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Research Article

Protective Effects of Dietary *Etlingera elatior* (Jack) Bud Flower Powder against *Edwardsiella tarda* Infection in African Catfish, *Clarias gariepinus*

Seong Wei Lee,[1] **Vui Kien Liew**,[2] **Zulhisyam Abdul Kari**,[1] **Muhammad Anamul Kabir**,[3] **M. N. Azra**,[4] **Martina Irwan Khoo**,[5] **and Wendy Wee**[6]

[1]*Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Jeli 17600, Kelantan, Malaysia*
[2]*Department of Johor State Fisheries Complex, Pendas Laut Road, Gelang Patah 81550, Johor, Malaysia*
[3]*Department of Aquaculture, Faculty of Fisheries, Sylhet Agricultural University, Sylhet 3100, Bangladesh*
[4]*Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu (UMT), Kuala Nerus 21030, Terengganu, Malaysia*
[5]*Department of Chemical Pathology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian 16150, Malaysia*
[6]*Center of Fundamental and Continuing Education, Universiti Malaysia Terengganu, Kuala Nerus 21030, Terengganu, Malaysia*

Correspondence should be addressed to Seong Wei Lee; leeseong@umk.edu.my and Wendy Wee; wendy@umt.edu.my

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This study investigates the impacts of dietary *Etlingera elatior* (Jack) bud flower powder (EE) supplementation on the growth and health status of African catfish, *Clarias gariepinus*. Each treatment group received different formulated diets: basal diet without EE (control), basal diet + 1% EE (EE1), basal diet + 2% EE (EE2), basal diet + 3% EE (EE3), and basal diet + 4% EE (EE4). After an 8-week feeding trial, fish supplemented with dietary EE exhibited significantly improved growth performance, such as weight gain (WG; 1,251.4%–1,604.3%; $p < 0.0001$), specific growth rate (SGR; 2.02%–2.20%; $p < 0.0001$), and final weight (FW; 141.7–178.9 g; $p < 0.0001$) than the control group (WG: 1,192.0%, SGR: 1.98%, and FW: 136.1 g), particularly EE2 and EE3. Furthermore, EE-treated fish recorded significantly lower feed conversion rate (FCR; 1.19–1.53; $p < 0.0001$), viscerosomatic index (VSI; 3.40%–4.50%; $p < 0.0001$), and hepatosomatic index (HSI; 3.13%–4.40%; $p < 0.0001$) than the control (FCR: 1.59, VSI: 5.48%, and HSI: 4.75%), particularly EE2 and EE3. The EE-treated fish also had significantly higher white blood cell (WBC) count (124.6–148.6/μL; $p < 0.0400$), red blood cell (RBC) count (2.43–4.03 $\times 10^3/\mu$L; $p < 0.0002$), hemoglobin (HGB) concentration (6.27–7.87 g/dL; $p < 0.0160$), and hematocrit (HCT; 26.8%–38.7%; $p < 0.0200$) compared to the control (WBC count: 112.1/μL, RBC count: 2.17 $\times 10^3/\mu$L, HGB concentration: 5.60 g/dL, and HCT: 23.7%), with the highest being EE2 and EE3. Dietary EE diets enhanced digestive enzyme activities, including amylase ($p < 0.0090$), protease ($p < 0.0040$), and lipase ($p < 0.0060$), significantly ($p < 0.05$) than the control, where EE2 and EE3 demonstrated the highest activities. The EE supplementation also significantly improved the fish's antioxidative responses, particularly catalase (CAT; $p < 0.0100$), glutathione peroxidase (GPx; $p < 0.0300$), and superoxide dismutase (SOD; $p < 0.0100$) in EE2 and EE3. Similarly, the cumulative survival rate of EE2 (66.7% ± 5.77%) and EE3 (66.7% ± 5.77%) were significantly ($p < 0.0001$) higher than other groups post-*Edwardsiella tarda* challenge. Therefore, this study findings highlighted the potential benefits of EE as a feed additive to boost the production in African catfish farming.

1. Introduction

African catfish, *Clarias gariepinus* culture, is gaining popularity in Malaysia due to the fish's rapid growth, adaptability in high stocking density, and high tolerance in nonoptimal environments [1]. Furthermore, their delectable flesh and affordable price are attributed to the high market demand, which is estimated to increase rapidly in the near future [1].

