Int Aquat Res (2021) 13:37–51
https://doi.org/10.22034/IAR.2021.1916077.1115

ORIGINAL RESEARCH

Effects of dietary tamarind (*Tamarindus indica L.*) leaves extract on growth performance, nutrient utilization, gut physiology, and susceptibility to *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus* L.)

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Received: 25 November 2020 / Accepted: 10 March 2021 / Published online: 28 March 2021

Abstract The rise in global concern on the adverse effects of uncontrolled utilization of synthetic antibiotics in the production of food fish necessitates search for alternative natural products in aquaculture. Tamarind (*Tamarindus indica*) leaf has great medicinal potentials but with scanty documentation of its utilization in fish production. Therefore, this study investigated the effect of tamarind leaves extract (TLE) on the growth performance, apparent nutrient digestibility, gut physiology, and resistance against *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*) fingerlings. The fish were fed experimental diets enriched with 0.0 (control), 5, 10, 15 or 20 g TLE/kg diet at 3% body weight daily for 12 weeks. Thereafter, a 4-week challenge test with *A. hydrophila* infection was done. The results showed that dietary TLE significantly ($P < 0.05$) enhanced fish growth, nutrient digestibility, and utilization, villi height and absorption area at 1.0-1.5% inclusion levels, compared to the control diet. Regression analysis showed 1.12% as the level of TLE for optimum weight gain. Post-challenge fish fed TLE-enriched diets showed higher survival rate, relatively to fish fed the control diet. The results from the present study demonstrated that dietary TLE promoted growth, nutrient digestibility and protection against *A. hydrophila* infection in Nile tilapia and its inclusion at 1.0% was therefore recommended for aquaculture use.

Keywords *Tamarindus indica* extract, Nile tilapia, fish growth, gut morphology, *Aeromonas hydrophila* infection

Introduction

Farming of finfishes and other inland aquatic animals grew consistently, contributing 66.1% and more than half of cultured fisheries, respectively, in 2018 (FAO 2020). Nile tilapia, *Oreochromis niloticus* is a major inland finfish, widely accepted food fish, intensively cultured globally and the mostly cultured in Africa. The major health challenge in intensive aquaculture is infectious diseases caused by both pathogenic and opportunistic bacteria, one of which is *Aeromonas* species. *Aeromonas hydrophila* has been reported to be

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a major pathogen associated with high mortality and economic losses in several fish species (Pachanawan et al. 2008; Zahran et al. 2018; Hardi et al. 2019; Adeniyi et al. 2020). To control the losses due to the bacterial infection, fish farmers apply synthetic antibiotics as prophylactic or therapeutics agents. However, the utilization of these synthetic agents has been under serious global scrutiny due to prevalence of bacteria-drug resistance and environmental hazards (Santos and Ramus 2018).

In the past few decades, efforts have been shifted to the application of herbal products used in human traditional medicine for the production of livestock and aqua products, in order to promote organic medicine and control the adverse effects of synthetic antibiotics. The utilization of herbal products is widely accepted as an alternative means of preventing the cumulative effects of synthetic drugs resulting to the development of bacteria resistance to drugs and the residues in fish meat for human consumption. Various herbal products have been applied for fish production as feed additives in diverse forms, including shoot (Kareem et al. 2016) or other parts such as pulp (Adeniyi et al. 2017a; 2018a; 2020), bud (Adeshina et al. 2019), leaf (Soltanian and Fereidouni 2016), root (Abdel-Tawwab 2012; Zahran et al. 2018) or seeds (Mukherjee et al. 2018; Nyadjeu et al. 2019) with proven remarkable output relating to promotion of nutrient digestion, appetite, growth, antimicrobial activity, anti-oxidation, wound-healing, immunity, and disease-resistance, among others.

