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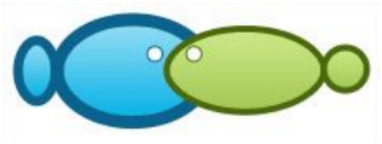

Antioxidant status and oxidative stress markers of white faeces syndrome-infected Pacific white shrimp (*Litopenaeus vannamei* Boone)

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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 (β-carotene, ascorbic acid, glutathione and α-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±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 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

Parameters	*Mean concentration*
Temperature (°C)	26.20±0.50
Dissolved oxygen (mg L^{-1})	7.24±0.16
pH	6.98±0.06
Ammonia (mg L^{-1})	0.13±0.01
Nitrate nitrogen (mg L^{-1})	1.60±0.02
Nitrite nitrogen (mg L^{-1})	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 & Ikbal (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_3. 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_2O_2 in a total

volume of 3 mL. Decomposition of H_2O_2 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 mL 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 mL 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 mL of 20% trichloroacetic acid (TCA) and washed with 5 mL of ethanol: ethyl acetate mixture (1:1). The protein pellet was dissolved in 2 mL 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 $U \cdot mg^{-1}$ 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 $O_2^{-}\cdot$. This suggests that the capacity of SOD to prevent cellular damage is decreased (Neves et al 2000).

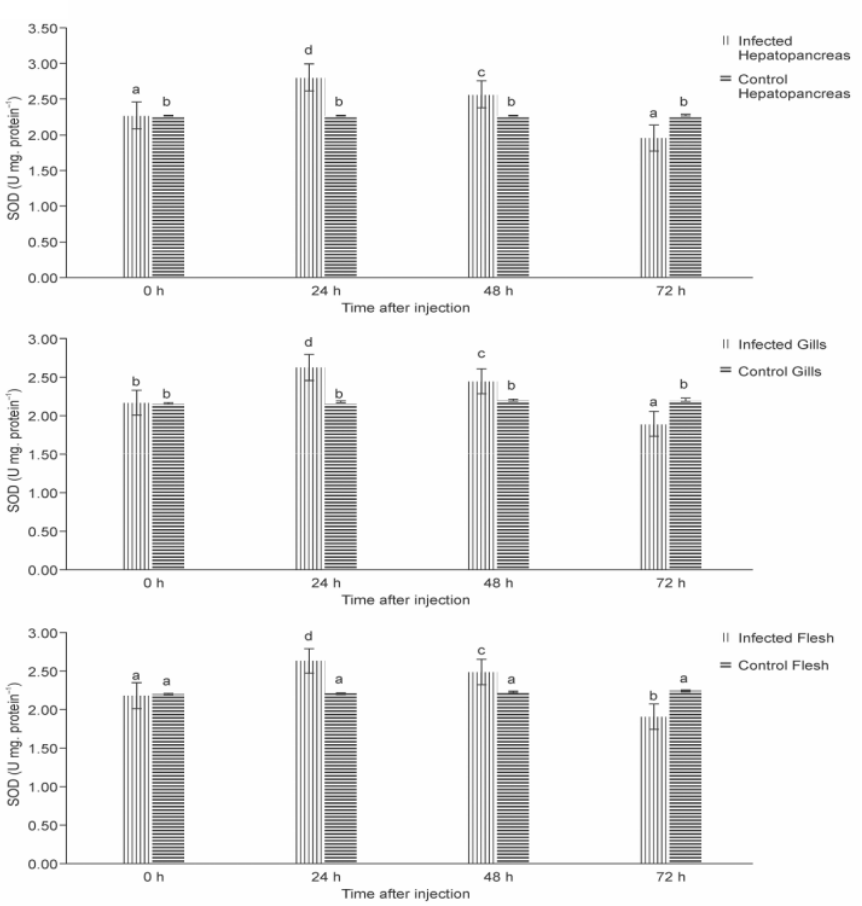

Figure 1. Variations in SOD activity in the hepatopancreas, gills, and flesh of WFS-infected *L. vannamei* and control shrimp. Same superscripts mean no significant differences ($p > 0.05$).

