



Research article

Dietary effect of *Xylopi aethiopica* supplementation on growth performance, hematology, serum biochemistry and tissue histology in African catfish hybrids (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂)

Wasiu Adeyemi Jimoh^{a,*}, Abimbola Kemisola Arise^b, Yusuf Olatunji Yusuf^a, Kabir Salman^a, Iretioluwa Oyiza Maliki^a, Aishat Abiola Aremu^a, Sekinat Omobonike Abdulrasaq^a, Luqman Alao Babatunde^a

^a Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State 240003, Nigeria

^b Department of Home Economics and Food Sciences, Faculty of Agriculture, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State 240003, Nigeria

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Abstract

Importance of the work: The incorporation of medicinal plants as natural feed additives is recognized increasingly as essential for improving productivity and profitability in catfish aquaculture.

Objectives: To evaluate the effects of dietary *Xylopi aethiopica* (Dunal) A. Rich. on growth performance, health indices, tissue histology and economic returns in hybrid catfish.

Materials and Methods: Hybrid catfish fingerlings with an initial mean weight of 15.95 ± 0.01 g were fed diets containing 0 to 2.0% *X. aethiopica* (XE) for 56 d and assessed for growth performance, hematological parameters, serum biochemistry, enzyme activities, histological alterations and profitability.

Results: Growth parameters differed significantly ($p < 0.05$), with 0.5% XE producing the highest final weight, weight gain, specific growth rate and feed efficiency. Conversely, 2.0% XE significantly ($p < 0.05$) reduced performance. Hematological indices (red and white blood cell counts, hemoglobin and packed cell volume) improved at 0.5% XE ($p < 0.05$). Based on serum biochemical markers, there were increased protein and globulin levels, reduced cortisol and glucose and enhanced hepatic and renal function at 0.5–1.0% XE. Plasma electrolytes remained stable at moderate XE inclusion but were significantly ($p < 0.05$) altered at 2.0% XE. Histological examination revealed minimal hepatic and renal changes at 0.5–1.0% XE, while 2.0% XE caused vacuolation and inflammation. Economic analysis indicated that 0.5% XE achieved the highest profit index and gross margin ($p < 0.05$).

Main finding: Moderate supplementation of *X. aethiopica* at 0.5–1.0% enhanced growth, health and profitability in hybrid catfish culture, while excessive inclusion at 2.0% had adverse effects.

* Corresponding author.

E-mail address: jimoh.wa@unilorin.edu.ng

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Introduction

Aquaculture is the fastest-growing sector in global food production, addressing the increasing demand for animal protein (Verdegem et al., 2023). Global aquaculture output now exceeds that of capture fisheries, with developing countries contributing a substantial share (Naylor et al., 2021). In Sub-Saharan Africa, aquaculture is recognized as a key strategy for improving food security, generating income and creating employment opportunities (Saidu, 2025). The African catfish [*Clarias gariepinus* (Burchell, 1822)] and its hybrids are among the most favored aquaculture species, attributed to their rapid growth, tolerance to challenging environmental conditions, ease of artificial propagation and strong consumer demand (Kwikiriza et al., 2025). Catfish hybrids, produced by crossing *C. gariepinus* with *Heterobranchius longifilis* Valenciennes, 1840, are especially valued in Nigeria and other West African nations for their accelerated growth, resilience and high market demand (Ataguba and Angela, 2023).

Although catfish aquaculture has achieved considerable progress, concerns persist regarding antimicrobial resistance, residue accumulation in fish tissue and associated public health risks (Okocha et al., 2018; Hossain et al., 2022). These challenges have prompted increased scrutiny of antibiotics and synthetic growth enhancers in aquafeeds previously used extensively to promote growth and disease resistance (Preena et al., 2020). Consequently, research has shifted toward identifying safe, natural and cost-effective feed additives that optimize feed utilization, support development, enhance health parameters and ensure economic sustainability in aquaculture systems (Madhulika et al., 2025). Phytogetic feed additives, derived from herbs, spices and plant extracts, have emerged as promising alternatives to antibiotics in fish nutrition due to their bioactive compounds (Firmino et al., 2021). These compounds have immunomodulatory, digestive, antioxidant and antibacterial activities (Caipang, 2020). Multiple studies have demonstrated that incorporating plant-based additives into the diets of aquaculture species, such as tilapia, carp and catfish, improves growth performance, hematological indices and serum biochemistry, while also reducing stress and disease incidence (Abdel-Tawwab et al., 2022; Sumana et al., 2025).

Xylopia aethiopica (Dunal) A. Rich (XE), commonly known as Ethiopian pepper or African guinea pepper, is a versatile plant indigenous to tropical Africa with a long history of use in traditional medicine and cuisine (Erhirhie and Moke, 2014). Phytochemical analyses have indicated

that its fruits contain alkaloids, flavonoids, tannins, saponins, terpenoids and essential oils, such as diterpenes and xylopic acid, all having therapeutic properties (Morakinyo et al., 2025). The pharmacological activities of XE include hepatoprotective, antibacterial, anti-inflammatory, hypolipidemic and antioxidant effects (Ogbuagu et al., 2020). These attributes support its potential as a natural growth stimulant and health promoter in aquaculture, further evidenced by its antibacterial efficacy as a prophylactic agent in aquaculture systems (Olusola et al., 2024). Therefore, XE may enhance physiological health, promote growth and mitigate disease risk in aquaculture. These attributes support its potential as a natural growth stimulant and health promoter in aquaculture.

Existing research on XE in aquaculture has predominantly examined growth and hematological parameters, often neglecting other important physiological aspects such as serum biochemistry (Ibrahim et al., 2024). This gap limits understanding of its practical relevance for commercial aquaculture. Additionally, the effects of XE on catfish hybrids remain underexplored. The possibility that hybrids have distinct physiological and metabolic responses compared to their parent species underscores the need for targeted research (Bartley et al., 2000). A thorough evaluation of XE as a phytogetic feed additive in hybrid catfish production requires an integrated assessment of growth, hematological, biochemical, enzymatic and economic parameters. Furthermore, no study has systematically assessed its efficacy in hybrid catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂). This gap highlights the need to examine optimal inclusion levels of XE that balance performance benefits with physiological safety and economic feasibility. Therefore, the current study was designed to investigate the effects of XE-supplemented diets on growth performance, hematological indices, serum biochemical parameters, activities and economic outcomes in African catfish hybrids. The study assessed both physiological and economic indicators, with the aim of providing comprehensive evidence regarding the efficacy of XE as a sustainable and natural feed supplement for catfish aquaculture.

Materials and Methods

Sources, processing of ingredients and experimental diets

X. aethiopica (XE) pods were obtained from a local spice market in Ilorin, Kwara State, Nigeria that had been sourced originally from North Central Nigeria during the

2023 harvest season. The XE pods were cleaned to remove debris and subsequently air-dried under shade at ambient laboratory temperature (27–30°C) for approximately 10 d until a constant weight was obtained before grinding. The dried XE pods were authenticated based on botanical characteristics by a plant taxonomist in the Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen was deposited in the departmental herbarium under voucher number UILH/001/1089/2026. The XE pods were dried and ground into a fine powder. Similarly, other feed ingredients, such as fish meal, soybean meal and fish oil, were sourced locally from Al Burock Feed Mill Odo Ire, Budo Esin Asa Dam Area, Ilorin, Nigeria (8°25'34"N, 4°30'59"E). They were properly processed and ground where necessary. Subsequently, all ingredients were passed through a 250 µm mesh sieve to obtain a uniform particle size before incorporation into

the experimental diets. The proximate composition of each feedstock was determined in triplicate according to Association of Official Analytical Chemists (2010) procedures. Using the results of these analyses, five isonitrogenous (40% crude protein), isolipidic (8% lipid) and isocaloric (18 MJ/kg) diets were formulated using a software-based tool developed by Network of Aquaculture Centers in Asia-Pacific (2008). The dietary mixtures contained XE powder at concentrations of 0% (control), 0.5% (0.5% XE), 1.0% (1.0% XE), 1.5% (1.5% XE) and 2.0% (2.0% XE), weight by weight, as shown in Table 1. The mixtures were moistened with hot water, pressed through a 2 mm mesh die to form pellets and sun-dried at 30–32°C for 72 hr. Once dried to a final moisture content below 10%, the diets were stored in Ziploc bags at 4°C. Representative samples from each dietary treatment were collected for proximate analysis to verify that the target protein and lipid levels had been achieved.

