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RESEARCH

Effects of dietary kaffir lime, *Citrus hystrix* DC, leaf powder on the growth performance, digestive enzyme, hematology, antioxidative response, and disease resistance against *Edwardsiella tarda* infection in African catfish, *Clarias gariepinus*

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Abstract
This study evaluated the effects of kaffir lime (KL), *Citrus hystrix*, leaf powder on growth performance, digestive enzyme activities, blood and antioxidant parameters, and disease resistance in African catfish, *Clarias gariepinus*. A total of 450 healthy juveniles (10.5 g) were randomly distributed into five groups and fed diets containing 0% (control), 1%, 2%, 3%, and 4% of KL for eight weeks. At the end of the feeding trial, 10 healthy fish from each group were challenged with *Edwardsiella tarda* infection. The study findings demonstrated significant differences in all growth parameters, including final weight, weight gain, specific growth rate, feed conversion rate, hepatosomatic percentage, and viscerosomatic index between dietary KL and control groups, with the group fed 2% and 3% KL exhibiting superior performance ($p < 0.05$). Dietary KL significantly increased white blood cells, red blood cells, hemoglobin, and hematocrit ($p < 0.05$) in African catfish, with the highest values observed in the 2% and 3% KL groups. Meanwhile, there were no significant differences in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations between treatments. Furthermore, dietary KL significantly increased digestive enzyme activities, including lipase, amylase, and protease ($p < 0.05$), and the highest activities were observed in fish fed 2% and 3% KL. Antioxidative responses, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in KL-treated fish, were also significantly higher than the control group ($p < 0.05$). Finally, the highest and the lowest cumulative survival rates following a challenge with *E. tarda* were 3% KL and control groups, respectively. Based on the study results, 2% or 3% dietary KL could improve the growth and health of African catfish, thus enhancing aquaculture production.

Keywords Feed additive · Growth · Health · Blood parameter · Immunostimulant · Phytobiotic

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Extended author information available on the last page of the article

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Introduction

African catfish, *Clarias gariepinus*, is a popular freshwater fish in Malaysia, which is highly demanded and affordable (Wei et al. 2024). This species is widely cultivated due to its high disease tolerance, adaptability to various environments, rapid growth, and dietary flexibility. In the economic context, African catfish have a short production cycle (2 to 3 months) reaching marketable size and can be farmed at high stocking density. Therefore, this aquaculture species is the best candidate to boost an aquaculturist's income. Nevertheless, fish farming at high stocking density causes crowding stress, impairing fish growth and health. Moreover, intensive farming renders fish vulnerable to disease outbreaks and causes major economic losses (Goh et al. 2023a, b). One of the critical diseases in aquaculture is Edwardsiellosis, caused by *Edwardsiella tarda*. This bacterium has major economic significance as the disease outbreak leads to high mortality and possibly the end of a fish farm operation. *E. tarda* is found in various hosts and environments, and the infection has been reported worldwide. Furthermore, *E. tarda* infection occurs in freshwater and marine aquatic animals, such as carp in Japan, India, and China (Pandey et al. 2021; Yamasaki et al. 2013); eels in China and South Korea (Mo et al. 2016; Park et al. 2017); American bullfrog, tilapia, catfish, golden pompano, and *Macrobrachium rosenbergii* in Malaysia (Lee et al. 2010a, b; Lee et al. 2009; Lee & Wendy 2017; Najiah et al. 2009); Nile tilapia in Egypt (Elgendy et al. 2022); channel catfish and chinook salmon in USA (Loch et al. 2017); and turbot, flounder, sturgeon, catfish, sea horse, farmed crocodile, and soft-shell turtle in China (Du et al. 2017; Liang et al. 2022; Wang et al. 2020; Wu et al. 2022). Several strategies have been applied to mitigate *E. tarda* infection in aquatic animals, such as vaccination in turbot (Castro et al. 2008). Other studies also revealed the potential of inactivated *E. tarda* in stimulating immune response in zebrafish, flounder, and tilapia (Kwon et al. 2006; Tang et al. 2017; Xu et al. 2019). However, vaccination is challenging due to the intensive labor, high cost, and species specificity.