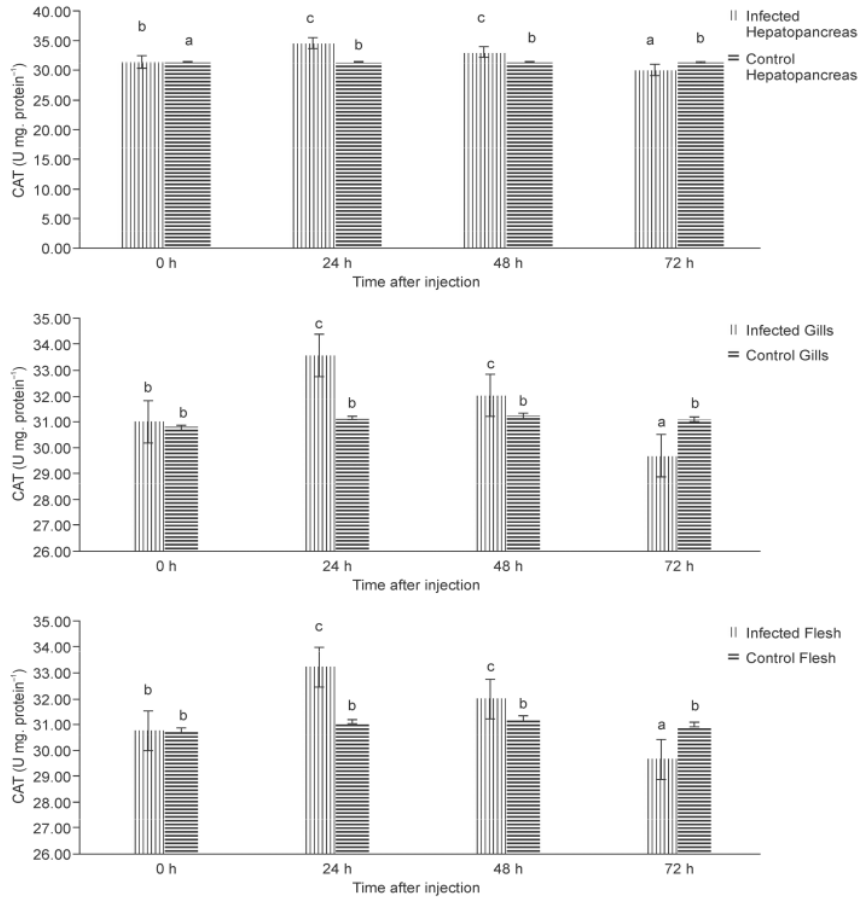

Figure 2. Variations in CAT activity in the hepatopancreas, gills, and flesh if WFS-infected *L. vannamei* and control shrimp. Same superscripts mean no significant differences ($p > 0.05$).

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^{-1} 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_2O_2 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 (1 O_2) and H_2O_2, which in turn form the hydroxyl radical (OH^-) 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.

