JURNAL PERIKANAN DAN KELAUTAN
Volume 31 No. 1, February 2026: 71-84

e-issn : 2721-8902
p-issn : 0853-7607

Tissue Structure of *Pangasianodon hypophthalmus* Fed on *Cosmos caudatus*-Enriched Pellets and Reared in Saline Media

Struktur Jaringan Ikan Patin (Pangasianodon hypophthalmus) yang Diberi Pakan yang Diperkaya dengan Daun Kenikir (Cosmos caudatus) dan Dipelihara pada Media Bersalinitas

Difa Syafwali[1*], Windarti[1], Efawani[1]
[1]Department of Aquatic Resources Management, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru 28293 Indonesia
email: dsyafwali@gmail.com

Received
20 January 2026

Accepted
25 February 2026

Abstract

Cosmos caudatus is known as an herb that may improve fish health in general. However, the effects of this herb provision on the fish tissue structure have never been studied. To determine any changes in fish tissue provided with C. caudatus-enriched pellets, a study was conducted in June - August 2024. A CRD with 4 treatments: T0 (control), T1 (10 g/kg), T2 (15 g/kg), and T3 (20 g/kg), and 3 replications per treatment was applied. Commercial fish feed pellets were mixed with C. caudatus powder, and this feed was given to the experimental fish 3 times/day, at satiation. The fish was reared in saline media (5 ppt) for 60 days. By the end of the experiment, the tissue was removed and processed for histological study (formalin-fixed, alcohol series processed, 5mm sliced, HE-stained). Results showed that the provision of *C. caudatus*-enriched pellets does not affect the tissue structure of the fish in general, as the tissue structure was almost normal. However, abnormalities were observed in several fish, including the control fish, including hyperplasia and hemorrhage in the gills, glomerular damage and hemorrhage in kidney tissue, and hepatocyte damage in the liver. As the control fish that were not fed C. caudatus showed abnormalities in their tissues, it is predicted that the provision of *C. caudatus* may not cause the damage; instead, it may be related to other factors, such as water salinity. T1 (10 g/kg feed) had the highest survival rate at 96.66%. Based on the data provided, it can be concluded that the provision of *C. caudatus*-enriched pellets does not negatively affect the fish's tissue structure.

Keywords: Herbal feed additives, Fish health, Tissue histology

Abstrak

Daun kenikir (*Cosmos caudatus*) dikenal sebagai herbal yang dapat meningkatkan kesehatan ikan secara umum. Namun, efek pemberian herbal ini pada struktur jaringan ikan belum pernah dipelajari. Untuk mengetahui perubahan jaringan ikan yang diberikan pelet yang diperkaya daun kenikir (*Cosmos caudatus*), penelitian dilakukan pada bulan Juni - Agustus 2024. CRD diterapkan dengan 4 perlakuan, yaitu P0 (kontrol), P1 (10 g/kg), P2 (15 g/kg), dan P3 (20 g/kg), dengan 3 kali ulangan untuk setiap perlakuan sehingga diperlukan 12 unit percobaan. Pelet pakan ikan komersial dicampur dengan bubuk daun kenikir dan pakan ini diberikan kepada ikan percobaan 3 kali/hari, pada saat kenyang. Ikan dipelihara selama 60 hari dan pada akhir percobaan, jaringan diambil dan diproses untuk studi histologis (difiksasi formalin, diproses seri alkohol, diiris 5mm, diwarnai HE). Hasil menunjukkan bahwa pemberian pelet yang diperkaya *C. caudatus* tidak mempengaruhi struktur jaringan ikan secara umum, karena struktur jaringan ikan hampir normal. Namun, ada kelainan pada beberapa ikan, termasuk ikan kontrol, seperti hiperplasia dan pendarahan pada insang, kerusakan glomerulus dan pendarahan pada jaringan ginjal dan juga kerusakan sel hepatosit di hati. Karena ikan kontrol yang tidak diberi makan dengan *C. caudatus* menunjukkan kelainan pada jaringan mereka, diperkirakan bahwa kerusakan tersebut mungkin tidak disebabkan oleh pemberian *C. caudatus*, sebaliknya, itu mungkin terkait dengan alasan lain seperti salinitas air. Tingkat kelangsungan hidup tertinggi (96,66%) ditemukan pada T1 (10 gr / kg pakan). Berdasarkan data yang diberikan, dapat disimpulkan bahwa pemberian pelet yang diperkaya *C. caudatus* tidak berdampak negatif terhadap struktur jaringan ikan.

Kata kunci: Adiftif pakan herbal, Kesehatan ikan, Struktur jaringan ikan

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is a freshwater aquaculture species of high economic importance in Indonesia, due to its favourable taste, thick flesh, and affordable market price, which has driven steady increases in consumer demand. To meet this demand, production has expanded through the use of alternative culture systems, including low-salinity rearing media. This approach offers a practical solution to freshwater scarcity, particularly in coastal regions and abandoned brackish ponds.

Appropriate salinity management has been reported to support fish growth and survival in improving water quality and environmental conditions. However, the transition from freshwater to saline conditions may induce physiological stress in Striped catfish, leading to reduced performance and increased susceptibility to pathogenic infections. Common diseases in pangasius aquaculture are caused by bacterial, viral, and parasitic agents, including *Flavobacterium columnare*, the causative agent of columnaris disease, characterized by lesions on the gills, skin, and fins, as well as *Aeromonas hydrophila*, which infects fish through different pathogenic mechanisms (Susanto et al., 2020; Sutrisno et al., 2022).

Disease prevention strategies in aquaculture commonly include water quality management, stress reduction, and the application of probiotics and immunostimulants (Hartami, 2024). Immunostimulants play a crucial role in enhancing the innate immune response of fish against pathogens (Rahman et al., 2018). Natural immunostimulants are increasingly preferred due to their low cost, availability, and absence of chemical residues (Wijayanto, 2019). Kenikir (*Cosmos caudatus*) leaves have been identified as a promising natural immunostimulant, containing various bioactive compounds including flavonoids, tannins, alkaloids, quinones, and polyphenols (Rahman et al., 2018; Saputra, 2017). In addition to their antioxidant properties, kenikir leaves are rich in vitamins and minerals that support fish growth and overall health. Previous studies have demonstrated that kenikir-based feed can enhance growth performance in fish; however, excessive inclusion levels may exert antinutritional effects and disrupt metabolic processes (Wijayanti et al., 2019).

Despite the growing interest in plant-based immunostimulants, limited information is available regarding the effects of kenikir-based feed on the tissue structure of Striped catfish reared in saline media. Therefore, this study aimed to evaluate the impact of kenikir-supplemented diets on the tissue structure of Striped catfish cultured under saline conditions, with particular emphasis on physiological balance, growth performance, and fish health. The findings of this study are expected to provide scientific evidence supporting the sustainable use of kenikir-based feed in pangasius aquaculture, especially in regions experiencing increasing salinity.

2. Material and Method

2.1. Time and Place

This study was conducted from June to August 2024 at the Integrated Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Riau, Indonesia. The preparation and histological examination of striped catfish samples were carried out at the Fisheries Biology Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

2.2. Methods

The study employed an experimental method using a Completely Randomised Design (CRD) with four treatment levels (T0, T1, T2, and T3). To reduce experimental error, each treatment was replicated three times, resulting in a total of 12 experimental units.

