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Research article

Effect of dietary supplementation of turmeric, *Curcuma longa* leaf on growth and health status of African catfish, *Clarias gariepinus*

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Abstract

High stocking density poses a risk to an aquaculture species' production and health status, increasing their vulnerability to diseases and infection. Feed additive reportedly enhances the growth and health of farmed fish. Therefore, this study evaluated the effects of turmeric, *Curcuma longa*, leaf powder on the growth performance, digestive enzyme activity, hematology, antioxidative response, and disease resistance of African catfish. A feeding trial was conducted using a control diet and three experimental diets containing powdered *C. longa* leaf at various levels (0.5%, 1.0%, and 1.5%). After eight weeks, the growth performance of African catfish supplemented with *C. longa* leaf powder improved significantly (p <0.05), particularly the fish fed with 1.5% turmeric leaf powder. Meanwhile, the FCR was significantly reduced in the treatment groups compared to the control group, where the CL15 group recorded the lowest FCR. Furthermore, the fish fed 1.5% powdered *C. longa* leaf had substantially higher (p <0.05) lipase, protease, and amylase activities than other groups. Powdered *C. longa* leaf supplementation also significantly enhanced (p <0.05) the antioxidative responses of African catfish compared to the control group, particularly glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). In addition, the cumulative survival rate was significantly (p <0.05) higher in fish that received powdered *C. longa* leaf, with the highest being the CL15 group. In summary, 1.5% powdered *C. longa* leaf is possibly the optimal dosage for African catfish feed to boost their productivity.

Keywords: Antioxidative response, Digestive enzyme, Disease resistance, *Edwardsiella tarda*, Phytobiotic, Plant based feed additive

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INTRODUCTION

African catfish, *Clarias gariepinus*, is one of Malaysia's most popular freshwater fish due to its high nutrition, unique taste, and affordable price (Zakaria et al., 2022). Furthermore, this fish is fast-growing, an unfussy feeder, and adaptable to high stocking density; thus, it is a profitable species to boost investors' income. This aquaculture species has been farmed in many other countries such as India, Bangladesh, Indonesia, and Thailand. However, high-intensity farming impairs the growth performance and health status of *C. gariepinus*, rendering them vulnerable to disease outbreaks.

Edwardsiella tarda is a pathogenic bacteria responsible for Edwardsiellosis worldwide (Goh et al., 2023), including carp farming in Japan and India (Yamasaki et al., 2013; Kumar et al., 2014; Pandey et al., 2021), eel in China and Korea (Xu and Zhang, 2014; Park et al., 2017), Nile tilapia in Egypt (Elgendy et al., 2022), Chinook salmon, channel catfish and barramundi in USA (Meyer and Bullock, 1973; Loch et al., 2017), and sturgeon, snakehead, yellow catfish, turbot and flounder in China (Xu and Zhang, 2014; Du et al., 2017; Yang et al., 2018; Liu et al., 2021; Guo et al., 2022).

E. tarda thrives in various environments and hosts, causing massive economic losses and mortality of aquaculture species. Several farm operations have been forced to shut down due to Edwardsiellosis outbreaks. Therefore, the stakeholders are looking for sustainable solutions to reduce the impact of the disease. Antibiotics and chemotherapeutic agents have been utilized to prevent disease infection, but the administration was accompanied by adverse impacts such as antibiotic residues, antibiotic resistance among pathogenic bacteria, environmental hazards, and public health concerns (Lee et al., 2016; Abdul Kari et al., 2022; Kari et al., 2022). Consequently, aquaculturists increasingly looking for alternative solutions, such as phytobiotics applications. Phytobiotics are alternatives to conventional treatments such as antibiotics, acting as a feed additive to promote the growth and health status of aquaculture species (Lee et al., 2024). Most phytobiotics are accessible, inexpensive, commercially available, and have lesser undesirable impacts on aquatic organisms and the environment (Mahmoud et al., 2017; Goh et al., 2023a; Goh et al., 2023b).