Despite that, intensive farming poses a risk of growth and health impairments in this aquaculture species [1], subjecting them to high stress and susceptibility to infectious diseases, such as Edwardsiellosis caused by *Edwardsiella tarda*. *Edwardsiella tarda* is a significant bacterial species in aquaculture that has devastating impacts on fish farmers, which could end their operation in severe cases [2]. This microorganism has a wide range of hosts, including marine, freshwater, terrestrial, and aquatic animals [3]. *Edwardsiella tarda* was reportedly isolated from various healthy, diseased, and moribund aquatic animals, such as African catfish (*C. gariepinus*) [4], Malaysia freshwater giant prawn (*Macrobrachium rosenbergii*) [5], silver catfish (*Pangasuis sutchi*) [6], American bullfrog (*Rana catesbeina*) [7, 8], Asian clam (*Corbicula fluminea*) [9], Asian seabass (*Lates calcarifer*) [10], hybrid snakehead (*Channa maculate* ♀ × *C. argus* ♂) [11], pompano (*Trachinotus blochii*) [6], Siamese crocodile (*Crocodylus siamensis*) [12], Japanese flounder (*Paralichthys olivaceus*) [13], red hybrid tilapia (*Oreochromis* spp.) [14, 15], and grass carp (*Ctenopharyngodon Idella*) [16]. Edwardsiellosis infection in African catfish caused histopathological alterations in the organs including kidney, spleen, muscles, and skin as well as swelling in the organs such as kidney, liver, spleen, intestine, and muscles, subsequently, Edwardsiellosis infection in African catfish end up with high mortality [17]. Consequently, treatment and prevention strategies against *E. tarda* infection in aquaculture species have been developed, such as antibiotics, probiotics, prebiotics, vaccines, and phytobiotics [18].

Antibiotic usage in aquaculture adversely impacts public health and the environment [19]. In addition, vaccination programs have been proven effective for disease control disease in aquaculture, but are costly and labor-intensive [18]. Alternatively, probiotics and prebiotics have recently been widely utilized as feed additive aquatic health management. Studies have revealed the potential of phytobiotics as a feed additive to enhance the growth and health of aquaculture species. For instance, dietary kelp powder stimulated disease resistance of hybrid snakeheads, *C. maculate* ♀ × *C. argus* ♂, against *Aeromonas hydrophila* [20]. Common carp, *Cyprinus carpio*, supplemented with ginger, *Zingiber officinale*, extract also demonstrated enhanced growth performance and health status [21]. Other phytobiotics such as essential oil from sweet orange, *Citrus sinensis*; bitter lemon, *C. limon* [22]; pineapple, *Ananas comosus*; waste [23]; curcumin; *Andrographis paniculata* leaf [24]; and ginger powder [25] also positively influenced the growth and health of Nile tilapia, *Oreochromis niloticus*; grass carp, *C. idella*; and stripped catfish, *Pangasianodon hypothalmus*; respectively.

Phytobiotics from the Zingiberaceae family promoted the growth and health of aquatic animals. Dietary ginger rhizome powder and extract, ginger oil, essential oil, and shogaol in this rhizome enhanced the growth performance and health status of striped catfish, *P. hypothalmus* [25]; rainbow trout, *Oncorhynchus mykiss* [26]; *Labeo rohita* [27]; common carp, *C. carpio* [28]; guppy, *Poecilia reticulata* [29]; tilapia, *Oreochromis* spp. [30]; and white leg shrimp, *Litopenaeus vannamei* [31]; respectively. Moreover, dietary ginger and its derivatives could relieve stress in largemouth bass, *Micropterus salmoides* [32] and stimulate disease resistance against Edwardsiellosis in African catfish, *C. gariepinus* [17]. Nonetheless, the potential of other plants in the Zingiberaceae family remained unexplored.

The torch ginger, *Elingera elatior* (Jack; EE) flower bud, or *bunga kan*tan in Malaysia is a common ingredient in Asian cuisine. This plant contains phenolic compounds, flavonoids, anthocyanin, and tannin, contributing to its superior antioxidant and antimicrobial properties [33]. Traditionally, torch ginger leaf is used for odor control, earache treatment, and wound healing [34]. No study has reported EE flower bud application as a feed additive in aqua feed. Therefore, this study is one of the first current to investigate the effects of EE as a feed additive on growth performance, digestive enzyme activity, hematology, antioxidative responses, and disease resistance against *E. tarda* infection in African catfish.