Tamarindus indica L. is a monotypic genus tree with a dense crown of feathery, alternate compound leaves, native to tropical Africa but are also grown widely in sub-tropical regions of the world (Odugbemi 2008; Bhadoriya et al. 2011; Fandohan et al. 2015). The plant is mostly cherished for its fruit pulp, as evident from scientific information on its constituents, medicinal and industrial usage (Khairunnuur et al. 2009; Julio et al. 2010; Nwodo et al. 2011; Jimoh and Onabanjo 2012; Adeola 2013; Menezes et al. 2016; Adeniyi et al. 2018a, 2018b, 2020). Tamarind leaves are edible and often used to make salads, curries and soups. Also various phytochemicals such as benzyl benzoate, flavonoids, limonene, luteolin, lupeol, orientin, palmitic acid, tartaric acids and terpenoids have been reported to be present in the plant and are associated with the antimicrobial, antioxidant, hepatoprotective and wound-healing properties of tamarind leaves (Pino et al. 2002; Imam et al. 2007; Khairunnuur et al. 2009; Havinga et al. 2010; Adeniyi et al. 2017b; Adeniyi et al. 2018a). Despite the valuable phytoconstituents and properties of tamarind leaves, there is scanty scientific information on its application in the production of food animals, including fish. Therefore, the present study aimed to investigate the effects of tamarind leaves extract on the performance of Nile tilapia.

Materials and methods

Preparations of tamarind leaves extract (TLE)

Fresh and mature tamarind green leaves were obtained from tamarind tree, washed with clean water, drained and shade-dried for 14 days at room temperature. The leaves were ground into fine powder and extracted with 96% ethanol and distilled water (ordinary, warm and hot) as described previously (Adeniyi et al. 2017b) at 1:10 (w/v), for 48 h during which it was agitated on mechanical shaker for 16 h. It was thereafter centrifuged at 4000g for 15 min and the supernatant was concentrated into paste using a rotary evaporator (SE-CF-TDZ-WS; Labkits U-Therm International Limited, Hong Kong), freeze-dried, and kept in freezer until use. The quantities of alkaloids (Gurinder and Daljit 2009), flavonoids (Edeoga et al. 2005) and saponins (Obadoni and Ochuko 2001) in TLE were determined in each extract.

Preparation of diets

Five isonitrogenous (≈ 30%) and isocaloric (≈ 1690 kj/100g gross energy) diets were prepared as shown in Table 1. The warm aqueous extract [based on our previous antimicrobial study (Adeniyi et al. 2017b) and phytochemical composition (Table 2)] was mixed with the basal diet at 0.0 (control), 0.5, 1.0, 1.5 or 2.0%. Cassava starch was used as binder and all ingredients were thoroughly mixed and then pelleted into 2mm sizes using a pelleting machine (Shuangying; SYSLJ-1, China). The pellets were sun-dried for 12 hours, hand-crumbled into smaller sizes, packed in separate airtight polythene bags, labeled, and stored at room temperature during the experimental period.

Table 1 Ingredients and proximate compositions of experimental diets (% dry matter) containing different levels of tamarind leaf extract (TLE)

Ingredients	**Levels of inclusion (%)**				
	0.0	0.5	1.0	1.5	2.0
Fish meal [a]	12.07	12.07	12.07	12.07	12.07
Soybean meal [b]	27.20	27.20	27.20	27.20	27.20
Groundnut cake [c]	25.16	25.16	25.16	25.16	25.16
Corn meal	25.57	25.57	25.57	25.57	25.57
Soy oil	2.00	2.00	2.00	2.00	2.00
Common salt	0.50	0.50	0.50	0.50	0.50
Bone meal	0.50	0.50	0.50	0.50	0.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix [d]	2.00	2.00	2.00	2.00	2.00
Chromium oxide	0.50	0.50	0.50	0.50	0.50
Starch	4.00	3.50	3.00	2.50	2.00
TLE	0.0	0.50	1.00	1.50	2.00
Total	100	100	100	100	100
Proximate composition					
Moisture	8.17	8.17	8.16	8.15	8.15
Crude protein	30.01	30.01	30.01	30.02	30.02
Crude fiber	4.39	4.39	4.38	4.40	4.39
Crude lipid	7.74	7.75	7.75	7.75	7.76
Ash	10.12	10.03	10.03	10.03	10.14
Nitrogen free extract (NFE)[e]	39.57	39.65	39.67	39.64	39.55
Gross energy (kj/100g)[f]	1686.81	1688.43	1688.69	1688.64	1687.04

