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GROWTH PERFORMANCE, GUT ECOLOGY, IMMUNOCOMPETENCE AND RESISTANCE OF *Oreochromis niloticus* JUVENILES FED DIETARY *Curcumin longa*

Emmanuel Kolawole Ajani[1], Olugbenga Orisasona*[2], Oladeji Kazeem Kareem[1], Friday Elijah Osho[1], Aminat Omosalewa Adeyemo[1], Bamidele Oluwarotimi Omitoyin[1], Abimbola Olumide Adekanmbi[3]

[1] *University of Ibadan, Department of Aquaculture and Fisheries Management, Ibadan, Nigeria*
[2] *Obafemi Awolowo University, Department of Animal Sciences, Ile-Ife, Nigeria*
[3] *University of Ibadan, Department of Microbiology, Ibadan, Nigeria*

*Corresponding Author, Email: osasonagbenga@gmail.com

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ABSTRACT

The growth, gut ecology and immunocompetence of *Oreochromis niloticus* and the resistance to *Aeromonas hydrophila* were investigated after been fed with diets containing dietary *Curcumin longa* for 12 weeks. Diets were formulated to contain 30% crude protein with diet TC1, TC2, TC3, TC4 and TC5 having 0% (control), 0.25%, 0.5%, 0.75% and 1.0% turmeric powder, respectively. Diets were allotted to groups of *O. niloticus* (mean weight of 1.29± 0.15 g) and replicated thrice for 84 days. Results showed that the highest mean final weight (4.79±0.04 g) was obtained in TC3 and corresponded to the treatment with the highest feed intake. A significantly high ($p<0.05$) specific growth rate (SGR) was observed in TC3 (0.73±0.03 %day^{-1}) while TC4 (0.57±0.02 %day^{-1}) gave the lowest value. The highest microbial load in the gut was observed in TC1 groups and the least in TC4 groups. Red blood cell count, hemoglobin, packed cell volume did not show significant variation ($p>0.05$) across treatments. However, white blood cell (WBC) count was significantly higher in TC1 (control). There was an improved immunocompetence, as aspartate aminotransferase (AST) progressively reduces in fish fed supplements. Similarly, there was a better oxidative response in the treated groups with reduced hydrogen peroxidase, increased total protein and glutathione peroxidase. Mortality ranged from 25% in TC4 to 95% in TC1 after the challenge test with *A. hydrophila*. This study showed that *C. longa* inclusion at 0.5% is more beneficial when growth and health status of *O. niloticus* juveniles are considered.

How to Cite

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INTRODUCTION

The need for increased fish production to meet the constantly growing human population has necessitated intensification of aquaculture. The increased intensification and a large-scale commercialization of aquaculture is accompanied by incidences of diseases and their management (Bondad-Reantaso et al., 2005). Medications such as antibiotics are frequently used to control disease or improve fish's immune system, and therefore help enhance resistance against invading pathogens (Stephen et al., 2006). The traditional feed additives, including antibiotics, used to improve fish growth performance and boost health status may result in side effects that are unfavourable to man and the environment (Anne-Rebecca, 2014). The problems associated with the use of chemosynthetic treatments include the emergence and proliferation of antimicrobial-resistant pathogens (Cabello, 2006). These pathogens may be transmitted from the aquaculture environments to humans and lead to the eventual emergence of human pathogens with antimicrobial-resistant genes (FAO, 2005). Also, there is a problem associated with the reduction or elimination of beneficial microbiota inhabiting gastrointestinal ecosystem (WHO, 2006). The exploration of nature via herbal plants as alternative growth promoters to deliver dietary supplements that will boost the immune system improve health status and life activity becomes imperative (Al-Salahy, 2002). In the report of Harikrishnan et al. (2011), herbal extracts or their products added to the diet or directly injected modify the gut microflora, ecology and promote the immune response of freshwater fish species against pathogenic microbes. Improved growth, better immunity against pathogenic bacteria and survival were reported when herbal leaf supplement was fed to fish (Adeshina et al., 2017; Abdel-Tawab et al., 2018; Omitoyin et al., 2019).

Turmeric (*Curcuma longa*), a perennial herbaceous rizhome according to Chan et al. (2009), belongs to the family Zingiberaceae. It contains curcumin, curcuminoids, turmerone, artumerone and zingiberene which are all antioxidants, as active ingredients (Selvam et al., 1995). According to El-Bashir et al. (2007) and Shagufta et al. (2010), the most prominent active ingredient in *C. longa* curcumin is a strong antioxidant with hepato-protective characteristics. According to Al-Sultan and Gameel (2004), curcumin and its oil (atsiri) in turmeric stimulates digestive enzyme production, digestion and nutrient metabolism, and causes an improvement in the functioning of the small intestine. Therefore, this study evaluates the result of growth performance, gut ecology, hematology of *O. niloticus* fed diets supplemented with *Curcuma longa* and the ability to resist *Aeromonas hydrophila* infection.

