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Full Length Research

The impact of heat stress on oxidative stress and histopathological dynamics in red hybrid tilapia experimentally infected with *Streptococcus agalactiae*

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ABSTRACT: The most common predisposing factor to Streptococcosis in the aquaculture industry is heat stress. The impact of heat stress on oxidative stress and histopathological changes in red tilapia with Streptococcosis is not well understood. This study aimed at evaluating the impact of heat stress on oxidative stress and histopathological dynamics in red hybrid tilapia experimentally infected with Streptococcus agalactiae. Apparently healthy 105 red hybrid tilapia (*Oreochromis nolyticus*) were used. The fish weighing 120 to 130 g were acclimatized for 2 days prior to onset of the experiment. The 105 tilapias were randomly separated to group 1, group 2 and group 3 of 35 fishes each in 200 litre tanks. Group 1 was inoculated with 0.1 mL of *S. agalactiae* at 10^9 CFU/mL intraperitoneally and subjected to heat stress (33°C). Group 2 was injected intraperitoneally with the same quantity of *S. agalactiae* in similar manner to group 1 without heat stress as positive control while group 3 served as negative control. Samples of blood was collected from five fish in every group before and at 6, 12, 24, 48, 72 h and 96 hours post-inoculation (pi) for lipid peroxidation [malondialdehyde (MDA)] and erythrocyte superoxide dismutase (SOD) analysis. The fish were necropsied and samples were taken from spleen, liver and kidney for microbiology and histopathology. Statistical analysis from the value of MDA and SOD revealed that there were significant differences ($p < 0.05$) between all the groups. Predominant lesions in the spleen were hemosiderin deposition, lymphoid depletion and haemorrhages. In the liver, main lesions were hepatocellular necrosis, mononuclear cell infiltration and haemorrhages while in the kidney, predominant histopathological lesions were mononuclear cell infiltration, necrosis, degeneration of tubular epithelial cells and haemorrhages. The lesions were significantly higher in spleen, liver and kidney of fish in group 1, followed by group 2 as compared to 3 which had no significant histological damage. Based on the findings of this study, heat stress alters significantly, the oxidative stress status and histopathology of Streptococcus infection in red hybrid tilapia.

Keywords: Heat, oxidative stress, red hybrid tilapia, Streptococcosis.

INTRODUCTION

Predominant predisposing factors to fish diseases leading to high mortality in aquaculture are physiological stress and stress induced by diseases (Rottmann et al., 1992; Nwunuji et al., 2013; Nadirah et al., 2016). Stress could be a physical and chemical factor that is capable of inducing body reactions which may influence the pathogenesis of a disease, consequently leading to high rate of death (Rottmann et al., 1992; Nwunuji et al., 2013; Nadirah et al., 2016). In Malaysia, the farming of red hybrid tilapia has become invaluable business in the aquaculture industry in view of its potential economic value with high international demand (Ng et al., 2013). However, numerous disease conditions in tilapia aquaculture have become threat to the industry and one of such common diseases is

Streptococcosis (Nadirah et al., 2016; Isiaku et al., 2017; Laith et al., 2017). Streptococcosis has been recognised as deleterious disease condition in view of the colossal loss resulting from mortality of adult fish as well as its massive negative impact on the economy (Ng et al., 2013; Laith et al., 2017). Streptococcus species are not only responsible for Streptococcus infections in diverse species of fish and mammals, they are also recognised as one of the main disease conditions resulting in substantial negative economic impact in the industry across the globe (Evans et al., 2002; Noraini et al., 2013).

Streptococcus agalactiae, which is a group b streptococcus, is pathogenic to a wide range of hosts, such as domestic and wild animals, terrestrial and aquatic animal species, not excluding human beings (Delannoy et al., 2013; Laith et al., 2017; Isiaku et al., 2017). Increased mortalities associated with Streptococcosis in tilapia have been linked to elevated water temperature (Mian et al., 2009; Rodkhum et al., 2011; Noraini et al., 2013). In the case of warm-water Streptococcosis associated with *S. agalactiae*, recent studies have demonstrated increased mortalities in fish owing to elevation in temperature of the water (Mian et al., 2009; Rodkhum et al., 2011; Nadirah et al., 2016).

Oxidative stress on the other hand is said to have occurred when there is a distortion in the balance between the generation of free oxygen radicals and the activities of antioxidants (Nadirah et al., 2016; Polycarp et al., 2017). Fish and other animals are usually protected from the adverse effect of reactive oxygen species by several defense mechanisms via antioxidant activity, one of which is superoxide dismutase (SOD) (Nadirah et al., 2016; Polycarp et al., 2020). Malondialdehyde and SOD are examples of biomarkers for oxidative stress (Nwunuji et al., 2014; Nadirah et al., 2016). Fluctuation in water temperature has been recognised as one of the environmental factors that is associated with stress in aquatic organisms especially fish (Mian et al., 2009; Nadirah et al., 2016). When fish are stressed, the level of production of harmful free radicals overwhelms that of the antioxidants, thereby leading to oxidative stress. One of such anti-oxidant enzyme is SOD, that catalyzes the breakdown of superoxide radicals to hydrogen peroxide (H_2O_2), a less harmful product (Nadirah et al., 2016). Although few studies have evaluated the effect of heat stress on fish diseases, inadequate information still exist with reference to how heat stress influences oxidative stress and histopathological changes in *Streptococcus agalactiae* infected red hybrid tilapia. The impact of heat stress and consequently oxidative stress on histopathological derangement in *Streptococcus agalactiae* infected red hybrid tilapia needs further investigation. Hence, this study aimed at the determination of the impact of heat stress and consequently oxidative stress on the histopathological changes in *Streptococcus agalactiae* infected red hybrid tilapia.

MATERIALS AND METHODS

Fish

The 105 red hybrid tilapia used in this study were procured from the Aquaculture Extension Centre, Department of Fishery Malaysia, located in Bukit Tinggi, Pahang. They were transferred and kept in a tank in the Pathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The tanks to be used for the study were thoroughly washed and cleansed. The fish weighing between 120 and 130 g were screened for *S. agalactiae* and thereafter divided into 3 groups of 35 fish each into 200 litre tanks. Aeration pumps were provided for all the tanks for continuous supply of oxygen and the fish were given commercial feed in the morning and evening.

Water quality

Water temperature, the pH as well as dissolved oxygen were taken every day in the morning and in the evening using YSI 556 (YSI, USA). The quantity of Nitrites, Sulphate and Ammonia were equally measured in the morning and in the evening every day using a Portable Spectrophotometer, DR 2800 (Hach, USA). The mean value of dissolved oxygen was 4.96 ± 0.2 mg L^{-1}, average temperature was 33.0 ± 0.3°C for the heat stressed group and 28.0 ± 0.3°C for the non-heat stressed groups, the average pH was 7.43 ± 0.2, Ammonia was 2.37 mg L^{-1} and mean nitrite concentration was 0.022 mg L^{-1}.

