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Research Article

Effect of Fasting Duration on Total Bacteria and Histopathology in Goldfish Infected with Aeromonas hydrophila

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Abstract

The purpose of the study was to evaluate the effect of fasting duration on total bacteria as well as changes in intestinal and gills of carp (Cyprinus carpio) after Aeromonas hydrophila infection. The experimental design consisted of five groups, namely negative control, positive control, one-day fasting, two-day fasting, and three-day fasting. Infection is carried out through immersion in a bacterial suspension with a density of 10^8 colonies per milliliter. The number of bacteria was calculated using the colony calculation technique in the Rimler–Shotts medium, while the histological picture of the intestine and gills was analyzed using hematoxilin–eosin staining. Statistical analysis includes normality tests, one-way variety analysis, and follow-up tests as appropriate. The results showed that fasting had a significant influence on total bacteria and tissue structure. A one-day fast results in the highest total bacteria in the intestines and gills, reflecting acute metabolic stress and decreased mucosal resistance to bacterial colonization. Fasting for two and three days led to a decline in the number of bacteria close to the negative control group, signaling physiological adaptation and stabilization of the microbiota. Intestinal histopathology shows mild lesions in the form of epithelial desquamas, decreased mucous producing cells, and immune cell infiltration, while the gills experience inflammation with reduced severity at a longer duration of fasting. Overall, the duration of the fast determines the effectiveness of the carp's physiological response to infection. Very short fasts increase the burden of bacteria and tissue disorders, while longer fasts provide a protective effect through histological adaptation and microbiota balance.

Keywords: Aeromonas hydrophila, fasting, goldfish, total bacteria, histopathology, gills, intestines

INTRODUCTION

The aquaculture sector plays a strategic role in supporting food security and the global economy. National conditions show the dominance of carp (*Cyprinus carpio*) as a leading commodity with a production of 355,742 tons in 2023 (Kementerian Kelautan dan Perikanan Republik Indonesia, 2024). Cultivation intensification increases environmental pressure and the opportunity for infectious diseases to appear, especially due to the bacteria Aeromonas hydrophila as a motile agent of aeromonas septicemia (MAS). A. *hydrophila infection* triggers skin bleeding, swelling of internal organs, damage to filaments and gill lamellae, and 70–100% mortality in extreme conditions. The use of synthetic antibiotics still dominates infection control, while repeated use increases bacterial resistance, drug residue accumulation, and ecological impact. Recent studies highlight fasting as a nonpharmacological strategy that strengthens the physiology and immune response of the gut and gills. The practice of fasting decreases the population of pathogenic bacteria, improves the histopathological integrity of the intestine, and maintains the histological structure of the gills that play a vital role in respiration and osmoregulation. Quantitative evidence on the effectiveness of fasting on intestinal parameters and gills of A. hydrophila infected carp is still limited (ANGGI et al., 2023; Saharia et al., 2024).

Previous studies have shown inconsistent results regarding the impact of fasting on fish health. Research by (Zhao et al., 2022) reported that short-term fasting increased the expression of noncellular immune genes in zebrafish (Danio rerio). Research on goldfish is still limited, especially studies that assess the relationship between fasting and total bacterial count, changes in intestinal histopathology, and hiphonetic conditions. The literature review also shows that there is no standard for optimal fasting duration and frequency to produce a protective response without causing nutritional stress. Quantitative research examining the association between fasting, total bacterial count, intestinal histopathology, and gilll structure in A. hydrophila-infected carp emerges as an urgent need in the development of sustainable fish health management and in line with the global agenda of antimicrobial resistance (AMR) reduction. The findings of the study are expected to expand the understanding of the mechanisms of fish's physiological adaptation to fasting during infection and strengthen the scientific basis of a feed management-based disease prevention strategy that includes key target organs such as the intestines and gills.

METHODS

Ethical approval

Ethical approval for the research has been granted by the Ethics Committee of Universitas Brawijaya with approval number 173-KEP-UB-2024.

Study period and location

This research was conducted from October 2024 to November 2025 at the Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia

Experimental design

This study applied a post-control group design by determining the number of samples through a complete random design. The research team conducted sample handling and analysis at several facilities, namely the Laboratory of Animal Microbiology and Immunology for bacteriological testing, the Laboratory of Anatomical Pathology for histopathological examination, and the Laboratory of Fish Diseases and Parasitology for fish rearing. All research activities take place within the Faculty of Veterinary Medicine, Universitas Brawijaya. Seventy-five *Cyprinus carpio* measuring 11–13 cm with an average weight of 35 g were placed in the aquarium during the seven-day acclimatization period. The acclimatization process takes place in clean, continuously aerated water at a temperature of 26–28°C and a pH of 7.0–7.5. After this period, the fish were randomly divided into four treatment groups with a total of 15 fish per group consisting of three replicas containing five fish. The treatment groups included negative control (no fasting and no infection), positive control (infection without fasting), P1 (fasting one day before infection), P2 (fasting two days before infection), and P3 (fasting three days before infection).

