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Comparative of Hepatocytes and Intestinal Villi in Zebra Fish (*Danio rerio*) and Guppy Fish (*Poecilia reticulata*): Novel Insights into Species-Specific Fish for Animal Model

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Authors' contributions

This work was carried out in collaboration among all authors. Author IWPS performed the experiments and collected the data. Author ZNF performed the experiments, collected, analyzed, interpreted the data. Author MMPS did data curation, and provided revision to scientific content, and provided stylistic/grammatical revisions of manuscript. Author FSP conceived the idea, and experimental design of the study, provided funding and stylistic/grammatical revisions of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The zebra fish (*Danio rerio*) is widely recognized as a prominent model organism in research. In contrast, Indonesia, with its abundant aquatic resources, frequently encounters the guppy (*Poecilia reticulata*) in its freshwater ecosystems. Notably, guppies exhibit reproductive rates akin to those of zebra fish. This study endeavors to compare the histomorphometric characteristics of the liver and intestinal organs between zebra fish and guppies. The research involved 9 zebra fish and 9 guppies obtained from local breeders in Malang, East Java. Following euthanasia, histological samples were prepared through a series of procedures including fixation, trimming, dehydration, clearing, paraffin embedding, sectioning, Hematoxylin-Eosin staining, and mounting. Histomorphometric observations focused on the diameter and area of hepatocytes, as well as the height and width of intestinal villi. The data were analyzed quantitatively using an Independent Sample T-Test at a 95% confidence level, facilitated by SPSS (Statistical Package for the Social Sciences). The findings revealed statistically significant differences in the histomorphometry of hepatocyte diameter and area, as well as the height and width of intestinal villi between zebra fish and guppies, with a significance value (2-tailed) of <0.05. These differences are attributed to various factors, including habitat, dietary selection and frequency, and physiological adaptations to the environmental conditions of the two species. The findings also contributed to novel insight utilizing these fish to be animal models that guppy fish is more suitable for aquaculture research model and zebra fish likely to more appropriate in human model.

Keywords: Animal models; guppy fish; Hepar; histomorphometry; intestine; zebra fish.

1. INTRODUCTION

In research, human subjects are typically reserved for highly controlled clinical trials, while animals, bacteria, fungi, and plants serve as model organisms due to their ability to represent broader species groups and their genetic modifiability. Model organisms commonly include mice, rats, rabbits, dogs, cats, and fishes, selected for their representational value, high reproductive rates, and ease of maintenance. Zebra fish (*Danio rerio*) have emerged as a prominent model organism owing to their manageable upkeep, rapid life cycle, and prolific reproduction rates [1,2]. Fish, in particular, are favored in research due to their compact size, transparent anatomy, ease of breeding, life cycle similarities, also genomic and homological similarities to humans [2-4]. However, certain limitations exist, such as the differences in organ systems and reproductive strategies compared to mammals, which can lead to disparities in the histology and pathology of human diseases. Zebra fish are characterized by their fusiform, laterally compressed bodies, adorned with distinctive stripe patterns extending from the gills to the tail [5,6].

Indonesia's vast aquatic territory fosters a rich diversity of ecosystems, including a wide variety of fish species. Among them, the guppy (*Poecilia reticulata*), prevalent in the nation's freshwater environments, is notable for its rapid reproductive rate and abundant population. This species inhabits freshwater regions such as ditches, swamps, river mouths, and lakes, and plays a crucial role in controlling malaria by consuming mosquito larvae [7]. Guppies are highly adaptable, thriving in varying weather conditions, temperatures, polluted waters, and different salinity levels. Their bodies are cylindrical and elongated, featuring a small head and a gradually widening midsection [8]. In contrast, the zebra fish (*Danio rerio*) is a well-established model organism in research, valued for its physiological similarities to mammals, particularly in cardiovascular, nervous, endocrine, exocrine, and digestive systems. It is widely utilized for studies on maintenance, disease diagnosis, parasitology, and the impact of parental factors on larval development [5]. Despite its morphological and size similarities to the zebra fish, the guppy, which is common in Indonesia's freshwater habitats, has not been extensively explored as a model organism. Thus, further research is warranted to investigate the morphological and histological characteristics of the guppy as a potential model organism [8].

This study aims to conduct a comparative histomorphometric analysis of the structure, thickness, and width of cells and tissues in the liver and intestines of zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*) [9]. Given their structural, size, and morphological similarities to zebra fish, guppy fish, commonly found in

Indonesia, present themselves as a promising alternative model organism. Fish are frequently employed in the study of hepatic and gastrointestinal disorders, including fatty liver, hepatotoxicity, and intestinal diseases, as the liver serves as a critical biomarker for health, reflecting damage and toxin exposure [3,10,11]. The objective of this study is to evaluate the potential of guppy fish (*Poecilia reticulata*) as an alternatives model for studying liver and gastrointestinal disease, alongside zebra fish (*Danio rerio*).

The selection of the intestines and liver is based on their pivotal roles in digestion and the overall health of the organism [12]. The intestines are often used as models for cancer or tumorigenesis, motility disorders, and detecting the effects of harmful agents that enter the body and affect intestinal function [13]. The liver is frequently used as a model to detect the presence of toxic substances, to study diseases caused by congenital defects, toxic injuries, fatty degeneration in the liver, cancer, and liver regeneration [14]. The use of fish as animal models is highly effective and appropriate for identifying toxic compounds before they are applied to humans. The genetic and digestive tract similarities between fish and humans offer a valuable basis for developing models of human diseases [10]. This study seeks to assess the potential of guppy fish (*Poecilia reticulata*) as an alternative model organism to zebra fish (*Danio rerio*) by comparing the histomorphometry of their liver and intestinal tissues. The findings are anticipated to provide essential insights for further research on guppy fish and to broaden the repertoire of available research models [10,7].

2. METHODOLOGY

2.1 Study Location

The study was conducted in Laboratory of Veterinary Histology, Faculty of Veterinary Medicine, Brawijaya University. The samples consisted of 9 Zebra fish and 9 Guppy fish obtained from Splendid Market, Malang. The fish were transported in plastic bags containing 1000 mL of freshwater.

