EFFECTS OF DIETARY ETHANOLIC EXTRACT OF *ALCHORNEA CORDIFOLIA* LEAF ON HAEMATO-IMMUNOLOGY AND ANTIOXIDANT STATUS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FINGERLINGS

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Abstract

The effects of dietary ethanolic extract of *Alchornea cordifolia* leaf (ACLE) on haematology, serum biochemistry, immune response and antioxidant capacity of African catfish, *Clarias gariepinus* was studied. A control diet ($ACLE_0$) containing 40% crude protein was formulated and the cellulose component of the diet was replaced with ACLE at 2.5 ($ACLE_{2.5}$), 5 ($ACLE_5$), 7.5 ($ACLE_{7.5}$) and 10 g/kg ($ACLE_{10}$). Three replicate groups of African catfish fingerlings (initial average weight, 6.11±0.02g) received the diets for sixty-three days. Highest weight gain (32.02±1.93g), daily weight gain (0.57±0.03g) and protein efficiency ratio (2.86±0.17) were observed in the group fed $ACLE_{10}$; the increases observed were however insignificant (p>0.05) relative to other groups. Dietary ACLE did not remarkably (p>0.05) alter white and red blood cell counts, haemoglobin and packed cell volume.$ACLE_{2.5}$ significantly (p<0.05) favoured increased lymphocyte (55.0±1.10%) relative to the control group. Higher levels (diets $ACLE_{7.5}$ and $ACLE_{10}$) of *A. cordifolia* leaf extract significantly (p<0.05) raised serum alanine aminotransferase of *C. gariepinus* fingerlings. The respiratory burst activity and total immunoglobulin was not influenced (p>0.05) by the treatment. Reduced glutathione and malondialdehyde in the serum were similar (p>0.05) across dietary groups. Significantly (p<0.05)higher activities of hepatic and serum superoxide dismutase was observed in ACLE fed groups compared to the control. This study revealed that ethanolic leaf extract of *A. cordifolia* can effectively enhance the antioxidative capacity of *C. gariepinus*.

Keywords: *Alchornea cordifolia* leaf extract; growth; diet utilization; immune response; superoxide dismutase; catalase; reduced glutathione

Introduction

Fish is an essential component of the human diet because of its high nutritional value and importance in improving human health.Fish supply in Nigeria is principally from capture fisheries sector, importation and aquaculture production. Supply from capture fisheries sector has been largely stagnated over the years due to declining fish stock (Olopade *et al*., 2017) whereas fish importation is currently being discouraged because of scarcity of foreign exchange; shifting thepolicy thrust of the Federal Government of Nigeria to local production rather than importation (Liverpool-Tasie *et al*., 2021). The current scenario has made aquaculture animportant sector in the supply of affordable animal protein to Nigeria's escalating population(Liverpool-Tasie *et al*., 2021).
The dominant farmed fish species in Nigeria is African mudcatfish, *Clarias gariepinus*(Dauda *et al*., 2018).Its success has been associated with its amenability to artificial propagation, rapid growth, tolerance to low oxygen and wide range of temperature and good market potential (Dauda *et al*., 2018; Oladele *et al*., 2021). It commands good commercial value with substantial production growth in the past three decades (Anetekhai, 2013; Dauda *et al*., 2018). However, a major factor limiting further production growth of *C. gariepinus* is regular outbreak of fish diseases (Dauda and Ibrahim, 2015). Such disease outbreaks typically result in significant financial loss, by raising production costs either through lost investment in dead fish or through direct cost of treatment; and through slower fish growth during period of diseased fish recuperation. Although, information regarding fish diseases are not well documented in Nigeria, Dauda and Ibrahim (2015) observed the preponderance of bacterial diseases in fish farms of Katsina State with economic loss in each farm studied ranging from ₦ 50,000 to as high as ₦1,000,000. To sustain aquaculture growth in Nigeria, there is therefore the need for adequate measure that can mitigate the problem of disease outbreak, especially with regards to bacterial infection which represents the highest economic loss on fish farms.