2.3. Procedures

2.3.1. Preparation of Fish Containers and Experimental Fish

The experimental units consisted of 12 plastic buckets with a total capacity of 100 L each. Fish were stocked at a density of 30 individuals per 90 L of water, equivalent to one fish per 3 L. Prior to stocking, all rearing containers were thoroughly cleaned and filled with 90 L of brackish water at 5 ppt salinity. The experimental fish used in this study were striped catfish juveniles measuring 6–7 cm in total length, obtained from a local hatchery in Pekanbaru, Indonesia. Prior to stocking, 30 fish were randomly selected and measured for total length and body weight using a ruler and an analytical balance. During the rearing period, sampling was conducted six times at 10-day intervals. Dissolved oxygen (DO), temperature, and pH were measured prior to feeding. Fish were fed three times daily (morning, afternoon, and evening) using an ad libitum feeding regime, in which feed was provided until the fish no longer exhibited feeding responses.

2.3.2 Preparation of Kenikir

The preparation of kenikir leaf meal–supplemented pellets consisted of two main stages: the production of kenikir leaf meal and its incorporation into fish feed. Kenikir leaves were first sun-dried until completely dry, then ground in a blender to obtain a fine powder. Kenikir-supplemented feed was prepared by mixing the kenikir leaf meal with commercial pellets. In this study, three inclusion levels of kenikir leaf meal were applied, namely 10 g, 15 g, and 20 g per kilogram of feed. The kenikir leaf meal was produced from dried kenikir leaves, where 1 kg of fresh kenikir leaves yielded approximately 30 g of dried leaf meal (equivalent to 3% of fresh weight). Accordingly, 10 g of kenikir leaf meal was equivalent to approximately 330 g of fresh kenikir leaves. The incorporation of kenikir leaf meal into the pellets was carried out using a coating method, in which the pellets were evenly coated with the kenikir leaf meal. This method allowed the powder to adhere firmly to the pellet surface without altering pellet size, thereby maintaining palatability and facilitating consumption by the fish. The coated pellets were air-dried and could be administered directly to the fish or stored in airtight containers under cool, dry conditions prior to use.

2.3.3. Preparation of Fish Tissue Structure

Histological sections of liver, kidney, intestine, gill, and skin were prepared using the method described by Windarti et al. (2017). Tissue samples were fixed in 10% formalin for 24–48 h and subsequently transferred to 4% formalin. The samples were then trimmed to approximately 2–3 mm in thickness. Each sample was placed in filter paper, wrapped with cotton, inserted into sealed plastic clips, and supplemented with 70% ethanol until the cotton was thoroughly moistened. The packaged samples were then placed in portable containers and transported to IPB University for histological slide preparation. The histological processing included the following steps: 1) Dehydration was performed by immersing the tissues in graded ethanol solutions (70% to absolute), each for 1 h, to remove water from the tissue, 2) Clearing was carried out by immersing the samples twice in pure xylene for 1 h each, allowing the ethanol to be replaced by a solvent compatible with paraffin.

3) Embedding began with immersion in a xylene–paraffin mixture (1:1) for 1 h, followed by two immersions in pure paraffin. The tissues were then embedded in moulds and allowed to solidify. 4) Sectioning was performed on a microtome at 5 µm thickness. The sections were floated on a water bath, mounted onto glass slides using glycerin–albumin adhesive, and dried at 45 °C for 24 h. 5) Staining involved re-immersion in xylene, rehydration through decreasing graded ethanol solutions, staining with hematoxylin for 4 min and eosin for 1.5 min, followed by dehydration and mounting with Entellan® New. The prepared slides were stored in an oven for 2–3 days prior to microscopic observation.

The histological parameters evaluated included abnormalities in the intestine, kidney, and gills. Kidney observations focused on the condition of the glomerulus and Bowman's capsule, as well as indications of tissue damage such as glomerulonephritis and tubulonephritis. Intestinal histology was assessed based on villus morphology and goblet cell distribution to compare the effects of kenikir leaf–based diets among treatments. Gill observations were conducted both visually and microscopically, including assessments of color and mucus production, measurements of the spacing and width of secondary lamellae, and the presence of tissue abnormalities. The degree of gill tissue damage was assessed using the Histopathological Alteration Index (HAI) following the method proposed by Poleksic & Mitrovic-Tutundzic (1994), as modified by Lopes & Thomaz (2011). The Histopathological Alteration Index (HAI) was calculated using the following equation:

$$HAI = (1x \sum I) + (10x \sum II) + (100x \sum III)$$

The Histopathological Alteration Index (HAI) values were interpreted according to Windarti et al. (2017): 0-10= Indicated normal organ function; 11-20= Indicated mild organ damage; 21-50= Indicated moderate organ damage ; 51-100= indicated severe organ damage and values >100= Indicated irreversible organ damage.

2.4. Measurement Parameters

2.4.1. Absolute Length Growth

The daily growth rate in length was calculated using a modified version of Busacker's *et al.* (1990) formula:

$$Ph = ((Lt – Lo)/T) \times 100\%$$

Information:

Ph	: Daily length growth (%)	Lt	: Final average length (cm)
Lo	: Initial average length (cm)	T	: Long maintenance (days)

2.4.2. Absolute weight (g)

The formula used to calculate weight growth according to Effendie (2002): W = Wt –Wo

Information:

W : Absolute weight growth (g)
Wt : Final fish weight (g)
Wo : Initial fish weight (g)

2.4.3. SGR (Specific Growth Rate)

Observation of the specific growth rate (SGR) of fish was calculated using the Ogunji et al. (2008) method as follows: SGR = (lnWt-ln Wo)/ t × 100

Information:

SGR	= Specific growth rate (%)	Wo	= Average initial weight of test fish (g),
Wt	= Average final test fish weight (g)	T	= Long maintenance (days).

2.4.4. FCR (Food Conversion Ratio)

According to Effendie (2003), FCR can be calculated using the following formula: FCR = F / (Wt – Wo)

Information:

FCR	: Food Conversion Ratio	F	: Amount of feed given during (kg)
Wt	: Final weight (g)	Wo	: Initial weight (g)

2.4.5. Survival Rate

Effendie (1997) stated that the survival rate can be calculated using the following formula: SR = $\frac{Nt}{No}$ x 100%

Information:

SR = Survival rate (%)
Nt = Number of fish surviving at the end of the experiment (individuals)
No = Number of fish at the beginning of the experiment (individuals)

2.4.6. Feed efficiency (FE)

Feed efficiency indicates the proportion of feed that is effectively utilised by fish relative to the total of feed supplied. Feed efficiency was calculated using the following formula (Zonneveld et al., 1991):

$$EP = \frac{(Wt+Wd)-W0}{F} \times 100\%$$

Information:

FE	: Feed efficiency (%)	Wt	: Final fish biomass (g)
W0	: Early Fish Biomass (g)	Wd	: Initial fish biomass (g)
F:	Total feed supplied (g)		

2.4.7. Water Quality

Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were measured every morning and afternoon, concurrently with fish sampling conducted at 10-day intervals.

2.5. Data Analysis

Histopathological data were analysed using SPSS software and subjected to analysis of variance (ANOVA). When significant differences among treatments were detected ($p < 0.05$), the Student–Newman–Keuls (SNK) post hoc test was applied to determine differences between individual treatments. Data on abnormalities in the liver, kidney, intestine, gills, and skin, as well as water quality parameters, were presented in tables, histological images, and descriptive analyses.

3. Result and Discussion

3.1. Growth Rate and Survival Rate

The rearing of Striped catfish fed kenikir-enriched diets under a recirculating system was conducted for 60 days. During this period, variations were observed in absolute weight gain, absolute length gain, and specific growth rate. On day 60, the absolute weight gain ranged from 20.99 to 24.23 g, while the absolute length gain ranged from 6.86 to 7.23 cm. The data on growth rate and survival rate are presented in the following Table 1.