[a, b, c] Nigerian Ingredients containing 51.70, 44.70, 36.35% crude protein , respectively
[d] Vitamin A = 20, 500.00IU, Vitamin B1 = 20, 000.00mg, Vitamin B2 =15, 000.00, Vitamin B3 = 90, 000mg, Vitamin = 4, 000. 00mcg, Vitamin B5 = 40.00mg Vitamin B6 = 20, 000.00, Vitamin B7 = 500.00mcg, Vitamin B12 = 15.00mcg, Vitamin C = 350, 000.00mg, Vitamin D3 = 4, 250, 000.00iu, Vitamin E = 250, 000.00iu, Vitamin K = 8, 000.00mg, Copper sulphate = 4, 000.00mg, Potassium Iodine = 2,000.00mg, Inositol = 50, 000.00mg, Methionine = 50, 000.00mg, Choline chloride = 600, 000.00mg, Ferrous sulphate = 40, 000.00mg, Manganese oxide = 30, 000.00mg, Magnesium = 60, 000.00mcg, Molybdenum = 100.00mg, Antioxidant125, 000.00mg, Lysine = 50, 000.00mg, Cobalt = 750.00mg, Sodium selenite 200.00mcg, Zinc oxide =40, 000.00mg.
[e] = 100 – (Crude protein + Crude fiber + Crude lipid + Ash)
[f]23.6, 39.5 and 17 kj/g for protein, lipid, and NFE, respectively (NRC 1993)

Experimental fish and feeding trial

The *O. niloticus* fingerlings were obtained from a local fish farm in Ilorin, Nigeria. After a two-week acclimation, fish (N = 450, 4.66 ± 0.15g) were randomly distributed into 100-L circular plastic tanks in three replicates of 30 fish per tank. The fish were hand-fed daily with the experimental diets at 3% body weight; the daily ration was divided into two and offered in the morning and evening for 12 weeks. The feed ration was adjusted fortnightly as the fish increased in weight. The culture water in each tank was completely renewed at three-day interval with gentle agitation with air-stone to maintain good water aeration. The water pH, temperature, and dissolved oxygen (DO) were measured and monitored with digital Hanna pH meter (pHep; HI98107, USA) and dissolved oxygen-temperature meter (AMT07; C. V. Java Multi Mandiri, Indonesia). The range of water temperature, pH, and DO were 25.2 – 27.5ºC, 7.0-7.3, 4.3 – 5.4mg/L, respectively.

Growth performance and feed utilization

Five fish were taken randomly from each replicate before feeding fortnightly and weighed to assess their growth rates and adjust feeding ration. Thereafter, the following parameters were calculated from the data obtained:

Weight gain (WG, g) = Final weight (FW) – Initial weight (IW);
Relative growth rate (RGR, %) = 100 (WG / Initial weight);
Specific growth rate (%/day) =100 (Ln FW – Ln IW) / Duration of feeding trial (days)
Feed conversion ratio (FCR) = Feed intake (FI) / WG;
Protein efficiency ratio = WG / Protein intake;

(Protein intake = Crude protein in diet × FI)
Nitrogen metabolism (Nm, %) = 0.549 × h (FW + IW) / 2;
Where, h = duration of feeding trial (days)
Apparent protein utilization (APU, %) = 100 (Protein gain in fish / Protein intake)
Apparent energy utilization (AEU, %) = 100 (Energy gain in fish / Energy intake)
(Energy intake = Gross energy in diet × FI)
Fish survival (FS, %) = 100 (Number of survived fish / number of fish stocked)
Fish productivity index (FPI) = (WG × FS) / (FCR × 10).

Collection of faeces

Collection of faecal samples commenced after 7 days of the feeding trial to allow complete evacuation of the previous feeds. The culture water was completely drained from each experimental tank and replaced after one hour of the last meal for the day, to ensure proper cleaning and prevent contamination with uneaten feeds. The faeces were siphoned from the tanks after 14-16 hours of the last feeding for the day (Belal 2005; Koprucu and Ozdemir 2005), filtered with Whatman filter paper (number 1), thrice per week for 12 weeks, oven-dried at 50ºC, and kept in freezer at -35ºC until use for digestibility analysis.

