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Effects of dietary *Jatropha gossypifolia* leaves extract on growth performance, antioxidant capacity, immune response, and disease resistance of African sharptooth catfish (*Clarias gariepinus*) infected with *Aeromonas hydrophila*

O.V. Adeniyi^a, M.R. Adedayo^b, and A.I. Afe^c

^aDepartment of Fisheries and Aquaculture, Kwara State University, Malete, Nigeria; ^bDepartment of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria; ^cDepartment of Crop Production, Kwara State University, Malete, Nigeria

ABSTRACT

This study investigated the effects of *Jatropha gossypifolia* leaf extract (JGE) on the performance and immune response of African sharptooth catfish. Fish (6.43 ± 0.26 g, $n = 450$, 30/replicate) were fed diets supplemented with 0, 0.5, 1.0, 1.5, or 2.0 g JGE/kg in triplicate for 84 days. Thereafter, the fish were injected with *Aeromonas hydrophila* and observed for 14 days. Fish fed 1.0–1.5 g JGE had higher ($P < 0.05$) weight gain, protein efficiency ratio, and villi absorption area, with highest performance at 1.0 g JGE. All the fish fed JGE-supplemented diets had enhanced ($P < 0.05$) activities of glutathione-s-transferase and glutathione peroxidase, compared to fish fed control diet, while the variations in erythrocyte and leucocyte counts were insignificant ($P > 0.05$). The post-challenge fish fed JGE-supplemented diets had higher lysozyme, phagocytic, and respiratory burst activities and survival than the control treatment.

KEYWORDS

African catfish; *Jatropha gossypifolia*; fish growth; immune response; *Aeromonas hydrophila*

Introduction

Aquaculture is intensified to meet the increasing demand for aquatic products. The intensification of aquaculture has however resulted to occurrence of stressors that trigger disease outbreaks, which include high water temperatures, fish stocking densities, poor handling of fish, and an organically polluted culture environment, resulting in infectious diseases and economic losses, and increasing use of antimicrobials (Adeniyi et al. 2021; Pridgeon et al. 2012). In order to prevent large economic losses due to fish diseases, various antibiotics and other chemotherapeutic agents are used for prevention and treatment of diseases and to improve productivity in aquaculture with great results. However, the application of these synthetic drugs leads to accumulation of

residues in fish tissue and adverse impacts on the aquatic environment; including development of resistant pathogen strains which can be transferred from fish to humans resulting in immune suppression, destabilization of helpful gut bacterial populations in fish as well as environmental pollution (Gent et al. 2012; Hwang et al. 2020; Santos and Ramos 2018).

Residues arising from utilization of antibiotic and development of antibiotic resistant bacteria have been reported in farm fish including *Clarias gariepinus* in Nigeria (Adah et al. 2024; Olatoye and Basiru 2013), Uganda (Wamala et al. 2018) and Benin (Mensah et al. 2019). The aforementioned adverse effects of synthetic antibiotics prompted many countries such as China, Denmark, Finland, Mexico, South Africa, United States as well European Union countries to ban (Cogliani, Goossens, and Greko 2011; Levy 2014) or place restrictions (Da Silva et al. 2023; Van et al. 2020) on the use of antibiotic growth promoters and some specific antibiotics in the production of fish/animal meant for consumption. The Council of European Union (European Union 2019; Simjee and Ippolito 2022) and Food and Drug Administration (Madelyn 2020; US FDA United State of America Food and Drug Administration 2023) have been at the forefront of strict regulation of antimicrobial drugs in food farm animals.

A high percentage (up to 89%) of global fish production is utilized for human consumption (FAO Food and Agriculture Organization 2022); hence it is highly significant to ensure that good management practices are put in place for production of safe and healthy fish and its products. In recent decades, the global scientific communities have focused on the utilization of natural alternatives to synthetic drugs; including prebiotics, probiotics, organic acid and medicinal plants. The application of plant-based extracts as prophylactic or chemotherapeutic agents of various fish diseases has been considered a sustainable alternative for controlling the spread of infection by increasing the antioxidative status of fish through cell protection, fish immunity, and enhancing positive innate behavior of fish (Abdel-Tawwab et al. 2018; Hoseinifar et al. 2020; Reverter et al. 2014). Medicinal plants are natural with many benefits including been widely available, accessible, less expensive, highly biodegradable, easily produced and eco-friendly, compared to synthetic drugs (Hoseinifar et al. 2020; Olusola, Emikpe, and Olaifa 2013; Reverter et al. 2014). Earlier studies on the utilization of medicinal plants such as *Aloe vera* (Mahdavi, Hajimoradloo, and Ghorbani 2013), *Rehmannia glutinosa* (Wang et al. 2014), *Pyropia yezoensis* (Choi, Bong-Joo, and Taek-Jeong 2015), *Avena sativa* (Baba et al. 2016), *Ocimum gratissimum* (Abdel-Tawwab et al. 2018), *Artemisia annua* (Soares et al. 2020) and *Tamarindus indica* (Adeniyi et al. 2021) showed that the herbal alternatives have growth-promoting and/or immune-stimulating potential.

Jatropha gossypifolia L., is a widely distributed shrub in the family Euphorbiaceae, commonly called “bellyache bush/black physic nut” (Auvin-Guette et al. 1997; Parvathi et al. 2012). The plant contains useful phytochemicals including saponins, tannins, flavonoids, steroids, alkaloids,

jatrophenone, and lignans such as gossypifan and gossypiline (Adeniyi, Adedayo, and Afe 2021; Felix-Silva et al. 2014a, 2014b; Okiti and Osuntokun 2020; Ravindranath et al. 2003) which might be responsible for its diverse biological activities and its utilization as antihypertensive (Abreu et al. 2003), anesthetic, antimalarial (Dhale and Birari 2010), anti-diarrheal (Apu et al. 2013; Jansen et al. 2010), analgesic (Apu et al. 2012), antioxidative (Kharat, Dolui, and Das 2011, Nazeema 2012), immunomodulatory (Deo, Chaudhari, and Inam 2012), wound-healing (Felix-Silva et al. 2014a), anti-inflammatory (Felix-Silva et al. 2014b; Xavier-Santos et al. 2018) and antimicrobial (Adeniyi, Adedayo, and Afe 2021; Ogundare 2007; Okiti and Osuntokun 2020) agents, among others. African sharptooth catfish (*Clarias gariepinus*) is a significant commercial farmed fish species in many parts of Europe and Asia, and widely cultured intensively in African countries, including Nigeria, because it is a fast-growing species, well accepted food fish of high market value and high adaptation to diverse environmental conditions (Adeniyi et al. 2023; Strauch et al. 2018). However, this species is also prone to bacterial infections, especially infection caused by *Aeromonas hydrophila* considered to be a limiting factor in the production of the species (Adah et al. 2024; Adeniyi et al. 2023; Adeshina et al. 2019; Mulia et al. 2023). Therefore, the present study aimed to investigate the effects of dietary *J. gossypifolia* on the performance of *C. gariepinus* and its resistance to *A. hydrophila* infection.

