

## ***Effect of palm kernel meal level on feed intake, body composition, and hepatic histopathology features of Kelabau (*Osteochilus melanopleurus*)***

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**Abstract.** *The effect of including palm kernel meal (PKM) on the feed intake, body composition, and hepatic histopathology of Kelabau (*Osteochilus melanopleurus*) was experimentally studied in a 50-day feeding trial. Four experimental diets were formulated in dry pellet form with a protein content of approximately 30%. The treatments consisted of varying PKM concentrations (0%, 5%, 15%, and 20%). The experimental fish were reared in 12 aquaria (30 x 30 x 40 cm<sup>3</sup>) and fed to satiation three times daily. A Completely Randomized Design (CRD) was employed for this research. Results indicated that the treatments had no significant effect ( $P > 0.05$ ) on total feed intake. However, feed consumption tended to decrease as PKM content increased. Furthermore, PKM inclusion significantly influenced body composition; higher PKM levels led to a sharp increase in Nitrogen-Free Extract (NFE), while simultaneously reducing body protein and lipid content. Additionally, increased PKM levels were observed to cause hepatic lipid accumulation and the displacement of cell nuclei to the periphery.*

**Keywords:** *Body composition, Histopathology, Kelabau, Palm Kernel Meal*

**Abstrak.** Pengaruh penyertaan bungkil inti sawit terhadap konsumsi pakan, komposisi tubuh, and histopatologi ikan Kelabau (*Osteochilus melanopleurus*) dipelajari secara eksperimental dalam uji coba pemberian makan selama 50 days.. Empat pakan uji dibuat dalam bentuk pelet kering dengan kandungan protein  $\pm 30$  %. Perlakuan dalam penelitian ini konsentrasi bungkil inti sawit (0%, 5%, 15, dan 20%). Ikan uji dipelihara dalam 12 akuarium berukuran 30 x 30 x 40 cm<sup>3</sup> selama 50 hari. Pakan uji diberikan secara at satiation tiga kali sehari Rancangan penelitian yang digunakan rancangan acak lengkap (RAL). Hasil analisis menunjukkan bahwa perlakuan tidak berpengaruh nyata ( $P > 0,05$ ) terhadap jumlah konsumsi pakan. Meskipun demikian, semakin tinggi kandungan bungkil sawit semakin rendah jumlah konsumsi pakan. Selain itu, bungkil inti sawit juga mempengaruhi komposisi tubuh dimana semakin tinggi kandungan bungkil inti, kandungan BETN tubuh ikan meningkat tajam. Sebaliknya, bungkil inti sawit dalam pakan menurunkan kandungan protein dan lipid tubuh ikan. Penemuan lain dalam penelitian ini adalah peningkatan kandungan bungkil sawit teramat menyebabkan penumpukan lemak di hati serta mendorong inti sel ke tepi atau bahkan keluar sel.

**Kata kunci :** *Bungkil Inti Sawit, Histopatologi, Kelabau, Komposisi Tubuh*

### **INTRODUCTION**

The distribution of Kelabau (*Osteochilus melanopleurus*) spans several freshwater systems in Asia, particularly the Mekong and Chao Phraya River basins and Peninsular Malaysia. In Indonesia, this species is frequently found in Kalimantan and Sumatra (Saenin, 1984; Roberts, 1989; Kottelat et al., 1993; Asiah et al., 2022; Syahputri et al., 2025). It is known by several local trade names, including Trey Krom (Cambodia), Pan nok khao (Laos), and Aro, Kalabau padi, or Kalabau (Indonesia) (Fishbase, 2018). This **species** has significant potential for large-scale cultivation due to its relatively high market price, coupled with its limited availability and production volume (Kristanto et al., 2018).

In aquaculture, feed availability is critical. Feed serves as the primary nutrient source; if nutrient content is insufficient, fish growth will be stunted. Artificial feed is formulated by mixing various animal and vegetable raw materials, taking into account the nutritional requirements and the nature of the target species. However, high production costs remain a major obstacle for farmers, as feed prices can account for 35–60% of total production costs (Sutikno, 2011). Therefore, it is necessary to identify alternative feed ingredients that are nutrient-dense, cost-effective, and abundant.