2.2 Histological Study

The guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*), acclimated in water at 26^{o}C, pH 7 with a light cycle, were then euthanized using the hypoxia method, induced by hypothermic shock at low temperatures (2-4°C) for approximately 10 minutes, until opercular movement ceased [15,16]. Following euthanasia, the fish were preserved in 10% formalin for 24 hours, after which they were decalcified using an 10% EDTA solution [17]. Histological preparations were conducted through a series of processes: fixation, trimming by excising the posterior portion of the body and placing it in a tissue cassette, dehydration using graded ethanol solutions (70%, 80%, 90%, 95%, and absolute ethanol I, II, and III) for 1 hour each, clearing with xylene solutions I, II, and III for 20 minutes each, and paraffin embedding with liquid paraffin at 56°C, repeated three times for 1 hour each. The embedding involved positioning the fish body in a tissue cassette within a metal mold, allowing it to solidify at room temperature. Sectioning was performed by cutting the paraffin block into 5 µm thick tissue sections using a microtome, with sections mounted on glycerin-coated slides. Staining was completed using Hematoxylin-Eosin, followed by mounting according to standard protocols [18,19].

2.2.1 Histomorphometry measurement

Histomorphometric analysis was conducted on the hepatocytes of the liver and the villi of the intestines in both guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*) [9]. Microscopic examinations were performed using an Olympus CX 21 light microscope (Olympus, Germany) at 100x to 400x magnification. Histological images were captured using the Optilab Advance plus® application (Miconos, Indonesia). The histomorphometric data were quantitatively presented, with measurements of the diameter and thickness of the tissues conducted using the ImageJ software (NIH, USA) [20] in micrometers (µm). ImageJ facilitated precise measurement of scale and size by calibrating the dimensions of the organs in the images, converting pixel data to micrometers.

The histomorphometric analysis of the liver included measurements of the hepatocyte diameter and area, calculated by averaging the longest and shortest diameters. The area was determined using the formula for the area of a circle (πr^2) [21]. For the intestinal tissue, histomorphometric data were obtained by measuring the height and width of the intestinal villi throughout the jejunum, ileum, and duodenum. Measurements were taken from the apical tip to the basal crypt of the villi to derive an average value that accurately represents the structure and function of the intestinal morphology. This serves as an indicator of the digestive organ's developmental effectiveness. Greater villi height and width correlate with enhanced nutrient absorption capacity [22-24].

2.3 Statistical Analysis

Data analysis in this study employed a non-experimental, quantitative descriptive approach. The data, derived from histomorphometric measurements of hepatocyte diameter and area, as well as intestinal villi height and width, were presented in a quantitative descriptive format. Each measurement was then analyzed to assess the similarities between guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*) using the Statistical Package for the Social Sciences (SPSS) software. The analyses were conducted using the Independent Sample T-test with a 95% confidence level.

3. RESULTS

3.1 Liver Histomorphometry

The hepatocyte of zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*) show histological differences. Hepatocytes of zebra fish (*Danio rerio*) are polyhedral with a clear cell membrane and organized arrangement with glycogen distribution in the hepatocyte cytoplasm, guppy fish shows polyhedral hepatocytes with varying sizes, irregular arrangement with lipid vacuolization in the hepatocyte cytoplasm or fatty degeneration with clear boundaries and hepatocyte nuclei pushed towards the periphery (Fig. 1). Histomorphometry results revealed significant differences between zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*). Fig. 2 show the histomorphometric depiction of hepatocytes diameters and areas between zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*). The average results of histomorphometric measurements of hepatocytes diameter for each zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*) are shown in Fig. 3 and Fig respectively. 4. Zebra fish (*Danio rerio*) generally displayed larger diameters and areas compared to guppy fish (*Poecilia reticulata*). These findings demonstrate a significant difference in the average hepatocyte diameter and area between zebra fish and guppy fish, whereas Table 1 show the average hepatocyte diameter being 10.47 ± 0.66 µm in zebra fish and 11.57 ± 0.21 µm in guppy fish, and the hepatocyte area being 87.56 ± 11.48 µm² in zebra fish and 105.24 ± 5.41 µm² in guppy fish.

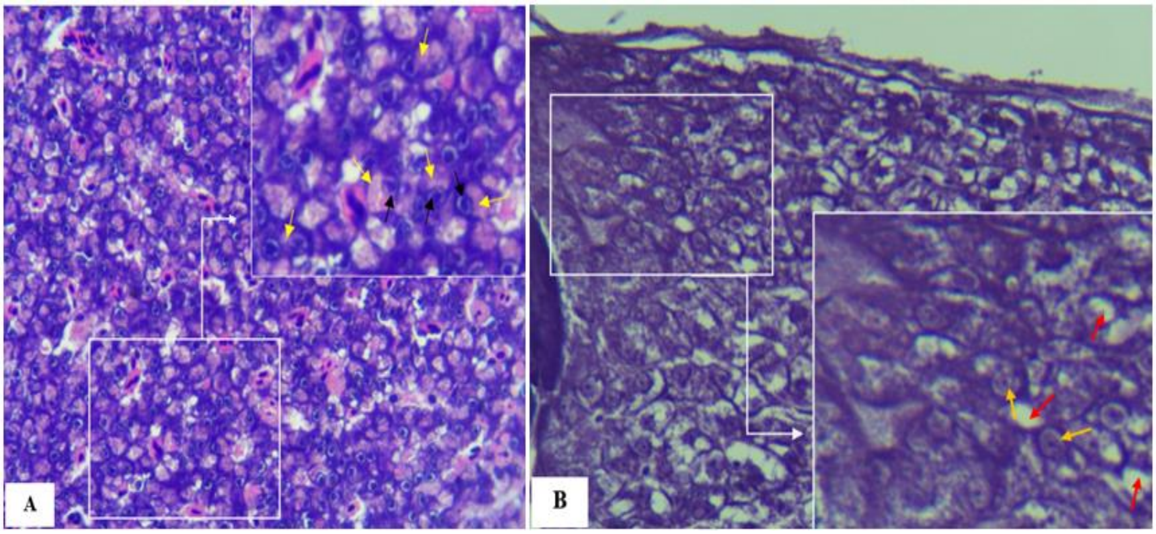

Fig. 1. Histological appearance of zebra fish (A) and guppy fish (B) hepatocytes. HE staining; Magnification 400x