Culture and inoculum preparation

Streptococcus agalactiae stock which was isolated from a previous outbreak was utilised in this study. The bacterial stock was sub-cultured on Brain Heart infusion Agar and incubated in incubator for 18 hours at 37^0C. The colonies obtained were then inoculated into brain heart infusion broth (BHIB) and incubated at 37°C in shaker incubator for yet another 18 hours. Following incubation, the culture was centrifuged at 10,000 X *g* for 15 minutes. The clear fluid on top (supernatant) was decanted and the sediment was re-suspended in phosphate buffered saline (PBS) and centrifuged again as above. The process was repeated and the bacterial concentration of 10^9 CFU was then determine using McFarland standards. The bacterial concentration was retrospectively determined using brain heart infusion agar.

Study design and sample collection

Group 1 was inoculated intraperitoneally with 0.1 mL 10^9 CFU of *S. agalactiae* and heat stress at 33°C water

temperature. Group 2 was similarly inoculated with 0.1 mL 10^9 CFU of *S. agalactiae* but not subjected to heat stress to serve as positive control group while Group 3 was neither exposed to *S. agalactiae* nor heat stress to serve as negative control group. Blood samples were collected at 6 hours post challenge, 12, 24, 48, 72 and 96 hours post challenge. Records of mortalities and time were kept every day and organs such as the spleen, liver and kidney of the fish that died were cultured for isolation. At 96 hours pi, the remaining fish from each group were sacrificed and post mortem examination was done while tissue samples from the spleen, liver and kidney were collected for bacterial detection and histopathological evaluation.

Malondialdehyde

The plasma MDA levels were determined following the procedure earlier described by Nwunuji et al. (2014) as modified by Nadirah et al. (2016).

Superoxide dismutase

The erythrocyte superoxide dismutase (SOD) was determined as earlier described Nwunuji et al. (2014) and modified by Nadirah et al. (2016).

Histopathology

The fish were sacrificed periodically as stated earlier and spleen, liver and kidney tissue samples were removed, fixed in 10% formalin. The fixed tissues were routinely processed and serially sectioned at 4 to 5 µM. Slides obtained thereof were stained following hematoxylin and eosin (H&E) staining procedure. Histopathological lesions from the spleen, liver and kidney were examined under compound microscope and significant histopathological lesions were captured as photomicrographs.

PCR

To confirm the presence of *S. agalactiae*, DNA was extracted using Promega DNA Purification Kit (Promega, USA), following the protocol provided by the manufacturer. The blood samples as well as colonies obtained from culture of the organs were evaluated using PCR. *Streptococcus agalactiae* specific primers STAUR 4 [ACGGAGTTACAAAGGACGAC] and STAUR 6 [AGCTCAGCCTTAACGAGTAC] were used, under the following cycling conditions; 1 cycle at 94°C for 4 minutes, followed by 34 cycles at 94°C for 1 minute, 52°C for 1 minute, 72°C for 1 minute and elongation for 10 minutes at 72°C as earlier described Noraini et al. (2013).

Statistical analysis

All data were recorded as mean plus or minus standard deviation. One-way analysis of variance (ANOVA) was done using MedCalc software, version 12.4.0.0 (MedCalc, Mariakerke, Belgium) and all p-values of less than 0.05 were considered statistically significant.

RESULTS

Clinical signs

The clinical signs observed following the inoculation and subsequent subjection to heat stress in group 1 include erratic swimming, trailing faeces (Figure 1), exophthalmia and distended abdomen. Similar signs were observed in group 2 but with lesser severity. Cumulative mortality of 20% was observed in group 1 at 72 h pi while no mortality was recorded from group 2 at 72 h pi. There was neither manifestation of clinical signs nor mortality in group 3 throughout the study period.

Mean MDA levels of all groups from zero hours to 96 hours Post inoculation.

The mean MDA levels for the three groups from zero hour to 96 h pi is shown in figure 2. From the figure, the MDA level of group 1 increased but less significantly compared to group 3 from zero hour through 12th hour pi after which it declined to normal. However, the MDA level rise again significantly above groups 2 and 3 from 48 h pi through 96 h pi. The MDA level of group 2 on the other hand, rose from zero hours to its peak at 6 h pi which was significantly higher compared to groups 1 and 3 and thereafter the MDA level declined steadily back to normal level at 48 h pi and later increased again towards the 96th hour pi. The MDA level of group 3 did not show any significant alteration from zero hour to 96 h pi.

Mean SOD levels for the three groups from zero to 96 hours post inoculation

The mean SOD levels obtained from each of the group during the study is presented in Figure 3. Based on the graph of mean values, the SOD activities in group 1 decreased significantly compared to group 3 from zero hour through the 6th hour pi where the least SOD activity was recorded for the group. Thereafter, the SOD activity of group 1 remained significantly below normal through 96th hour pi where the SOD activity rose to values within normal range. The SOD activity of group 2 followed similar trend as that of group 1 even though lower compared to groups 1 and 3. The SOD activity for this group however,

Figure 1. Picture of red hybrid tilapia inside the glass aquarium in group 1 showing trailing faeces following *S. agalactiae* infection.

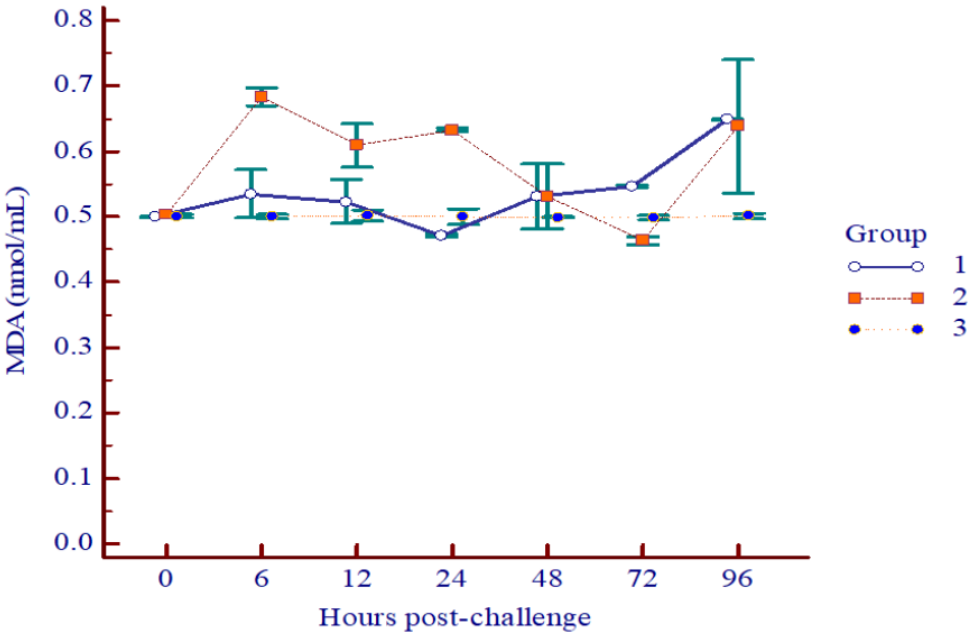

Figure 2. Mean MDA levels for the groups from zero to 96 hours post inoculation showing increased levels of MDA, with group B having the highest between zero to 48 hours pi while group 1 MDA level was higher from 48 to 96 hours pi.

increased to values within normal range at 72 h pi. There was no significant alteration in SOD activity in group 3 throughout the study period.