Conventional approach to bacterial disease management in fish involves the use of antibiotics (Ikpi and Offem, 2011; Okocha *et al*. 2018). However, there are several problems associated with the uncontrolled use of antibiotics which include the emergence of antimicrobial-resistant bacterial strains, rendering many antibiotics ineffective; environmental safety concerns; change in microflora structure and diversity of aquacultural environments; bioaccumulation of antibiotics in fish tissue and its biomagnification up the food chain, among others (Hong *et al*. 2018). A pragmatic approach to disease management would, therefore, be those that prevent disease occurrence on fish farms. In this regard, vaccination has proven to be quite effective but it is costly, pathogen-specific, with limited large-scale farm application (Gudding, 2014).An alternative strategy is the use of medicinal plants (comprising of herbs, seaweeds, spices, plant-derived products) which are known to contain natural products such as alkaloids, polyphenols, phenols, flavonoids, among others.They are known to promote the capacity of fish to resist diseases because of raised immunity and can also increase feed palatability as well as stimulate fish appetite (Hai, 2015; Wang *et al*., 2017; Olude *et al*., 2022). They are also environmentally friendly, cheap and effective, with fewer side-effects (Doan *et al*., 2019). This strategy of disease management can be easily adopted by small- and large-scale fish farmers.

One of such medicinal plants whose health benefits as antimicrobial and antioxidant had previously been established is *Alchornea cordifolia,* a straggling shrub that grows in wet or dry areas (Nyananyo, 2006). Kouakou *et al.* (2013) investigated the immunostimulatory properties of polysaccharides from *A. cordifolia* and established positive therapeutic properties. Bioactive components that inhibited the growth of common pathogens such as *Escherichia coli* and *Staphylococcus aureus*were previously documented by Kigigha *et al.* (2012)**.** Olude *et al*. (2022) also recently affirmed the antimicrobial and antioxidant potential of ethanolic extract of *A. cordifolia* leaf.Despite its potential therapeutic factors, to our knowledge,*A. cordifolia* is yet to be evaluated in fish diet.The present study thus investigated the effects of feeding diets containing ethanolic extractof *A. cordifolia*leaf on growth, haemato-immunological responses and activity of antioxidant enzymes in *Clarias gariepinus* fingerlings.

Materials and Methods

Collection of leaves and preparation of extracts

The leaves of *Alchornea cordifolia* were collected from the Botanical Garden, University of Lagos, Akoka. It was identified and authenticated by Dr. A.B. Kadiri of the Department of Botany, University of Lagos Akoka, Lagos. The leaves were cleaned and air-dried as previously detailed in Olude *et al*. (2022) after which they were extracted in ethanol according to the method of Azlim-Almey *et al*. (2010) and preserved in refrigerator (Haier Thermocool HR134MBS R6) at 4ºC prior to use.

Experimental site and fish

The experiment was carried out at the Aquaculture Unit, Botanical Garden, University of Lagos. University of Lagos lies between latitude 6º30´ 59.99´´ N and longitude 3º23´ 5.99´´ E. Two hundred and fifty (250) *C. gariepinus* fingerlings were procured from a fish farm in Akoka, Lagos, Nigeria. They were transported from the fish farm in a black plastic keg to the experimental site. The fingerlings were acclimatized in a circular plastic tank for a period of 2 weeks. The fish were fed 1mm Skretting feed during the acclimatization period.

Feed preparation and formulation

The ingredients used in the preparation of the diets are presented in Table 1. The major ingredients were procured from Iceberg Agricultural Consult, Ikotun, Lagos, Nigeria. The ingredients were used to formulate acontrol diet containing 40% crude protein, 10.5% crude lipid, 7% total ash, 8.4% crude fibre and 19 kJ/g gross energy. The cellulose component of the control diet ($ACLE_0$) was progressively replaced with ethanolic extract of *A. cordifolia* leaf at 2.5 ($ACLE_{2.5}$), 5 ($ACLE_5$), 7.5 ($ACLE_{7.5}$) and 10 g/kg ($ACLE_{10}$). The feeds were pelletized into 2mm size, dried using blower and stored in plastic bottles prior to the commencement of the experiment.