One potential ingredient is palm kernel meal (PKM), which is widely available. East Kalimantan is one of the largest palm oil-producing provinces in Indonesia. According to the Indonesian Central Statistics Bureau (2015), there are 1.02 million hectares of oil palm plantations in East Kalimantan, a figure that increases annually. Data from the East Kalimantan Plantation Service shows that 17.7 million tons of Fresh Fruit Bunches (FFB) were processed in 2020, equivalent to 3.8 million tons of Crude Palm Oil (CPO). The palm oil industry generates a massive amount of waste that is currently underutilized, including palm kernel meal. Mathius and Sinurat (2001) estimate that each

bunch produces approximately 2% palm kernel cake. Given its nutritional profile, PKM serves as a viable alternative ingredient for fish feed.

According to Pamungkas et al. (2011). PKM is a byproduct of the palm oil industry that has been successfully used as animal feed, including for several fish species. It is available in large quantities, is inexpensive, and contains adequate nutrients. Reported protein content ranges from 13.6% to 17.45% (Orunmuyi et al., 2006), with fat content between 1% and 21.55% (Hadadi et al., 2007). Proximate analysis of the PKM used in this research showed a protein content of 18.64% and a fat content of 10.74%.

Experiments using PKM as a feed substitute have been conducted on several species. In catfish, the inclusion of up to 8% PKM resulted in feed digestibility, protein retention, and growth rates that were not significantly different from the control (Abidin, 2006). Similar results were observed in Tilapia (*Oreochromis niloticus*) by Rusmayati et al. (2017). In milkfish, feed containing 16% PKM (with 26.56% protein) resulted in better growth compared to commercial feed (Zulfahmi et al., 2019). Furthermore, Kader et al. (2018) reported that 30% of fish meal could be replaced with PKM in giant prawn (*Macrobrachium rosenbergii*) feed without adverse effects. Recent studies have also demonstrated the efficacy of fermented PKM in tilapia (Simarmata et al., 2024) and giant gourami (Inez et al., 2024; Zakariyah et al., 2025).

However, research on the utilization of PKM specifically for Kelabau (*O. melanopleurus*) has not yet been conducted. This study, therefore, aims to analyze the effects of PKM inclusion on the feed intake, body composition, and hepatic histopathology of Kelabau.

## MATERIALS AND METHODS

### Fish Rearing

The experiment was conducted from October 2022 to December 2022 at the Local Fish Development Laboratory, Faculty of Fisheries and Marine Sciences, Mulawarman University. The Kelabau fry were obtained from the freshwater hatchery in Mandiangin, South Kalimantan. The fish were transported to the laboratory and acclimated in  $2 \times 1 \text{ m}^2$  tanks for feed and environmental adaptation. After acclimation, twenty fish were randomly stocked in each  $30 \times 30 \times 40 \text{ cm}^3$  aquarium. The feeding trial was conducted for fifty days using a recirculating aquaculture system (RAS). During the experimental period, the fish were fed to apparent satiation three times daily.

### Experimental Design

This study employed a Completely Randomized Design (CRD) with four treatments and three replicates. The treatments consisted of different concentrations of palm kernel meal (PKM) in the feed formulations: P1 (5% PKM), P2 (10% PKM), P3 (15% PKM), and P4 (20% PKM).

### Experimental Diet

A proximate analysis of the feed ingredients was conducted prior to formulation. All ingredients were sieved to achieve a fine texture before weighing. The ingredients were mixed according to the specific formulation for each treatment. Subsequently, the dough was processed into pellets and dried in an oven. The ingredient composition of the experimental diets is shown in Table 1, and the results of the proximate analysis are presented in Table 2.