Materials and methods

Plant extraction

The leaves of *J. gossypifolia* were harvested from herbal garden, rinsed with tap water, drained and air-dried in laboratory at 30–33°C with close sanitary measures for 14 days, and thereafter blended into uniform fine powder using a kitchen blender. A known weight of the plant material was dissolved in ethanol (96%) at a ratio of 1:10 (weight/volume; 50 g plant powder:500 mL ethanol) in a bottle and sealed; the mixture was left for 24 h on a mechanical orbital shaker (Dlab model SK-0330-pro, Dual life Science Private Limited, Surat, India) to agitate at the speed of 200 rpm (Adeniyi, Adedayo, and Afe 2021). The agitated mixture was thereafter filtered through No 1 Whatman paper (42 µm pore size); the filtrate (crude extract) was concentrated at 45°C using rotary evaporator (IKA® RV10 digital, Artisan Technology Group, Champaign, USA) to remove the solvent (Adeniyi, Adedayo, and Afe 2021; Zhang et al. 2014). The resulting concentrated *J. gossypifolia* extract (JGE, dry matter 20.01%) was kept at –20°C until use. The extraction yield of JGE was calculated to be $5.91 \pm 0.03\%$ using the following formula:

$$\text{Yield of extract (\%)} = 100 \times (\text{Dry weight of extract} / \text{Dry weight of original plant powder})$$

Preparation of experimental diets and fish culture technique

Five isonitrogenous ($\approx 40\%$) and isocaloric (≈ 4600 kcal/kg) diets were formulated and produced based on the nutritional composition of the main ingredients and to meet the nutrient requirement of the experimental fish (Table 1). All ingredients (excluding the test ingredient) were homogenized, steamed to produce 2 mm dried basal diet sinking pellets using ring die pelletizer (HKJ250, CAP'S Feed Ltd, China). The extract was added to the basal diet, by spraying, at 0.0 (control), 0.5, 1.0, 1.5 or 2.0 g JGE/kg, as presented in Table 1, in a completely randomized design fortnightly during the study. The diets were air-dried, labeled accordingly and stored in airtight bag in a dry place at room temperature during the experimental period.

The fish, *C. gariepinus* fingerlings, were obtained from a local farm and acclimatized under indoor condition for three weeks during which they were fed with

Table 1. Formulation (g/kg dry basis) and proximate composition (g/kg, as fed basis) of experimental diets containing *Jatropha gossypifolia* extract (JGE) fed to *Clarias gariepinus*.

Ingredients	JGE inclusion levels (g/kg diets)				
	0.0	0.5	1.0	1.5	2.0
Fish meal (72% CP) ^a	200.0	200.0	200.0	200.0	200.0
SBM (44% CP) ^b	260.0	260.0	260.0	260.0	260.0
GNC (41.5% CP)	300.0	300.0	300.0	300.0	300.0
Cornflour	208.0	207.5	207.0	206.5	206.0
Fish oil ^c	20.0	20.0	20.0	20.0	20.0
JGE	0.0	0.5	1.0	1.5	2.0
Dicalcium phosphate	2.5	2.5	2.5	2.5	2.5
Premix ^d	2.5	2.5	2.5	2.5	2.5
Methionine	1.0	1.0	1.0	1.0	1.0
Lysine	1.0	1.0	1.0	1.0	1.0
Common salt (NaCl)	5.0	5.0	5.0	5.0	5.0
Total	1000.0	1000	1000	1000	1000
Proximate composition					
Moisture	86.7 $\pm$ 1.7	86.9 $\pm$ 1.3	86.7 $\pm$ 1.7	86.8 $\pm$ 4.4	86.7 $\pm$ 4.4
Crude protein	400.3 $\pm$ 3.3	400.3 $\pm$ 3.3	400.2 $\pm$ 6.7	400.2 $\pm$ 6.7	400.2 $\pm$ 4.3
Crude fiber	46.1 $\pm$ 0.4	46.0 $\pm$ 0.3	46.1 $\pm$ 0.4	45.7 $\pm$ 0.6	46.0 $\pm$ 0.3
Ether extract	94.7 $\pm$ 0.2	95.0 $\pm$ 0.1	94.7 $\pm$ 0.1	94.8 $\pm$ 0.3	94.6 $\pm$ 0.3
Ash	100.0 $\pm$ 2.9	100.3 $\pm$ 1.7	100.5 $\pm$ 2.9	100.5 $\pm$ 2.9	100.5 $\pm$ 2.9
NFE ^e	358.9 $\pm$ 2.8	358.4 $\pm$ 2.8	358.5 $\pm$ 3.0	358.8 $\pm$ 3.1	358.7 $\pm$ 5.8
GE (kcal/kg) ^f	4631.7 $\pm$ 12.9	4632.5 $\pm$ 8.2	4629.5 $\pm$ 11.1	4631.7 $\pm$ 10.9	4629.4 $\pm$ 19.6

^aFF Skagen, Denmark.

^bSBM (soy bean meal), Gerawa Oil Mills, Nigeria.

^cSONAC, Netherlands.

^dVitamin-mineral premix (per kg premix): Vitamin A, 22000 IU; vitamin B1, 20 mg; vitamin B2, 25 mg; vitamin B6, 10 mg; vitamin B12, 0.05 mg; vitamin C, 300 mg; vitamin D3, 5,000 IU; vitamin E, 300 mg; vitamin K3, 10 mg; niacin, 120 mg; folic acid, 5 mg; biotin, 1 mg; inositol, 50 mg; calcium pantothenate, 60 mg; choline chloride, 500 mg; manganese, 30 mg; iron, 35 mg; zinc, 45 mg; copper, 3 mg; cobalt, 2 mg; iodine, 5 mg; selenium, 0.15 mg.

^eNFE, (nitrogen free extract) = 100 – (crude protein + crude fiber + ether extract + ash).

^fGE (gross energy) was calculated as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively (NRC. National Research Council 1993).

CP, crude protein; GNC, groundnut cake.

commercial floating feeds (Skretting, 1.8 mm, 52% crude protein, 15% crude fat and 1% crude fiber). The acclimated fish ($n = 450$) of relatively similar weights (6.43 ± 0.26 g) were randomly distributed into 15 plastic aquaria (500-L water capacity) in triplicates per treatment at 30 fingerlings per replicate using a static renewal system. Each aquarium was supplied with borehole water, which was completely renewed with well aerated water from a water storage tank at three day intervals to maintain good water quality. The fish were hand-fed the predesigned diets to apparent satiation thrice daily from 8:30 to 9:00, 13:30 to 14:00, and 17:00 to 17:30 h for 84 days. Fish not moving or responding to touch were identified as dead fish and were removed and recorded during the feeding trial.

Water samples were also taken biweekly from the aquaria to determine concentration of some water quality parameters. The dissolved oxygen (DO), water temperature and pH were measured using the dissolved oxygen meter, mercury-in-glass thermometer, and pH meter, while ammonia and nitrite were determined using commercial kits (LaMotte, USA). The DO, temperature and pH were found to fall within 4.9 to 5.5 mg/L of water, 25.6 to 27.7°C and 7.0 to 7.2, respectively while the concentration of ammonia and nitrite were below 0.2 ppm.

Fish performance and feed utilization

During the feeding trial the feed fed to the fish in each of the aquaria were weighed, recorded and the sum was expressed as the feed intake at the end of the feeding trial. At the beginning and end of the feeding trial fish in each tank were counted and batch-weighed. The weight gain (WG), specific growth rate (SGR), feed intake, protein efficiency ratio (PER), feed conversion ratio (FCR) were calculated from the data obtained as shown in [Table 2](#).

Proximate composition

The proximate composition of the experimental diets and the whole-body of samples ($n = 3$) of fish in each of the aquaria was analyzed (AOAC Association of Official Analytical Chemistry International 2005). Concisely, to estimate the moisture content samples were dried to constant weight at 105°C in an oven (Mini/50/SS, Genlab, England) and calculated from the loss in weight. The crude protein was determined using Kjeldahl system consisting of automated digester (8 Hole, Foss Tecator digester, Denmark) and Kjeltex auto distillation unit (Kjeltex 8200, Denmark), followed by titration and estimation of crude protein by multiplying the nitrogen content by 6.25. Crude lipid was determined in a soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA), using petroleum ether as extraction solvent. Crude fiber of diets was estimated from dried samples after digestion of fat-free samples using acid and base while the ash content of samples was determined by using an electric muffle furnace (Shangai Gwangdi Geological instrument

equipment Co. Ltd) at 550°C for 6 h for complete combustion. The gross energy in the experimental diets was calculated according to the NRC (National Research Council) (1993).