Turmeric, or *Curcuma longa*, is a well-known herb widely used as a traditional medicine, cooking, yellow coloring agent, and food preservative (Kim et al., 2020; Zou et al., 2022). This rhizome contains one of the most-studied bioactive compounds called curcumin, that its role has been highlighted in growth promotion, antioxidant enhancement, immunomodulation, gut microbiota improvement, and disease resistance (Giri et al., 2019; ; Ming et al., 2020; Pereira et al., 2020; Bhoopathy et al., 2021; Hong et al., 2022). For instance, dietary curcumin in white-leg shrimp improved their growth performance and stimulated disease resistance against vibriosis caused by *Vibrio harveyi* (Bhoopathy et al., 2021). Likewise, Nile tilapia supplemented with curcumin exhibited significantly improved growth performance and resistance against *Aeromonas hydrophila* infection (Mahmoud et al., 2017). Moreover, rainbow trout fed with curcumin demonstrated superior growth performance and antioxidant capacity (Yonar et al., 2019). Dietary turmeric also modulates gut microbiota in Nile tilapia and gilthead seabream (Pereira et al., 2020; Ashry et al., 2021).

Despite the extensive research on curcumin as a feed additive, studies on the potential therapeutic role of turmeric leaf remain lacking. Several studies have reported on the advantages of *C. longa* leaf in enhancing immunity, antioxidant activities, and anti-aging (Choi and Lee, 2014; Kim and Lee, 2014; Kim et al., 2020). The utilization of *C. longa* leaf could also prevent the improper disposal of this agricultural by-product, thus reducing environmental concerns.

Therefore, the current study explores the benefits of powdered *C. longa* leaf on the growth performance, digestive enzyme activity, antioxidant capacity, and disease resistance against *E. tarda* of African catfish, *C. gariepinus*. The study outcomes pose a guideline for farmers in managing *C. longa* by-products and provide African catfish farmers with an alternative feed additive that could boost their farm revenues and productivity.

MATERIALS AND METHODS

Ethical statement

This study has been approved by the animal care and use committee of Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia (UMK/FIAT/ACUE/PG/03/2023) and conducted according to the ethical protocol and guidelines for experimental animals.

Feeding experiment

Four types of diets were formulated and prepared for this study (Table 1). Three diets were incorporated with different levels of powdered *C. longa* leaf (0.5%, 1.0%, and 1.5%). The *C. longa* leaf was obtained from a wet market at Tanah Merah, Kelantan, Malaysia. The leaves were oven-dried at 60°C for 72 h and powdered using a blender (Panasonic, Malaysia). All the feed ingredients were homogenized and pelleted using a lab pelletizer. The diets were then air-dried and kept in the freezer at -20°C until further use. The proximate analysis was conducted for all diets to determine the moisture, lipid, protein, and ash contents (Latimer, 2023).

A total of 500 African catfish juveniles were purchased from a commercial farm at Tanah Merah, Kelantan. The fish were acclimatized in a 300 L holding tank for one week. Subsequently, healthy fish were selected and distributed into 50 L aquaria for every dietary treatment in triplicates (n = 30 fish/ aquarium). The feeding trial was conducted for eight weeks, where the fish were fed once daily in the morning, followed by 100% water change in the afternoon using dechlorinated tap water. The experimental fish were monitored and weighed weekly. In addition, the water parameters were recorded weekly and maintained as follows: water temperature 25.8 - 28.4 °C, ammonia < 0.03 ppm, dissolved oxygen 5.3 - 6.5 ppm, and pH 6.4 - 7.2.

Determination of growth performance

The fish were sampled and weighed at the end of the feeding trial. Their growth performance was determined as described by previous studies (Kabir et al., 2023; Rahman et al., 2023):

Total weight gain	= Final body weight – initial body weight
Weight gain (WG, %)	= (Total weight gain/ initial body weight) ×100
Specific growth rate (SGR, %)	= (Total weight gain / experimental duration) ×100
Hepatosomatic index (HSI)	= Total liver weight / total body weight × 100
Viscerosomatic index (VSI)	= Total viscera weight / total body weight × 100
Feed conversion ratio (FCR)	= Total feed intake / total weight gain

Hematological analysis

Fish (n = 3) from each treatment group were selected randomly for blood sampling. First, the fish were anesthetized using clove oil, followed by blood collection, and placed in heparin tubes for further analysis. The blood analysis was conducted using a hematology analyzer (Mythic 18 Vet, USA) (Zakaria et al., 2022).