2. Materials and Methods

2.1. Plant Preparation. The EE flower buds were purchased from a wet market in Jeli, Kelantan, Malaysia. The flower buds were rinsed under running tap water and oven-dried at 50°C until completely dried [34]. The dried flower buds were ground into powder form and stored in a freezer until further use.

2.2. Torch Ginger (EE) Flower Bud Diet Preparation. A starter commercial pellet for African catfish was purchased and ground into powder form. The nutritional profile of the commercial diet is as follows: crude protein = 34%, fat = 4%, and moisture = 11%. The powdered commercial pellet was homogenized with EE at different levels: control (no EE), EE1 (1% EE), EE2 (2% EE), EE3 (3% EE), and EE4 (4% EE), and water was added to form a dough. The dough was passed through a hand meat processor and oven-dried at 40°C until completely dried. Finally, all the prepared feed was broken down into smaller pieces (±2 mm) and stored in the freezer for further use.

2.3. Experimental Fish. Healthy African catfish fry ($n = 1{,}000$) was purchased from a commercial farm in Tanah Merah, Kelantan, and acclimatized in a 500 L tank for a week. Subsequently, 450 healthy fries with an average weight of 10.5 g were distributed equally into 15 units of 50 L aquarium. The experiment was carried out in triplicates (30 fry/tank), and the feeding trial was conducted for 8 weeks. The fish were fed once daily *ad libitum* in the morning, and 100% water change was carried out in the afternoon. The water parameters, such as temperature, pH, dissolved oxygen and ammonia, of the aquaria, were monitored weekly using multiparameter (YSI ProQuatro, USA) and ammonia kit (API, Malaysia).

2.4. Determination of Growth Parameters. At the end of the feeding trial, all the fish were weighed to determine their weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), viscerosomatic index (VSI), and hepatosomatic index (HSI), as described in earlier studies [35, 36].

Table 1: Growth performance parameters of experimental fish fed torch ginger, *Etlingera elatior*, flower bud powder diets for 8 weeks.

Parameters	Control	EE1	EE2	EE3	EE4
Initial weight (IW; g)	10.5 ± 0.06	10.5 ± 0.06	10.5 ± 0.00	10.5 ± 0.10	10.4 ± 0.12
Final weight (FW; g)	136.1 ± 4.50^{a}	157.3 ± 1.56^{b}	177.6 ± 4.02^{c}	178.9 ± 7.16^{c}	141.7 ± 4.99^{a}
Weight gain (WG; %)	$1{,}192.0 \pm 39.94^{a}$	$1{,}402.6 \pm 16.31^{b}$	$1{,}591.1 \pm 38.30^{c}$	$1{,}604.3 \pm 61.11^{c}$	$1{,}251.4 \pm 44.36^{a}$
Specific growth rate (SGR; %)	1.98 ± 0.024^{a}	2.10 ± 0.008^{b}	2.19 ± 0.018^{c}	2.20 ± 0.028^{c}	2.02 ± 0.025^{a}
Hepatosomatic index (HSI; %)	4.75 ± 0.130^{a}	3.98 ± 0.122^{b}	3.19 ± 0.278^{c}	3.13 ± 0.254^{c}	4.40 ± 0.262^{ab}
Visceral somatic (VSI; %)	5.48 ± 0.117^{a}	4.15 ± 0.136^{b}	3.40 ± 0.210^{a}	3.43 ± 0.089^{a}	4.50 ± 0.295^{b}
Feed conversion ratio (FCR)	1.59 ± 0.058^{a}	1.36 ± 0.014^{b}	1.20 ± 0.029^{c}	1.19 ± 0.050^{c}	1.53 ± 0.057^{a}

Note. Data expressed as mean ± standard deviation. EE1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of torch ginger, *Etlingera elatior*, flower bud powder diets. Values in the same row with different letters showed significant difference at $p < 0.05$.

2.5. Hematological Analysis. Experimental fish from each treatment ($n = 3$) were sampled for blood collection at the end of the feeding trial. First, the selected fish were anesthetized using clove oil. The blood was withdrawn, and the samples were kept in heparinized tubes. Finally, the blood samples were subjected to hematological analysis in the laboratory using a hematology analyzer (Mythic 18 Vet, USA) [37]. The hematological parameters were measured in the current study, including white blood cell (WBC) count, lymphocytosis (LYM), monocytes (MON), red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

2.6. Determination of Digestive Enzyme Activities. The fish from Section 2.5 were dissected, and their intestines were harvested. The samples were homogenized with phosphate-buffered saline (PBS) and centrifuged at 8,000 rpm for 10 min. The supernatants were used for the determination of digestive enzyme activities. The iodine solution and Folin–Ciocalteu phenol reagent were used to analyze amylase and protease activities, respectively [38]. Meanwhile, lipase activity was determined, as described by Borlongan [39].

