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Histopathology of liver, kidney, intestine, spleen, and bile of catfish with Jaundice

Histopatologi organ hati, ginjal, usus, limpa, dan empedu ikan lele yang mengalami penyakit kuning Jaundice

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ABSTRACT

One of the diseases that attack catfish is jaundice, also known as yellowing, which can result in low or even unsalable selling prices. The yellow color in catfish is associated with tissue/organ disorders, especially bile or liver function. This study aimed to compare the histopathological structure with organ parameters of liver, kidney, intestine, spleen, and bile of jaundiced catfish both natural and injected with *Aeromonas* sp. bacteria associated with jaundice. This study use the observation method with three variables: healthy catfish as control (K), yellow catfish treated with bacteria associated with jaundice, namely *Aeromonas* sp. bacteria (KP), and yellow catfish from the farm (KL) from Ciomas District, Bogor Regency, West Java. Then it will be categorized by the level of organ damage. The number of fish used was 9 fish with 3 samples for each treatment. The observation results obtained show that the field scale variable (KL) has the highest level of necrosis damage in each organ compared to the laboratory scale variable (KP). The yellow color in catfish is related to the disruption of organ tissue, especially bile or liver function. It can be seen in the organs of the liver, intestines, kidneys, spleen, and bile that the field variable always scores a high level of necrosis damage with a range of scoring values of 1.11-2.48 which is classified as a moderate degree of damage, with a percentage of necrosis 40%≤P<60% in the liver, kidneys, and bile.

Keywords: *Clarias* sp., histopathology, jaundice, necrosis

ABSTRAK

Salah satu penyakit yang menyerang ikan lele adalah penyakit kuning atau dikenal juga dengan penyakit kuning, yang dapat mengakibatkan harga jual menjadi rendah atau bahkan tidak dapat dijual. Warna kuning pada ikan lele dikaitkan dengan gangguan jaringan/organ, terutama empedu atau fungsi hati. Penelitian ini bertujuan untuk membandingkan struktur histopatologi dengan parameter organ hati, ginjal, usus, limpa, dan empedu ikan lele yang sakit kuning baik yang alami maupun yang diinjeksi dengan bakteri *Aeromonas* sp. yang berasosiasi dengan penyakit kuning. Penelitian ini menggunakan metode observasi dengan tiga variabel yaitu ikan lele sehat sebagai kontrol (K), ikan lele kuning yang diberi perlakuan injeksi dengan bakteri yang berasosiasi dengan penyakit kuning yaitu bakteri *Aeromonas* sp. (KP), dan ikan lele kuning yang berasal dari farm (KL) berasal dari, Kecamatan Ciomas, Kabupaten Bogor, Jawa Barat. Kemudian akan dikategorikan dengan tingkat kerusakan organ. Jumlah ikan yang digunakan sebanyak 9 ekor ikan dengan setiap perlakuan terdapat 3 sampel. Hasil pengamatan yang diperoleh menunjukkan bahwa variabel skala lapangan (KL) memiliki tingkat kerusakan nekrosis paling tinggi pada setiap organnya dibandingkan dengan variabel skala laboratorium (KP). Warna kuning pada ikan lele berhubungan dengan terganggunya jaringan organ terutama empedu atau fungsi hati. Terlihat pada organ hati, usus, ginjal, limpa, dan empedu bahwa variabel lapangan selalu mendapatkan skor tingkat kerusakan nekrosis yang tinggi dengan kisaran nilai skoring 1,11-2,48 yang tergolong sebagian derajat kerusakan sedang, dengan persentase nekrosis 40% ≤ P<60% pada organ hati, ginjal, dan empedu.

Kata kunci: *Clarias* sp., histopatologi, nekrosis, penyakit kuning

INTRODUCTION

Catfish, Clarias sp., is one of the leading freshwater fish commodities that has been cultivated in Indonesia. Catfish has various advantages, including a relatively fast growth rate, can be maintained in various cultivation containers, and is relatively resistant to disease (Anis & Hariani, 2019). Telaumbanua *et al*. (2018) also stated that catfish is one of the commodities in demand by farmers because it can be kept in a narrow area with a high stocking density, and the market demand is quite high. Therefore, this fish can be declared as one of the freshwater commodities that has high potential to continue to be developed (Kristiany, 2020). Catfish is known as a fishery commodity that is currently cultivated by many Indonesians, both on a large scale and household scale, because of its increasing demand (Kesuma *et al*., 2019).

Especially for catfish commodities, the demand for catfish which tends to always increase also causes an increase in the value of catfish production to adjust to market needs (Utomo & Himawanto, 2021). Therefore, catfish production over the past five years (2018-2022) has fluctuated, with an average increase in production of 10.9% (DJPB, 2022). Based on the catfish production trend chart by KKP (2022), the total value of the latest catfish production volume in 2022 is 343,414 tons, an increase of 0.14% from the previous year of 2021. Based on the data listed above, it can be concluded that catfish farming has prospects that will continue to be sustainable because its production always increases along with market needs. In this case, KKP (2020) targets catfish production in 2023 at 1.57 million tons and in 2024 at 1.65 million tons. This has spurred catfish farmers in a more developed intensive system.

However, fish farming with high stocking densities can cause a decrease in water quality, resulting in obstacles that occur in organisms susceptible to disease infection (Anwar *et al*., 2022). One of the diseases that attack catfish is yellow catfish, also known as jaundice. Yellow catfish disease in its marketing results in low or even unsold selling prices, thus disrupting the development of the aquaculture industry (Luu, 2013). Jaundice in catfish is characterized by the appearance of yellow color throughout the body such as skin, internal organs including liver, kidneys, spleen, intestines, and bile. Although the movement of fish in the water is still agile, after harvesting it will look limp and die, resulting in mass death of fish in a relatively short time (Hastuti & Subandiyono, 2012).