Determination of digestive enzyme activity

Digestive enzyme activities in the fish gut were determined following the procedures described in previous studies (Kari et al., 2023; Suma et al., 2023). Fish (n = 3) from each treatment group were sampled to harvest their intestines, homogenized in phosphate-buffered saline (PBS), and centrifuged at 8000 rpm for 10 mins at 4 ^{o}C. The supernatants were stored in the refrigerator until use. The amylase and protease activities were detected using the iodine solution and the Folin-Ciocalteu phenol reagent, respectively (Lowry et al., 1951). Meanwhile, the lipase activity was evaluated using olive oil as a substrate, following the methods of (Borlongan, 1990).

Antioxidative response

Antioxidative response assays were performed according to previous studies (Paray et al., 2020; El Basuini et al., 2022; Wei, 2024). Fish (n = 3) from each treatment group were sampled to harvest their livers and homogenized in saline, followed by centrifugation at 10000 rpm for 10 min. The resulting supernatants were used to determine superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities via colorimetric kits (Nanjing, China). The results were obtained using a microplate reader (BioRad, USA) at 280 nm.

Disease resistance assay

At the end of the feeding trial, fish (n = 3) from each treatment were selected for the *E. tarda* challenge. The fish were inoculated with the bacteria (1×10^8 cfu/mL) via an intraperitoneal injection (Lee et al., 2016). The survival rate of the experimental fish was monitored and recorded weekly for four weeks. Finally, the cumulative survival rate was determined at the end of the experiment.

Statistical analysis

All data were checked for normality before being analyzed using Statistical Package for Social Sciences (SPSS) version 20.1 (IBM, USA). A one-way analysis of variance (ANOVA) was performed to determine significant differences between the treatments, followed by grouping via Tukey's post hoc test. The significance level was set at p <0.05, and the results were presented as mean ± standard deviation (SD).

Table 1 Nutritional profile and feed formulations with *Cucurma longa* leaf supplementation for the feeding trial of African catfish, *Clarias gariepinus*

Raw materials		Diets (%)			
		Control	CL 5	CL 10	CL 15
Soybean meal		22	22	22	22
Fish meal		50	50	50	50
Wheat bran		17	16.5	16.0	15.5
Premix		2	2	2	2
Fish oil		3	3	3	3
Vegetable oil		3	3	3	3
Carboxymethyl cellulose (CMC) binder		3	3	3	3
Powdered *Curcuma longa* L. leaf		0	0.5	1.0	1.5
	Total	100	100	100	100
Nutritional profiles					
Carbohydrate		43.3	44.2	44.1	42.5
Protein		32.1	32.8	32.9	32.3
Ash		6.7	4.8	6.7	6.8
Lipid		6.6	6.7	6.1	7.6
Fiber		4.6	4.7	5.2	5.1
Moisture		6.7	6.8	5.0	5.7

* CL5 = 0.5% of *C. longa* leaf; CL10 = 1.0% of *C. longa* leaf; CL15 = 1.5% of *C. longa* leaf

RESULTS

The growth performance of African catfish supplemented with powdered *C. longa* leaf is detailed in Table 2. The final weight, WG, and SGR increased significantly (p <0.05) in the turmeric leaf-treated groups, with the highest being in fish supplemented with 1.5% powdered *C. longa* L. leaf. Conversely, HSI, VSI, and FCR demonstrated a decreasing trend, with the lowest being the CL15 group.