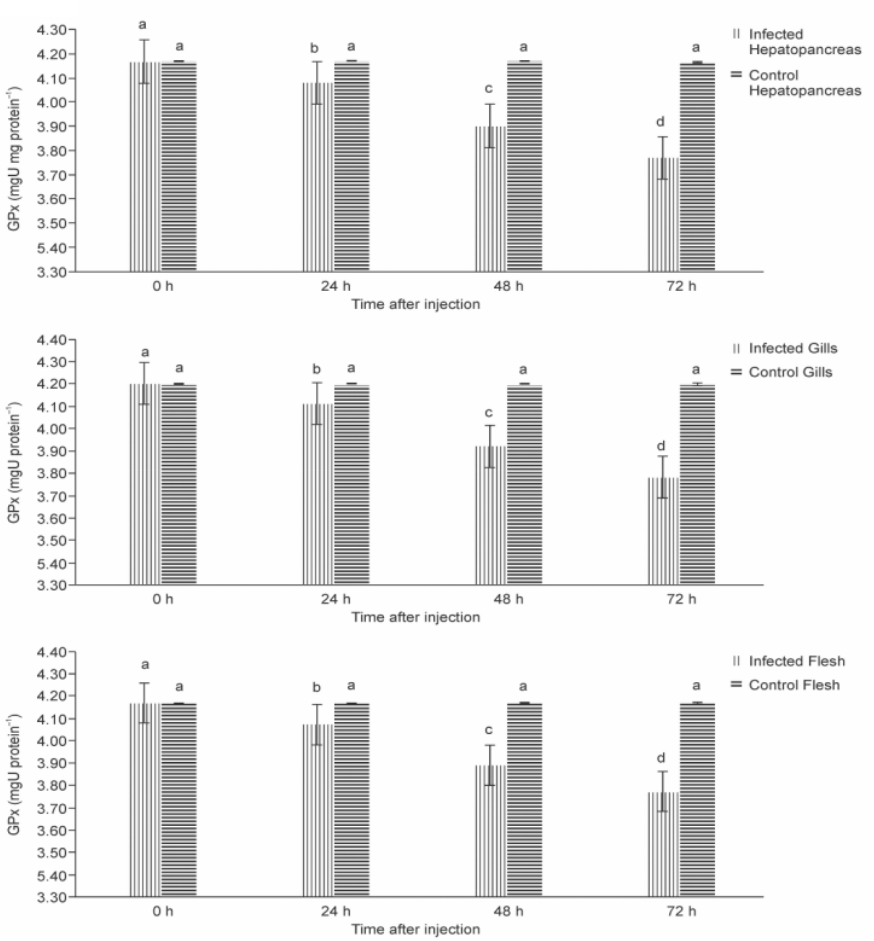

Figure 3. Variations in GSH-Px activity in the hepatopancreas, gills, and flesh of WFS-infected *L. vannamei* and control shrimp. Same superscripts mean no significant differences ($p > 0.05$).

TBARS is an indicator of oxidative damage in the hepatopancreas, gills, and flesh in infected and uninfected shrimp. Formation of TBARS (nmol MDA·mg^{-1} 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.