Table 1. Survival Rate of Striped Catfish treated with Kenikir and Reared in Saline Media

Treatment	Absolute weight (g)	Absolute length (cm)	SGR (%/day)	SR
T0	20.99±0.21[a]	6.87±0.20	2.92±0.10[a]	96.66±3.33[a]
T1	24.23±0.17[d]	7.03±0.27	3.16±0.02[b]	96.11±0.96[b]
T2	22.48±0.18[c]	6.86±0.10	3.01±0.05[a]	94.44±1.93[a]
T3	21.73±0.29[b]	7.23±0.2	2.99±0.01[a]	91.11±3.84[a]

The administration of kenikir leaves at different dietary levels had a significant effect on the length and weight growth of Striped catfish. The group without kenikir supplementation (T0) exhibited the lowest mean body weight and total length, measuring 20.99 ± 0.21 g and 6.87 ± 0.20 cm, respectively. These results indicate that, in the absence of immunostimulant supplementation, fish were unable to achieve optimal growth due to reduced stress-coping capacity under saline rearing conditions. According to Suprayudi et al. (2015), environmental stress without additional nutritional intake can decrease growth efficiency in fish. In contrast, fish fed diets supplemented with kenikir leaf meal demonstrated higher survival rates.

In treatment T1 resulted in the highest absolute length and weight gains (7.03 ± 0.27 cm and 24.23 ± 0.17 g), indicating that dietary supplementation with kenikir leaf powder positively affected the growth of striped catfish. The flavonoid and antioxidant content of kenikir leaves is presumed to enhance immune function and improve nutrient absorption. Appropriate use of herbal additives may also accelerate cell regeneration and support metabolic processes, thereby promoting more optimal fish growth. In treatment T2, the length and weight growth of pangasius catfish were higher than those observed in T0 but remained lower than in T1. This indicates that the dose of kenikir leaf supplementation applied in T2 was not as optimal as that in T1 in stimulating growth performance. The effectiveness of plant-based immunostimulants is highly dependent on dosage and the physiological response of fish (Kusumawati et al., 2020).

In treatment T3, the highest fish length was recorded at 7.23 ± 0.20 cm, with a body weight of 21.73 ± 0.29 g. Although the body weight was lower than that observed in treatment T1, length growth was more pronounced. This indicates a shift in the growth pattern, with fish exhibiting greater increases in body length than in body weight. The metabolic response under treatment T3 was presumed to favour linear growth over weight gain. Overall, treatment T1 performed better than the other treatments, indicating that the optimal dose was achieved with T1. This finding highlights the importance of determining optimal inclusion levels when using feed additives such as kenikirleaves, in order to maximise their physiological benefits without inducing adverse effects. The ANOVA analysis revealed a highly significant difference among treatments ($p < 0.05$). This pattern indicates that the inclusion of kenikir leaves in the diet was not only safe but also enhanced fish growth performance, particularly at the T1 dosage.

The specific growth rate (SGR) reflects the efficiency of fish growth, measured as changes in body weight over time. The results showed that treatment T1 (moderate dose of kenikir leaves) produced the highest SGR on day 60, reaching 3.16 ± 0.02% day^{-1}, which was significantly higher than that of the control group (T0) and treatment T3, which recorded values of 2.92 ± 0.10 and 2.99 ± 0.01% day^{-1}, respectively. The Specific Growth Rate (SGR) reflects the efficiency of fish growth, measured as daily weight gain. The treatment without kenikir supplementation (T0) resulted in the lowest SGR (2.92 ± 0.10 % day^{-1}), indicating suboptimal growth, as fish allocated more energy to coping with environmental stress within the rearing system. This finding suggests that, in the absence of functional feed additives such as immunostimulants, growth efficiency tends to decline (Effendie, 2002).

Treatment T1 exhibited the highest daily growth rate of striped catfish (3.16 ± 0.02 % day^{-1}), which was significantly higher than those of the other treatments. This result indicates that the kenikir leaf dosage applied in T1 was the most effective, as its flavonoid and vitamin C contents may enhance immune responses, improve metabolic processes, and reduce osmotic stress, thereby promoting optimal fish growth. In treatment T2, the SGR reached 3.01 ± 0.05 % day^{-1}. Although this value was higher than the control, it was not statistically different from T0 or T3. This suggests that, while growth improvement was observed, the effectiveness of kenikir bioactive compounds at this dosage may have plateaued and was not as pronounced as in T1.

In treatment T3, the SGR was recorded at 2.99 ± 0.01 % day^{-1}, comparable to that of T2. Although fish length in this treatment temporarily showed higher values, the daily growth rate was not as optimal as in T1. This condition was likely not caused by kenikir supplementation itself, but rather by external factors, particularly water quality conditions with salinity levels that induced osmoregulatory stress. Such environmental stress likely led fish to allocate more energy to physiological adaptation and maintenance of internal homeostasis rather than to weight gain. Consequently, although kenikir-based feed did not exert direct toxic effects, unstable water quality appeared to play a more dominant role in suppressing growth rate in treatment T3. The ANOVA results indicated a significant difference among treatments on day 60. Treatment T1 resulted in the highest specific growth rate (SGR), presumably due to the presence of bioactive compounds in kenikir leaves, such as flavonoids and saponins, which enhance feed intake and digestive enzyme activity. These effects likely improved nutrient absorption efficiency and optimised energy utilisation for growth.

The survival rate (SR) is the proportion of fish that survive over a 60-day experimental period. The results showed high SR values across all treatments, ranging from 91.11% to 96.66%, with no statistically significant differences observed. These findings indicate that the use of kenikir leaves, even at the highest inclusion level, did not negatively affect the survival of striped catfish. The ANOVA results indicated that differences among treatments did not have a significant effect on the survival rate of Striped catfish ($p = 0.188$). This finding suggests that kenikir leaf supplementation did not significantly affect fish survival. In other words, all treatments, including both low and high dosage levels, were equally capable of supporting fish survival at comparable rates. These results are consistent with those of Yilmaz et al. (2020), who reported that survival is more strongly influenced by extreme environmental conditions or disease occurrence than by dietary treatments alone, particularly under relatively stable environmental conditions.

3.2. FCR and Feed Efficiency

The food conversion rate (FCR) indicates the efficiency with which feed is converted into fish body weight; lower FCR values reflect higher feed utilisation efficiency. The results showed that the lowest FCR was observed in treatment T1 (1.20 ± 0.02) on day 60, indicating that feed supplemented with a moderate dose of kenikir leaves was the most efficient. In contrast, the highest FCR value was recorded in the control group (T0) at 1.73 ± 0.08 and was significantly higher than those of the other treatments ($p < 0.05$) (Table 2).

Table 2. FCR and Feed Efficiency

Treatment	FCR	Feed efficiency (%)
T0	1.73±0.08[d]	57.62±2.68[a]
T1	1.20±0.02[a]	83.11±1.81[d]
T2	1.36±0.46[b]	73.72±2.37[c]
T3	1.45±0.01[c]	68.77±0.39[b]

In the control treatment (T0) without kenikir leaf supplementation, the feed conversion ratio (FCR) reached 1.73 ± 0.08%, the highest among all treatments. This result indicates low feed efficiency, as more feed was required to increase fish body weight. The low efficiency may be attributed to the absence of bioactive compounds from kenikir leaves that could support digestive processes. According to Sari et al. (2022), compounds such as flavonoids and tannins play an important role in enhancing digestive enzyme activity and intestinal microflora, thereby improving feed utilisation efficiency.

