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Effects of Surfactants on Biological, Physiological, and Histological Performance of Mahseer Seeds, *Neolissochilus soro*

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ABSTRACT

Mahseer (*Neolissochilus soro*) grows and develops in Indonesian lakes and rivers. However, in line with the increase in population, the pollution load that enters water bodies is increasing due to anthropogenic activities along the river. One of the chemicals that can potentially be a source of water pollution is Linear Alkylbenzene Sulfonate (LAS). This study aims to determine the lethal toxicity of LAS surfactant to mahseer and to analyze the sub-lethal effect of this surfactant on the biological, physiological, and histological conditions of mahseer seeds. Parameters studied included lethal toxicity (LC_{50}), sub-lethal toxicity, and biological, histological, and hematological conditions. The results showed that the LAS surfactant had an LC_{50}-96 hour value of 6.14 mg/L in mahseer and was classified as highly toxic. Fish exposed to LAS for 40 days experienced a decrease in specific growth rate and feed efficiency as the surfactant concentration increased. Exposure to LAS also decreases the number of erythrocytes, hemoglobin, hematocrit, and blood sugar levels while increasing the number of leukocytes. There was also damage to the gills of fish exposed to LAS. In general, the sub-lethal concentration of LAS negatively affected mahseer seeds.

1. Introduction

Mahseer (*Neolissochilus soro*) is a freshwater fish distributed in several areas in Indonesia, such as Sumatra and Java. Previously, this fish was described as the Tor soro species. However, recently, the Tor soro was declared not a member of the Tor genus but instead included in *Neolissochilus* and classified as *Neolissochilus soro* (Scharpf 2015). This fish also has local names in several areas, such as tambra fish in Java, semah (Sumatra), garing (West Sumatra), keureling (Aceh), and sapaan (Borneo) (Muchlisin *et al.* 2022). Several Asian countries have become targets for exporting this fish, including Malaysia, Singapore, Hong Kong, and Korea. The price range for this fish outside Indonesia is between 65–380 USD/kg, and domestically, it is between IDR 800,000–1,000,000/kg in early 2020. Apart from its relatively high value on the market, mahseer fish is also closely related to cultural values. In North Sumatra, this fish is cooked as food in traditional ceremonies (Yuhana *et al.* 2021).

Naturally, mahseer grows and develops in their habitat, such as lakes and rivers. This fish is also an umbrella species because it has a role in the cycle of freshwater ecosystems and contributes to the nutrient cycle (Everard *et al.* 2021). Mahseer also has low fecundity, ranging from 2,000-5,000 eggs (Haser *et al.* 2021). It can be a threat of extinction for this fish. However, as the population grows, the pollution load entering water bodies increases due to anthropogenic activities along the river. Surfactant is one of the chemicals that can be a source of water pollution. Linear Alkylbenzene Sulfonate (LAS) is a type of surfactant that is commonly found in Indonesian waters. This substance is commonly used as a mixture of detergents and household cleaners (Porter 1993). This component has the potential to inhibit biogeochemical cycles and significantly affect ecosystems (Gheorghe *et al.* 2020), so if left for a long time, it can cause the extinction of various aquatic organisms.

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Several previous studies have been conducted regarding the effect of surfactants on fish survival, one of which showed that exposure to LAS concentrations of 2,500 µg/L for 96 hours to carp (*Cyprinus carpio*) caused physiological disturbances in the form of a decrease in the number of red blood cells thereby disrupting the process of oxygen transport (Tasaki *et al.* 2015). In addition, detergents containing LAS have been shown to affect lipid and protein content in carp's gills, liver, and muscles (*C. carpio*) after 30 days of rearing (Gopal *et al.* 2008, 2013). Previous research on toxicology in mahseer fish shows that the increase in the percentage of wastewater is directly proportional to the increase in the mortality of *Tor tambra* (Ihsan *et al.* 2021). However, research on the sublethal effect of LAS surfactant on the fry of mahseer (*N. soro*) has not been widely studied, so further studies are needed.