Table 1 Ingredients and chemical composition of *Xylopia aethiopica* (XE)-supplemented diets fed to hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchius longifilis* ♂)

Ingredient and Chemical composition	Dietary XE Supplementation				
	Control	0.5% XE	1% XE	1.5% XE	2% XE
Ingredient composition (%)					
Fishmeal@65% CP	15.30	15.30	15.30	15.30	15.30
SBM@42% CP	35.71	35.71	35.71	35.71	35.71
GNC@45% CP	31.11	31.11	31.11	31.11	31.11
Corn@10% CP	10.20	10.20	10.20	10.20	10.20
Negro pepper	0.00	0.50	1.00	1.50	2.00
*Fish premix	2.00	2.00	2.00	2.00	2.00
Fish oil	1.00	1.00	1.00	1.00	1.00
Soy oil	1.00	1.00	1.00	1.00	1.00
Starch	3.68	3.18	2.68	2.18	1.68
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Moisture	9.69	9.67	9.65	9.63	9.61
Crude protein	41.32	41.34	41.36	41.38	41.40
Lipid	6.83	6.84	6.85	6.86	6.87
Ash	6.64	6.65	6.67	6.69	6.70
Fiber	5.50	5.69	5.88	6.08	6.27
NFE	30.03	29.81	29.58	29.36	29.14
Total	100.00	100.00	100.00	100.00	100.00
Gross energy (MJ/kg)	18.71	18.63	18.56	18.48	18.41
Amino acid profile (%)					
Arginine	3.50	3.50	3.50	3.50	3.50
Histidine	1.02	1.02	1.02	1.02	1.02
Isoleucine	1.75	1.75	1.75	1.75	1.75
Leucine	3.06	3.06	3.06	3.06	3.06
Lysine	2.28	2.28	2.28	2.28	2.28
Methionine	0.67	0.67	0.67	0.67	0.67
Methionine + Cysteine	1.21	1.21	1.21	1.21	1.21
Phenylalanine	2.03	2.03	2.03	2.03	2.03
Phenylalanine + Tyrosine	3.58	3.58	3.58	3.58	3.58
Threonine	1.48	1.48	1.48	1.48	1.48
Tryptophan	0.49	0.49	0.49	0.49	0.49
Valine	2.07	2.07	2.07	2.07	2.07

Table 1 Continued

Ingredient and Chemical composition	Dietary XE Supplementation				
	Control	0.5% XE	1% XE	1.5% XE	2% XE
Fatty acid profile (%)					
LOA (18:2n-6)	1.91	1.91	1.91	1.91	1.91
LNA (18:3n-3)	0.19	0.19	0.19	0.19	0.19
ARA (20:4n-6)	0.03	0.03	0.03	0.03	0.03
EPA (20:5n-3)	0.14	0.14	0.14	0.14	0.14
DHA (22:6n-3)	0.31	0.31	0.31	0.31	0.31
Total n-3	0.64	0.64	0.64	0.64	0.64
Total n-6	1.94	1.94	1.94	1.94	1.94
n3:n6	0.33	0.33	0.33	0.33	0.33

SBM = Soybean Meal; GNC = Groundnut Cake; CP = Crude Protein; NFE = Nitrogen Free Extract; LOA = linoleic acid; LNA = linolenic acid; ARA = arachidonic acid; EPA = eicosapentanoic acid; DHA = docosahexanoic acid

*Each kilogram of HI-MIX fish premix contains vitamin A 1.600.00 international units (UI); vitamin D3 300.000 UI; vitamin E, 12.000mg; vitamin K3 1.200mg; vitamin B1 3.000 mg; vitamin B2 4.000 mg; vitamin B6 3.000 mg; vitamin B12 4 mg; niacin 14.000 mg; biotin 100 mg; calcium D-panthothenate 9.000 mg; folic acid 1.000 mg; vitamin C (protected) 30.000 mg; iron (iron (II) sulphate monohydrate) 4.00 mg; zinc 18.000 mg; copper (copper (II) sulphate pentahydrate) 1.000 mg; iodine (potassium iodide) 200 mg; manganese (manganese(II) oxide) 4.000 mg; selenium (sodium selenite) 40 mg; antioxidants; calcium carbonate.

Proximate, fatty acid and amino acid profiles of experimental diets calculated using Excel software (Excel; Microsoft Corp.; Redmond, WA, USA) developed by Network of Aquaculture Centers in Asia-Pacific (2008).

Biochemical analysis

Following the procedure of the Association of Official Analytical Chemists (2010), 3 g of each sample was heated to 105°C for 24 hr in a hot-air oven ((UN 75; Memmert GmbH + Co. KG; Büchenbach, Bavaria, Germany).) to determine the moisture content. The amount of ash in the sample was determined by burning the samples in a furnace (LMF-3550; OMEGA Engineering, Inc. Stamford, CT, USA) at 600 °C for 4 hr. Using a Kjeldahl protein auto-analyzer (FOSS Analytical A/S; Hillerød, Denmark), the crude protein in each sample was assessed following acid digestion. The nitrogen was multiplied by 6.25 to produce the crude protein content. The crude lipid content of each sample was measured using a Soxhlet extraction apparatus (Soxtec 2050; FOSS Analytical A/S; Hillerød, Denmark). The raw fiber was examined using a hot extraction fiber analyzer (FT 122 Fibertec™; FOSS Analytical A/S; Hillerød, Denmark). Trial data were analyzed in triplicate.

Phytochemical and fiber quantification

The tannin content was determined using the Association of Official Analytical Chemists (1984) method, with extracts mixed in 50% methanol, heated and analyzed spectrophotometrically at 760 nm. The alkaloid content was

measured according to Rivier (1981), involving alcohol digestion and nitrogen quantification, with conversion to total alkaloids using a factor of 3.26. Flavonoids were assessed according to Mabry et al. (1970), using ethanol extraction and measuring absorbance at 520 nm. The saponin content was determined spectrophotometrically, as described by Brunner (1984), using isobutyl alcohol and measuring the absorbance at 380 nm. Cardiac glycosides were identified based on chloroform extraction and subsequent chemical reactions, as indicated using absorbance at 510 nm. Steroids were quantified using the method of Wall et al. (1952), involving chloroform-methanol extraction and measurement of absorbance at 620 nm. Triterpenes were analyzed by dissolving the extracts in a chloroform-methanol mixture, followed by centrifugation and measurement of absorbance at 510 nm. The phenolic content was assessed by mixing the extracts with acetone and measuring the absorbance at 510 nm. Dietary fiber differentials were determined using the methods of Goering and Van Soest (1970). For each sample, hemicellulose was calculated as the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) and cellulose as the difference between acid detergent fiber (ADF) and acid detergent lignin (ADL), as shown in Table 2. All analyses were conducted in triplicate at the University of Ilorin Central Laboratory, Kwara State, Nigeria using standard solutions for calibration.

Table 2 Differential (Δ) phytochemical and fiber contributions relative to control diet following *Xylopia aethiopica* (XE) supplementation

	Dietary XE supplementation				
	Control	0.5% XE	1% XE	1.5% XE	2% XE
Phytochemical content (ppm)					
Steroid	0.00	0.02±0.01 ^a	0.03±0.01 ^a	0.030±0.01 ^a	0.04±0.01 ^a
Triterpenoids	0.00	0.04±0.00 ^d	0.08±0.00 ^c	0.13±0.01 ^b	0.17±0.01 ^a
Glycosides	0.00	0.03±0.01 ^c	0.07±0.01 ^b	0.10±0.01 ^a	0.12±0.01 ^a
Total tannin	0.00	0.10±0.00 ^d	0.20±0.01 ^c	0.31±0.01 ^b	0.41±0.00 ^a
Saponin	0.00	0.04±0.00 ^c	0.05±0.00 ^{bc}	0.08±0.01 ^b	0.11±0.01 ^a
Flavonoid	0.00	0.03±0.01 ^b	0.06±0.01 ^b	0.09±0.01 ^a	0.11±0.01 ^a
Total phenolics	0.00	0.68±0.01 ^d	1.34±0.00 ^c	2.02±0.01 ^b	2.68±0.01 ^a
Fiber (%)					
Hemicellulose	0.00	0.07±0.01 ^d	0.17±0.01 ^c	0.25±0.01 ^b	0.34±0.01 ^a
Cellulose	0.00	0.07±0.01 ^d	0.12±0.01 ^c	0.17±0.01 ^b	0.24±0.00 ^a
Acid detergent lignin	0.00	0.10±0.00 ^d	0.20±0.00 ^c	0.31±0.00 ^b	0.39±0.00 ^a
Acid detergent fiber	0.00	0.17±0.01 ^d	0.32±0.01 ^c	0.47±0.01 ^b	0.63±0.01 ^a
Neutral detergent fiber	0.00	0.25±0.01 ^d	0.50±0.00 ^c	0.72±0.01 ^b	0.97±0.00 ^a

ppm = parts per million.