Evaluation

Fish fasting in the positive control groups, P1, P2 and P3 for 0, 1, 2, and 3 days before infection. Fish infection by immersion in *suspension of A. hydrophila* concentrated 10^8 CFU/mL by aeration. The negative control group received immersion in pathogen-free well water. Researchers monitored all fish for three days post-infection. A. *hydrophila cultures* were grown on Rimler–Shotts medium (Merck, Germany) containing yeast (3.0 g/L), maltose (3.5 g/L), L-cysteine hydrochloride (0.3 g/L), L-lysine hydrochloride (5.0 g/L), L-ornithine hydrochloride (6.5 g/L), sodium thiosulfate (6.8 g/L), ferri ammonium citrate (0.8 g/L), sodium deoxycolate (1.0 g/L), sodium chloride (5.0 g/L), blue bromothymol (0.03 g/L), and agar (13.5 g/L). Culture incubation lasts for 24 hours at a temperature of 27°C. Clinical monitoring is carried out after infection by assessing clinical symptoms, behavioral disorders, fin erosion, and mortality. Intestinal and gills samples were taken after euthanasia procedures through the overdose method of tricaine methanesulfonate MS-222 anesthesia at a dose of 100 mg/L and sodium bicarbonate at a dose of 200 mg/L as a metabolite neutralizer for 1-2 minutes after infection, then the fish were necropsy and sample collection.

Data collection

10% intestinal and gills samples were diluted in stages in nutrient broth (NB) media by transferring 1 mL of initial suspension as a 10^{-1} dilution, then continuing dilution to 10^{-9} using sterile micropipettes and microtips. Dilution is done to reduce the concentration of microbes by a ratio of 1:9 in the initial stage and a reduction of 1/10 in each subsequent stage. The last three dilutions (10^{-7}, 10^{-8}, 10^{-9}) were cultured on the RS medium using *the spread plate technique*. A total of 0.1 mL of suspension is dripped onto the surface to be then leveled with *a spreader* that has been sterilized using alcohol and light heating in triples. The cups were incubated at 27.5°C for 48 hours to calculate the total plate count (TPC) (Artanti et al., 2024). Observation of bacterial growth was carried out after 48 hours of incubation in the hospital medium. All colonies formed are counted, both single colonies and colonies that are chained to each other. The identification *of A. hydrophila* was established based on the number of yellow colonies in the RS medium (Figure 4.2). The calculation of total bacteria followed *the simplified agar plate* method by taking the average value of several dilution levels (Semwal et al., 2023).

The intestinal organs and gills of the carp that had been collected were then preserved in *10% neutral buffered formalin (NBF)* for 24 hours for histological fixation. The next process includes multi-stage dehydration using 70%, 80%, 90%, 95%, and absolute ethanol (three stages), each for 1 hour, followed by a clearing stage in xylol 1–3 for 10 minutes per stage. The sample was then infiltrated with liquid paraffin (58–60°C) in three stages, 1 hour each, before being embedded into a metal mold until the paraffin hardened at room temperature. Paraffin blocks are cut with a 5 µm thick microtom, and each slice is widened in a 40°C waterbath before being placed on the glass of the object. Staining is carried out by the hematoxilin–eosin method through the stages of deparafinition, rehydration, staining, dehydration, and clearing. The preparation is then mounted using strainers as an antifungal and adhesive (Mora et al., 2022). Histopathological observation takes place microscopically by assessing changes in the structure of intestinal tissue through a descriptive approach.

Data analysis

The Shapiro–Wilk test is used to assess the normality of data. Comparisons between treatments were analyzed using one-way ANOVA, then followed by a *Tukey post hoc* test to identify differences between group pairs. The significance limit was set at $p < 0.05$. All results are presented in the form of a standard deviation ± average. Statistical analysis was performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

RESULT AND DISSCUSSION

Clinical symptoms and mortality

Exposure to *A. hydrophila* causes typical clinical signs of infection in C. carpio, including difficulty breathing, hemorrhagic lesions of the skin, superficial wounds, and impaired balance while swimming. These clinical manifestations are consistent