**Table 1.** Experimental diets composition (%)

Ingredients	Treatments			
	T1	T2	T3	T4
Fish meal	48	46	45	44
Rice Bran	29	26	22	20
Palm Kernel Meal	5	10	15	20
Tapioca	10	10	10	10
Fish oil	2	2	2	2
Vitamin Mix	3	3	3	2
Mineral Mix	3	3	3	2
Total	100	100	100	100

**Table 2.** Results of proximate analysis of experimental diets (% dry weight basis)

Treatments	Protein	Lipid	Nitrogen Free Extract	Crude Fibre	Ash
P1	30.27	24.93	34.44	5.24	24.93
P2	30.60	24.42	34.59	5.77	24.42
P3	30.06	23.61	35.10	6.03	23.61
P4	29.85	23.36	34.65	7.48	23.36

### Body Composition Analysis

For body composition analysis, fish samples from each treatment were randomly collected. Total crude protein was determined using the Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25. Lipid content was determined using the Soxhlet method with hexane as the solvent for six hours. Total ash content was determined by mineralizing the samples in a muffle furnace at 550°C for five hours. Crude fiber was determined through acid-base digestion using and NaOH, followed by drying at 130°C and incineration at 600°C in a furnace.

### Hepatic Histopathological Assay

Histopathological analysis was conducted according to the procedures described by Handari (1983). Liver tissues were fixed by immersion in Bouin's solution for three to twenty-four hours, followed by a washing process using 70% alcohol. The samples were then dehydrated using a graded series of alcohol concentrations. Subsequently, the specimens were cleared in xylol for 24 hours until transparent, infiltrated with a xylol-paraffin mixture, and embedded in paraffin blocks. The blocks were sectioned and stained before being photographed for observation.

### Measurements and Statistical Analysis

Feed consumption for each meal was recorded and calculated as the total amount of feed consumed by the fish during the 50-day trial. The data were subjected to a one-way Analysis of Variance (ANOVA) using Microsoft Excel 2010. Body composition was evaluated based on the proximate analysis of protein, lipids, and ash from the whole body of the fish. Results from the hepatic histopathological observations were analyzed qualitatively and described descriptively.

## RESULTS AND DISCUSSION

### Feed Intake

The experiment, which evaluated diets with varying levels of palm kernel meal (PKM), was conducted over a fifty-day period. Results from the Analysis of Variance (ANOVA) indicated that the inclusion levels of PKM in the experimental diets had no significant impact on total feed intake ( $P > 0.05$ ). Despite the lack of statistical significance, the study observed that T1 (5% PKM) resulted in the highest feed intake (220.06 g), followed by T2 (196.72 g), T3 (192.57 g), and T4 (190.37 g). These findings suggest a downward trend, where feed intake tends to decrease as the palm kernel meal content increases.

A fish's response to feed consumption is governed by both internal and external factors. Fish possess specialized receptors that process information from these factors to determine their feeding behavior. The characteristics of the feed provided are external factors that significantly influence hunger and the drive to forage. Specifically, the flavor and aroma of the feed play a crucial role in palatability and the physiological reaction to consumption. Furthermore, the natural feeding habits of the species also dictate how the fish reacts to the specific diet offered.

