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PROOF
RESEARCH PAPER

Promoting Growth and Health of African Catfish, *Clarias gariepinus*, Through Dietary Novel Supplement, Ginger, *Zingiber officinale* Rosc, Leaf Powder

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Abstract

African catfish is a popular freshwater aquaculture species as it can be farmed at high stocking density. However, high stocking density caused growth and health impairment. Hence, nutritional approach such as feed additive can be applied in fish farming to overcome this issue. This study evaluated the impacts of ginger (*Zingiber officinale*) leaf powder (GLP) on growth performance, digestive enzymes activities, antioxidative response, and disease resistance towards *Edwardsiella tarda* infection in African catfish. A feeding trial was performed using a control diet and four formulated diets with GLP at 1, 2, 3, and 4%. Ginger is an herb used in cuisine and traditional medicine. It was reported possesses medicinal values such as antimicrobial, anti-oxidant, and immune system modulator. After eight weeks, the growth performance of GLP-treated African catfish was significantly improved than control, and the best results were observed in the 2 and 3% groups ($p<0.05$). Meanwhile, fish supplemented with 1 and 4% GLP demonstrated comparable growth performance. There was a significant decreasing trend in FCR, HSI, and VI, where the lowest values were recorded by the 2 and 3% GLP diet groups, followed by 1% GLP, 4% GLP, and control groups ($p<0.05$). The digestive enzymes activity, including amylase, lipase, and protease, was significantly higher in dietary GLP groups than in control, where the highest activity was exhibited by groups 2 and 3% GLP ($p<0.05$), followed by 1% and 4% GLP groups. There was an increasing trend in the antioxidative response, where the GLP-treated groups had significantly higher catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities than the control, and the highest was observed in fish fed with 2 and 3% GLP ($p<0.05$). Furthermore, the GLP-treated groups had a significantly higher cumulative survival rate than the control, where 2 and 3% GLP groups demonstrated the highest survival ($p<0.05$), followed by 1 and 4% GLP groups. In summary, this study revealed that dietary GLP potentially enhanced African catfish production at recommended doses of 2 and 3%.

Introduction

Aquaculture is rapidly growing an industry that provides affordable protein sources for humans. The African catfish, *Clarias gariepinus,* is gaining popularity in Malaysia due to its flavor, rapid growth, and flexible feeding habits. The short production cycle, high demand, and the ability to flourish under high stocking density (Abraham et al., 2015; Singh & Lakra, 2011) have made African catfish a targeted aquaculture species among investors to improve their return of investment. Nonetheless, high stocking density is a stress factor for an aquaculture species, leading to an increased risk of disease infection. Edwardsiellosis caused by *Edwardsiella tarda* is an economically significant disease in aquaculture that results in high mortality and devastation in an aquaculture farm (K. W. Goh et al., 2023). Increased organic matter in aquaculture ponds triggers Edwardsiellosis infection, particularly in high stocking density. *E. tarda* thrives in a wide range of environments and hosts. This disease has been reported in tilapia, catfish, golden pompano, Malaysian freshwater giant prawn, and bullfrog in Malaysia (Seongwei Lee et al., 2010; S. W. Lee et al., 2010; Lee et al., 2009; Lee & Wendy, 2017; Najiah et al., 2009), eels, carp, turbot, sturgeon, flounder, sea horse, crocodile, and turtle in China (Du et al., 2017; Liang et al., 2022; Mo et al., 2016; Wang et al., 2020; Wu et al., 2022), catfish and salmon in US (Loch et al., 2017) and various aquatic animals in Korea, Japan, and Egypt (Elgendy et al., 2022; Park et al., 2017; Yamasaki et al., 2013).