This study aims to analyze the lethal toxicity effect of LAS surfactants on mahseer fish and the sub-lethal effect of these chemicals on the biological, physiological, and histological conditions of mahseer seeds. Knowing the risks that LAS surfactant brings to the mahseer fish, including its toxicity, to protect the mahseer fish population in nature, it is predicted that it would become fundamental knowledge in aquaculture and restocking activities.

2. Materials and Methods

The test animals in this study were the seeds of mahseer sized 4.38±0.22 cm, obtained from hatcheries at the Aquaculture and Toxicology Environmental Research Installation, Cibalagung, Bogor, West Java, Indonesia. The method used in this study consisted of several steps: acclimatization, lethal toxicity test, sub-lethal test, and analysis. Acclimatization was carried out to adapt the test fish to their new environment. This stage is carried out in a fiber tank with freshwater as a medium for seven days before the fish are spread to each aquarium to be treated. The duration of acclimatization is referred to previous research by Gouda *et al.* (2022). During the acclimatization period, the fish were fed at satiation with commercial feed PF500 and monitored for their health and feasibility as test animals.

Pure LAS surfactant in liquid form was used in the lethal toxicity test and sub-lethal test. Before being applied to the maintenance medium, pure LAS surfactant was diluted using aqua pro injection and acetone until the required concentration was obtained. The lethal toxicity test was carried out using the bioassay method consisting of two stages of the test (Busvine 1971): (1) Preliminary test to find the toxicity threshold (critical range) of surfactants in the test fish by determining the upper lethal concentration (LC_{100}-24 hours) and the lower lethal concentration (LC_0-48 hours); (2) Advanced test, by exposing test fish for 24, 48, 72, and 96 hours in surfactant solution at six concentration levels which are between the upper and lower lethal concentration.

In the sub-lethal test, a completely randomized design (CRD) was used with four treatments in the form of differences in surfactant concentrations in the test medium, i.e., 0, 10, 30, and 50% of the LC_{50}-96 hour values with three replication for each. The test fish were stocked at a density of 1 fish per 2 liters of water and fed at satiation (Taufik *et al.* 2009). The rearing media volume was 60 L, with 30 fish in each treatment. Maintenance was carried out for 40 days. Referring to previous research by Widyastuti *et al.* (2021), the effect of rearing media on the seeds of mahseer fish can be seen after 40 days of rearing. Therefore, a maintenance period of 40 days was chosen. Water changes were carried out every two days (48 hours) as much as 90% with the appropriate surfactant concentration for each treatment. During maintenance, the fish were fed with PF500 with a protein content of 39% with a frequency of 3 times per day, with feeding times at 07:00 AM, 12:00 PM, and 05:00 PM.

The biological parameters observed included survival rate (SR), specific growth rate (SGR), feed conversion ratio (FCR), and feeding efficiency (FE) (Handayani *et al.* 2014). Blood samples were taken from mahseer fish seed, which had been treated for 40 days using a sterile syringe in the caudal vein. Blood preparation was carried out by mixing the blood sample with EDTA solution. The analysis included calculating the number of erythrocytes, leukocytes, hemoglobin, hematocrit, and blood glucose (Fitria *et al.* 2019). Blood glucose measurement is carried out by dropping a blood sample onto the EasyTouchGCU glucometer strip, and the blood glucose level will appear on the glucometer screen. Histological analysis of the gills was carried out following standard techniques and stained with hematoxylin and eosin (Roberts 2001).

Data on the mortality of mahseer in the lethal toxicity test was carried out by probit analysis using the EPA Probit Analysis Program Version 1.5 to determine the LC_{50} value at 24, 48, 72, and 96 hours of exposure. Other quantitative data were analyzed statistically using RStudio.