Vaccines and antibiotics are used as treatments and preventive measures in aquaculture operations. Nonetheless, vaccination is labor-intensive and expensive (Wei et al. 2022), while antibiotics are banned in numerous countries due to environmental and public health concerns. As a result, feed additives such as probiotics, prebiotics, and phytobiotics are gaining attention in aquaculture as a preventive and treatment strategy (Wee et al. 2024). Phytobiotics, in particular, are inexpensive, abundant, accessible, and effective, offering fish farmers an alternative source of feed additives. Thus, researchers have embarked on a journey to discover the full potential of phytobiotics in aquaculture. This alternative feed additive has shown promising results in preventing and treating infectious diseases (Abdel-Tawwab et al. 2018, 2021; Adeshina et al. 2019) and improving growth and health status of aquatic animals. For instance, dietary Spirulina enhanced the growth and health of stinging catfish, *Heteropneustes fossilis* (Rahman et al. 2023). Various plant-based polysaccharides also boosted the production of multiple carp species (Goh et al. 2023a Goh et al. 2023b). In addition, fermented water spinach modulated the growth and health of stinging catfish, *H. fossilis* (Nandi et al. 2023). Dietary papaya leaf extract (Hamid et al. 2022), pineapple waste (Anis Mohamad Sukri et al. 2022, 2023), olive oil by-product (Hazreen-Nita et al. 2022), soybean lecithin (Wee et al. 2023), *Peperomia pellucida* (Lee et al. 2016), and other phytobiotics (Kari et al. 2023a, b; Kari et al. 2022) positively impacted the growth and health of aquatic animals. Therefore, phytobiotics are a cost-effective, convenient, and effective approach to reducing vaccines and antibiotic usage and boosting aquaculture productivity.

Kaffir lime (KL), *Citrus hystrix*, is a native lime species in Southeast Asia, including Malaysia. This fruit reportedly contains 78 bioactive compounds, including β-pinene, limonene, sabinene, and citronellal, which benefit human health (Zhao et al. 2023). Phytochemical analysis also demonstrated that KL has terpenoids, phenolic acids, flavonoids, and coumarins as the main constituents (Zhao et al. 2023). Earlier studies revealed that this plant has antimicrobial (Panthong et al. 2013), antioxidant (Panthong et al. 2013), anti-mosquito (Nararak et al. 2017), anti-tumor (Anuchapreeda et al. 2020; Borusiewicz et al. 2017; Sun et al. 2018), anti-inflammatory (Anuchapreeda et al. 2020), and neural-protective properties (Sammi et al. 2016). Furthermore, its fresh, frozen, or dried leaf is widely used in Asian cuisines as a flavoring agent and tea. Nevertheless, limited literature describes the potential of KL leaf as a feed additive in animals, particularly aquatic species. Therefore, this study evaluated the impacts of dietary KL leaf powder on the growth performance, digestive enzyme, hematology, antioxidative response, and disease resistance to *E. tarda* infection in African catfish, *C. gariepinus*.

Materials and methods

Kaffir lime leaf powder preparation

Kaffir lime leaves were purchased from a wet market in Tanah Merah, Kelantan. The leaves were oven-dried at 60 °C for 48 h and powdered using a blender (Panasonic, Malaysia). The KL leaf powder was stored in a freezer for further use.

Medicated feed formulation

Five different diets were formulated and prepared, as detailed in Table 1. Four diets were added with 1%, 2%, 3%, and 4% KL leaf powder and labeled KL1, KL2, KL3, and KL4, respectively. All the ingredients were mixed, homogenized, and pelleted using an extruder. All diets were air-dried, placed in sealed, labeled plastic bags, and stored in a freezer for further use. Proximate analyses were carried out to determine carbohydrate, protein, lipid, moisture, and ash content in each diet (Latimer & International 2023). Carbohydrates and protein of the formulated diets ranged from 42.5% to 44.2% and 32.1% to 32.9%, respectively.