Notes : A) Polyhedral hepatocyte with a clear cell membrane and organized arrangement (→). Glycogen distribution in the hepatocyte cytoplasm is more evenly spread, indicated by eosinophilic-stained masses (→). B) Polyhedral hepatocytes with varying sizes and irregular arrangement (→). The liver exhibits lipid vacuolization in the hepatocyte cytoplasm or fatty degeneration with clear boundaries and appears larger, with hepatocyte nuclei pushed towards the periphery (→)

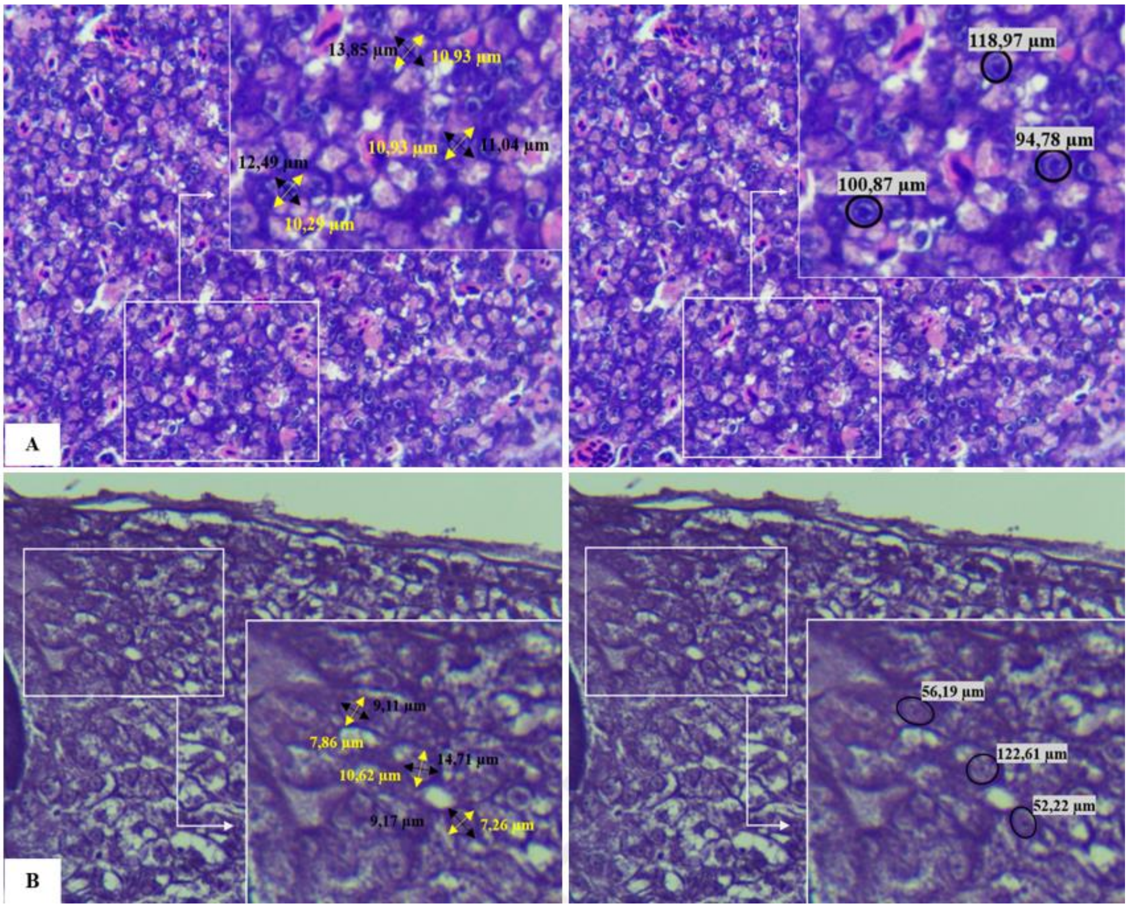

Fig. 2. Histomorphometric view of hepatocytes diameter (left) and hepatocytes area (right) in zebra fish (A) and guppy fish (B). HE staining; Magnification 400x

Notes : → (yellow arrow)=short axis; → (black arrow)=long axis; O(round)=areas

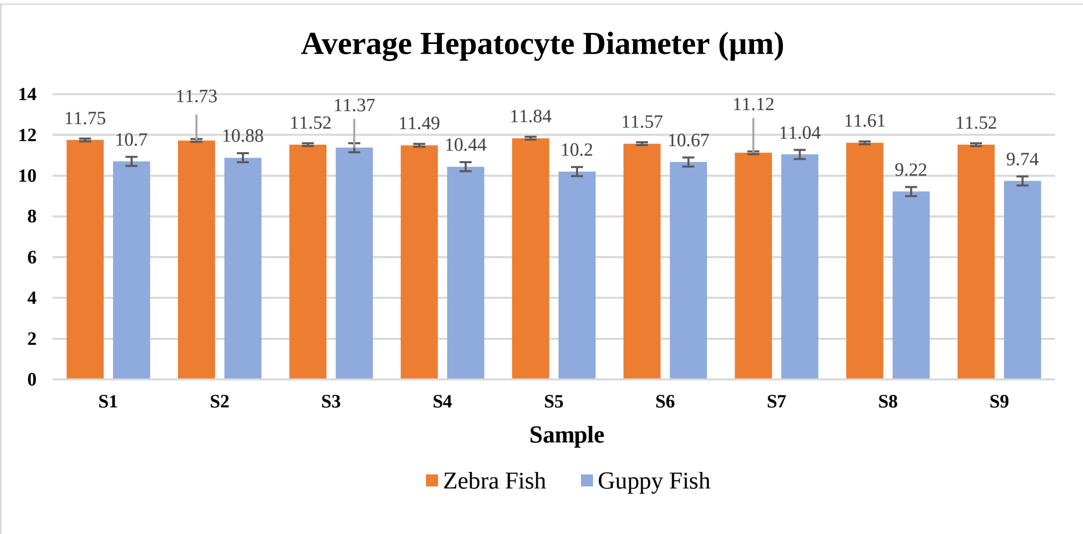

Fig. 3. Graph of the average results of histomorphometric measurements of hepatocytes diameter in the liver for each zebra fish and guppy fish