Bacterial isolation and confirmation through PCR

The details of the percentages of positivity of the sampled

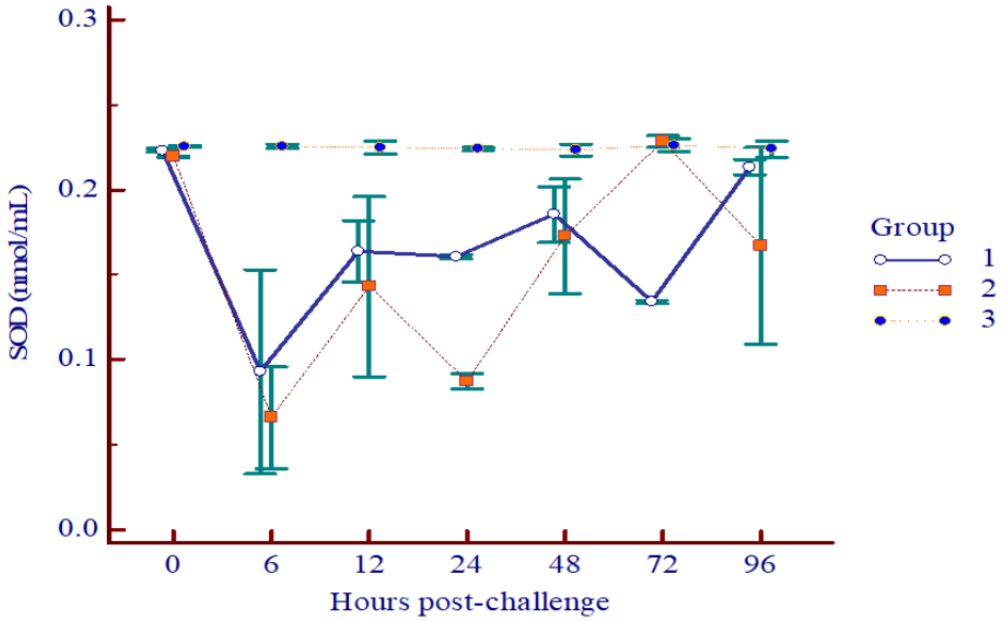

Figure 3. Mean SOD activities for the groups from zero hour to 96 hours post inoculation, showing significantly reduced SOD activities from groups A and B, with group B having lowest SOD activities from zero hour to 96 hours post inoculation.

Table 1. Percentage of isolation of *S. agalactiae* from the Spleen, Liver and the kidney

Groups	Hours post-infection	6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours
		(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
	Spleen	100%	100%	100%	50%	50%	40%
Group 1	Liver	80%	100%	100%	50%	100%	40%
	Kidney	100%	100%	100%	50%	100%	20%
	Spleen	100%	100%	50%	40%	40%	60%
Group 2	Liver	100%	100%	75%	40%	40%	40%
	Kidney	100%	100%	75%	40%	40%	60%
	Spleen	0%	0%	0%	0%	0%	0%
Group 3	Liver	0%	0%	0%	0%	0%	0%
	Kidney	0%	0%	0%	0%	0%	0%

organs (spleen, liver and kidney) to *S. agalactiae* is shown in Table 1. Almost all the isolates from spleen, liver and kidney were presumptive of Streptococcal spp. The isolates were all confirmed by PCR to be *S. agalactiae*. The bacterium was not detected in any organ in group 3 throughout the study period. The generality of the result showed that higher percentage of the organs in group 1 were positive for *S. agalactiae* compared to groups 2 and 3. All sampled organs in group 1 were positive for *S. agalactiae* at 12 h and 24 h pi

Gross lesions at post mortem

Lesions observed during the post mortem examination were opaque eye, exophthalmia and haemorrhages. Haemorrhagic areas were also seen on the head, particularly around the mouth, operculum and fins.

Histopathology

The histopathological changes observed were captured in photomicrographs and are presented in Figures 4 to 15. The lesions were generally more severe in groups 1 and 2 compared to group 3 which was the control group and most severe lesions were from group 1 followed by group 2. Predominant histopathological lesions in the spleen of group 1 included moderate to severe hemosiderin

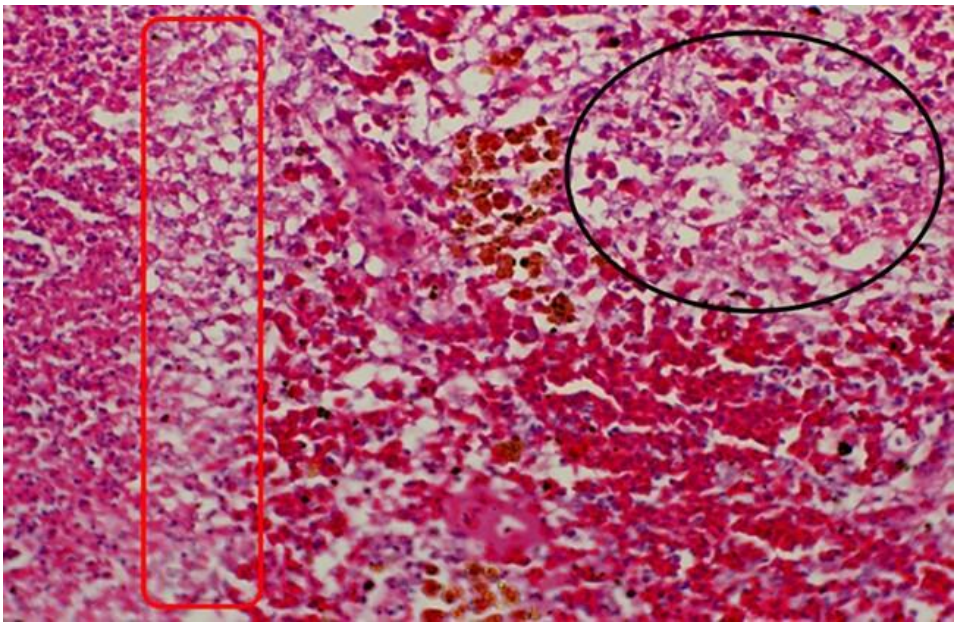

Figure 4. Representative photomicrograph of the spleen of group 1 at 24 hours pi showing moderate hemosiderin deposition, moderate haemorrhages, moderate vacuolation (area covered by rectangle) and depletion of lymphocytes (area covered by circle).