3. Results

3.1. Lethal Toxicity (LC_{50})

Based on the preliminary test results, the lethal surfactant concentration threshold for mahseer fish was obtained; the lower threshold (LC_0-48 hours) was 3 mg/L, and the upper threshold (LC_{100}-24 hours) was 10 mg/L. Logarithms were calculated from the two lethal concentration threshold values, and the concentration was 3.57, 4.25; 5.06, 6.01, 7,14; and 8.49 mg/L for the advanced test. The observations at 24, 48, 72, and 96 hours after application showed that fish mortality was higher with increasing exposure time.

The probit analysis of mahseer fish showed that the LC_{50} value at 24, 48, 72, and 96 hours was 6.51 mg/L, 6.36 mg/L, 6.25 mg/L, 6.14 mg/L, respectively. The decreasing LC_{50} value with an increasing exposure period of 6.14 mg/L after 96 hours suggested that the LAS surfactant was more harmful to mahseer fish. Based on the lethal toxicity rating, the toxicity of surfactants for mahseer fish is highly toxic, with LC_{50}-96 hour values ranging from 1-10 mg/L.

3.2. Sub-lethal Toxicity

The sub-lethal concentration was used during the 40-day rearing period of the test fish. The concentrations used were 0, 0.61, 1.84, and 3.07 mg/L. After the rearing period, the fish were analyzed biologically, haematologically, and histologically.

3.3. Biological Parameter Analysis

Mahseer seeds during the study had a survival rate (SR) of 100% for each treatment (Table 1), which means that all fish survived until the end of the rearing period. The highest SGR value was obtained at a concentration of 0.61 mg/L (2.16%), followed by the control (2.03%), where both were significantly ($P<0.05$) higher than the concentration of 3.07 mg/L (1.84%) and 1.84 mg/L (1.80%). In contrast, the concentrations of 1.84 and 3.07 mg/L were not significantly different ($P>0.05$). According to the analysis, the concentration of 0.61 mg/L has the greatest FE value, 35.55±0.80%, and the lowest FCR value, 2.81±0.06. Meanwhile, the FE values at concentrations of 1.84 and 3.07 mg/L were lower.

3.4. Hematological Conditions

This research showed that erythrocytes, hemoglobin, hematocrit, and blood glucose decreased despite increasing leukocyte counts (Table 2). The LAS concentration of 0.61 mg/L caused a significant reduction in erythrocytes and an increase in leukocytes ($P<0.05$). A significant decrease in hemoglobin levels occurred when LAS was given at 3.07 mg/L. The percentage of hematocrit decreased significantly at 1.84 mg/L LAS concentration. Blood glucose at a concentration of 0.61 mg/L had a higher value than the control, and

Table 1. Analysis of biological parameters of mahseer fish after 40 days of rearing

Linear alkylbenzene sulfonate concentration (mg/L)	Survival rate (%)	Specific growth rate (%)	Feed conversion ratio	Feeding efficiency (%)
0.00	100	2.03^a	$3.34±0.32^a$	$30.11±3.03^b$
0.61	100	2.16^a	$2.81±0.06^b$	$35.55±0.80^a$
1.84	100	1.80^b	$3.61±0.07^a$	$27.70±0.57^b$
3.07	100	1.84^b	$3.39±0.27^a$	$29.66±2.37^b$

Numbers in the same column followed by the same superscript letter are not significantly different ($P>0.05$)

Table 2. Analysis of the hematological condition of mahseer fish after 40 days of rearing

Linear alkylbenzene sulfonate concentration (mg/L)	Total erythrocytes (10^5 cells/mm^3)	Total leukocytes (10^3 cells/mm^3)	Hemoglobin (grams/100 ml)	Hematocrit (%)	Blood glucose (mg/dl)
0.00	$15.03±1.42^a$	$13.97±2.00^b$	$9.73±0.42^a$	$27.14±2.42^a$	$213.33±3.06^b$
0.61	$10.50±1.18^b$	$24.50±0.50^a$	$9.27±0.42^a$	$23.49±3.20^{ab}$	$224.83±1.61^a$
1.84	$9.10±0.85^b$	$27.17±2.52^a$	$8.18±0.99^{ab}$	$17.87±2.97^b$	$157.83±2.57^c$
3.07	$8.30±1.57^b$	$25.50±1.50^a$	$7.02±0.90^b$	$18.36±1.34^b$	$145.83±1.61^d$