with those reported by (Patil, 2025) . who observed that rainbow trout (*Oncorhynchus mykiss*) showed lethargy, slow movement, and panting before death from infection. In this study, the symptoms were severe in the positive control group, mild in the P1, P2, and P3 groups and no clinical symptoms in the negative control group. These findings suggest that short-term fasting before infection may reduce the severity of symptoms of motile aeromonas septicemia (MAS) disease. This trend is in line with (Domínguez-Andrés et al., 2023) which states that short-term fasting prepares the immune system and increases resistance to bacterial infections. In particular, fish in the P2 group showed better clinical outcomes than fish in the P1 group, which supports the idea that longer fasting duration improves adaptive readiness. This observation is also in line with the research conducted by (Wang et al., 2019) which shows short-term fasting (3 days) before infection may increase the resistance of unvaccinated tilapia to Streptococcus agalactiae. However, this is different from (Caruso et al., 2012) which found that fasting did not significantly affect nonspecific immune responses in red porgyfish. In contrast, refeeding in a short period of time (7 days) was associated with increased hemaglutination titers. The health of fish during infection is highly dependent on the innate immune system, especially in suboptimal thermal conditions, as T and B cell proliferation and antibody production are disrupted, depending on factors such as the duration of the fast, species, and age.

Total bacteria

The total number of intestinal bacteria of goldfish that were given fasting treatment and infected *with A. hydrophila* bacteria (108 CFU/ml) can be seen in **Figure 1.** Statistical analysis at the last two dilution levels (10^{-8} and 10^{-9}) with five replicas showed significant differences in the total number of bacteria (TPC) of *Cyprinus carpio fish* intestines between treatments. The Shapiro–Wilk normality test showed that most of the data groups were normally distributed (K–, K+, P2, and P3), while P1 did not meet the normality assumption so the analysis was continued with the Kruskal–Wallis test. The test yielded a value of $p = 0.0138$ ($p < 0.05$), indicating a significant difference between treatments. Dunn's follow-up test showed that the 1-day fasting group (P1) differed significantly from the negative control (K–) and the 2-day fasting (P2) group, while the rest of the comparison showed no significant difference. These findings indicate that fasting for 1 day triggers a significant increase in the number of gut bacteria, while extending the duration of fasting to two and three days lowers the bacterial population back to close to the control condition. The duration of fasting has been shown to affect the dynamics of gut bacterial communities, where short fasts increase bacterial growth while longer fasts suppress their populations.

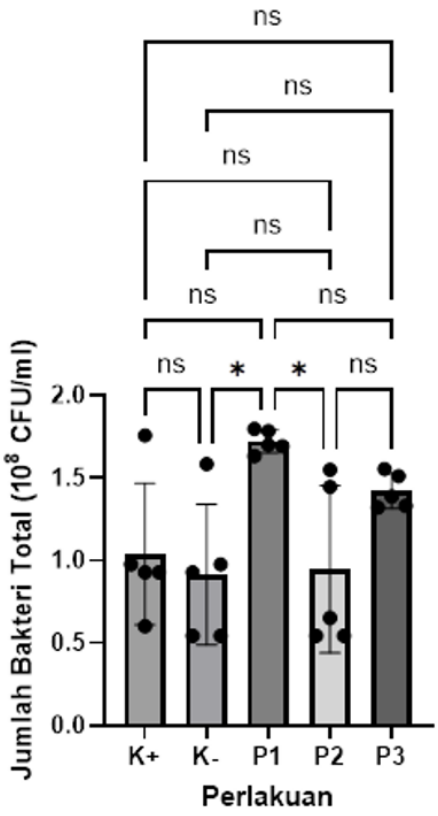

Figure 1. Graph of the total number of bacteria in the intestines of carp infected *with A. hydrophila* (108 CFU/ml) and fed with an oil mixture according to the treatment group. The sign (*) indicates a significant difference (P=0.01-0.05). The (ns) sign indicates that there is no significant difference between the two treatments.

The increase in total bacteria (TPC) in *Cyprinus carpio* fish that underwent a one-day fast (P1), then a decrease on the second and third days, showed physiological changes and intestinal microbial dynamics during the adaptation phase to feed deficiency. Very short-term fasting triggers a rapid shift in the microbiota community, as stress-tolerant opportunistic bacteria gain an advantage when competition from feed-dependent bacteria decreases. Studies on rainbow trout (*Oncorhynchus mykiss*) show that short-feed restriction changes the structure of the microbiota through an increase in the Firmicutes and Bacteroidetes groups as well as a decrease in Actinobacteria, which reflects early dysbiosis when the body is just entering the hunger phase (Messina et al., 2023). A similar phenomenon is suspected to occur in goldfish that have just been fasted for one day because the intestinal lumen still contains endogenous mucus and feed substrate residues that bacteria use for rapid growth.