Several prevention measures and treatment approaches have been proposed to combat *E. tarda* infection, including the utilization of vaccines, probiotics, prebiotics, and antibiotics (K. W. Goh et al., 2023; Kari et al., 2022; Lee & Wendy, 2017; Wee et al., 2024; Wei et al., 2022). However, the high labor, cost, and species-specific application have limited vaccine use in aquaculture (Wee et al., 2024). Furthermore, consumers are reluctant to purchase aquaculture products exposed to antibiotics due to residues seeping into the food chain and adversely impacting public health and the environment (Kari, Sukri, et al., 2023; Kari et al., 2024). Alternatively, probiotics, prebiotics, and phytobiotics are widely applied in aquaculture for prophylactic purposes. The potential of phytobiotics as a feed additive for aquaculture was highlighted in recent studies with promising outcomes. Examples of phytobiotics in aquaculture are plant-based polysaccharides, fermented water spinach, papaya leaf extraction, pineapple wastes, olive oil by-products, soybean lecithin, and *Peperomia pellucida* that have positively impacted the health and growth of aquatic animals (Anis Mohamad Sukri et al., 2022; Khang Wen Goh et al., 2023; Hamid et al., 2022; Hazreen-Nita et al., 2022; Lee et al., 2016; Nandi et al., 2023; Wee et al., 2023). Therefore, phytobiotics are viable feed additives for fish farmers to boost their farm production.

Ginger, *Zingiber officinale*, is a staple ingredient in cooking, health care, and cosmetics owing to the array of beneficial bioactive compounds in this rhizome, including gingerol, zingiberol, zingerone, and zingiberene (Özcan, 2022). These bioactive compounds are responsible for ginger's antimicrobial, antioxidant, and anti-inflammatory properties (Abdelmagid et al., 2023). Moreover, ginger can be a flavoring agent to enhance palatability and digestion (Lai et al., 2022). Recent studies revealed that dietary ginger enhanced the growth and health status of numerous aquatic animals, such as the common carp, tilapia, *Labeo rohita*, rainbow trout, striped catfish, and black rockfish (Ashry et al., 2023; Mohammadi et al., 2020; Naliato et al., 2021; Nya & Austin, 2009; Oh et al., 2022; Rawat et al., 2022). Dietary ginger also stimulates resistance against Edwardsielosis in rockfish (Kim et al., 2017). Nevertheless, no study has explored the effects of dietary ginger leaf on the growth and health of African catfish. In addition, ginger leaf is an agricultural by-product that requires proper disposal to avoid environmental pollution. Therefore, this study evaluated the effects of dietary ginger leaf powder (GLP) on the growth performance, digestive enzyme, hematology, antioxidative response, and disease resistance against *E. tarda* infection in African catfish.

Materials and methods

Preparation of Ginger Leaf Powder

Ginger, *Zingiber officinale* Rosc, leaves were collected from the compounds of several residents in Jeli, Kelantan. The ginger leaves preparation was followed as described in the previous studies (S Lee et al., 2011; SW Lee et al., 2011; SW Lee et al., 2010; Seong Wei et al., 2011). The leaves were oven-dried using oven dryer (Memmert, Germany) at 60°C until completely dried, ground into powder form, and stored at -20°C until use.

Experimental Diets

This study used a commercial African catfish feed (Star, Malaysia) comprising 32% crude protein, 3% crude fat, and 12% moisture as the basal diet. The basal diet was grounded into powder form and homogenized with 1, 2, 3, and 4% GLP, respectively. The prepared diets were stored at -20°C until use.

Feeding Trial

Juvenile African catfish were purchased from a commercial farm in Tanah Merah, Kelantan. The fish were acclimatized in a 500 L tank for a week and fed with a commercial diet (Star, Malaysia) *ad libitum*. Subsequently, 450 healthy fish were distributed evenly into 15 aquaria (50 L) at 30 pieces/tank. A health fish was defined as no wound on the fish body and swim actively. The fish were given the control and experimental feed daily *ad libitum* in the morning based on their respective treatments (GLP 1 – 4), while the water change was conducted in the evening. Each treatment was performed in triplicates. The water parameters in the aquaria were maintained in optimum ranges as follows: temperature, 26-28°C, dissolved oxygen >6ppm, pH 6-7, and ammonia <0.01 ppm. The feeding trial was carried out for eight weeks.