2.7. Determination of Antioxidative Responses. Liver samples were obtained from the fish in Section 2.5 to analyze their antioxidative responses. The fish livers were homogenized with PBS and centrifuged at 8,000 rpm for 10 min. The supernatants were collected and subjected to catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities analysis using commercial kits (Elabscience, USA) via colorimetric method. The results were determined using a microplate reader (BioRad, USA) at a wavelength of 560 nm [25].

2.8. Edwardsiella tarda Infection. At the end of the feeding trial, 10 fish from each replicate from every treatment were challenged with *E. tarda* infection. Edwardsiella tarda strain C7 used in this study was sourced from an earlier research [40]. The fish were exposed to the bacteria (1×10^{8} cfu/mL) via intraperitoneal injection at a concentration recommended by Lee et al. [41]. The infected fish were continually fed with the designed diets for 4 weeks. The fish mortality was monitored daily, and their cumulative survival was calculated weekly. All the moribund and died due to the bacterial infection fish have symptoms such as abnormal swimming behavior, pale skin, and gross lesions on skin. A necropsy was conducted on the dead fish by reisolating the bacteria from the fish liver by using xylose lysine deoxycholate (XLD, Hi Media, India) [42]. After 48 hr incubation period, the suspected clear colony bacteria with black at the center and reddish peripheral ring in diameter 1–2 mm was further confirmed with Gram staining, indole production, motility, oxidase, and catalase test. All the isolated bacteria were Gram negative, positive in indole production, motility, and catalase tests whereas they were negative in oxidase test. Therefore, they were identified as *E. tarda* that used in the bacterial challenge assay.

2.9. Statistical Analysis. All the data were checked using Kolmogorov–Smirnov test of normality and homogeneity of variances was checked using Levene's test for equality of variances prior to the one-way analysis of variance (ANOVA) followed by grouping using the Tukey post hoc test. Cumulative survival rate was analyzed through Kaplan–Meier and log rank tests. The significance level was set at $p < 0.05$, and data was presented as mean ± standard deviation (SD). The ANOVA and Kaplan–Meier tests were analyzed using Statistical Package for Social Sciences (SPSS) version 20.1 (IBM, USA).

3. Results

The effects of dietary EE on the growth performance of African catfish are shown in Table 1. After the 8-week feeding trial, all growth performance parameters (WG, $p < 0.0001$; SGR, $p < 0.0001$; HIS, $p < 0.0001$; VSI, $p < 0.0001$; and FCR, $p < 0.0001$) for EE-treated groups were significantly higher than the control group, particularly EE2 and EE3. However, experimental fish fed EE4 diet showed significantly lower than in all tested growth parameters compared to fish fed EE2 and EE3. The dietary EE treatment groups exhibited significantly lower HSI, VSI, and FCR than the control group, with the lowest being those fed with 2% and 3% EE, followed by 1% and 4% EE. Furthermore, EE-treated groups recorded significantly higher WBC count ($p < 0.0400$), RBC count ($p < 0.0002$), HGB concentration ($p < 0.0160$), and HCT ($p < 0.0200$) compared to the control (Table 2), led by EE2 and EE3 and followed by EEI and EE4. No significant differences were observed in MCHC, MCH, LYM, and MON for all treatment groups in the current study. The water parameters of the aquaria during feeding trial were recorded as follows: temperature = 24–26°C, pH = 6.4 −7.2, dissolved oxygen = 5.8–6.3 ppm, and ammonia <0.1 ppm.

Table 2: Blood parameters of experimental fish fed torch ginger, *Etlingera elatior*, flower bud powder diets for 8 weeks.