The hematological analysis findings are presented in Table 3. The white blood cell (WBC) count was significantly higher (p <0.05) in fish that received powdered *C. longa* leaf than in the control group, with the highest being the CL15 group. A similar pattern was observed in the lymphocyte (LYM) levels. In addition, powdered *C. longa* leaf groups had significantly higher (p <0.05) levels of hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Meanwhile, no significant differences were recorded for monocyte (MON) and red blood cell (RBC) levels between the treatments.

Digestive enzyme activity was significantly higher (p <0.05) in fish that received powdered *C. longa* leaf, with the highest being in the CL15 group (Figure 1). The fish fed 1.5% powdered *C. longa* leaf had the highest amylase, protease, and lipase activities.

Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were also significantly higher in African catfish fed powdered *C. longa* leaf (p <0.05) than the control group (Figure 2). The CL15 group demonstrated the highest SOD, GPx, and CAT, followed by CL5 and CL10 treatment groups.

Following the *E. tarda* challenge, cumulative survival rate of fish supplemented with powdered *C. longa* leaf was significantly higher (p <0.05) than the control group (Figure 3). The highest cumulative survival rate was recorded by the CL15 group, followed by the CL5 and CL10 treatment groups. A 100% mortality rate was observed in the control group four weeks post-*E. tarda* infection.

Table 2 Growth performance of African catfish, *Clarias gariepinus*, fed feed supplementation with 0.5, 1.0, and 1.5% of *Curcuma longa* leaf for eight weeks

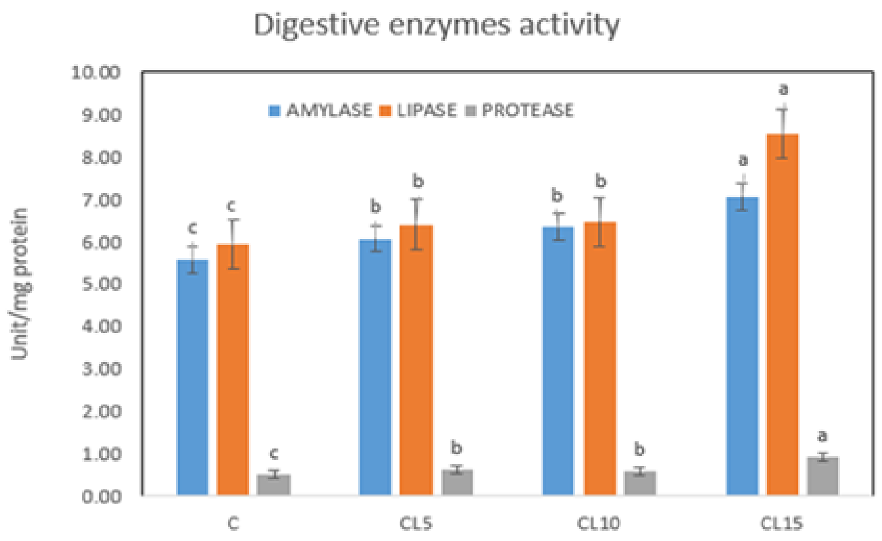
Parameters	Control	CL5	CL10	CL15
Initial weight (IW) (g)	10.8±0.40	11.1±0.76	10.9±0.52	10.7±0.50
Final weight (FW) (g)	206.9±4.76^{c}	240.3±4.09^{b}	229.1±2.017^{b}	274.5±6.57^{a}
Weight gain (WG) (%)	1818.5±113.11^{c}	2077.2 ±138.12^{b}	2004.3 ±87.92^{b}	2462.3 ±172.49^{a}
Specific growth rate (SGR) (%)	2.29±0.046^{c}	2.39±0.049^{b}	2.36±0.032^{b}	2.51±0.051^{a}
Hepatosomatic index (HSI)	3.05±0.28^{c}	2.43±0.124^{b}	2.41±0.155^{b}	2.15±0.059^{a}
Viscerosomatic index (VSI)	3.90±0.208^{c}	3.57±0.175^{b}	3.67±0.122^{b}	2.82±0.120^{a}
Feed conversion ratio (FCR)	1.38±0.037^{c}	1.18±0.020^{b}	1.24±0.010^{b}	1.02±0.027^{a}