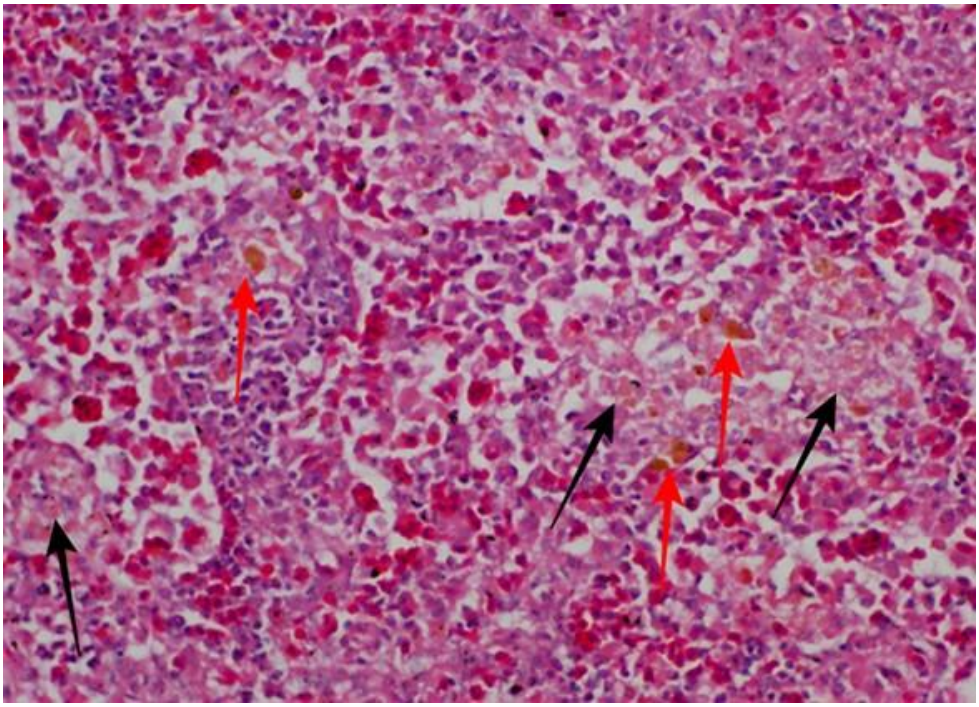

Figure 5. Representative photomicrograph of the spleen of group 1 at 96 hours pi showing moderate hemosiderin deposition (red arrows), necrosis and depletion of lymphocytes (black arrows), severe haemorrhages.

deposition, moderate to severe haemorrhages, moderate to marked depletion of lymphocytes, vacuolation and mild necrosis (Figures 4 to 7). In the liver, most common lesions were mild to severe hepatocellular necrosis, marked to focal mononuclear cell infiltration, moderate to severe haemorrhages, pyknosis and moderate vacuolation

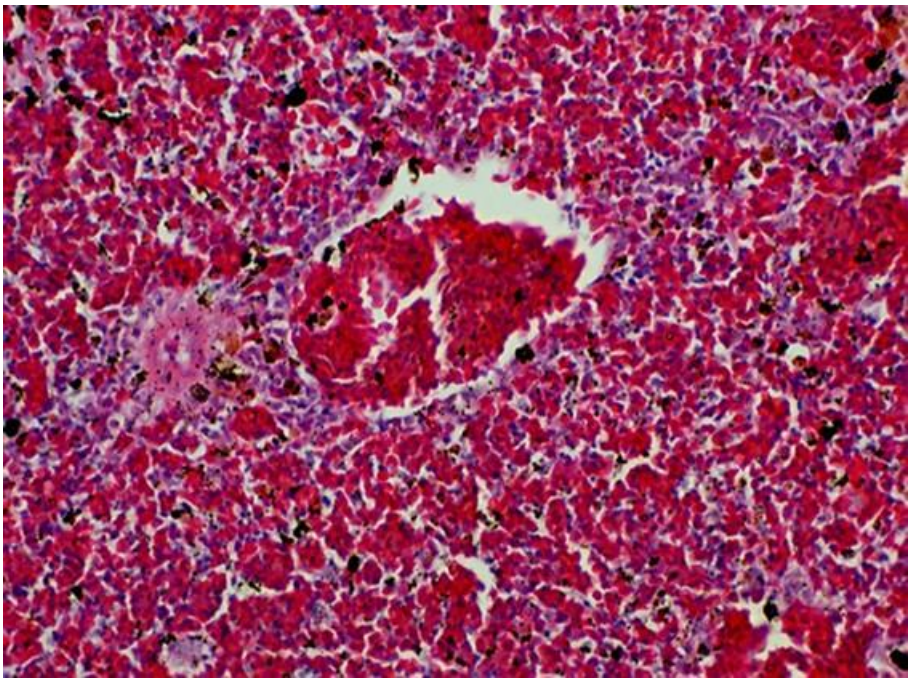

Figure 6. Representative photomicrograph of the spleen of group 2 at 24 hours pi showing marked hemosiderin deposition, severe haemorrhages

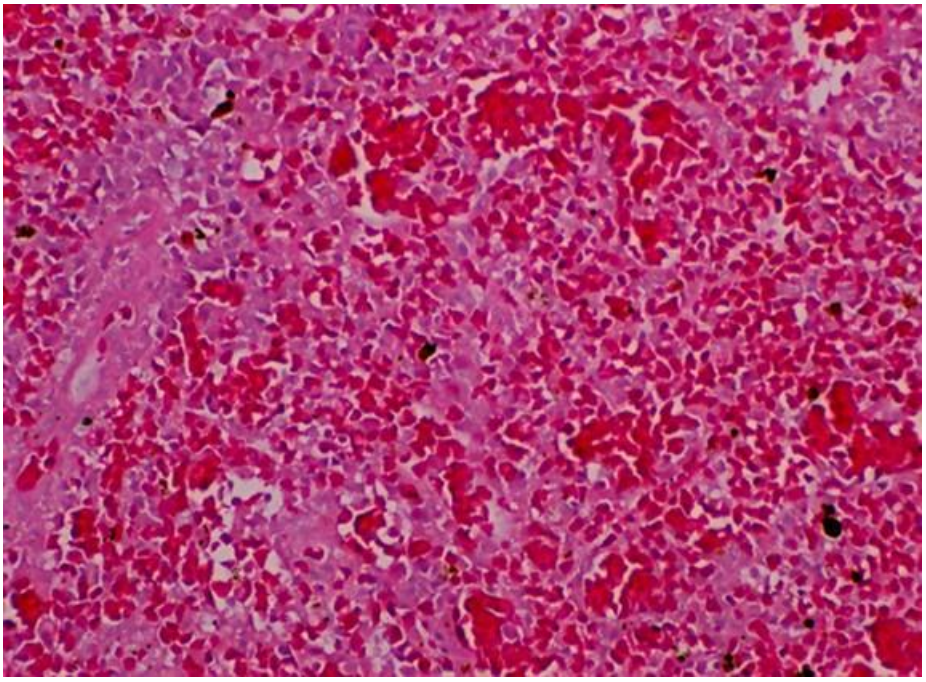

Figure 7. Representative photomicrograph of the spleen of group 1 at 96 hours pi showing severe haemorrhages, marked depletion of lymphocytes and obliteration of the sinusoids

(Figures 8 to 11). In the kidney however, predominant lesions were moderate to marked mononuclear cell infiltration, severe degeneration of the tubular epithelial cells, necrosis, vacuolation of tubular epithelial cells, moderate to severe haemorrhages, mild congestion of renal capillaries, glomerular atrophy and disintegrated