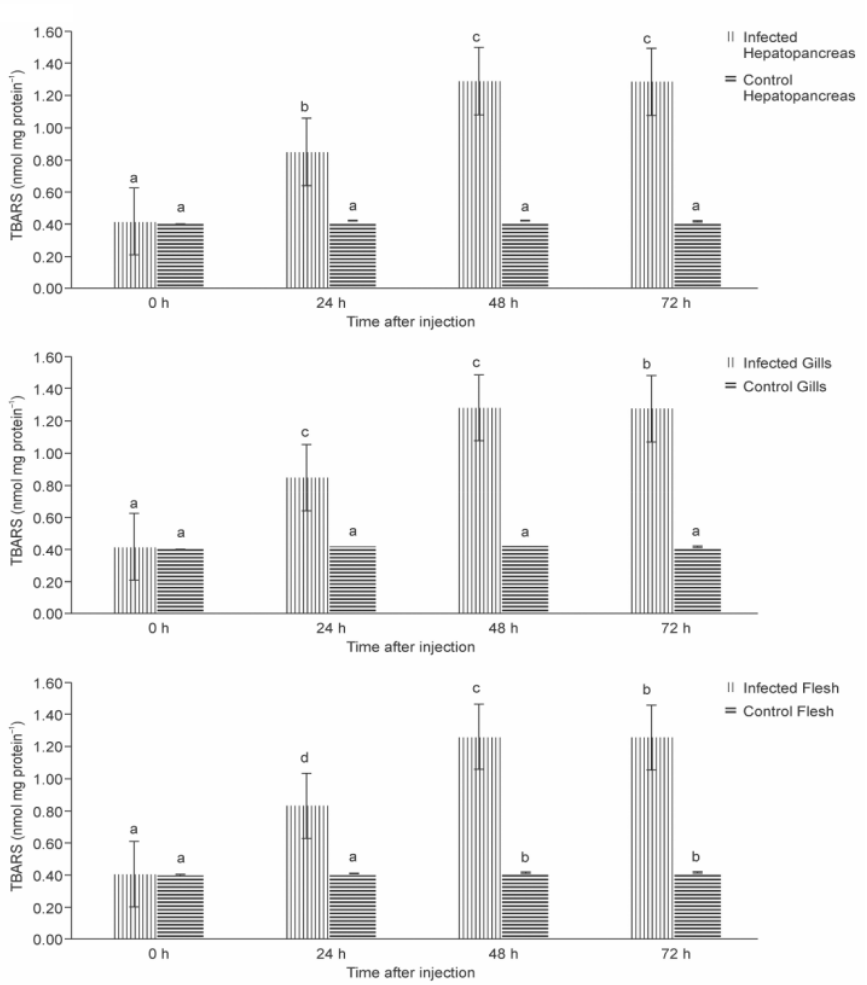

Figure 4. Formation of TBARS as an indicator of oxidative damage in response to WFS infection in the hepatopancreas, gills, and flesh of *L. vannamei*. Same superscripts mean no significant differences ($p > 0.05$).

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^{-1}. 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.

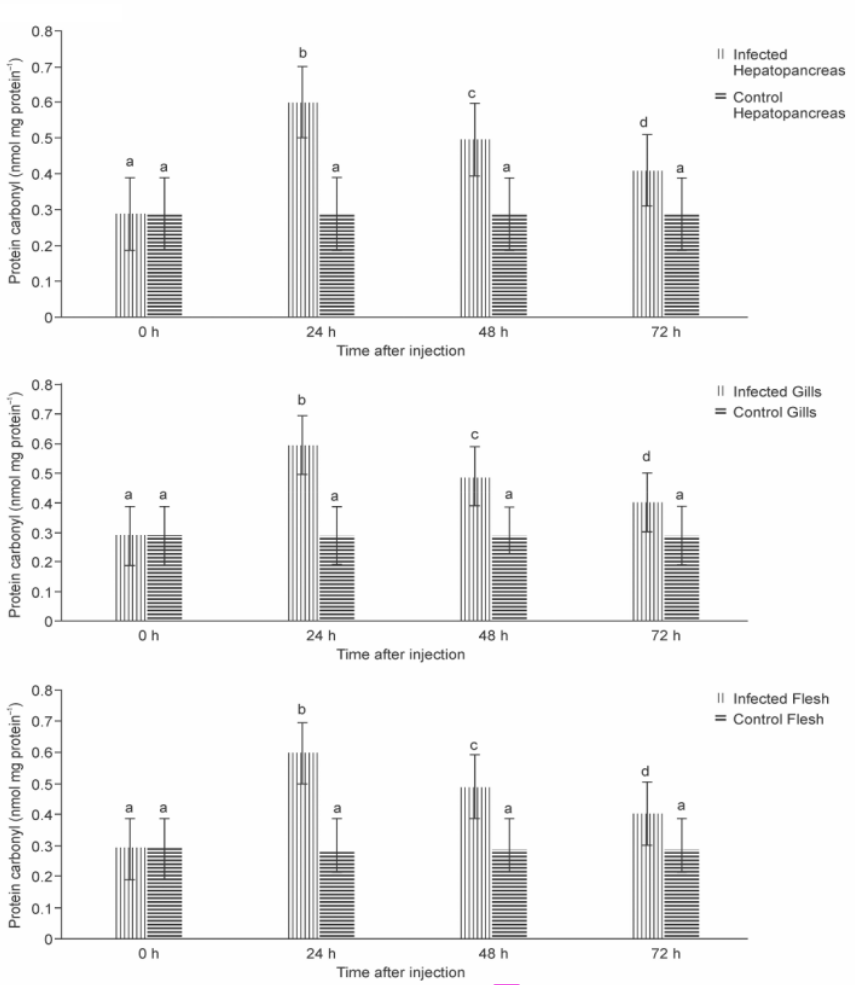

Figure 5. Formation of protein carbonyls in response to WSS infection in the hepatopancreas, gills, and flesh of *L. vannamei*. Same superscripts mean no significant differences ($p > 0.05$).

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 not 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.

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