Table 1: Ingredient (g/kg) composition of the experimental diets

Ingredients (g/kg)	$ACLE_0$	$ACLE_{2.5}$	$ACLE_5$	$ACLE_{7.5}$	$ACLE_{10}$
Groundnut cake	300	300	300	300	300
Soybean meal	300	300	300	300	300
Fish meal	200	200	200	200	200
Cellulose	22.8	20.3	17.8	15.3	12.8
ACLE extract[1]	0	2.5	5	7.5	10
Wheat meal	82	82	82	82	82
Vegetable oil	50	50	50	50	50
Vit.-Min. Premix[2]	20	20	20	20	20
CMC[3]	10	10	10	10	10
BHT[4]	0.2	0.2	0.2	0.2	0.2
Choline chloride	5	5	5	5	5
Lysine	5	5	5	5	5
Methionine	5	5	5	5	5
Total	1000	1000	1000	1000	1000

[1]Ethanolic extractof *Alchornea cordifolia* leaf[2]Complete nutrients Vitamin-Mineral premix (quantity/kg): Vitamin A, 4,000,000IU Vitamin D3, 400,000IU, Vitamin E, 40,000mg Vitamin K3, 1000mg, Vitamin B1, 4000mg, Vitamin B2, 3000mg, Vitamin B6, 3000mg Vitamin B12, 3mcg, Nicotonic Acid, 18,00mg, Panthothenic Acid, 8000mg, Folic Acid, 800mg, Biotin, 100mcg Choline Chloride, 120,000mg, Ion, 8000mg, Copper, 800mg, Manganese, 6000mg, Zinc, 8000mg, Iodine, 400mg, Selenium, 40mcg, Vitamin C (coated), 60,000mg, Cobalt, 150mg, Antioxidant, 25,000mg; [3]Carbxymethyl Cellulose; [4]Butylated Hydroxytoluene**.**

Table 2: Growth performance and nutrient utilization of *Clarias gariepinus* fed diets containing graded levels of ethanolic extractof *Alchornea cordifolia* leaf.

	ACLE$_{0}$	ACLE$_{2.5}$	ACLE$_{5}$	ACLE$_{7.5}$	ACLE$_{10}$
IWT (g)	6.06±0.03	6.09±0.04	6.12±0.04	6.11±0.05	6.12±0.01
FWT (g)	35.61±0.19	35.75±0.23	37.61±2.15	37.12±1.72	38.13±1.94
WG (g)	29.55±0.17	29.66±0.26	31.49±2.12	31.00±1.74	32.02±1.93
DWG (g/day)	0.53±0.00	0.53±0.00	0.56±0.04	0.55±0.03	0.57±0.03
SGR (%/day)	2.81±0.00	2.81±0.02	2.88±0.08	2.86±0.08	2.90±0.08
FCR	0.91±0.01	0.94±0.01	0.89±0.06	0.91±0.05	0.88±0.02
PER	2.73±0.02	2.65±0.02	2.82±0.19	2.77±0.16	2.86±0.17
SUR (%)	93.33±3.33	93.33±3.33	93.33±3.33	93.33±3.33	93.33±3.33

Data are mean values ± SE (n = 3). Values in the same row having different superscript letters are significantly different ($P < 0.05$). IWT, Initial Weight, FWT, Final Weight, WG, Weight Gain, DWG, Daily Weight Gain, SGR, Specific Growth Rate, FCR, Feed Conversion Ratio, PER, Protein Efficiency Ratio and SUR, Survival.

Table 3: Haematological parameters of *Clarias gariepinus* fingerlings fed diets containing graded levels of ethanolic extractof *Alchornea cordifolia* leaf.