Proximate chemical analyses

Samples of the whole fish, diets, and dried faeces were analyzed for proximate composition (AOAC 2005). Briefly, the moisture content was determined by drying the samples to constant weight at 105°C using oven (Mini/50/SS, Genlab, England) while the crude lipid contents were determined by ether extraction using soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA). The crude fiber contents of the lipid-free samples were estimated after digestion with acid and base followed by oven-drying. Ash content was estimated by combustion in electric muffle furnace (Shangai Gwangdi Geological Instrument Equipment Co Ltd.) for 6 h at 550°C. The nitrogen contents of the samples were determined with automated digester (8 Holes, Foss Tecator digester, Denmark) and Kjeltec auto distillation unit (Kjeltec 8200, Denmark) and then multiplied by 6.25 to estimate the crude protein, while the gross energy was calculated according to NRC (1993).

Nutrient digestibility

The samples of diets and faeces were digested with concentrated Trioxonitrate (V) and 70% perchloric acids and then cooled to allow complete oxidation to determine the quantities of Cr_2O_3 in the samples (Furukawa and Tsukahara 1966). Thereafter, the oxidized solutions were diluted with distilled water to make 100 mL. The absorbance of the resulting solutions was determined by spectrophotometry (Jenway 6305 model, Keison International Ltd., UK) at 350 nm. The apparent digestibility coefficient (ADC) of nutrients was calculated from the ratio of the chromium oxide in the diets or faeces as shown in the formula (Koprucu and Ozdemir 2005) below:

$$ADC = \{100 \times [(1 - (D_{Cr2O3} / F_{Cr2O3}) \times (F_{Nutrient / Energy} / D_{Nutrient / Energy})]\}$$

Where, F = Faeces, D =Diets

Gut microflora and morphology

Fish guts were aseptically removed from the sampled fish in each replicate and examined. To examine the microflora, the guts were weighed and homogenized with mortar and pestle; the homogenates were properly mixed with sterile peptone water to 10 mL in McCartney bottles and serially diluted to 10^{-10}. Thereafter 0.1 mL of each dilution was put in sterile Petri dish and seeded with sterilized differential agars; plate count agar (for total viable count), potato dextrose agar (to enumerate mould growths), MacConkey agar (for *Escherichia coli* and other enteric bacteria count), and yeast extract agar (for yeast, *Saccharomyces* sp. count) using pore plate technique. Each of the samples was plated in triplicate and incubated at 37ºC for 24 hours for bacteria and 3 to 5 days for moulds. The total number of cells of samples was counted to estimate the total colony forming units (CFU) on the plates, CFU/g was calculated, expressed as Log_{10}CFU/g and