Feeding trial and experimental design

The experimental fish were purchased from a commercial farm at Tanah Merah, Kelantan, and kept for a week in a holding tank (300 L). The fish were acclimatized for a week and given a control diet (Table 1) once daily in the morning with the feeding rate at 5% of body weight. A complete water exchange was done in the afternoon. At the end of the acclimatization period, only the healthy fish were selected and kept in 50 L aquaria. Each aquarium housed 30 fish and the experiment was conducted in triplicates. The experimental fish received the formulated diets for eight weeks. The experimental fish were given the formulated diets once daily in the morning (feeding rate: 5% of body weight) and 100% water change was carried out in the afternoon. The water quality in the aquaria was maintained as follows: water temperature = 26.8–28.8 °C, ammonia > 0.05 ppm, dissolved oxygen = 6.3–6.8 ppm, and pH 6.1–7.1.

Table 1 Feed formulations and nutritional profile of formulated diets

	Diet formulation in different treatments (%)				
	Control	KL 1	KL 2	KL 3	KL 4
Raw materials					
Soybean meal	22	22	22	22	22
Fish meal	50	50	50	50	50
Wheat bran	17	16	15	14	13
Premix	2	2	2	2	2
Fish oil	3	3	3	3	3
Vegetable oil	3	3	3	3	3
Carboxymethyl cellulose (CMC) binder	3	3	3	3	3
Citrus hystrix, leaf powder	0	1	2	3	4
Total	100	100	100	100	100
Nutritional profiles					
Carbohydrate	43.3	44.2	44.1	43.5	42.5
Protein	32.1	32.8	32.9	32.3	32.3
Ash	6.7	4.8	6.7	6.8	6.8
Lipid	6.6	6.7	6.1	6.6	7.6
Fibre	4.6	4.7	5.2	5.1	5.1
Moisture	6.7	6.8	5.0	5.7	5.7

Premix (Aquavita, Indonesia) contains vitamins A, D_3, E, K, B_1, B_2, B_{12}, C, calcium pantothenate, folic acid, lactose acid, biotin, amino acids, inositol, manganese sulphate, copper sulphate, and cobalt chloride

C control, *KL1 2*, *3*, and *4*, 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder

Growth performance determination

At the end of feeding trial, the experimental fish from each treatment were weighed and the growth performance parameters were determined as described in the previous studies: final weight (FW) (final body weight − initial body weight), weight gain (WG) ([total weight gain/initial body weight] × 100), specific growth rate (SGR) (total weight gain × 100/experimental days), feed conversion rate (FCR) (total feed intake/total weight gain), viscerosomatic index (VSI) (total viscera weight/total body weight), and hepatosomatic index (HSI) (total liver weight/total body weight) (Abdul Kari et al. 2021; Kari et al. 2023a, b; Zakaria et al. 2022).

Hematology analysis

Once the feeding trial ended, three fish per replicate from each treatment were anesthetized using clove oil for blood collection. The blood samples were kept in heparinized tubes and later analyzed using a hematology analyzer (Mythic 18 Vet, USA) (Zakaria et al. 2022).

Digestive enzyme activities

The digestive enzyme activity was determined as described in previous studies. The intestines of three experimental fish per replicate from each treatment were harvested and homogenized in phosphate-buffered saline (PBS). The mixture was centrifuged at 7 168 g for 10 min to obtain the supernatant and subjected to digestive enzyme activity assay. The protease and amylase activities were determined through the Folin-Ciocalteu phenol reagent and iodine solution, respectively (Lowry et al. 1951). Meanwhile, the lipase activity was determined, as described by Borlongan (1990).

Antioxidative response determination

The liver of experimental fish ($n=3$) per replicate from each treatment was harvested and homogenized in saline. The supernatant of the sample was obtained via centrifugation at 11 200 g for 10 min. Subsequently, the supernatant was used to determine superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities through the colorimetric method via commercial kits (Elabscience, Malaysia). The results were analyzed using a microplate reader (BioRad, USA) at 560 nm (Ashry et al. 2023).

Edwardsiella tarda infection assay

At the end of the feeding trial, 10 healthy fish per replicate from each treatment were challenged with *E. tarda* infection. The experimental fish was exposed to the bacteria (1×10^8 cfu/mL) via intraperitoneal injection (Lee et al. 2016). The cumulative survival rate of the experimental fish was observed and recorded after four weeks.