Numbers in the same column followed by the same superscript letter are not significantly different ($P>0.05$)

its levels decreased at concentrations of 1.84 mg/L and 3.07 mg/L.

3.5. Histological Conditions

Changes in histological conditions in the gills were found at a concentration of 0.61, 1.84, and 3.07 mg/L (Figure 1). Mahseer gill in the control treatment showed primary and secondary lamellae under normal conditions. At 0.61 mg/L, inflammation was found in the primary lamella. In the 1.84 mg/L treatment, there was damage in the form of blood vessel congestion and incomplete lamella fusion. Mahseer gill at a concentration of 3.07 mg/L experienced some damage, including secondary lamella fusion, aneurysm, inflammation, and blood vessel congestion.

3.6. Water Quality Analysis

Based on the results of water quality measurements, it can be seen that the physico-chemical properties of water during maintenance are within the threshold value for fisheries (Table 3). This is because, during maintenance, water changes are carried out in a controlled manner to maintain water quality. So, the results of observations of biological, physiological,

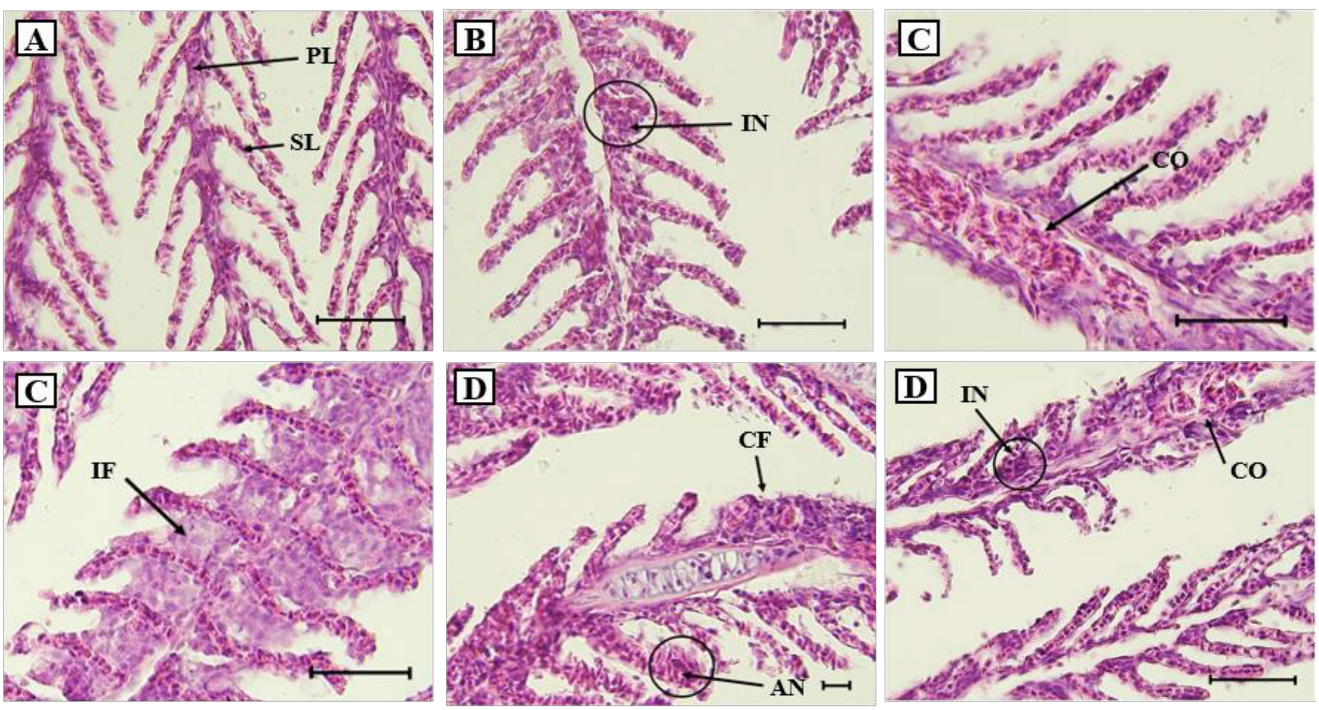

Figure 1. Histological condition of mahseer gills after 40 days of rearing. (A) 0 mg/L (control), (B) 0.61 mg/L, (C) 1.84 mg/L, (D) 3.07 mg/L of linear alkylbenzene sulfonate concentration. PL = Primary lamella, SL = Secondary lamella, IN = Inflammation, CO = Congestion of blood vessel, IF = Incomplete lamella fusion, CF = Complete lamella fusion, AN = Aneurysm. H and E staining, 50 μm scale

Table 3. Results of water quality measurements on rearing media after 40 days of maintenance

Linear alkylbenzene sulfonate concentration (mg/L)	Parameters observed					
	Temperature (°C)	pH	Dissolved oxygen (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
0.00	26.3-26.6	7.09-7.21	6.38-6.94	0.03-0.18	0.04-0.16	6.94-9.79
0.61	26.3-26.6	7.21-7.47	6.40-6.68	0.00-0.30	0.02-0.15	7.62-11.14
1.84	26.3-26.6	6.71-6.93	5.88-6.11	0.00-0.40	0.02-0.22	6.67-9.67
3.07	26.3-26.6	6.87-6.99	5.54-5.59	0.00-0.35	0.01-0.18	6.91-9.97

