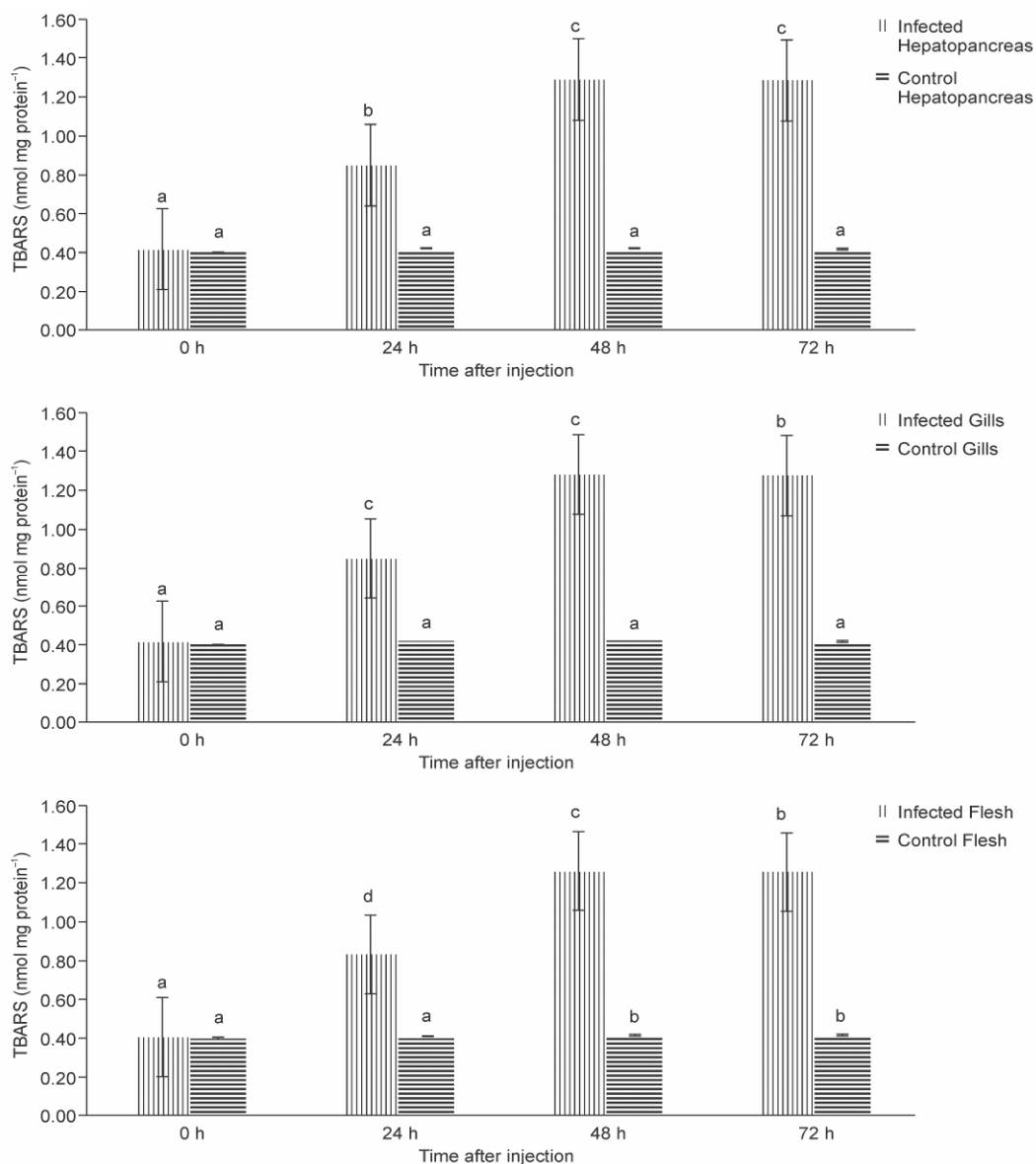


Figure 4. Formation of TBARS as an indicator of oxidative damage in response to WFS infection in the hepatopancreas, gills, and flesh of *L. vannamei*. Same superscripts mean no significant differences ( $p > 0.05$ ).