*Data expressed as mean ± SD
* CL5 = 0.5% of *C. longa* leaf; CL10 = 1.0% of *C. longa* leaf; CL15 = 1.5% of *C. longa* leaf
*Values in the same row with different superscripts indicate significant differences at p < 0.05

Table 3 Blood parameters of African catfish, *Clarias gariepinus*, fed feed supplementation with 0.5, 1.0, and 1.5% of *Curcuma longa* leaf for eight weeks

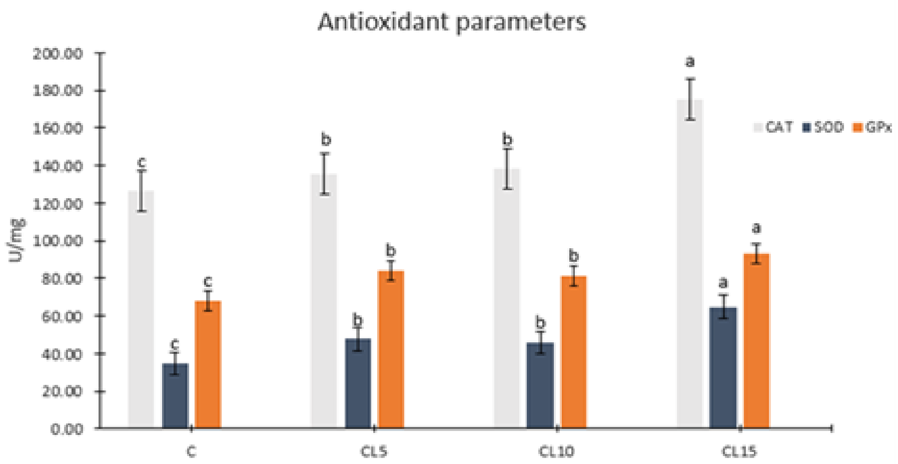
Blood parameters	Control	PO	WH	RC
WBC (/µL)	112.4±3.70^{b}	131.5±5.76^{a}	127.8±3.93^{a}	139.9±2.82^{a}
LYM (%)	90.9±2.31^{c}	108.1±9.6^{b}	112.4±8.33^{b}	130.4±19.90^{a}
MON (%)	12.6±0.61	13.3±2.21	12.9±0.21	13.1±1.73
RBC (10^3/ µL)	2.2±0.12	2.3±0.15	2.4±0.31	2.4±0.25
HGB (g/dL)	6.4±0.31^{b}	8.8±0.40^{a}	8.9±0.23^{a}	8.9±0.25^{a}
HCT (%)	26.6±2.18	29.3±1.08^{a}	29.6±0.61^{a}	29.0±1.01^{a}
MCH (pg)	35.3±3.25^{b}	47.3±1.71^{a}	48.0±1.59^{a}	47.7±0.87^{a}
MCHC (g/dL)	27.7±0.40^{b}	31.7±0.92^{a}	30.9±0.86^{a}	31.8±0.31^{a}

*Data expressed as mean ± SD
* CL5 = 0.5% of *C. longa* leaf; CL10 = 1.0% of *C. longa* leaf; CL15 = 1.5% of *C. longa* leaf
* Values in the same row with different superscripts indicate significant differences at p <0.05
*WBC = white blood cell, LYM = lymphocyte, MON = monocyte, RBC = red blood cell, HGB = hemoglobin, HCT = hematocrit, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration



*C= Control; CL5 = 0.5% of *C. longa* leaf; CL10 = 1.0% of *C. longa* leaf; CL15 = 1.5% of *C. longa* leaf
*Values in the same row with different superscripts indicate significant differences at $p<0.05$

Figure 1 Digestive enzyme activity of African catfish, *Clarias gariepinus*, fed feed supplementation with 0.5, 1.0, and 1.5% of *C. longa* leaf for eight weeks



*C= Control; CL5 = 0.5% of *C. longa* leaf; CL10 = 1.0% of *C. longa* leaf; CL15 = 1.5% of *C. longa* leaf
*Values in the same row with different superscripts indicate significant differences at $p<0.05$