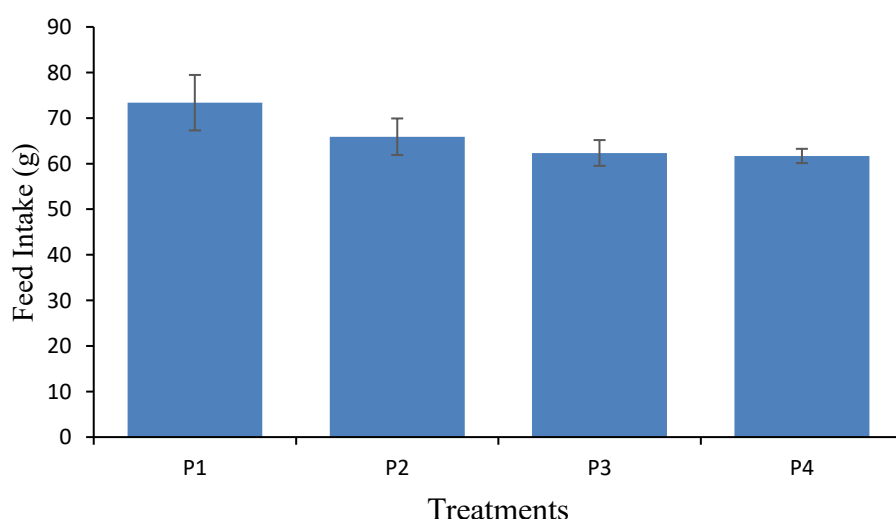


Figure 1. Feed intake for 50 days experiment

Fish do not always consume all the provided feed. The nutritional profile or ingredient composition of a diet is a crucial element that significantly influences the development of appetite, a phenomenon demonstrated in numerous studies. For instance, experiments on rainbow trout (*Oncorhynchus mykiss*) showed that this species can distinguish between diets with differing essential amino acid contents (Yamamoto et al., 2001). Similarly, research

by Vivas et al. (2006) indicated that sharpsnout seabream (*Diplodus puntazzo*) preferred feed with higher protein content and adjusted consumption to meet their energy requirements. Furthermore, Rubio et al. (2009) observed that Senegalese sole (*Solea senegalensis*) selected feeds containing specific macronutrients based on individual physiological needs.

In the present study, the experimental diets contained varying levels of palm kernel meal (PKM). Feeding was conducted to apparent satiation, a method employed to minimize unconsumed feed. Although statistical analysis showed that feed consumption was not significantly affected by the treatments, a clear trend was observed: feed intake decreased as the concentration of PKM increased. This mirrors findings by Abidin (2006), who reported that catfish reduced their feed intake in response to higher PKM concentrations.

As PKM levels rise, the proportional protein contribution from fishmeal decreases while the contribution from PKM increases. It is widely recognized that fish-derived protein typically possesses a superior amino acid profile for meeting the nutritional requirements of species like Kelabau. Additionally, higher PKM inclusion increases the lipid and carbohydrate content, thereby raising the total energy density of the feed. The addition of PKM is also believed to alter the flavor and aroma of the diet. It is hypothesized that these variables—nutritional quality, energy density, and palatability—collectively contributed to the observed decrease in feed intake at higher PKM levels.

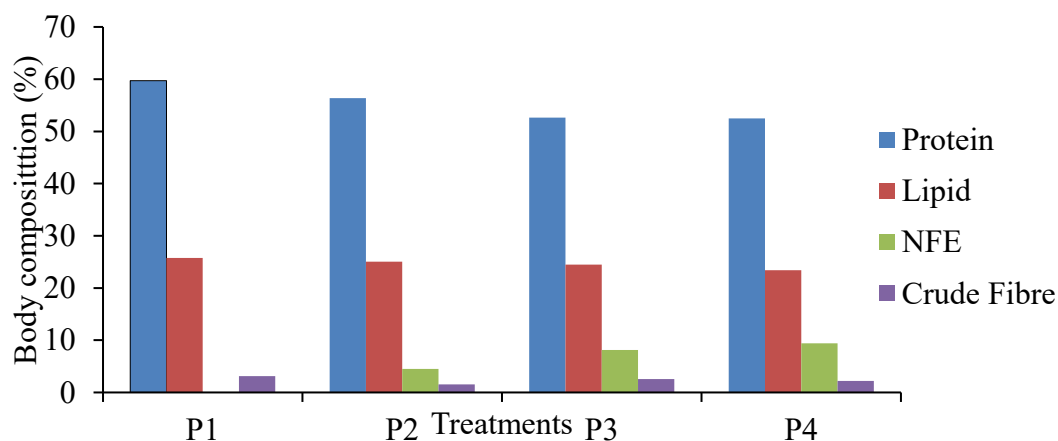
### Body Composition

Table 3 and Figure 2 present the results of the proximate body analysis, detailing the body composition of the experimental fish. The analysis indicates that fish fed diets with higher levels of palm kernel meal (PKM) exhibited lower body protein content. The treatment with 5% PKM (T1) resulted in the highest body protein content (59.71%), followed by T2 (56.37%), T3 (52.62%), and T4 (52.50%), showing a clear downward trend in protein accumulation as PKM inclusion increased.