MATERIALS AND METHODS

Tumeric rhizome and feed

Tumeric rhizome was procured and authenticated at the Herbarium, Botany Department, University of Ibadan. Rhizomes were rinsed, grated, dried in air at room temperature and reduced to fine particle size in an electric blender. Five isonitrogenous diets (Table 1) were formulated using Pearson's square method (FAO, 1990) with turmeric rhizome powder supplemented at 0, 0.25, 0.50, 0.75 and 1.00% for diets TC1 (control), TC2, TC3, TC4 and TC5, respectively (Adeshina et al., 2017; Mohammad, 2017 with slight modification). After milling and mixing of ingredients, diets were manufactured as pellets with a flat-die pelletizer (Model; OAZL150, Capacity: 60 – 80 kg per hour, with 2 mm diameter die). Each diet was air-dried and packed separately in well-labelled polythene bags until ready for use.

Experimental procedure and design

Oreochromis niloticus fingerlings obtained from Durante farm in Ibadan were acclimatized to conditions in the laboratory using aerated 1 m^3 tanks for 14 days. 300 *O. niloticus* (1.29±0.01 g mean weight) were thereafter randomly selected and stocked into 15 (30 L capacity) plastic aquaria at 20 fish per unit. Aquaria were supplied with air via electric pumps through an air compressor throughout the experimental period. Feeding was done twice daily between 7.00 hours-7.30 hours and 16.00 hours-16.30 hours for 12 weeks, with fish fed to satiation. During the feeding trials, wastes in each aquarium were siphoned out 30 mins before the first daily feeding. The quantity of feed given during each feeding is recorded and uneaten feed siphoned, weighed and used to calculate daily feed intake according to Helland et al. (1996).

About two-thirds of each aquarium water was replaced at an interval of three days throughout the duration of the trial. A Combined Digital Probe (YSI Model 57, New Jersey) was used to measure dissolved oxygen and temperature in culture units, while pH, ammonia and nitrite were measured with pH meter (Photoic 20; Labtech International, Heathfield, UK) and fish farming testing kit (Model FF-1A; HACH, Loveland, CO, USA), respectively. Pooled weekly range of water parameters recorded were 4.8-4.92 mg/L for dissolved oxygen, 6.3-6.5 for pH, 25.0°C–27.0°C for temperature, 0.1-0.2 mg/L for nitrite and 0.5-0.55mg/L for ammonia.

Chemical analysis

Samples of diets fed were analyzed for proximate content using the official method (A.O.A.C. 2005). Crude protein content was determined by digesting samples in sulfuric acid, distillation and titration (Kjeldahl method); the Soxhlet apparatus was used for ether extract determination using petroleum ether (40-60°C) for 3 hrs;

Table 1. Gross and chemical composition of experimental diets with graded level of tumeric rhizome (g/100 g DM)

Ingredients	TC1	TC2	TC3	TC4	TC5
Fishmeal	8.77	8.77	8.77	8.77	8.77
Soybean	26.31	26.31	26.31	26.31	26.31
Ground nut	17.54	17.54	17.54	17.54	17.54
Corn bran	20.69	20.69	20.44	20.19	20.14
Biscuit waste	20.69	20.44	20.44	20.44	20.24
Starch	2.00	2.00	2.00	2.00	2.00
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Premixes*	1.50	1.50	1.50	1.50	1.50
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
C. longa	0.00	0.25	0.50	0.75	1.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Crude protein	30.11	30.14	30.24	30.08	30.02
Crude fibre	7.82	7.31	7.11	6.98	6.85
Ether extract	6.18	6.15	6.32	5.92	6.12
Ash	5.58	5.50	5.15	5.62	5.29
Moisture	6.10	6.15	6.02	6.13	6.01
NFE	44.21	44.75	45.16	45.27	45.71

Note: Abbreviation: NFE, nitrogen free extract. *One kilogram contains: vitamin A, 4,000,000 IU; vitamin D3, 6,00,000 IU; vitamin E, 12,000 IU; vitamin K3, 15 mg; vitamin B1, 2,500 mg; vitamin B2, 1,750 mg; vitamin B6, 800 mg; vitamin B, 1,250 mg; nicotinic acid 3,750 mg; pantothenic acid, 5,000 mg; folic acid, 250 mg; biotin, 100.1 mg; choline chloride 1,20,000 mg; iron, 11,000 mg; copper, 1,800 mg; manganese, 6,000 mg; zinc, 20,000 mg; iodine, 400 mg; selenium, 40 mg; vitamin C (coated), 60,000 mg; inositol, 10,000 mg; cobalt, 150 mg

moisture contents were analyzed by drying samples in the oven at 50°C for 24 hrs and the muffle furnace used for ash determination at 550°C for 4 hrs.