Gut morphometry and microflora

Six fish out of those sampled for blood analysis were randomly selected from each replicate and sacrificed on ice to remove the guts for morphometry and microflora analyses. For gut morphometry, three sections of the gut were prepared to determine the histomorphometry: the proximal region encompassed the region within 1 cm after the pyloric section of the stomach; the distal part was identified as the region within 1 cm before the anus, while the region in between proximal and distal regions was identified as the mid-gut (Anguiano et al. 2013). Three guts were prepared for histomorphological analysis following the descriptions of Awad et al. (2009) and Adeniyi et al. (2021). Briefly, the guts were fixed in 10% buffered formalin, sectioned to 5 µm thickness, stained with hematoxylin and eosin on slides, and examined using light microscopy. Three intact well oriented villi were selected for each cross section per slide. Villus height, villus width, and cryptal width of the proximal, mid and distal sections of the guts were measured using a binocular microscope with a micrometer rule and digital camera (Olympus Cx21, Tokyo, Japan). The absorption area was calculated from the product of villus height and width.

The other set of guts were prepared for microbial analysis under aseptic condition following the description of Ahmad et al. (2017). Briefly, 1 g of each of the gut samples were homogenized in 9 mL of sterile distilled water and 10-fold serial dilutions were made to 10^{-2} . One milliliter of 10^{-2} homogenate from each sample was plated on nutrient agar and MacConkey agar plates in triplicates using pour plate technique. The plates were incubated at 37°C for 48 h. After incubation, number of distinct bacterial colonies in each plate was observed and recorded. Colonies developed on the plate were counted and expressed as cfu/g. The isolates on nutrient agar plates were recorded as total heterotrophic count (THC) while those on MacConkey agar plates were recorded as total coliform count (TCC). The bacterial growths were identified based on their morphological characteristics, gram staining (Bello et al. 2014) and biochemical tests (Cheesbrough 2008; Izah, Kigigha, and Okowa 2016).

Fish hematological and antioxidant assays

After the feeding trial all fish were fasted for 24 h, after which eight fish were taken from each aquarium and anesthetized with sodium bicarbonate buffered tricaine methane sulfonate (MS222; 30 mg/L). Thereafter, blood samples were collected from fish through the caudal vein using a 2 mL plastic syringe. A set

of blood samples from three fish/aquarium were pooled and dispensed into ethylenediaminetetraacetic acid (EDTA) bottles for determination of hematocrits, hemoglobin concentration, red blood cell (RBC), erythrocyte sedimentation rate, white blood cell (WBC), lymphocyte, neutrophils, neutrophil-lymphocyte ratio (NLR), eosinophil, monocyte and platelet counts. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated (Al-Dohail, Hashim, and Paiko 2009) as shown in Table 5. Another set of blood samples from five fish/aquarium were pooled and dispensed into non-EDTA bottles, allowed to clot for 30 min and centrifuged (SE-CF-TDZ-WS, Labkits, U-Therm International (Hong Kong) Limited) at 4000 g for 15 min at room temperature to get sera samples. The sera were used to determine the antioxidative status of the experimental fish, including concentration of malondialdehyde (MDA) and reduced glutathione (GSH) and activities of glutathione-s-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD) with commercial assay kit using ELISA microplate reader (Diatech DR-ZOOPS, UK).

Fish challenge protocols

A preserved pathogenic *A. hydrophila* (ATCC 23,211) previously isolated and identified from a diseased fish was obtained and grown on nutrient broth and incubated at 35°C for 24 h and thereafter centrifuged at 4000 g for 15 min to obtain bacterial pellets; the pellets were dissolved and thoroughly mixed with physiological saline water to make 10 mL. Serial dilutions were made from the mixture to prepare several concentrations for determination of LD₅₀, after which a sub-lethal dose (Abdel-Latif et al. 2020) of 0.2 mL (1.0×10^6 cells/mL) was used for the challenge test. Thirty fish were randomly selected and pooled from each treatment, divided into three replicates; and each fish was aseptically injected with the same sub-lethal dose of the prepared inoculum by intraperitoneal injection (Abdel-Latif et al. 2020; Wang et al. 2014). The challenged fish were then transferred into culture tanks and observed for 14 days, using same culture system earlier described. Cumulative mortality (CM) and relative survival (RS) were calculated as presented in Figure 4.

Nonspecific immune responses

After the 14 days challenge test, blood samples were obtained from three fish in each tank for immune assays. The phagocytic activity was performed using the method of Yoshida and Kitao (1991) described by Srichaiyo et al. (2020). Concisely, 200 μ L of cell (2×10^6 cells/mL)

suspension in phosphate buffer saline (PBS) were placed on a coverslip in triplicate and incubated at 25°C for 2 h and then washed. A solution of 200 μL of fluorescence latex beads (2×10^7 of beads/mL, Sigma-Aldrich, USA) was put into each coverslip, re-incubated at 25°C for 1.5 h and rewashed, fixed with methanol and stained with Giemsa stain (Sigma-Aldrich, USA). The coverslips were cleaned with PBS (pH 7.4), dried at room temperature, and attached to slides to view microscopically. The number of phagocytic cells was counted (200 cells/coverslip) and expressed as the percentage of cell with phagocytic activity (cells containing microparticles of polystyrene inside the cytoplasm). Respiratory burst activity (RBA) was determined using nitro blue tetrazolium (NBT) reduction assay (Secombes 1990). In brief, 175 μL cells suspension in PBS containing 6×10^6 cells/mL was put inside the 96-well microplates in triplicates, after which 25 μL NBT at 1 mg/mL was added to each well and incubated at 25°C for 2 h. The supernatants were discarded, while cells were fixed with 125 μL of 100% methanol five minutes and later cleaned up twice with 125 μL of 70% methanol and allowed to dry. Solutions of 2 M potassium hydroxide (125 μL) and dimethyl sulfoxide (150 μL) were added and the plates were measured at 640 nm via microplate-reader (Diatech DR-ZOOPS, UK). The RBA was expressed as the NBT reduction of the sample. Serum lysozyme activity was determined using the turbidometric assay (Ellis 1990) based on lysis of *Micrococcus luteus* (Sigma, ATCC 4698). In brief, 25 μL each of the samples were mixed with 175 μL of *M. luteus* suspension at 0.2 mg/mL in PBS (pH 6.4) and 1% agarose gel in microplates. The mixtures were incubated at 25°C, and its OD was measured after 0.5 and 4.5 min at 540 nm using spectrophotometry (UV/VIS, Mettler Toledo, USA). One unit of lysozyme activity was defined as the amount of the enzyme producing a decrease in absorbance of 0.001/min.

Statistical analysis

Prior to analysis homogeneity of variances among different treatments was tested using Levene's test. All data were analyzed using one-way analysis of variance (ANOVA) to determine the effects dietary JGE on the parameters investigated, while Duncan's multiple range tests were used to compare differences among means and recorded as mean $\pm$ standard error (SE). Polynomial regression analysis was performed to determine the optimum inclusion level of JGE for weight gain of *C. gariepinus*. Post-challenge survival was assessed using Kaplan–Meier analysis. The level of significance was 0.05 in all analyses.

