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Impact of Dietary Coconut Oil (*Cocos nucifera L.*) on *Aeromonas hydrophila* Colonization and Lactic Acid Bacteria Populations in Zebrafish (*Danio rerio*): Implications for Aquaculture Health

Dahliatul Qosimah
Laboratory of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia

Izuwa Iwanegbe
Department of Food Science and Nutrition, University of Benin, PMB 1154, Benin City, Nigeria

Achmad Efendi
Statistics Study Program, Department of Mathematics, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia

Angellica Christine Nasution
Undergraduate Students, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia

Ameilia Hardiana
Undergraduate Students, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia

Naura Nisrina Zahrany
Undergraduate Students, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia

Pramadhita Wika Satria Putra
Undergraduate Students, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia

Respati Satya Daniswara
Undergraduate Students, Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia

Address: Jl. Veteran, Ketawanggede, Malang City
Corresponding author: dahlia_qosimah@ub.ac.id

Abstract*. The Aeromonas hydrophila (A. hydrophila) Aeromonas hydrophila (A. hydrophila) is an opportunistic pathogen causing significant losses in aquaculture. The overuse of antibiotics has led to antimicrobial resistance, necessitating alternative control strategies. This study evaluates the potential of coconut oil to inhibit A. hydrophila colonization in gills, brain, and gut microbiota of zebrafish while enhancing lactic acid bacteria (LAB) populations. A completely randomized design was used with five groups: a negative control (healthy), a positive control (A. hydrophila-infected), and three treatment groups (1000 mg/kg, 2000 mg/kg, 4000 mg/kg coconut oil) for 60 days. Bacterial load in gills and brain was quantified using the pour plate method on Rimler-Shotts agar, while LAB in the gut was assessed using de Man, Rogosa, and Sharpe Agar (MRSA). 1000 mg/kg coconut oil significantly reduced A. hydrophila in the gills (0.3 ± 0.09 × 10^7 CFU/ml) compared to the positive control (138.13 ±*

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*Corresponding author, dahlia_qosimah@ub.ac.id

11.26 × 10^7 CFU/ml). 4000 mg/kg was most effective in reducing A. hydrophila in the brain (1.78 ± 0.51 × 10^7 CFU/ml vs. 4.53 ± 0.40 × 10^7 CFU/ml in the positive control). LAB populations significantly increased ($p < 0.0001$), with the highest count at 1000 mg/kg (9.20 ± 0.87 × 10^7 CFU/ml), followed by 2000 mg/kg (3.16 ± 0.13 × 10^7 CFU/ml), and 4000 mg/kg (1.78 ± 0.35 × 10^7 CFU/ml). Coconut oil effectively enhances LAB populations, inhibits A. hydrophila colonization, and acts as a natural antimicrobial and probiotic agent. The 1000 mg/kg dose is optimal for gut microbiota improvement, while 4000 mg/kg protects brain tissues, highlighting coconut oil's potential as an eco-friendly alternative to antibiotics in aquaculture.

Keywords: *Aeromonas hydrophila, Coconut oil, Danio rerio, Immunomodulator*

INTRODUCTION

Aeromonas hydrophila is an opportunistic pathogen posing a serious threat to the global aquaculture industry, accounting for up to 40% economic losses in total production (Santos & Ramos, 2018). This bacterium infects various freshwater fish species, causing Motile Aeromonas Septicemia (MAS), characterized by hemorrhages, tissue necrosis, and high mortality rates reaching 80–100% in acute cases (Abdelhamed et al., 2017; Qosimah et al., 2023).

The control of *A. hydrophila* infections currently relies heavily on antibiotic use; however, this approach has led to multifaceted challenges. Antibiotic resistance has increased significantly, with over 70% of *A. hydrophila* strains showing resistance to at least three different classes of antibiotics (Nhinh et al., 2021). Antibiotic residues in aquaculture products further pose risks to consumer health and the environment (Okeke et al., 2022). More alarmingly, antibiotic resistance genes in A. hydrophila can be transferred to human pathogens via plasmids, elevating public health risks (Freitas et al., 2018; Stratev & Odeyemi, 2016)

This complexity necessitates the search for effective and safe natural alternatives. Natural immunomodulators, like coconut oil, have gained attention for their potential to enhance host defenses without resistance risks (Wang et al., 2017). This study uniquely explores the dose-specific effects of coconut oil on zebrafish's gills and brain, providing critical insights into organ-specific colonization and its implications for sustainable aquaculture. Coconut oil, rich in medium-chain fatty acids, has emerged as a promising candidate. Lauric acid (45–52%) and capric acid (5–10%) in coconut oil have demonstrated antimicrobial and immunomodulatory properties (Abel Anzaku et al., 2017; Silalahi, 2020). Recent studies indicate that these fatty acids can enhance macrophage phagocytic activity (Schumann, 2016). However, the use of coconut oil in aquaculture is still limited due to insufficient understanding of its mechanisms of action and optimal dosage. Additionally, the distribution and effectiveness of its active components, particularly in organs protected by the blood-brain barrier such as the brain, remain poorly understood.

Zebrafish (*Danio rerio*) was chosen due to its genetic similarity to humans (70%) and transparent immune system, allowing detailed host-pathogen interaction studies (Torraca & Mostowy, 2018). This model is ideal for examining *Aeromonas hydrophila* colonization in the gills and brain, which differ in accessibility and immune response (Li et al., 2024). Coconut oil, rich in lauric, capric, and caprylic acids, was selected for its demonstrated immunomodulatory properties. These active compounds enhance macrophage activity, modulate cytokines, and strengthen mucosal barriers

This study aims to evaluate the potential of coconut oil in controlling *A. hydrophila* colonization in the gills and brain of zebrafish and to elucidate its mechanisms of action. Our hypothesis is that coconut oil will exhibit varying effectiveness in different target organs due to differences in tissue accessibility and characteristics. This understanding is critical for optimizing the application of coconut oil in sustainable aquaculture.