Mean $\pm$ SD values (n = 3) within row with different lowercase superscripts differ significantly ($p < 0.05$).

Δ = difference relative to control diet (0.00 XE). Values represent net phytochemical and fiber contributions attributable to XE inclusion.

Culture conditions

The experiment took place in the wet laboratory of the Aquaculture and Fisheries Department at the University of Ilorin, Nigeria. *Heteroclinus* fingerlings, hybrids of African catfish (*C. gariepinus* ♀ $\times$ *H. bidorsalis* ♂), were sourced from Yesha Yahu Farms Nigeria Limited (8° 23' 22"N, 4° 28' 30"E), Ilorin, Nigeria and fed a standard 1.8 mm Skretting® catfish starter feed (Skretting Nigeria Limited; Ibadan, Oyo State, Nigeria). After acclimation to laboratory conditions for 15 d, 15 fish with an average weight of 15.95 $\pm$ 0.01 g were placed in each of the 15 replicate tanks. Subsequently, the tanks were randomly assigned to the five dietary treatments, with three replicate tanks per treatment. To prepare for the feeding trial, the fish underwent fasting for 24 hr to standardize digestive conditions, stimulate appetite and reduce stress during measurement and stocking. Each rectangular plastic tank (84 L capacity; 80 cm $\times$ 35 cm $\times$ 30 cm) was filled with 70 L of gently aerated water. Manual feedings to apparent satiation occurred twice daily at 0900 hours and 1700 hours each day for 65 d, with biweekly bulk weighing to allow for feed adjustments and ongoing growth monitoring. Throughout the trial, water temperature (27.26 $\pm$ 1.29°C), dissolved oxygen (6.55 $\pm$ 1.23 mg/L) and pH (6.57 $\pm$ 1.17) remained within optimal levels. Water quality parameters were monitored every second day using a portable multiparameter water quality meter (Hanna HI-9829 Multiparameter Portable Meter; Hanna Instrument Inc; Woonsocket, RI, USA).

Growth performance assessment

The growth response was assessed using the methodology described by Jobling (2016) as described in Equations 1–5, with all weights measured in grams:

$$\text{Weight gain} = \frac{[(\text{Final mean weight} - \text{Initial mean weight}) / \text{Initial mean weight}] \times 100}{1} \quad (1)$$

$$\text{Specific growth rate (\% d}^{-1}\text{)} = \frac{[\ln \text{ final mean weight} - \ln \text{ initial mean weight}] / \text{time of culture} \times 100}{2} \quad (2)$$

$$\text{Feed conversion ratio} = \text{Total feed fed (g)} / \text{Weight gain (g)} \quad (3)$$

$$\text{Protein efficiency ratio} = \text{Weight gain (g)} / \text{Total protein fed (g)} \quad (4)$$

$$\text{Survival (\%)} = \frac{[\text{Initial number of fish} - \text{Number of dead fish}] / \text{Initial number of fish} \times 100}{5} \quad (5)$$

Economic performance analysis

Economic performance indices were calculated to evaluate the cost-effectiveness of the experimental diets based on the feed utilization and biomass gain recorded during the feeding trial. The profit index and incidence of cost were derived directly from the experimental data obtained for feed intake, feed cost and fish weight gain. These indices were used to compare the relative economic efficiency of the dietary treatments

within the experimental conditions. Therefore, the economic evaluation reflected the cost-effectiveness of the feeds tested during the juvenile growth phase, rather than projections of commercial profitability at market size.

The production economics analysis utilized the indices defined in Equations 6–10, as described by Jimoh et al. (2019) and Jimoh et al. (2020), after obtaining the cost per kilogram of each of the dietary treatments as shown in Table 3. Notably, all costs were initially recorded in Nigerian naira but were converted to US dollars, where USD 1 = NGN 1369.97 at 12 May, 2026

$$\text{Incidence of Cost} = \text{Cost of Feed/Weight of Fish} \quad (6)$$

$$\text{Profit Index} = \text{Value of Fish/Cost of Feed} \quad (7)$$

$$\text{Profit/kg} = \text{Value of 1 kg fish} - \text{Incidence of cost} \quad (8)$$

$$\text{Value of fish produced} = \frac{\text{Final weight of fish in kg} \times \text{Market price of 1 kg fish}}{\text{Market price of 1 kg fish}} \quad (9)$$

$$\text{Cost of Feeding} = \frac{\text{Total feed fed} \times \text{Cost/kg of the experimental diet}}{\text{experimental diet}} \quad (10)$$

In use “Profit per kilogram = Value of 1 kg of fish – Incidence of cost”

Blood sampling for hematology and serum biochemistry

Following the feeding period, six fish were chosen randomly from each test tank for blood tests. The fish were humanely put to sleep using a 25 mg/L clove oil solution. For blood tests, 1 mL of blood was drawn from the caudal peduncle vein using a 2 mL heparinized syringe fitted with a 23-gauge

needle. The blood samples were transferred immediately into ethylenediaminetetraacetic acid anticoagulant tubes to prevent coagulation and kept under chilled conditions before laboratory analysis. A 5 Parts Hematology Analyser (DF52; Shenzhen Dymind Biotechnology Co., Ltd.; Shenzhen, Guangdong, China) was used to test the blood samples for red blood cell (RBC), white blood cell (WBC) and its differentials, hemoglobin concentration (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelets. For other tests, 2.5 mL of blood from fish on each dietary treatment were placed in plain bottles and left to clot during storage in the refrigerator at 4°C. After 30 min, the clotted blood samples were centrifuged at high speed (8,000 revolutions per minute) for 6 min using a Centrifuge 90-4 (Remi Elektrotechnik Limited; Mumbai, Maharashtra, India) to separate the serum for biochemical analysis. Blood minerals (sodium, potassium, calcium, magnesium) were measured using a flame photometer (PFP7; Jenway, a Cole-Parmer Company; Stone, Staffordshire, UK). Serum cortisol and glucose, as blood stress biomarkers, were determined using commercially available diagnostic kits from Randox Laboratories Ltd. (Crumlin; UK). Cortisol levels were measured using an enzyme-linked immunosorbent assay and glucose concentrations were determined using a colorimetric enzymatic method, following the manufacturer’s protocols. All tests were carried out at the Tanke Ilorin Central Laboratory, Ilorin, Nigeria (8° 28’54.1”N, 4° 36’25.5” E). Blood performance was calculated using Equation 9 (Esmaeili, 2021):

$$\text{Blood Performance} = \text{Ln}(\text{Hgb, g dL}^{-1}) + \text{Ln}(\text{RBC} \cdot 10^6 \mu\text{L}^{-1}) + \text{Ln}(\text{Hct, (\%)}) + \text{Ln}(\text{WBC} \cdot 10^3 \text{uL}^{-1}) + \text{Ln}(\text{Total p, mg dL}^{-1}) \quad (9)$$

Table 3 Cost per kilogram of experimental diets containing *Xylopia aethiopica* (XE) fed to hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchius longifilis* ♂)

Ingredient	Cost (USD /kg)	Dietary XE supplementation				
		Control	0.5% XE	1% XE	1.5% XE	2% XE
Fishmeal@65% CP	2.19	0.34	0.34	0.34	0.34	0.34
SBM@42% CP	0.47	0.17	0.17	0.17	0.17	0.17
GNC@45% CP	0.28	0.09	0.09	0.09	0.09	0.09
Corn@10% CP	0.40	0.04	0.04	0.04	0.04	0.04
Negro pepper	2.19	0.00	0.00	0.01	0.01	0.02
Fish premix	2.92	0.06	0.06	0.06	0.06	0.06
Fish oil	1.75	0.02	0.02	0.02	0.02	0.02
Soy oil	1.24	0.01	0.01	0.01	0.01	0.01
Starch	0.73	0.03	0.02	0.02	0.02	0.01
Total		0.74	0.75	0.75	0.75	0.75

SBM = Soybean Meal; GNC = Groundnut Cake; CP = Crude Protein. Values originally in Nigerian naira (NGN) currency converted to US dollars, where USD 1 = NGN 1369.97 at 12 May, 2026

where HgB = Hemoglobin content (measured in grams per deciliter), RBC = Red Blood Cell (measured in microliters), HcT = Hematocrit (measured as a percentage), WBC = White Blood Cell (measured in microliters) and Total p = Total Protein (measured in milligrams per deciliter).