Blood parameters	Control	EE1	EE2	EE3	EE4
WBC count/μL	112.1 ± 9.88^{a}	124.6 ± 12.86^{ab}	144.6 ± 4.59^{b}	148.6 ± 9.54^{b}	127.9 ± 7.47^{ab}
LYM (%)	82.3 ± 10.24	80.8 ± 8.61	80.7 ± 9.38	81.7 ± 9.46	81.3 ± 9.64
MON (%)	12.2 ± 1.21	12.3 ± 1.22	12.4 ± 1.42	12.5 ± 1.62	12.2 ± 1.37
RBC ($\times 10^3/\mu$L)	2.17 ± 0.21^{a}	2.43 ± 0.38^{a}	3.90 ± 0.53^{b}	4.03 ± 0.42^{b}	2.67 ± 0.15^{a}
HGB concentration (g/dL)	5.60 ± 0.44^{a}	6.27 ± 1.01^{ab}	7.87 ± 0.40^{b}	7.83 ± 0.76^{b}	6.30 ± 1.04^{ab}
HCT (%)	23.7 ± 2.91^{a}	27.7 ± 1.69^{ab}	38.7 ± 4.42^{c}	36.9 ± 4.07^{bc}	26.8 ± 4.63^{a}
MCH (pg)	29.7 ± 3.75	32.1 ± 3.55	30.7 ± 1.98	32.4 ± 4.09	30.7 ± 7.84
MCHC (g/dL)	23.9 ± 2.80	23.5 ± 2.76	22.7 ± 1.93	22.7 ± 2.04	22.9 ± 2.55

Note. Data expressed as mean $\pm$ standard deviation. EE1, 2, 3, and 4 = 1%, 2%, 3%, and 4% of torch ginger, *Etlingera elatior*, flower powder diets. Values in the same row with different letters showed significant difference at $p < 0.05$. WBC, white blood cell; LYM, lymphocytosis; MON, monocytes; RBC, red blood cell count; HGB, hemoglobin concentration; HCT, hematocrit; MCH, mean corpuscular hemoglobin; and MCHC, mean corpuscular hemoglobin concentration.

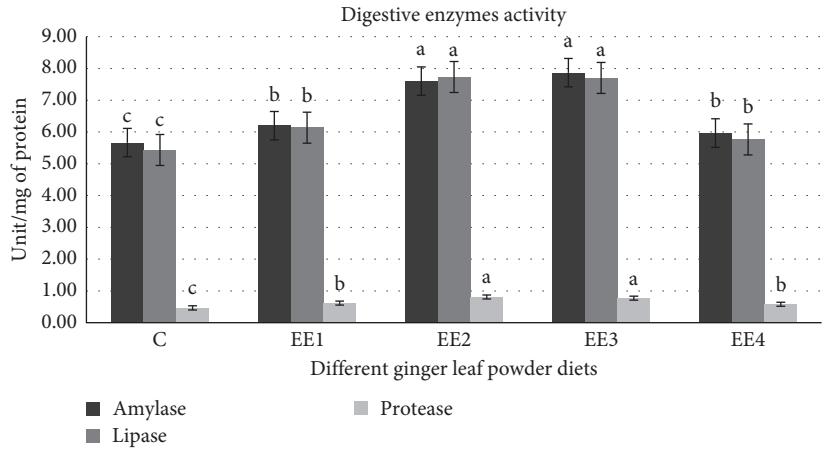

Figure 1: Comparative digestive enzymes activity of fish fed *Etlingera elatior* flower bud powder diets and control group for 8 weeks. Different letters on the bar are significantly different ($p < 0.05$).

Figure 1 illustrates the comparative digestive enzymes activities of EE-treated fish and the control group after 8 weeks. Overall, fish supplemented with dietary EE demonstrated significantly higher amylase (6.91 ± 0.83 unit/mg of protein; $p < 0.0090$), protease (0.70 ± 0.10 unit/mg of protein; $p < 0.0040$), and lipase (6.83 ± 0.89 unit/mg of protein; $p < 0.0060$) activities than the control group, particularly EE2 and EE3, followed by EE1 and EE4 (Figure 1). Antioxidative responses, including catalase (CAT; $p < 0.0100$), glutathione peroxidase (GPx; $p < 0.0300$), and superoxide dismutase (SOD; $p < 0.0100$), were significantly higher in EE-supplemented fish compared to the control group, with the highest being the EE2 and EE3, followed by fish fed EE1 and EE4 diet groups (Figure 2). Meanwhile, EE groups (EE1, 23.3% $\pm$ 5.77%; EE2, 66.7% $\pm$ 5.77%; EE3, 66.7% $\pm$ 5.77%; and EE4, 23.3% $\pm$ 5.77%) exhibited significantly ($p < 0.0001$) superior cumulative survival rates post-*E. tarda* infection compared to the control group (3.3% $\pm$ 5.77%; Figure 3). *Edwardsiella tarda* was successfully isolated from liver of all the dead fish.