Determination of growth and utilization of nutrient in experimental fish

Biweekly weight of fish per experimental unit and weight of feed consumed were monitored (Orisasona et al., 2017) using a weighing scale (OHAUS model C5000). After 84 days of feeding, growth in fish and nutrient utilization parameters (mean weight gain, MWG; specific growth rate, SGR; feed conversion ratio, FCR and protein efficiency ratio, PER) and survival rate (%) were calculated (Castell and Tiews, 1980) using bi-weekly data averages of measured weight and feed intake.

Analysis of blood and serum parameters of O. niloticus fed Curcumin longa diets

Nine fish from each treatment were selected randomly after the 84-day feeding trials and serially bled as described by Omitoyin et al. (2019). Two separate bottles received blood for each treatment. The first bottle had 20 U/L sodium heparinate to prevent coagulation for the analysis of platelets, WBC (white blood cells), RBC (red blood cells), Hb (haemoglobin) and PCV (packed cell volume). The second bottles without anticoagulant were allowed to clot at 4°C for serum biochemical analysis. For the estimation of PCV, samples of fresh blood were centrifuged in a microhaematocrit centrifuge for 10 min and measured in the tube reader (Hawkley and Sons, Lancing, UK). For Hb, 2

microlitres of the sample were taken using a micropipette, added to 5 ml of Drabkin solution and allowed to stand for 5 min. Colorimetric readings were taken by estimating the formation of cyanomethaemoglobin as described by Vankampen and Zijlstra (1961). Erythrocytic indices were determined according to Jain (1986), while WBCs were determined using a Neubauer haemocytometer according to Kaplow (1955). The colorimetric methods (Reitman and Frankel, 1957) were used to estimate AST (aspartate aminotransferase) and ALT (alanine aminotransferase), while ALP (alkaline phosphate) was estimated according to Tietz et al. (1983).

Determination of indices for oxidative stress

The liver of six fish randomly selected from each treatment were taken and held on ice for oxidative stress indices analyses. Liver samples weighing 0.5 g from each treatment were macerated with physiological saline and centrifuged (3,000 rpm) for 10 min (Ilavazhahan et al., 2012). Supernatants from the samples were then collected and put in plain bottles at −20°C for analysis. Superoxide dismutase (SOD) was measured according to Misra and Fridovich (1972) with slight modifications. The assay contained 1.0 ml of NBT 33 µmol/L, 50 mM sodium carbonate buffer pH 10.2, 0.25 ml of riboflavin 0.0033 mmol/L, 0.25 ml of methionine 10 mmol/L, 0.5 ml of EDTA 0.66 mmol/L and 50 µl of supernatant. The inhibition of nitroblue tetrazolium (NBT) was measured using a spectrophotometer at 560 nm. Reduced glutathione (GSH) was estimated in the liver using the method of Jollow et al. (1974). The addition of 5,5-dithiobis (2-nitrobenzoic acid), Ellman reagent to sulfohyryl compound produces a relatively stable yellow colour. The absorbance was read at 412 nm and the equivalent GSH was estimated from the standard GSH curve supplied in the kits.

The thiobarbituric acid reactive substance (TBARS) described by Varshney and Kale (1990) was used to spectrophotometrically estimate lipid peroxidation and malondialdehyde (MDA). This is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA). In the presence of heat at acidic pH, a pink complex was formed which absorbs maximally at 532 nm. The MDA level of liver was estimated from the absorbance. A modification to Lowry et al. (1951) in Hartree (1972) was used to determine total protein. The assay contained supernatant diluted in 1 mL H_2O and 0.9 mL of solution A (2 g L^{-1} potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 100 g L^{-1} sodium carbonate (Na_2CO_3) in 0.5 M NaOH) added before incubation for 10 min at 50°C. Samples were then cooled and 1 mL of solution B (0.2 g L−1 $KNaC_4H_4O_6 \cdot 4H_2O$ and 0.1 g L^{-1} copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in 0.1 M NaOH) was added and left for 10 min. Finally, 3 mL of solution C (Folin–Ciocalteu phenol reagent in H_2O (1:16 v/v)) was added before incubation for 10 min at 50°C. A standard curve was made of bovine serum albumin (BSA; 0, 0.0625, 0.125, 0.25, 0.5 and 1 g L^{-1}) and absorbance was read at 650 nm. Nitric oxide (NO) content, glutathione peroxide (GPx) and hydrogen peroxide (H2O2) were determined as described by Aebi (1984), Rajaraman et al. (1998) and Beutler et al. (1963), respectively.

Evaluation of gut bacteria and morphometric of O. niloticus fed experimental diets

Aseptically collected guts (intestine) from three fish per experimental unit were weighed into sterile bottles containing 0.1% peptone water for 2 hours for the release of the available bacteria. For serial dilution, 1 ml of sample from each bottle was diluted 10-folds and then using a dilution factor of $x10^{-4}$ subsequently. Thereafter, 1 ml was taken and dispensed into disposable Petri dishes and molten sterile medium of each agar was poured aseptically into each Petri dish corresponding to the label on the Petri dishes. The poured plates were swirled gently for even spread of inoculums, and allowed to cool and gel before incubation was carried out at 37°C (NL-9052-1 Newlife Laboratory Incubator). After 24-48 hours of incubation, organisms grew into visible separate colonies. The pure plate method was used for Total Heterotrophic Count (THC) and Total Coliform Count (TCC) as described by APHA (1985) and Bello et al. (2012). A Wincom Colony Counter with 16W, 220V±10% and 50Hz was used to count the colonies formed. All measurements were in triplicates and the TVC and TEB presented as Log_{10}CFU/g.