Jaundice in catfish is associated with yellow skin pigmentation. Yellow pigmentation is an indicator of increased bilirubin production or decreased bilirubin elimination by the liver. Bilirubin is a yellow orange crystalline pigment derived from the metabolism of old or damaged heme (Lubis *et al*., 2013). Fish that experience health problems with yellow catfish disease are related to liver function. The condition of liver function is reflected by transaminase enzymes (Hastuti *et al*., 2019). The yellow color of catfish is associated with impaired bile tissue or liver function. The occurrence of the phenomenon of changes in the histology structure of yellow catfish liver is caused by high toxic substances in the water medium in the form of ammonia, which causes catfish to experience impaired liver function, thus affecting the histology structure of catfish liver.

This is in accordance with Hastuti and Subandiyono (2011), which states that the liver is the organ that hoards the most toxic substances that enter the body, so the liver is unable to process the breakdown of red blood cells efficiently because it is easily affected by the effects of these toxins. This can be seen in the journal Hartanti *et al*. (2013) with many abnormalities that occur in the histopathology of yellow catfish liver compared to normal fish liver, as well as in research Siswoyo (2021) with suspected jaundice in catfish characterized by histopathological abnormalities such as vacuolar degeneration and necrosis characterized by loss of tissue structure later. Degeneration is characterized by swelling which progresses to necrosis. Degeneration can be caused by pathogen infection and is reversible (can recover after the source of damage is eliminated) (Latifah *et al*., 2014).

The difference in organ condition in this study was determined by histopathology tests. Histopathology is the science that focuses on tissue and organ damage to enhance macroscopic diagnosis (Faulana *et al*., 2024). Accuracy of diagnosis is needed in the treatment of fish diseases. Diagnosis of fish infectious diseases needs to be done by paying attention to clinical signs that include external and internal characteristics and pathological changes. Histopathological examination is carried out to see tissue changes that occur due to pathogen infection that causes tissue abnormalities (Safratilofa, 2017). Histopathological analysis can be an important and more specific parameter in determining the condition of cell structures that occur in internal organs focusing on the liver, intestines, kidneys, spleen, and bile for jaundice.

MATERIALS AND METHODS

Research design

This study used an observational method that focused on a specific case for descriptive analysis to obtain accurate conclusions, as described by histopathology tissues. The study consisted of three variables, each with three replicates. The variables consisted of healthy catfish as control (K), yellow catfish treated with *Aeromonas* sp. bacteria injection at a dose of 0.1 mL (KP), and yellow catfish from the field (KL). The study lasted ±30 days starting from September to November 2022.

Fish preparation

The test fish used in this study were *Clarias* sp. catfish with jaundice, either from the field or healthy fish conditioned for jaundice by injecting bacteria associated with the condition. They were compared with healthy catfish used as a control group. The yellow catfish had an average length of 24 ± 11.26 cm and an average weight of 244 ± 116.63 g, obtained from catfish farms originating from Ciomas District, Bogor Regency, West Java. Catfish injected with jaundice-associated bacteria were compared with a healthy control group.

Yellow catfish samples from Bogor, West Java, were injected with 0.1 mL of *Aeromonas* sp. bacteria at a concentration of 10^7 CFU/mL. The catfish had an average length of 19 ± 6.55 cm and weighed 48 ± 17.08 g. Histopathological analysis was performed on the tissues of the catfish. Histopathological analysis was performed on tissues and organs from naturally and artificially infected yellowfish, and compared with healthy catfish tissues and organs as controls.

Histopathology preparation

Histopathology preparation refers to the method of Nuryati *et al.* (2023) by preparing liver, kidney, intestine, spleen, and bile organs from catfish samples that will enter the fixation, dehydration, cleaning, embedding, tissue blocking, and staining stages for histopathological analysis. During the fixation stage, the liver and kidney were immersed in 10% BNF (buffered normal formalin) fixative solution for 24 to 48 hours. Tissue samples were dehydrated using a series of alcohol concentrations. Samples were immersed in 70%, 80%, 90%, and 95% alcohol for two hours each, followed by 12 hours in 100% alcohol concentration. Tissue samples were prepared for sectioning by first soaking them in a 1:1 mixture of alcohol and xylol for 30 minutes. This was followed by three immersions in xylol for 30 minutes each.

The samples were then subjected to paraffinization by immersing them in a mixture of xylol and paraffin (1:1) at 50-60°C. After paraffinization, the samples were placed on printing blocks and immersed in paraffin for 45 minutes. The tissues were cut into 0.5 micrometer sections using a microtome. The staining process begins with the dewaxing step, which entails immersing the tissue in xylol twice for three to five minutes. The tissue is hydrated twice with 100% alcohol for three minutes each time. Next, it is immersed in solutions containing 95%, 90%, 80%, 70%, and 50% alcohol for three minutes and then washed with distilled water.

Hematoxylin and eosin were used for staining of tissue preparations. The preparations were immersed in hematoxylin for three to five minutes, followed by washing with distilled water. The tissues are immersed in eosin for three to seven minutes and then washed off with distilled water. Next, they were progressively submerged in 50%, 70%, 85%, 90%, and 100% alcohol for two minutes each. Finally, the preparations were mounted using Entellan Rapid Mounting Medium and covered with cover glass. After drying for a full day, the preparations were ready for examination (Nuryati *et al.*, 2023).

Histopathology observation

Histopathology tissue observations were recorded using an Olympus CX31 microscope with 40×10 magnification, an optilab camera, and Image Raster software on the first, second, third, fifth, tenth, and fourteenth-days post-test. The proportion of kidney and liver necrosis in each of the five fields of view was calculated using the formula provided by Izwar *et al.* (2020):

$$P\ (\%) = \frac{\Sigma\ KS}{\Sigma\ TS} \times 100$$

Note:
P (%) = Percentage of cells with necrosis
Σ KS = Total number of necrotizing cells in five fields of view
Σ TS = Total number of cells in 5 fields

The maximum level is one, and the normal condition is five. To aid identification of each level, the definition of each score is given in paragraph form. The scoring system uses whole numbers to evaluate the liver and kidney organs of juvenile sea bass. The scoring system used is based on Wolf *et al*. (2015) division of scores, as shown in Table 1.

RESULTS AND DISCUSSION

Result

Liver histopathology

Moderate damage resulted in the highest level of damage in variable KL (fish from the field). The degree of damage in variable K (control fish) was normal, while in variable KP (fish injected with bacteria), the degree of damage was mild. The results of the observation of catfish liver histopathology related to jaundice from different variables are presented in Figure 1. The results of histopathological scoring observations on the catfish liver organ are presented in Figure 2.