LITERATURE REVIEW

Aeromonas hydrophila is a significant pathogen in aquaculture, causing high morbidity and mortality in freshwater fish that experience symptoms like hemorrhagic septicemia, ascites, and skin ulcers (Ofek et al. 2023), and also leading to substantial economic losses (Abdul Kari et al. 2022). *A. hydrophila* frequently harbors genes such as aerolysin (aerA), hemolysin (hlyA), and cytotonic enterotoxin (act), which are associated with its pathogenicity in fish (Mahmood 2024). This bacterium is challenging to control due to its resistance to multiple antibiotics and its ability to cause severe disease outbreaks (Nhinh et al. 2021). Therefore, a safe and environmentally friendly alternative strategy is needed, one of which is by using natural ingredients that have immunomodulatory properties, such as coconut oil.

Coconut oil is rich in medium-chain fatty acids (MCFAs), including lauric acid, capric acid, and caprylic acid, has been recognized for its potential health benefits, including antimicrobial, anti-inflammatory (Deen et al. 2021), and immunostimulatory properties. Coconut oil acts as a natural antibiotic and helps modulate immunity due to its medium chain fatty acids and monoglycerides, which are effective and safe immune-nutritive actives. oconut oil is 50% lauric acid, to which antibacterial, and is attributed, especially to its monoglyceride, monolaurin (Vereau and Méndez 2021).

Capric acid and lauric acid both exhibit bactericidal and anti-inflammatory activities against *Propionibacterium acnes*, with the anti-inflammatory effect partially occurring through inhibition of NF-β activation and MAP kinases (Huang et al. 2014). The antibacterial action of lauric acid involves the generation of reactive oxygen species and damage to bacterial cell membranes, leading to cell death. This mechanism is effective against both Gram-positive and some Gram-negative bacteria (Fischer et al. 2012; Yang et al. 2018). Lauric acid supplementation in broiler chicken diets improves growth and immune functions by regulating lipid metabolism and gut microbiota, potentially reducing antibiotic usage and improving food safety (Wu et al. 2021). It reduces inflammation by decreasing pro-inflammatory cytokines such as IL-6 and TNF-α, and increasing anti-inflammatory cytokines like IL-4 and IL-10, which can be beneficial in conditions like type II diabetes and bacterial infections (Mustafa et al. 2023).

RESEARCH METHODS

This experimental study was conducted using a Completely Randomized Design (CRD). Adult zebrafish were divided into five treatment groups: a negative control group (without infection), a positive control group (inoculated with *A. hydrophila* at a concentration of 10^7 CFU/ml via immersion), and three treatment groups (T1, T2, and T3) receiving coconut oil at doses of 1000 mg/kg, 2000 mg/kg, and 4000 mg/kg feed based on previous research (Qosimah et al., 2024) for 60 days prior to bacterial inoculation. On day 62, the gills and brain were collected for enumeration of *A. hydrophila bacteria* (Table 1). The study was conducted at the Microbiology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, from July to September 2024. Zebrafish maintenance was carried out at the Faculty of Fisheries and Marine Science, Universitas Brawijaya.

Table 1. Experimental Design and Treatment Groups for Coconut Oil Administration in Zebrafish

Group	Description	Dose of Coconut Oil (mg/kg) BW	Duration
Negative Control	No infection, no coconut oil	0	60 days
Positive Control	Infection with *A. hydrophila*	0	60 days
T1	Infection + coconut oil	1000	60 days
T2	Infection + coconut oil	2000	60 days
T3	Infection + coconut oil	4000	60 days

Preparation of Test Animals

Adult zebrafish (4–6 months old, 3–4 cm in length) were obtained from the Faculty of Fisheries and Marine Science, Universitas Brawijaya. The fish were acclimatized for 7 days in aquaria containing dechlorinated water (15 L per aquarium, 10 fish per aquarium) at a temperature of 26±1°C, pH 7.0 ± 0.2, and a photoperiod of 14 hours light/10 hours dark. The study received ethical approval from the Research Ethics Committee of Universitas Brawijaya (No. 118-KEP-UB-2024).

Preparation and Administration of Coconut Oil

Virgin coconut oil was obtained from a certified producer and analyzed for its fatty acid composition using GC-MS (Agilent 7890B, USA) (Agilent 7890B, USA) (Kumar et al., 2023).

Commercial feed (PF500) was ground and mixed with coconut oil according to the treatment doses, then dried at 60°C (Matin et al., 2024) or 15 minutes. The feed was administered at 3% of body weight per day, split into two feedings.

Bacterial Culture and Characterization

The *A. hydrophila* strain was obtained from the Fish Health and Environmental Testing Center (BPKIL), Serang, Indonesia, and identified using the Vitek® 2 Compact system (Elbehiry et al., 2019). The bacteria were grown on Tryptic Soy Agar (TSA, Merck) and incubated at 26°C for 24 hours. Identification and characterization were performed through colony morphology observation, Gram staining, and biochemical tests.