Growth Performance

The growth performance parameters of the experimental fish, including final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), hepatosomatic index (HIS), and viscerosomatic index (VSI) were determined at the end of feeding trial. The formulas for the growth performance parameters were adapted from previous studies (Anis Mohamad Sukri et al., 2022; Kari, Téllez-Isaías, et al., 2023; Rahman et al., 2023).

Determination of Blood Parameters

Once the feeding trial ended, blood samples from each treatment were collected from the experimental fish (n=3). The fish were first anesthetized using clove oil. The caudal peduncle of the fish was cut to withdrawn blood. The blood was drawn and kept in heparinized tubes for further analysis. The blood parameters, including white blood cell (WBC), lymphocytosis (LYM), monocytes (MON), red blood cell (RBC), haemoglobin (HGB), hematocrit (HCT), mean corpuscular heemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using a hematology analyzer (Mythic 18 Vet, USA) (Zakaria et al., 2022).

Determination of Digestive Enzymes Activity

After the blood was withdrawn from the fish, the same fish were further dissected to obtained the fish intestinal for digestive enzymes activity assay. The intestines experimental fish (n=3) from each were harvested, cut into small pieces, and homogenized in 10 mM HCl at 4°C. Subsequently, the tissue homogenate was centrifuged at 10000 rpm for 10 min at 4°C, and the supernatant was used in the digestive enzyme activities assay. The total protein content was determined using the Bradford assay, where bovine serum albumin was utilized as a standard (Bradford, 1976; Cardoso et al., 2021). The digestive enzymes activities were conducted using an ELISA plate, and the reading was visualized via an ELISA reader (BioRad, USA) at a wavelength of 595 nm.

Determination of Antioxidative Response

Determination of the antioxidative response was conducted as described in previous studies (Ashry et al., 2023; Cai et al., 2020). The liver of the experimental fish (n=3) was sampled and subjected to protein extraction. First, the liver was homogenized in saline and centrifuged at 10000 rpm for 10 mins at 4°C. The obtained supernatant to determine catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities through colorimetric method via an antioxidative enzymes kit (Elabscience, Malaysia). The results were analyzed using a microplate reader (BioRad, USA) at a wavelength of 560 nm.

***Edwardsiella tarda* Challenge**

Only healthy fish (n=10) with no wound on the fish body and swim actively were selected for *E. tarda* infection assay at the end of the feeding trial. The fish were injected with 1 X 10 10^8 cfu/mL bacterial suspension intraperitoneally (Lee et al., 2016) and monitored weekly for four consecutive weeks. The cumulative survival rate of each treatment was recorded.

Statistical Analysis

All statistical analyses in this study were performed using the Statistical Package for Social Science (SPSS) version 20.1 (IBM, USA). The data was first tested for normality before being subjected to a one-way analysis of variance (ANOVA) followed by Tukey post hoc grouping. The significance level was set at p<0.05, and the results were expressed as mean ± standard deviation (SD).

Results

Growth performance parameters such as FW, WG, and SGR were significantly higher in the GLP-treated groups compared to the control group, where the best performance was recorded by the 2 and 3% GLP treatments (p<0.05) (Table 1). Meanwhile, the 1 and 4% GLP groups demonstrated comparable FW, WG, and SGR. In contrast, GLP-supplemented fish exhibited lower FCR, HSI, and visceral somatic index (VSI) than the control group, where fish fed 2 and 3% GLP demonstrated superior performance (p<0.05). The 1 and 4% GLP groups performed similarly in FCR, HSI, and VSI.