Statistical analysis

All data were tested for normality before being analyzed using Statistical Package for Social Sciences (SPSS) version 20.1 (IBM, USA). One-way analysis of variance (ANOVA) was used to determine significant differences among the treatments at $p<0.05$, followed by grouping with the Tukey post hoc test. The results were expressed as mean $\pm$ standard deviation (SD).

Results

Table 2 demonstrates the growth performance of African catfish after the feeding trial. Dietary KL leaf powder increased the FW, WG, and SGR, with the highest values recorded by the 2% and 3% KL treatment groups ($p<0.05$). Furthermore, fish fed with 2% and 3% KL leaf powder exhibited significantly lower FCR values than other groups, while the control group recorded the highest FCR. Meanwhile, the 1% and 4% dietary KL groups demonstrated similar FCR values. Likewise, the HSI and VSI of the 2% and 3% KL groups were the lowest compared to other treatments.

The hematological profile of fish fed with 2% and 3% KL was significantly superior ($p<0.05$) in WBC, RBC, HGB, and HCT, followed by 1% and 4% (Table 3). Conversely,

Table 2 Growth performance of experimental fish fed with different percentages of kaffir lime, *Citrus hystrix*, leaf powder for eight weeks

Parameters	Control	KL1	KL2	KL3	KL4
Initial weight (IW) (g)	10.4 ± 0.12	10.3 ± 0.06	10.0 ± 0.00	10.4 ± 0.10	10.4 ± 0.10
Final weight (FW) (g)	202.8 ± 4.43^{a}	217.3 ± 1.56^{b}	234.2 ± 4.02^{c}	235.6 ± 3.91^{c}	217.7 ± 1.12^{b}
Weight gain (WG) (%)	1843.9 ± 63.55^{a}	2002.6 ± 19.95^{b}	2130.8 ± 38.30^{c}	2165.7 ± 34.01^{c}	1993.1 ± 28.59^{b}
Specific growth rate (SGR) (%)	2.30 ± 0.025^{a}	2.36 ± 0.007^{b}	2.41 ± 0.013^{c}	2.42 ± 0.012^{c}	2.36 ± 0.011^{b}
Hepatosomatic index (HSI) (%)	1.97 ± 0.129^{a}	1.50 ± 0.106^{b}	1.21 ± 0.007^{c}	1.12 ± 0.088^{c}	1.58 ± 0.183^{b}
Visceral somatic index (VSI) (%)	3.50 ± 0.158^{a}	3.04 ± 0.060^{b}	2.58 ± 0.100^{c}	2.60 ± 0.048^{c}	3.09 ± 0.058^{b}
Feed conversion ratio (FCR)	1.30 ± 0.030^{a}	1.21 ± 0.009^{b}	1.12 ± 0.020^{c}	1.11 ± 0.019^{c}	1.21 ± 0.007^{b}

Data expressed as mean ± SD. Values in the same row with different superscript letters showed significant differences at $p < 0.05$

C control, *KL1 2*, *3*, and *4*, 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder

Table 3 Blood parameters of experimental fish fed with different percentages of kaffir lime, *Citrus hystrix*, leaf powder for 8 weeks

Blood parameters	Control	KL1	KL2	KL3	KL4
WBC/µl	106.2 ± 4.29^{a}	121.5 ± 3.50^{b}	134.8 ± 4.00^{c}	137.4 ± 2.88^{c}	121.6 ± 2.56^{b}
LYM (%)	86.7 ± 1.56	87.5 ± 1.93	84.0 ± 2.23	86.1 ± 0.69	87.2 ± 0.50
MON (%)	12.9 ± 0.06	12.8 ± 0.30	13.0 ± 0.35	13.6 ± 0.31	13.3 ± 0.45
$RBC10^3/\mu l$	2.27 ± 0.15^{a}	2.77 ± 0.15^{b}	3.40 ± 0.30^{c}	3.27 ± 0.21^{c}	2.67 ± 0.15^{b}
HGB (g/dl)	5.23 ± 0.12^{a}	6.33 ± 0.15^{b}	7.30 ± 0.20^{c}	7.30 ± 0.26^{c}	6.37 ± 0.31^{b}
HCT (%)	25.3 ± 0.32^{a}	28.7 ± 1.25^{b}	36.1 ± 0.55^{c}	36.3 ± 1.01^{c}	29.5 ± 1.30^{b}
MCH (pg)	33.7 ± 2.52	35.5 ± 2.57	34.0 ± 4.42	31.7 ± 1.05	31.1 ± 0.93
MCHC (g/dl)	26.3 ± 2.18	27.8 ± 2.06	26.4 ± 2.53	26.7 ± 3.75	27.6 ± 4.11