Bacterial Colonization Enumeration

On day 62 post-infection, zebrafish were euthanized using cooled temperature (Islamet al., 2021). The gills and brain were aseptically collected, weighed, and homogenized in sterile PBS. The bacterial count was determined using the spread plate method. The homogenates were serially diluted and cultured on Rimler-Shotts agar ((Semwal et al., 2023). Colonies were counted after 24 hours of incubation at 26°C and expressed as CFU/ml. The colony forming units per milliliter of inoculant (CFU.ml-1) were counted taking into account the dilutions which presented a range of 30–300 colonies per plate in the spreading method (Dong 2018). Additionally, lactic acid bacteria (LAB) were cultured on de Man, Rogosa, and Sharpe Agar (MRSA) medium to assess gut microbiota composition. The plates were incubated at 37°C for 48 hours, and the resulting colonies were enumerated and expressed as CFU/ml.

Data Analysis

Data were analyzed using one-way ANOVA followed by Tukey's test at a 5% significance level using GraphPad Prism 8. Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

The results showed that the group receiving coconut oil at a dose of T1 (1000 mg/kg) exhibited a significant reduction in *A. hydrophila* counts in the gills and brain compared to the positive control group. Higher doses of coconut oil showed varying trends in bacterial reduction, with decreased efficacy observed at T3 (4000 mg/kg).

Colonization of *A. hydrophila* in the Gills

Coconut oil administration significantly ($p<0.05$) influenced the number of *A. hydrophila* colonies in the gills. The negative control group had the lowest bacterial count ($1.5 \pm 0.43 \times 10^8$ CFU/g), whereas the positive control group had the highest count ($138.13 \pm 11.26 \times 10^8$ CFU/g). Coconut oil at a dose of 1000 mg/kg feed (T1) showed the best effect in reducing bacterial colonization ($0.3 \pm 0.09 \times 10^8$ CFU/g), followed by by dose of 2000 mg/kg feed (T2) ($19.71 \pm 2.58 \times 10^8$ CFU/g) and dose of 2000 mg/kg feed (T2) ($58.46 \pm 3.41 \times 10^8$ CFU/g) in Fig. 1.

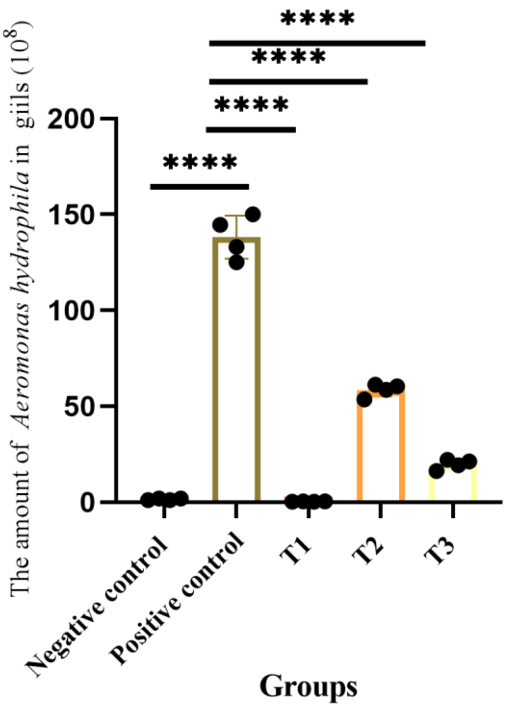

Figure 1. The number of *A. hydrophila* colonies in the gills across different treatment groups. The figure shows the mean bacterial counts in the gills of zebrafish. Groups include the negative control (no infection), positive control (infection without coconut oil), and three treatment groups receiving coconut oil at doses of 1000 mg/kg (T1), 2000 mg/kg (T2), and 4000 mg/kg (T3). Data are presented as mean ± standard deviation. $P<0.05$ indicates significant differences between groups. (****) denotes highly significant differences ($P<0.001$), while the absence of asterisks indicates no significant differences.

Colonization of *A. hydrophila* in the Brain

In brain tissues, colonization patterns differed from those observed in the gills. The negative control group had a bacterial count of $4.53 \pm 0.40 \times 10^8$ CFU/g, while the positive control group exhibited a count of $1.46 \pm 0.50 \times 10^8$ CFU/g. The dose of 4000 mg/kg (T3) was most effective in reducing bacterial colonization ($1.78 \pm 0.51 \times 10^8$ CFU/g), while dose of 1000 mg/kg feed (T1) and dose of 1000 mg/kg feed (T2) showed variable results (2.41 ± 0.53 and $4.59 \pm 0.80 \times 10^8$ CFU/g, respectively) in Fig. 2.

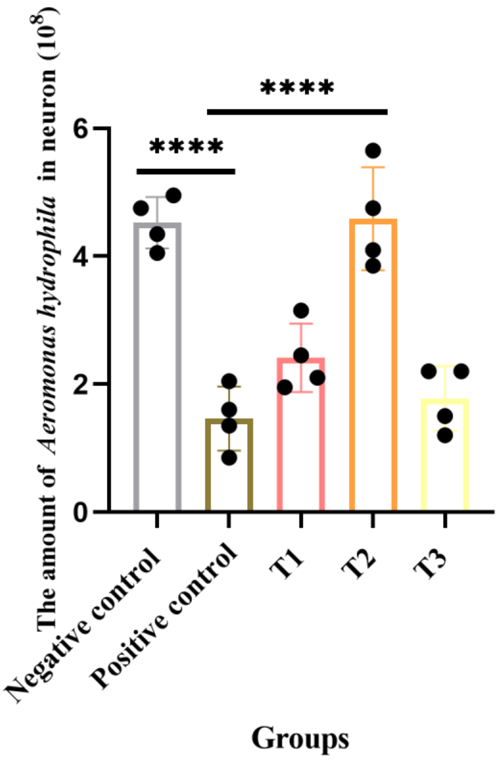

Figure 2. The number of *Aeromonas hydrophila* colonies in the brain across different treatment groups. Description: The graph illustrates the mean bacterial counts in the brains of zebrafish. Groups include the negative control, positive control, and three treatment groups with varying doses of coconut oil. Data are presented as mean ± standard deviation. (****) denotes highly significant differences (*P*<0.001), while the absence of asterisks indicates no significant differences.