Figure 2 Antioxidative response of African catfish, *Clarias gariepinus*, fed feed supplementation with 0.5, 1.0, and 1.5% of *C. longa* leaf for eight weeks

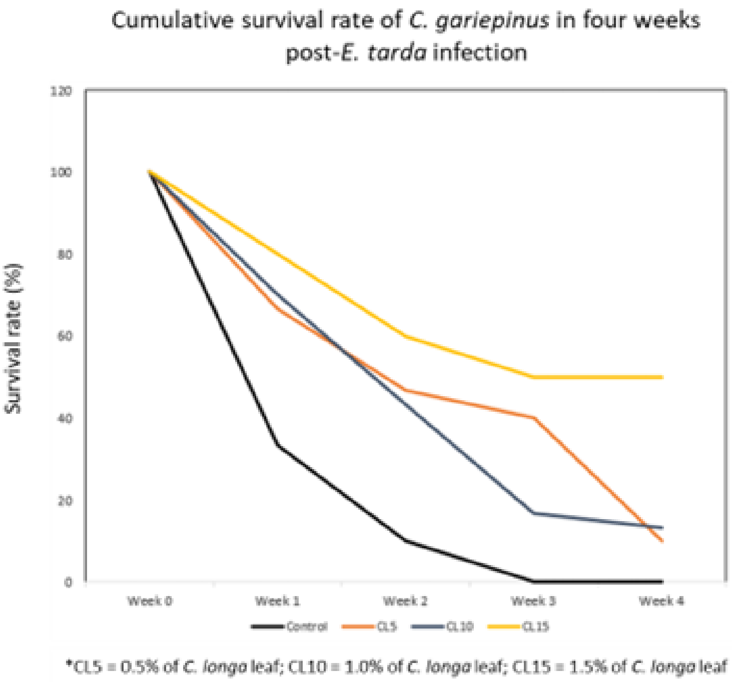


Figure 3 The cumulative survival rate four weeks post-*E. tarda* infection of the African catfish, *Clarias gariepinus*, fed feed supplementation with 0.5, 1.0, and 1.5% of *C. longa* leaf for eight weeks.

DISCUSSION

This study revealed the advantages of powdered *C. longa* leaf supplementation on the growth, health, and disease resistance of African catfish, *C. gariepinus*. Several analyses were conducted in this study, including a feeding trial, hematological profiling, determination of digestive enzyme activities and antioxidative response, and disease resistance against *E. tarda*.

Phytobiotics are promising feed additives as they can improve the growth performance and health status of aquatic animals (Kari et al., 2022; Wei et al., 2022). Nevertheless, earlier studies have highlighted the importance of determining phytobiotic dosage and its impacts on different species. For instance, dietary cinnamon enhanced growth performances of juvenile heteroclarias (Jimoh et al., 2023). In this study, powdered *C. longa* leaf supplementation enhanced African catfish's growth rate, SGR, and FCR. This improvement was most evident in the CL15 group, indicating that powdered *C. longa* leaf supplementation at 1.5% is optimal and beneficial as a feed additive in African catfish to boost their productivity.

The current study findings are consistent with earlier reports on curcumin supplementation that enhanced the growth and health of gilthead seabream (Ashry et al., 2021), common carp (Giri et al., 2019), Nile tilapia (Mahmoud et al., 2017), grass carp (Ming et al., 2020), large yellow croaker (Ji et al., 2021), rainbow trout (Akdemir et al., 2017; Yonar et al., 2019), and crucian carp (Jiang et al., 2016). However, the optimum curcumin dosages vary between studies as feed additive doses rely on fish size, fish stage, feeding behavior, farming system, and duration (Zhu, 2020).