Notes : S1=Sample 1; S2=Sample 2; S3=Sample 3; S4=Sample4; S5=Sample 5; S6=Sample 6; S7=Sample 7; S8=Sample 8; S9=Sample 9

Table 1. Results of SPSS calculations and Independent Sample T-test for the diameter and area of hepatocytes between zebra fish and guppy fish

SPSS Calculation and Independent Sample T-test Results for Hepatocytes			
Test Parameter	***Mean* ± SD**		***p*-Value**
Hepatocyte Diameter	Zebra fish	11.57 ± 0.21 µm	0.001*
	Guppy fish	1.47 ± 0.66 µm	
Hepatocyte Area	Zebra fish	105.24 ± 5.41 µm	0.001*
	Guppy fish	87.56 ± 11.48 µm	

*Notes : *p <0.05 indicates a significant difference in hepatocytes between zebra fish and guppy fish*

Table 2. Results of SPSS calculations and Independent Sample T-test for the height and width of intestinal villi between zebra fish and guppy fish

SPSS Calculation and Independent Sample T-test Results for Intestinal Villi			
Test Parameter	***Mean* ± SD**		***p*-Value**
Villi Height	Zebra fish	106.64 ± 10.02 µm	0.028*
	Guppy fish	93.40 ± 12.97 µm	
Villi Width	Zebra fish	24.28 ± 1.35 µm	0.001*
	Guppy fish	29.58 ± 3.55 µm	

*Notes : *p <0.05 indicates a significant difference in intestinal villi between zebra fish and guppy fish*

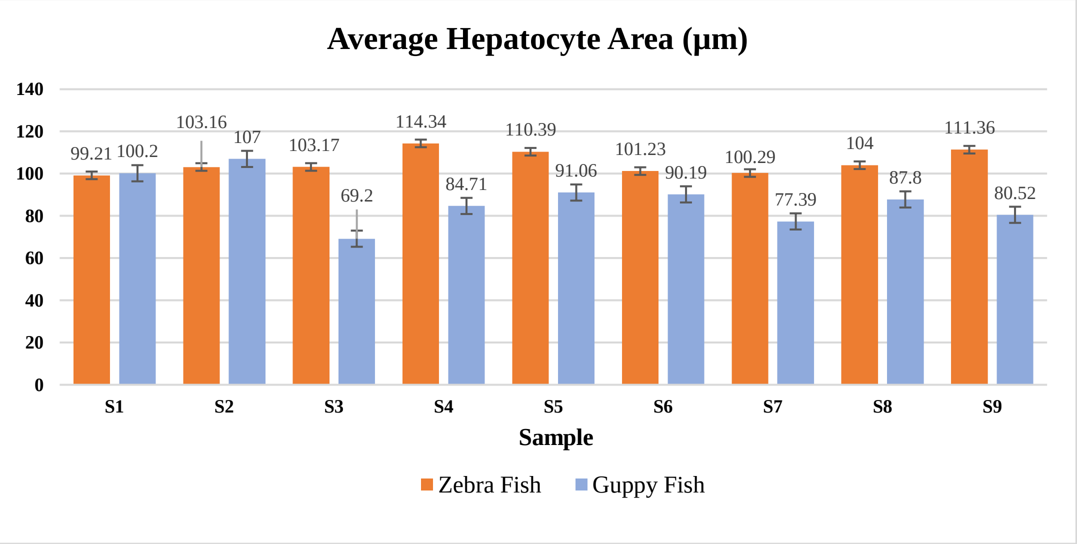

Fig. 4. Graph of the average histomorphometric measurements of the hepatocytes area in the liver of each zebra fish and guppy fish

Notes : S1=Sample 1; S2=Sample 2; S3=Sample 3; S4=Sample 4; S5=Sample 5; S6=Sample 6; S7=Sample 7; S8=Sample 8; S9=Sample 9

3.2 Intestinal Histomorphometry

Histomorphometric view of villi height and width in the intestinal organs of zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*) are shown in Fig. 6. The average histomorphometric measurements of intestinal villi width between guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*) are presented in Fig. 7 and Fig. 8 respectively. The analysis of the average histomorphometric measurement of intestinal villi height and width between these two fish are revealed significant differences. Histologically, zebra fish exhibit more complex, longer, branched, and convoluted intestinal villi accompanied by muscularis consisting of a circular muscle layer, whereas guppy fish display shorter, wider villi with a non-convoluted structure accompanied by a muscularis consisting of a circular muscle layer and a longitudinal muscle layer (Fig. 5). The results of homogeneity and normality tests confirmed that

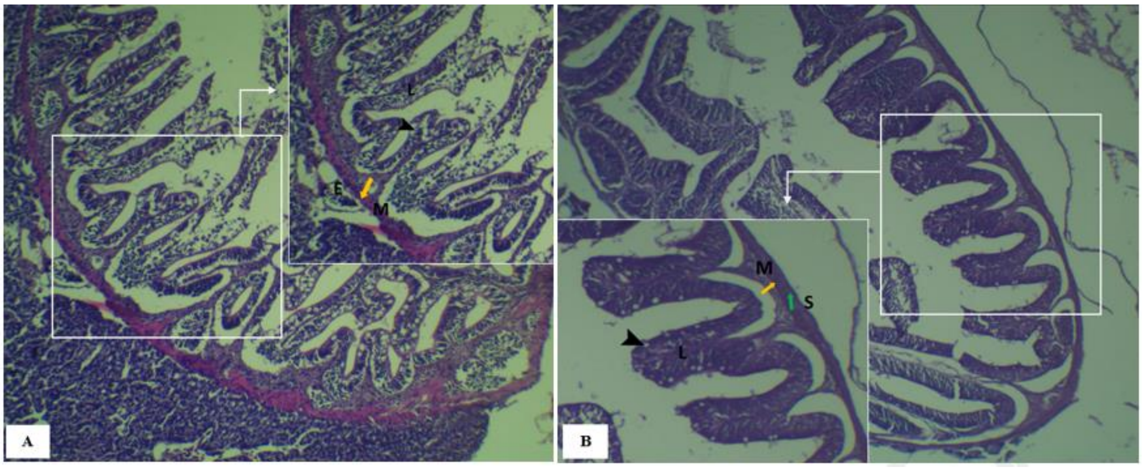

Fig. 5. Histological appearance of intestinal villi in zebra fish (A) and guppy fish (B). HE staining; Magnification 100x