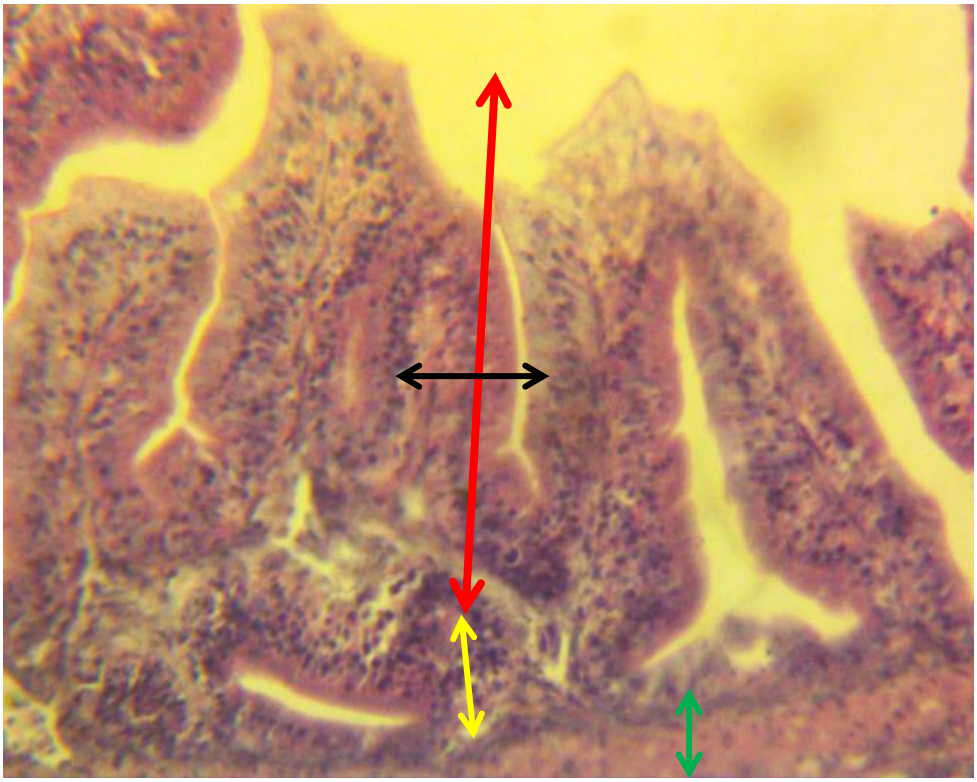

Plate 1 A representative photomicrograph of villi of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks, showing villus height (red arrow), villus width (black arrow), crypt depth (yellow arrow), and the *Mucosa muscularis* thickness (green arrow)

identified using Bergy's manual of bacteriology (Holt et al. 1994 ; Rahimi et al. 2011; Ramiah et al. 2014). To determine gut morphometrics, fish guts were removed and immediately placed in 10% formaldehyde and thereafter processed for histology following the procedure described by Takashima and Hibiya (1995). Briefly, the guts were dehydrated in graded levels of ethanol, cleared in xylene and embedded in paraffin wax and were thereafter sectioned at random to 5 µm thickness from proximal, mid, and distal parts; stained with haematoxylin and eosin on slides and viewed microscopically. Gut histomorphological parameters were measured according to the description of Awad et al. (2009) and Adeniyi et al. (2020). Villus height, villus width, crypt depth, and the *Mucosa muscularis* thickness (Plate 1) were measured using a binocular microscope (Olympus, USA), with a micrometer ruler and digital camera. The goblet cells were counted while the absorption area was calculated from the product of villus height and its width. The mean for the proximal, mid and distal regions of the gut was used for all parameters in this study.

Challenge with pathogenic bacterium (*Aeromonas hydrophila*)

The stock of *A. hydrophila* was obtained from the Department of Microbiology, University of Ibadan. The bacterium was isolated from diarrhoeagenic stool, characterized using biochemical and molecular tools (Adegoke and Ogunbanwo 2016) and with virulence properties (Adegoke and Ogunbanwo 2018). It was stored in glycerol medium, resuscitated using *Aeromonas* agar and maintained on slants at 4ºC. The bacterium was sub-cultured on nutrient agar at 37ºC for 24 hours; colonies were aseptically picked, dissolved in sterilized peptone water and standardized with 0.5 McFarland standard (1 x 10^8 CFU/mL) as described by CLSI (2012). The standardized *A. hydrophila* was added to tanks containing 20 L of water at density of 0.2 mL/litre of water for bath challenge. Fish from each replicate were immersed in the inoculated water at 15 fish / tank for one hour (Adelmann et al. 2008; Emmenegger et al. 2013). The challenged fish were transferred into culture tanks, fed with their respective diets, and observed for four weeks during which mortality were recorded. The cumulative mortality (CM) and relative survival (RS) were also calculated (Amend 1981) as follows:

Table 2 Quantity (%) of selected groups of phytochemicals in tamarind leaf extracts (TLE)

TLE	Alkaloids	Flavonoids	Saponins
Ordinary aqueous	4.73 ± 0.04^{b}	9.05 ± 0.06^{b}	3.20 ± 0.01^{c}
Warm aqueous	5.51 ± 0.04^{a}	9.51 ± 0.04^{a}	2.91 ± 0.02^{d}
Hot aqueous	4.07 ± 0.04^{d}	7.47 ± 0.04^{c}	3.41 ± 0.01^{b}
Ethanol	4.99 ± 0.03^{c}	7.27 ± 0.03^{d}	3.47 ± 0.02^{a}

Means with different superscripts in the same column are significantly different at $P < 0.05$.