Kidney histopathology

The highest level of damage occurred in the KL variable with a moderate damage assessment category. The KP variable is classified as lightly damaged, while the K variable for the degree of damage is still classified as normal. The results of the observation of catfish kidney histopathology related to jaundice from different

Table 1. Histopathological assessment and extent of damage.

Necrosis	Score	Damage level	Description
P<20%	0	Ordinary	The tissue structure of the organ is still normal and functioning properly
20% ≤ P < 40%	1	Lightly damaged	Tissue structure and function are still relatively normal, but there are some minor changes under the microscope.
40% ≤ P < 60%	2	Medium damage	Tissue structure begins to change significantly, potentially leading to decreased tissue function
60% ≤ P < 80%	3	Heavy damage	Loss of most of the normal structure of the tissue
P≥80%	4	Heavy damage	Tissue structure that is almost or completely missing

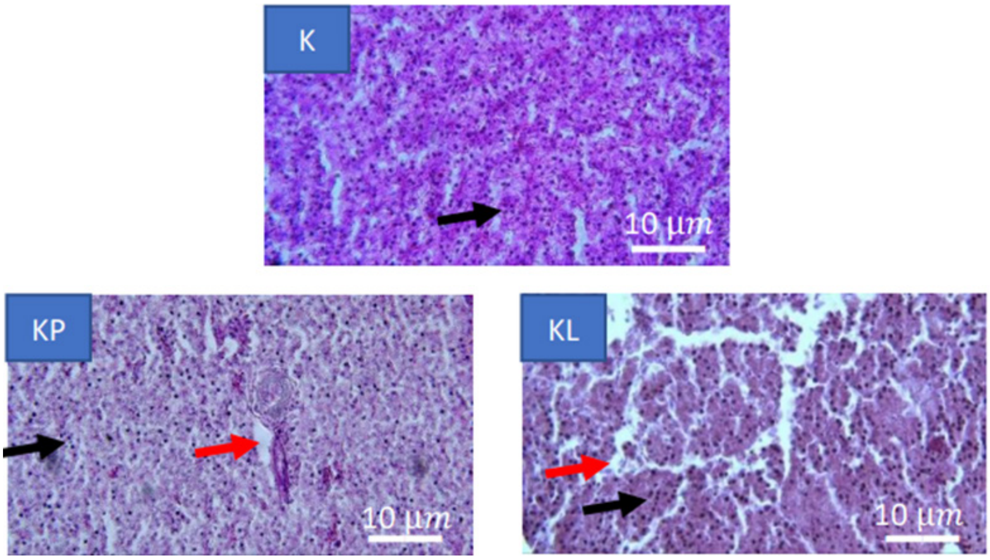

Figure 1. Microscopic condition of catfish liver from three different variables: K (control fish), KP (fish injected with bacteria), and KL (fish from the field).
Note: necrosis (red), normal cells (black). The liver was observed under 40×10 magnification.

variables are presented in Figure 3. The results of histopathological scoring observations on catfish kidney organs are presented in Figure 4.

Intestinal histopathology

Histopathology scores of intestinal organs in KP and KL variables were not significantly different

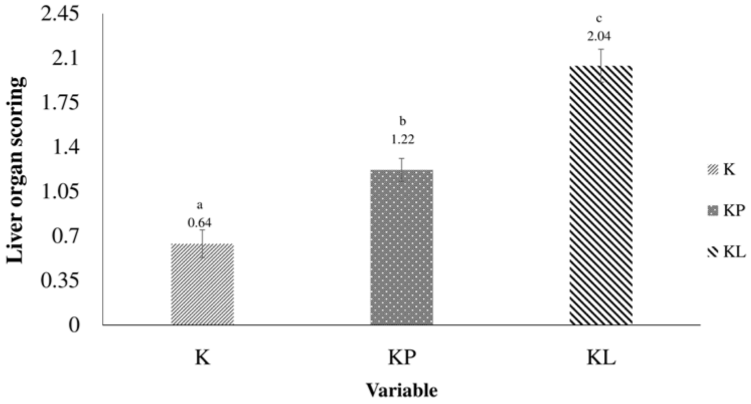

Figure 2. The total score of catfish liver histopathology test analyzed using Kruskall Wallis.
Note: Different letters in each variable above the bar indicate significantly different results (Kruskal Wallis P<0.05).

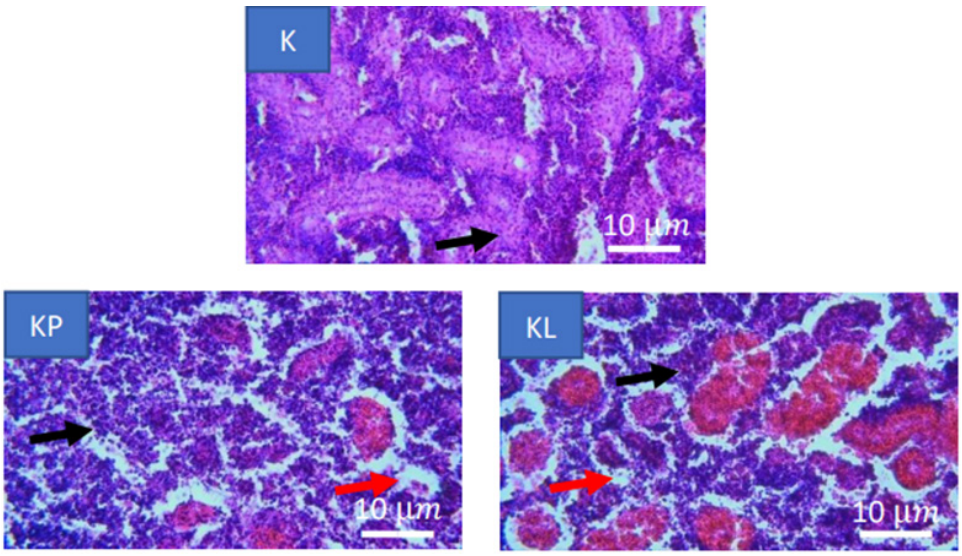

Figure 3. Microscopic condition of catfish kidneys from three different variables: K (control fish), KP (bacteria-injected fish), and KL (fish from the field).
Note: (red) necrosis, (black) normal cells. Kidney observed under 40×10 magnification.