Notes : A) Exhibit more complex, longer, branched, and convoluted intestinal villi accompanied by tunica muscularis (M) consisting of a circular muscle layer (➞), the lamina propria (L), serosa (S), and goblet cells surrounding the villi (➤). B) Exhibit more shorter, wider villi with a non-convoluted structure accompanied by a tunica muscularis consisting of a circular muscle layer (➞) and a longitudinal muscle layer (➞), the lamina propria (L), serosa (S), and goblet cells surrounding the villi (➤)

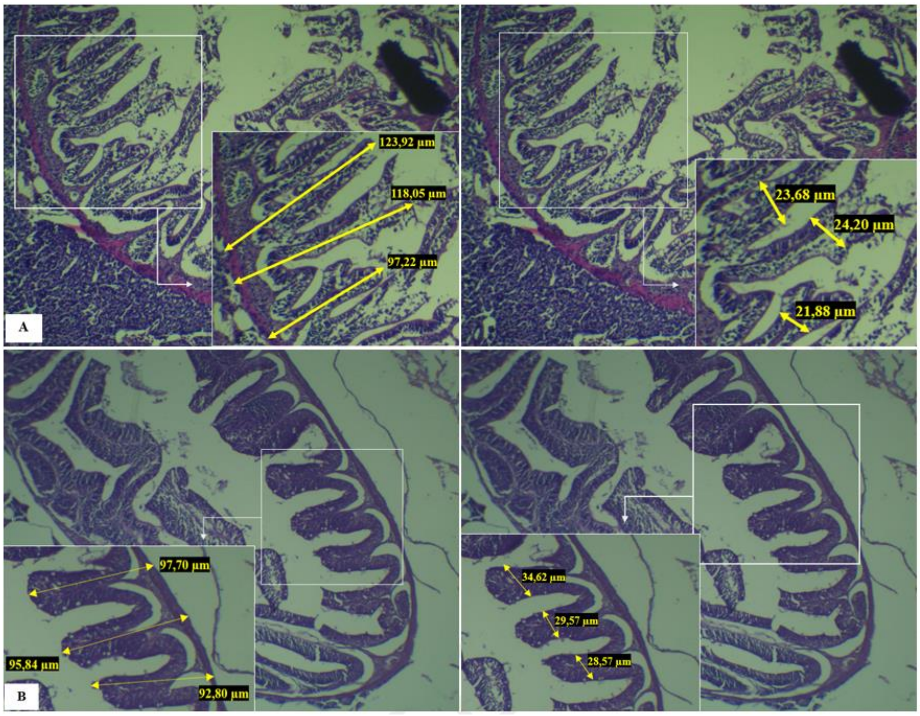

Fig. 6. Histomorphometric view of villi height (left) and villi width (right) in the intestine of zebra fish (A) and guppy fish (B). HE staining; Magnification 100x

all parameters were normally distributed ($p>0.05$). Given this normal distribution, the data were further assessed for homogeneity, which was also confirmed ($p>0.05$). These findings underscore a significant difference in the average height and width of villi between zebra fish and guppy fish, with the average villi height being 24.28 ± 1.35 µm in zebra fish and 93.40 ± 12.97 µm in guppy fish, and the average villi width being 87.56 ± 11.48 µm in zebra fish and 29.58 ± 3.55 µm in guppy fish (Table 2).

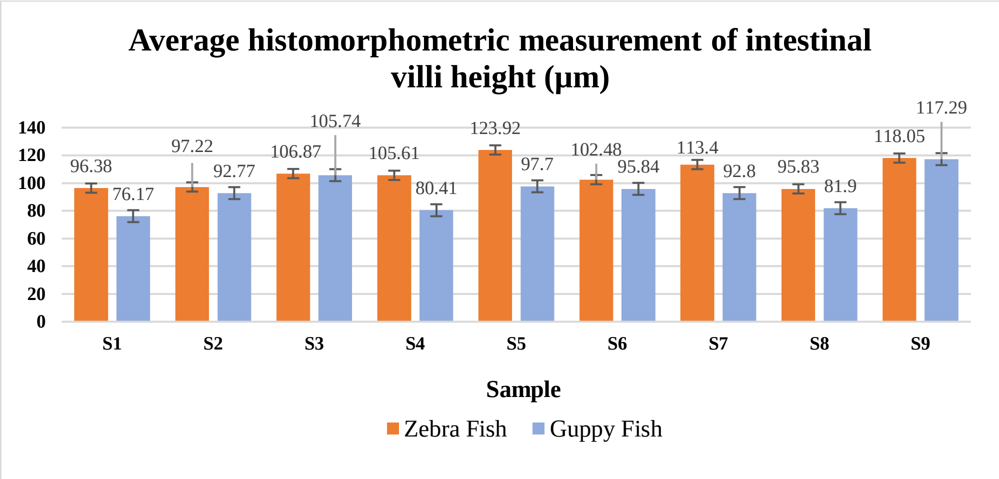

Fig. 7. Graph of the average histomorphometric measurements of intestinal villi height in zebra fish and guppy fish

Notes : S1=Sample 1; S2=Sample 2; S3=Sample 3; S4=Sample 4; S5=Sample 5; S6=Sample 6; S7=Sample 7; S8=Sample 8; S9=Sample 9