and histological parameters of fish are not affected by environmental conditions but only due to the influence of surfactants given during rearing.

4. Discussion

LAS surfactant at a particular concentration can stimulate the growth of mahseer fish. In this study, it is suspected to be the cause of the higher SGR value at a concentration of 0.61 mg/L than the control treatment. It is supported by previous research, where there was an increase in the growth of sea bass larvae (*Lates calcarifer*) after being given the addition of LAS surfactant to the rearing medium (Rejeki *et al.* 2005). In addition, carp (*C. carpio*) exposure to LAS with a concentration of 10% of the LC_{50} value for 30 days increased lipid and protein levels in the gills, liver, and muscles (Gopal *et al.* 2008, 2013). The presence of LAS in the maintenance medium can induce lipid mobilization by stimulating lipase activity thereby breaking down lipids into free fatty acids. These free fatty acids enter the utilization cycle in fish tissue (Gopal *et al.* 2008). Increased protein levels are thought to be caused by the induction of microsomal enzymes to detoxify foreign materials and other constituent enzymes from various metabolic segments (Gopal *et al.* 2013).

The results of this study indicate that 0.61 mg/L LAS concentration can produce the highest feeding efficiency (FE) of mahseer seeds (35.55±0.80%). It means that at this concentration, the efficiency level of fish is higher in utilizing feed as a source of energy for growth compared to control. When all the energy needed for biological processes has been used, the body will use the extra energy from food to form new tissue and promote growth processes (Setijaningsih and Puspaningsih 2022). LAS surfactant contamination still within tolerance limits can increase thyroid hormone, affecting fish growth (Heath 2000). In addition, the high FE value is caused by surfactants that reduce surface tension and can emulsify fats and proteins in water (Manik and Edward 1987). Feed in water is broken down into simpler molecules with the help of surfactants, so fish more easily absorb that feed and increase the FE value.