Table 3 Growth performance, nutrient utilization and survival of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks

Parameters	TLE levels (%)				
	0.0	0.5	1.0	1.5	2.0
Initial weight (g/fish)	4.66±0.02	4.69±0.01	4.64±0.04	4.59±0.02	4.60±0.02
Final weight (g/fish)	14.00 ± 0.10^{e}	14.72 ± 0.03^{c}	15.30 ± 0.10^{a}	14.99 ± 0.16^{b}	14.37 ± 0.10^{d}
Weight gain (g/fish)	9.34 ± 0.11^{e}	10.03 ± 0.04^{c}	10.69 ± 0.07^{a}	10.39 ± 0.14^{b}	9.77 ± 0.10^{d}
Specific growth rate (%/day/fish)	1.31 ± 0.01^{c}	1.36 ± 0.01^{b}	1.42 ± 0.01^{a}	1.41 ± 0.01^{a}	1.36 ± 0.01^{b}
Feed intake (g/fish)	16.84±0.31	17.27±0.70	16.87±0.36	17.26±0.19	16.58±0.42
Feed conversion ratio	1.80 ± 0.05^{a}	1.73 ± 0.07^{b}	1.58 ± 0.02^{c}	1.66 ± 0.01^{b}	1.70 ± 0.03^{ab}
Protein efficiency ratio	1.85 ± 0.06^{c}	1.94 ± 0.08^{b}	2.11 ± 0.02^{a}	2.01 ± 0.01^{b}	1.96 ± 0.03^{b}
Apparent protein utilization (%)	61.19 ± 0.64^{bc}	61.34 ± 0.40^{abc}	62.11 ± 0.25^{a}	61.89 ± 0.44^{ab}	61.03 ± 0.31^{c}
Apparent energy utilization (%)	23.75±0.75	23.90±1.09	23.81±1.07	23.68±1.00	24.07±1.06
Fish Survival (%)	92.22±5.09	93.33±3.34	95.55±3.85	93.33±3.34	93.33±0.00

Means with different superscripts within a row are significantly different at $P < 0.05$.

i. $CM = Dt_1 + Dt_2 + Dt_3 + \text{---------- } Dt_n$

Where, D is the number of dead fish at the given time (days) t_1, t_2, t_3. ---------- t_n

ii. $RS = [1 – (\%\text{ mortality in treatment} / \%\text{ mortality in control})] \times 100$

Statistical analysis

Statistical significance was determined by one-way analysis of variance while comparison among means was by Duncan multiple range test. The quadratic regression was used to determine the optimum inclusion levels of dietary TPE levels. The analysis was done using statistical package for social sciences (IBM SPSS Statistics for Windows, IBM Corp., Version 23, Armonk, NY). The statistical significance was established at $P < 0.05$.

Results

The preliminary phytochemical screening of tamarind leaf extracts showed flavonoids as the highest components, followed by the alkaloids while the least was saponins. Significantly highest flavonoids, alkaloids and lowest saponins ($P < 0.05$) were present in tamarind warm aqueous extract, compared to the other extracts (Table 2).

Growth performance

The growth of Nile tilapia fed dietary TLE were significantly enhanced ($P < 0.05$), when compared to the fish fed the control diet (Table 3). The FW, WG, and SGR rose to the peak at 1.0% TLE and started decreasing, although the SGR of fish fed 1.0 and 1.5 % TLE-enriched diets did not differ ($P < 0.05$). The feed intake was not significantly affected ($P > 0.05$). However, the significantly lower FCR and higher PER were obtained in fish fed TLE-enriched diets, with the lowest FCR and highest PER at 1.0% TLE inclusion level. The APU of fish fed 1.0% was also higher ($P < 0.05$), compared to the fish fed the control and 2.0%