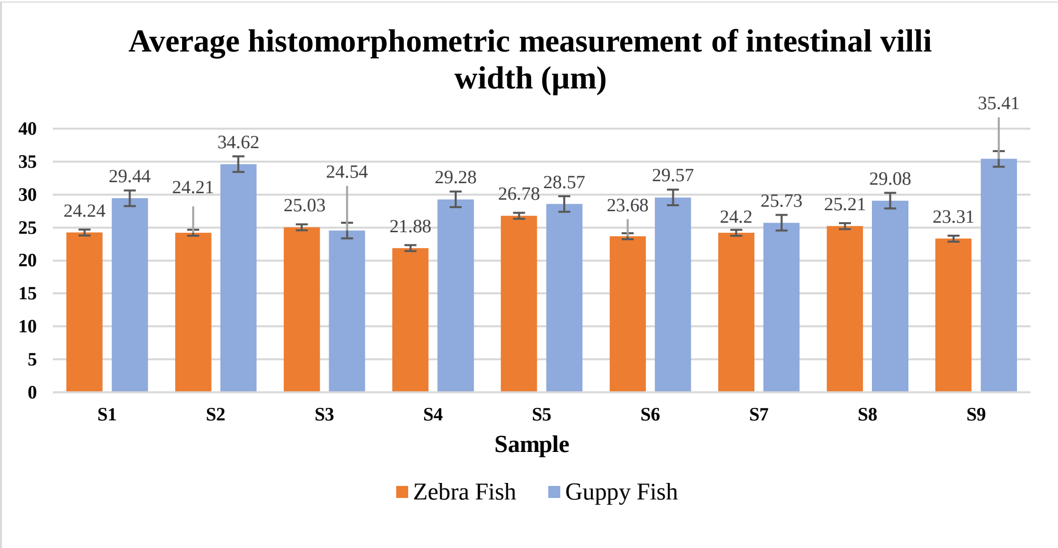

Fig. 8. Graph of the average histomorphometric measurements of intestinal villi width in zebra fish and guppy fish

Notes : S1=Sample 1; S2=Sample 2; S3=Sample 3; S4=Sample 4; S5=Sample 5; S6=Sample 6; S7=Sample 7; S8=Sample 8; S9=Sample 9

4. DISCUSSION

The liver, responsible for toxin accumulation and digestive metabolism, is highly sensitive to environmental changes, which can significantly impact its histological structure [25,26]. Comparative findings of hepatocytes between zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*) from this study revealed notable differences, with zebra fish exhibiting higher glycogen content and guppy fish displaying greater lipid content. These variations influence the outcomes of the Independent Sample T-test, highlighting significant structural differences in the liver between the two species [27,28]. Another distinguishing feature of the liver in these two fish species can be observed based on the position of the hepatocyte nucleus. In guppy fish (*Poecilia reticulata*), the nucleus appears displaced towards the periphery due to the cytoplasm being vacuolated by lipids, accompanied by an irregular arrangement [29]. In zebra fish (*Danio rerio*), the nucleus is centrally located as the cytoplasm is primarily distributed by glycogen, with a regular arrangement [30].

We hypothesize that the differences in hepatocyte histological features and histomorphometry (diameter and area) between these two fish species are influenced by factors such as body size, environmental conditions, diet, and age. In particular, low feeding frequency can impair hepatocyte metabolic performance. Hepatocyte histomorphometry, which reflects the size associated with glycogen or lipid content, serves as an indicator of the fish's nutritional status [27,31]. Zebra fish (*Danio rerio*) hepatocytes exhibit a more organized size and structure, likely due to their stable aquatic environment, which reduces the necessity for extreme adaptive responses. Additionally, zebra fish, with their larger livers, demonstrate more efficient hepatocyte function in carbohydrate and glycogen metabolism. This organized liver structure facilitates efficient substance exchange and metabolic processes [32,30].

Hepatocytes in guppy fish (*Poecilia reticulata*) exhibit an irregular structure, varying in shape and size, with smaller diameters and areas compared to those in zebra fish (*Danio rerio*) (Fig. 5). The smaller size of guppy hepatocytes corresponds to their relatively small liver, a feature influenced by their habitat [33]. Guppy fish frequently inhabit fluctuating environments, which impacts hepatocyte performance, leading to the presence of more lipid vacuoles as an adaptive response. Environmental stress and pollution exposure also contribute to hepatocyte hypertrophy in guppy fish [34]. Their lipid-rich diet results in the accumulation of lipids for energy storage, particularly under stress or during periods of starvation [35,36]. The hepatocytes in guppy fish are actively involved in lipid metabolism, causing the hepatocyte membrane to appear larger due to lipid accumulation. In contrast, zebra fish hepatocytes are larger and primarily store glycogen. The metabolism of guppy hepatocytes is more flexible and resilient compared to that of zebra fish, reflecting their ability to adapt to varying environmental conditions [29,37].

Comparative differences were also investigated in the histological appearance of the intestines. Histologically, the intestinal wall comprises four layers: the mucosa, submucosa, muscularis, and serosa [12]. In zebra fish (*Danio rerio*), the intestinal villi are taller, branched, and slender, with infrequent and random epithelial folds, which enhance nutrient absorption. In contrast, guppy fish (*Poecilia reticulata*) have shorter, wider, and non-coiled villi [38-40]. The histologically of the intestinal villi between zebra fish (*Danio rerio*) and guppy fish (*Poecilia reticulata*) has been shown in Fig. 5. These structural differences reflect each species' adaptation to their respective diets and habitats, with guppy fish possessing a simpler intestinal structure suited to a simpler diet, whereas zebra fish exhibit a more complex intestinal morphology to accommodate a more varied diet [37].

The variation in the length or height of intestinal villi between guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*) is also reflected in the muscularis layer, which comprises both circular and longitudinal muscles. Guppy fish possess both muscle layers, facilitating dynamic food movement and mixing within the intestines [34,35]. In contrast, zebra fish exhibit only circular muscles, which are sufficient for efficient digestion given their more consistent diet. This distinction suggests that the histological structure of the muscularis and intestinal villi influences the length of the villi and their absorptive function in both species [39,40].