Longer fasting durations decrease the availability of energy and carbon in the intestinal lumen thereby suppressing the growth of feed-dependent heterotrophic bacteria. Physiological adaptations to hunger, such as decreased digestive enzyme

activity, reduced mucus secretion, and changes in epithelial permeability, result in conditions that are less favorable for microbial proliferation (Frohn et al., 2024). Other research suggests that fasting acts as a selection factor that only preserves bacteria with the ability to utilize endogenous sources, such as mucopolysaccharides or host cell residues (Sato et al., 2025). A spike in TPC on a one-day fast reflects the microbiota's response to sudden changes in nutrient availability, while a decrease in TPC at a two- to three-day fast signals a reorganization of microbial communities toward more stable physiological conditions. A systematic study by (Saglam et al., 2023) reported a consistent pattern, namely initial fluctuations in the microbiota during intermittent fasting followed by the formation of a new balance after the adaptation phase. The overall dynamics suggest that the duration of fasting serves as an important regulator in the stability of the fish intestinal microbiota community, with the effect of population increase on short fasting and population repression on prolonged fasting.

The total number of bacteria in goldfish gills that were given fasting treatment and infected *with A. hydrophila* bacteria (108 CFU/ml) can be seen in **Figure 2.** Statistical analysis at the last two dilution levels (10^{-8} and 10^{-9}) with five replicas showed significant differences in the total number of bacteria (TPC) of *Cyprinus carpio* fish gills between treatments. The Shapiro-Wilk normality test showed that all normally distributed data groups (K-, K+, P1, P2, and P3) obtained *a p*> value of 0.05, indicating that the data met the assumption of normal distribution. Thus, parametric procedures such as ANOVA can be applied appropriately. The test was followed by *One Way* ANOVA which showed a value of $F = 14.69$ with $p < 0.0001$, which means that there was a very significant difference between the average total bacteria in the five treatment groups. An R^2 value of 0.7461 indicates that about 74.6% of the total bacterial variation can be explained by treatment, so the treatment has a strong influence on the number of *cyprinus carpio* fish gills.Tukey's follow-up test showed a more specific pattern of difference, showing that P1 differed significantly from almost all groups (K+, K-, P2, and P3), confirming that the 1-day fasting treatment produced the highest number of bacteria consistently. Overall, these findings show that the 1-day fasting treatment (P1) actually significantly increased the total gill bacteria compared to other treatments and controls. This condition can indicate a decrease in gill mucosal immunity due to acute metabolic stress, changes in the gill microbiota during early fasting, an inflammatory response that increases susceptibility to bacterial colonization. Fasting of 2 to 3 days did not show a significant increase in bacteria, which was associated with more stable physiological adaptation in longer fasting periods.

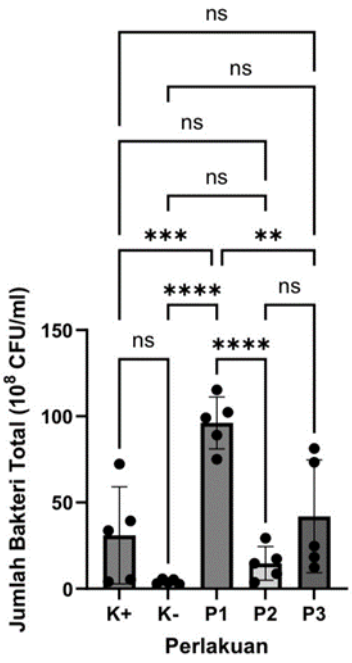

Figure 2. Graph of the total number of bacteria in goldfish gills infected *with A. hydrophila* (108 CFU/ml) and given positive control treatment, negative control, 1-day fasting treatment, 2-day fasting treatment, 3-day fasting treatment.

The sign (*) indicates a significant difference (P=0.01-0.05). The (ns) sign indicates that there is no significant difference between the two treatments.

Increased total bacteria (TPC) in fish *Cyprinus carpio* who underwent a one-day fast (P1), then decreased on the second and third days, showed physiological changes and microbial dynamics of the gills during the adaptation phase to feed deficiency. Fasting, both short-term and long-term, has different biological effects on total bacteria in the gills of goldfish (*Cyprinus carpio*) through mechanisms related to metabolic stress, mucosal immunology, and microbiota balance. In short-term fasting, fish experience acute metabolic stress that triggers internal energy mobilization as well as temporary physiological adaptation, which can alter the balance of microbiota on the surface of the mucosa including the gills (Qasimah *et al*., 2025). In some cases, this response can weaken mucus production and decrease local immune activity so that opportunistic bacterial colonization is easier to occur and leads to a total increase in bacteria in the gills. However, some studies have also shown that short fasting can stimulate positive adaptive mechanisms in the form of increased microbiota homeostasis and strengthening of the immune response, so it does not always lead to an increase in bacteria if environmental conditions favor (Li et al., 2019). In contrast, long-term fasting tends to have a more detrimental impact on the integrity of mucosal defenses. Too long fasting duration can decrease the activity of the innate immune system, damage the homeostasis of the microbiota, and disrupt the structure of the mucosa through increased oxidative stress and decreased protective mucus secretion. This condition facilitates dysbiosis in the gills, which is

an imbalance of microbial communities characterized by the overgrowth of commensal and opportunistic bacteria so that the total number of bacteria increases significantly (Gou et al., 2023). Thus, short-term fasting can produce changes in the microbiota that are adaptive or maladaptive depending on the complications of acute stress, while long-term fasting consistently tends to increase the burden of bacteria due to decreased mucosal protective function and immune system disruption, as supported by various literature related to the physiological and immunological response of fish to feed restrictions.