Five fish were also randomly selected per experimental unit for gut morphometric analysis. 30 mg/L of tricaine methane sulfonate (buffered) solution were used to tranquillize fish samples and the intestines were removed immediately for villi length, width and crypt depth measurements, using an Olympus CX21 light microscope (HE ×40) (CX21, Japan) with a micro-meter rule. Gut preparation on slides was according to Culling (1974) and Drury et al. (1967). Area of absorption (AA, cm^2) was calculated according to Eyarefe et al. (2008):

Area of absorption (cm^2) = Villus lenght (cm) × Villus width (cm)

Challenge test

The isolation, culture and maintenance of a virulent strain of *Aeromonas hydrophilla* were done using methods described by Collins et al. (1991). A strain of *A. hydrophila* from diseased *O. niloticus* was isolated and then inoculated on a blood agar plate. This was incubated according to Elgendy et al. (2015) at 37°C for 24 hr. An adjustment of bacteria culture was done to $1x10^7$CFU/ml using (PBS) phosphate-buffered saline. After feed withdrawal for 24 hr, 20 fish randomly selected per treatment were grouped into 2 (10 fish per group). 0.2 ml PBS containing $1x10^7$CFU/ml virulent *A. hydrophila* were injected intraperitoneally into a group (Misra et al., 2006). The second group serving as control was injected with 0.2 ml saline solution intraperitoneally. Experimental diets were given to fish after infection for 14 days, and daily mortalities and abnormal behaviours recorded.

The determination of Relative Percentage Survivals (RPS) of challenged fish was as described by Kocour et al. (2005):

RPS (%) = (Number of surviving fish after challenge) / (Number of fish injected with bacteria) x 100

Statistical analysis

Homogeneity of variance was performed on data generated by Bartlett's test. To establish the effect of *Curcumin longa* on experimental fish, data were further analysed using descriptive statistics and one-way ANOVA, and differences in means were separated using Duncan's Multiple Range Test at 95% Confidence Interval. The optimum inclusion level of *Curcumin longa* for growth was determined using polynomial regression.

RESULTS

Growth performance and nutrient utilization by cultured fish

Parameters for growth and utilization of nutrients in *O. niloticus* administered diets supplemented with *Curcumin longa* are as presented in Table 2. Fish fed diets containing 0.25% and 0.5% *C. longa* supplements showed significantly higher ($p<0.05$) final weights in comparison to treatments that received 0.0%, 0.75% and 1.00% supplements. Result showed higher weight gain in groups fed *C. longa* supplemented diets. However, the observed mean weight gain exhibited significantly higher ($p<0.05$) value in TC3 (3.62 g) while other treatments were statistically similar. This same trend was observed for the feed intake. Feed conversion ratio was highest in TC5 compared to other groups with the least value of 1.29 recorded in TC3. Fish survival rate was not significantly affected by treatment and ranged from 89.33% in TC4 to 94.66% in T1 and T2.

The relationship between *C. longa* inclusion level and weight gain using fourth-order polynomial regression is presented in Figure 1.

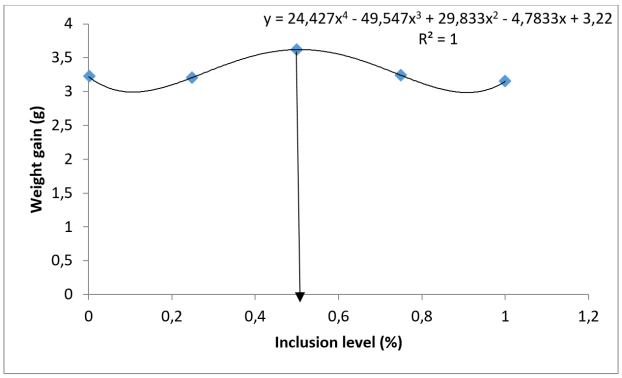

Fig 1. Optimum inclusion level of *Curcuma longa* in relation to mean weight gain

The curve showing inclusion level and growth is similar to a normal distribution curve and the optimal inclusion for weight gain is estimated as 0.56%. The relationship is best expressed by the equation within each plot area.