**Table 3.** Comparison of whole-body composition (% dry weight basis) of experimental fish groups fed diets containing various levels of palm kernel meal.

Treatments	Protein	Lipid	NFE	Crude Fibre
P1	59.71280104	25.76943482	undetected	3.133251791
P2	56.36886774	25.03414194	4.491169011	1.558788275
P3	52.6170606	24.47822721	8.107908039	2.585758614
P4	52.49361372	23.38870493	9.424556433	2.198124064

Observations of body lipid content revealed a decline as the inclusion of palm kernel meal (PKM) in the diet increased. T1 (5% PKM) resulted in the highest lipid content (25.77%), while T4 (20% PKM) exhibited the lowest (23.39%). In contrast, a different trend was observed regarding the Nitrogen-Free Extract (NFE) of the fish body. The NFE content increased sharply in correlation with higher PKM levels. Specifically, the 20% PKM treatment produced the highest NFE content (9.4%), followed by 15% PKM (8.12%), and 10% PKM (4.5%), whereas NFE was undetected in the 5% PKM treatment.



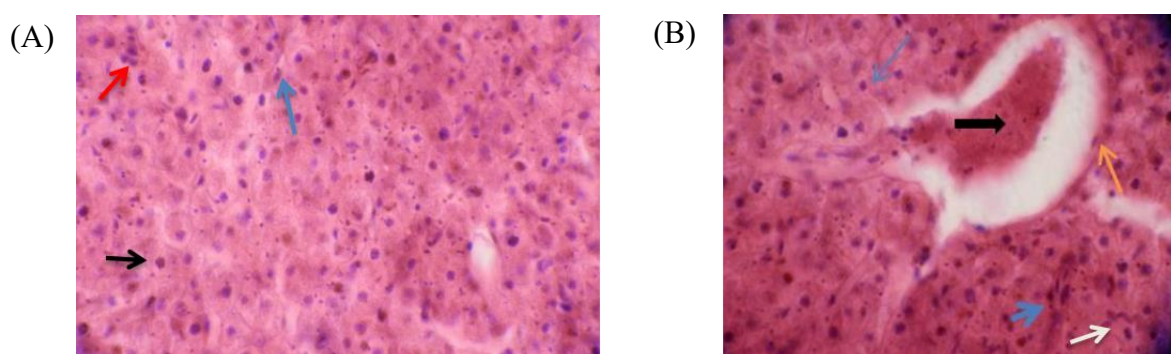
**Figure 2.** Body composition of experimental fish after 50 days rearing



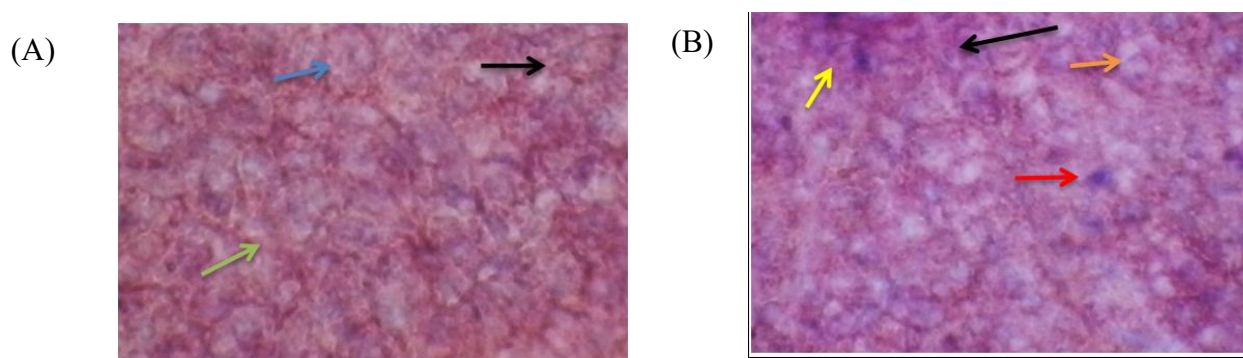
Numerous factors, such as species (Ali et al., 2006), temperature, body size (Cui and Wootton, 1988), and feed (Usman et al., 2010), affect the body composition of fish. In addition to body composition, diet affects fish growth and carcass quality (Roberts & Bullock, 1989; Abbas et al., 2021). The treatment in this research was the inclusion of palm kernel meal (PKM). The observed differences in body composition are thought to be due to differences in the chemical composition of the feed. Even though the experimental feed contains relatively the same protein and lipids, there are differences in the composition of amino acids or fatty acids from animal or vegetable sources.