Results

Fish growth performance and body composition

The growth performance and nutrient utilization of *C. gariepinus* fingerlings fed with diets enriched with JGE is shown on Table 2. Fish fed diets enriched with 0.5 to 1.5 g JGE/kg diet had enhanced ($P < 0.05$) final weight and WG, compared to the fish fed control diet. The quadratic regression analysis with respect to the WG (Figure 1) of the fish showed 0.945 g JGE/kg diet ($Y = 46.589 + 14.185x - 7.503x^2$; $R^2 = 0.820$) as the optimum dietary inclusion level. The results also showed that the fish fed 1.0 g JGE had significantly higher SGR than the fish fed control and other JGE-enriched diets. The *C. gariepinus* fed the control diet had higher ($P < 0.05$) feed intake than those fed JGE-enriched diets, but in contrast, fish fed JGE-based diets had higher PER and lower FCR, compared to those fed the control diet. Higher ($P > 0.05$) fish survival was observed in 1.0 to 2.0 g JGE treatments, compared to the control and 0.5 g JGE treatments. Dietary supplementation with JGE did not affect ($P > 0.05$) the whole-body moisture content, crude protein, crude lipid and the ash content of *C. gariepinus* (Table 3).

Table 2. Growth performance and feed utilization of *Clarias gariepinus* fed diets containing *Jatropha gossypifolia* extract (JGE) for 84 days.

Parameters	Inclusion levels of JGE (g/kg diet)				
	0.0	0.5	1.0	1.5	2.0
Initial weight (g)	6.68 ± 0.16	6.24 ± 0.17	6.31 ± 0.20	6.53 ± 0.32	6.55 ± 0.34
Final weight (g)	53.17 ± 0.45 ^d	57.57 ± 1.00 ^b	62.60 ± 0.79 ^a	55.30 ± 0.55 ^c	52.27 ± 0.78 ^d
Weight gain (g)	46.49 ± 0.31 ^d	51.33 ± 1.41 ^b	56.29 ± 1.64 ^a	48.77 ± 1.59 ^c	45.72 ± 1.48 ^d
SGR (%/day)	2.49 ± 0.01 ^d	2.64 ± 0.00 ^b	2.72 ± 0.02 ^a	2.55 ± 0.03 ^c	2.51 ± 0.01 ^d
Feed intake (g)	70.24 ± 0.51 ^a	65.59 ± 2.07 ^b	70.21 ± 1.99 ^a	62.66 ± 1.41 ^b	63.85 ± 0.67 ^b
Feed conversion ratio	1.51 ± 0.00 ^a	1.28 ± 0.04 ^c	1.25 ± 0.01 ^{cd}	1.31 ± 0.05 ^c	1.40 ± 0.01 ^b
Protein efficiency ratio	1.65 ± 0.01 ^c	1.96 ± 0.04 ^a	2.00 ± 0.01 ^a	1.95 ± 0.05 ^a	1.79 ± 0.02 ^b
Survival (%)	90.00 ± 1.11 ^a	90.00 ± 1.92 ^a	95.56 ± 2.94 ^a	95.67 ± 3.02 ^a	92.22 ± 2.94 ^a

^{a,b,d,e}Data represented as mean ± SE of triplicate aquaria ($n = 3$). Different superscripts in the same row indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

Weight gain (WG, g) = final weight (FW) – initial weight (IW).

Specific growth rate (%/day) = $100 \times (\ln FW - \ln IW) / \text{time of feeding trial (days)}$.

Feed conversion ratio (FCR) = feed intake (FI)/WG.

Protein efficiency ratio = WG/protein intake.

(Protein intake = crude protein in diet × FI).

Fish survival (FS, %) = $100 \times (\text{number of survived fish} / \text{number of fish stocked})$.

Fish gut morphometry and microflora

The fish fed JGE diets showed significant variations ($P < 0.05$) in the gut morphometrics among the three segments of the gut; the highest villi height and width were obtained at the mid-gut section, compared to the proximal and distal sections (Table 4). Fish fed the control diet had lower ($P < 0.05$) villi height and width, compared to those fed JGE-based diets. Among the JGE

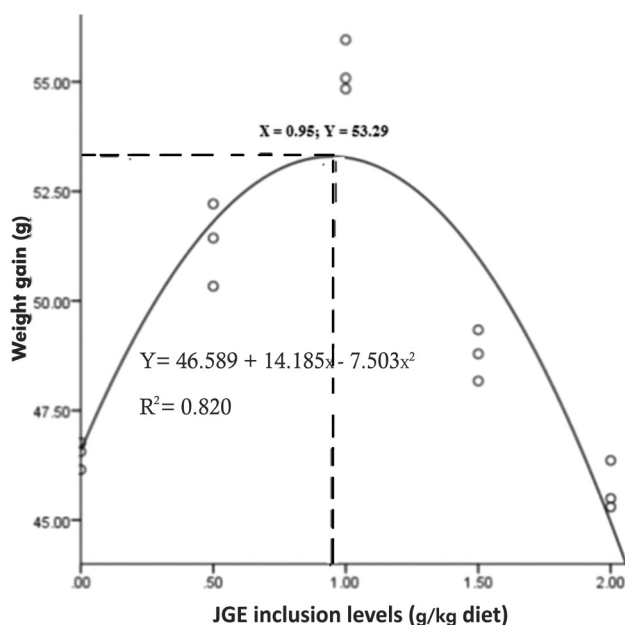


Figure 1. Quadratic regression analysis showing the relationship between dietary *Jatropha gossypifolia* extract (JGE) and weight gain of *Clarias gariepinus* in triplicate aquaria ($n = 3$).

Table 3. Whole-body composition (g/100 g wet weight) of *Clarias gariepinus* fed diets containing different levels of *Jatropha gossypifolia* extracts for 84 days.

Diets (g/kg diets)	Moisture	Crude protein	Crude lipid	Ash
0.0	74.58 ± 0.17	16.36 ± 0.03	5.69 ± 0.15	3.37 ± 0.07
0.5	75.04 ± 0.20	16.29 ± 0.07	5.30 ± 0.16	3.37 ± 0.07
1.0	74.72 ± 0.12	16.34 ± 0.04	5.45 ± 0.17	3.49 ± 0.10
1.5	74.92 ± 0.33	16.40 ± 0.03	5.44 ± 0.23	3.25 ± 0.13
2.0	74.89 ± 0.10	16.36 ± 0.04	5.15 ± 0.21	3.41 ± 0.14

Data represented as mean ± SE of triplicate aquaria ($n = 3$). Data in the same column without letters indicate insignificant differences among treatments according to the Duncan test ($P < 0.05$).

Table 4. Gut morphometrics (μm) of *Clarias gariepinus* fed diets containing different levels of *Jatropha gossypifolia* extracts (JGE) for 84 days.