The Number of Lactic Acid Bacteria in the Gut of Fish Infected with *A. hydrophila*

Based on the data on lactic acid bacteria counts in the intestines of fish treated with coconut oil at various doses, a significant difference (p < 0.0001) was observed among the treatment groups. According to the Tukey test, the T1 group (1000 mg/kg feed) exhibited the highest number of lactic acid bacteria, which was significantly different from both the positive and negative control groups. The negative control group (0.58 ± 0.05) had a higher bacterial count compared to the positive control group (0.00 ± 0.00). The T1 group (9.20 ± 0.87) showed the highest lactic acid bacteria count compared to T2 (3.16 ± 0.13) and T3 (1.78 ± 0.35). The addition of coconut oil resulted in an increase in lactic acid bacteria in all treatment groups compared to the positive control group in Fig. 3.

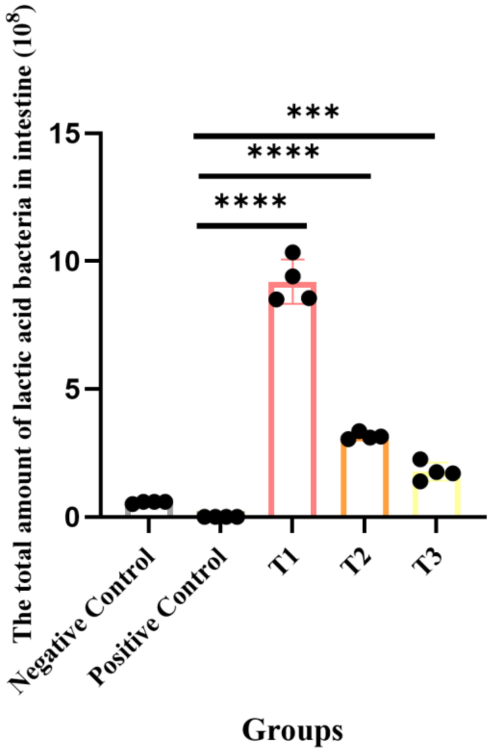

Figure 3. The number of Lactic Acid Bacteria in the gut of fish infected with *A. hydrophila*

In the T1 group (1000 mg/kg feed), there was a significant increase in lactic acid bacteria compared to other treatment groups. This finding suggests that 1000 mg/kg feed is the optimal dose for supporting the population of beneficial bacteria in the gut, which plays a crucial role in inhibiting the colonization of *A. hydrophila*. Lactic acid bacteria produce organic acids, which lower the intestinal pH, making the environment less conducive for pathogen growth. These bacteria are essential for maintaining gut microbiota balance and enhancing the local immune response against pathogenic bacterial infections.

Regarding the colonization of *A. hydrophila* in other organs (muscle, brain, and gills), it was observed that coconut oil at certain doses (T1 and T 3) effectively reduced pathogen colonization in vital organs. Notably, the lower dose (1000 mg/kg feed) was more effective in increasing lactic acid bacteria counts. This effect presents a promising disease management strategy by optimizing gut microbiota balance and reducing pathogenic bacterial loads. The combination of increased lactic acid bacteria and decreased Aeromonas counts in target organs demonstrates the potential of coconut oil as both an antimicrobial and probiotic agent.

This study revealed an intriguing pattern in the effectiveness of coconut oil against *A. hydrophila* colonization, with distinct responses observed in the gills and brain. The primary findings indicate that a low dose of T1 (1000 mg/kg) is optimal for the gills, while a high dose of T3 (4000 mg/kg) is more effective for the brain. The gills, as an organ in direct contact with the environment, showed a positive response to the low dose (0.3 ± 0.09 × 10^8 CFU/g) compared to the positive control (138.13 ± 11.26 × 10^8 CFU/g). Additionally, results indicated that at a dose of T2 (2000 mg/kg), bacterial counts in the intestine were higher than in other doses, significantly exceeding the count observed at dose of T3 (4000 mg/kg) (unpublished data). This finding suggests the potential for bacterial adaptation or tolerance at moderate doses. The reduction in colonization at dose of T3 demonstrates the effectiveness of coconut oil in enhancing intestinal protection through inflammation modulation and mucosal barrier strengthening (Table 2). This is supported by Firmino et al. (2021), who reported that bioactive compounds can directly interact with the gill mucosal surface. Conversely, the brain, protected by the blood-brain barrier, requires a higher dose for optimal effects. Schönfeld et al. (2016), explained that medium-chain fatty acids require sufficient concentrations to penetrate this barrier, corroborating our findings that a dose of 4000 mg/kg was most effective in reducing brain colonization (1,78±0,51 x 10^8 CFU/g).