Treatment T1 exhibited the lowest and most favourable FCR value of 1.20 ± 0.02%, which was statistically significantly lower than those of the other treatments, indicating superior feed utilisation efficiency. This improvement is presumably associated with the bioactive components of kenikir leaves, including flavonoids, tannins, saponins, and vitamin C, which function as immunostimulants and antioxidants. These compounds may enhance digestive function and reduce physiological stress, leading to more efficient nutrient absorption (Sari et al., 2022). In treatment T2, the FCR value of 1.36 ± 0.46% indicated an improvement in feed efficiency compared with the control, although it remained inferior to that of treatment T1. This result suggests that kenikir supplementation began to exert a positive effect, although not yet at an optimal level. The relatively high data variability indicates an unstable fish response, possibly due to suboptimal dosage or environmental factors associated with saline rearing conditions (Kusumawati et al., 2020). Treatment T3 showed an FCR value of 1.45 ± 0.01%, which was lower than that of the control (T0) but higher than those of T1 and T2. This finding suggests a decline in feed efficiency at higher supplementation levels. Appropriate dosing is essential to maintain metabolic balance and minimise stress in fish, thereby allowing more efficient feed utilisation (Suprayudi et al., 2015). The consistent significance values supported the formation of homogeneous subsets.

Feed efficiency (FE) indicates the effectiveness of feed conversion into fish body weight. The results showed that treatment T1 exhibited the highest FE on day 60 (83.11%), followed by T2 (73.72%) and T3 (68.77%). In contrast, the control treatment (T0) showed the lowest FE value, at 57.62%. The control treatment (T0) exhibited the lowest feed efficiency, at approximately 57.6%, indicating that more than half of the dietary nutrients were not utilised for growth. This result suggests suboptimal feed utilisation due to the absence of growth-promoting bioactive compounds; moreover, under salinity-induced stress conditions, standard feed alone appears insufficient to support efficient growth.

In treatment T1, kenikir leaves significantly enhanced nutrient utilisation, resulting in the highest feed efficiency (83.1%), which was significantly higher than in the other treatments. This finding indicates that nearly all dietary nutrients were effectively absorbed and allocated to growth. These results are consistent with previous studies reporting that herbal additives such as kenikir leaves can improve digestive function, nutrient absorption, and overall fish growth (Dien et al., 2024). Treatment T2 achieved a feed efficiency of 73.7%, which was higher than the control but remained lower than T1. This suggests that kenikir leaves began to affect feed utilisation positively; however, the response was not yet optimal. This pattern aligns with the dose-dependent nature of herbal additives, whereby intermediate doses produce measurable effects but may not reach maximum efficacy (Fujaya et al., 2023).

Although treatments T2 and T3 showed higher feed efficiency than the control, the highest efficiency was observed in T1, indicating an optimal dosage that maximizes the beneficial effects of kenikir supplementation. Excessive supplementation, as in T3, may reduce feed efficiency by disrupting digestive processes. This observation is in agreement with Sari et al. (2022), who reported that the effectiveness of herbal additives is strongly influenced by dosage and that excessive inclusion does not necessarily enhance performance. Overall, kenikir leaves improved feed efficiency, particularly at the T1 dosage, and are therefore suitable for application in Striped catfish culture.

3.3. Tissue Structure of Striped Catfish

Histological observations revealed that the liver tissue structure of pangasius catfish exhibited varying degrees of damage across treatments. The observed alterations included necrosis, hemorrhage, and changes in the shape and position of hepatocyte nuclei. The severity of tissue damage increased with higher doses of kenikir leaves and was also influenced by water salinity. T0 (kontrol): Liver tissue exhibited severe damage in the absence of kenikir leaf supplementation, including necrosis, hemorrhage, non-centrally located nuclei, and irregular sinusoidal structures.

T1 (10 g/kg): Mild tissue damage was observed. One fish showed a slight hemorrhage, while two individuals exhibited more pronounced alterations. This dosage remained tolerable for the majority of the fish. T2 (15 g/kg): Mild necrosis, tissue disorganization, and hemorrhage were evident. The disturbances became more pronounced, likely due to the combined effects of salinity stress and antinutritional compounds present in the diet. T3 (20 g/kg):The most severe hepatic damage was observed at this treatment level, characterised by extensive necrosis, severe hemorrhage, and perforated tissue structures. These findings indicate substantial physiological stress and metabolic impairment resulting from the interaction between dietary supplementation and water quality. The histological observations are presented in Figure 1. Alterations in liver tissue stricter are presented in Table 3.

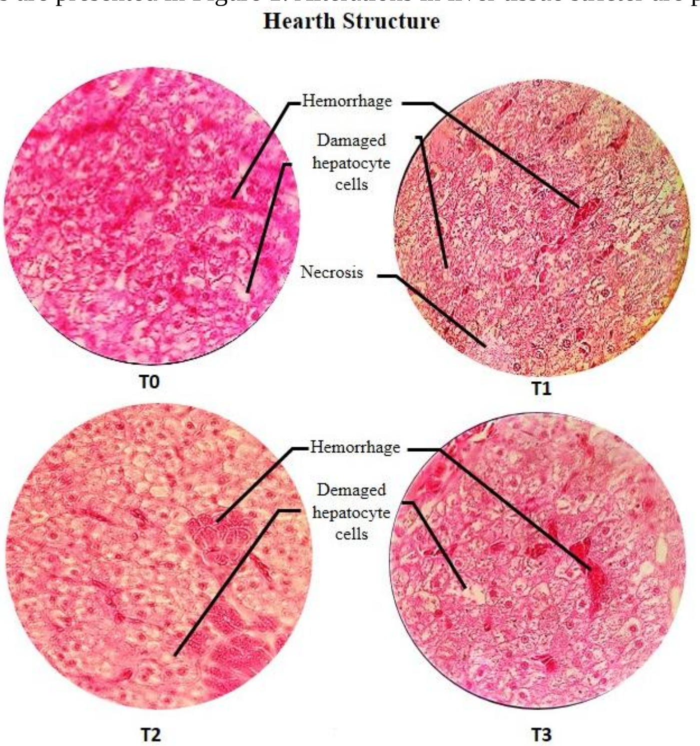

Figure 1. Liver Tissue Structure of Striped Catfish at Day -60; (1000X magnification)
Notes**:** T0: Untreated (control), T_1: 10 g/kg feed, T_2: 15 g/kg feed, T_3: 20 g/kg feed

Table 3. Damage to the Structure of the Liver Tissue of Striped Catfish

No	Abnormal	Treatment			
		T0	T1	T2	T3
1.	Normal liver	-	-	-	-
2.	Nekrosis	-	✓	✓	-
3.	Hemoragi	✓	✓	✓	✓
4.	Damage hepatocyte cells	✓	✓	✓	✓✓

Note: ✓ : Experiencing ilness, - : Not experiencing disease

Histological observations of the liver tissue of Striped catfish revealed that all treatments (T0–T3) exhibited hepatic tissue damage with varying degrees of severity. In T0 (control), despite the absence of kenikir supplementation, necrosis and hepatocyte damage were still observed. Treatment T1 (10 g kg^{-1}) showed mild tissue damage; one sample exhibited mild hemorrhage, while the other two displayed more pronounced alterations, indicating that this dosage was still tolerable. In T2 (15 g kg⁻¹), necrosis, hemorrhage, and tissue disorganisation began to appear, suggesting that stress associated with salinity and antinutritional effects had started to influence liver structure. The most severe damage was observed in T3 (20 g kg⁻¹), characterised by extensive necrosis, severe hemorrhage, and vacuolated hepatocytes, indicating high physiological stress resulting from excessive kenikir dosage combined with elevated salinity. According to Arfah et al. (2022), a healthy kidney structure in freshwater fish is characterised by compact, non-ruptured glomeruli and parallel-arranged tubules without the presence of vacuolisation or inflammatory cell infiltration. This condition indicates that the kidney is still functioning properly as the primary organ for excretion and osmoregulation. The histological observations are presented in Figure 2. Alterations in kidney tissue strictures are presented in Table 4.