Data expressed as mean ± SD. Values in the same row with different superscript letters indicate significant differences at $p < 0.05$

C control, *KL1*, *2*, *3*, and *4*, 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder; *WBC*, white blood cell; *LYM*, lymphocytosis; *MON*, monocytes; *RBC*, red blood cell; *HGB*, hemoglobin; *HCT*, hematocrit; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration

the control group exhibited significantly lower ($p < 0.05$) WBC, RBC, HGB, and HCT than other treatments. There were no significant differences in MCH, MCHC, LYM, and MON between the groups in the present study. Dietary KL leaf powder also enhanced digestive enzyme activities significantly ($p < 0.05$), with the highest activities observed in fish fed with 2% and 3% KL (Figs. 1, 2 and 3), followed by 1% and 4% KL groups. In contrast, the control group exhibited the lowest digestive enzyme activity for amylase, protease, and lipase.

Antioxidant activities (CAT, SOD, and GPx) were significantly higher in the KL-treated groups ($p < 0.05$) (Fig. 4, 5 and 6). Fish fed with 2% and 3% KL leaf powder demonstrated the highest activities, followed by the 1% and 4% KL treatment groups. The control group recorded the lowest antioxidative responses. In addition, the 2% and 3% KL groups exhibited superior cumulative survival rates post *E. tarda* infection (Fig. 7) compared to 1% and 4% KL treatments and the control group.

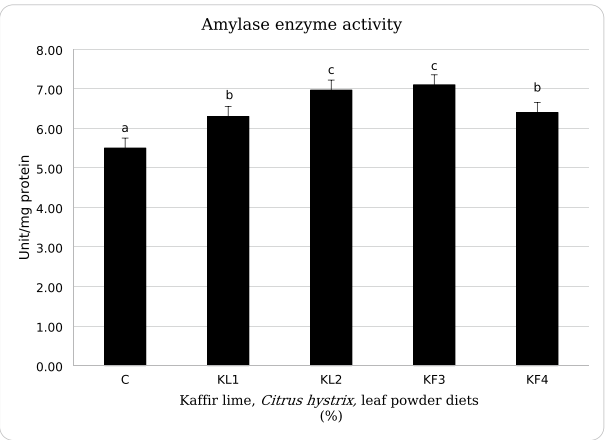

Fig. 1 Amylase enzyme activity of African catfish fed percentage of kaffir lime, *Citrus hystrix*, leaf powder for 8 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder. Values in the same row with different superscript letters indicate significant differences at $p < 0.05$

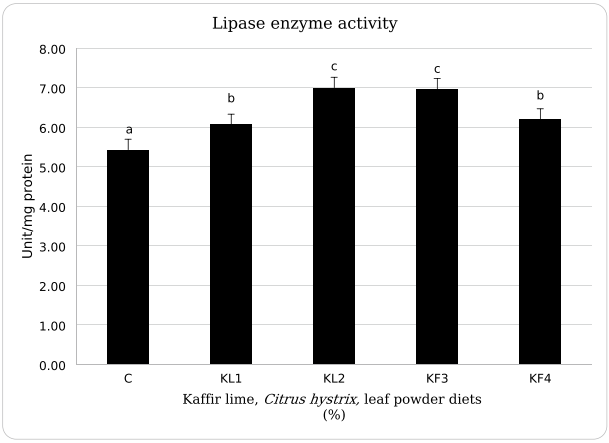

Fig. 2 Lipase enzyme activity of African catfish fed percentage of kaffir lime, *Citrus hystrix*, leaf powder for 8 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder. Values in the same row with different superscript letters indicate significant differences at $p < 0.05$