Histological examination

After the feeding trial, three fish were randomly selected from each treatment replicate tank for histological examination. This sample size was consistent with common practice in fish nutrition and histopathological studies, where representative specimens are used to evaluate tissue architecture and detect potential pathological alterations (Vastos, 2021). The fish were humanely euthanized using clove oil (25 mg L⁻¹). The liver and kidney tissues were dissected carefully and trimmed into small pieces (approximately 3–5 mm thickness) to facilitate proper fixation. The tissues were fixed in 10% neutral-buffered formalin (pH $\approx$ 7.0) at a fixative-to-tissue ratio of at least 10:1 for 72 hr. Following fixation, the tissues were dehydrated using a graded ethanol series (50%, 70%, 90%, 100%), cleared in xylene and infiltrated with molten paraffin wax at 58–60°C before embedding in paraffin blocks. The paraffin-embedded tissues were sectioned at 5 μ m thickness using a rotary microtome and the sections were mounted on adhesive glass slides and dried in an oven at 58–60°C for 30–60 min to ensure proper adhesion. For staining, the sections were deparaffinized in xylene and rehydrated through a descending ethanol series (100%, 95%, 70%) to distilled water. The tissues were stained with Harris hematoxylin, differentiated in acid alcohol and rinsed in running tap water to achieve bluing. Then, each section was counterstained with eosin Y, dehydrated through ascending grades of ethanol, cleared in xylene and mounted using DPX mounting medium (Thermo Fisher Scientific Inc.; Waltham, MA, USA). The stained slides were examined under a light microscope at $\times$ 400 magnification for kidney sections and $\times$ 100 magnification for liver sections. Histological interpretation and evaluation were conducted at the Histology Unit of the Veterinary Teaching Hospital, University of Ilorin, Nigeria.

Statistical analysis

Prior to statistical analysis, data were assessed for homogeneity of variance and normality using the Levene test and the Shapiro-Wilk test, respectively. Growth, hematology,

antioxidants, oxidative stress biomarkers and plasma electrolytes were analyzed using one-way analysis of variance. When significant differences ($p < 0.05$) were detected among treatments, Tukey's b post hoc test was applied. Analyses were conducted using the SPSS software (version 17.0; SPSS Inc.; Chicago, IL, USA). Results were presented as mean $\pm$ SD. Statistical significance was set at $p < 0.05$.

Ethical statement

Throughout the study, there was strict adherence to the ethical guidelines for animal research outlined in the University of Ilorin's research policy on the use and care of animals. The experimental protocol and procedures received approval from the Departmental Ethical Review Committee (DERC), Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, Nigeria (protocol ID: DERC/AQF/0029; DERC approval number: DERC/AQF/2024/1229).

Results

Phytochemical and fiber composition of the experimental diets

There was a gradual increase in the phytochemical and fiber contents of the experimental diets with increased inclusion of XE (Table 2). The total phenolics recorded the most pronounced rise relative to the control diet.

Growth performance of fish fed X. aethiopica-supplemented diets

There were no significant differences in initial mean body weights among treatments. There were significant differences among treatments all growth performance indicators: final mean weight, mean weight gain, percentage weight gain (PWG) and specific growth rate (SGR), as shown in Table 4. The XE diets were dose-dependent: Specifically, the fish fed the diets containing 0.5% and 1% XE had significantly higher growth performance parameters than the control group, while there was no significant difference between the 0.5% and 1% XE treatments. However, a further increase in the XE concentration (1.5%) reduced the growth to a level comparable to the control, while 2% produced the lowest value, which was significantly lower than for the other treatments.

Table 4 Growth and economic performance of hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchus longifilis* ♂) fed *Xylopi* *aethiopica*-supplemented diets.

	Dietary XE Supplementation				
	Control	0.5% XE	1% XE	1.5% XE	2% XE
Growth performance					
Initial mean weight (g)	15.93±0.03 ^a	15.94±0.01 ^a	15.98±0.02 ^a	15.98±0.02 ^a	15.91±0.02 ^a
Final mean weight (g)	48.78±1.34 ^b	53.55±0.39 ^a	52.04±0.24 ^a	48.11±0.26 ^b	36.64±0.83 ^c
Mean weight gain (g)	32.84±132 ^b	37.61±0.38 ^a	36.07±0.24 ^a	32.14±0.26 ^b	20.74±0.82 ^c
PWG (%)	206.12±8.15 ^b	235.94±2.37 ^a	225.74±1.64 ^a	201.14±1.63 ^b	130.33±5.09 ^c
SGR (%/day)	2.00±0.05 ^b	2.16±0.01 ^a	2.11±0.01 ^a	1.97±0.01 ^b	1.49±0.04 ^c
FCR	1.70±0.02 ^{ab}	1.50±0.06 ^c	1.59±0.05 ^{bc}	1.69±0.01 ^{ab}	1.81±0.02 ^a
PER	1.40±0.00 ^{bc}	1.60±0.06 ^a	1.50±0.06 ^{ab}	1.40±0.01 ^{bc}	1.30±0.01 ^c
Survival (%)	84.44±2.22 ^b	93.33±0.00 ^a	86.67±0.00 ^b	86.67±0.00 ^b	86.67±0.00 ^b
Economic Performance					
VoFP (USD × 10 ⁻²)	8.90±0.25 ^b	9.77±0.07 ^a	9.50±0.04 ^a	8.78±0.05 ^b	6.69±0.15 ^c
CoF (USD × 10 ⁻²)	4.16±0.22 ^a	4.23±0.18 ^a	4.35±0.15 ^a	4.16±0.02 ^a	2.90±0.15 ^b
IoC (USD)	1.24±0.01 ^{ab}	1.09±0.05 ^c	1.16±0.03 ^{bc}	1.23±0.01 ^{ab}	1.32±0.02 ^a
Profit index	2.10±0.06 ^a	2.27±0.09 ^a	2.17±0.07 ^a	2.07±0.03 ^a	2.23±0.07 ^a
Gross margin (USD)	0.58±0.01 ^{bc}	0.73±0.05 ^a	0.67±0.03 ^{ab}	0.59±0.01 ^{bc}	0.507±0.02 ^c

WG: Percentage Weight Gain; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio; PER: Protein Efficiency Ratio; VoFP: Value of fish produced; CoF: Cost of feeding; IoC: Incidence of cost, Market value of 1kg Fish = ₦2550 where USD 1 = NGN 1369.97 at 12 May, 2026. Mean ± SD values within row with different lowercase superscripts differ significantly ($p < 0.05$).

There was a comparable pattern among the feed utilization indices, where the 0.5% XE diet produced the lowest FCR (1.50 ± 0.06), which was significantly better than the control. The FCR of the 1% dietary group and the control-fed group were not significantly different. Fish fed the 2% XE diet had the highest FCR. The PER peaked at 0.5% XE, significantly exceeding both the control and 2% XE diets. The survival rate was significantly higher in the 0.5% XE group than in the control, whereas the survival rates of the other dietary groups did not differ significantly from the control.

Dietary inclusion of 0.5% XE resulted in optimal growth performance, feed utilization, survival and economic returns. In contrast, higher inclusion levels of 2% or more adversely affected both growth and profitability.

A second-order polynomial regression (Fig. 1) was used to examine the relationship between weight gain and dietary supplementation level of the XE in hybrid African catfish. The fitted equation ($y = -9.9196x^2 + 13.902x + 32.857$; coefficient of determination (R^2) = 0.9628) indicated that maximum weight gain was achieved at approximately 0.7% inclusion. Beyond this point, growth performance declined, demonstrating a clear dose-dependent effect with an optimal inclusion level below 0.7%.

Hematological responses

There were significant differences RBC counts, Hb, PCV, WBC counts and differential leukocyte counts across treatments (Table 5). RBC, Hb and PCV showed the same trends, with values that were highest in fish fed 0.5% XE, significantly exceeding both the control and 2% XE, but they were not significantly different from the 1% and 1.5% groups.