Table 2. Bacterial Colonization in Gills and Brain Across Treatment Groups

Organ	Negative Control (CFU/g)	Positive Control (CFU/g)	T1 (CFU/g)	T2 (CFU/g)	T3 (CFU/g)
Gills	1.5 ± 0.43 × 10^8	138.13 ± 11.26 × 10^8	0.3 ± 0.09 × 10^8	58.46 ± 3.41 × 10^8	19.71 ± 2.58 × 10^8
Brain	4.53 ± 0.40 × 10^8	1.46 ± 0.50 × 10^8	2.41 ± 0.53 × 10^8	4.59 ± 0.80 × 10^8	1.78 ± 0.51 × 10^8
Intestine	0,58 ± 0,05 × 10^8	0,00 ± 0,00 × 10^8	9,20 ± 0,87 × 10^8	3,16 ± 0,13 × 10^8	1,78 ± 0,35 × 10^8

Results from Gas Chromatography-Mass Spectrometry (GC-MS) analysis indicated that the coconut oil used in this study contained lauric acid (C12:0) (45.98%), myristic acid (C14:0) (17.41%), capric acid (C10:0) (5.76%), and caprylic acid (C8:0) (4.32%) as the main components. Coconut oil functions through complex mechanisms involving various active compounds: a) Lauric acid enhances natural killer (NK) cell activity and Interleukin (IL-2) production (Garcia et al., 2023), and modulates Tumor necrosis factor (TNF) -α, IL-1β expression via the NF-κB pathway (Khan et al., 2021; Zlatanova et al., 2024) to express inflammation. Its effects on the gills are more prominent due to direct mucosal contact (Emam et al. 2022); b) Capric acid activates PPAR-γ for inflammation regulation (Lee & Kang, 2017), strengthens mucosal barriers by upregulating tight junction proteins (Usuda et al., 2021), and protects the brain through neuroinflammation modulation (Shekhar et al., 2023); c). Caprylic acid modulates T-cell differentiation and regulatory cytokines (Hung et al., 2023), and enhances antimicrobial peptide production (Grimsey et al., 2020).

The differences in bacterial counts between the positive control in the gills (138.13 ± 11.26 × 10^8 CFU/g) and the brain (1.46 ± 0.50 × 10^8 CFU/g) align with Abdelhamed et al. (2017), who described the organ tropism of *A. hydrophila* in the brain and gills. Interestingly, increasing the dose does not always yield a linear effect, particularly in the gills. Wan et al., (2024), explained this phenomenon using the concept of hormesis, where excessive doses can trigger oxidative stress (Pavlek et al. 2020). The effectiveness of the low dose in the gills highlights the critical role of the mucosal immune system s essential for protecting fish against pathogen (Hopo et al. 2024). Medium-chain fatty acids can enhance protective mucus production, optimize mucosal microbiota composition, and strengthen barrier function through tight junction regulation (Barbara et al., 2021; Liu et al., 2024).

The higher dose required for brain protection is associated with the complexity of the blood-brain barrier (Dong 2018). The transport of medium-chain fatty acids to the brain requires specific carrier systems (Tsuji, 2005), and neuroinflammation modulation necessitates higher threshold concentrations, involving protective effects mediated by microglia and astrocyte activation (Watanabe & Tsujino, 2022)

This study showed mortality rates of 80% in the positive control group and 0% in the negative control group, while mortality in the T1, T2, and T3 treatment groups was 20%, 25%, and 15%, respectively. The reduced mortality in the treatment groups indicates the immunomodulatory effects of coconut oil, which enhances phagocytic activity and anti-inflammatory cytokine production (Intahphuak et al. 2010). However, higher doses (2000 and 4000 mg/kg feed) may induce mild toxic effects, which warrant further evaluation.

Lauric acid, which is present in high concentrations in coconut oil, is a medium-chain fatty acid known for its antibacterial and antiviral properties. In the body, lauric acid is converted into monolaurin, which is effective in combating pathogenic bacteria. Studies have shown that monolaurin can disrupt bacterial cell membranes (Nitbani et al. 2022), leading to cell death and a reduction in pathogen infectivity, including *A. hydrophila*. In addition to lauric acid, capric acid also exhibits strong antimicrobial activity, particularly against Gram-positive and some Gram-negative bacteria. Capric acid works by disrupting the lipid membrane of microorganisms, leading to alterations in membrane permeability and ultimately causing bacterial cell lysis (Guimarães and Venâncio 2022). Oleic acid possesses anti-inflammatory properties and enhances cell membrane stability, which is beneficial in maintaining cell integrity during infections (Bilal et al. 2021). Additionally, oleic acid contributes to immune responses by enhancing phagocytic activity, assisting the immune system in eliminating pathogens (Santa-María et al. 2023). Vitamin E (Tocopherol), present in coconut oil, functions as an antioxidant, which helps reduce oxidative stress in infected fish cells and tissues. The presence of vitamin E protects cells from bacterial-induced damage, thereby enhancing overall immune resilience and reducing pathological effects caused by infections (Lewis et al. 2019).

The bioactive compounds in coconut oil provide synergistic effects in strengthening immune responses and protecting fish tissues from pathogen attacks. This supports the findings of this study, where fish groups supplemented with coconut oil showed a reduction in *A. hydrophila* counts in specific organs compared to the positive control group.

The findings of this study have practical implications for aquaculture, emphasizing the importance of dose titration according to the target protection organ, the potential for coconut oil as a safer antibiotic alternative, and the necessity of pharmacokinetic considerations in its application. Future research should evaluate long-term effects on growth and reproduction parameters, explore interactions with the adaptive immune system, conduct pharmacokinetic studies to optimize dosing and frequency, and develop formulations that enhance the bioavailability of active components. Additionally, further studies are needed to elucidate the molecular mechanisms underlying the immunomodulatory activity of coconut oil.

CONCLUSION

This study demonstrates that coconut oil has potential as an immunomodulatory agent for controlling *A. hydrophila* infections in zebrafish, with varying effectiveness across different target organs. It functions by reducing *A. hydrophila* colonization and stimulating the growth of beneficial bacteria, such as lactic acid bacteria in the gut. A coconut oil dose of 1000 mg/kg feed is optimal for supporting gut microbiota health, while a dose of 4000 mg/kg is more effective in suppressing pathogenic bacteria in vital organs. This dual action can be optimized as a health management strategy for farmed fish.