JGE (g/kg diet)	Gut region	Villi height	Villi width	Crypt width
0.0	Proximal	1094.65 ± 29.48 ^d	313.58 ± 9.74 ^{b,c}	210.85 ± 4.80 ^c
0.5		1895.51 ± 36.49 ^c	282.27 ± 8.47 ^c	244.16 ± 8.89 ^b
1.0		1993.39 ± 4.56 ^b	395.18 ± 6.66 ^a	263.75 ± 8.75 ^a
1.5		2186.99 ± 39.71 ^a	323.84 ± 8.63 ^b	262.43 ± 8.89 ^a
2.0		2185.11 ± 43.80 ^a	243.08 ± 6.46 ^d	214.67 ± 6.04 ^c
0.0	Middle	2027.64 ± 10.27 ^c	327.17 ± 3.09 ^b	224.66 ± 2.33 ^{b,c}
0.5		2107.30 ± 9.28 ^c	330.84 ± 5.30 ^b	209.62 ± 9.29 ^c
1.0		2311.54 ± 6.31 ^a	400.53 ± 5.89 ^a	256.06 ± 12.47 ^a
1.5		2241.49 ± 8.35 ^b	327.87 ± 6.31 ^b	235.25 ± 6.50 ^{a,b}
2.0		2215.60 ± 15.07 ^b	320.16 ± 5.06 ^b	235.09 ± 5.04 ^{a,b}
0.0	Distal	1931.42 ± 8.69 ^a	344.73 ± 8.02 ^b	231.20 ± 4.74 ^{b,c}
0.5		1946.90 ± 18.64 ^a	295.56 ± 5.20 ^c	211.31 ± 4.68 ^{b,c}
1.0		1960.93 ± 28.01 ^a	388.59 ± 1.18 ^a	274.28 ± 7.99 ^a
1.5		1982.11 ± 19.16 ^a	307.25 ± 6.89 ^c	221.31 ± 6.71 ^{b,c}
2.0		2035.91 ± 35.32 ^a	282.90 ± 9.82 ^d	246.00 ± 2.89 ^b

^{a,b,c,d}Data represented as mean ± SE of triplicate aquaria ($n = 3$). Different superscripts in the same column indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

treatments, fish fed 1.0 g JGE/kg diet showed the highest villi height, villi width, crypt width, and area of absorption (Figure 2) at the mid-gut in the present study. Fish fed JGE-based diets had higher total gut heterophilic and coliform counts (Figure 3), compared to those fed the control diet. Morphological and biochemical characterization of the fish gut microflora showed presence of *Bacillus* sp., *Micrococcus* sp., and *Staphylococcus aureus*, *Pseudomonas* sp.

Hematological and antioxidant profiles

Fish fed 1.0 to 1.5 g JGE had significantly higher hematocrits and hemoglobin concentrations, compared to the value obtained from group of fish fed the control diet (Table 5). The *C. gariepinus* fed JGE diets seemed to have increase in MCH at 0.5 to 1.5 g levels; while the variations in MCV among the treatments were not significant. Furthermore, all experimental fish did not have significant variations in MCHC across the treatments. Fish fed JGE-enriched diets had higher ($P > 0.05$) WBC counts, while lower ($P < 0.05$) platelet counts were found in the control treatments. Fish fed JGE-based diets did not differ ($P > 0.05$) in RBC, lymphocyte and neutrophil counts, and the NLR among the treatments compared to the values obtained in the

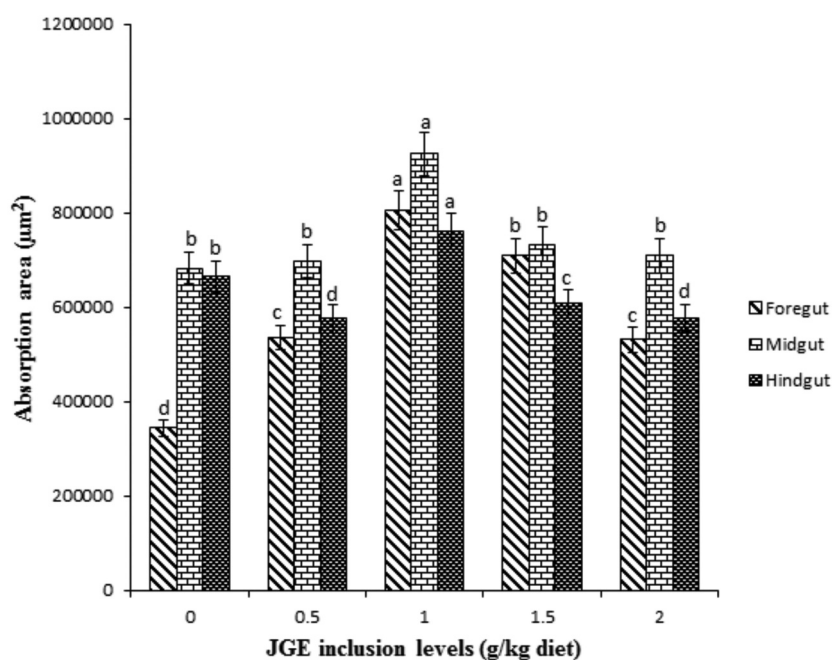


Figure 2. The villi absorption areas of *Clarias gariepinus* fed diets containing *Jatropha gossypifolia* extract for 84 days. Data represented as mean $\pm$ SE of triplicate aquaria ($n = 3$). Different letters on similar bars indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

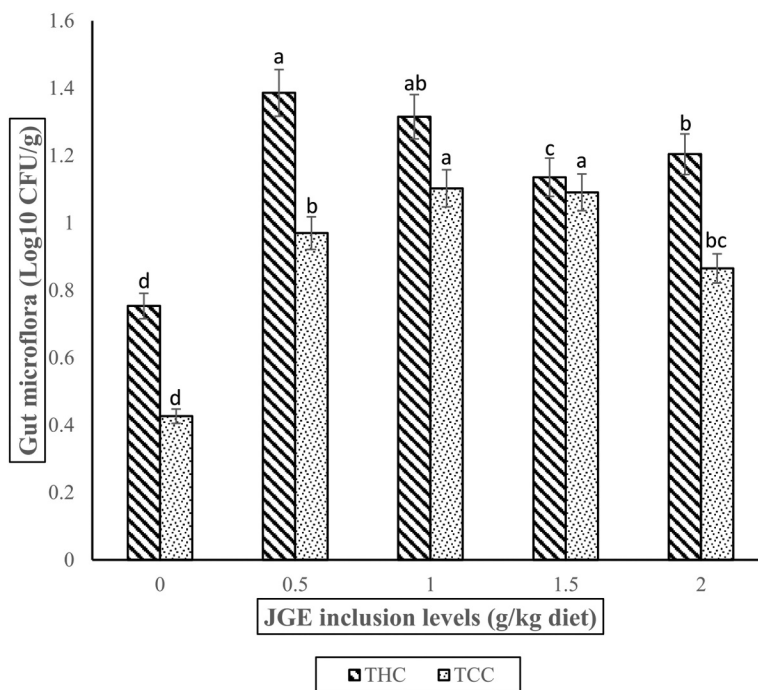


Figure 3. Total heterotrophic counts (THC) and total coliform counts (TCC) in *Clarias gariepinus* fed diets containing different levels of *Jatropha gossypifolia* extracts (JGE) for 84 days. Data represented as mean $\pm$ SE of triplicate aquaria ($n = 3$). Different letters on similar bars indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

Table 5. Hematological profile of *Clarias gariepinus* fed diets containing different levels of *Jatropha gossypifolia* extracts (JGE) for 84 days.