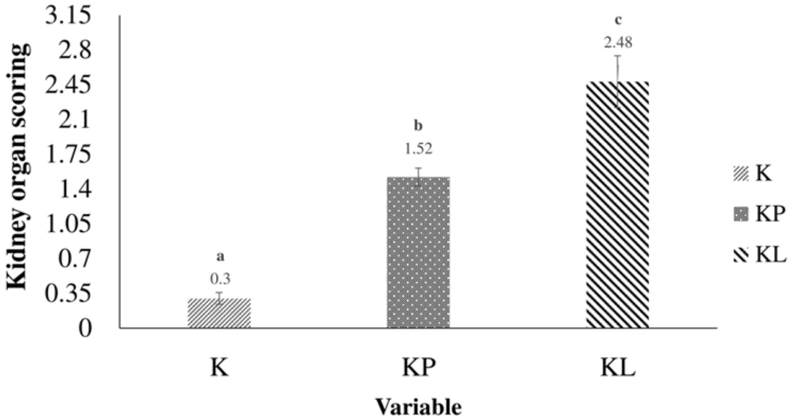

Figure 4. The total score of catfish kidney histopathology test analyzed using Kruskall Wallis.
Note: Different letters in each variable above the bar indicate significantly different results (Kruskal Wallis P<0.05).

(P>0.05) while between K and KL and KP there were significantly different scores. Therefore, the degree of damage that occurs between the KP and KL variables is still classified as the same, namely mild damage, while the K variable obtained a degree of damage that is still classified as normal. The results of histopathological observations of catfish intestine associated with jaundice from different variables are presented in Figure 5. The results of histopathological scoring observations on catfish intestinal organs are presented in Figure 6.

Spleen histopathology

The histopathology score of the spleen organ between variables K and KP was not significantly different (P>0.05), but the degree of damage was different with K obtaining a normal degree of damage while KP obtained the same degree of damage as KL, which was lightly damaged. This happened because the scoring results on the control almost touched the percentage value of 20%, so the value was not significantly different from the KP variable. Significantly different results (P<0.05) occurred in K with KL and KP with KL variables. The results of histopathology observation of catfish spleen associated with jaundice from different variables are presented in Figure 7. The results of histopathological scoring observations on catfish spleen organs are presented in Figure 8.

Bile histopathology

The highest level of damage occurred in the KL variable with a medium damage assessment category. Variable KP is classified as lightly damaged, while variable K for the degree of damage is still classified as normal. The results of histopathological observations of catfish bile associated with jaundice from different variables are presented in Figure 9. The results of histopathological scoring observations on catfish bile organs are presented in Figure 10.

Further test of Mann Whitney method

The next advanced test carried out is the Mann Whitney method with the aim of knowing the specific differences by comparing each variable in each organ, so as to answer the hypothesis whether the average assessment level between the variables of each organ being compared is significantly different or not (Utomo, 2021). The following Mann Whitney method test results are presented in Table 2.

Discussion

Jaundice is a common occurrence in catfish farming, as noted by Hartanti *et al.* (2013). This condition is characterized by the yellow appearance of the external and internal organs of the fish, resulting from the dispersion of bilirubin during metabolic processes. This can lead to organ abnormalities, including necrosis. According to Wahyuni *et al.* (2017), organ tissues are assumed to have necrosis abnormalities when cell borders are visible and the cell nucleus is not due to the loss of some parts of the cell. Necrosis is also characterized by picnosis, which is the shrinking of the cell nucleus, and karyolysis, which is the fading of the cell nucleus, as described by Meidiza *et al.* (2017).

The severity of organ disruption increases with the amount of necrosis that occurs. Necrosis is used as a parameter to determine the percentage of cell damage, also known as grading, in organ histopathology. The calculation of the assessment is based on research Syawali (2020). The average level of damage can be determined through data analysis using the Kruskal-Wallis method and the Mann Whitney follow-up test.

The Kruskal-Wallis test is named after William Kruskal and Wilson Allen Wallis. It is used to compare the average rating levels of more than two variables for each organ (Siebert & Siebert, 2017). Kruskal-Wallis converts interval data into an ordinal or rating form. In other words, data with higher values will receive higher rating scores (Ostertagova *et al.*, 2014).

The percentage necrosis formula with five fields of view was used to assess each variable and determine the average score for the control variable (K) and treatment variable (KP) for each organ. The average score for the control variable (K) ranged from 0.30-0.99 for each organ, while the treatment variable (KP) ranged from 0.30-0.99 for each organ. The average score for the treatment variable (KP) ranged from 1.02-1.59, and for the field variable (KL) ranged from 1.11-2.48. At first glance, there seems to be a difference, but it cannot be concluded directly as it may be due to sampling error (Jamco & Balami, 2022).

Therefore, it was necessary to test for statistical significance using the Kruskal Wallis method. The main function of the liver is to maintain homeostasis through metabolism and detoxification. In fish, it achieves this by producing enzymes that function in biotransformation (Sari *et al.*, 2016). This study found that the liver scored 0.64 ± 0.11 on the K variable, which is still within