Haematology and serum biochemistry

The values for RBC, WBC, Hb and PCV did not vary significantly ($p>0.05$) among groups (Table 3) with values ranging from 1.77-2.49, 12.85-17.10, 7.70-7.87 and 22.66-25.66, respectively. WBC count lowered significantly ($p<0.05$) in groups fed *C. longa*. Differential leukocyte (%) counts in *O. niloticus* fed turmeric diets showed no significant variation ($p>0.05$), as presented in Table 4. Values of aspartate aminotransferase in sera were lower in fish that consumed *C. longa* diets compared with the TC1 group (control). However, alanine transferase values were statistically similar in TC1, TC2 and TC4, while TC5 had a significantly higher value. The percentage of leukocyte counts was not affected by *C. longa* supplement (Table 4).

Oxidative markers

The effect of *Curcumin longa* on the total protein and other oxidative stress indices in the liver are shown in Table 5.

Table 2. Growth performance and nutrient utilization of *Oreochromis niloticus* fed diets supplemented with *Curcumin longa*

Ingredients	TC1	TC2	TC3	TC4	TC5
Initial weight (g)	1.17±0.04	1.30±0.00	1.17±0.04	1.28±0.02	1.29±0.01
Final weight (g)	4.39±0.01^{c}	4.51±0.03^{b}	4.79±0.02^{a}	4.42±0.02^{bc}	4.44±0.04^{bc}
Weight gain (g)	**3.22±0.04^{b}**	**3.21±0.03^{b}**	**3.62±0.03^{a}**	**3.24±0.02^{b}**	**3.15±0.06^{b}**
Feed intake (g)	4.25±0.02^{b}	4.42±0.00^{b}	4.65±0.10^{a}	4.37±0.25^{b}	4.44±0.11^{b}
Feed Conversion Ratio	1.39±0.04^{b}	1.36±0.01^{b}	1.29±0.08^{c}	1.34±0.04^{b}	1.40±0.01^{a}
Specific growth rate (%day^{1})	0.64±0.32^{b}	0.62±0.10^{b}	0.73±0.03^{a}	0.57±0.02^{c}	0.60±0.03^{bc}
Protein efficiency ratio	2.39±0.08^{b}	3.13±0.03^{a}	3.30±0.03^{a}	3.14±0.22^{a}	3.06±0.13^{a}
Survival (%)	94.66±2.30^{a}	94.66±6.11^{a}	92.00±0.00^{a}	89.33±6.11^{a}	94.06±4.39^{a}

Mean values with same subscripts on same row are not significantly different ($p>0.05$)

Table 3. Haematological parameters of *Oreochromis niloticus* fed experimental diets containing different levels of *C. longa*

Parameters	Diets				
	TC1	TC2	TC3	TC4	TC5
RBC ($10^6 \mu l^{-1}$)	2.01±1.16^a	1.77±0.66^a	1.87±0.59^a	2.49±1.01^a	2.07±0.61^a
WBC (10^6 μl^{-1})	17.10±0.34^a	15.56±0.00^b	12.85±0.00^d	13.51±0.01^c	15.28^b
Hb (g dl^{-1})	7.73±0.92a	7.77±0.66^a	7.87±0.59^a	7.49±1.01^a	7.07±0.61^a
PCV (%)	24.33±3.21*a*	23.66±2.08^a	22.66±2.51^a	25.66±3.05^a	23.66±3.21^a
PLT (10^3 μl^{-1})	171.29±1.03^a	170.47±0.57^a	171.99±0.00^a	153.44±0.29^b	151.74±0.85^b
MCV (fl)	139.59±49.85^a	141.92±34.93^a	126.21±24.54^a	112.73±37.23^a	119.14±24.37^a
MCH (pg)	44.47±15.98^a	44.81±9.61^a	41.21±7.24^a	35.00±10.90^a	38.74±7.88^a
MCHC (g dl^{-1})	31.82±0.78^a	31.77±1.32^a	32.76±1.36^a	31.15±0.78^a	32.55±1.81^a
AST (µL)	208.44±5.46^a	190.51±5.31^b	184.95±1.67^b	191.66±1.20^b	190.66±0.33^b
ALT (µL)	27.33±0.16^c	27.59±0.03^c	29.91±0.30^b	27.14±0.12^c	30.92±0.34^a
ALP (µL)	189.14±0.12^b	179.33±0.38^c	173.88±0.11^c	191.15±0.25^{ab}	196.56±4.77^a

Mean values with same subscripts on same row are not significantly different (p>0.05)

RBC, red blood cell; WBC, white blood cell; Hb, hemoglobin; PCV, packed cell volume; PLT, platelets; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphate

Table 4. Different leukocyte (%) count in Nile tilapia fed diets supplemented by different levels of *C. longa*

Parameters	TREATMENTS				
	TC1	TC2	TC3	TC4	TC5
Basophils	0.33±0.57^a	0.33±0.57^a	0.66±0.57^a	0.33±0.57^a	0.00±0.00^a
Lymphocytes	63.33±6.42^a	60.00±6.00^a	62.00±3.60^a	65.33±5.85a	65.66±4.04^a
Heterophils	30.33±8.38^a	32.33±8.08^a	30.66±2.08^a	27.00±7.21^a	27.00±4.58^a
Monocytes	3.00±1.73^a	3.66±1.15^a	3.00±1.00^a	3.00±1.00^a	2.66±1.15^a
Eosinophils	3.00±1.00^a	3.66±1.52^a	3.66±1.52^a	4.33±1.15^a	4.66±1.52^a