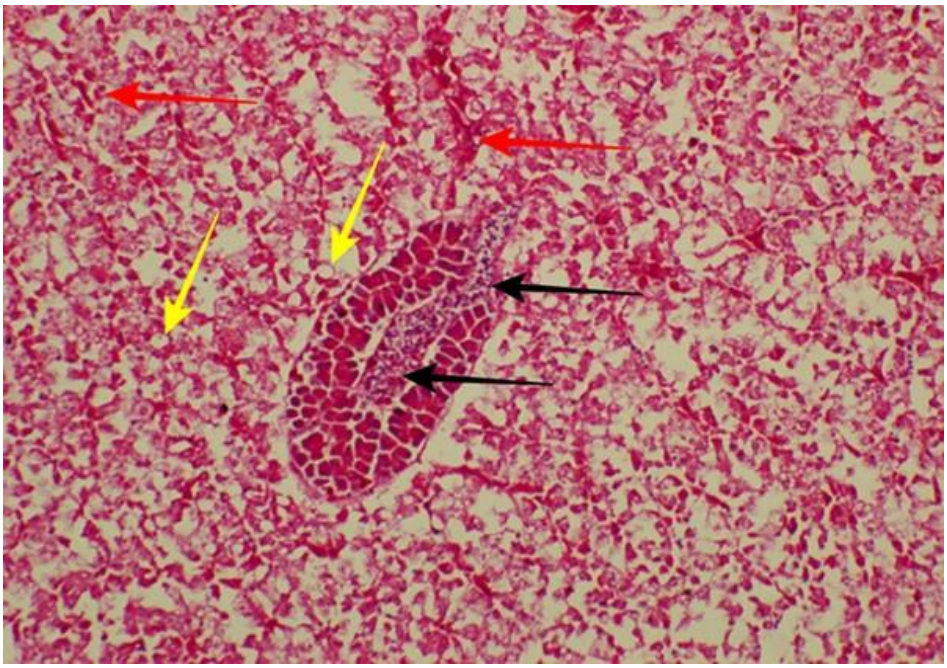

Figure 8: Representative photomicrograph of the liver of group 1 at 24 hours pi showing severe hepatocellular necrosis, marked mononuclear cell infiltration of the hepatopancreas (black arrows), haemorrhages (red arrow) and moderate vacuolation (yellow arrows).

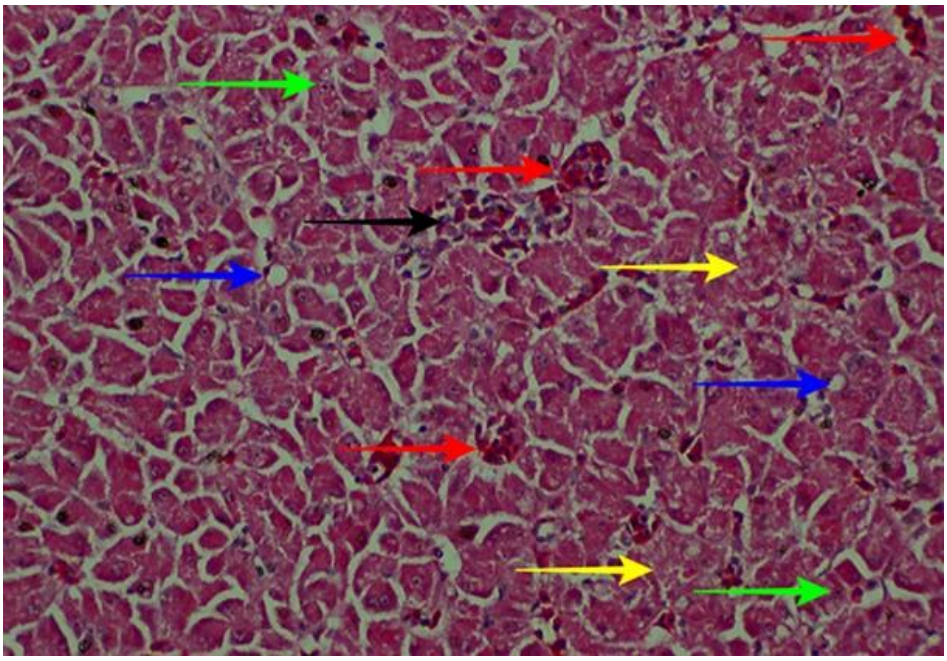

Figure 9. Representative photomicrograph of the liver of group 1 at 96 hours pi showing hepatocellular necrosis (yellow arrows), focal mononuclear cell infiltration (black arrow), haemorrhages (red arrows), pyknosis (green arrows) and moderate vacuolation (blue arrows).

filtration membrane (Figures 12 to 15). These lesions were less at 6 to 12 h pi and become significantly more from 48 h pi and most severe at the 72nd hours pi.

DISCUSSION

Streptococcus agalactiae has been reported to be very

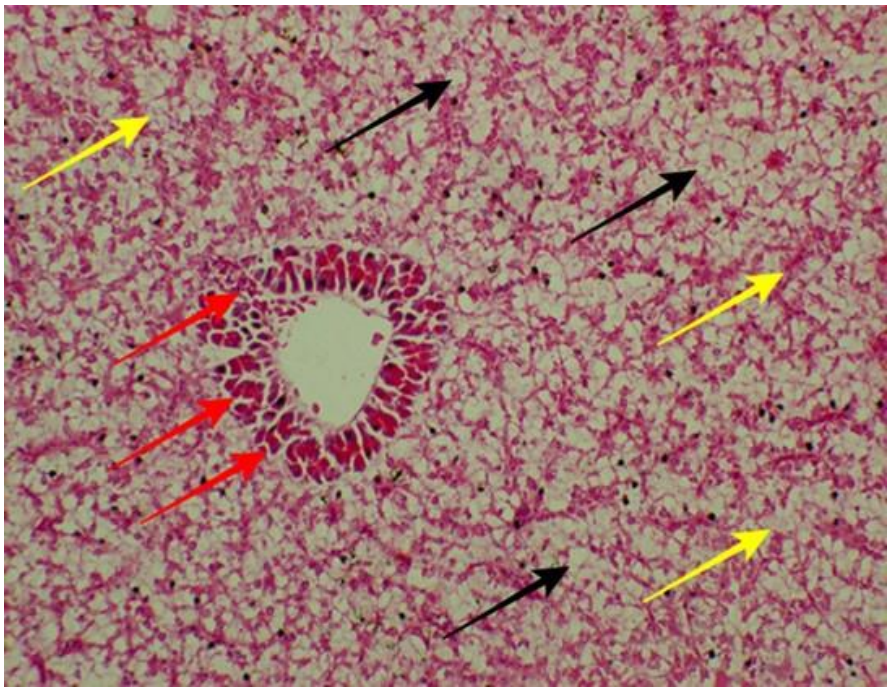

Figure 10. Representative photomicrograph of the liver of group 2 at 24 hours pi showing severe hepatocellular necrosis (yellow arrows), marked vacuolation (black arrows) and congested hepatopancreas (red arrows).