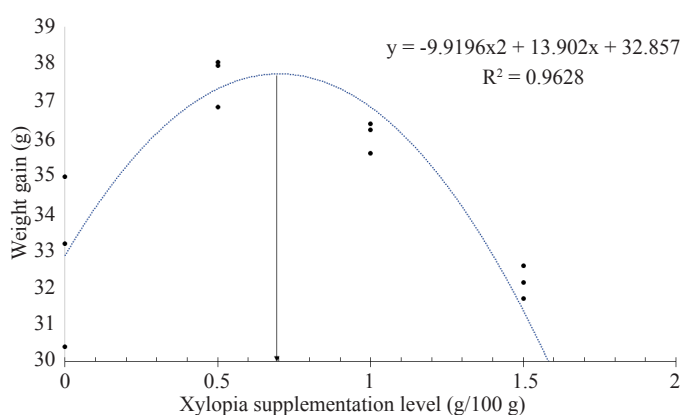


Fig. 1 Second-order polynomial relationship between weight gain and dietary *Xylopi aethiopica* supplementation level in hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂), where R^2 = coefficient of determination

Table 5 Hematological profile of African catfish hybrid (*Clarias gariepinus* ♀ × *Heterobranchus longifilis* ♂) fed *Xylopi aethiopica* (XE)- supplemented diet.

Parameter	Dietary XE Supplementation				
	Control	0.5% XE	1% XE	1.5% XE	2% XE
Hematological profile					
RBC ($\times 10^{12}/L$)	5.74 $\pm$ 0.25 ^b	6.53 $\pm$ 0.01 ^a	6.08 $\pm$ 0.01 ^{ab}	5.91 $\pm$ 0.01 ^{ab}	4.69 $\pm$ 0.30 ^c
Hb (g/dl)	11.47 $\pm$ 0.50 ^b	13.15 $\pm$ 0.01 ^a	12.20 $\pm$ 0.00 ^{ab}	11.82 $\pm$ 0.01 ^{ab}	9.29 $\pm$ 0.60 ^c
PCV (%)	34.40 $\pm$ 1.51 ^b	39.36 $\pm$ 0.03 ^a	36.53 $\pm$ 0.03 ^{ab}	35.50 $\pm$ 0.00 ^{ab}	27.93 $\pm$ 1.82 ^c
MCV (fl)	59.95 $\pm$ 0.04 ^c	60.25 $\pm$ 0.01 ^a	60.17 $\pm$ 0.00 ^b	60.16 $\pm$ 0.01 ^b	59.75 $\pm$ 0.02 ^d
MCH (pg)	19.75 $\pm$ 0.0 ^d	20.10 $\pm$ 0.01 ^a	20.05 $\pm$ 0.00 ^b	20.01 $\pm$ 0.01 ^c	19.86 $\pm$ 0.01 ^e
MCHC (g/dl)	33.24 $\pm$ 0.07 ^a	33.72 $\pm$ 0.19 ^a	33.62 $\pm$ 0.17 ^a	33.5455 $\pm$ 0.14 ^a	33.24 $\pm$ 0.01 ^a
WBC ($\times 10^9/L$)	1.90 $\pm$ 0.09 ^b	2.97 $\pm$ 0.14 ^a	2.75 $\pm$ 0.15 ^a	1.98 $\pm$ 0.25 ^b	1.62 $\pm$ 0.12 ^b
Heterophils (%)	21.12 $\pm$ 1.61 ^a	12.44 $\pm$ 1.23 ^b	10.29 $\pm$ 1.46 ^b	11.29 $\pm$ 2.37 ^b	13.07 $\pm$ 1.27 ^b
Lymphocytes (%)	74.07 $\pm$ 1.23 ^c	82.63 $\pm$ 0.64 ^a	85.13 $\pm$ 1.03 ^{ab}	80.50 $\pm$ 0.12 ^b	81.90 $\pm$ 0.91 ^{ab}

RBC: Red Blood Corpuscle; WBC: White Blood Corpuscle; Hb: Hemoglobin content; MCH: Mean Cell Hemoglobin; MCV: Mean Cell Volume; MCHC: Mean Cell Hemoglobin Concentration.

Mean $\pm$ SE values (n = 3) within row with different lowercase superscripts differ significantly ($p < 0.05$).

MCV and MCH were significantly higher in fish fed the 0.5 XE diet than in the control and the group receiving higher XE concentrations (Table 5). MCHC remained stable across treatments, suggesting that the hemoglobin concentration per cell was not substantially affected. The leukocyte indices indicated that WBC counts were significantly higher at 0.5% and 1% XE than in the control; however, they declined with further increases in the XE concentration and were significantly different from the control. Heterophils significantly increased in all the XE-fed groups compared to the control. Lymphocyte percentages increased significantly in the fish fed the XE diets, although the dose-dependent effects were unclear, with the highest lymphocyte level observed at 1%, followed by 0.5%, 2% and 1.5%.

The dietary XE significantly affected the blood performance index (Fig. 2). Fish fed 0.5% XE had the highest value, although it was not significantly different from that of the 1.0% XE. Increasing XE inclusion to 1.5% significantly reduced the

index compared to the 0.5% XE group, while the 2% XE group also had a lower value. Overall, the 0.5% and 1% XE groups had higher blood performance index than the control and the 2% XE group.

Physio-immunological, hepatorenal and stress responses

XE supplementation significantly affected total protein in a dose-dependent manner. Specifically, the fish fed 0.5 and 1% had higher values than the control. In contrast, values were significantly lower at 1.5% and 2% and (Table 6). The maximum total protein and globulin values were observed at 1% XE. At the same time, albumin levels remained consistent across all treatments with only minor fluctuations.

Cortisol concentrations (Table 6) were significantly lower in the groups fed the XE-supplemented diets than in the control group. The lowest cortisol value occurred at 1% XE. In addition, glucose concentrations significantly decreased in all XE-supplemented groups relative to the control.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase activities were reduced in the XE-supplemented groups compared to the control, particularly at 1–1.5% XE. Creatinine and blood urea nitrogen (BUN) concentrations were significantly lower in the XE-supplemented groups, particularly at 1.5% and 2% XE. Based on these results there could have been enhanced renal clearance and reduced metabolic stress relative to the control. Fish receiving the XE-supplemented diets had reduced cholesterol and triglyceride concentrations, with the lowest values recorded at 2% XE (Table 6).

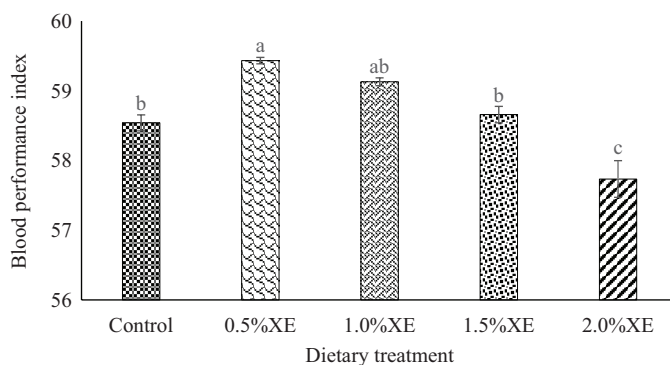


Fig. 2 Blood performance index of hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) fed diets supplemented with *Xylopi aethiopica* (XE), where error bars = $\pm$ D

Table 6 Physio-immunological, hepatorenal and stress responses of African catfish hybrid (*Clarias gariepinus* ♀ × *Heterobranchus c*