Protein oxidation, as measured by protein carbonyl (PC) content, is an important indication of cellular injury. Different trends were observed in the levels of PC in organs from infected shrimp. The initial level of protein oxidation was around 0.287 nmol·mg protein<sup>-1</sup>. This basal level of PC content was also observed in uninfected organs. Increased PC levels were present at 24 hours after virus challenge, reached a maximum level of nearly twice that of the control, and then slightly declined until 72 hours post-infection (Figure 5). No significant differences ( $p > 0.05$ ) were detected in the control group. Subramanian & Philip (2013) reported a similar increase in oxidative stress in



terms of hydroperoxide, conjugated diene, and MDA concentrations in various tissues of *Fenneropenaeus indicus*. This may be indicative of increased production of peroxides that damage all of the biomolecules present in cellular and subcellular membranes, including lipids. Increased formation of lipid peroxides is recognized to damage the integrity of cellular membranes, which in turn causes the leakage of cytoplasmic enzymes (Bagchi et al 1995). In this study, increased CAT-SOD and decreased GSH-Px activities were followed by the induction of lipid peroxidation. This supports these previous works.

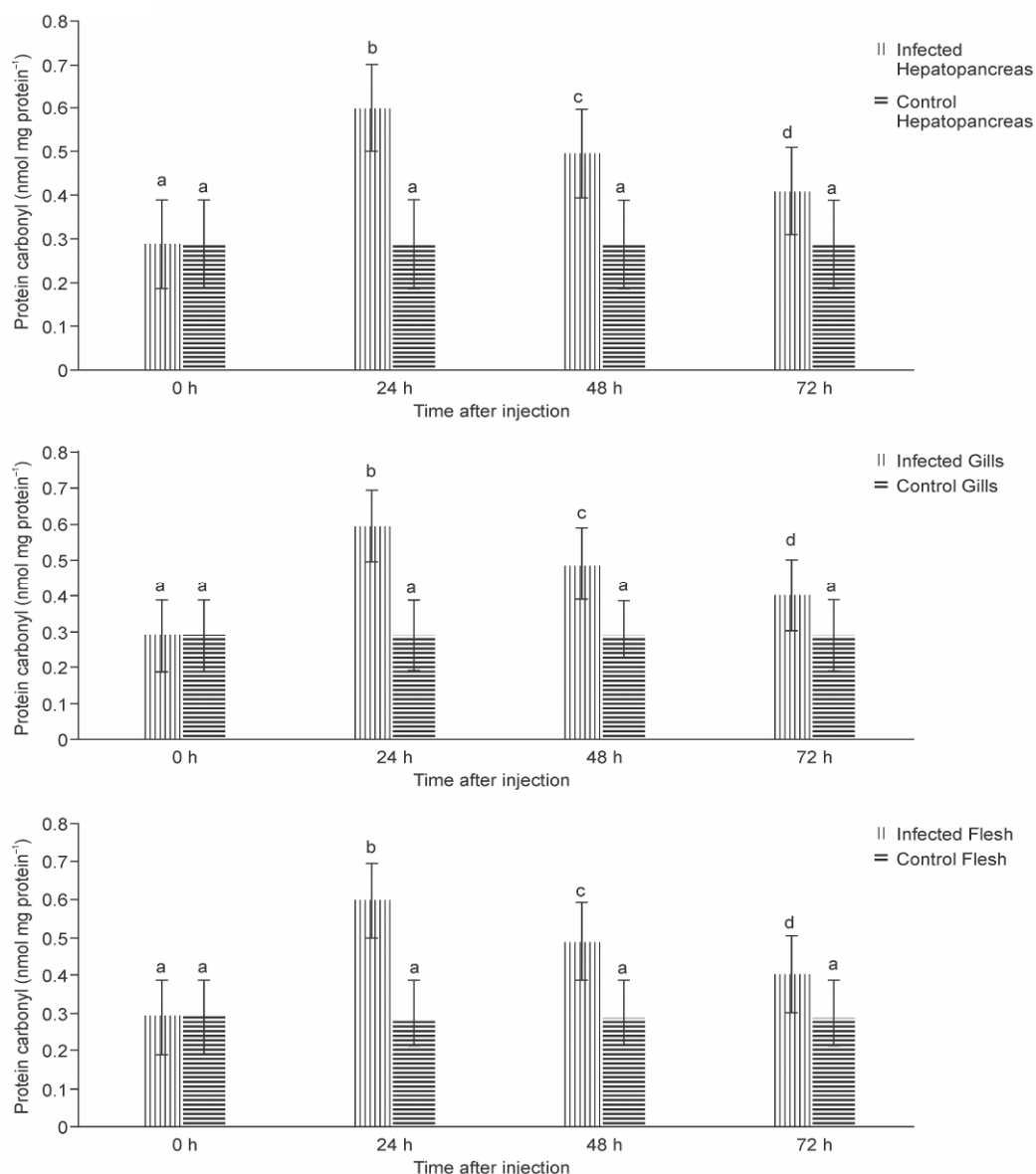


Figure 5. Formation of protein carbonyls in response to WFS infection in the hepatopancreas, gills, and flesh of *L. vannamei*. Same superscripts mean no significant differences ( $p > 0.05$ ).

High concentrations of peroxide, coupled with the failure of the antioxidant system, caused increasing damage; this process was initiated by viral infections (Dandapat et al 2003; Abele & Puntarulo 2004). Failure of the antioxidant system led to increased lipid peroxidation and tissue damage. This finding is important because lipid peroxides themselves are free radicals with large reaction constants and can therefore cause oxidative damage associated with cell death (Kidd 1991; Gaschler et al 2017). Lipid oxidation, which is initiated by ROS, is a free radical chain reaction that has very dangerous effects on the survival of the organism. Therefore, it is important to

understand how lipid peroxidation contributes to the clinical signs and mortality in WFS-infected shrimp.