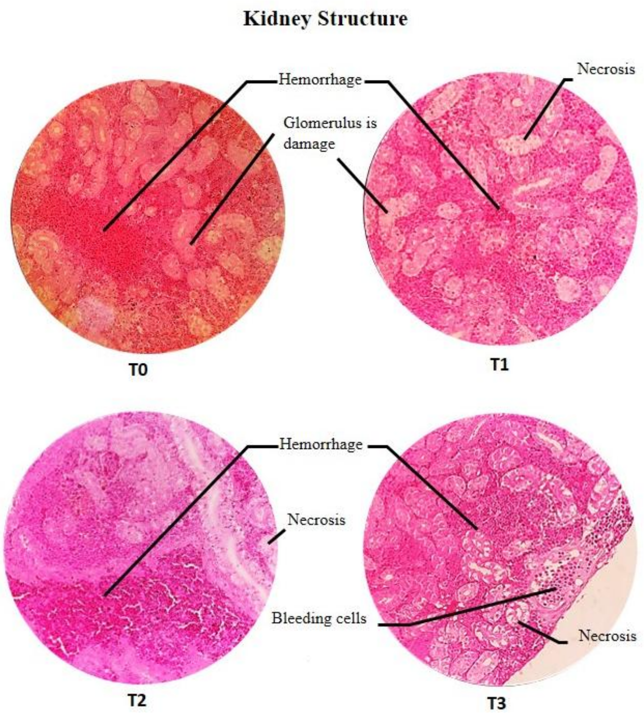

Figure 2. Kidney Tissue Structure of Striped Catfish at day-60; (1000X magnification)
Note: T0: Notes: T0: Untreated (control), T_1: 10 g/kg feed, T_2: 15 g/kg feed, T_3: 20 g/kg feed.

Table 7. Damage to the Structure of the Kidney Tissue of Striped Catfish

No	Abnormal	Treatment			
		T0	T1	T2	T3
1.	Normal kidney	-	-	-	-
2.	Bleeding	✓	✓	✓	✓
3.	Mikrosis	✓	✓	-	-
4.	Damage hepatocyte cells	✓	-	✓	✓

Note: ✓ : Experiencing ilness, - : Not experiencing disease

In T0 (10 g kg⁻¹) at day 60 (H-60), the renal histological structure of striped catfish in the control group (T0) exhibited consistent damage across all three samples. Histological observations revealed the presence of hemorrhage and necrosis, accompanied by bleeding within the glomerular cells, with partial damage to the glomerular structure. This hemorrhagic condition indicates a disruption of the renal blood circulation system, which may impair glomerular filtration function, particularly in saline environments.

In T1 (10 g kg⁻¹ kenikir leaf), mild renal abnormalities were observed, similar to those in the control group (T0), including hemorrhage in the glomerulus and partial tissue damage. These findings indicate that high salinity conditions likely induce physiological stress. In treatment T2 (15 g kg⁻¹), renal damage was more pronounced, characterised by ruptured blood vessels, inflammation, and tissue discolouration. This condition was presumably caused by the combined effects of salinity stress and the irritative properties of bioactive compounds present in the feed, including antinutritional substances such as flavonoids found in kenikir leaves (Yilmaz et al., 2020). Treatment T3 (20 g kg⁻¹) resulted in the most severe renal damage, including intense inflammation, congestion, and extensive necrosis. The high inclusion level of kenikir leaf exacerbated the toxic effects on the kidney under saline water pressure.

The inclusion of kenikir leaf meal in the diet did not show adverse effects on renal tissue condition. The structure of the glomerulus, Bowman's capsule, and renal tubules remained intact in with organisation and cellular integrity preserved. These results indicate that the use of kenikir leaves as an immunostimulant feed ingredient does not induce toxic effects or cause renal tissue damage.

On day 60 of the rearing period, histological observations of the intestinal tissue of striped catfish revealed differences in tissue condition among treatments. In the control treatment (T0, day 60), the intestines of all three observed samples examined at 1000× magnification appeared normal, indicating that in the absence of additional treatments (kenikir leaves or salinity exposure), the intestinal structure remained in a healthy physiological condition. The visualisation of the intestinal condition is presented in Figure 3.

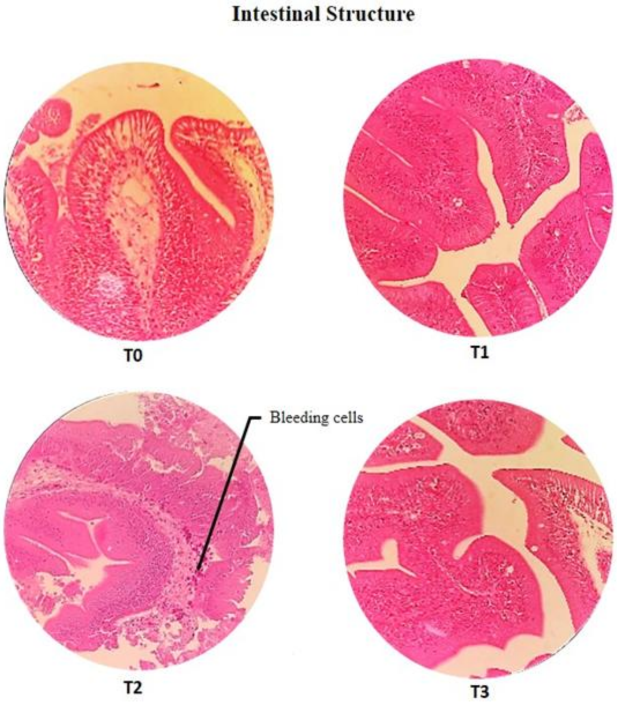

Figure 3. Intestinal Tissue Structure of Striped Catfish at Day -60; (1000X magnification)

Table 5. Damage to the Structure of the Intestinal Tissue of Striped Catfish

No	Abnormal	Treatment			
		T0	T1	T2	T3
1.	Normal	✓	✓	✓	✓
2.	Wound	-	-	-	-

In T1 (10 g kg⁻¹), the intestinal structure of pangasius catfish was observed to be normal. This finding is consistent with the study of Hamid et al. (2023), which reported that low doses of herbal additives can maintain intestinal tissue integrity without inducing pathological alterations. The flavonoid and antioxidant compounds present in kenikir leaves are believed to contribute to maintaining intestinal health, even under varying salinity levels. In T2 (15 g kg⁻¹), one out of three samples exhibited mild hemorrhage, while the remaining two samples showed normal tissue structure. According to Rahman et al. (2023), such hemorrhagic responses may represent an adaptive reaction to a novel diet and do not necessarily indicate pathological conditions. In treatment T3 (20 g kg⁻¹), all samples exhibited normal intestinal structure. This finding indicates that a high dietary inclusion level of up to 20 g kg⁻¹ remains safe and well tolerated by the fish, consistent with the report of Nurjanah et al. (2022), which stated that herbal-based ingredients support intestinal function and nutrient absorption.

Overall, the administration of kenikir at a dose of 20 g/kg did not cause significant intestinal damage. Bioactive compounds such as flavonoids, tannins, alkaloids, and saponins exhibit antioxidant and antimicrobial properties, maintaining the integrity of the intestinal mucosa and reducing inflammation (Wahyuni et al., 2024). The preservation of villi structure and goblet cells further supports efficient nutrient absorption, indicating that kenikir leaves are a safe feed additive for pangasius catfish cultured in saline media (Suhardi et al., 2023).