Fish receiving GLP diets had significantly higher white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), and hematocrit (HCT) in comparison to the control group (p<0.05) (Table 2). Fish fed with 2 and 3% GLP diets recorded the best WBC, RBC, HGB, and HCT, followed by the 1 and 4% GLP groups. However, there are no significant differences among the groups for the

Table 1. Growth performance parameters of experimental fish fed with different percentage of ginger, *Zingiber officinale* Rosc, leaf powder diets

Parameters	Control	GL1	GL2	GL3	GL4
Initial Weight (IW) (g)	10.5±0.00	10.47±0.06	10.5±0.00	10.5±0.10	10.5±0.10
Final Weight (FW) (g)	203.8±4.21^c	227.3±1.56^b	254.2±9.70^a	255.6±3.91^a	227.7±1.12^b
Weight Gain (WG) (%)	1840.6±40.09^c	2071.4±18.19^b	2321.3±92.42^a	2334.5±14.15^a	2068.3±10.70^b
Specific Growth Rate (SGR) (%)	2.30±0.016^c	2.39±0.007^b	2.47±0.030^a	2.48±0.005^a	2.39±0.004^b
Hepatosomatic Index (HSI) (%)	3.37±0.112^c	2.76±0.092^b	2.29±0.161^a	2.26±0.064^a	2.72±0.104^b
Visceral Somatic (VS) (%)	3.66±0.176^c	2.87±0.098^b	2.34±0.117^a	2.40±0.047^a	2.78±0.080^b
Feed Conversion Ratio (FCR)	1.35±0.029^c	1.20±0.009^b	1.07±0.043^a	1.06±0.017^a	1.20±0.006^b

*Data expressed as mean ± standard deviation
*C = control; GL1, 2, 3, and 4 = 1, 2, 3, and 4% of ginger, *Zingiber officinale* Rosc, leaf powder
*Values in the same row with different superscript showed significant difference at p<0.05

value of monocytes, lymphocytosis, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

Amylase, protease, and lipase activities were significantly higher in African catfish that received GLP than the control group (p<0.05) (Figure 1). Fish fed 2 and 4% GLP recorded the highest digestive enzymes activities, the 1 and 4% GLP groups. A similar pattern was also observed in the antioxidative response. Fish fed with GLP had significantly higher superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) than control (p<0.05) (Figure 2). Fish fed 2 and 3% GLP diets demonstrated the highest SOD, CAT, and GPx activities, followed by 1 and 4% GLP groups. In the *E. tarda* infection challenge assay, the GLP treatment groups exhibited a significantly higher cumulative survival rate than control (p<0.05) (Figure 3). Fish fed 2 and 3% of GLP had the highest cumulative survival rate. Meanwhile, fish fed 1 and 4% GLP shared similar cumulative survival rates.

Discussion

The application of plant-based feed additives in aquatic animals has been widely explored in recent studies. For instance, a spirulina-supplemented diet enhanced the growth and health of stinging catfish (Rahman et al., 2023). Other plant-based feed additives that benefit aquatic animals are pineapple wastes (Anis Mohamad Sukri et al., 2022), papaya leaf (Hamid et al., 2022), and soybean lecithin (Wee et al., 2023). In the present study, the effects of dietary GLP as a feed additive on the growth performance and health of African catfish, *C. gariepinus* was investigated by conducting a feeding experiment, and analyzing their blood parameters, digestive enzymes, and antioxidative activities, and disease resistance against *E. tarda* infection.

The present study discovered that dietary GLP significantly enhanced the growth performance of African catfish after eight weeks. Previous studies

Table 2. Blood parameters of experimental fish fed different percentage of ginger, *Zingiber officinale* Rosc, leaf powder diets