Parameters	ACLE$_{0}$	ACLE$_{2.5}$	ACLE$_{5}$	ACLE$_{7.5}$	ACLE$_{10}$
WBC($\times 10^3/\mu l$)	6.00 ± 1.15	4.87± 0.47	6.13 ± 0.90	4.40 ± 0.30	4. 30 ± 0.85
Lymph (%)	36.00 ± 0.00 ^{c}	55.0 ± 1.10^{a}	44.43 ± 2.77^{abc}	39.00 ± 4.50^{bc}	50.47 ± 6.84^{ab}
HGB(g/dl)	9.00 ± 3.61	9.93 ± 0.52	13.00 ± 1.25	12.33 ± 0.67	12.17 ± 0.73
RBC($\times 10^6/\mu l$)	3.47 ± 1.64	3.80 ± 0.17	4.71 ± 0.33	3.50 ± 0.50	4.78 ± 0.27
PCV(%)	37.33 ± 2.33	33.10 ± 0.67	39.87 ± 3.02	35.60 ± 0.31	37.50 ± 2.75
MCV(fl)	83.67 ± 4.33^{ab}	87.36 ± 2.22^{ab}	85.63 ± 8.57^{ab}	105.41 ± 12.72^{a}	78.58 ± 5.13^{b}
MCH(pg)	30.67 ± 4.81	26.11 ± 0.20	27.87 ± 3.21	38.53 ± 7.16	25.43 ± 0.22
MCHC(g/dl)	32.33 ± 0.33^{ab}	29.94 ± 0.96^{b}	32.43 ± 0.64^{ab}	35.72 ± 2.83^{a}	32.60 ± 1.97^{ab}

Data are mean values ± SE (n = 3). Values in the same row having different superscript letters are significantly different ($P < 0.05$). WBC; white blood cell, Lymph; lymphocytes, HGB; hemoglobin, RBC; red blood cell, PCV; hematocrit, MCV; mean cell volume, MCH; mean cell hemoglobin, MCHC; mean cell hemoglobin concentration

Table 4: Serum Biochemistry of *Clarias gariepinus* fingerlings fed graded levels of ethanolic extractof *Alchornea cordifolia* leaf.

Parameters	ACLE$_{0}$	ACLE$_{2.5}$	ACLE$_{5}$	ACLE$_{7.5}$	ACLE$_{10}$
Total protein (g/l)	77.13±4.10	71.10±3.13	76.03±2.79	76.97±3.35	79.33±5.46
Albumin (g/l)	41.10 ± 2.20	39.80 ± 0.93	39.80 ± 0.53	40.00 ± 1.90	40.00 ± 0.90
Globulin (g/l)	36.03 ± 2.77	31.30 ± 2.20	36.23 ± 2.60	36.96 ± 2.90	39.33 ± 5.93
AST (u/l)	150.20±3.00	187.50±15.12	176.73±22.24	156.70±20.74	181.80±43.42
ALT (u/l)	31.57± 3.87^{b}	56.57± 8.81^{ab}	48.53± 7.49^{ab}	59.90± 11.11^{a}	56.30 ± 9.47^{a}
Creatinine (µmol/L)	49.43± 5.77	40.97± 7.00	56.70± 7.69	43.37± 4.68	54.90± 3.00
Urea (mg/dL)	6.60 ± 0.70^{ab}	5.93 ± 0.48^{b}	6.33 ± 0.86^{ab}	7.90 ± 0.10^{a}	6.40 ± 0.30^{ab}
Cholestorol (mg/dl)	1.94 ± 0.22	1.71 ± 0.17	1.82 ± 0.11	1.52 ± 0.17	2.00 ± 0.17
Triglyceride	0.92 ± 0.17	0.57 ± 0.19	0.55 ± 0.21	0.74 ± 0.13	0.84 ± 0.14

Data are mean values ± SE (n = 3). Values in the same row having different superscript letters are significantly different ($P < 0.05$). AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

Table 5: Immune response, serum antioxidant enzymes activities and oxidative stress biomarker of *Clarias gariepinus* fed graded levels of *Alchornea cordifolia* leaf extract.