This study evaluated histopathological changes in the gills of striped catfish induced by the administration of kenikir leaf flour-based feed in saline medium. Due to their direct contact with the environment, gills are highly susceptible to structural damage from salinity stress or from active compounds in the feed. According to Anjani et al. (2023), lesions such as hyperplasia, hypertrophy, and lamellar bending serve as early indicators of osmoregulatory and respiratory disturbances. Although the flavonoid and tannin components in kenikir leaves function as immunostimulants and antioxidants, high doses may induce oxidative stress, damaging the gill epithelium (Yuliani et al., 2022). The results indicated that high-dose kenikir treatments in saline medium caused more severe gill damage than the control. The condition of the gill preparations for each treatment is presented in Figure 4.

In the control group (T0) on day 60, the gills of pangasius catfish generally appeared normal, with lamellae arranged neatly and free from obvious parasites. However, a small proportion of individuals exhibited mild hyperplasia. This condition indicates a mild physiological stress response due to environmental factors, particularly the saline water quality. Hyperplasia represents an adaptive response characterised by an increase in cell number in response to irritation or mild stress, whereas hypertrophy involves cell enlargement under similar conditions (Anjani et al., 2023). Although not considered severe damage, both conditions suggest that the fish were still experiencing stress from the rearing environment. These findings are consistent with those of Yuliani et al. (2022), who reported that suboptimal water quality can induce structural changes in the gills, especially under high-salinity conditions.

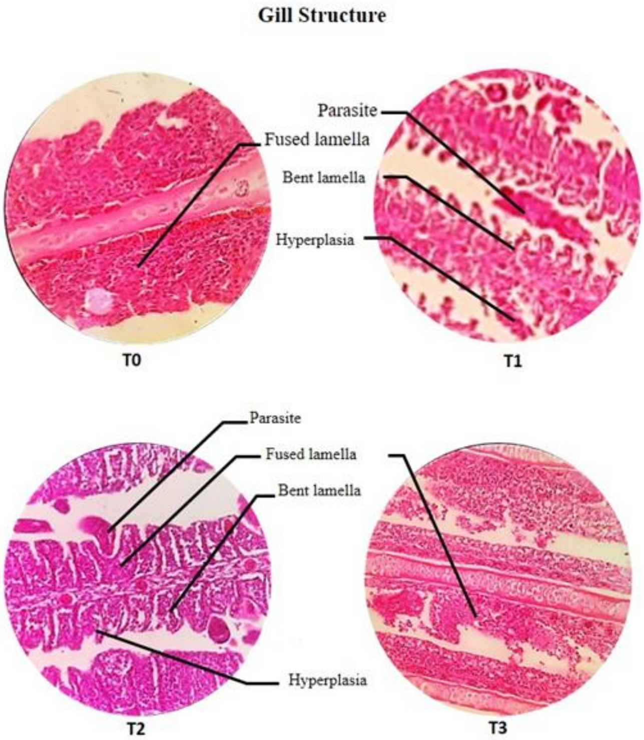

Figure 4. Intestinal Tissue Structure of Striped Catfish at Day-60; (1000X magnification)

Table 6. Damage to the Structure of the Gill Tissue of Striped Catfish

No	Abnormal	Treatment			
		T0	T1	T2	T3
1.	Normal	-	-	-	-
2.	Hyperplasia	✓	-	✓	✓
3.	Hypertropes	✓	✓	✓	✓
4.	Parasit	-	-	-	-
5.	Mikrosis	-	✓	-	-
6.	Fused Lamella	-	-	✓	✓

Histological examination of pangasius catfish gills revealed varying levels of damage across the kenikir leaf supplementation treatments. In T1 (10 g/kg), gill damage was moderately severe, with samples exhibiting hyperplasia, hypertrophy, microsis, and parasites. The observed microsis indicated significant disruption of epithelial cells, potentially impairing respiratory function. T2 (15 g/kg) was dominated by hyperplasia, with no parasites detected in samples 1 and 3. However, sample 2 showed severe hyperplasia and hypertrophy, along with lamellar bending, which could hinder efficient gas exchange. T3 (20 g/kg) showed the most severe damage, including a combination of hyperplasia, hypertrophy, lamellar bending, and heavy parasite infestation in some samples, except for sample 3, in which no parasites were detected.

These gill structural damages suggest that high doses of kenikir leaves may induce physiological stress and impair the respiratory function of pangasius catfish (Windarti et al., 2017). Observed tissue alterations included hyperplasia, hypertrophy, and lamellar deformation. Across treatments to T3, these abnormalities were likely caused by a combination of salinity stress, parasite presence, and adaptive responses to the environment.

Hyperplasia was interpreted as a defensive response of the gills to chronic irritation, characterised by thickening of secondary lamellar epithelial cells, potentially reducing gas exchange efficiency (Nugroho et al., 2023). Hypertrophy reflected epithelial cell adaptation to environmental stressors, including exposure to toxins or parasites (Supriatna et al., 2022). Lamellar bending was associated with excessive osmotic pressure, which disrupted supporting gill tissue and thereby impaired respiration. The presence of parasites, particularly in treatments T0 to T3, supports findings by Wijaya et al. (2022) that parasitic infections not only damage tissue but also trigger inflammation and hyperplasia as a defence mechanism. According to Permana & Santoso (2024), the combination of hyperplasia, hypertrophy, and lamellar structural changes is indicative of advanced physiological disturbances, potentially reducing respiratory efficiency by 40–60%. The gill alteration index (GAI) for each treatment is presented in Table 7.

Table 7. PAI Gill Tissue Structure day-60

Treatment	Number HAI
T0	2
T1	34
T2	14
T3	46

Based on the Histopathological Alteration Index (HAI), differences in gill tissue damage were observed among the treatments on day 60. The normal control group (T0) exhibited healthy tissue with the lowest HAI value (2). Treatment T1 (10 g/kg feed) showed moderate to severe damage (HAI = 34), whereas T2 (15 g/kg feed) exhibited the mildest tissue damage among the treatment groups (HAI = 14). In contrast, T3 (20 g/kg feed) showed damage approaching severe levels (HAI = 46). This phenomenon is supported by the findings of Ibrahim et al. (2023), who reported that high doses of kenikir leaves may act as pro-oxidants and induce oxidative stress in gill tissues. Furthermore, Rahman and Hadiroseyani (2023) stated that the bioactive compounds in kenikir leaves can accumulate and become toxic at high doses over time. Therefore, the optimal dose to minimise gill damage was observed in T2.

Histological examination of striped catfish skin on day 60 revealed normal, uniform tissue across all treatments. In the control group (T0), all samples exhibited intact epidermal and dermal structures without abnormalities such as necrosis, edema, or inflammatory cell infiltration. This indicates that the applied treatments, including kenikir leaf supplementation and salinity exposure, did not cause microscopic damage to the skin tissue. Figure 5 presents representative skin preparations from each treatment, supporting these findings (Windarti et al., 2017).