Many studies have demonstrated that PC content is closely related to lipid oxidation in aquatic animals (Heise et al 2006; Castex et al 2010; Pazos et al 2011; Estévez 2011). Elevated oxidative stress in the cell membrane is followed by higher levels of lipid and protein oxidation. In the present study, even though viral challenge caused an upward trend in lipid peroxidation over 72 hours, PC contents did not follow the same trend. However, because the test was generally used for evaluation of PC levels of protein damage (Mercier et al 2004; Estévez & Xiong 2019), this test has limited ability to detect the destruction of certain amino acids. Therefore, our results indicated that the formation of PC was limited to a specific carbonyl group on certain amino acids and did not represent the complete oxidative phenomena. Oxidatively modified proteins may change the structural arrangement of protein, leading to inactivation of many enzymes (Reznick & Pecker 1994). Thus, some of the virus-induced changes in protein structure and function may not be detected using the PC assay, suggesting that other amino acid groups may not be oxidised to form carbonyls.

Data demonstrated that organs from infected shrimp had higher levels of lipid and protein oxidation, as well as CAT-SOD activities, than those in the control group. Differences in the antioxidant defence status and oxidative damage between the two groups of shrimp were significant. Moreover, the observation of increased lipid peroxidation over the 72 hour study period may indicate a significant increase in oxidative damage in the cell membrane. These results were confirmed by increasing protein oxidation levels in infected organs, although the PC content showed an opposite trend at 24 hours after viral challenge. Moreover, the activities of SOD and CAT in the hepatopancreas, gills and flesh of WFS-infected *L. vannamei* were rapidly increased following WFS injection, possibly due to the increase in the number of virus particles in the infected ectodermal and mesodermal tissues. However, the reduced activity of GSH-Px in the organs of infected shrimps may be explained by the observation that various hydroperoxides produced in the cell membrane are initially and mainly metabolised by GSH-Px.