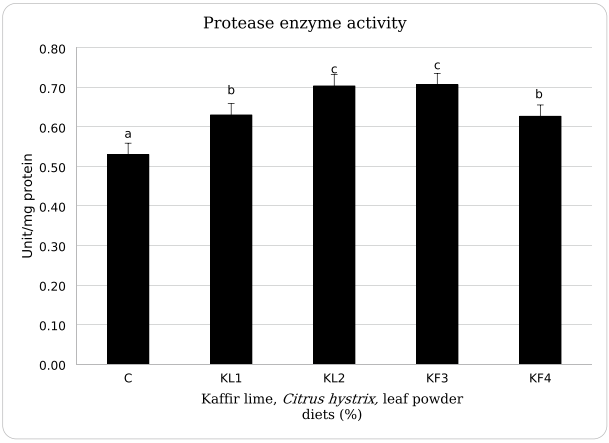

Fig. 3 Protease enzyme activity of African catfish fed percentage of kaffir lime, *Citrus hystrix*, leaf powder for 8 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder. Values in the same row with different superscript letters indicate significant differences at $p < 0.05$

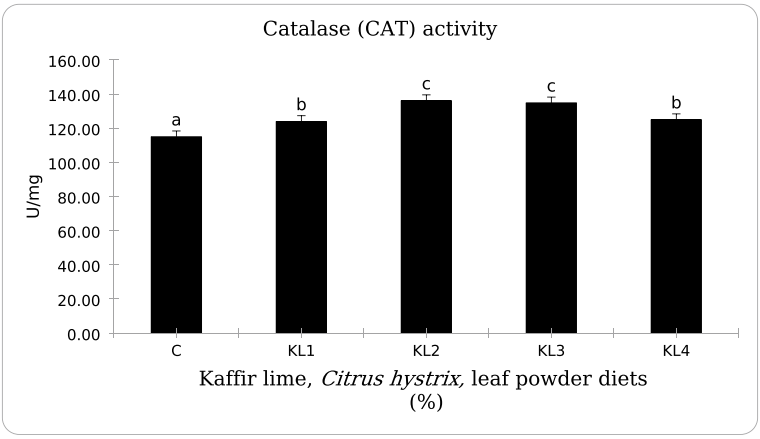

Fig. 4 Catalase (CAT) activity of fish fed different percentages of kaffir lime, *Citrus hystrix*, leaf powder after 8 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder

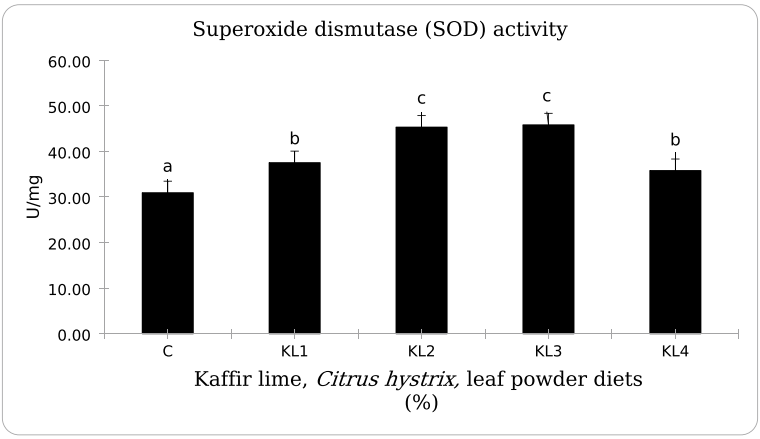

Fig. 5 Superoxide dismutase (SOD) activity of fish fed different percentages of kaffir lime, *Citrus hystrix*, leaf powder after 8 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder

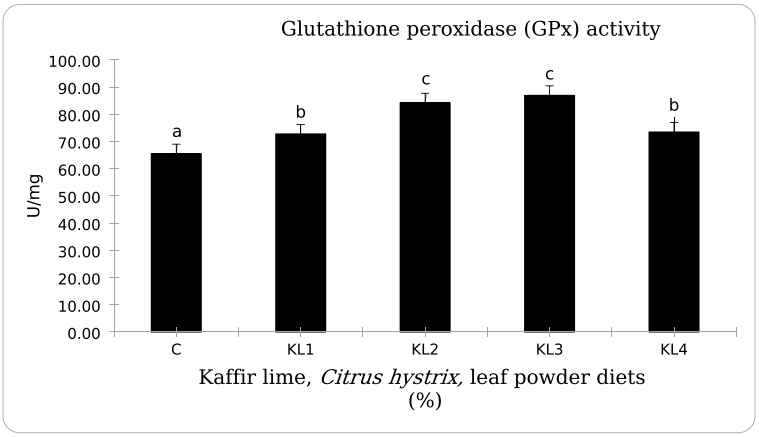

Fig. 6 Glutathione peroxidase (GPx) activity of fish fed different percentages of kaffir lime, *Citrus hystrix*, leaf powder after 8 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix*, leaf powder