	Dietary XE supplementation				
	Control	0.5% XE	1% XE	1.5% XE	2% XE
Physio-immunological marker					
Total protein(g/dL)	6.19±0.02 ^b	6.46±0.01 ^a	6.43±0.01 ^a	6.16±0.02 ^b	6.10±0.01 ^c
Albumin (g/dL)	1.65±0.03 ^a	1.69±0.04 ^a	1.51±0.01 ^b	1.46±0.02 ^b	1.51±0.02 ^b
Globulin (mmol/L)	4.55±0.04 ^c	4.78±0.04 ^b	4.93±0.01 ^a	4.71±0.02 ^b	4.59±0.01 ^c
Physiological stress marker					
Cortisol (ng/mL)	335.99±1.21 ^a	275.94±14.30 ^b	142.41±1.40 ^c	184±184.42±9.63 ^c	180.08±20.98 ^c
Glucose (mmol/L)	1.46±0.02 ^a	1.31±0.02 ^b	1.38±0.02 ^b	1.36±0.02 ^b	1.39±0.02 ^b
Liver function test marker					
Aspartate aminotransferase (U/L)	171.67±5.65 ^a	158.87±5.06 ^{ab}	144.93±3.67 ^b	145.59±4.28 ^b	150.43±2.74 ^b
Alanine aminotransferase (U/L)	74.07±0.78 ^a	39.93±5.52 ^b	68.77±1.77 ^a	63.87±1.16 ^a	68.51±2.10 ^a
Alanine phosphate (U/L)	57.75±0.82 ^a	56.78±0.95 ^a	51.41±0.39 ^b	51.96±0.73 ^b	51.31±1.40 ^b
Kidney function test marker					
Creatinine (μmol/L)	67.90±1.76 ^a	53.09±0.88 ^b	50.93±3.65 ^b	37.60±4.26 ^c	33.82±1.86 ^c
Blood urea nitrogen (mg/dL)	21.30±3.11 ^a	18.83±2.23	12.87±1.41 ^{ab}	12.50±0.76 ^{ab}	9.79±1.62 ^b
Lipid function test marker					
Total cholesterol (mmol/L)	4.77±0.17 ^{ab}	5.06±0.20 ^a	4.01±0.19 ^b	4.81±0.06 ^{ab}	3.21±0.29 ^c
Triglycerides (mmol/L)	4.44±0.46 ^a	3.76±0.27 ^a	2.65±0.51 ^a	2.56±0.73 ^a	2.07±0.71 ^a

Mean ± SE values within row with different lowercase superscripts differ significantly ($p < 0.05$).

Plasma electrolyte responses of XE diets

Dietary treatments significantly affected the potassium, chloride and sodium ion levels but not plasma calcium (Fig. 3). Plasma calcium concentrations remained stable across the control, 0.5%, 1.0% and 1.5% XE groups. In contrast, fish receiving 2% XE had significantly lower calcium levels, indicating potential impairment of calcium regulation at

excessive supplementation. Plasma potassium concentrations were highest at 0.5% XE, with similar values ($p \geq 0.05$) in the control and 1.5% XE groups that were significantly less. Plasma chloride levels peaked at 0.5% XE and decreased progressively with higher supplementation, reaching the lowest concentration at 2% XE. Similarly, plasma sodium concentrations were significantly higher at 0.5% and 1.5% XE than in the control, but declined sharply at 2% XE.

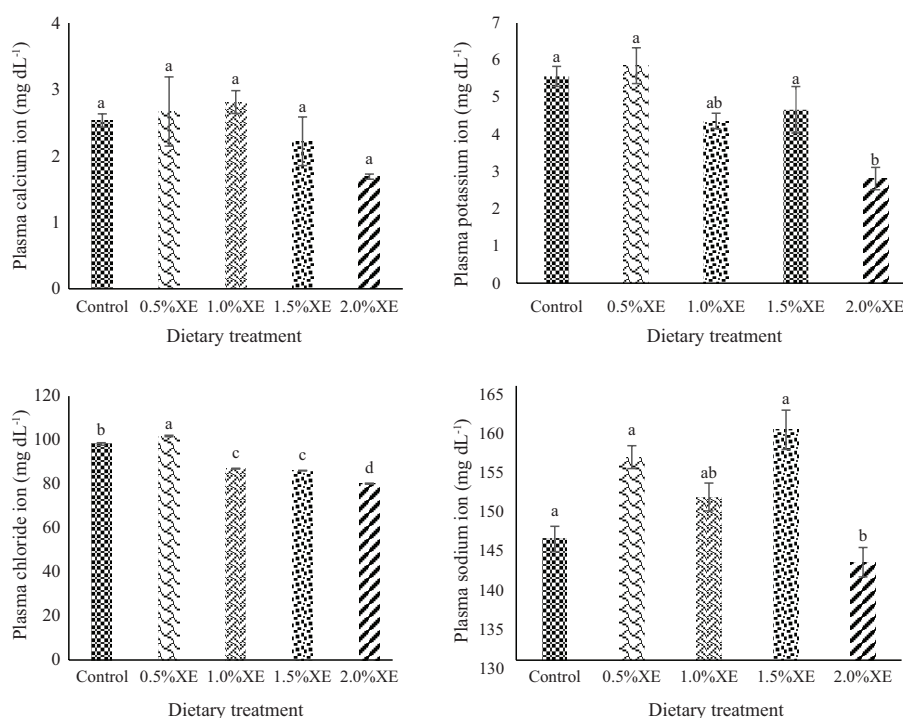


Fig. 3 Plasma electrolytes of African catfish hybrid (*Clarias gariepinus* ♀ × *Heterobranchus longifilis* ♂) fed *Xylopi aethiopica*-supplemented diets. Different superscripts above bars denote significant difference ($p < 0.05$) among means, error bars indicate standard error.

Histological examination of the liver

In the control group, the liver had normal tissue organization, with clearly defined central veins, hepatocyte nests and cords and interspersed sinusoids (Fig. 4A). At 0.5% XE supplementation, the liver maintains this typical morphology, with only slight vacuolation and the presence of resident Kupffer cells (Fig. 4B). These minor alterations do not suggest significant pathological changes. At 1.0% XE supplementation, the liver structure remained largely intact, with the normal central vein morphology. The hepatocyte nests and surrounding sinusoids were slightly more distended, accompanied by increased vacuolation and visible Kupffer cells (Fig. 4C). Based on these observations, there might have been an early adaptive response to supplementation, possibly reflecting enhanced metabolic or detoxification activity. No major cellular degeneration was evident. At 1.5% XE supplementation, the liver had a similar architecture, though the increased vacuolation (red arrows) and more prominent

sinusoids indicated a mild physiological stress response. Kupffer cells were present and the central vein was well-formed (Fig. 4D). At 2.0% XE supplementation, the liver tissue had more pronounced vacuolated hepatocytes surrounding the central vein, while the overall architecture remained intact (Fig. 4E).

Histological examination of the kidney

The control group had normal histological architecture, with no observable cellular alterations (Fig. 5A), indicating a healthy kidney under standard dietary conditions. At 0.5% XE supplementation, mild distortion and irregular morphology of the glomeruli and tubules were evident, along with some interstitial inflammation. (Fig. 5B) These findings indicated an initial adaptive response to supplementation, with no substantial pathological changes at this concentration. At 1.0% XE supplementation, the kidneys had moderate alterations in the glomeruli and tubules and interstitial inflammation was more pronounced (Fig. 5C).

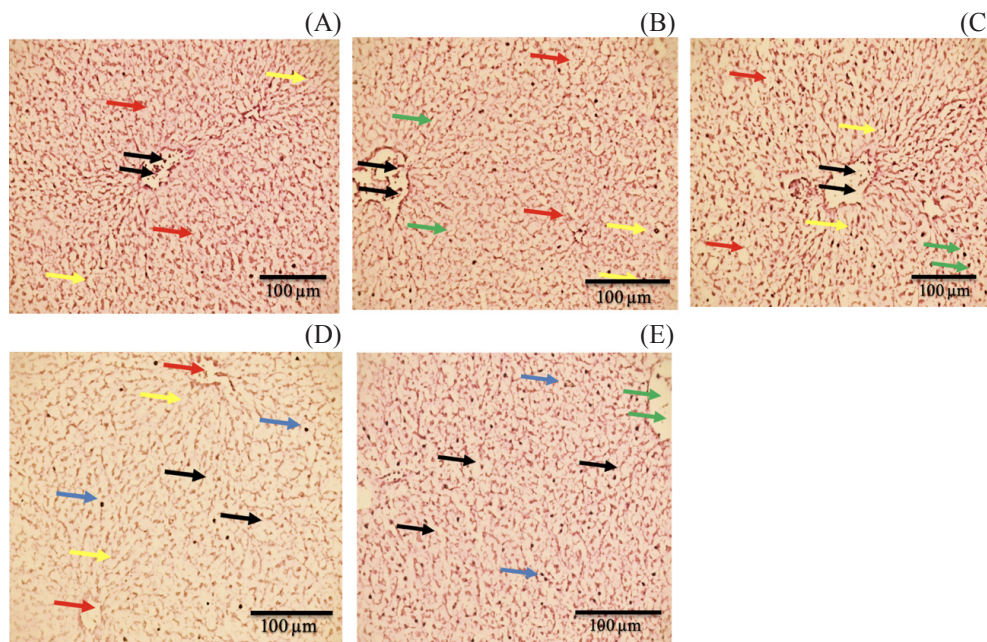


Fig. 4 Histological sections (5 μ m, Hematoxylin & Eosin stained, $\times 100$) of liver in hybrid African catfish (*Clarias gariepinus* $\text{♀} \times \text{Heterobranchus bidorsalis}$ ♂) fed diet supplemented with *Xylopi aethiopica*: (A) control showing normal liver architecture, with central vein (black arrows) surrounded by hepatocytes nests and cords (red arrows) with interspersing sinusoids (yellow arrow); (B) 0.5% XE showing normal morphology, with central vein (black arrows), surrounded by hepatocyte nests and cords (green arrows), interspersing sinusoids and vacuoles (red arrows) and resident Kupffer cells (yellow arrows); (C) 1.0% XE, with normal liver architecture of a central vein (black arrows), surrounded by cords and nests of hepatocytes (yellow arrows), sinusoidal channels, vacuoles (red arrows) and Kupffer cells (green arrows); (D) 1.5% XE, with normal liver architecture showing central vein (red arrows), hepatocyte nests (yellow arrows), surrounded by sinusoidal channels and vacuoles (black arrows) and Kupffer cells (blue arrow). (E) 2.0% XE showing normal appearing central vein (green arrows) surrounded by nests and cords of vacuolated hepatocytes (black arrows), interspersed with resident Kupffer cells (blue arrows)