LIMITATION

This study has several limitations that should be addressed in future research:

1. Short-term Focus: The study primarily assessed the short-term effects of coconut oil on *A. hydrophila* colonization without evaluating its long-term impact on growth performance, immune function, and reproductive parameters of zebrafish.
2. Specificity to Zebrafish: While zebrafish are a valuable model organism, the findings may not fully translate to other aquaculture species due to interspecies differences in physiology and immune responses.

3. Absence of Molecular Analysis: The study did not investigate the molecular mechanisms underlying the immunomodulatory effects of coconut oil, such as gene expression changes or signaling pathways.
4. Limited Dose Range: The study only explored three doses of coconut oil. Intermediate doses or alternative administration methods (e.g., injection or immersion) could provide further insights into its optimal use.

REFERENCES

Abdelhamed, H., Ibrahim, I., Baumgartner, W., Lawrence, M. L., & Karsi, A. (2017). Characterization of Histopathological and Ultrastructural Changes in Channel Catfish Experimentally Infected with Virulent Aeromonas hydrophila. *Frontiers in Microbiology*, *8*, 1519. https://doi.org/10.3389/fmicb.2017.01519

Abel Anzaku, A., Ernsets bassey, A., Martins, A., Uplah, P., & Terungwa, K. (2017). Antimicrobial Activity of Coconut Oil and its Derivative (Lauric Acid) on Some Selected Clinical Isolates. *International Journal of Medical Science and Clinical Inventions*, *4*(8). https://doi.org/10.18535/ijmsci/v4i8.12

Abdul Kari Z, Wee W, Mohamad Sukri SA, et al (2022) Role of phytobiotics in relieving the impacts of Aeromonas hydrophila infection on aquatic animals: A mini-review. Front Vet Sci 9:1023784. https://doi.org/10.3389/fvets.2022.1023784

Barbara, G., Barbaro, M. R., Fuschi, D., Palombo, M., Falangone, F., Cremon, C., Marasco, G., & Stanghellini, V. (2021). Inflammatory and Microbiota-Related Regulation of the Intestinal Epithelial Barrier. *Frontiers in Nutrition*, *8*, 718356. https://doi.org/10.3389/fnut.2021.718356

Bilal RM, Liu C, Zhao H, et al (2021) Olive Oil: Nutritional Applications, Beneficial Health Aspects and its Prospective Application in Poultry Production. *Front Pharmacol*, 12:723040. https://doi.org/10.3389/fphar.2021.723040

Deen A, Visvanathan R, Wickramarachchi D, et al (2021) Chemical composition and health benefits of coconut oil: an overview. *J Sci Food Agric* 101:2182–2193. https://doi.org/10.1002/jsfa.10870

Dong X (2018) Current Strategies for Brain Drug Delivery. Theranostics 8:1481–1493. https://doi.org/10.7150/thno.21254

Elbehiry, A., Marzouk, E., Abdeen, E., Al-Dubaib, M., Alsayeqh, A., Ibrahem, M., Hamada, M., Alenzi, A., Moussa, I., & Hemeg, H. A. (2019). Proteomic characterization and discrimination of *Aeromonas* species recovered from meat and water samples with a spotlight on the antimicrobial resistance of *Aeromonas hydrophila*. *MicrobiologyOpen*, *8*(11), e782. https://doi.org/10.1002/mbo3.782

Emam M, Caballero-Solares A, Xue X, et al (2022) Gill and Liver Transcript Expression Changes Associated With Gill Damage in Atlantic Salmon (Salmo salar). Front Immunol 13:806484. https://doi.org/10.3389/fimmu.2022.806484

Firmino, J. P., Galindo-Villegas, J., Reyes-López, F. E., & Gisbert, E. (2021). Phytogenic Bioactive Compounds Shape Fish Mucosal Immunity. *Frontiers in Immunology*, *12*, 695973. https://doi.org/10.3389/fimmu.2021.695973

Freitas, M. R., Freire, N. B., Peixoto, L. J. E. S., Oliveira, S. T. L. D., Souza, R. C. D., Gouveia, J. J. D. S., Costa, M. M. D., & Gouveia, G. V. (2018). The presence of plasmids in Aeromonas hydrophila and its relationship with antimicrobial and heavy metal-resistance profiles. *Ciência Rural*, *48*(9). https://doi.org/10.1590/0103-8478cr20170813

Fischer CL, Drake DR, Dawson DV, et al (2012) Antibacterial Activity of Sphingoid Bases and Fatty Acids against Gram-Positive and Gram-Negative Bacteria, *Antimicrob Agents Chemother* 56:1157–1161. https://doi.org/10.1128/AAC.05151-11

Garcia, C., Andersen, C. J., & Blesso, C. N. (2023). The Role of Lipids in the Regulation of Immune Responses. *Nutrients*, *15*(18), 3899. https://doi.org/10.3390/nu15183899

Grimsey, E., Collis, D. W. P., Mikut, R., & Hilpert, K. (2020). The effect of lipidation and glycosylation on short cationic antimicrobial peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1862*(8), 183195. https://doi.org/10.1016/j.bbamem.2020.183195

Guimarães A, Venâncio A (2022) The Potential of Fatty Acids and Their Derivatives as Antifungal Agents: A Review. *Toxins*, 14:188. https://doi.org/10.3390/toxins14030188

Hopo MG, Mabrok M, Abu-Elala N, Yu Y (2024) Navigating Fish Immunity: Focus on Mucosal Immunity and the Evolving Landscape of Mucosal Vaccines. Biology 13:980. https://doi.org/10.3390/biology13120980