Histopathology

The histopathological picture of the intestines of goldfish that were given fasting treatment and were infected *with A. hydrophila* bacteria (108 CFU/ml) can be seen in **Figure 3.**

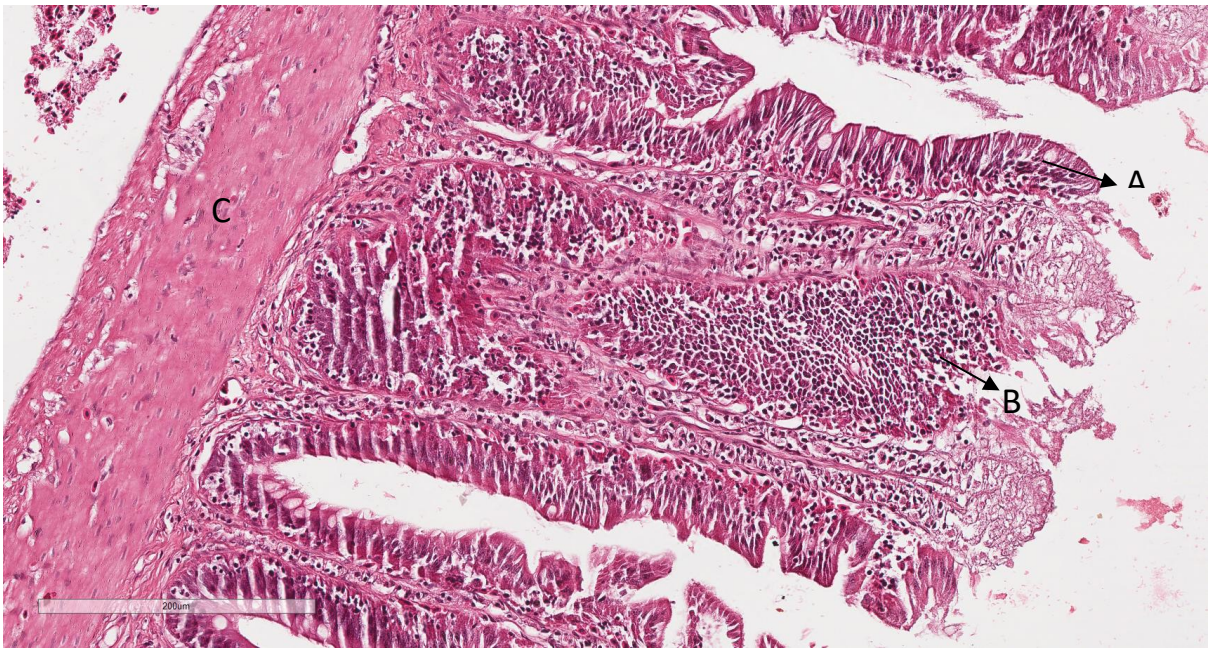

Figure 3. Intestinal histopathology *of C. caprio* who was given 2-day fasting treatment. (A) mild disqualifying of the columnar epithelium. (B) infiltration of inflammatory cells. (C) tunica muscularis (Personal Documentation, 2025).

Microscopic examination of the intestine of Cyprinus carpio after a two-day fast showed a completely composed intestinal wall, consisting of mucosa, submucosa, muscularis, and serosa. The villi appeared shorter with mild degrees of atrophy and a not-so-sharp apex as the control group. Columnar epithelium shows mild desquammation and cytoplasmic vacuolization of the apical part. The number of goblet cells decreases, which signals a decrease in mucous secretion during nutrient deficits. The lamina propria contains mild to moderate amounts of lymphocyte and macrophage infiltration, accompanied by a widening of the intercellular space indicating minimal edema. No necrosis, hemorrhage, or ulceration is visible. The

structure of the Lieberkühn crypt remains orderly with well-defined oval-nucleated basal cells. The submucosa shows no noticeable changes, while the muscular layer retains the orientation of the muscle fibers and the integrity of their structure. The overall findings illustrate mild lesions that are adaptive to short-term fasting. Epithelial desquammation and immune cell infiltration reflect early metabolic and immunological responses, while goblet cell reduction and cytoplasmic vacuolization show adjustment to reduced feed stimuli to maintain mucosal barrier function.