Blood parameters	Control	GL1	GL2	GL3	GL4
WBC / µl	118.8±1.72[c]	131.2±3.06[b]	137.9±1.84[a]	138.6±2.25[a]	131.3±3.43[b]
LYM (%)	88.3±1.32	86.5±1.28	85.3±4.07	87.0±1.40	87.7±1.34
MON (%)	13.2±0.52	13.3±0.53	13.4±0.35	13.8±0.70	13.2±0.40
RBC10^3/ µl	2.33±0.21[c]	2.70±0.10[b]	3.57±0.12[a]	3.70±0.20[a]	2.67±0.15[b]
HGB (g/dl)	5.93±0.15[c]	6.93±0.15[b]	7.53±0.25[a]	7.50±0.20[a]	6.97±0.12[b]
HCT (%)	25.4±0.55[c]	29.3±1.37[b]	35.4±1.47[a]	33.6±2.36[a]	30.2±1.19[b]
MCH (pg)	33.0±2.10	35.5±2.57	34.0±4.42	35.7±2.10	34.1±3.23
MCHC (g/dl)	26.6±1.82	25.5±1.19	24.4±103	25.1±2.21	25.3±2.36

*Data expressed as mean ± standard deviation
*C = control; GL1, 2, 3, and 4 = 1, 2, 3, and 4% of ginger, *Zingiber officinale* Rosc, leaf powder
* Values in the same row with different superscript showed significant difference at p<0.05
*WBC = White Blood Cell, LYM = Lymphocytosis, MON = Monocytes, RBC = Red Blood Cell, HGB = Haemoglobin, HCT = Hematocrit, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration

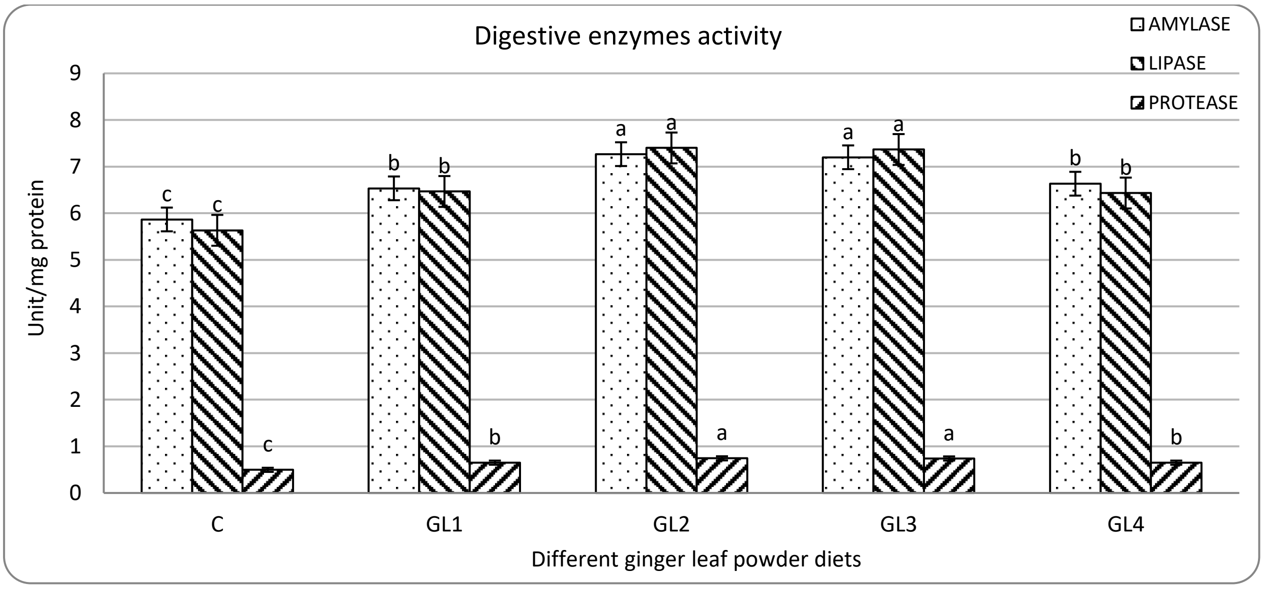

Figure 1. Digestive enzymes activity of African catfish fed with different percentage of *Zingiber officinale* Rosc, leaf powder diets.
*C = control; GL1, 2, 3, and 4 = 1, 2, 3, and 4% of *Zingiber officinale* Rosc, leaf powder.
*Values in the same row with different superscript showed significant difference at p<0.05