Parameters	Inclusion levels of JGE (g/kg diet)				
	0.0	0.5	1.0	1.5	2.0
Ht (%)	32.33 $\pm$ 0.67 ^d	34.00 $\pm$ 0.58 ^{c,d}	37.67 $\pm$ 0.67 ^{a,b}	38.00 $\pm$ 1.05 ^a	33.00 $\pm$ 0.58 ^{c,d}
Hg (g/dL)	10.75 $\pm$ 0.18 ^c	11.53 $\pm$ 0.12 ^b	12.90 $\pm$ 0.15 ^a	13.07 $\pm$ 0.19 ^a	10.83 $\pm$ 0.24 ^{b,c}
RBC ($\times 10^6/\mu\text{L}$)	3.28 $\pm$ 0.06	3.22 $\pm$ 0.03	3.31 $\pm$ 0.06	3.40 $\pm$ 0.07	3.36 $\pm$ 0.05
WBC ($\times 10^6/\mu\text{L}$)	16.80 $\pm$ 0.06 ^b	16.93 $\pm$ 0.19 ^b	17.02 $\pm$ 0.15 ^{a,b}	17.13 $\pm$ 0.12 ^{a,b}	17.18 $\pm$ 0.13 ^{a,b}
PLT ($\times 10^6/\mu\text{L}$)	114.33 $\pm$ 3.22 ^c	122.33 $\pm$ 2.02 ^{b,c}	132.75 $\pm$ 1.71 ^{a,b}	138.00 $\pm$ 1.08 ^a	135.67 $\pm$ 0.33 ^{a,b}
LYM (%)	64.33 $\pm$ 1.75 ^{a,b,c}	64.67 $\pm$ 0.33 ^{b,c}	62.00 $\pm$ 0.87 ^{b,c}	66.67 $\pm$ 1.45 ^{a,b}	62.33 $\pm$ 1.20 ^{b,c}
Neutrophils (%)	29.00 $\pm$ 1.21 ^{a,b}	28.67 $\pm$ 0.88 ^{a,b}	30.33 $\pm$ 1.03 ^a	26.00 $\pm$ 1.73 ^{b,c}	30.67 $\pm$ 1.45 ^a
Monocytes (%)	3.00 $\pm$ 0.58	3.00 $\pm$ 0.58	4.00 $\pm$ 0.58	4.00 $\pm$ 0.00	3.00 $\pm$ 0.58
Eosinophils (%)	3.33 $\pm$ 0.58	3.67 $\pm$ 0.33	3.33 $\pm$ 0.58	3.33 $\pm$ 0.33	4.00 $\pm$ 0.58
Basophils (%)	0.33 $\pm$ 0.33	0.00 $\pm$ 0.00	0.33 $\pm$ 0.33	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
MCHC (g/L) ^a	33.48 $\pm$ 0.75	33.24 $\pm$ 0.20	34.26 $\pm$ 0.21	34.52 $\pm$ 0.90	33.92 $\pm$ 0.66
MCH (pg) ^b	33.06 $\pm$ 1.02 ^d	34.10 $\pm$ 0.68 ^{b,c}	39.02 $\pm$ 0.38 ^{a,b}	38.54 $\pm$ 1.89 ^{a,b}	33.31 $\pm$ 0.88 ^{c,d}
MCV (fL) ^c	98.64 $\pm$ 2.03 ^b	102.58 $\pm$ 1.48 ^{a,b}	113.92 $\pm$ 0.51 ^{a,b}	112.22 $\pm$ 1.91 ^a	98.18 $\pm$ 0.81 ^b
NLR	0.43 $\pm$ 0.06 ^{a,b}	0.44 $\pm$ 0.02 ^{a,b}	0.49 $\pm$ 0.03 ^a	0.39 $\pm$ 0.01 ^{b,c}	0.49 $\pm$ 0.03 ^a
ESR	2.53 $\pm$ 0.01 ^{a,b}	2.52 $\pm$ 0.01 ^{a,b}	2.53 $\pm$ 0.02 ^{a,b}	2.57 $\pm$ 0.02 ^a	2.56 $\pm$ 0.02 ^a

^{a,b,c,d}Data represented as mean $\pm$ SE of triplicate aquaria ($n = 3$). Different superscripts in the same row indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

Ht, hematocrit; Hg, hemoglobin; RBC, red blood cells; WBC, white blood cells; PLT, platelets; LYM, lymphocytes; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; NLR, neutrophil- lymphocyte ratio; ESR, erythrocyte sedimentation rate.

^a = $100 \times (\text{hemoglobin/hematocrits})$.

^b = $10 \times (\text{hemoglobin/RBC count})$.

^c = $10 \times (\text{hematocrits/RBC count})$.

control treatment. Fish in all treatments did not have significant variations in the other WBC differential cells (monocyte, eosinophil and basophil). The fish exhibited higher ($P > 0.05$) ESR values as the JGE dosage increased, but the values obtained in all JGE treatments did not differ from the control treatment.

The sera antioxidant capacity of *C. gariepinus* fed diets containing different levels of JGE is presented in Table 6. Fish fed JGE-supplemented diets had lower ($P < 0.05$) concentration of MDA, compared to the fish fed the control diet. Conversely, fish fed 1.0 to 2.0 g JGE had higher ($P < 0.05$) activities of SOD and GST, compared to the other treatments. Fish fed diets supplemented with JGE from 0.5 to 2.0 g also showed higher ($P < 0.05$) activities of GPx with increasing inclusion levels while concentrations of GSH did not differ ($P > 0.05$) among the treatments.

Table 6. Serum antioxidant status of *Clarias gariepinus* fed diets containing different levels of *Jatropha gossypifolia* extracts for 84 days.

Diets (g/kg diets)	MDA ($\mu\text{mol}/\text{mg}$ protein)	GSH ($\mu\text{mol}/\text{L}$)	SOD (U/mg protein)	GPx (U/mg protein)	GST (U/mg protein)
0.0	0.84 ± 0.00^a	155.26 ± 0.62	240.88 ± 1.88^b	153.84 ± 4.03^e	$636.97 \pm 8.47^{d,e}$
0.5	0.54 ± 0.00^b	156.49 ± 2.62	243.09 ± 5.40^b	168.35 ± 1.46^d	650.93 ± 10.34^d
1.0	0.54 ± 0.01^b	162.20 ± 2.21	262.69 ± 5.52^a	185.72 ± 4.20^c	682.24 ± 8.01^c
1.5	0.53 ± 0.00^b	160.75 ± 2.63	263.06 ± 8.16^a	244.72 ± 4.37^b	739.64 ± 9.05^a
2.0	0.53 ± 0.00^b	157.50 ± 1.87	262.97 ± 5.84^a	266.90 ± 7.00^a	$723.30 \pm 6.53^{a,b}$

^{a,b,c,d,e}Data represented as mean $\pm$ SE of triplicate aquaria ($n = 3$). Different superscripts in the same column indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; GST, glutathione-s-transferase.

Fish challenge and immune response

The challenged fish fed JGE had lower cumulative mortality ($P < 0.05$) while the relative survival increased ($P < 0.05$) in the present study (Figure 4). Fish fed diets containing JGE had a significant post-challenge survival log-rank (Mantel–Cox) test ($P < 0.05$) as shown in Figure 5. Fish survival was enhanced with increase in the inclusion levels of JGE, compared with the survival in fish fed the control diet. Mortality started from day 2 in fish fed control diets and extended to day 10, while mortality commenced at the later days and ended earlier in JGE-treated groups (Figure 5); fish survival increased with rise in dietary inclusion of JGE. Fish fed JGE-supplemented diets had enhanced post-challenge respiratory burst, phagocytic and lysozyme activities ($P < 0.05$) than the fish fed control diet (Table 7). Among the JGE-administered treatments, fish fed 1.0 to 2.0 g JGE/kg diets had higher ($P < 0.05$) respiratory burst, phagocytic and lysozyme activities than the fish fed 0.5 g JGE/kg diet.