4. Discussion

This study investigated the impacts of EE flower buds as a feed additive on African catfish's growth performance and health status by conducting a feeding trial, digestive enzyme activities analysis, hematological profiling, antioxidative responses, and disease resistance against *E. tarda* infection. Numerous studies have reported ginger as a feed supplement in aquatic animals. For instance, ginger supplementation enhanced the growth performance of Nile tilapia, *O. niloticus* [43]; black rockfish, *Sebastes schlegelii* [44]; common carp, *C. carpio* [21]; *L. rohita* [45]; and striped catfish, *P. hypothalmus* [25]. This study is the first to report dietary EE as a feed additive in aquaculture.

The study findings revealed increased FW, WG, and SGR in African catfish after the 8-week EE treatment, possibly related to the activation of digestive enzymes, such as amylase, lipase, and protease, which promote digestion and absorption of amino acids. As a result, feed utilization is enhanced, and the fish growth rate is improved [25, 46]. This finding was supported by the significantly lower FCR in dietary EE groups compared to the control. The Zingiberaceae family, including EE, possesses bioactive compounds such as terpene and zingiberene, contributing to their distinct odor and flavor and enhanced palatability [47]. Gingerol, in particular, improved EE feed palatability and efficiency in the fish intestine [25], which explains the higher growth performance in EE-treated African catfish compared to the control group.

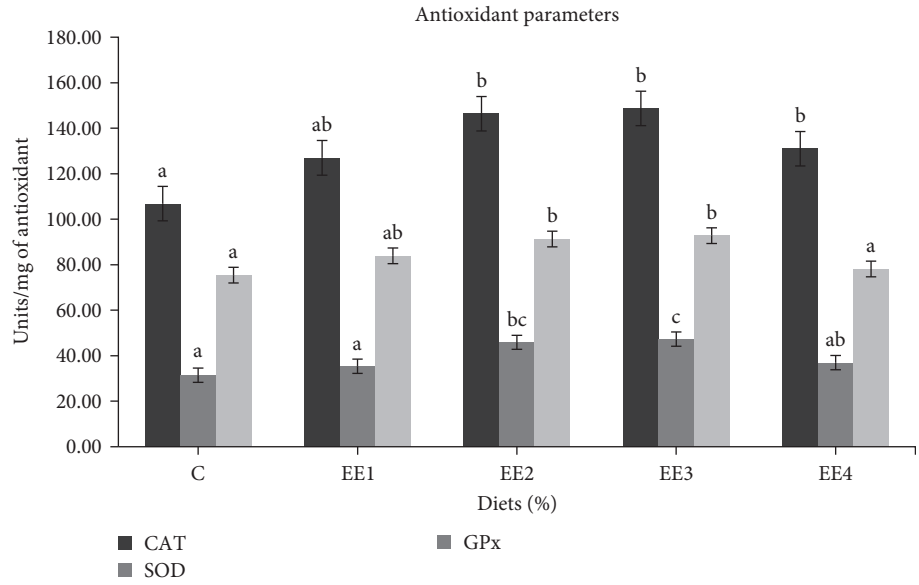

FIGURE 2: Comparative antioxidative response of fish fed *Etlingera elatior* flower bud powder diets and control group for 8 weeks. Different letters on the bar are significantly different ($p < 0.05$).