### Hepatic Histopathological Features

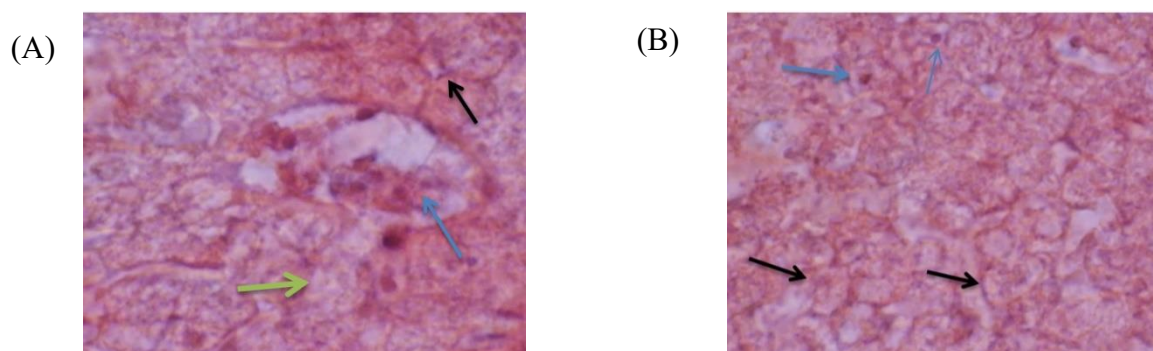
The liver serves as a primary indicator of the nutritional status and overall health of fish, as it plays a central role in metabolism, nutrient storage, and detoxification. In this study, hepatic histopathological examinations were conducted to evaluate the physiological impact of varying levels of palm kernel meal (PKM) inclusion on Kelabau (*O. melanopleurus*). While the liver typically maintains a consistent structure under optimal nutritional conditions, the introduction of alternative feed ingredients can trigger morphological changes, such as lipid accumulation or cellular shifts. The following observations detail the transition from normal hepatic architecture to the specific cellular alterations—including hepatocyte swelling and nuclear displacement—observed across the different experimental treatments.



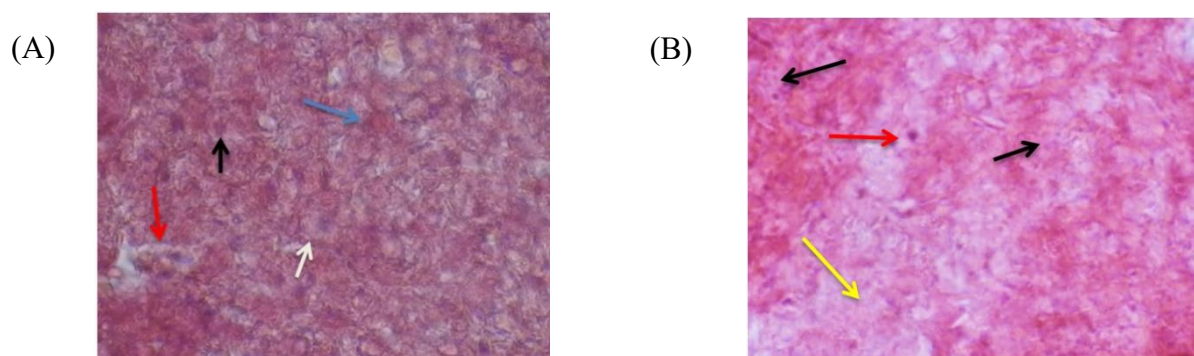
**Figure 3.** Hepatic histology of normal Kelabau (Treatment P1). (A) Typical appearance of normal hepatic architecture: hepatocytes (black arrow), intraluminal blood cells (red arrow), and Kupffer cells (blue arrow). (B) Normal hepatocytes (blue arrow) and erythrocytes (white arrow) within the sinusoids. (Bouin's fixative; H&E staining; magnification 400x; section thickness 6  $\mu\text{m}$ ).