Discussion

This study evaluated the impacts of KL, *Citrus hystrix*, leaf powder as a feed additive on the growth performance and health status of African catfish, *C. gariepinus*. Several assays were conducted in the present study, including a feeding trial (Enis Yonar et al. 2012). Recent research on phytobiotics as a feed additive in aquatic animals revealed promising results in improving the growth and health of aquatic animals. In this study, dietary *C. hystrix* leaf powder improved the growth and health status of African catfish, *C. gariepinus*, particularly in FW, WG, and SGR. To the best of our knowledge, this study is the first to

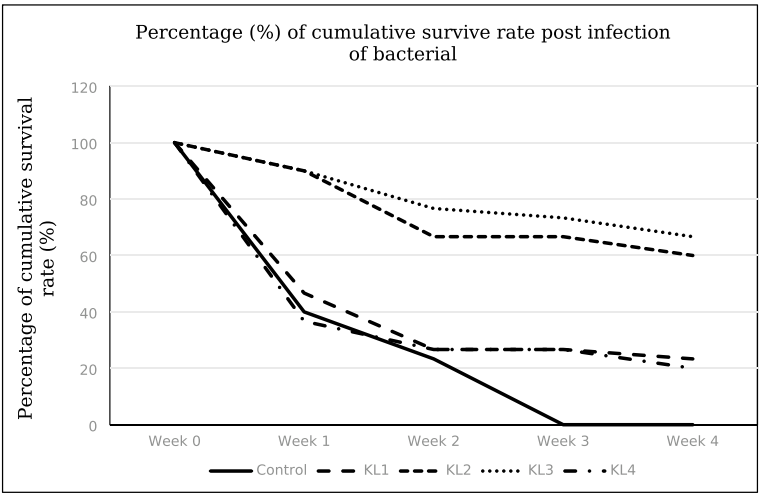

Fig. 7 Cumulative survival rate of African catfish post-*Edwardsiella tarda* infection after 4 weeks. C = control; KL1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of kaffir lime, *Citrus hystrix,* leaf powder

report on the effects of KL leaf powder on African catfish. Nonetheless, previous studies have reported the benefits of *Citrus* products on the growth performance of aquatic animals, particularly *Citrus* spp. essential oil (EO). For instance, *Citrus limon* extract (CLE) promoted the growth of Nile tilapia (Oliveira e Silva et al. 2024), limonene and thymol in Nile tilapia (Aanyu et al. 2018), *C. sinensis* EO in Mozambique tilapia (Acar et al. 2015), bergamot, *C. bergamia* EO, in European sea bass (Acar et al. 2019), and bitter orange, *C. aurantium* EO, in juvenile carp (Acar et al. 2021).

The beneficial effects of *Citrus* spp. on aquatic animals are primarily attributed to the bioactive compounds such as pectin, flavonoids, polysaccharides, and EO (García Beltrán et al. 2017; González-Molina et al. 2010; Macedo et al. 2023). These bioactive compounds are essential in nutrient absorption and promote the protein synthesis of growth factors (Mohamed et al. 2021; Morante et al. 2021; Souza et al. 2023). Moreover, dietary *C. hystrix* leaf powder significantly enhances fish growth performance and improves digestive enzyme activities, including lipase, amylase, and protease. This positive regulation enhances digestion and amino acid absorption and, subsequently, the fish growth performance (Ashry et al. 2023; Lai et al. 2022). Moreover, *C. hystrix* leaf powder is a flavoring agent in fish feed to boost their appetite and feed intake. Nevertheless, excess dietary *C. hystrix* leaf powder may compromise African catfish's growth performance. *C. hystrix* leaf powder at 4% reduced the growth performance of African catfish. Similarly, excessive CLE also adversely impacted the growth performance of Nile tilapia (Mohamed et al. 2021).