**Conclusions.** Oxidative stress occurred over the course of WFS infection, as demonstrated by increased lipid peroxidation and PC content. Oxidative stress induced biochemical changes in the hepatopancreas, gills and flesh of WFS-infected *L. vannamei*. Increased lipid peroxidation and PC content altered antioxidant defences and triggered changes in SOD, CAT and GSH-Px activities. Alterations in antioxidant defences can either induce or suppress enzymes. Data showed that CAT-SOD and GSH-Px activity were inversely proportional. Alterations in antioxidant defences may also be relevant to the ability of the hepatopancreas and other investigated organs to cope with oxidative stress during the viral infection. Higher CAT-SOD activities were observed in the hepatopancreas, while higher GSH-Px activity was recorded in the gills. Elevated oxidative stress appeared to increase the amount of carbonylation present in the investigated tissues. Differences were noted in the extent of carbonylation between infected and uninfected control groups. However, our study showed that upward trends in lipid peroxidation were not correlated to the measurements of protein oxidation. This demonstrated that oxidative modification of proteins might generate various oxidized products that may not be detected through PC assays. Thus, further studies are required to identify other, more comprehensive approaches that can be used to precisely measure oxidized proteins during viral infections.

**Acknowledgements.** We would like to thank I-MHERE Project B.1 Batch III of the Pangkep State Polytechnic of Agriculture, Indonesia for the financial support. We also appreciate our coworkers' assistance with shrimp collection and sampling.

## References

- Abele D., Puntarulo S., 2004 Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comparative Biochemistry and Physiology, Part A* 138(4):405-415.
- Ahmad I., Hamid T., Fatima M., Chand H. S., Jain S. K., Athar M., Raisuddin S., 2000 Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. *Biochimica et Biophysica Acta* 1523(1):37-48.
- APHA, 2012 Standard methods for the examination of water and wastewater. 22<sup>nd</sup> edition, American Public Health Association, Washington, D.C, USA, 1360 pp.
- Ardiansyah, Indrayani, 2007 Natural antioxidants dietary and lipid oxidation analysis in zebrafish (*Brachydanio rerio*) tissue. *Hayati Journal of Bioscience* 14(3):87-92.
- Arthur J. R., 2000 The glutathione peroxidases. *Cellular and Molecular Life Sciences* 57(13-14):1825-1835.
- Arun S., Subramanian P., 1998 Antioxidant enzymes in fresh water prawn *Macrobrachium malcolmsonii* during embryonic and larval development. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 121(3):273-277.
- Athar M., Iqbal M., 1998 Ferric nitrilotriacetate promotes N-diethylnitrosamine-induced renal tumorigenesis in the rat: implications for the involvement of oxidative stress. *Carcinogenesis* 19(6):1133–1139.
- Bagchi M., Bagchi D., Adickes E., Stohs S. J., 1995 Chronic effects of smokeless tobacco extract on rat liver histopathology and protection of HSP-90. *Journal of Environmental Pathology, Toxicology and Oncology* 14(2):61-68.
- Bannister J. V., Calabrese L., 2006 Assays for superoxide dismutase. *Methods of Biochemical Analysis* 32:279-312.
- Byadgi O. V., Shankar K. M., Naveen Kumar B. T., Patil R., Ahmed I., 2014 Passive immunity in tiger shrimp (*Penaeus monodon*) fed with monoclonal antibody to white spot syndrome virus. *Aquaculture International* 22:887-900.
- Campa-Cordova A. I., Hernandez-Saavedra N. Y., De Philippis R., Ascencio F., 2002 Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp (*Litopenaeus vannamei*) as a response to beta-glucan and sulphated polysaccharide. *Fish and Shellfish Immunology* 12(4):353-366.
- Castex M., Lemaire P., Wabete N., Chim L., 2010 Effect of probiotic *Pediococcus acidilactici* on antioxidant defences and oxidative stress of *Litopenaeus stylirostris* under *Vibrio nigripulchritudo* challenge. *Fish and Shellfish Immunology* 28:622-631.
- Chang C. F., Su M. S., Chen H. Y., Liao I. C., 2003 Dietary  $\beta$ -1,3-glucan effectively improves immunity and survival of *Penaeus monodon* challenged with white spot syndrome virus. *Fish and Shellfish Immunology* 15(4):297-310.
- Chen I. T., Aoki T., Huang Y. T., Hirono I., Chen T. C., Huang J. Y., Chang G. D., Lo C. F., Wang K. C., 2011 White spot syndrome virus induces metabolic changes resembling the warburg effect in shrimp hemocytes in the early stage of infection. *Journal of Virology* 85(24):12919-12928.
- Dandapat J., Rao J. K., Chainy G. B. N., 2003 Lipid peroxidation and antioxidant defence status during larval development and metamorphosis of giant prawn, *Macrobrachium rosenbergii*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 135(3):221-233.
- Durand S. V., Tang K. F. J., Lightner D. V., 2000 Frozen commodity shrimp: potential avenue for introduction of white spot syndrome virus and yellow head virus. *Journal of Aquatic Animal Health* 12(2):128-135.
- El-Beltagi H. S., Ahmed O. K., El-Desouky W., 2011 Effect of low doses  $\gamma$ -irradiation on oxidative stress and secondary metabolites production of rosemary (*Rosmarinus officinalis* L.) callus culture. *Radiation Physics and Chemistry* 80(9):968-976.
- Espinosa-Diez C., Miguel V., Mennerich D., Kietzmann T., Sánchez-Pérez P., Cadenas S., Lamasa S., 2015 Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biology* 6:183-197.