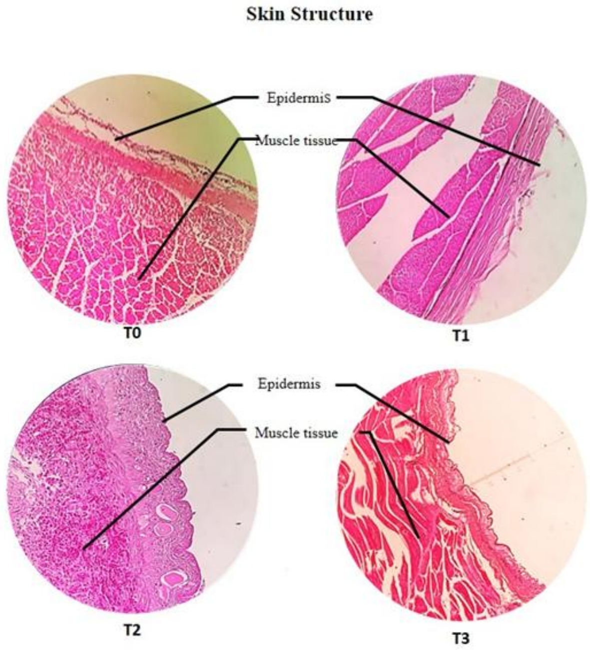

Figure 5. Tissue Structure of Striped Catfish Skin Day-60; (1000X magnification)

On day 60, the histological condition of the skin in pangasius catfish from all treatments (various doses of kenikir leaf powder and different salinity levels) showed normal epidermis, dermis, and hypodermis structures without any damage. This indicates that the treatments did not compromise skin integrity. According to Suryaningsih et al. (2022), healthy fish skin is characterised by an intact epidermal layer, well-organised structure, and absence of mucosal cell or pigment damage. These findings are supported by Rahmawati & Fauzi (2023), who reported that pangasius skin is resistant to environmental changes due to its thick structure and protective mucosal layer.

Therefore, the doses of kenikir leaf powder used in this study did not have a negative impact on skin tissue. However, the lack of significant structural changes suggests that the skin is not the primary target organ in response to these treatments. This aligns with Hartono & Suhartono (2023), who noted that histopathological alterations largely depend on the exposure pathway, duration, and target organ. In this study, organs such as the gills and digestive system are more likely to exhibit changes because they directly interact with the feed and the surrounding environment.

3.4. Water Quality

During the study period, water quality parameters, including temperature, pH, and dissolved oxygen (DO), remained within ranges suitable for the maintenance of Striped catfish. Detailed water quality data are presented in Table 8.

Table 8. Water Quality

Parameters	Days-1	Days-30	Days-60
Temperature ^{0}C	26-27	27-28	27-29
pH	7,2-7,5	7,2-7,7	7,2-7,6
DO (ppm)	6,4-7,6	3,7-5,4	3,8-5,2

During the study, water temperature ranged from 26 to 28°C, pH from 6.8 to 7.5, and dissolved oxygen (DO) from 5.1 to 6.7 mg/L. These values were still within the optimal range for maintaining healthy striped catfish, according to Effendi (2022). No significant differences in water quality were observed among the treatments (T0, T1, T2, T3), indicating that the administration of kenikir leaves did not affect the environmental conditions. However, by day 60, although temperature, pH, and DO appear stable, histopathological analysis revealed severe damage to the liver and kidneys, primarily due to reduced DO levels (down to 3.8 mg/L), salinity adaptation, and the fish's physiological response to feed compounds.

This indicates that water quality, even when physically and chemically optimal, does not necessarily reflect the fish's overall internal health. Therefore, evaluations should also include physiological and histological observations. Stable water quality further supports maximum growth and survival, particularly in treatments T1, T2, and T3, which were fed kenikir leaves (Rahman et al., 2023; Yilmaz, 2020). Under uniform environmental conditions, the growth-promoting effects observed in the treatment groups can be more directly attributed to the feed rather than differences in water quality.

3.5. Fisheries Resources Management

This study highlights the importance of fisheries resource management that considers the interaction between environmental quality, particularly salinity, and natural feed such as kenikir leaves in striped catfish aquaculture systems. The results indicated that the inclusion of kenikir leaf powder up to 20 g/kg was non-toxic to intestinal tissues, whereas liver and kidney damage was primarily influenced by increased salinity rather than by the feed. This suggests that physiological stress induced by salinity is the main factor affecting organ integrity (Suprapto et al., 2023). Kenikir leaves, which contain bioactive compounds such as flavonoids and antioxidants, help mitigate oxidative stress and enhance fish immune function. Furthermore, water quality parameters, including temperature, pH, and dissolved oxygen, remained within optimal ranges; however, fluctuating salinity continued to exert negative effects on vital organs (Purwanti & Idris, 2022).

The use of kenikir leaves is also considered economical, as it reduces costs and reliance on commercial feed, aligning with locally adaptive approaches in sustainable aquaculture (Fitriani et al., 2022). This study reinforces the concept that aquaculture efficiency should be achieved through the synergy of natural feed and environmental control within an ecosystem-based management strategy.

4. Conclusions

Based on the results of this study, it can be concluded that the inclusion of kenikir leaves in the feed had a positive effect on the tissue structure of striped catfish, particularly the intestinal and skin tissues, as all treatments showed normal tissue conditions. The intestines exhibited well-organised villi and round-to-oval-shaped goblet cells. However, some abnormalities were observed in the liver and kidney tissues, including inflammation, hemorrhage, and cell necrosis, in both fish fed kenikir leaf powder and those without it. These findings indicate that salinity plays a more significant role in organ damage than the inclusion of kenikir leaf powder in the feed. The administration of kenikir leaves at a dose of 10 g/kg feed (T1) was found to be the most effective in maintaining organ health and feed efficiency without causing tissue damage. However, given the water quality conditions in the management of saline rearing media, the kenikir leaf dosage in the feed must be carefully balanced to achieve optimal, sustainable culture outcomes

5. References

Anjani, N. P., Widodo, A., & Lestari, S.D. (2023). Kerusakan histopatologi insang ikan akibat peningkatan salinitas dan paparan bahan toksik. *Jurnal Akuatik Tropis,* 11(1): 45–53.

Arfah, N.R., Prasetyo, D.A., & Yuliani, E. (2022). Histopatologi organ ginjal ikan nila (*Oreochromis niloticus*) akibat pajanan pestisida organofosfat. *Jurnal Biologi Tropis,* 22(1): 15–22.

Busacker, G.P., Adelman, I.R., & Goolish, E.M. (1990). Growth performance, nutrient utilization of Nile tilapia (*Oreochromis niloticus*) fed housefly manggot meal diets. *Turkish Journal of Fisheries and Aquatic Science*, 8(1): 141-147.

Dien, N.T., Le., T.T., Doan, T.T., & Tran, M. T. (2024). Effects of herbal ghytobiotics on growth performance, feed efficiency, and immunity in freshwater fish: A meta-analysis. *Science of the Total Environment, 905*, 167957.

Effendi, I. (2002). *Probiotics for marine organism disease protection*. Fakultas Perikanan dan Ilmu Kelautan Universitas Riau. Pekanbaru.

Effendi, I., Yoswaty, D., Syawal, H., Austin, B., Lyndon, A.R., Kurniawan, R., & Wahyuni, S. (2022). Effects of herbal growth promoters on common carp (*Cyprinus carpio*). *AACL Bioflux*, 15: 1096-1103.

Effendie, M.I. (1997). *Biologi perikanan*. Yayasan Pustaka Nusatama.Yogyakarta.

Effendie, M.I. (2002). *Biologi perikanan*. Yayasan Pustaka Nusatama. Yogyakarta.

Effendie, M.I. (2003). *Metode biologi perikanan*. Yayasan Dewi Sri Bogor. 112 p.

Fitriani, D., Saputra, A., & Hamdani, H. (2022). Analisis Histopatologi Hati Ikan Lele (*Clarias sp.*) yang Terpapar Limbah Cair Industri. *Jurnal Akuakultur Tropis*, 10 (1), 45-52.

Fujaya, Y., Hamka, N., & Muthmainnah, D. (2023). Pemanfaatan fitobiotik dalam meningkatkan efisiensi pakan dan daya tahan tubuh ikan. *Jurnal Akuakultur Tropis*, 11(2): 85–93.