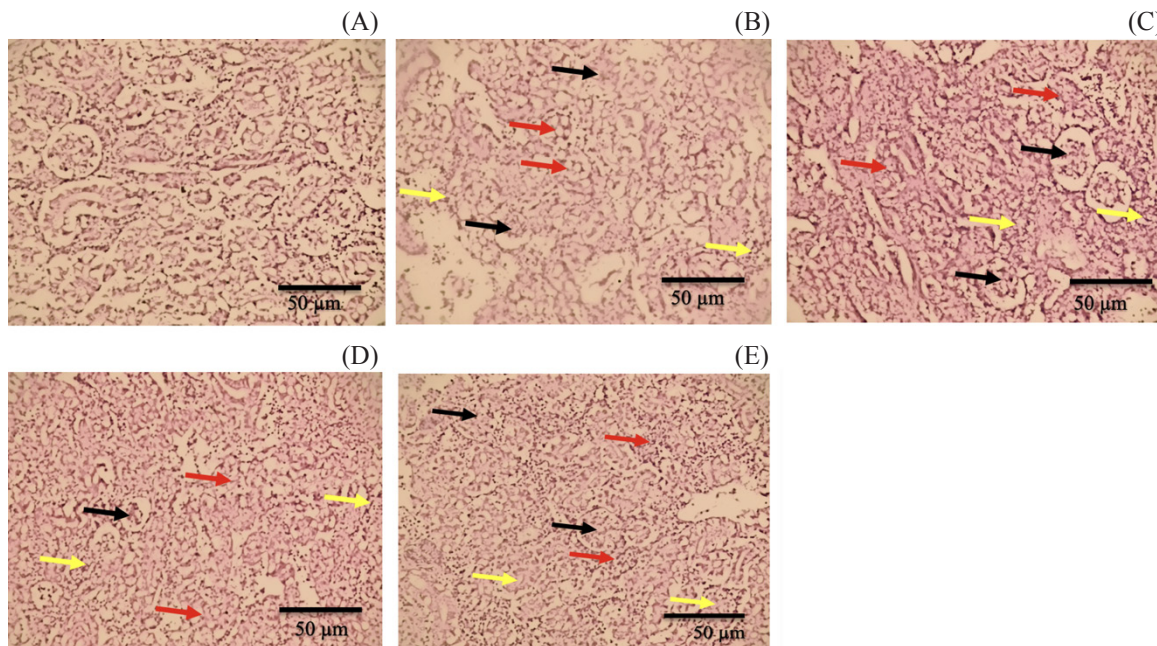


Fig. 5 Histological sections (5 μ m, Haematoxylin & Eosin stained, $\times 400$) of kidney of hybrid African catfish (*Clarias gariepinus* $\times$ *Heterobranchius bidorsalis*) fed diets supplemented with *Xylopi aethiopica*. (a) control showing normal kidney architecture, with no observable cellular changes; (B) 0.5%XE showing distorted and irregular morphology of glomeruli (black arrow) and renal tubules (red arrows), with mild interstitial inflammation (yellow arrows); (C) 1.0%XE showing glomeruli (black arrows) and renal tubules (red arrows) surrounded by interstitial inflammation (yellow arrows); (D) 1.5%XE showing glomeruli (black arrows) and renal tubules (red arrows) surrounded by interstitial inflammation (yellow arrows); (E) 2.0%XE showing glomeruli (black arrows) and renal tubules (yellow arrows) surrounded by interstitial inflammation (red arrows).

The overall kidney structure remained largely preserved, with no evidence of severe damage. These findings suggested that moderate supplementation induced mild inflammation without causing overt injury. At 1.5% XE supplementation, the kidney had more pronounced interstitial inflammation surrounding the glomeruli and tubules (Fig. 5D). These alterations were more pronounced than those observed in the 1.0% XE group, potentially indicating increased physiological stress or a threshold at which supplementation had a more adverse effect on kidney morphology. At 2.0% XE supplementation, the kidney displayed changes comparable to those in the 1.5% XE group, with glomeruli and tubules surrounded by more severe interstitial inflammation (Fig. 5E). These findings suggested that higher levels of XE supplementation led to more pronounced inflammatory responses in the kidney, although there was no evidence of tissue necrosis or severe pathology. Moderate XE supplementation (up to 1.5%) resulted in mild histological changes in the kidney, while higher supplementation (2.0%) led to more pronounced inflammation. Based on these results, there was a dose-dependent response, highlighting the need for further investigation to determine potential long-term effects.

Discussion

A wide range of bioactive metabolites can be used to characterize medicinal plants. In more recent years, these plants have emerged as promising alternatives in aquaculture (Zhu, 2020). In the current study, supplementation with XE significantly enhanced growth performance, feed utilization, survival and economic returns in cultured fish. According to Dawood et al. (2018), an effective feed additive should promote both growth performance and overall fish health. The 0.5% XE inclusion level produced the highest final weight, weight gain, percent weight gain, specific growth rate and the lowest feed conversion ratio. These findings indicated that moderate supplementation optimized nutrient utilization and growth efficiency. These results were consistent with those reported by Hoseini et al. (2021a) in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with roselle. Comparable outcomes were observed in *C. gariepinus* fed diets based on XE pod powder (Ibrahim et al., 2024). Diets enriched with plant-derived compounds have been shown to improve growth, digestive enzyme activity, antioxidant capacity and

immune function in fish (Yousefi et al., 2020). Furthermore, the enhanced protein efficiency ratio and survival rate recorded in the current study at this level highlight its positive impact on metabolism and health. These results were consistent with other research indicating that bioactive compounds in phytogetic feed additives stimulate appetite, improve gut health and enhance nutrient assimilation (Caipang, 2020). The economic performance indices obtained in the current study provided an indication of the relative cost-effectiveness of the experimental diets based on the growth performance and feed utilization observed during the feeding trial. Although the experiment was conducted under controlled laboratory conditions using juvenile fish, these indices remain useful for comparing the economic efficiency of the dietary treatments because they are derived directly from the measured experimental data. However, economic outcomes under commercial production conditions may differ due to variations in stocking density, production scale, management practices and the market size of fish (Shamsuddin et al., 2022). Therefore, the economic indices presented in the current study should be interpreted primarily as comparative indicators of dietary cost efficiency among the treatments tested. Notably, this is believed to be the first published study to evaluate the effect of phytochemicals, specifically XE, on the economic performance of African catfish hybrids to the best of the current authors' knowledge. The improved growth performance and economic returns observed at 0.5% XE supplementation may be associated with reduced physiological stress and the improved physiological status of the fish, as suggested by the lower cortisol and glucose concentrations recorded in the supplemented groups, which are commonly used indicators of stress in fish (Odhiambo et al., 2020). In addition, the higher serum total protein and globulin levels observed in the XE-fed fish may indicate improved immune competence. Furthermore, the liver biochemical indices showed favorable trends in the supplemented groups. The reduced activities of AST, ALT and ALP, particularly at 1–1.5% XE inclusion, were consistent with improved hepatic condition and reduced hepatocellular disturbance. Notably, this interpretation was supported by the generally normal hepatic architecture observed in the liver photomicrographs of the XE-fed fish, suggesting that XE supplementation had not induced major structural alterations in liver tissues. Similarly, the lower creatinine and BUN values in the supplemented groups may indicate improved metabolic regulation and kidney function relative to the control group. However, the histological observations revealed more pronounced interstitial inflammatory changes at higher inclusion levels (1.5–2.0% XE),

suggesting that although the biochemical indicators were lower, renal responses at higher XE levels may reflect early inflammatory changes, indicating that the beneficial effects of XE on kidney physiology may not be unequivocal at higher dietary inclusion levels. Therefore, moderate inclusion levels may be more physiologically favorable. These findings suggest that dietary XE supplementation, particularly at moderate levels, may support improved physiological status in hybrid African catfish. However, to the best of the current authors' knowledge, only limited information is available currently regarding the effects of XE supplementation on the physiological and histological responses in hybrid catfish; further studies are required to elucidate the underlying mechanisms. These findings demonstrated that there was an optimal level of dietary inclusion of *X. aethiopica* that improved growth, health, productivity and economic sustainability in aquaculture. The quadratic regression model ($y = -9.9196x^2 + 13.902x + 32.857$; $R^2 = 0.9628$) indicated a strong dose-response relationship between *X. aethiopica* supplementation and weight gain, with peak performance predicted at approximately 0.7% inclusion.