- Estévez M., 2011 Protein carbonyls in meat systems: a review. *Meat Science* 89(3):259-279.
- Estévez M., Xiong Y. 2019 Intake of oxidized proteins and amino acids and causative oxidative stress and disease: recent scientific evidences and hypotheses. *Journal of Food Science* 84(3): 387-396.
- Eze J. I., Anene B. M., Chukwu C. C., 2008 Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index, in *Trypanosoma brucei*-infected rats. *Comparative Clinical Pathology* 17(2):67–72.
- FAO, 2008 State of world fisheries and aquaculture. Food and Agricultural Organisation of the United Nations, Rome, Italy, 192 pp.
- FAO, 2009 Fishstat Plus. Food and Agricultural Organisation of the United Nations, Rome, Italy, 295 pp.
- FAO, 2013 Cultured aquatic species information programme. FAO Fisheries and Aquaculture Department, Technical Papers, Rome, Italy, 13 pp.
- FAO, 2016 The state of world fisheries and aquaculture 2016, contributing to food security and nutrition for all. Food and Agriculture Organization of the United Nations, Rome, Italy, 204 pp.
- Fijałkowski P., Jędrzejczak-Pospiech K., Błaszczak J., 2018 Do catalase and glutathione peroxidase protect blood platelets from lipid peroxidation in multiple sclerosis? *Advances in Psychiatry and Neurology* 27(1):49-53.
- Fridovich I., 2004 Mitochondria: are they the seat of senescence? *Aging Cell* 3(1):13-16.
- Gaschler M. M., Stockwell B. R., 2017 Lipid peroxidation in cell death. *Biochemical and Biophysical Research Communications* 482(3):419-425.
- He N. H., Qin Q. W., Xu X., 2005 Differential profile of genes expressed in hemocytes of white spot syndrome virus-resistant shrimp (*Penaeus japonicus*) by combining suppression subtractive hybridization and differential hybridization. *Antiviral Research* 66(1):39-45.
- Heise K., Puntarulo S., Nikinmaa M., Abele D., Portner H. O., 2006 Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. *The Journal of Experimental Biology* 209:353-363.
- Huang C. H., Zhang L. R., Zhang J. H., Xiao L. C., Wu Q. J., Chen D. H., Li J. K. K., 2001 Purification and characterization of white spot syndrome virus (WSSV) produced in an alternate host: crayfish, *Cambarus clarkii*. *Virus Research* 76(2):115-125.
- Johnson K. N., Van-Hulten M. C. W., Barnes A. C., 2008 "Vaccination" of shrimp against viral pathogens: phenomenology and underlying mechanisms. *Vaccine* 26:4885-4892.
- Kidd P. M., 1991 Natural antioxidants-the first line of defences. In: Living with the AIDS virus: a strategy for long term survival. Kidd P. M., Huber W. (eds), PMK Biomedical Nutritional Consulting, Albany, CA, pp. 115-142.
- Kobeasy M. I., El-Beltagi H. S., El-Shazly M. A., Khattab E. A. H., 2011 Induction of resistance in *Arachis hypogaea* L. against *peanut mottle virus* by nitric oxide and salicylic acid. *Physiological and Molecular Plant Pathology* 76(2):112-118.
- Liu C. H., Tseng M. C., Cheng W., 2007 Identification and cloning of the antioxidant enzyme, glutathione peroxidase, of white shrimp, *Litopenaeus vannamei*, and its expression following *Vibrio alginolyticus* infection. *Fish and Shellfish Immunology* 23(1):34-45.
- Liu J. Q., Zhang K., Ren X. J., Luo G. M., Shen J. C., 2004 Bioimprinted protein exhibits glutathione peroxidase activity. *Analytica Chimica Acta* 504:185-189.
- Liu K. F., Yeh M. S., Kou G. H., Cheng W., Lo C. F., 2010 Identification and cloning of a selenium-dependent glutathione peroxidase from tiger shrimp, *Penaeus monodon*, and its transcription following pathogen infection and related to the moult stages. *Development and Comparative Immunology* 34(9):935-944.
- Lo C. F., Ho C. H., Peng S. E., Chen C. H., Hsu H. C., Chiu Y. L., Chang C. F., Liu K. F., Su M. S., Wang C. H., Kou G. H., 1996 White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Diseases of Aquatic Organisms* 27:215-225.

- Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 1951 Protein measurement with folin phenol reagent. *The Journal of Biological Chemistry* 193(1):265-275.
- Martins D., English A. M., 2014 Catalase activity is stimulated by H<sub>2</sub>O<sub>2</sub> in rich culture medium and is required for H<sub>2</sub>O<sub>2</sub> resistance and adaptation in yeast. *Redox Biology* 2:308-313.
- Mercier Y., Gatellier P., Renerre M., 2004 Lipid and protein oxidation *in vitro*, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. *Meat Science* 66(2):467-473.
- Miller D. S., 2002 Xenobiotic export pumps, endothelin signalling, and tubular nephrotoxics - a case of molecular hijacking. *Journal of Biochemical and Molecular Toxicology* 16(3):121-127.
- Mohankumar K., Ramasamy P., 2006 White spot syndrome virus infection decreases the activity of antioxidant enzymes in *Fenneropenaeus indicus*. *Virus Research* 115(1):69-75.
- Narayan O. P., Verma N., Singh A. K., et al, 2017 Antioxidant enzymes in chickpea colonized by *Piriformospora indica* participate in defense against the pathogen *Botrytis cinerea*. *Scientific Reports* 7:13553.
- Neves C. A., Santos E. A., Bainy A. C. D., 2000 Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Palaemonidae), infected by *Probopyrus ringueleti* (Isopoda, Bopyridae). *Diseases of Aquatic Organisms* 39:155-158.
- Ozturk C., Batcioglu K., Karatas F., Hazneci E., Genc M., 2008 Comparison of plasma malondialdehyde, glutathione, glutathione peroxidase, hydroxyproline and selenium levels in patients with vitiligo and healthy controls. *Indian Journal of Dermatology* 53(3):106-110.
- Pacheco R., Ascencio F., Zarain M., Gómez G., Campa A., 2011 Enhancement of superoxide dismutase and catalase activity in juvenile brown shrimp, *Farfantepenaeus californiensis* (Holmes, 1900), fed  $\beta$ -1.3 glucan vitamin E, and  $\beta$ -carotene and infected with white spot syndrome virus. *Latin American Journal of Aquatic Research* 39(3):534-543.
- Pan C. H., Chien Y. H., Hunter B., 2003 Alterations of antioxidant capacity and hepatopancreatic enzymes in *Penaeus monodon* (Fabricius) juveniles fed diets supplemented with astaxanthin and exposed to *Vibrio damsela* challenge. *Journal of the Fisheries Society of Taiwan* 30(4):279-290.
- Parilla-Taylor D. P., Zenteno-Savin T., Magallon-Barajas F. J., 2013 Antioxidant enzyme activity in Pacific whiteleg shrimp (*Litopenaeus vannamei*) in response to infection with white spot syndrome virus. *Aquaculture* 380-383:41-46.
- Pazos M., da Rocha A. P., Roepstorff P., Rogowska-Wrzesinska A., 2011 Fish proteins as targets of ferrous-catalyzed oxidation: identification of protein carbonyls by fluorescent labeling on two-dimensional gels and MALDI-TOF/TOF mass spectrometry. *Journal of Agricultural and Food Chemistry* 59(14):7962-7977.
- Peraza-Gómez V., Luna-González A., Campa-Córdova A., López-Meyer M., Fierro-Coronado A., Alvarez-Ruiz P., 2009 Probiotic microorganisms and antiviral plants reduce mortality and prevalence of WSSV in shrimp (*Litopenaeus vannamei*) cultured under laboratory conditions. *Aquaculture Resource* 40(13):1481-1489.
- Power A., Sheehan D., 1996 Seasonal variation in the antioxidant defense systems of gill and digestive gland of the blue mussel, *Mytilus edulis*. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* 114(2):99-103.
- Pradeep B., Rai P., Mohan S. A., Shekhar M. S., Karunasagar I., 2012 Biology, host range, pathogenesis and diagnosis of white spot syndrome virus. *Indian Journal of Virology* 23(2):161-174.
- Rahman M. M., Escobedo-Bonilla C. M., Corteel M., Dantas-Lima J. J., Wille M., Alday-Sanz V., Pensaert M. B., Sorgeloos P., Nauwynck H. J., 2006 Effect of high water temperature (33°C) on the clinical and virological outcome of experimental infections with white spot syndrome virus (WSSV) in specific pathogen-free (SPF) *Litopenaeus vannamei*. *Aquaculture* 261:842-849.