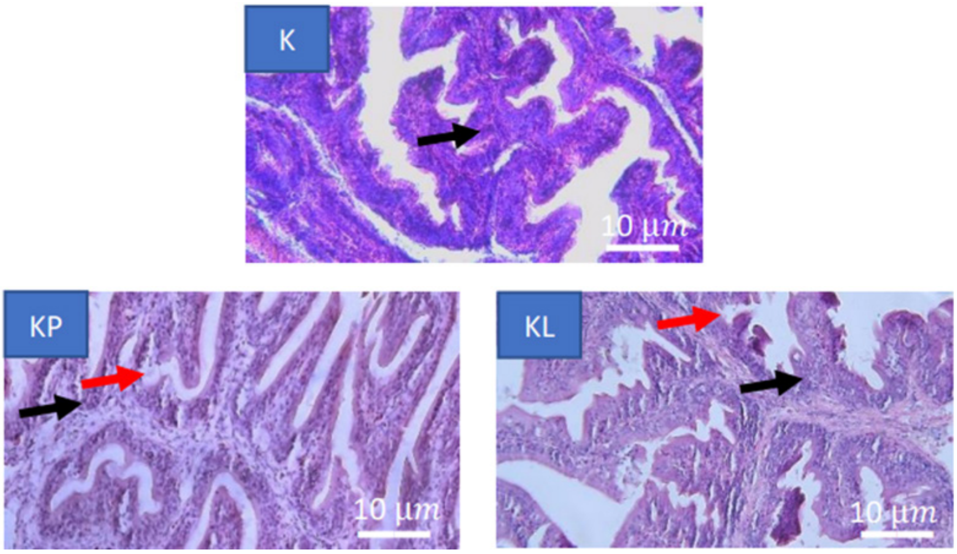

Figure 5. Microscopic condition of catfish intestine from three different variables: K (control fish), KP (fish injected with bacteria), and KL (fish from the field).
Note: (red) necrosis, (black) normal cells. Intestine observed under 40×10 magnification.

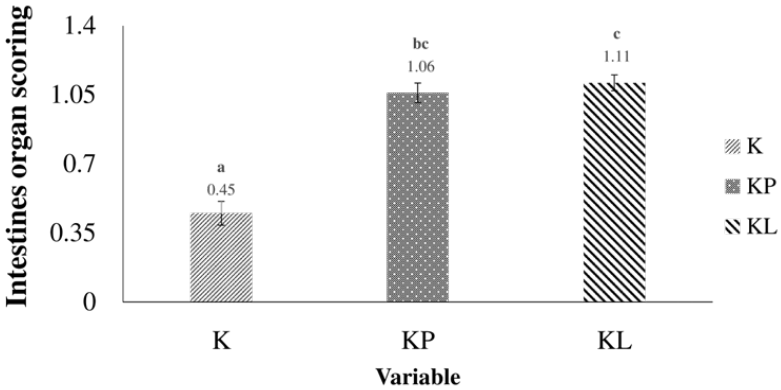

Figure 6. The total score of catfish intestinal histopathology test analyzed using Kruskall Wallis (Figure 6).
Note: Different letters in each variable above the bar indicate significantly different results (Kruskal Wallis P<0.05).

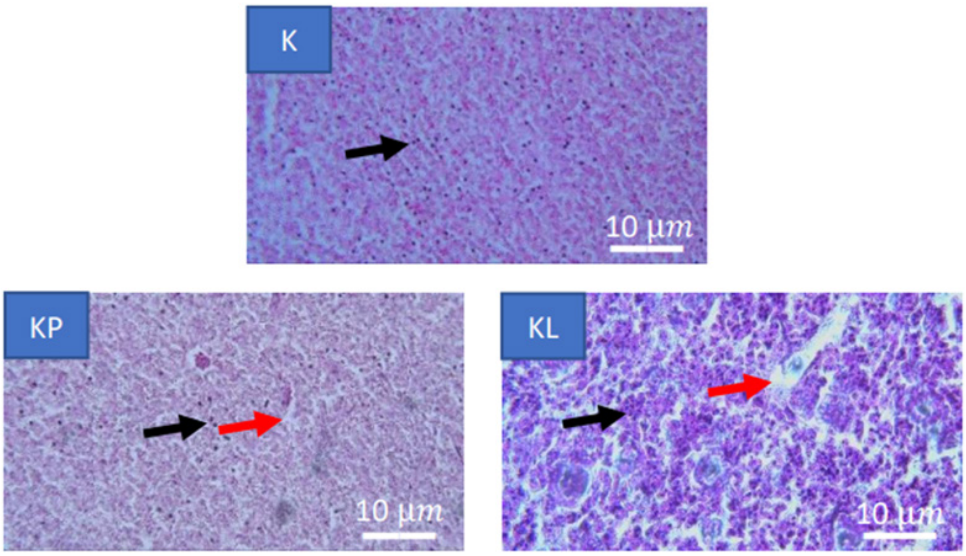

Figure 7. Microscopic condition of catfish spleen from three different variables: K (control fish), KP (fish injected with bacteria), and KL (fish from the field).
Note: (red) necrosis, (black) normal cells. Spleen observed under 40×10 magnification.

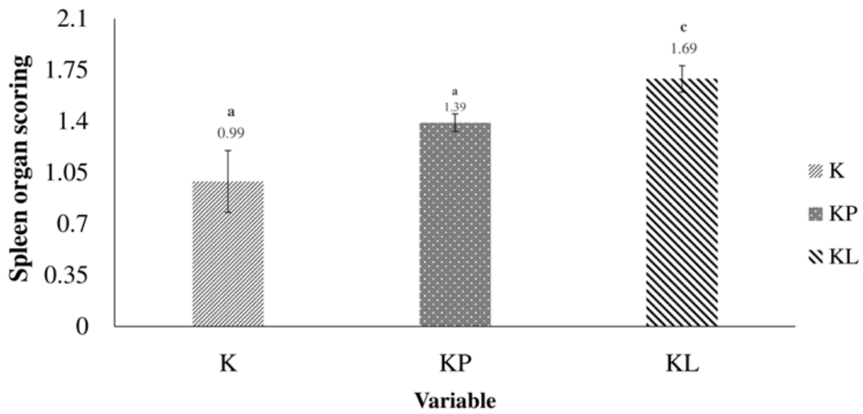

Figure 8. The total score of catfish spleen histopathology test analyzed using Kruskall Wallis.
Note: Different letters in each variable above the bar indicate significantly different results (Kruskal Wallis $P<0.05$).