Meanwhile, the FE values at concentrations of 1.84 and 3.07 mg/L were lower. The accumulation of toxic substances causes the organs of the fish to experience disturbances, thereby reducing feed consumption, (Supriyono *et al.* 2013). Fish tend to utilize energy from feed to defend themselves from environmental stress, so the feed absorbed becomes very little body weight (Magfirah *et al.* 2015).

The decreased levels of erythrocytes, hemoglobin, and hematocrit in this study are in line with the results of previous studies (Gouda *et al.* 2022) that showed exposure to high concentrations of LAS surfactant caused a significant decrease in erythrocytes hemoglobin, and hematocrit in tilapia (*Oreochromis niloticus*). LAS is an anionic surfactant that can disrupt the fluidity of cell membranes (Tan *et al.* 2010), induce denaturation of hemoglobin (Hayashi *et al.* 1995), and cause structural changes in red blood cells (Bielinska and Terlecki 1984). The surfactant can also change the structure of hemoglobin and reduce its ability to bind and transport oxygen. This situation can cause a decrease in oxygen transport so that the fish experience hypoxia (Tasaki *et al.* 2015).

A significant increase in the number of leukocytes and the addition of surfactant concentration, indicates that the presence of surfactant induces a fish defense mechanism against toxic stress. Under stress or exposure to toxins, changes in the number of white blood cells are a natural response (Narra *et al.* 2017). A significant increase in the number of leukocytes along with the addition of surfactant concentration, indicates that the presence of surfactant induces a fish defense mechanism against toxic stress. This also occurred in studies conducted on tilapia (*O. niloticus*) exposed to lethal concentrations of LAS (Gouda *et al.* 2022), pangas catfish (*Pangasius* sp.) exposed to latex waste (Susanto *et al.* 2014), and streaked prochilod (*Prochilodus lineatus*) exposed to chemical pollutants (Pereira *et al.* 2012).

Blood glucose levels decreased in line with higher surfactant concentrations. This decrease indicates that fish use energy from glucose to adapt to environmental stress. In addition, the high blood glucose level in the control treatment and 0.61 mg/L was caused by more feed consumption. During rearing, the fish were fed at-satiation, resulting in the highest feed efficiency (FE) value at a concentration of 0.61 mg/L, followed by the control treatment. Food deprivation generally causes a decrease in plasma glucose levels in many fish species (Blasco *et al.* 1996). This is also supported by the results from the previous study (Soengas *et al.* 2006), which showed that the re-feeding of rainbow trout (*Oncorhynchus mykiss*) caused an increase in blood glucose of more than 100%.

Gills are one of the fish organs that are directly exposed to the surrounding environment. In this study, exposure to LAS surfactant was proven to be able to cause gill damage such as inflammation, blood vessel congestion, lamella fusion, and aneurysm. Inflammation is a form of basic protective response to tissue damage experienced by vertebrates (Roberts and Rodger 2012). Blood vessel congestion is thought to be caused by injury to the pillar cells and blood vessels, thereby increasing blood flow in the lamellae. In addition, blood vessel congestion is also caused by edema of the gill filaments (Chen *et al.* 2011). An increase in the number of cells or hyperplasia caused lamella fusion. Aneurysms are formed due to the rupture of pillar cells in the gills caused by greater blood flow (Martinez *et al.* 2004). These changes in the structure of the gills result in inhibiting the process of gas exchange in the fish's body.

Several fundamental aspects of aquaculture are important to consider, including water quality control and environmental sustainability. The results of this study provide information regarding the impact of LAS surfactant in certain concentrations on the seed of mahseer fish. This information can be used to optimize environmental conditions before aquaculture activities. If the maintenance of mahseer fish is carried out with a water source containing LAS surfactant, the maximum concentration in the water is 0.61 mg/L. For future research, analysis of the effect of LAS surfactant on protein and lipid contents, and the action of enzymes in the body of mahseer fish is still needed. Study is also required at higher fish stadia to determine the impact of LAS on fecundity and reproduction.

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