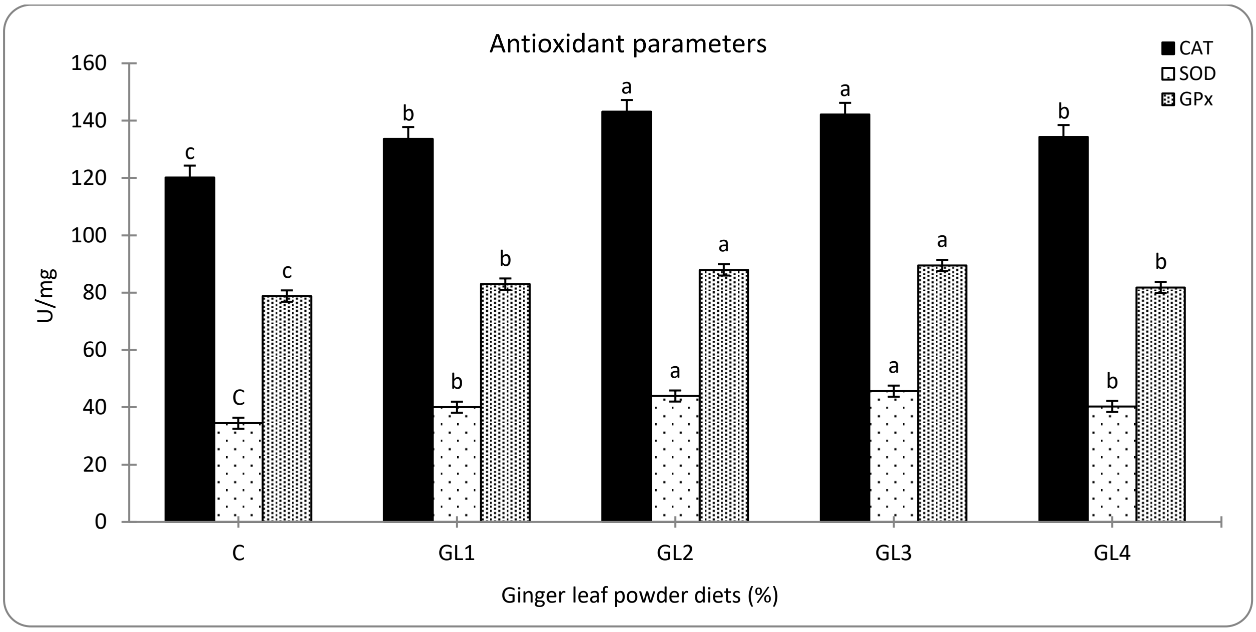

Figure 2. Antioxidative parameters response among African catfish fed with different percentage of *Zingiber officinale* Rosc, leaf powder diets. *C = control; GL1, 2, 3, and 4 = 1, 2, 3, and 4% of *Zingiber officinale* Rosc, leaf powder