Mean values with same subscripts on same row are not significantly different (p>0.05)

Table 5. Antioxidative effect on the liver of *Oreochromis niloticus* fed *C. longa* supplemented diets

Parameters	TREATMENTS				
	TC1	TC2	TC3	TC4	TC5
Total Protein (U/mg)	0.87±0.01^c	1.01±0.00^{ab}	0.94±0.02^b	1.02±0.01^{ab}	0.91±0.00^{bc}
H_2O_2 (µmol/mg)	125.5±0.09^a	112.96±1.41^c	90.43±0.29^e	115.53±0.36^b	92.90±0.05^d
MDA (U/mg tissue)	1.16±0.00^b	0.95±0.00^e	1.08±0.00^c	1.02±0.00^d	1.19±0.00^a
GSH (U/mg tissue)	81.85±0.36^c	82.45±0.02^c	104.38±0.28^b	103.62±0.31^b	148.90±0.37^a
GPx (U/mg tissue)	228.26±0.22^d	222.11±0.33^e	285.17±0.49^b	256.57±0.66^c	305.11±1.94^a
SOD (U/mg tissue)	9.86±0.01^c	8.10±0.00^e	10.55±0.00^b	8.37±0.00^d	11.12±0.05^a
NO (µmol/mg)	7.15±0.00^b	7.53±0.06^a	6.79±0.01^c	7.25±0.00^b	5.35±0.04^d

Mean values with same subscripts on same row are not significantly different (p>0.05)

H_2O_2, hydrogen peroxide; MDA, malondialdehyde; GSH, reduced glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; NO, nitric oxide

Significantly ($p<0.05$) lower total protein was observed in TC1 (0.87 µ/mg) and TC5 (0.91 µ/mg) groups compared with the other groups. Peroxidation values were significantly ($p<0.05$) higher in the liver of fish without supplement (TC1) compared to others. In this study, glutathione peroxidase ranged from 222.11 in TC2 to 305.11 U/mg tissue in TC5. Significantly higher SOD was observed in TC5 (11.12 U/mg tissue) with the least value recorded in TC2 (8.10 U/mg tissue). MDA was significantly higher in the control and TC5 groups (1.16 and 1.19 U/mg tissue, respectively), while the least was observed in TC2 (0.95 U/mg tissue).

Gut microbiota and morphometry

The heterotrophic count was highest in the control (TC1) with 26.13±4.20 X10^{4}cfu/g whereas TC4 had the lowest count of 20.30±2.11 X104cfu/g but showed no significant difference among treatments (Table 6).

A significant reduction was observed in the TCC as the inclusion of *C. longa* increased with the highest count recorded in TC1 (21.80±2.44 X104cfu/g) and TC2 (21.73±2.42 X104cfu/g), respectively.

Villi heights were marginally higher in fish fed *C. longa* diets as shown in Table 6 and this resulted in increased absorption area (AA) in fish fed the diets. The highest value was recorded in TC3 (1.68 x 10-2cm2), with TC1 having the lowest value (1.36 x 10^{-2}cm^2).

Challenge test

As shown in Table 7, fish survival against *A. hydrophila* was least in the fish not fed *C. longa* extract diets compared to the groups that received it. The least mortality of 20% was observed in TC4 while diet TC1 without *C. longa* supplementation recorded 100% mortality at the end of 14 days.

Table 6. Gut ecology and morphometric analysis of *O. niloticus* fed diets supplemented with *C. longa*

Growth performance	Diets				
	TC1	TC2	TC3	TC4	TC5
THC (X10^{4}cfu/g)	2.63±4.20	2.54±4.99	2.34±5.61	2.03±2.11	2.16±1.91
TCC (X10^{4}cfu/g)	2.18±2.44^a	2.17±2.42^a	1.94±4.92^{ab}	1.39±1.56^b	1.42±3.6^b
TEC (X10^{4}cfu/g)	ND	ND	ND	ND	ND
TLC (X10^{4}cfu/g)	ND	ND	ND	ND	ND
VH (cm)	0.17±0.16^b	0.19±0.05^{ab}	0.21±0.03^a	0.18±0.02^{ab}	0.19±0.03^{ab}
VW (cm)	0.08±0.03^a	0.08±0.03^a	0.08±0.12^b	0.08±0.01^a	0.08±0.01^a
AA (x 10^{-2}cm^2)	1.36±0.05^d	1.52±0.05^b	1.68±0.03^a	1.44±0.03^c	1.52±0.05^b

Mean values with same subscripts on same row are not significantly different ($p>0.05$)

Keys: ND: not detectable, THC: Total heterotrophic count, TCC: Total coliform count, TEC: Total E. coli count, TLC: Total Lactobacilli count, ND: not detected; VH; Villi height; VW, Villi width; AA, Absorption area