- Rajan P. R., Ramasamy P., Purusothaman V., Brennan G. P., 2000 White spot baculovirus syndrome in the Indian shrimp *Penaeus monodon* and *Penaeus indicus*. *Aquaculture* 184(1-2):31-44.
- Reznick A. Z., Packer L., 1994 Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods in Enzymology* 233:357-363.
- Rout N., Kumar S., Jaganmohan S., Murugan V., 2007 DNA vaccines encoding viral envelope proteins confer protective immunity against WSSV in black tiger shrimp. *Vaccine* 25(15):2778-2786.
- Sánchez-Paz A., 2010 White spot syndrome virus: an overview on an emergent concern. *Veterinary Research* 41:43.
- Schill R. O., Kohler H. R., 2004 Energy reserves and metal-storage granules in the hepatopancreas of *Oniscus asellus* and *Porcellio scaber* (Isopoda) from a metal gradient at Avonmouth, UK. *Ecotoxicology* 13:787-796.
- Searle A. J., Wilson R. L., 1980 Glutathione peroxidase: effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine* 37(2):213-217.
- Siddique Y. S., Ara G., Afzal M., 2012 Estimation of lipid peroxidation induced by hydrogen peroxide in cultured human lymphocytes. *Dose-Response* 10(1):1-10.
- Siddique T., Deng H. X., Ajroud-Driss S., 2013 Motor neuron disease. In: Emery and Rimoin's principles and practice of medical genetics. Sixth edition, Rimoin D., Pyeritz R., Korf B. (eds), Academic Press, pp. 1-22.
- Sriurairatana S., Boonyawiwat V., Gangnonngiw W., Laosutthipong C., Hiranchan J., Flegel T. W., 2014 White faeces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. *PLoS ONE* 9(6):e99170.
- Subramanian S., Philip R., 2013 Antioxidant defence profile of hepatopancreas, gill and muscle tissue of *Fenneropenaeus indicus* subjected to acute salinity change and WSSV challenge. *International Journal of Research in Zoology* 3(4):32-40.
- Trachootham D., Lu Wq., Ogasawara M. A., Rivera-Del Valle N., Huang P., 2008 Redox regulation of cell survival. *Antioxidants and Redox Signaling* 10(8):1343-1374.
- Takahashi Y., Itami T., Maeda M., Suzuki N., Kasornchandra J., Supamattaya K., Khongpradit R., Boonyaratpalin S., Kondo M., Kawai K., Kusda R., Hirono I., Aoki T., 1996 Polymerase chain reaction (PCR) amplification of bacilliform virus (RV-PJ) DNA in *Penaeus japonicus* bate and systemic ectodermal and mesodermal baculovirus (SEMBV) DNA in *Penaeus monodon* Fabricius. *Journal of Fish Diseases* 19(5):399-403.
- Walker P. J., Mohan C. V., 2009 Viral disease emergence in shrimp aquaculture: origins, impacts and the effectiveness of health management strategies. *Reviews in Aquaculture* 1:125-154.
- Walker P. J., Winton J. R., 2010 Emerging viral diseases of fish and shrimp. *Veterinary Research* 41(6):51.
- Wang K. C., Tseng C. W., Lin H. Y., Chen I. T., Chen Y. H., Chen Y. M., Chen T. Y., Yang H. L., 2010 RNAi knock-down of the *Litopenaeus vannamei* Toll gene (*LvToll*) significantly increases mortality and reduces bacterial clearance after challenge with *Vibrio harveyi*. *Developmental and Comparative Immunology* 34(1):49-58.
- Wang N., Meng M. X., Wang M. N., Meng X. L., 2017 Immunological responses of *Procambarus clarkii* to MAP30 and its efficacy to protect shrimp against white spot syndrome virus and *Vibrio Alginolyticus*. *Aquaculture and Marine Biology* 6(4):00160.
- Watanabe T., Shimbo S., Moon C. S., Zhang Z. W., Ikeda M., 1996 Cadmium contents in rice samples from various areas in the world. *Science of the Total Environment* 184(3):191-196.
- Weber D., Davies M. J., Grune T., 2015 Determination of protein carbonyls in plasma, cell extracts, tissue homogenates, isolated proteins: focus on sample preparation and derivatization conditions. *Redox Biology* 5:367-380.