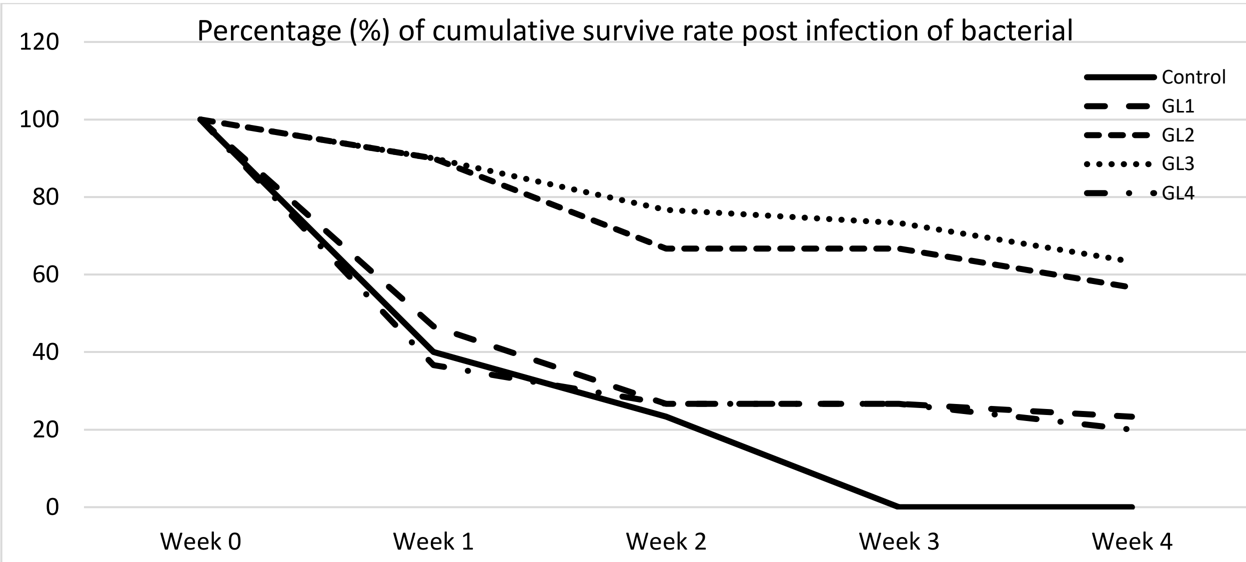

Figure 3. Cumulative survival rate of post infection of *Edwardsiella tarda* in African catfish, *Clarias gariepinus* fed *Zingiber officinale* Rosc, leaf powder diets. *C = control; GL1, 2, 3, and 4 = 1, 2, 3, and 4% of *Zingiber officinale* Rosc, leaf powder

revealed similar results where ginger and its derivatives improved the growth performance of other aquatic animals such as striped catfish, Nile tilapia, common carp, black rockfish, and *Labeo rohita* (Ashry et al., 2023; Mohammadi et al., 2020; Naliato et al., 2021; Oh et al., 2022; Sukumaran et al., 2016). Conversely, dietary ginger did not positively impact the growth performance of zebrafish, *Danio rerio* (Ahmadifar et al., 2019), which may be attributed to the different ginger derivatives, species, feeding regime, and doses (Ashry et al., 2023). Dietary ginger activates digestive enzymes in aquatic animals, boosting their digestion and amino acid absorption (Ashry et al., 2023; Lai et al., 2022). The enhancement of growth performance of African catfish is supported by the finding of digestive enzymes activity enhancement, including lipase, amylase, and protease. Similar observations were reported in striped catfish, black rockfish, rainbow trout, and zebrafish fed ginger derivatives (Ahmadifar et al., 2019; Ashry et al., 2023; Nya & Austin, 2009; Oh et al., 2022).

Dietary GLP increased the feed utilization efficiency by lowering the FCR in GLP-treated African catfish, enhancing their feed intake and growth performance. The bioactive compounds in ginger, such as gingerol, terpene, and zingiberene, may have added flavor and smell to the fish feed and enhanced the palatability (Özcan, 2022). Other studies have reported that ginger derivatives enhanced feed intake in other aquatic animals, including striped catfish, Nile tilapia, common carp, black rockfish, and *Labeo rohita* (Ashry et al., 2023; Naliato et al., 2021; Oh et al., 2022; Sukumaran et al., 2016). In addition, fish supplemented with GLP had significantly lower hepatosomatic index (HIS) and viscerosomatic index (VSI), indicating more flesh on its body. This finding also suggests that ginger inclusion in fish feed enhanced lipid metabolism and reduced visceral fat deposition effectively (Oliveira e Silva et al., 2024; Weil et al., 2013). The positive outcomes could be explained by the presence of the anti-lipogenic bioactive compound, flavonoid, in the ginger leaf (Abdel Rahman et al., 2019). On the contrary, excessive ginger leaf inclusion in the fish diet adversely impacted the African catfish growth performance, as observed in the 4% GLP group. In conclusion, GLP inclusion at 2 and 3% in African catfish diets are the optimum doses to boost farm productivity.