Dietary *C. hystrix* leaf powder also decreased HSI and VSI significantly at all inclusion levels, indicating more flesh on the fish's body. This finding indicated that dietary *C. hystrix* enhanced lipid metabolism effectively and reduced fat deposition in lipids and viscera (Oliveira e Silva et al. 2024; Weil et al. 2013), possibly due to the presence of flavonoids that are known to exert anti-lipogenic properties (Abdel Rahman et al. 2019). Excessive *C. hystrix* may trigger an anti-lipogenic effect, resulting in a decline in fish growth performance. Previous studies also reported similar findings (García Beltrán et al. 2017; Macedo et al. 2023; Oliveira e Silva et al. 2024). Therefore, fish

farmers should avoid including high doses of *C. hystrix* leaf powder (4%) in the African catfish diet. Dietary *C. hystrix* leaf positively regulated the hematological indices of African catfish in this study. The KL treatment groups exhibited significantly higher WBC, HBG, HCT, and RBC. High HBG and HCT indicate that the fish is not anemic with optimal metabolism and nutrient availability in the fish blood (Ashrafizadeh et al. 2020). Furthermore, higher RBC, HCT, and HBG indicate effective hemosynthesis and erythropoiesis activities in the fish body, preventing malnutrition and anemia (Enis Yonar et al. 2012). Meanwhile, there were no significant differences in MCH and MCHC, representing the absence of anemia in the experimental fish (Yonar et al. 2019). Fish fed with *C. hystrix* leaf have significantly higher WBC, indicating improved health status. However, the WBC levels may be influenced by sex, feeding behavior, season, stress, and pollutants (Ahmed et al. 2020).

Dietary *Citrus* spp. substantially promoted African catfish's health, manifested through a higher cumulative survival rate after *E. tarda* infection. The antioxidative response outcomes in the present study may have contributed to the positive outcomes. Additionally, *C. hystrix* leaf powder enhanced antioxidative responses, including CAT, SOD, and GPx in KL-treated fish. High antioxidant capacity mitigates stress caused by bacterial infection, resulting in a higher cumulative survival rate in fish supplemented with *C. hystrix* leaf. Likewise, previous studies reported that Nile tilapia and African catfish that received 1% and 2% lemon peel have higher survival rates after *Aeromonas hydrophila* infection (Abdel Rahman et al. 2019). In addition, 5% lemon peel EO in the diet of *Labeo victorianus* can stimulate their disease resistance towards *A. hydrophila* (Ngugi et al. 2017). Moreover, dietary *C. aurantifolia* peels at 1.5% and 3% combined with probiotic *Bacillus licheniformis* protected common carp from *A. hydrophila* infection (Sadeghi et al. 2021). The dietary tangerine, *C. depressa*, leaf supplementation in the barramundi diet also promoted their tolerance against *A. hydrophila* infection (Shiu et al. 2016). On the contrary, dietary *C. limon* extract did not exert any protective effects in striped catfish against *A. hydrophila* infection (Macedo et al. 2023).

Conclusion

This study demonstrated that dietary *C. hystrix* leaf positively impacted African catfish's growth performance and health status. Their enhanced growth performance can be attributed to the superior digestive enzymes activities, while the disease resistance stimulation is possibly linked to the activation of SOD, CAT, and GPx activities. In summary, 2% or 3% dietary *C. hystrix* leaf is highly recommended in the African catfish diet to improve their productivity.

Author contribution Lee Seong Wei, Kon Yeu Hooi, Martina Irwan Khoo: conceptualization, methodology, and investigation. Mohamad Nor Azra and Wendy Wee: resources, data curation, and visualization. All authors: writing—original draft and supervision. All authors have read and agreed to the published version of the manuscript.

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Data availability The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethics approval The experimental design has been registered and approved under the Faculty of Agro Based Industry, Universiti Malaysia Kelantan animal care and use committee with the code UMK/FIAT/ACUE/PG/04/2023.

Consent for publication Not applicable.

Competing interests All authors declare that they have unknown competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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