Improvements in African catfish growth performance in this study could also be contributed by curcumin, which promotes digestive enzyme activities in fish. In addition, dietary *C. longa* leaf possibly activates digestive enzymes such as protease, lipase, and amylase, promoting digestion and amino acid absorption (Gao et al., 2022; Lai et al., 2022). Similar effects were also reported in climbing perch fed spray-dried hog plum, *Spondias pinnata* (Promprom et al., 2023). Besides, dietary *C. longa* leaf significantly reduced VSI and HSI, as reported in previous studies, indicating more flesh on the fish body. Moreover, curcumin reportedly modulated gut microbiota propagation against harmful microorganisms in fish (Fernández-Lázaro et al., 2020; Stohs et al., 2020). Curcumin may also add flavor that increases feed palatability, thus improving feed intake, utilization, and growth (Alagawany et al., 2021).

Hematological indices are reliable in assessing the impacts of feed additives on aquatic animal's health status (Vazirzadeh et al., 2017). In the present study, the hematological analysis revealed that dietary *C. longa* leaf positively impacted African catfish's health by significantly enhancing the HCT and HGB levels. This outcome was aligned with previous studies that dietary curcumin supplementation in gilthead sea bream and rainbow trout improved HCT and HGB levels (Ashry et al., 2021; Yonar et al., 2019). High HCT and HGB values indicate the absence of anemia in an aquatic species (Ashry et al., 2021). Furthermore, African catfish supplemented with *C. longa* leaf demonstrated significantly higher WBC and LYM levels. This finding indicated that *C. longa* leaf promoted metabolism and nutrient availability in the blood of African catfish. Nevertheless, WBC levels are often influenced by sex, season, feeding behavior, stress levels, and pollutants (Ahmed et al., 2020).

E. tarda is a virulent bacterium that causes high mortality of aquatic species and thus has a significant economic impact on aquaculture. This devastating outcome was demonstrated by the 100% mortality of the control group in this study at three weeks post-*E. tarda* infection. On the contrary, African catfish fed 1.5% *C. longa* leaf recorded significantly higher survival rates than other treatment groups when challenged with *E. tarda*.

An earlier report claimed that curcumin, the main bioactive compound in *C. longa,* exhibited inhibitory activity against harmful bacteria (Banez et al., 2020). In addition, dietary curcumin enhanced CAT, SOD, and GPx activity as reported in abalone (Zou et al., 2022), grass carp (Ming et al., 2020), Nile tilapia (Mahmoud et al., 2017), and rainbow trout (Yonar et al., 2019). Therefore, curcumin is also potentially responsible for the significantly higher cumulative survival rate of African catfish fed with *C. longa* leaf in this study. Furthermore, *C. longa* leaf reportedly possesses high antioxidant activities (Braga et al., 2018), which could have enhanced the antioxidant capacity of African catfish by increasing CAT, SOD, and GPx activities in this study.

The efficacy of a feed additive in enhancing aquatic animal's health status could be assessed by monitoring their survival rate under pathogenic stress. This study findings indicated that dietary *C. longa* leaf is a potential immunostimulant, as the cumulative survival rate of the treatment groups was significantly higher than the control group. Similar findings have been reported in common carp (Giri et al., 2019), grass carp (Ming et al., 2020), and abalone (Zou et al., 2022) supplemented with dietary curcumin. Therefore, dietary *C. longa* leaf can mitigate stress caused by bacterial infection and stimulate disease resistance against *E. tarda* infection, particularly in African catfish.

CONCLUSIONS

Findings of this study revealed the benefits of powdered *C. longa* leaf supplementation on African catfish growth performance and health status. Their superior growth performance could be linked to enhanced digestive enzyme activities, including lipase, protease, and amylase. Meanwhile, their enhanced health status is possibly associated with significantly higher antioxidative responses via GPx, SOD, and CAT activation and a substantially higher cumulative survival rate after *E. tarda* infection. Future work should investigate the impacts of dietary *C. longa* leaf on various species of aquatic animals. In summary, powdered *C. longa* leaf is a promising feed additive that potentially enhancing African catfish productivity.

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AUTHOR CONTRIBUTIONS

Lee Seong Wei, Kon Yeu Hooi: Conceptualization, Methodology, Investigation. Martina Irwan Khoo, Azra MN, 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.

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