Two-day fasting triggers changes in mucosal morphology within the limits of physiological adaptation. Reduced surface epithelium and thinning mucus appear due to decreased metabolic activity and epithelial protein synthesis during energy deficits. Immune cell infiltration reflects a homeostatic response to changes in microbial communities in the gut lumen. Studies on rainbow trout (*Oncorhynchus mykiss*) show that short-term fasting for 24–48 hours lowers vili height, reduces the number of goblet cells, and increases mucosal lymphocyte infiltration as part of tissue adaptation to starvation conditions (Messina et al., 2023). The findings are in line with the report of Frohn et al. (2024) which states that the early phases of fasting decrease digestive enzyme activity, mucous secretion, and epithelial permeability without causing permanent structural damage. The condition of the gut during the two-day fast also forms a microbial environment that supports the growth of stress-resistant bacteria. The findings of Sato et al. (2025) suggest that changes in the microbiota during the short fasting phase increase local immune stimulation, which may explain the infiltration of mild lymphocytes in the lamina propria. Saglam et al. (2023) reported a similar phenomenon in mammalian models, where intermittent fasting caused temporary fluctuations in microbial communities that then shifted towards a new equilibrium after a few days. The overall findings confirm that the histopathological changes in the two-day fasting treatment reflect a nonpathological adaptive response that maintains epithelial integrity as well as microbiota stability during energy deficit conditions.

Histopathological picture of gills of goldfish that have been given fasting treatment and identified bacteria *A. hydrophila* who were given fasting treatment and were infected with bacteria *A. hydrophila* (108 CFU/ml) is seen in **Figure 4. and Figure 5.**

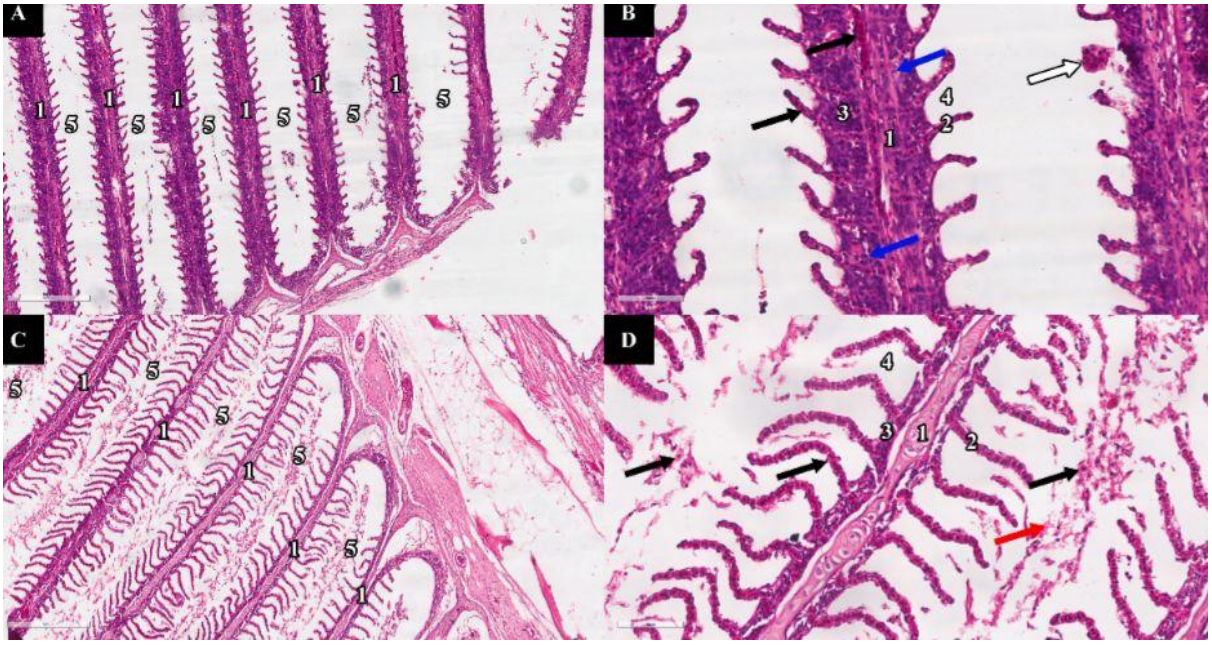

Figure 4. Histopathological picture of gill organs from the negative (A&B) and positive control (C&D) control groups.

Figure A (100x magnification) shows that the primary lamella (figure 1) has a symmetrical secondary lamellae. The gap between the primary lamellae looks quite clean (figure 5). Figure B (400x magnification) shows the epithelial cells supporting the secondary lamella (figure 3). The secondary lamella appears to have a firm boundary without any damage (figure 2). The lacuna (figure 4) or the gap between the secondary lamellae appears clean and symmetrical. Erythrocytes (black arrows) appear to fill the blood vessels in the primary lamella as well as the secondary lamellae. Chloride cells (blue arrows) are seen among epithelial cells. There is a pathological change that appears to be in the form of telangiectasis (white arrow). Figure C (100x magnification) shows that the gap between the primary lamellae shows that there is an accumulation of cells with the primary lamellae that appear normal. Figure D (400x magnification) shows the accumulation of cells in the gap between the primary lamellas, erythrocytes and fibrin (red arrow). The epithelial cells supporting the secondary lamellae appear much less or are called hypoplasia (Personal documentation, 2025).