Table 7. Survival and mortality rate in treatment groups of *O. niloticus* fed different concentration of *Curcuma longa* for 84 days challenged with *Aeromonas hydrophila*

Parameter	TC1	TC2	TC3	TC4	TC5
Level of supplemented Tumeric	0	0.25	0.50	0.75	1.00
No. of challenged fish	20	20	20	20	20
Total no of dead fish at the end of 14 days	19	12	8	5	8
Mortality (%)	95	60	40	25	40

DISCUSSION

In this present study, fish that received 0.5% *Curcumin longa* extract diets had higher mean weight gain and specific growth rate. Similarly, intake of feed was higher in fish that consumed *C. longa* in comparison with the control group. Georgieva (2018) gave a similar result when common carp was fed diets supplemented with extracts of curcumin. The increase in feed intake may be attributed to the effect of curcumin which causes the release of gut hormones associated with digestion and nutrient utilization. This assertion is supported by Pransin (2006) who observed high protease and lipase activities and increased growth in goldfish fed turmeric supplemented diets. According to Prasad and Aggarwal (2011), turmeric improves the activities of lipase, amylase and chymotrypsin, thus functioning as a digestive stimulant. Turmeric has been reported to contain phenolic compounds, terpenoids, alkaloids and sterols, with curcuminoids and essential oil being the major bioactive ingredients (Li et al., 2011). These compounds cause the secretion of digestive enzymes in the fish digestive tract which enhances nutrient utilization and growth (Omitoyin et al., 2019; Kocour et al., 2005). In contrast, Abo-State and El-Deen (2017) and Peterson et al. (2014) reported that essential phytochemical products did not significantly affect the utilization of feed and growth in fish. Result of the polynomial regression shows a very strong positive regression exists between fish weight gain and inclusion levels of turmeric. The villus is a specialized tissue with a thin wall (one-cell thick) for the absorption of nutrients in the small intestine (Oxley et al., 2007). The morphometric configuration of the intestine in this present study shows an enlarged area of absorption in all fish fed turmeric diets. A similar result was reported by Omitoyin et al. (2019) feeding *Psidium guajava* leaf extract to juveniles of *O. niloticus* for 84 days. The increased surface area caused by the marginal increases in villi heights may have increased the nutrient absorption area and hence improved growth, as corroborated by Merrifield et al. (2010).

Haematological parameters expose the changes in animal physiology as a result of exposure to different stress conditions including poor feed and water qualities, hence, they are important indices pointing to the health condition of fish (Nwanna, et al., 2013; Olanrewaju, 2017). In this study, RBC, Hb and PCV values were similar across treatments. Although all investigated parameters fall within those recommended by Das et al. (2012) for a positive health status, *C. longa* had no significant effect on the RBC, Hb and PCV of *O. niloticus*. This contradicts earlier reports which indicated that bioactive substances such as contained in *C. longa* increased blood cell counts causing the authors to conclude that they enhanced immune system and improved natural defense in fish (Ajeel and Al-Faragi, 2013; Talpur et al., 2013; Das et al., 2013). Significantly lower white blood cell (WBC) counts were recorded in *O. niloticus* that received turmeric diets in comparison to the control group. Although *C. longa* did not cause an increase in red blood cells in this present study, the supplement did not result in any damage to cells and organs as evident in the reduced white blood cells.

Serum biochemistry profile is a very sensitive indicator for assessing liver and kidney function, fatty acid components in the blood, iron intake and storage. They are also indispensable in the study of immunity level and the adequacy of dietary intake. The data obtained revealed that ALT was statistically similar in experimental fish except in the TC5 group which had a significantly higher value. Aspartate transaminase was lower in fish fed *C. longa* diets. Lower values in fish fed *C. longa* diets are indicative of healthy liver, as reported by Jahanjoo et al. (2018) where reduced AST implied zero damage to the liver and a suppressed hepatic amino acid utilization. The increased values of ALP obtained in the present study at 0.75% and 1.00% turmeric inclusion levels indicate that extracts were better absorbed in the fish, and that build-up of liver cells was good and cell function was normal when compared with the control and lower levels of inclusion. This is affirmed by the observation of Omitoyin et al. (2019) feeding extracts of guava leaf to fish.

Carvalho et al. (2012) reported that indicators of oxidative stress and the first line of defense in fish are the oxidative biomarkers. The hydrogen peroxide value was lower ($P<0.05$) in the group of fish fed *C. longa* supplemented diets than the control. Also, MDA which is a final product in the peroxidation of lipid reduced significantly in treatment groups that received *C. longa* supplemented feed except in the group fed 1% inclusion. Thus, it may be inferred that supplementation improved the immunocompetence of the fish as a reduction in the liver values of malondialdehyde and hydrogen peroxide are indicators of no-damage to body cells (Yagi, 1984). In this study, peroxidase (GPx) content in livers was higher in fish fed *C. longa* supplemented diets and therefore are expected to have more innate immune-defense to wade off pathogenic organisms (Stosik et al., 2001). Also, increased GPx is known as a reliable indicator for antimicrobial activity of white blood cells, most especially the primary granule cellular molecule transport (Quade and Roth, 1997). Similarly, in this present study, the activities of plasmatic glutathione (GSH) increased significantly in fish fed *C. longa* supplements while there was a reduction in the values of SOD (superoxide dismutase). Total protein content increased slightly in all fish that received diets containing curcumin, compared to the control. However, the ranges of total protein in this study fell below the recommended standard reported for most freshwater teleost (Das et al., 2012).