	ACLE$_0$	ACLE $_{2.5}$	ACLE $_5$	ACLE $_{7.5}$	ACLE $_{10}$
RBA	0.73±0.04	0.67±0.05	0.89±0.15	0.83±0.30	0.74±0.18
Total Ig	0.09±0.00	0.08±0.02	0.05±0.01	0.08±0.02	0.08±0.02
SOD	1.10±0.01^a	1.65±0.11^b	1.48±0.13^{ab}	1.69±0.12^b	1.64±0.29^b
CAT	11.56±0.28^b	9.35±0.83^{ab}	7.11±0.69^a	8.60±0.84^a	8.66±1.10^a
GSH	10.39±0.14	9.81±1.34	12.13±2.24	13.54±1.69	13.88±1.75
MDA	4.85±0.85	4.94±1.55	4.46±1.66	6.93±0.79	4.51±1.94

Data are expressed as mean ± SE (n = 3). Mean values in the same row with the same superscript were not significantly different (*P*> 0.05). RBA, Respiratory burst activity; Total Ig, Total Immunoglobulin (g/dL); SOD, superoxide dismutase was expressed as micromole/ml protein/min; CAT, Catalase as micromole H_2O_2 decomposed/ml protein/min; GSH, Reduced glutathione as micromoles/ml of protein; MDA, malondialdehyde as micromole/ ml of protein.

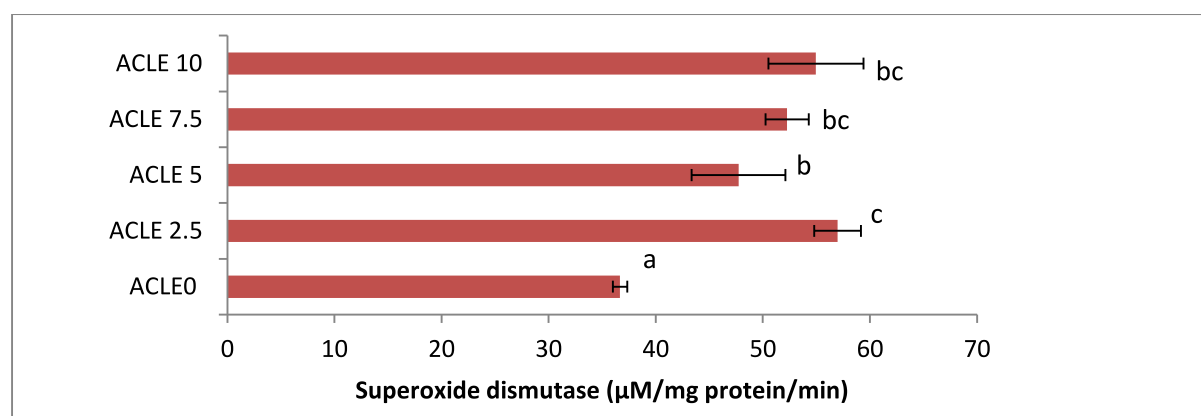

Figure 1: Hepatic superoxide dismutase enzyme activity of *Clarias gariepinus* fed diets containing different levels of ethanolic leaf extract of *Alchornea cordifolia*