**Figure 4.** Hepatic histology of Kelabau (Treatment P2). (A) Hepatic tissue showing normal architecture with glycogen storage within hepatocytes (blue arrow) and erythrocytes (black arrow). The sinusoids appear normal (green arrow). (B) Increased visibility of lipid droplets; presence of erythrocytes (black arrow), hepatocytes (yellow arrow), and a bile duct (red arrow). Edematous hepatocytes are observed with the cell nuclei displaced to the periphery (brown arrow). (Bouin's fixative; H&E staining; magnification 400x; section thickness 6  $\mu\text{m}$ ).



**Figure 5.** Hepatic histology of Kelabau (Treatment P3). (A) Hepatocytes exhibiting swelling due to lipid accumulation (green arrow); the central vein is congested with erythrocytes (blue arrow); presence of Kupffer cells (black arrow). (B) A marked increase in lipid droplets within hepatocytes (black arrow) alongside areas of relatively normal hepatocytes (blue arrow). (Bouin's fixative; H&E staining; magnification 400x; section thickness 6  $\mu\text{m}$ ).



**Figure 6.** Hepatic histology of Kelabau (Treatment P4). (A) View of the central vein (red arrow); hepatocytes exhibit cell wall thickening, with a high concentration of glycogen storage within the cytoplasm. (B) Presence of pyknotic nuclei (red arrow) indicating early stages of cell death, cloudy swelling (black arrow) as a sign of cellular injury, and fatty degeneration (yellow arrow) within the hepatic tissue. (Bouin's fixative; H&E staining; magnification 400x; section thickness 6  $\mu\text{m}$ ).

Figures 3 to 6 illustrate how varying concentrations of palm kernel meal (PKM) affect the hepatic histology of Kelabau. Beyond fatty degeneration, which causes cell nuclei to be displaced to the periphery and replaced by lipid deposits, additional changes were observed in the liver, such as an increase in glycogen accumulation. These conditions became more pronounced in treatments with higher PKM inclusion.

It is well established that fish, particularly many freshwater species, do not efficiently utilize high levels of carbohydrates for energy. Roberts (2012) states that excess carbohydrates can be difficult to metabolize; when they exceed the fish's energy requirements, they are stored as lipids. Furthermore, PKM contains both fats and carbohydrates that may exceed the physiological needs of the fish, leading to lipid accumulation within the hepatocytes. This buildup causes nuclear displacement to the cell periphery, creating the appearance of tissue vacuoles. These findings suggest that high levels of PKM inclusion may negatively impact liver health by promoting hepatic steatosis (fatty liver), characterized by excessive lipid storage and structural changes in liver cells.

## CONCLUSION

In summary, untreated palm kernel meal (PKM) can be incorporated into the diet of *Kelabau (Osteochilus melanopleurus)* only in limited quantities. The study found that total feed intake decreased as the dietary concentration of PKM increased. Furthermore, higher PKM inclusion levels resulted in a decline in body protein content, while simultaneously increasing the concentration of body carbohydrates (NFE). Histological analysis confirmed these physiological changes, revealing a significant accumulation of glycogen and lipid droplets within the liver tissue as the proportion of PKM in the diet rose

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