The hematological results revealed that dietary GLP enhanced African catfish health status by significantly increasing the RBC, WBC, HCT, and HBG levels. The RBC, HCT, and HBG improvements indicated better erythropoiesis and hemosynthesis activities in the fish (Ashrafizadeh et al., 2020). These findings also reflected that the fish were in good health, without issues such as anemia and malnutrition (Enis Yonar et al., 2012). Likewise, the lack of significant differences in MCH and MCHC for all treatment groups reflected their satisfactory health status. The positive outcomes, particularly the absence of anemia, were consistent with a previous study (Yonar et al., 2019). The higher WBC values in GLP-treated groups are also an indicator of good health among the African catfish in this study. Despite that, various factors could contribute to higher WBC, such as gender, season, feeding regime, stress level, and pollutants in the fish environment (Ahmed et al., 2020).

The GLP supplementation in this study promoted disease resistance of African catfish against *E. tarda*, indicated by the higher cumulative survival rate post-infection. Ginger and its derivatives reportedly possess antibacterial properties that improve aquatic animal's disease resistance against *Streptococcus agalactiae*, *Aeromonas hydrophila*, *Vibrio harveyi*, *A. salmonicida*, and Gram–negative bacterial infections (Brum et al., 2017; Fazelan et al., 2020; Lee et al., 2021; Naliato et al., 2021; Rawat et al., 2022; Sukumaran et al., 2016; Talpur et al., 2013). Bioactive compounds such as gingerols may be responsible for this enhancement, as reported in an earlier study (Wang et al., 2015). In addition, the rise in disease resistance in GLP-treated African catfish could be attributed to SOD, CAT, and GPx activation. Ginger is well-known for its antioxidant properties that could reduce lipid peroxidation and eliminate free radicals (Zhang et al., 2022). The enhanced antioxidative responses in African catfish fed with dietary GLP help mitigate stress caused by *E. tarda* infection, resulting in a higher cumulative survival rate post-infection. Previous studies also reported that SOD, GPx, and CAT activation yielded positive outcomes in striped catfish, *Labeo rohita*, Nile tilapia, and zebrafish (Ahmadifar et al., 2019; Ashry et al., 2023; Naliato et al., 2021; Oh et al., 2022; Sukumaran et al., 2016). Bioactive compounds such as saponins, flavonoids, polyphenols, and tannins in ginger may be responsible for SOD, GPx, and CAT activation, thus boosting the antioxidant capacity of the aquaculture species (Zhang et al., 2022).

Conclusion

This study revealed that dietary GLP at 2 and 3% in African catfish enhanced their growth performance, feed digestion, and antioxidative response, stimulating disease resistance against *E. tarda* infection. The positive outcomes are possibly associated with significant improvements in digestive enzymes activities. Meanwhile, the improved fish health status could be attributed to significant enhancements in SOD, CAT, and GPx activities, blood parameters, and disease resistance. Therefore, farmers could include 2 and 3% dietary GLP in their African catfish feeding regime to increase farm productivity. Future studies could also explore the impacts of GLP at the recommended levels in other aquaculture species.

Ethical Statement

The study was approved with the animal ethics of Universiti Malaysia Kelantan with registration code UMK/FIAT/ACUE/PG/05/2023.

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Author Contribution

Lee Seong Wei, Zulhisyam Abdul Kari, Muhammad Anamul Kabir: Conceptualization, Methodology, Investigation. Martina Irwan Khoo, Mohamad Nor Azra, Wendy Wee: Resources, Data Curation, Visualization. All authors: Writing—original draft, Supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

All the authors declare no conflict of interest regarding this work.

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