Fish that had higher levels of *C. longa* extract supplementation had lower bacteriological profile as both total heterotrophic and coliform counts showed significantly higher values ($p<0.05$) in the control groups and TC2. This desired result from turmeric may

be attributed to its constituent antioxidants that are potent (Salama and El-Bahr, 2007; El-Bahr et al., 2007). A modification of the gut flora of fish with beneficial microbiota and microbial enzymatic activities is reported in Adeshina et al. (2017). Cao et al. (2015) stated that *C. longa* is an anti-inflammatory and immunomodulatory agent.

Fish survival rate and protection against *A. hydrophila* infection were significantly improved with fish groups supplemented with curcumin compared to the control. The challenge with infection resulted in 100% mortality in TC1 group, while significantly lower mortality was recorded in TC3 group. The result was in accord with the findings of Saly et al. (2016) where reduced mortality in *Oreochromis niloticus* fed curcumin diets and post-challenged with *Aeromonas hydrophila* was reported. The same phenomenon was also observed by Amany Diab et al. (2014) in *Oreochromis niloticus* fed curcumin supplementation and challenged with *Pseudomonas fluorescens*. Similar trend was also reported by Mahmoud et al. (2014) for Nile tilapia fed turmeric extract supplementation. Saad et al. (2013) also observed lower mortality in sea bass fed *Nigella sativa* (black cumin seeds) with or without *Curcumin longa* (turmeric) when injected with *Pseudomonas fluorescence*.

CONCLUSION

From this study, it can be deduced that the supplementation of *C. longa* extract resulted in a significant improvement in the utilization of nutrients in feed and growth of *Oreochromis niloticus*. Fish fed the extract inclusion level of 0.52% performed better than other treatments in terms of weight gain. Higher levels of *C. longa* extract inclusion were also found to reduce microbial loads in the gut of *O. niloticus* and enhanced the survival of *O. niloticus* against *A. hydrophila*.

SAŽETAK

UČINKOVITOST RASTA, EKOLOGIJA CRIJEVA, IMUNOKOMPETENCIJA I OTPORNOST MLAĐI *Oreochromis niloticus* HRANJENIH HRANOM S DODATKOM *Curcumin longa*

U radu je istraživana ekologija crijeva i imunokompetencija nilske tilapije *Oreochromis niloticus* te njena otpornost na *Aeromonas hydrophila* nakon 12 tjedana hranidbe hranom koja sadrži kurkumu *Curcumin longa*. Smjese su formulirane da sadrže 30% sirovog proteina (TC1, TC2, TC3, TC4 i TC5) s 0% (kontrola), 0,25%, 0,5%, 0,75% i 1,0% praha kurkume. Hrana je ponuđena skupinama *O. niloticus* (prosječna masa 0,5 ± 0,15 g) u triplikatima kroz trajanje od 84 dana. Rezultati su ukazali da je skupina T3 imala najveću završnu masu (4,79 ± 0,04 g) i odgovara skupini s najvećim unosom hrane. Značajno viša ($p<0,05$) specifična stopa rasta (SGR) zabilježena je u TC3 (0,73 ± 0,03% dan^{-1}) dok je skupina TC4 (0,57 ± 0,02% dan^{-1}) dala najnižu vrijednost. Najviša koncentracija mikroorganizama u crijevima zabilježena je u TC1 najmanje u TC4. Broj eritrocita, hemoglobina, hematokrit nije pokazao značajne razlike ($p>0,05$) u različitim tretmanima. Međutim, broj bijelih krvnih stanica (WBC) bio je značajno viši u TC1 (kontrola). Utvrđena je poboljšana imunokompetentnost kod skupina sa suplementiranom hranom. Slično tome, došlo je i do boljeg oksidativnog odgovora u skupinama tretiranim kurkumom i to u obliku smanjene hidrogen peroksidaze, povećanim ukupnim proteinima i glutation-peroksidazom. Smrtnost riba se kretala od 25% u TC4 do 95% u TC1 nakon ispitnog testa s *A. hydrophila*. Ovo istraživanje je ukazalo kako je uključivanje *C. longa* od 0,5% korisnije kada se uzmu u obzir rast i zdravstveno stanje mlađi *O. niloticus*.

Ključne riječi: Flora crijeva, *Curcumin longa*, rast, imunološki sustav, *O. niloticus*, *Aeromonas hydrophila*

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