Hung, H.-C., Tsai, S.-F., Chou, H.-W., Tsai, M.-J., Hsu, P.-L., & Kuo, Y.-M. (2023). Dietary fatty acids differentially affect secretion of pro-inflammatory cytokines in human THP-1 monocytes. *Scientific Reports*, *13*(1), 5511. https://doi.org/10.1038/s41598-023-32710-5

Intahphuak S, Khonsung P, Panthong A (2010) Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. Pharmaceutical Biology 48:151–157. https://doi.org/10.3109/13880200903062614

Islam, J., James Slater, M., Thiele, R., & Kunzmann, A. (2021). Influence of extreme ambient cold stress on growth, hematological, antioxidants, and immune responses in European seabass, Dicentrarchus labrax acclimatized at different salinities. *Ecological Indicators*, *122*, 107280. https://doi.org/10.1016/j.ecolind.2020.107280

Khan, H. U., Aamir, K., Jusuf, P. R., Sethi, G., Sisinthy, S. P., Ghildyal, R., & Arya, A. (2021). Lauric acid ameliorates lipopolysaccharide (LPS)-induced liver inflammation by mediating TLR4/MyD88 pathway in Sprague Dawley (SD) rats. *Life Sciences*, *265*, 118750. https://doi.org/10.1016/j.lfs.2020.118750

Kumar, S. S., Manasa, V., Madhubalaji, C. K., Tumaney, A. W., & Giridhar, P. (2023). GC/MS quantification of individual fatty acids of selected green leafy vegetable foliage and their biodiesel attributes. *Grasas y Aceites*, *74*(2), e499. https://doi.org/10.3989/gya.0907212

Lee, S. I., & Kang, K. S. (2017). Function of capric acid in cyclophosphamide-induced intestinal inflammation, oxidative stress, and barrier function in pigs. *Scientific Reports*, *7*(1), 16530. https://doi.org/10.1038/s41598-017-16561-5

Lewis ED, Meydani SN, Wu D (2019) Regulatory role of vitamin E in the immune system and inflammation. *IUBMB Life*, 71:487–494. https://doi.org/10.1002/iub.1976

Li, Z., Chen, F., Wei, M., Zhi, L., Su, Z., Chong, Y., Xiao, Z., & Wang, J. (2024). Concurrent impacts of polystyrene nanoplastic exposure and Aeromonas hydrophila infection on oxidative stress, immune response and intestinal microbiota of grass carp (Ctenopharyngodon idella). *Science of The Total Environment*, *912*, 169225. https://doi.org/10.1016/j.scitotenv.2023.169225

Liu, H., Lu, H., Wang, Y., Yu, C., He, Z., & Dong, H. (2024). Unlocking the power of short-chain fatty acids in ameliorating intestinal mucosal immunity: A new porcine nutritional approach. *Frontiers in Cellular and Infection Microbiology*, *14*, 1449030. https://doi.org/10.3389/fcimb.2024.1449030

Mahmood S (2024) Molecular characterization of Aeromonas hydrophila detected in Channa marulius and Sperata sarwari sampled from rivers of Punjab in Pakistan. *PLoS ONE*, 19:41524 Bytes. https://doi.org/10.6084/M9.FIGSHARE.25329460.V3

Matin, A., Brandić, I., Gubor, M., Pezo, L., Krička, T., Matin, B., Jurišić, V., & Antonović, A. (2024). Effect of conduction drying on nutrient and fatty acid profiles: A comparative analysis of hazelnuts and walnuts. *Frontiers in Sustainable Food Systems*, *8*, 1351309. https://doi.org/10.3389/fsufs.2024.1351309

Mustafa A, Indiran MA, Shanmugham R, Ramalingam K (2023) Anti-inflammatory activity of lauric acid, thiocolchicoside and thiocolchicoside-lauric acid formulation. *Bioinformation*, 19:1075–1080. https://doi.org/10.6026/973206300191075

Nhinh, D. T., Le, D. V., Van, K. V., Huong Giang, N. T., Dang, L. T., & Hoai, T. D. (2021). Prevalence, Virulence Gene Distribution and Alarming the Multidrug Resistance of Aeromonas hydrophila Associated with Disease Outbreaks in Freshwater Aquaculture. *Antibiotics*, *10*(5), 532. https://doi.org/10.3390/antibiotics10050532

Nitbani FO, Tjitda PJP, Nitti F, et al (2022) Antimicrobial Properties of Lauric Acid and Monolaurin in Virgin Coconut Oil: A Review. *ChemBioEng Reviews*, 9:442–461. https://doi.org/10.1002/cben.202100050

Ofek T, Izhaki I, Halpern M (2023) Aeromonas hydrophila infection in tilapia triggers changes in the microbiota composition of fish internal organs. *FEMS Microbiology Ecology*, 99:fiad137. https://doi.org/10.1093/femsec/fiad137

Okeke, E. S., Chukwudozie, K. I., Nyaruaba, R., Ita, R. E., Oladipo, A., Ejeromedoghene, O., Atakpa, E. O., Agu, C. V., & Okoye, C. O. (2022). Antibiotic resistance in aquaculture and aquatic organisms: A review of current nanotechnology applications for sustainable management. *Environmental Science and Pollution Research*, *29*(46), 69241–69274. https://doi.org/10.1007/s11356-022-22319-y

Pavlek LR, Dillard J, Rogers LK (2020) The role of oxidative stress in toxicities due to drugs of abuse. Current Opinion in Toxicology 20–21:29–35. https://doi.org/10.1016/j.cotox.2020.04.003