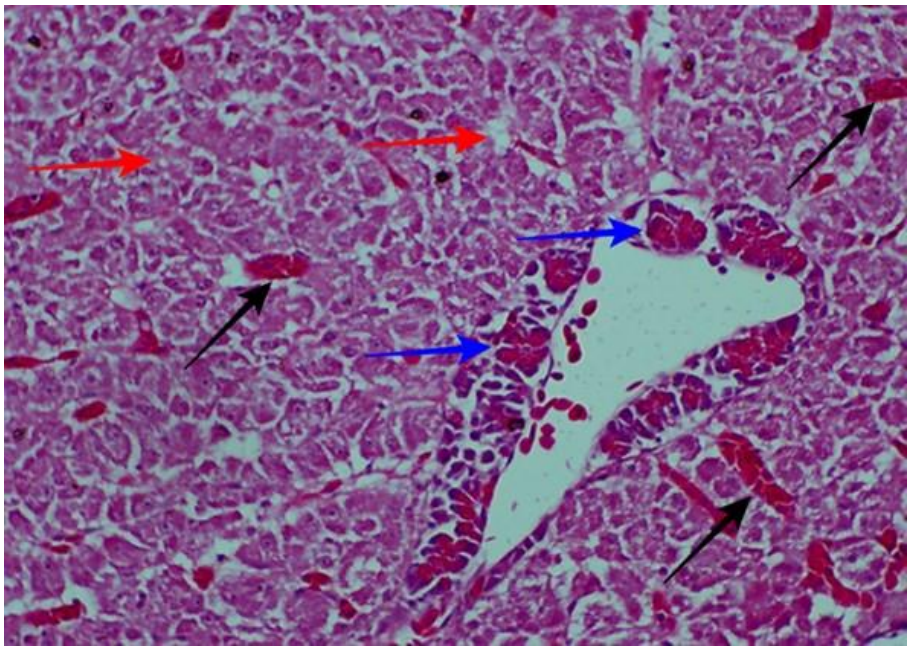

Figure 11. Representative photomicrograph of the liver of group 2 at 96 hours pi showing marked congestion of hepatic veins (black arrows), congested hepatopancreas (blue arrows) and necrosis (red arrows).

pathogenic to red hybrid tilapia with very high mortalities in experimental settings and during outbreaks. Outbreaks of the disease are reported frequently, most especially during the hot seasons when the water temperature is usually elevated (Mian et al., 2009: Rodkhum et al., 2011; Noraini et al., 2013). In this study, the fish were observed to be

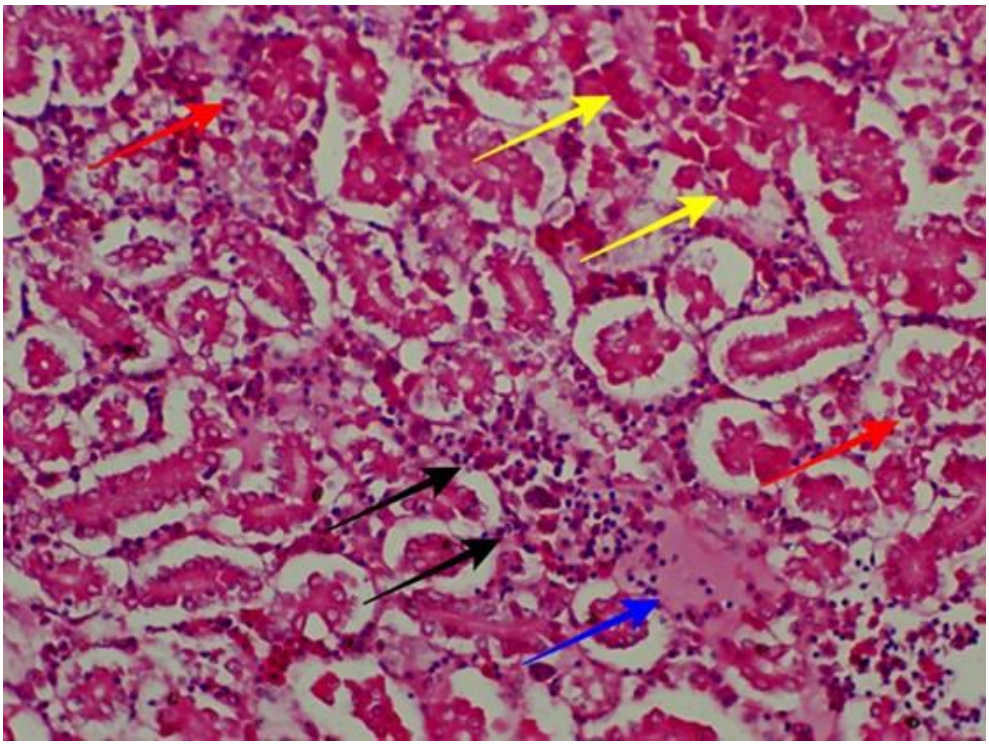

Figure 12. Representative photomicrograph of the kidney of group 1 at 24 hours pi showing marked mononuclear cell infiltration (black arrows), severe necrosis and degeneration of tubular epithelial cells (red arrows), moderate haemorrhages (yellow arrows) and focal deposition of proteinaceous materials (blue arrows).

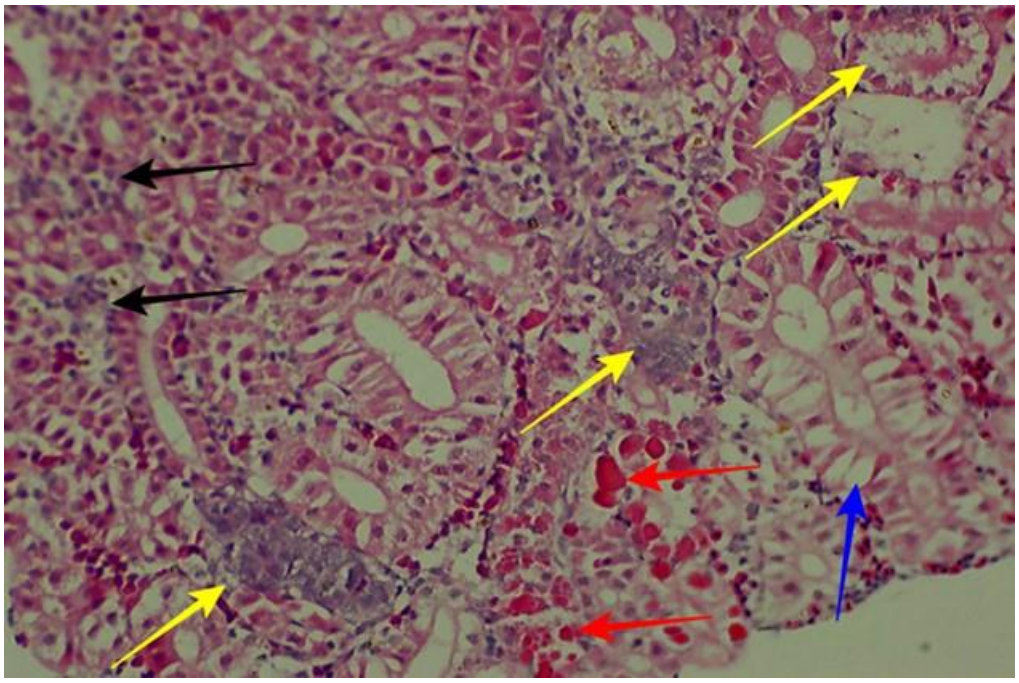

Figure 13. Representative photomicrograph of the kidney of group 1 at 96 hours pi showing moderate mononuclear cell infiltration (black arrows), severe necrosis and degeneration of tubular epithelial cells (yellow arrows), vacuolation of tubular epithelial cells (blue arrow) and moderate haemorrhages (red arrows).