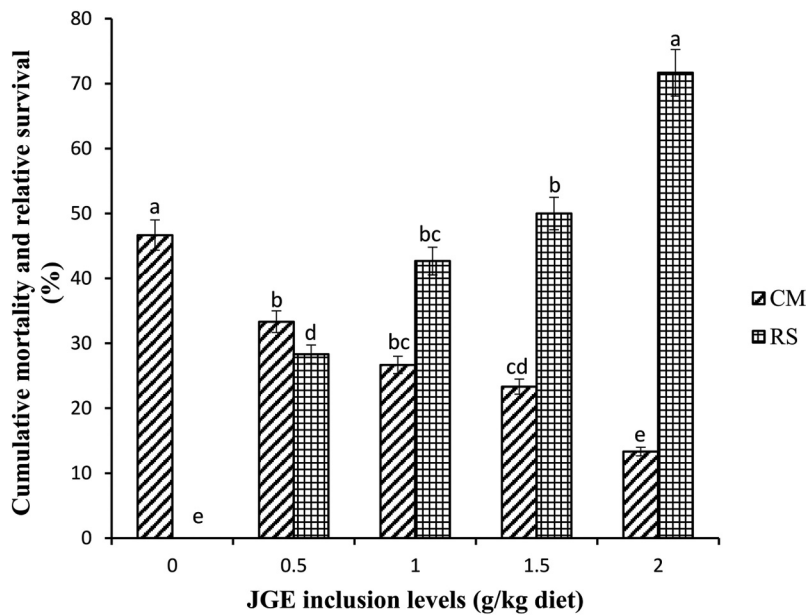


Figure 4. Cumulative mortality (CM) and relative survival (RS) of *Clarias gariepinus* fed diets containing different levels of *Jatropa gossypifolia* extracts (JGE) for 84 days and then challenged with *Aeromonas hydrophila* for 14 days. Data represented as mean $\pm$ SE of triplicate aquaria ($n = 3$). Different super-scripts on similar bars indicate significant differences among treatments according to the Duncan test ($P < 0.05$). $CM = Dt_1 + Dt_2 + Dt_3 + \dots + Dt_n$ (D is the number of dead fish at the given time (days), $t_1, t_2, t_3, \dots, t_n$). $RS = 1 - (\% \text{ mortality in treatment} / \% \text{ mortality in control}) \times 100$

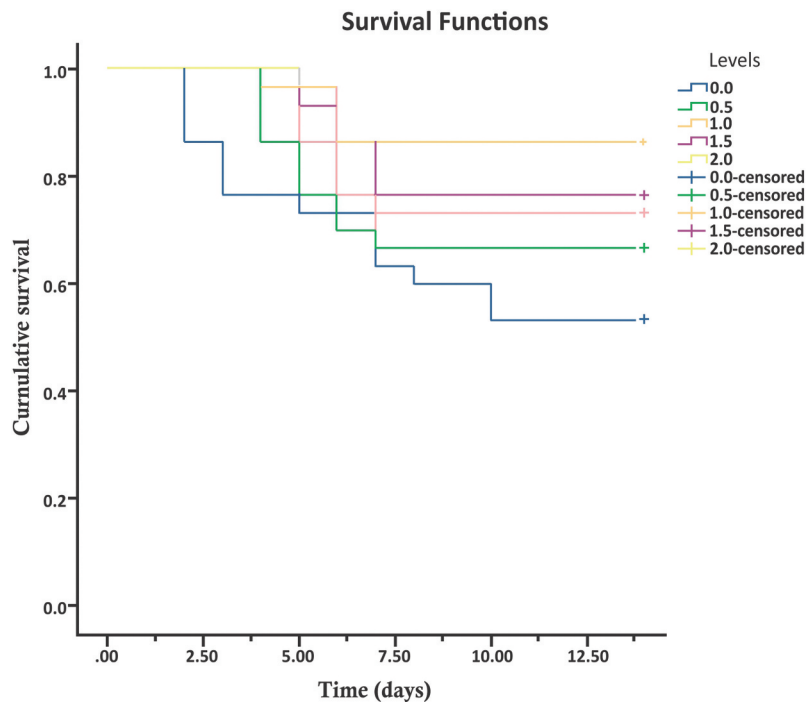


Figure 5. Post challenge Kaplan–Meier survival of *Clarias gariepinus* fed diets containing different levels of *Jatropa gossypifolia* extracts for 84 days and then experimentally infected with *Aeromonas hydrophila* for 14 days (means of triplicate, $n = 30$, $P < 0.05$ for Mantel–Cox test).

Table 7. Blood immune response of *Clarias gariepinus* infected with *Aeromonas hydrophila* and fed diets containing different levels of *Jatropha gossypifolia* extracts for 14 days.

Diets (g/kg diets)	Respiratory burst (OD)	Phagocytic activity (%)	Lysozyme activity (U/mg protein)
0.0	0.38 ± 0.00 ^c	21.58 ± 0.36 ^c	15.16 ± 0.21 ^d
0.5	0.51 ± 0.01 ^b	22.40 ± 0.29 ^b	17.05 ± 0.23 ^{b,c}
1.0	0.67 ± 0.03 ^a	24.77 ± 0.66 ^a	18.67 ± 0.19 ^{a,b}
1.5	0.61 ± 0.03 ^a	24.91 ± 0.37 ^a	18.45 ± 0.20 ^{a,b}
2.0	0.64 ± 0.02 ^a	24.34 ± 0.28 ^a	19.67 ± 0.63 ^a

^{a,b,c,d}Data represented as mean ± SE of triplicate aquaria ($n = 3$). Different superscripts in the same column indicate significant differences among treatments according to the Duncan test ($P < 0.05$).

Discussion

The results on the growth performance of *C. gariepinus* in the present study has demonstrated the growth-enhancing effects of dietary 0.5 to 1.5 g JGE/kg diet, with higher performance at 1.0 g inclusion level, while the regression analysis showed 0.945 g. The enhanced growth might be ascribed to the enhanced feed utilization efficiency as expressed in FCR and PER in the present study. Although there are paucity of information on the effects of JGE in fish culture to compare with but the enhanced growth observed in the present study is similar to many previous studies (Abdel-Tawwab et al. 2018; Adeniyi et al. 2021; Choi, Bong-Joo, and Taek-Jeong 2015; Huang et al. 2022; Mahdavi, Hajimoradloo, and Ghorbani 2013; Wang et al. 2014) which demonstrated growth-promoting effects of herbal feed additives in aquaculture. The significant decrease in growth performance observed in fish fed 2.0 g dose of JGE could be attributed to the presence of tannins reported in our previous study (Adeniyi, Adedayo, and Afe 2021) as well as gossypifan, and gossypiline (Felix-Silva et al. 2014a, 2014b; Okiti and Osuntokun, 2020) which are anti-nutritional factors in JGE. Therefore, caution must be taken to use appropriate dose (1.0 g) of JGE in practical fish culture. Similar trend of decreased growth performance at higher level of dietary herbal additives were observed in fish fed *Tribulus terrestris* extract (Yilmaz et al. 2014); *Aspilia mossambicensis* and *Azadirachta indica* leaves (Kapinga et al. 2018), tea leaf extract (Zhang et al. 2015), lotus leaf extract (Zhu et al. 2019) and tamarind leaf extract (Adeniyi et al. 2021).

The enhanced growth could also be attributed to the influence of JGE on the intestinal morphometry resulting to increased villi height and width with the resultant increased area of absorption, which might have contributed to higher absorption of digested nutrients and increase in the growth performance in *C. gariepinus* fed JGE-based diets in the present study. The positive correlation between the intestinal morphometry and growth performance observed in the current study coincides with earlier reports (Abdel-Latif et al. 2020; Adeniyi et al. 2021, 2023; Adeshina et al. 2019, 2021) on the beneficial roles of herbal supplements on the gut of fish species. The higher area of absorption of villi from the mid-gut could be an indication that higher digestion and absorption

of nutrients occurred in the mid-gut (Rønnestad and Conceic, 2005; Rønnestad et al. 2013). The increased number of microflora in the gut of the fish fed JGE-based diets in the present study could have enhanced digestion of nutrients and limited interaction of the fish with pathogenic bacteria. Gut microbes have been reported to play significant roles in the digestion of herbal products into absorbable active molecules and absorption of nutrients, which implies enhancement of physicochemical activity of the herbal medicine (Tarnecki et al. 2017; An et al. 2019). Fish gut contains enormous number of microflora, which are much higher than in the surrounding water as a result of favorable ecological niches for these microbes in the gut (Denev et al. 2009). The resident bacteria in fish gut epithelial surface serve as a first natural defense which restricts the attachment and colonization of pathogenic bacteria, maintain the structural and physiological integrity of the gut and enhance nutrient metabolism (Clark, Haas, and Sivamani 2017). Higher richness in gut microflora was also observed in fish fed diets enriched with plant extracts in previous studies (Souza et al. 2020; Ye et al. 2019).