Hamid, A., Kusnandar, F., & Pratama, R.I. (2023). The Effect of herbal supplementation on intestinal histopathology and growth performance of *Pangasianodon hypophthalmus*. *Aquaculture Research*, 54(4): 1242-1256.

Hartono, R., & Suhartono, E. (2023). Evaluasi histologi organ target pada ikan air tawar yang mengalami stress lingkungan: Sebuah tinjauan komprehensif. *Jurnal Iktiologi Indonesia*, 23 (1): 67-82.

Kusumawati, E. (2020). Efektivitas ekstrak herbal terhadap imun dan pertumbuhan ikan nila. *Jurnal Akuatika*, 11 (2): 112–118.

Lopes, F. & Thomaz, A.T. (2011). Histopathological alterations observed in fish gills as an environmental monitoring. Braz. *J. Biol*., 71(1):179-188.

Nugroho, E., Hadiaty, R.K., & Rahardjo, M.F. (2023). Perubahan histopatologi insang ikan nila (*Oreochromis niloticus*) yang terpapar logam berat: Analisis mikroskopik dan implikasinya dalam budidaya berkelanjutan. *Jurnal Iktiologi Indonesia*, 23(2): 189-203.

Nurjanah, S., Widanarni, W., & Setiawati, M. (2022). Histological evaluation of digestive organs of striped catfish (*Pangasianodon hypophthalmus*) fed with an herbal supplemented diet under different salinity levels. *Indonesian Journal of Aquaculture*, 21 (2): 98-107.

Ogunji, J., Toor, RS., Schulz, C., & Kloas, W. (2008). Growth performance, nutrient utilization of Nile Tilapia (*Oreochromis niloticus*) fed housefly manggot meal diets. *Turkish Journal of Fisheries and Aquatic Science*, 8 (1), 141-147.

Pandala, C. (2018). *Efektivitas ekstrak daun kenikir dan daun sirih sebagai biofungisida terhadap penyebab penyakit antraknosa (Colletotrichum capsici) pada tamanam cabai merah (Capsicum annuum L.) secara in vitro.* Universitas Medan Area.

Permana, A., & Santoso, L. (2024). Evaluasi Histopatologi Insang Sebagai Indikator Kualitas Air Dalam Sistem Budidaya Ikan Air Tawar. *Jurnal Perikanan dan Kelautan*, 29(1): 45-58.

Purwanti, D., & Idris, M. (2022). hubungan kualitas air terhadap pertumbuhan dan kelangsungan hidup ikan air tawar dalam sistem budidaya intensif. *Jurnal Akuakultur Nusantara*, 8 (1): 33–40.

Rahman, A., & Hadiroseyani, Y. (2023). Antioxidant properties of *Cosmos caudatus* and its effects on intestinal health of striped catfish under different salinity levels. *Journal of World Aquaculture Society*, 54 (1): 234-247.

Rahman, A., Prasetyo, H., & Nugraha, D. (2018). *Efektivitas tanaman herbal sebagai imunostimulan alami dalam akuakultur.* Pustaka Akuakultur. Yogyakarta.

Rahmawati, D., & Fauzi, A. (2023). Respons histologis kulit ikan patin (*Pangasianodon hypophthalmus*) terhadap perubahan parameter lingkungan budidaya. *Jurnal Perikanan dan Kelautan*, 28 (1): 112-123.

Saputra, D. (2017). *Tanaman herbal Indonesia dan pemanfaatannya dalam pangan dan kesehatan*. Penerbit Bahari. Jakarta.

Sari, D.P., Yuliani, E., & Kurniawan, A. (2022). Pengaruh senyawa bioaktif daun kenikir terhadap efisiensi pencernaan ikan air tawar. *Jurnal Biotek Akuatika*, 9(2): 67–74.

Setiawan, D. (2018). *Kandungan gizi dan keunggulan daging ikan patin sebagai sumber protein*. Penerbit Samudera. Yogyakarta.

Suhardi, S., Agustono, A., & Lamid, M. (2023). Intestinal histology and absorptive capacity of *Pangasianodon hypophthalmus* fed with different dietary supplements: A comprehensive review. *Reviews in Aquaculture*, 15(2): 689-705.

Suprapto, R., Lestari, T.N., & Firmansyah, M.R. (2023). Pemanfaatan pakan fungsional berbasis herbal dalam pengelolaan budidaya perikanan ramah lingkungan. *Jurnal Teknologi Akuakultur*, 11(1): 22–30.

Suprayudi, M.A. (2015). Strategi pakan dan manajemen stres pada ikan budidaya. *Jurnal Perikanan*, 6(2): 123–130.

Supriatna, I., Setiawati, M., & Jusadi, D. (2022). Kajian perubahan struktur lamela insang ikan lele (*Clarias sp*.) akibat paparan amonia pada sistem budidaya intensif. *Jurnal Akuakultur Indonesia*, 21(2): 112-123.

Suryaningsih, S., Windarti, W., & Yusfi, M. (2022). Struktur Histologi normal kulit dan insang pada beberapa jenis ikan air tawar konsumsi. *Jurnal Biologi Tropis,* 22(1): 169-177.

Susanti, D., Putri, W.P., & Ramadhan, F. (2021). Efektivitas ekstrak herbal dalam meningkatkan LPS ikan nila (*Oreochromis niloticus*). *Jurnal Perikanan dan Kelautan Nusantara*, 13(1): 45–52.

Susanto, D., Nugroho, B., & Saputra, F. (2020). *Patogen ikan air tawar dan strategi pengendaliannya*. Penerbit Laut Biru. Malang

Sutrisno, D., Kurniawan, T., & Lestari, S. (2022). Infeksi *Flavobacterium columnare* pada ikan budidaya. Penerbit Samudera. Jakarta.

Wahyuni, D., Setiawati, M., & Suprayudi, M.A. (2024). Antioxidant and antimicrobial properties of *Cosmos caudatus* leaf extract and its Application in Aquafeed. *Journal of Applied Psychology*, 36(1): 45-58.

Wijaya, A., Lusiastuti, A.M., & Taukhid. (2022). Identifikasi parasit dan dampaknya terhadap histopatologi insang pada budidaya ikan air tawar di Jawa Barat. *Jurnal Riset Akuakultur*, 17(3): 203-214.

Wijayanti, L., Kurniawan, B., & Prasetyo, H. (2019). *Peningkatan performa ikan melalui suplementasi pakan berbasis estrak daun kenikir.* Universitas Perikanan Nusantara. Surabaya

Wijayanti, M. (2021). *Identifikasi dan penanggulangan penyakit MAS pada ikan patin*. Penerbit Akuakultur Sejahtera. Medan.

Wijayanto, B. (2019). *Keunggulan imunostimulan alami dalam meningkatkan imunitas ikan budidaya*. Penerbit Laut Biru. Bandung.

Windarti, W., Simarmata, A.H., & Eddiwan, E. (2017). *Buku ajar histologi*. UR Press. Pekanbaru.

Yilmaz, M., Ergün, S., & Tural, S. (2020). The effects of herbal feed additives on survival and growth performance in fish: A meta-analysis. *Aquaculture*, 529

Yuliani, A., Rosyid, A.N., & Nurrahman, R. (2022). Efek toksik tanin terhadap sel tubulus ginjal pada ikan nila (*Oreochromis niloticus*). *Jurnal Bioteknologi & Akuakultur,* 10(1): 22–29.

Zonneveld, N., Huisman, E.A., & Boon, J.H. (1991). *Budidaya ikan.* Gramedia