Qosimah, D., Amri, I. A., Pratama, D. A. O. A., Permata, F. S., Noorhamdani, N., Widasmara, D., & Sabri, J. (2024). Hexane extract from black soldier fly prepupae: A novel immunomodulatory strategy against Aeromonas hydrophila infection in zebrafish. *Veterinary World*, 1655–1660. https://doi.org/10.14202/vetworld.2024.1655-1660

Qosimah, D., Santoso, S., Maftuch, M., Khotimah, H., Fitri, L. E., Aulanni'am, A., & Suwanti, L. T. (2023). Aeromonas hydrophila induction method in adult zebrafish (Danio rerio) as animal infection models. *Veterinary World*, 250–257. https://doi.org/10.14202/vetworld.2023.250-257

Santa-María C, López-Enríquez S, Montserrat-de La Paz S, et al (2023) Update on Anti-Inflammatory Molecular Mechanisms Induced by Oleic Acid. *Nutrients* ,15:224. https://doi.org/10.3390/nu15010224

Santos, L., & Ramos, F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents*, *52*(2), 135–143. https://doi.org/10.1016/j.ijantimicag.2018.03.010

Schönfeld, P., & Wojtczak, L. (2016). Short- and medium-chain fatty acids in energy metabolism: The cellular perspective. *Journal of Lipid Research*, *57*(6), 943–954. https://doi.org/10.1194/jlr.R067629

Schumann, J. (2016). It is all about fluidity: Fatty acids and macrophage phagocytosis. *European Journal of Pharmacology*, *785*, 18–23. https://doi.org/10.1016/j.ejphar.2015.04.057

Semwal, A., Kumar, A., & Kumar, N. (2023). A review on pathogenicity of Aeromonas hydrophila and their mitigation through medicinal herbs in aquaculture. *Heliyon*, *9*(3), e14088. https://doi.org/10.1016/j.heliyon.2023.e14088

Semwal, A., Kumar, A., & Kumar, N. (2023). A review on pathogenicity of Aeromonas hydrophila and their mitigation through medicinal herbs in aquaculture. *Heliyon*, *9*(3), e14088. https://doi.org/10.1016/j.heliyon.2023.e14088

Shekhar, N., Tyagi, S., Rani, S., & Thakur, A. K. (2023). Potential of Capric Acid in Neurological Disorders: An Overview. *Neurochemical Research*, *48*(3), 697–712. https://doi.org/10.1007/s11064-022-03809-4

Silalahi, J. (2020). Nutritional Values and Health Protective Properties Of Coconut Oil. *Indonesian Journal of Pharmaceutical and Clinical Research*, *3*(2), 1–12. https://doi.org/10.32734/idjpcr.v3i2.4065

Stratev, D., & Odeyemi, O. A. (2016). Antimicrobial resistance of Aeromonas hydrophila isolated from different food sources: A mini-review. *Journal of Infection and Public Health*, *9*(5), 535–544. https://doi.org/10.1016/j.jiph.2015.10.006

Torraca, V., & Mostowy, S. (2018). Zebrafish Infection: From Pathogenesis to Cell Biology. *Trends in Cell Biology*, *28*(2), 143–156. https://doi.org/10.1016/j.tcb.2017.10.002

Tsuji, A. (2005). *Small Molecular Drug Transfer across the Blood-Brain Barrier via Carrier-Mediated Transport Systems*. *54–82*.

Usuda, H., Okamoto, T., & Wada, K. (2021). Leaky Gut: Effect of Dietary Fiber and Fats on Microbiome and Intestinal Barrier. *International Journal of Molecular Sciences*, *22*(14), 7613. https://doi.org/10.3390/ijms22147613

Vereau GV, Méndez G (2021) Antibacterial Effect of Coconut Oil (Cocus nucifera) on Streptococcus mutans ATCC 25175: An In vitro Study. nt J Odontostomat 15:922–927

Wan, Y., Liu, J., Mai, Y., Hong, Y., Jia, Z., Tian, G., Liu, Y., Liang, H., & Liu, J. (2024). Current advances and future trends of hormesis in disease. *Npj Aging*, *10*(1), 26. https://doi.org/10.1038/s41514-024-00155-3

Wang, W., Sun, J., Liu, C., & Xue, Z. (2017). Application of immunostimulants in aquaculture: Current knowledge and future perspectives. *Aquaculture Research*, *48*(1), 1–23. https://doi.org/10.1111/are.13161

Watanabe, S., & Tsujino, S. (2022). Applications of Medium-Chain Triglycerides in Foods. *Frontiers in Nutrition*, *9*, 802805. https://doi.org/10.3389/fnut.2022.802805

Wu Y, Zhang H, Zhang R, et al (2021) Serum metabolome and gut microbiome alterations in broiler chickens supplemented with lauric acid. *Poultry Science* ,100:101315. https://doi.org/10.1016/j.psj.2021.101315

Yang H-T, Chen J-W, Rathod J, et al (2018) Lauric Acid Is an Inhibitor of Clostridium difficile Growth in Vitro and Reduces Inflammation in a Mouse Infection Model. Front Microbiol 8:2635. https://doi.org/10.3389/fmicb.2017.02635

Zlatanova, M., Nešić, A., Trbojević-Ivić, J., Četić, D., & Gavrović-Jankulović, M. (2024). Targeting NF-κB Signaling: Selected Small Molecules Downregulate Pro-Inflammatory Cytokines in Both Food Allergen and LPS-Induced Inflammation. *International Journal of Molecular Sciences*, *25*(11), 5798. https://doi.org/10.3390/ijms25115798.