The hematological responses observed in hybrid African catfish indicated that XE exerted a modulatory effect on blood physiology and immune function. Supplementation with 0.5% XE resulted in significantly higher RBC counts, Hb concentration and PCV levels. These enhancements suggested increased erythropoiesis and oxygen-carrying capacity, likely due to phytochemicals, such as flavonoids, alkaloids and phenolic compounds, that stimulate hematopoiesis and protect erythrocytes from oxidative stress (Muscolo et al., 2024). Enhanced oxygen transport at this supplementation level may account for the improved growth performance observed. Similar hematological responses have been documented in other fish species. For example, Hoseini et al. (2021b) reported comparable effects in common carp fed Russian olive (*Elaeagnus angustifolia*) leaf extract, although the growth rates were not significantly different from the control group. Additionally, Hoseini et al. (2021a) found that supplementation with 0.5% roselle improved both blood parameters and growth performance. The blood performance index offered an empirical insight and a comprehensive assessment of hematological health in hybrid African catfish. Notably, the significantly higher index in the fish receiving 0.5% *X. aethiopica* corresponded with increased RBC, Hb and PCV values, indicating enhanced erythropoiesis and oxygen transport, which supported improved metabolic efficiency and growth. Elevated WBC counts and increased lymphocyte proportions at 0.5–1% XE in the current study indicated enhanced immune competence.

This leukocytosis suggests that bioactive compounds in XE function as immunostimulants, thereby strengthening the cellular immune response. The simultaneous reduction in heterophil percentages may indicate decreased physiological stress or infection risk, accentuating the role of XE in maintaining immune balance. The observed increase in lymphocytes and decrease in heterophils at 0.5–1% XE was consistent with the findings of Hoseini et al. (2021b), supporting the conclusion that moderate supplementation enhanced adaptive immunity. While several studies have reported that plant supplementation increased both the lymphocyte and neutrophil counts in fish (Poolsawat et al., 2022; Zare et al., 2023), contrasting evidence exists. For example, Adel et al. (2017) observed higher neutrophil percentages following phytogetic supplementation.

Growth performance declined markedly at the higher supplementation levels, particularly at 2% XE, indicating a negative impact on weight gain. Growth suppression at higher inclusion levels, particularly at 2% XE, as recorded in the current study, was likely attributable to antinutritional or phytotoxic compounds in *X. aethiopica*. A similar result was reported by Hoseini et al. (2021b), who observed slight toxicity in carp fingerlings fed a diet containing 3% Russian olive (*Elaeagnus angustifolia*) leaf extract. This treatment affected several physiological parameters. At elevated concentrations, these compounds may reduce nutrient digestibility, lower feed palatability, or induce metabolic stress in fish (Ciji and Akhtar, 2021). The dose-dependent effects of phytogetic feed additives are well established, with lower concentrations promoting performance and higher levels causing inhibition (Caipang, 2020). The reduction in the hematological parameters at 2% XE, specifically the lower RBC, Hb, PCV and WBC, suggested that excessive supplementation may exert hematotoxic effects as observed by Ahmed et al. (2020). This outcome may result from overexposure to phytochemicals that impair nutrient assimilation or induce oxidative stress at high doses (Blomhoff, 2010). These findings aligned with the dose-dependent effects observed in many plant-derived additives, where moderate inclusion is beneficial but higher concentrations are inhibitory or toxic (Sethi et al. 2025). Elevated WBC counts and lymphocyte percentages at 0.5% XE further supported the higher blood performance index, demonstrating that moderate supplementation improved both oxygen-carrying capacity and immune competence. The lowest blood performance index at 2% XE was associated with reductions in RBC, Hb, PCV and WBC, indicating that excessive supplementation could compromise hematological health, leading to impaired oxygen transport and reduced immune function. Furthermore,

the intermediate values at 1.0% and 1.5% XE supported a dose-dependent effect, with moderate inclusion promoting hematological stability and higher levels causing hematological depression.

Lipid-lowering effects were more pronounced at a supplementation level of 2% XE. Concurrent declines in hematological and growth indices indicated that excessive inclusion may have disrupted metabolic balance. The observed hypocholesterolemic activity was likely due to the phytochemical constituents of XE. Histological analysis showed that fish fed 2% XE had hepatocytes with increased vacuolation around the central vein, while the overall hepatic architecture remained intact. This vacuolation may reflect an adaptive response to enhanced processing of bioactive compounds (Ganesan et al., 2018). Furthermore, the presence of Kupffer cells in the photomicrographs suggested ongoing immune modulation induced by supplementation, similar to what was reported in Huang et al. (2010). These findings indicated that moderate supplementation of *X. aethiopica* at levels up to 1.5% XE did not cause substantial liver damage, as only mild hepatocyte vacuolation and inflammatory responses were observed, which are suggestive of adaptive detoxification processes. In contrast, supplementation at 2% XE resulted in more severe alterations, indicating a threshold beyond which phytochemical overload induces tissue stress. In addition, the renal tissue responses mirrored the hepatic changes. At supplementation levels of 0.5 to 1.5% XE, there was mild interstitial inflammation around glomeruli and tubules, likely reflecting an immune or stress-related adjustment. At 2% XE, the inflammation was more pronounced, paralleling the hepatic response to high supplementation. Collectively, these findings suggested that while XE provided beneficial effects at moderate levels, excessive supplementation imposed systemic strain on detoxification and filtration organs, leading to compromised tissue integrity and physiological stress.

Moderate dietary inclusion of XE (0.5–1.5%) in hybrid African catfish maintained stable plasma electrolyte levels, supporting osmoregulation and physiological stability (Greenwell et al., 2003). At 0.5% XE, the increased plasma sodium and potassium concentrations indicated improved ion regulation, which is associated with enhanced growth performance and greater resilience to environmental stressors (Zhou et al., 2020). In contrast, supplementation at 2% XE led to significant reductions in plasma calcium, potassium, sodium and chloride levels, indicating electrolyte imbalance, impaired osmoregulation and systemic stress, as further evidenced by the decreased hematological indices and increased metabolic stress

markers in the current study. The histopathological analysis aligned with these physiological findings. For example, the fish fed 0.5–1.5% XE displayed only mild-to-moderate adaptive changes in the liver and kidney, whereas those fed 2% XE showed pronounced inflammatory stress in both organs. Based on these results, exceeding a certain threshold of XE supplementation imposed harmful effects. The concurrent inflammatory responses in the hepatic and renal tissues highlighted the necessity of a dose-dependent application, since excessive supplementation impaired organ function and disrupted homeostasis (Abd-Elaziz et al., 2023)

The current study has demonstrated that dietary supplementation with XE significantly affected growth, hematological health, immune competence, physiological stability and economic returns in hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂). Optimal performance was observed consistently at 0.5–1.0% inclusion, as indicated by superior growth and feed utilization, enhanced oxygen transport and immune responses, stable plasma electrolytes and reduced stress markers. In contrast, excessive supplementation at 2% led to depressed growth, disrupted electrolyte balance and impaired hematological and physiological health. For aquaculture production, XE could be incorporated into catfish diets at 0.5% (approximately 0.7% optimum) to maximize productivity, improve fish health and enhance economic sustainability, while avoiding higher inclusion levels that may be detrimental.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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