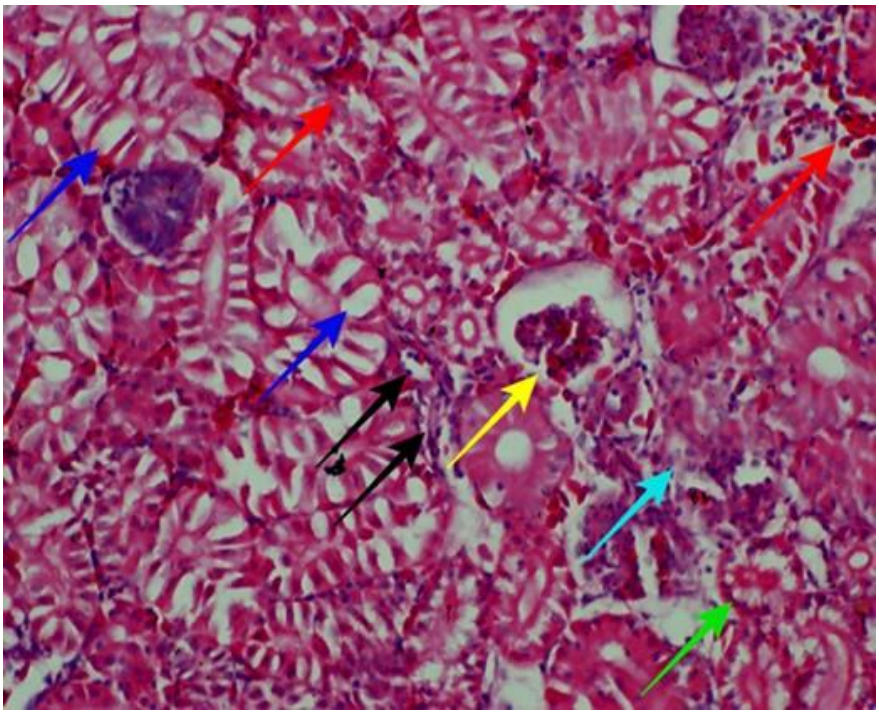

Figure 14. Representative photomicrograph of the kidney of group 2 at 24 hours pi showing moderate mononuclear cell infiltration (black arrows), degenerated tubular epithelial cells (green arrow), necrosis (light blue arrow), vacuolation of tubular epithelial cells (blue arrows), moderate haemorrhages (red arrows), mild congestion of renal capillaries, glomerular atrophy and disintegrated filtration membrane (yellow arrow).

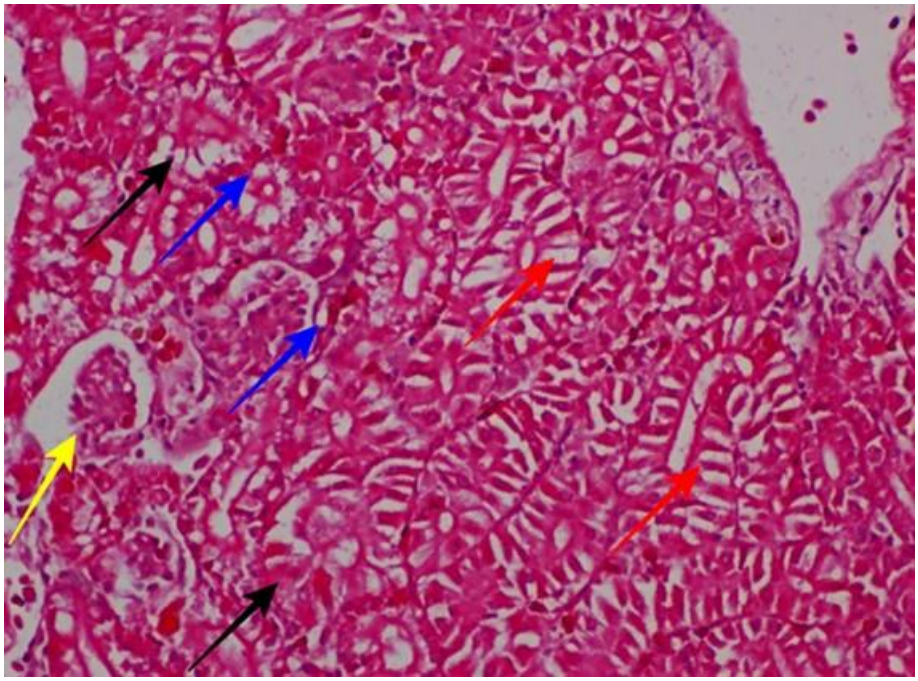

Figure 15. Representative photomicrograph of the kidney of group 2 at 96 hours pi showing degenerated and necrotic tubular epithelial cells (black arrows), vacuolation of tubular epithelial cells (red arrows), moderate haemorrhages (blue arrows) and glomerular atrophy (yellow arrow).

swimming in an erratic manner, with trailing faeces, exophthalmia and distended abdomen following the inoculation of the fish with *S. agalactiae.* These observed clinical signs are not different from the clinical signs reported in other related studies (Mian et al., 2009; Rattanachaikunsopon and phumkhachorn, 2009; Isiaku et al., 2017). It was observed that the severity of clinical signs were significantly higher in group 1 compared to the mild clinical manifestation observed in group 2 and this observed difference in the severity of clinical signs associated with the disease could be attributed to the heat-induced stress resulting from the elevated water temperature as similarly observed by other scholars (Mian et al., 2009; Rodkhum et al., 2011; Nadirah et al., 2016) in different experimental studies where severe clinical signs were noted on red hybrid tilapia subjected to heat-induced stress. In several other studies (Bromage and Owens, 2009; Mian et al., 2009: Rodkhum et al., 2011), severe clinical signs and increased mortality were reported in tilapia subjected to heat-induced stress following *S. agalactiae* infection as similarly observed in this study. These observed difference in disease severity associated with heat-induced stress perhaps explains the role of stress in exacerbating pathogenicity of *Streptococcus agalactiae* in red hybrid tilapia.