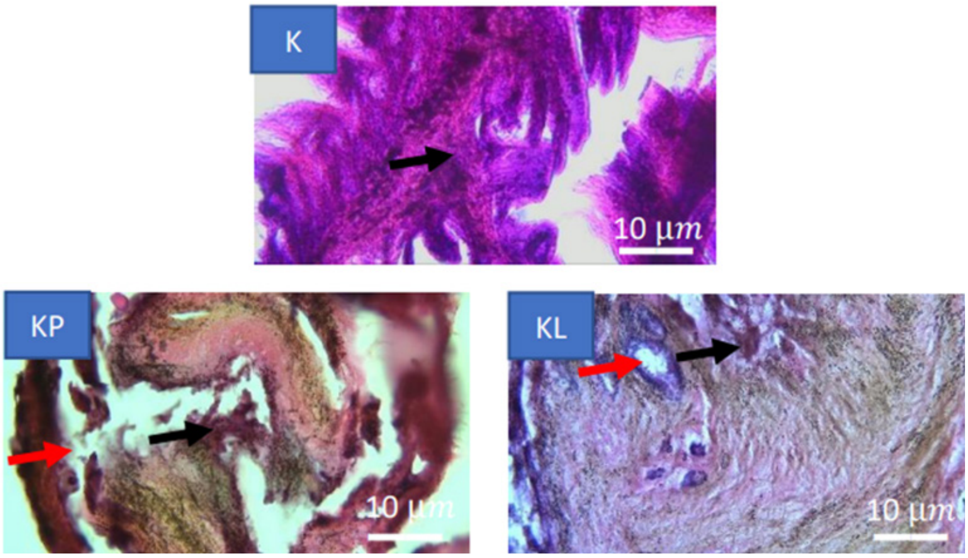

Figure 9. Microscopic condition of catfish bile from three different variables; K (control fish), KP (bacteria-injected fish), and KL (fish from the field).
Note: (red) necrosis, (black) normal cells. Bile was observed under 40×10 magnification.

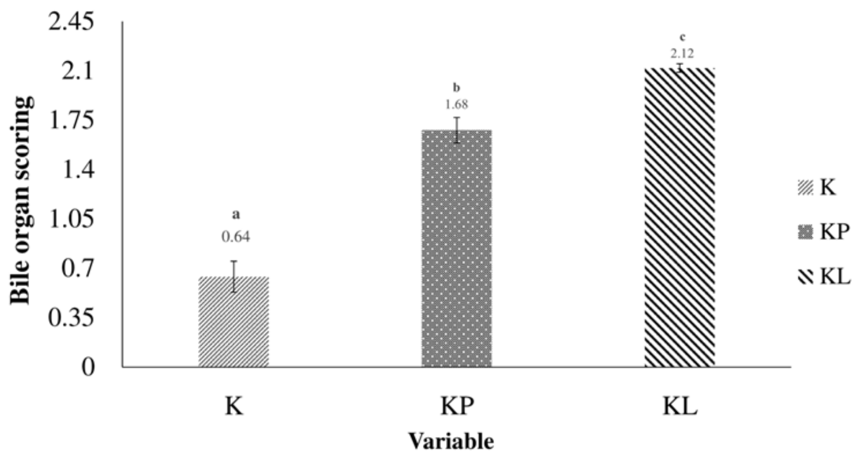

Figure 10. The total score of catfish bile histopathology test analyzed using Kruskall Wallis.
Note: Different letters in each variable above the bar indicate significantly different results (Kruskal Wallis $P<0.05$).

the normal range. The KP variable produced a score of 1.22 ± 0.09, indicating mild damage. The KL variable scored 2.04 ± 0.13, indicating moderate damage.

These results are expected, as the liver works hard to collect, neutralize, and remove toxins from the bloodstream, which can lead to yellowing of the liver, as noted by Ashari *et al*. (2014). The liver may become discolored and swollen due to the increased workload caused by the neutralization and elimination of toxins entering the circulatory system. The gut is a vital digestive organ responsible for absorbing nutrients. Damage to intestinal cells, such as necrosis, can disrupt the digestive process and absorption of nutrients (Siswoyo, 2021).

The intestinal organ of variable K was considered healthy, scoring 0.45 ± 0.06. However, the KP and KL variables, with scores of 1.06 ± 0.05 and 1.11 ± 0.04 respectively, belonged to the mildly damaged group. However, the KP and KL variables, with scores of 1.06 ± 0.05 and 1.11 ± 0.04 respectively, belong to the lightly damaged group. It should be noted that the difference in values between the KP and KL variables was not significant. However, KP and KL variables, with scores of 1.06 ± 0.05 and 1.11 ± 0.04, respectively, belong to the mildly damaged group.

Although the difference in scores between KP and KL is not significant, both are equally affected. Pratiwi and Manan (2015) state that the gut is an organ that is often exposed to pathogenic agents and parasites that enter through food, spreading toxins throughout the digestive tract. Histopathology test scores of yellow catfish kidney organs showed mild to moderate necrosis. Variable K had no damage because the score obtained was only 0.30 ± 0.06, while variable KP had mild damage with a score of 1.52 ± 0.09. The highest level of damage was observed in the KL variable with a score of 2.48 ± 0.26, included in the moderate damage category.

Assessment of the degree of damage to each variable by the kidneys experiencing mild to moderate levels of damage indicates severe damage to the internal organs due to bacterial infection, which causes abnormalities of damaged glomerular cells. According to Juanda and Edo (2018), the damage found in many fish organs is necrosis caused by acute toxin exposure, as well as the presence of bacteria, viruses, and parasites. Yellow catfish disease (jaundice) causes increased bilirubin levels in the blood, which leads to inflammation of the spleen organ. The spleen organ score in the K variable remained normal at 0.99 ± 0.21, which was not significantly different (P>0.05) from that of the lightly damaged KP variable with a score of 1.39 ± 0.06.

The reason for this is that the K score is very close to the percentage range for mild damage. As a result, the KL variable showed a significant difference (P<0.05) between the two treatments. The score obtained for the KL variable was 1.69 ± 0.09 in the lightly damaged condition. According to Suhermanto *et al*. (2018), spleen is the main site of phagocytic cell activity, and any abnormalities in this organ are likely due to the role of bacteria and toxins in infected fish. The bile organ plays a crucial role in removing waste substances and pigments, such as bilirubin, from the body (Siswoyo, 2021).