The histopathological picture between the positive control group and the negative control group showed quite diverse differences. The negative control group had pathological changes in the form of congestence and telangiectasis. Congenital and telangiectasis are side effects of the euthanasia method that uses a combination of tricaine methanesulfonate MS 22 dose 50-100 mg/L and sodium bicarbonate dose ratio of 2:1 with MS-22. The administration of the drug triggers an increase in heart rate so that the fish's blood circulation appears full. The gill is an organ that will be significantly congested considering that the circulation of the heart to the gills is quite close and very crucial. Cell accumulation in the gap between the primary lamellae in

the negative control group remained visible although slightly. The positive control group showed contrasting differences in the presence of hemorrhage or erythrocyte discharge as well as fibrin accumulation. Capillaries in the secondary lamellae of the positive control group were slightly damaged characterized by unevenness of the lining of the secondary lamellae (Mokhtar, 2021; Zachary & McGavin, 2012).

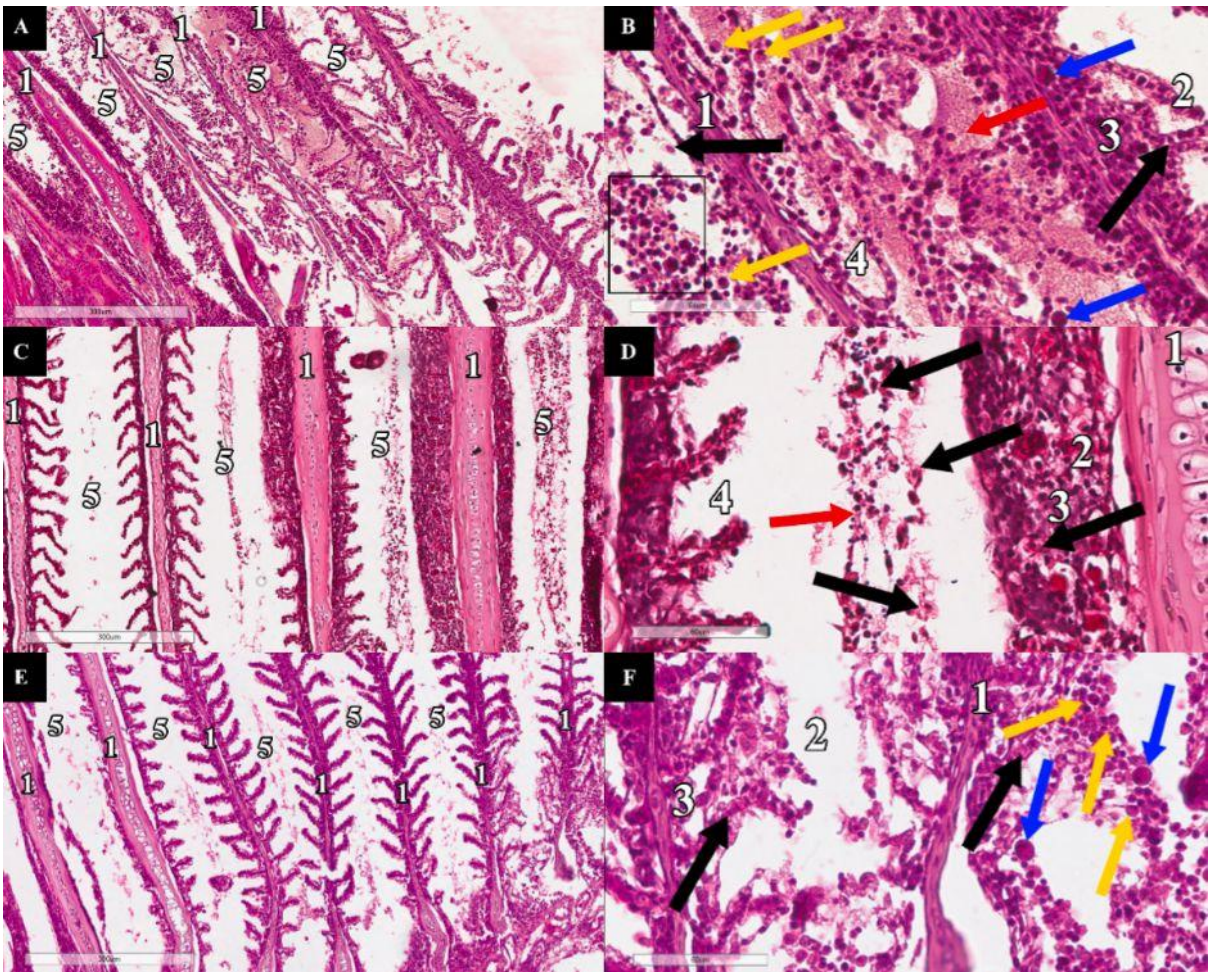

Figure 5. Histopathological overview of treatment group 1 (A&B), treatment 2 (C & D), treatment 3 (E&F).