Witteveldt J., Vlak J. M., Van Hulten M. C. W., 2004 Protection of *Penaeus monodon* against white spot syndrome virus using a WSSV subunit vaccine. *Fish and Shellfish Immunology* 16(5):571-579.

Received: 10 December 2019. Accepted: 31 January 2020. Published online: 06 March 2020.

Authors:

Ardiansyah, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: ardi\_kimsan@yahoo.com

Andi Asdar Jaya, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: andiasdar\_bdp@yahoo.com

Amrullah, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: ulla\_285@yahoo.com

Dahlia, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: unga-dahlia@yahoo.co.id

Mohamad A. Baiduri, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: mabaiduri@yahoo.co.id

Hartinah, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: tinatayibu@gmail.com

Wahidah, Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: ida\_wahidah@yahoo.co.id

Indrayani, Department of Aquatic Resource Management, Faculty of Fisheries and Marine, Haluoleo University, South East Sulawesi, Indonesia, e-mail: indrayani-tajudin@yahoo.com.au

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Ardiansyah, Jaya A. A., Amrullah, Dahlia, Baiduri M. A., Hartinah, Wahidah, Indrayani, 2020 Antioxidant status and oxidative stress markers of white faeces syndrome-infected Pacific white shrimp (*Litopenaeus vannamei* Boone). *AAFL Bioflux* 13(2): 503-517.