No significant changes were observed in the whole-body proximate composition of *C. gariepinus* in the present study, implying that the nutritional quality of the fish is maintained. Similar to our observation in the current study, insignificant differences were observed in the proximate composition of fish fed herbal additives (Dada 2012; Erfan et al. 2015; Jeong, Cho, and Lee 2020). The insignificant differences in the moisture and ash contents of the fish also coincided with previous studies on fish fed herbal-supplemented diets; although the authors reported differences in crude protein or lipid contents of the fish (Mohammadiazarm et al. 2021; Tan et al. 2018; Zhu et al. 2019).

Hematological parameters are very significant and have been widely used to evaluate effects of diets and stressors on the physiological and health status of fish (Adeshina et al. 2019, 2021; Fagbenro, Adeparusi, and Jimoh 2013; Vazquez and Guerrero 2007). The increased values of hematocrit, hemoglobin, and platelets with dietary JGE in the current study were similar to earlier studies (Adeshina et al. 2019, 2021; Hoseini et al. 2018; Ngugi et al. 2015; Zemheri-Navruz, Acar, and Yilmaz 2019) and could imply that JGE enhanced tissue oxygenation and blood clotting. The insignificant variations in the red blood cells, MCH, MCHC, or white blood cells, compared to the control support the good health status of the fish and were in line with previous observations (Nhu et al., 2019; Rajabiesterabadi, Yousefi, and Hoseini 2020) on the dietary supplementation with herbal products in fish culture.

Oxidative stress is a serious imbalance between production of reactive oxygen species and antioxidant defenses when free radicals are produced faster than they are scavenged by the antioxidant system resulting to damage varieties of biomolecules (Halliwell 2007; Xing et al. 2012). Reduced glutathione (GSH) is a multifunctional, intracellular, low molecular mass thiol-containing tripeptide and non-enzymatic antioxidant considered to play significant roles

in redox homeostasis in conjunction with antioxidant enzymes such as SOD, GPx and GST (Adeshina et al. 2021; Baldissera et al. 2018; Kohen and Nyska 2002; Masella et al. 2005; Swaran 2009). The antioxidant enzymes and GSH reduce lipid peroxidation and protecting the fish from tissue damage and infectious diseases (Hoseinifar et al. 2021). The current study has shown that dietary JGE enhanced activities of SOD, GPx and GST, while MDA was reduced in *C. gariepinus*, which implies the protective effects of JGE in the fish from oxidative stress. There is paucity of information on the antioxidant activity of dietary JGE in fish, but literature (Kharat, Dolui, and Das 2011; Nazeema 2012) established antioxidant properties of JGE in the vitro studies. Similar significant roles of herbal products in enhancing antioxidant capacity and preventing oxidative stress in fishes were reported in several previous studies (Adeniyi et al. 2023; Adeshina et al. 2021; Almarri et al. 2023; Huang et al. 2022; Jiang et al. 2016; Baldissera et al. 2018; Paray et al. 2020; Tan et al. 2018; Vazirzadeh, Jalali, and Farhadi 2019; Zhu et al. 2019). The antioxidants activities of JGE in the present study might be related to its phytoconstituents, especially flavonoids (Kharat, Dolui, and Das 2011; Felix-Silva et al. 2014a, 2014b; Felix-Silva et al. 2018; Adeniyi, Adedayo, and Afe 2021), which is widely known for antioxidant capability of herbal products.

Aeromonas hydrophila is a widely spread pathogen in varieties of freshwater fishes, causing motile aeromonas septicemia, resulting to economic loss in aquaculture enterprise (Adeniyi et al. 2021; Noga 2012; Plumb and Hanson 2011). The higher level of protection against *A. hydrophila* infection demonstrated in *C. gariepinus* fed JGE-supplemented diets in the current study showed dose-dependent prophylactic and chemotherapeutic role of JGE. The enhanced survival levels of the experimentally infected *C. gariepinus* with the dietary levels of JGE coincided with the earlier reports on the utilization of herbal products against *Aeromonas* infection (Adeniyi et al. 2021; Adeshina et al. 2019; Baba et al. 2016; Ngugi et al. 2015; Nugroho et al. 2017). The increasing resistance of the fish against *A. hydrophila* infection observed at increasing levels of JGE could be an indication of the roles of the antioxidant enzymes in ameliorating the deleterious effects of ROS on the fish cell membrane, protecting the cells and stimulation of the immune system, which resulted in the higher protection against the pathogenic bacteria (Hoseinifar et al. 2021) observed in the current study.

The serum lysozyme activity increased considerably with increasing level of dietary JGE in *A. hydrophila*-infected *C. gariepinus* in the present study, which indicates the immuno-stimulating effects of JGE in the fish at varying levels of inclusion with the significant fish survival at 2.0 g JGE level, among JGE treatments. Lysozyme plays significant roles in fish nonspecific defense mechanism by activating the complement system and phagocytes as well as hydrolyzing the N-acetylmuramic acid and N-acetylglucosamine, which are important components of the peptidoglycan layer of bacterial cell wall (Abd-El

-Rhman 2009; Magnadottir 2006). The increase in the activity of lysozyme as well as the complementary roles of phagocytic and respiratory burst activities in the current study showed the immunostimulatory activity of JGE and could have contributed to the in vivo antibacterial activity against *A. hydrophila* as indicated with low mortality of challenged *C. gariepinus*. Many previous studies (Adeniyi et al. 2023; Adeshina et al. 2021; Almarri et al. 2023; Choi, Bong-Joo, and Taek-Jeong 2015; Masoud, Pourmoghimi, and Rohani 2018; Mohammadiarzam et al. 2021; Ngugi et al. 2015; Rajabiesterabadi, Yousefi, and Hoseini 2020; Soares et al. 2020; Sutthi et al. 2020) have shown that herbal-medicated diets enhanced the activities of lysozyme, respiratory burst or phagocytic activities in farmed fish, which have been associated with higher protection against *Aeromonas* and other pathogens. The role of herbal products as immunostimulants in the enhancement of immune system by stimulating activities of phagocytic cells, lysozyme and respiratory burst has been emphasized (Abarike et al. 2018, 2019; Hoseinifar et al. 2020).

Conclusion

In conclusion, the results of the present study have shown that dietary supplementation with 1.0 g *Jatropha gossypifolia* leaf extract/kg diet significantly enhanced growth performance, villi absorption area, antioxidation, lysozyme, phagocytic, and respiratory burst activities and protection against *Aeromonas hydrophila* infection in *Clarias gariepinus*. Its application at this level will enhance growth and health status in the fish; hence, 1.0 g *Jatropha gossypifolia* per kg diet is therefore recommended in the culture of the fish. Further study on the bioactive ingredient in *Jatropha gossypifolia* leaf is recommended.

Disclosure statement

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ORCID

O.V. Adeniyi  <http://orcid.org/0000-0002-0964-8300>

Authors' contributions

Adeniyi O.V. made the conceptualization, participated in the investigation and data collection, general supervision, and drafted the manuscript. Adedayo M.R. participated in the gut microflora analysis, while Afe A.I. participated in text ingredient preparation, data collection and analysis. All authors were involved in funding acquisition, editing and approval of the final manuscript.

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