Pictures A, C, and E are 100x magnification. Pictures B, D, and F are 400x magnification. Treatment 1 showed the most accumulation of fibrin (red arrow) compared to other treatments, even the positive control group. Hemorrhage is seen throughout treatment. The secondary lamella (figure 2) in treatment 1 has the most pronounced capillary membrane compared to treatment 2 and 3. Although it is clear that there are several broken lamellas. Inflammatory cells are clearly visible accumulating (black boxes) in the gaps between the primary lamellas. Chloride cells are seen to be detached or detached. Treatment group 2 underwent lamellar fusion in the vicinity of the secondary lamellae supporting cells (figure 3). Capillary membrane of the secondary lamella has undergone structural changes so that it is not interpreted firmly. Erythrocytes (black arrows) in treatment 1 and 2 were seen to still

have a normal structure, namely oval shape and oval cell nucleus. Erythrocytes in treatment 3 experienced poikilocytosis. The cell size of erythrocytes has varied and the cell nucleus does not show an oval shape but is curved. Yellow arrows indicate the presence of macrophage cells (Personal documentation, 2025).

Treatment 1, namely fasting for one day, showed the highest accumulation of fibrin compared to the entire group. Fibrin accumulation is a side effect of the occurrence of hemorrhage. Hemorrhage will trigger coagulation factors to cause blood clotting. Unsuccessful freezing will lead to an accumulation of fibrin. Fibrin is a protein found in blood plasma. Inflammation is the body's natural response when damage occurs. The whole treatment is inflamed. Inflammation can occur due to the presence of necrosis cells that secrete patterns called DAMPs (damage associated molecular patterns). DAMPs can be recognized by the body through PRR (Pattern Recognition Receptor) which then triggers inflammation (Zachary & McGavin, 2012). Treatment 2 shows that the secondary lamella has an attachment called *lamellar fusion. Lamellar fusion* is the fish's response to decrease diffusion by limiting the secondary lamellae. This method is expected to prevent infections that occur through air (Malheiros et al., 2023). Treatment 3 is the histopathological picture whose erythrocyte identification is the most difficult. Erythrocytes in treatment 3 suffer from *poikilositosis. Poikilositosis* is the difference in the size and nucleus of the cell in erythrocytes. Erythrocytes in fish have special characteristics, namely oval cell membranes and oval cell nuclei. *Poikilositosis* can occur due to several factors, namely viral infections, anemia, and oxidative stress (Bardhan et al., 2024).

CONCLUSION

This study aims to evaluate the effectiveness of fasting of different durations on total bacteria as well as histopathological changes in the intestines and gills of carp infected *with Aeromonas hydrophila*. The results of the analysis showed that fasting treatment had a real influence on microbiological and histopathological parameters. One-day fasting (P1) significantly increased total bacteria in the intestines and gills compared to other groups, indicating that acute metabolic stress in the early phases of fasting lowered mucosal resistance to bacterial colonization. In contrast, two- and three-day fasts (P2 and P3) lower the number of bacteria close to the control condition, indicating physiological adaptations during longer fasting periods. The intestinal histopathological picture shows that the two-day fasting only causes mild lesions that are adaptive in nature, in the form of epithelial desquamation, decreased goblet cells, and minimal inflammatory cell infiltration without severe structural damage. Gill histopathology also showed differences between groups, with P1 showing the highest inflammation and fibrin deposition, while P2 and P3 showed milder and protective changes, including lamella fusion in an adaptive response to infection. Overall, the study confirms that the effects of fasting are duration-dependent: fasting that is too short exacerbates the burden of bacteria and tissue lesions, while moderate fasting (2–3 days) provides a protective effect through microbiota stabilization and tissue adaptation responses. These findings support the potential of fasting as a nonpharmacological strategy in fish health management with

the determination of optimal duration to maximize benefits without causing nutritional stress.

DECLARATIONS

Availability of Data and Materials

All data generated is included in the manuscript

Acknowledgements

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Author Contributions

All authors conceptualize and design research, conduct experiments, collect data, and revise manuscripts, extract data and statistical analysis, and draft manuscripts. All authors have also read and approved the final manuscript.

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