The distinct differences in hepatic and intestinal histology between guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*) offer novel insights into the potential of these species as specific animal models. Despite variations in the height and width of intestinal villi, as well as the diameter and area of hepatocytes, both species serve as valuable model organisms. Guppy fish, with their wide and simple villi, are often utilized to study nutrient absorption, supplement efficacy, toxicology, and gene expression [37,41,42]. Their lipid-filled hepatocytes make guppy fish particularly useful in toxicological research, especially in studies examining the effects of pollution and pesticides, also lipid metabolism and liver fat degeneration studies [35]. This makes guppy fish an ideal model for aquaculture research, particularly with smaller subjects, as opposed to using larger species like Tilapia. Conversely, zebra fish, with their more complex intestinal structure, are employed in various studies, including nutrient absorption, feed formulation tests, toxicology, gene expression, and human disease models, making them more suitable for research related to human health [40]. With glycogen-filled hepatocytes, zebra fish are better suited for studies on carbohydrate and glycogen metabolism, as well as toxicology and environmental biology, serving as a mimicry model for human liver function [31].

5. CONCLUSION

This study highlights the significant histological differences between guppy fish (*Poecilia reticulata*) and zebra fish (*Danio rerio*), particularly in their hepatic and intestinal structures. Zebra fish hepatocytes are larger and predominantly filled with glycogen, reflecting their more stable environment and efficient carbohydrate metabolism. In contrast, guppy fish exhibit smaller, lipid-rich hepatocytes, an adaptation to their fluctuating habitats and diet. Additionally, the intestinal villi of zebra fish are taller and more complex, enhancing nutrient absorption, while guppy fish have simpler, shorter villi. These findings suggest that guppy fish are well-suited for aquaculture research focused on nutrient absorption and toxicology, especially in smaller species, whereas zebra fish are more appropriate for studies related to human disease models, carbohydrate metabolism, and environmental biology. The distinct histological features of both species provide valuable insights for their use as specific animal models in various research contexts. The use of fish as experimental animals is highly effective and suitable for identifying and detecting the negative effects of pollutants on health before applying them to humans. Zebra fish (*Danio rerio*) are appropriate for studying glycogen metabolism, whereas guppy fish (*Poecilia reticulata*) are suitable for investigating lipid metabolism and fatty degeneration.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologie such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

The study has received Ethical Clearance Certificate No. 140 – KEP – UB – 2023 from the Institutional Animal Care and Use Ethics Committee of Brawijaya University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Intan PR, Khariri. Utilization of Appropriate laboratory animals for drug and vaccine testing. Proceedings of the national seminar on biology in the Era of the COVID-19 Pandemic. 2020;48–53.
2. Karimah U. Maintenance of Zebrafish (*Danio rerio*) in the Laboratory as Preparation for Research Animal Models. Bandung: Bandung: Widina Bhakti Persada Publisher Bandung; 2021.
3. Shimizu N, Shiraishi H, Hanada T. Zebrafish as a useful model system for human liver disease. Cells. 2023;12(18):1–34.
4. Hanyang L, Xuanzhe L, Xuyang C, Yujia Q, Jiarong F, Jun S, Zhihua R. Application of zebrafish models in inflammatory bowel disease. Frontiers in Immunology. 2017;8(501):1-10.
5. Khotimah H, Ali MM. Zebrafish (*Danio rerio*) as Animal models in biomedical research and how to maintain them. Sanus Medical Journal. 2020;1(1):1–7.
6. Utami N. Zebrafish (*Danio rerio*) as Animal Models for Diabetes Mellitus. BioTrends. 2018;9(1):15–19.
7. Edfaniar VA, Ncemar BR, Krisnawati W, Iza N. Characteristics of the morphological structure of female gatul fish in the kendedes-malang River as a 'Candidate Animal Model' in the Field of Developmental Biology. Journal of Philosophy, Science, Technology, and Socio-Culture. 2022;28(3):68–74.
8. Chairunnisa RA, Windarti, Efizon D. Reproductive biology of Guppy Fish (*Poecilia reticulata*) from the dam of the faculty of fisheries and marine sciences, university of Riau. Journal of Aquatic Resources and Environment. 2020;1(2):1–11.
9. Siswandy E, Rahmi D, Masyitha, Fitriani, Fadli AG, Zuhrawaty, Akmal M. Histology, histomorphometry, and histochemistry of native Chicken Liver (*Gallus gallus domesticus*) During the Pre-Hatching and Post-Hatching Periods. Agripet Journal. 2020;20(2):193–202.
10. Yuniarto A, Sukandar EY, Fidrianny I, Ketut Adnyana I. Application of Zebrafish

(*Danio rerio*) in several experimental disease models. Media Pharmaceutica Indonesiana. 2017;1(3):116–126.