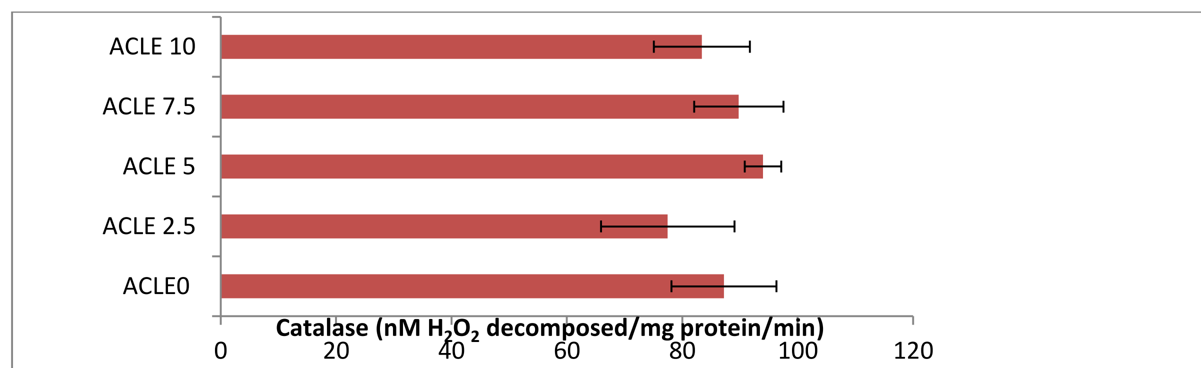

Figure 1: Hepatic catalase enzyme activity of *Clarias gariepinus* fed diets containing different levels of ethanolic leaf extract of *Alchornea cordifolia*

Experimental design and feeding

C. *gariepinus* fingerlings (average weight, 6.11 ± 0.02g) of the same agewere distributed into fifteen (15) rectangular plastic tanks (43×29×24) cm^3 at a stocking density of 10 fish per tank. The experimental diets were assigned to three clusters of experimental fish in a completely randomized design. They were fed to satiation twice daily at 9:00am and 5:00pm for 63 days.Rearing water was changed at 3-day interval throughout the experimental period to sustain optimal rearing environment. pH (7.2–8.1), temperature (30-31°C) and dissolved oxygen (6.4–7.5 mg/l) in the experimental tanks were monitored regularly using a pH meter, mercury-in-glass thermometer and a dissolved oxygen meter, respectively. Fish were regularly monitored for mortality;which were removed, counted and recorded to estimate survival.

Growth performance and nutrient utilization

At the end of the experimental period, the following growth performance and diet utilization indices were calculated using the following formulae:

Weight Gain, WG (g) = Final Weight (g) − Initial Weight (g);

Daily Weight Gain, DWG (g) = Final Weight (g) − Initial Weight (g) / Experimental Period;

Specific Growth Rate, SGR (%) = (Log$_e$ Final Weight – Log$_e$ Initial Weight) / Experimental Period × (100);

Feed Conversion Ratio, FCR = Feed Intake (g) / Fish Weight Gain (g) and

Protein Efficiency Ratio, PER = Fish Weight Gain (g) / Protein Intake (g).

Collection of blood and tissue

Three fish from each treatment replicate were randomly chosen, and about 4ml of blood was collected from their caudal vein with the aid of a disposable plastic syringe and needle. Collected blood was immediately transferred into two different vials; sterile ethylene diamine tetra-acetic acid (EDTA) bottle and sterile plain bottle for haematological and biochemical analysis, respectively.

Liver was collected from three fish per replicate and weighed into centrifuge bottles. The tissues were submerged into 0.25M chilled sucrose solution and homogenized (Omni-Mixer Homogenizer17106, International, Waterbury, CT, USA). The homogenate was centrifuged (5000 g for 10 mins at 4ºC) and the resulting supernatant decanted into labeled universal vials and kept at -20ºC prior to further analysis.

Haematology and serum biochemistry

The haematology of the experimental fish in terms of packed cell volume (PCV), haemoglobin (Hb), red blood cells count (RBC), white blood cell count (WBC),mean corpuscular haemoglobin concentration(MCHC),mean corpuscular haemoglobin(MCH), and mean corpuscular volume (MCV) of the experimental fish were studied at the Central Research Laboratory of Lagos University Teaching Hospital (LUTH) using automatic analyser (Mindray, BC 3200). Similarly, total protein, albumin, globulin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), triglycerides and cholesterol were determined with Erba Mannheim (XL 200/640) automatic biochemical analyser.

Determination of immune responses

The respiratory burst activity followed the modified method of Stasiak and Baumann (1996) using Nitroblue Tetrazolium (NBT). The protocol of Anderson and Siwicki (1995) which estimated the difference in total serum protein and the concentration of protein absorbed by 12% polyethylene glycol was used to assay for total immunoglobulin (Ig).