Under normal physiological conditions, fish have adequate antioxidant defense system, which employs both enzymatic as well as non-enzymatic mechanisms to moderate the effects of oxidants, thus maintaining homeostasis. Oxidative stress is said to have occurred when the activity of these antioxidant defense systems decreases or the production of oxidants is increased (Valavanidis et al., 2006; Nwunuji et al., 2014). Lipid peroxidation determined via Malondialdehyde has been recognised and used as a biomarker for oxidative stress in fish (Prieto et al., 2007; Nadirah et al., 2016). In this study, the levels of lipid peroxidation (MDA) of group 1 increased above that of the control group (group 3) but less significantly compared to group 2 from zero hour through 12th hour pi after which it declined to normal. This is contrary to the observation of Nadirah et al. (2016) who reported that the lipid peroxidation for the heat stressed group increased significantly following bacterial inoculation and heat-induced stress. The real mechanism behind the insignificant increase in lipid peroxidation of the heat stressed group could only be speculated to be due to stress response dynamics of this group of red hybrid tilapia as documented by Lenartova et al. (1997), that fish tend to adapt to oxidative conditions to which they are exposed. It could be as a result of the gradual rise in the water temperature in which case there was attempt to adjust systemically by producing more antioxidants (SOD) to break down the reactive oxygen metabolites generated. However, the lipid peroxidation rises again significantly above groups 2 and group 3 from 48 h pi through 96 h pi. This later significant increase in lipid peroxidation from 48 h pi through 96 h could be attributed to the heat-induced stress which exacerbated the pathogenicity of the Streptococcus infection. It could be said that at this point the antioxidant defense system of the fish was overwhelmed following several hours of heat-induced stress, hence the rise in lipid peroxidation level. This corroborated with the mortality observed at 72 h pi in group 1 compared to group 2 which had no mortality. These findings of elevated lipid peroxidation level in the heat stressed fish is in accord with results obtained in an earlier related study (Nadirah et al., 2016). The level of lipid peroxidation of group 2 on the other hand, was observed to increase significantly at 6 h and 96 h pi, implying infection with *S. agalactiae* is also associated with increased lipid peroxidation and consequently oxidative stress in red hybrid tilapia. This also corroborate with the findings in other studies (Prieto et al., 2007; Nadirah et al., 2016), where stress was linked with increase in lipid peroxidation.

Antioxidant assays such as the determination of SOD has been used by several scholars as biomarker for the determination of the response of fish to stress (Prieto et al., 2007; Nadirah et al., 2016). Superoxide dismutase enzyme has an antioxidant activity that ensures balance between reactive oxygen species production and antioxidant activity (Parihar et al., 1997; Tanko et al., 2019). In this study, SOD activities in group 1 decreased significantly compared to group 3 from zero hour through the 6th hour pi where the least SOD activity was recorded for the group. The decrease in SOD activities in the heat stressed group compared to group 3 could be attributed to the stress induced by the heat stress, during which the production of oxidant possibly overwhelmed the activities of the antioxidant enzymes and other antioxidant mechanisms, thereby resulting in oxidative stress in the fish. Similar findings have been reported in related studies where heat stress was linked to oxidative stress in tilapia (Prieto et al., 2007; Nadirah et al., 2016). The SOD activity of group 2 followed similar trend as that of group 1 even though lower compared to group 3. This is also in tandem with findings in other related studies that reported less significant decrease in SOD in non-heat stressed group compared to heat-stressed group (Prieto et al., 2007; Nadirah et al., 2016). The SOD activity for this group however, increased to values within normal range at 72 h pi. The increase in the value of SOD to normal range toward 96 h pi in both groups 1 and 2 implies recovery from the stress induced by both heat stress as well as stress induced by *S. agalactiae* infection and this was similarly suggested in other studies in both ruminants as well as red hybrid tilapia (Nwunuji et al., 2014; Nadirah et al., 2016).

Outbreaks of *Streptococcus agalactiae* infections has been linked to elevated water temperature and it does frequently result in huge mortalities and substantial impact on commercial fish farm industries globally (Musa et al., 2009; Rodkhum et al., 2011; Noraini et al., 2013). These bacteria have been isolated in different organs of both naturally and experimentally infected tilapia (Hernandez et

al., 2009; Rattanachaikunsopon and phumkhachorn, 2009; Nadirah et al., 2016). In this study, the kidney, liver and spleen tissues were positive for the bacterium in both group 1 and group 2 with higher percentage from group 1 using PCR technique. The isolation of the bacteria in these organs of infected red hybrid tilapia corroborates with reports in other related studies (Musa et al., 2009; Rattanachaikunsopon and phumkhachorn, 2009; Abuseliana, 2011) where *Streptococcus agalactiae* was isolated in different organs of infected tilapia. The detection of higher percentage of the bacterium in group 1 as compared to group 2 could only be attributed to the effects of heat-induced stress via the elevated water temperature which is normally associated with outbreaks of *Streptococcus agalactiae* infection (Mian et al., 2009; Rodkhum et al., 2011; Nadirah et al., 2016).

Most studies on histopathological lesions associated with *S. agalactiae* infection in red tilapia focused more on the pathology of the brain, the eye and the gills without much interest on the spleen, liver and kidney (Hernandez et al., 2009; Noraini et al., 2013; Isiaku et al., 2017). The current experiment focused more on the spleen, liver and the kidney and predominant lesions observed in the spleen were hemosiderin deposition, necrosis and depletion of lymphocytes and haemorrhages of varying severity, with highest severity in group 1 compared to group 2. These observed histopathological lesions are not significantly different from those observed in an earlier study (Laith et al., 2017) while the increased or higher severity of lesions in group 1 could be stress-induced (Noraini et al., 2013; Mian et al., 2009).

The liver is seldom used in pathological studies of *S. agalactiae* infection in tilapia even though significant pathological damages could be induced by the bacteria (Noraini et al., 2013; Isiaku et al., 2017). In this investigation, hepatocellular necrosis, vacuolation, congestion and mononuclear cell infiltration were observed and the manifestation of these lesions could only be induced by the *S. agalactiae* seeing that the control group (group 3) did not manifest any of those lesions. These observed lesions corroborate with the lesions observed in the liver of *S. agalactiae*-infected red tilapia in earlier studies (Evans et al., 2002; Abuseliana, 2011; Laith et al., 2017). However, it was noted that the severity of the lesions was higher in group 1 compared to group 2 and the difference could be linked to the heat-induced stress (Rodkhum et al., 2011; Noraini et al., 2013; Mian et al., 2009). The marked infiltration of the kidney by mononuclear cells, necrosis, vacuolation of tubular epithelial cells, degeneration of tubular epithelial cells and haemorrhage observed in this study could be attributed to the *S. agalactiae* infection as similarly reported in earlier studies on *S. agalactiae* infection in red tilapia (Evans et al., 2002; Abuseliana, 2011; Laith et al., 2017). Furthermore, other lesions such as glomerular atrophy, congestion of renal capillaries and disintegration of the filtration membrane observed in this study were not reported in earlier studies. The observed higher severity of the lesions in group 1 as compared to group 2 revealed the impact of heat-induced stress on the pathology of *S. agalactiae* in red hybrid tilapia. This is in agreement with findings by other scholars on *S. agalactiae* in red hybrid tilapia (Mian et al., 2009; Rodkhum et al., 2011; Noraini et al., 2013).

Conclusion

This study demonstrated the impact of heat-induced stress on oxidative stress and the pathology of *S. agalactiae* in red hybrid tilapia. Heat-induced stress was demonstrated in this study to have exacerbated the impact of *S. agalactiae* on red hybrid tilapia with highest impact around 72 h pi where mortality was recorded only in the heat-stressed group. Based on these findings, it was then concluded that heat-induced stress has the capacity to exacerbate oxidative stress in red hybrid tilapia in addition to enhancing the pathology of the disease. Other parameters of stress such as hematological parameters and antioxidants such as glutathione, catalase and biomarkers of stress such as heat shock protein should also be investigated in future studies

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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