11. Sarvestani FS. Comparative histology of liver in *Pangusius Pangusius* (Herbivore Fish) And *Notopetrus Chitals* (Carnivore Fish). International Journal of Pharmaceutical Research and Allied Sciences. 2017;6(4):28–31.
12. Emha R, Iskandar CD, Rahmi E. Histology of the intestine of Gourami Fish (*Osphronemus gouramy* Lac.) in the seed and adult phases. Journal of Veterinary Students (JIMVET). 2018;2(1):56–63.
13. Akmal Y, Devi CMS, Muliari, Humairani R, Zulfahmi I. Digestive system morphometrics of Tilapia (*Oreochromis niloticus*) Exposed to Palm Oil Mill Effluent. Journal of Galung Tropika. 2021;10(1):68-81.
14. Goessling W, Sadler KC.. Zebrafish: An important tool for liver disease research. In Gastroenterology. 2015;149(6):1361-1377.
15. McGill University. Fish and Aquatic Amphibian Euthanasia. Montreal: McGill University; 2022
Available:https://www.mcgill.ca/research/files/research/303-fish_and_aquatic_amphibian_euthanasia_-_march_2022_v2.pdf.
16. Matthews M, Varga ZM. Anesthesia and euthanasia in zebrafish. ILAR Journal. 2012;53(2):192–204.
17. Rakhmawati H, Situmeang A, Nurhidayat, Lubis AMT, Murti H, Boediono A. Effectiveness of Decalcification Solution on Tibia Bone of Garut Sheep (*Ovis aries*). Veterinary Journal. 2019;20(3):403–408.
18. Arya IMC, Piraksa IW, Besung INK, Suwiti NK. The Effect of Pegagan (*Centella asiatica*) administration on microscopic images of the small intestine of mice infected with *Salmonella typhi.* Udayana Veterinary Bulletin. 2012;4(2):73–79.
19. Putra RP, Rahmi E, Masyitha D, Zainuddin, Wahyuni S, Salim MN. Histological Structure and histomorphometry of Turkey Heart (*Meleagris gallopavo*) at Different Age Levels. Scientific Journal of Veterinary Students (JIMVET). 2022;6(3):143–152.
20. Image J. Version 2.3. Bethesda (MD): National Institutes of Health; 2023.
Available: https://imagej.nih.gov/ij/.
21. Latifah AN, Djaelani MA, Kasiyati. Bulletin of Anatomy and Physiology Histomorphometry of Pekin Duck Liver (*Anas platyhrynchos*) after Giving Moringa Leaf Flour (*Moringa oleifera* Lam.) as Feed Additive Combined with Light Exposure. Bulletin of Anatomy and Physiology. 2022;7(1):1–10, 2022.
22. Alfina S, Febriani H, Syukriah. Effectiveness test of cosmos caudatus kunth. Leaf extract on duodenal epithelial damage of Male White Rats (*Rattus norvegicus*) Induced by Aspirin. Journal of Agromedicine and Medical Sciences. 2022;8(2):108–114.
23. Dwijayanti B, Rahmi E, Balqis U, Fitriani F, Masyitha D, Aliza D, Akmal M. Histology, Histomorphometry, and histochemistry of native Chicken Intestine (*Gallus gallus domesticus*) During the Pre-Hatching and Post-Hatching Periods. Agripet Journal. 2021;21(2):128–140.
24. Wang Z, Du J, Lam SH, Mathavan S, Matsudaira P, Gong Z. Morphological and molecular evidence for functional organization along the rostrocaudal axis of the adult zebrafish intestine. BMC Genomics. 2010;11(392):1–13.
25. Lubis UF, Marusin N, Zakaria IJ. Histological analysis of Milkfish Liver (*Osteochilus hasseltii* C. V.) in Lake Maninjau and Lake Singkarak, West Sumatra. Journal of Biology, Andalas University. 2014;3(2):162–167.
26. Firmani U. Histology of milkfish liver from traditional ponds in Ujung Pangkah District, Gresik. Pantura Fisheries Journal. 2021; 4(1):50–58.
27. Rodrigues RA, Saturnino KC, Fernandes CE. Liver histology and histomorphometry in hybrid Sorubim (*Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans*) Reared on Intensive Fish Farming. Aquaculture Research. 2017;48 (9):5083–5093.
28. Pradana GW, Ma'ruf MF, Eprilianto DF. Application of student t-test to assess the effectiveness of fiscal decentralization course textbook development in the public administration department of Unesa. Journal of Educational and Learning Dimensions. 2022;10(2):182–190.
29. Al-Kawaz JM. Morphological description and histological structure of the liver in adult guppy fish. Journal University of Kerbala. 2019;17(2):43–51.
30. Oliveira LB, De AS, Leonardo, De Lima EMM. Macro- and microstructural descriptions of the Zebrafish (*Danio rerio*) Liver. Folia Morphologica (Warsz). 2016;75 (3):382–387.

31. Goessling W, Sadler KC. Zebrafish: An important tool for liver disease research. Gastroenterology. 2015;149(6):1361-1377.
32. Menke AL, Spitsbergen JM, Wolterbeek A. PM, Woutersen RA. Normal Anatomy and Histology of The Adult Zebrafis. Journal of Toxicology Pathology. 2011;39 (5):759–775.
33. Sousa S. Microscopic Morphology of the Liver of the Guppy (*Poecilia reticulata*). Porto: Institute of Biomedical Sciences; 2018.
34. Paulo DV, Fontes FM, Flores-Lopes F. Histopathological alterations observed in the Liver of *Poecilia vivipara* (Cyprinodontiformes: *Poeciliidae*) as a Tool for The Environmental Quality Assessment of The Cachoeira River, BA. Brazilian Journal of Biology. 2012;72(1):131–140.
35. Sharon G, Fridman S, Reiss- Hevlin N, Sinai T, Boisot P, Zilberg D. Effects of different commercial diets on growth performance, health and resistance to *Tetrahymena* sp. Infection in Guppies, *Poecilia reticulata* (Peters). Aquaculture Research. 2014;47(7):2276–2286.
36. Soto E, LaFrentz BR, Yun S, Megarani D, Henderson E, Piewbang C, Johnston AE, Techangamsuwan S, Ng TFF, Warg J, Surachetpong W, Subramaniam K. Diagnosis, Isolation and description of a novel amnoonvirus recovered from diseased fancy guppies, *Poecilia reticulata*. Journal of Fish Disease. 2024;47(6):1–13.
37. Monsefi M, Gholami Z, Esmaeili HR. Histological and morphological studies of digestive tube and liver of the persian Tooth-carp, *Aphanius persicus* (Actinopterygii: *Cyprinodontidae*). IUFS Journal of Biology. 2010;69(1):57–64.
38. Erian V, Zainuddin, Balqis U. The description of Villi Surface area of adult male local Catfish (*Clarias batrachus*). Journal of Veterinary Students (JIMVET). 2018;2(3):283–287.
39. Fizikri SL, Ziainuddin, Winaruddin, Jalaluddin M. Histological structure of the oesophagus, ventriculus, and intestines in Garing Fish (*Tor tambroides*) in the Jorong Ikan Banyak River, Lima Puluh Kota Regency, West Sumatra. Journal of Veterinary Students (JIMVET). 2018;2(1): 124–129.
40. Nikiforov-Nikishin A, Smorodinskaya S, Kochetkov N, Nikiforov-Nikishin D. Effects of three feed additives on the culturable microbiota composition and histology of the anterior and Posterior Intestines of Zebrafish (*Danio rerio*). Animals. 2022;12 (18):1–22.
41. Jannah SN, Adityani R, Purwantisari S. The effect of probiotic Lactobacillus paracasei on The Performance of Guppy Fish (*Poecilia reticulata* var. Mosaic). Journal of Biology and Biology Education. 2023;15(1):75–86.
42. Mohammed RS, King SD, Bentzen P, Marcogliese D, Van Oosterhout C, Lighten J. Parasite Diversity and ecology in a model species, the Guppy (*Poecilia reticulata*) in Trinidad. Royal Society Open Science. 2020;7(1):1–8.

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