Bilirubin is a by-product of the breakdown of red blood cells, which is produced in the liver and then transported to the bile organ to be secreted into the intestine as part of bile. Histopathology test results of yellow catfish bile organs showed mild to moderate necrosis. Variable K had no damage because the score was only 0.64 ± 0.11, while variable KP had a mild degree of damage with a score of 1.68 ± 0.09. The highest degree of bile organ damage occurred in the KL variable with a score of 2.48 ± 0.26, placing it in the moderate damage category. Factors that cause moderate damage to the bile organ include problems with removing bilirubin, which is the main cause of jaundice.

Hastuti and Subandiyono (2012) stated that the bile organ plays an important role in this process. Therefore, any problems with the bile organ can affect the health of the body and trigger jaundice. A Mann Whitney test was performed to compare the mean difference in organ grading levels between the two variables. Several variables, including intestine and spleen organs, showed no significant differences (P>0.05) based on the Mann Whitney follow-up test. The results obtained showed a value of 0.599 between the variables KP and KL for the intestinal organ, and a value of 0.175 between the variables K and KP for the spleen organ.

This phenomenon was also observed in research Syawali (2020), where histological analysis of baronang fish reared at different salinities showed no significant differences (P>0.05) in cell conditions between treatments. Histopathological damage in each organ of the injection-treated variable (KP) was classified as mild. These results are consistent with the study of Yanti (2018), who injected V. *Harveyi* to artificially induce high mortality values indicating the pathogenicity of the bacteria. However, after scoring the histopathological damage, it was still classified as mild. However, Ratnawati *et al*. (2013) and A'yunin *et al*. (2020) conducted research using artificial infection and histopathology approach which is the gold standard method for disease diagnosis. Variables derived from the field scale (KL) showed moderate damage to several organs, including the liver, kidneys and bile. Jaundice is often associated with impaired liver and biliary function when hyperbilirubin occurs. In addition, damaged kidneys can affect the function of other organs, as noted by Siswoyo (2021). This phenomenon is a natural occurrence, as supported by the literature of Kwo *et al*. (2017) and Yang *et al*. (2014).

The yellow catfish (jaundice) variable from the field scale (KL) showed the highest level of necrosis. This observation is due to uncontrollable environmental factors. The aquatic environment, which is difficult to control, can harbor different types of pathogens that can easily infect fish and impair their organ functions due to direct exposure to toxic substances, leading to large amounts of necrosis. As noted by Jamin and Erlangga (2016), necrosis can be caused by bacterial, viral, and even toxin infections from the aquatic environment, which starts with cell swelling and protein denaturation.

CONCLUSIONS

Comparison of histopathological structures between catfish with jaundice from the field (KL) and those injected with *Aeromonas* sp. in the laboratory (KP) revealed that the field variable (KL) caused the highest degree of necrosis damage to each organ of the liver, kidney, intestine, spleen, and bile, with a scoring range of 1.11-2.48. This may be due to less controlled environmental factors.

REFERENCES

Anis MY, Hariani D. 2019. The administration of commercial feed with addition of EM4 (effective microorganism 4) to increase catfish (*Clarias* sp.) growth rate. Jurnal Riset Biologi dan Aplikasinya 1: 1–8.

Anwar AU, Subroto IMI, Taufik M. 2022. Expert system for diagnosing tilapia disease based on bayes method. Metamorfosa Journal of Biological Sciences 4: 285–292.

Ashari C, Tumbol RA, Kolopita MEF. 2014. Diagnosis of bacterial disease in tilapia (*Oreochromis niloticus*) cultured in capture nets in Lake Tondano. Budidaya Perairan 2: 24–30.

A'yunin, Budianto Q, Andayani S, Yuwanita R. 2020. Histopathological analysis o f *Pangasius* sp. Infected by *Edwardsiella tarda* causes Edwardsiellosis disease. IOP Conference Series Earth and Enviromental Science 441: 012031.

[DJPB] Direktorat Jenderal Perikanan Budidaya Kementrian Kelautan dan Perikanan. 2022. Performance Report (LKj) Directorate General of Aquaculture Year 2022. Jakarta (ID): DJPB.

Faulana AF, Empra DEP, Rahmadian D, Shalihah F, Nuriliani A, Retnoaji B, Saragih HTSS, Rohma Z, Septriani NI. 2024. Review: aplikasi histopatologi untuk praktik forensic. Borneo Journal of Medical Laboratory Technology 6: 529–539.

Hartanti S, Hastuti S, Sarjito. 2013. Blood profile performance of jaundiced dumbo catfish (*Clarias gariepinus*) after rearing with vitamin c addition to the diet. Journal of Aquaculture Management and Technology 2: 113–125.

Hastuti S, Subandiyono S, Windarto S. 2019. Blood performance of jaundice catfish *Clarias gariepinus*. AACL Bioflux 12: 480–489.

Hastuti S, Subandiyono. 2012. Osmotic effects of media salinity and satiation on the recovery process of jaundiced dumbo catfish (*Clarias gariepinus*, Burch). In: Junior MZ, Sudrajat AO, Jusadi D, Alimuddin, Widanarni, Yuhana M, Nuryati S, Diatin I, editor. *Prosiding Simposium N a s i o n a l Bioteknologi Akuakultur IV*; 2012 Oktober 18; Bogor, Indonesia. Bogor (ID): IPB lnternational Convention Center. pp 95–104.

Hastuti S, Subandiyono S. 2011. Hematological performance of sangkuriang catfish (*Clarias gariepinus*) and media water quality in culture system with biofiltration pond implementation. Jurnal Saintek Perikanan 6: 1–5.

Izwar A. 2018. Isolasi, identifikasi, dan uji patogenisitas bakteri penyebab penyakit pada ikan kakap putih *Lates calcarifer* B [Tesis]. Bogor (ID): Institut Pertanian Bogor.

Jamco JCS, Balami AM. 2022. Kruskal-Wallis analysis to determine student learning concentration based on the field of interest of the statistics study program FMIPA Unpatti. Jurnal Matematika, Statistika dan Terapannya 1: 39–44.

Jamin, Erlangga. 2016. Effects of organophosphate class insecticides on gift tilapia (*Oreochromis niloticus*, Bleeker) fry: histological analysis of liver and gills. Acta Aquatica 3: 46–53.

Juanda SJ, Edo SI. 2018. Histopathology of gills, liver and intestine of catfish (*Clarias gariepinus*) in Kupang city, East Nusa Tenggara. Journal of Fisheries Science and Technology 14: 23–29.