Serum and hepatic antioxidant enzymes activities and stress biomarker

The procedure of Aebi (1974) which measured the decomposition of H_2O_2at 240nm was followed to determine catalase (CAT) activity. Serum superoxide dismutase (SOD) activity was estimated according to the method of McCord and Fridovich (1969). Reduced glutathione (GSH) was estimated by following the method of Moron *et al*. (1979) while determination of serum lipid peroxidation followed the method described by Ohkawa *et al.* (1979).The methods described in Takahara *et al*. (1960) and Misra and Fridovich (1972) were used to assay the hepatic catalase and SOD activities, respectively.

Statistical analysis

Experimental data obtained were expressed as mean ± standard error (SE) and were statistically analyzed using Statistical Package for Social Science (SPSS, version 22). The generated data were subjected to one-way ANOVA and where necessary, Duncan's multiple range test was used to resolve differences between the means at 5% probability level.

Results and Discussion

Growth and diet utilization of *C. gariepinus*that received different quantities of ACLE are presented in Table 2. Generally, WG, DWG, SGR, FCR and PER of the treatment groups showed insignificant ($p>0.05$) enhancement over the control. The results showed that $ACLE_{10}$ fed group recorded the highest growth performance in terms of WG (32.02±1.93g), DWG (0.57±0.03g) and best nutrient utilization in terms of FCR (0.88±0.02) and PER (2.86±0.17); the increases were however insignificant ($p>0.05$) relative to other groups. The observed measurable increase in growth could probably have resulted from beneficial effects of *Alchornea cordifolia*extract on gut microbiota of *C. gariepinus* as well as stimulation of intestinal digestive enzymes which could have aided nutrient digestion as previously elucidated by different authors (Mohammadi *et al*., 2020; Zhang *et al*. 2020). Survival was similar across dietary groups and the mortalities recorded could not be associated with the dietary treatments.This shows that dietary supplementation of ACLE, even at highest level of 10 g/kg, was not toxic as much to cause mortality of*C. gariepinus*fingerlings. The few mortalities recorded cut across the experimental groups and could have resulted from handling stress.

The results of the haematological study are presented in Table 3. White blood cell count varied insignificantly ($p>0.05$) from 4.30 ± 0.85 $\times10^3$/µl in $ACLE_{10}$ to 6.13 ± 0.90 $\times10^3$/µl in $ACLE_5$. Lower content of lymphocyte was observed in the group fed $ACLE_0$ relative to other dietary groups; the observed variation was significant($p<0.05$) when the control treatment was compared to those fed with $ACLE_{2.5}$ and $ACLE_{10}$. Lymphocytes are component of white blood cell whose increase is usually linked with enhanced adaptive immunity through the generation of antibody (Secombes and Wang, 2012). Nhu *et al*. (2019) reported elevated lymphocytes after the administration of different plant extracts in the diet of stripped catfish. They explained that the increase could have been induced by polysaccharides usually associated with plant extracts; a factor which could also have been responsible for our result.Thissuggests that ACLE has the capacity to trigger the adaptive immunity of *C. gariepinus*.Dietary treatment did not substantially ($p>0.05$) affect red blood cell, haemoglobin and packed cell volume of *C. gariepinus*. This shows that supplementation of ACLE in *C. gariepinus* diet did not impair blood formation and its capacity to carry oxygen. Herein, our result supports the report of Pakravan *et al*. (2012) who observed that dietary willow herb did not alter RBC, Hb and PCV of *Cyprinus carpio*. Our finding is however at variance with the report of Aluta *et al*. (2020) who documented remarkable decreases in RBC, Hb and PCV when they fed diets containing onion peel powder to *C. gariepinus*.