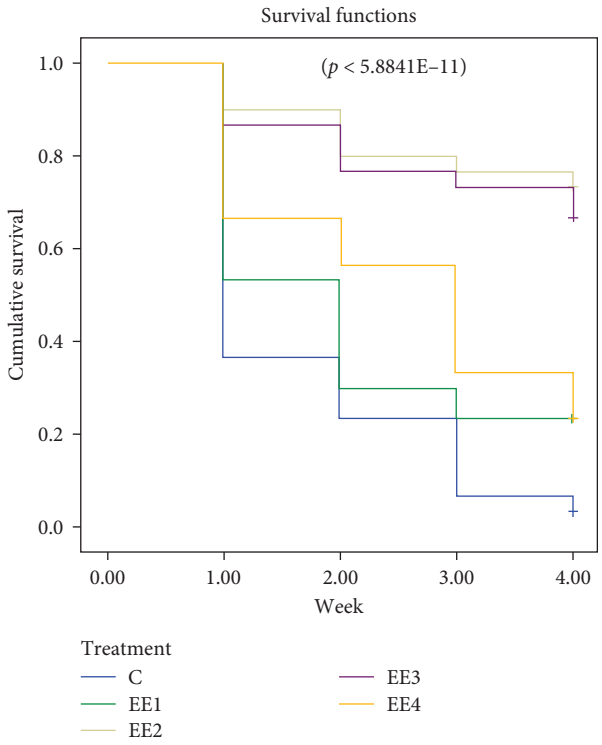

FIGURE 3: Kaplan–Meier cumulative survival plot of post-bacterial infection fish fed *Etlingera elatior* flower powder diets and control group for 8 weeks.

An earlier study found no significant difference in zebrafish growth performance after dietary ginger supplementation [48], possibly attributed to the different ginger parts used, species, dose, and feeding period. In addition, the highest EE dose in the current study reduced African catfish's growth performance, which could be attributed to the antilipogenic effect. Other studies have shown that excessive *Citrus* spp. extract as a feed additive resulted in a growth decline in gilthead seabream, *Sparus aurata* [49]; Nile tilapia, *O. niloticus* [24, 50]; and rainbow trout, *O. mykiss* [51]. In contrast, the lipogenic effect could benefit African catfish, as reflected by the low HSI and VSI in EE2 and EE3 treatment groups. Low HSI and VSI indicate less fat deposition in the fish liver and digestive system, which can be translated as the fish having more flesh. Flavonoid is the bioactive compound responsible for the antilipogenic effect, which is abundant in EE [50, 52, 53]. Therefore, the optimum dose of EE as a feed additive in African catfish is 2%–3%.

Dietary EE also enhanced the RBC count, HCT, and HBG of African catfish, indicating that EE catalyzes erythropoiesis and hemosynthesis activities in fish [54]. In addition, RBC count, HCT, and HBG enhancement are evidence of the absence of anemia and malnutrition in an aquatic species [48, 55, 56]. Furthermore, the good health status was confirmed by similarities in MCH and MCHC values among the experimental fish [56]. The EE diets also stimulated disease resistance against *E. tarda* infection, consistent with earlier studies that highlighted phytobiotics as a stress mitigation approach to prevent disease infection [18].

The antioxidative responses (SOD, CAT, and GPx) were significantly higher in fish fed with EE diets compared to the control group, indicating the role of EE in mitigating stress resulting from *E. tarda* infection in African catfish. The EE-treated groups exhibited a significantly higher cumulative survival rate post-*E. tarda* infection compared to the control group. Moreover, the findings were supported by the significantly higher WBC count readings in the blood samples of EE-treated groups than in the control group. An elevated WBC count is imperative in boosting the fish immune system against bacterial invasion [55]. Likewise, previous studies discovered that dietary ginger and its derivatives enhanced the

antioxidative capacity of striped catfish, *P. hypothalmus* [25]; *L. rohita* (Sukumaran et al., 2016); Nile tilapia, *O. niloticus* (Naliato et al., 2021); black rockfish, *S. schlegelii* [44]; and zebrafish, *Danio rerio* [48]. Furthermore, dietary ginger and its derivatives promoted the immunity of various fish species against other pathogens, including *A. hydrophila* (Naliato et al., 2021) and *Streptococcus inaie* [44]. In conclusion, dietary EE potentially enhances antioxidative responses and WBC count values in the fish blood to mitigate stress due to bacterial infection.

5. Conclusion

The EE flower bud diets improved the growth performance and health status of African catfish in this study. The optimum EE inclusion ranges from 2% to 3%, while excessive EE supplementation (4%) is detrimental for the African catfish. This study is the first to highlight the potential of EE as a feed additive in aquaculture, thus requiring further investigations to explore the benefits of EE on the growth and health of other aquatic species.

Data Availability

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

Ethical Approval

The experimental design has been registered and approved under Faculty of Agro Based Industry, Universiti Malaysia Kelantan animal care and use committee with the code UMK/FIAT/ACUE/PG/07/2023.

Conflicts of Interest

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.

Authors' Contributions

Seong Wei Lee and Muhammad Anamul Kabir contributed to conceptualization, methodology, and investigation. Zulhisyam Abdul Kari, M. N. Azra, and Wendy Wee contributed to resources, data curation, and visualization. All authors contributed to writing the original draft and supervision. All authors have read and agreed to the published version of the manuscript.

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