[KKP] Kementerian Kelautan dan Perikanan. 2020. Strategic Plan 2020-2024. Jakarta (ID): Direktorat Jenderal Perikanan Budidaya (DJPB).

[KKP] Kementerian Kelautan dan Perikanan. 2022. Marine and Fisheries Data Release Quarter I 2022. Jakarta (ID): Pusat Data, Statistik, dan Informasi Sekretariat Jenderal KKP.fff

Kesuma BW, Budiyanto, Brata B. 2019. Effectiveness of probiotics in feed on water quality and growth rate in sangkuriang catfish (*Clarias gariepinus*) rearing in tarpaulin system. Jurnal Penelitian Pengelolaan Sumberdaya Alam dan Lingkungan 8: 21–27.

Kristiany MGE. 2020. Economic assessment of catfish (*Clarias* sp.) rearing with boster system and conventional rearing method. Jurnal Kelautan dan Perikanan Terapan 3: 45–50.

Kwo PY, Cohen SM, Lim JK. 2017. ACG clinical guideline: evaluation of abnormal liver chemistries. The American Journal of Gastroenterology 112: 18–35.

Latifah DL, Sarjito, Prayitno SB. 2014. Karakterisasi bakteri dan gambaran histopatologi pada ikan gurami (*Osphronemus gouramy*) yang terserang penyakit "mata belo". Journal of Aquaculture Management and Technology 3: 93–100. (In Indonesia).

Lubis BM, Rasyidah, Syofiani B, Sianturi P, Azlin E, Tjipta GD. 2013. Bilirubin albumin ratio in neonates with hyper bilirubinemia. Sari Pediatri 14: 292–297.

Luu TTT. 2013. Investigation into jaundice in farmed catfish (*Pangasianodon hypophthalmus*, Sauvage) in the Mekong Delta, Vietnam [Thesis]. Mekong (VN): University of Stirling.

Meidiza R, Arimbi, Hastutiek P. 2017. Image of hepatic pathology of dumbo catfish (*Clarias gariepinus*) infected with bacteria *Edwardsiella tarda*. Jurnal Ilmiah Perikanan dan Kelautan 9: 47–56.

Nuryati S, Yanti M, Rahman, Wahyuwardani S. 2023. Kidney and liver histopathology of sea bass *Lates calcarifer* infected with black body syndrome-associated bacteria. Jurnal Akuakultur Indonesia 22: 170–178.

Ostertagova E, Ostertag O, Kovac J. 2014. Methodology and application of the kruskal-wallis test. Applied Mechanics and Materials 611: 115–20.

Pratiwi HC, Manan A. 2015. Basic histology techniques in gourami (*Osphronemus gouramy*). Jurnal Ilmiah Perikanan dan Kelautan 7: 153–158.

Ratnawati A, Purwaningsih U, Kurniasih. 2014. Histopathologically suspected Edwardsiella tarda as the cause of mascot fish mortality (*Crassius auratus*): Postulat Koch. Jurnal Sain Veteriner 31: 55–65.

Safratilofa. 2017. Histopathology of liver and kidney of catfish (*Pangasionodon hypopthalmus*) injected with *Aeromonas hydrophila* bacteria. Jurnal Akuakultur Sungai dan Danau 2: 83–88.

Sari W, Okavia IW, Cerianna R, Sunarti. 2016. Liver microscopic structure of seurukan fish (*Osteochilus vittatus*) from Krueng Sabee river, Aceh Jaya district polluted by gold ore milling waste. Jurnal Biotik 4: 33–40.

Siebert CF, Siebert DC. 2017. Data analysis with small samples and non-normal data: Non parametrics and other strategies. United Kingdom (UK): Oxford University Press.

Siswoyo AB. 2021. Histology Analysis of Diseased Intestines, Kidneys, and Liver Catfish from Parung, Bogor [Thesis]. Bogor (ID): Institut Pertanian Bogor.

Suhermanto A, Sukenda, Zairin JRM, Lusiastuti AM, Nuryati S. 2018. Whole cell and extracellular product (ECP) toxicity of hemolytic and non-hemolytic *Streptococcus agalactiae* in tilapia fish (*Oreochromis niloticus*). Jurnal Riset Akuakultur 13: 317–328.

Utomo H. 2021. Comparison of Indonesian mortality table and CSO mortality table using mann-whitney test and kruskal-wallis test. Jurnal Ilmiah Indonesia 6: 1210–1215.

Syawali A. 2020. Histological analysis of baronang fish *Siganus guttatus* reared in different salinities [Skripsi]. Bogor (ID): Institut Pertanian Bogor.

Telaumbanua NH, Rusliadi, Pamukas NA. 2018. Growth and survival of dumbo catfish (*Clarias gariepinus*) with different stocking densities using probiotic boster aquaenzyms in the diet. Jurnal Online Mahasiswa Bidang Perikanan dan Ilmu Kelautan 5: 1–13.

Utomo TA, Himawanto DA. 2021. Empowerment of community potential through the introduction of inland fisheries as a start of an integrated farming system in Karangdowo and Juwiring sub-districts of Klaten district:

some problems in terms of pond construction. Jurnal Budimas 3: 435–441.
Wahyuni S, Windarti, Putra RM. 2017. Comparative study of gill and kidney tissue structure of cork fish (*Channa striata,* Bloch 1793) from Sibam River and Kulim river, Riau province. JOM Universitas Riau 7: 1–14.
Wolf JC, Baumgartner WA, Blazer VS, Camus AC, Engelhardt J A, Fournie JW, Frasca SJ, Groman DB, Kent M, Khoo LH. 2015. Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: A Guide for Investigators, Authors, Reviewers, and Readers. Toxicologic Pathology 43: 297–325.
Yanti M. 2018. Kidney and liver histopathology of *Lates calcarifer* Bloch snapper fry injected with five different bacterial isolates [Thesis]. Bogor (ID): Institut Pertanian Bogor.
Yang X, Schnackenberg LK, Shi Q, Salminen WF. 2014. Hepatic toxicity biomarkers. Biomarkers In Toxicology 241–259.