The results of the present study showed that serum total protein, albumin and globulin were not significantly ($p>0.05$) promoted by dietary treatment (Table 4)which is in agreement with the observation of Laith *et al*. (2017) and Aluta *et al*. (2020). Serum total protein is indicative of nutritional and immune status of fish; hence, its enhancement is desirable. In the present study, serum aspartate aminotransferase were similar($p>0.05$) across dietary groups whereas alanine aminotransferase in the serum increased substantially ($p<0.05$) in the groups that received $ACLE_{7.5}$ and $ACLE_{10}$relative to the control. Increased transaminases in fish serum are typically a result of liver injury, which causes the

enzymes to seep into the bloodstream. The result of the present study is similar to that of Nurudeen *et al*. (2022) who documented increased ALT and AST consequent of *Eucalyptus globulus* leaf extract in *Oreochromis niloticus* diet. Our result suggests that caution must be taken when supplementing ACLE levels of 7.5 g/kg and beyond in *C. gariepinus* diet in order not to compromise the liver integrity.There was no significant (p>0.05) difference in serum creatinine, urea as well as total cholesterol and triglyceride among the experimental groups. The lowest urea (5.93 ± 0.48 mg/dL) and creatinine (40.97±7.00 µmol/L) levels were recorded in $ACLE_{2.5}$ fed group. Urea and creatinine are parameters associated with kidney function whose increase may suggest impairment of renal function (Mansour*et al*. 2022). The similarity observed in the urea and creatinine levels and those of total cholesterol and triglyceride, under current investigation, showed that dietary ACLE neither induced kidney dysfunction nor impaired lipid metabolism in*C. gariepinus* fingerlings.

The respiratory burst activity and total immunoglobulin varied from0.67±0.05– 0.89±0.15and 0.05±0.01–0.09±0.01g/dL, respectively (Table 5) and did not differ significantly (p> 0.05) across dietary groups. Immunoglobulins are known for their role in protecting fish against pathogenic invasions both internally and externally (Bulfon *et al*., 2015; Mashoof and Criscitiello, 2016). Respiratory burst is another mechanism through which fish can defend itself against pathogenic invasion by rapidly releasing toxic superoxide anion to deal with any pathogenic condition (Laith *et al*. 2017). The results on immunoglobulins and respiratory burst activity are consistent with those of WBC, total protein and its fractions and reasonably point to the fact that ACLE did not substantially boost the immunity of *C. gariepinus* unlike the results of some similar studies (Alambra *et al* 2012; Mohammadi *et al*., 2020) utilizing medicinal plants in which non-specific immunity of fish was boosted via elevated immunoglobulins, respiratory burst and lysozyme activities.

The serum and hepatic (Figure 1)SOD activityremarkably (p< 0.05) increased in ACLE fed groups relative to the control whereashepatic catalase (Figure 2) and reduced gluthathione of the serum were similar (p> 0.05) in treatedand control groups. Lipid peroxidation measured via serum malondialdehyde was similar (p > 0.05) in the control and treated groups. The actions of SOD, CAT and GSH in neutralizing free radicals into non-toxic chemicals constitute the primary line of defense in fish against oxidative stress (Wang *et al*., 2018; Zhang *et al*., 2019). SOD catalyzes the dismutation of superoxide into hydrogen peroxide (H_2O_2), which is then converted to oxygen and water by catalase. The bioactive phytochemical components of ACLE known to have free radical scavenging properties as previously reported by Olude *et al*. (2022) might be responsible for the increased activities of SOD in the serum and liver of *C. gariepinus*. Aluta *et al*. (2020) similarly documented significant increaseinthe expression of SOD gene after feeding *C. gariepinus* with diets containing onion peel.

Conclusion

This study clearly revealed that ethanolic leaf extract of *Alchornea cordifolia* is largely beneficial for the enhancement of antioxidant capacity of *C. gariepinus* fingerlings. Conclusively, a dietary level of 2.5 g/kg ethanolic leaf extract of *Alchornea cordifolia* was recommended to improve antioxidant capacity and generate antibody without compromising liver and kidney integrity of *Clarias gariepinus* fingerlings. There is need to pursue further studies using more concentratedproducts such as essential oil or polysaccharides isolated from *A. cordifolia* leaf.

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Conflict of interest

The authors declare no conflict of interest

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