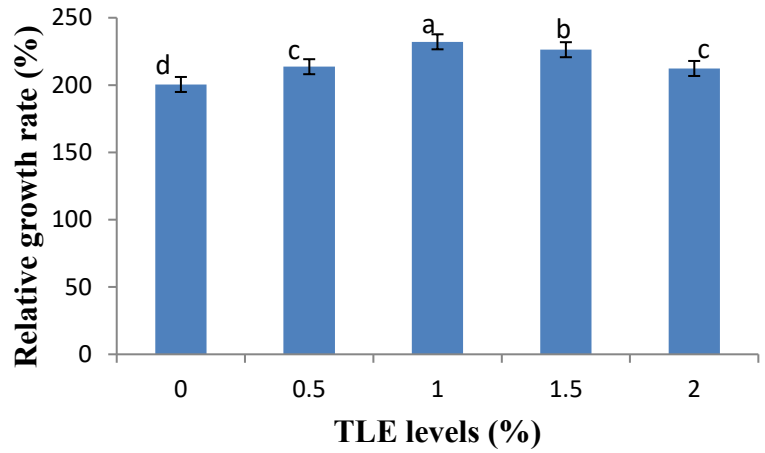

Fig. 1 The relative growth rate of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks

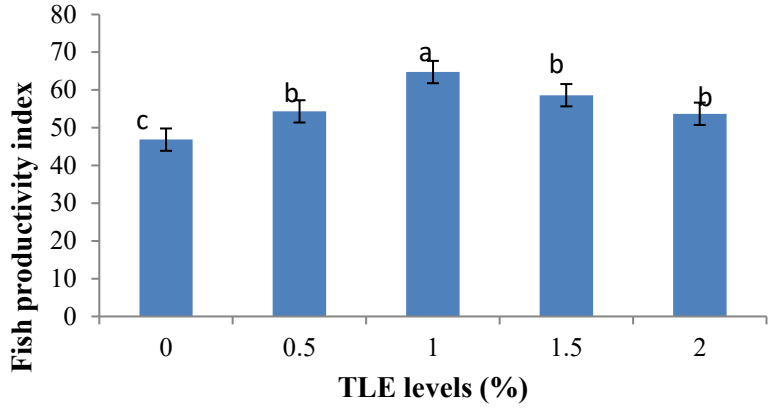

Fig. 2 The fish productivity index of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks

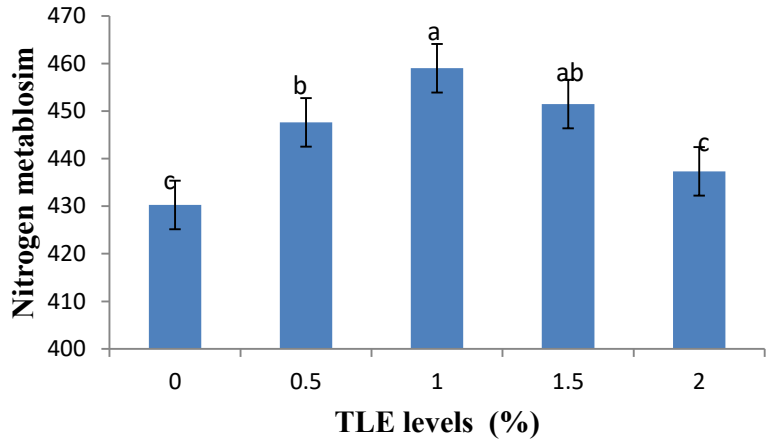

Fig. 3 The nitrogen metabolism of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks

TLE-enriched diets, while the AEU and fish survival were not affected ($P > 0.05$) by dietary TLE, although highest values of these parameters were obtained at 1.0% TLE level. Similar to the observations on WG and SGR, the RGR (Figure 1) and FPI (Figure 2) of the fish were also significantly higher at 1.0% TLE level, when compared to other treatments. Higher ($P < 0.05$) Nm (Figure 3) was obtained in fish fed 0.5-1.5 TLE-enriched diets, compared to those fed the control and 2.0% TLE-enriched diets. The regression analyses of the relationship between dietary TLE levels and FW (Figure 4), WG (Figure 5), and RGR (Figure 6)

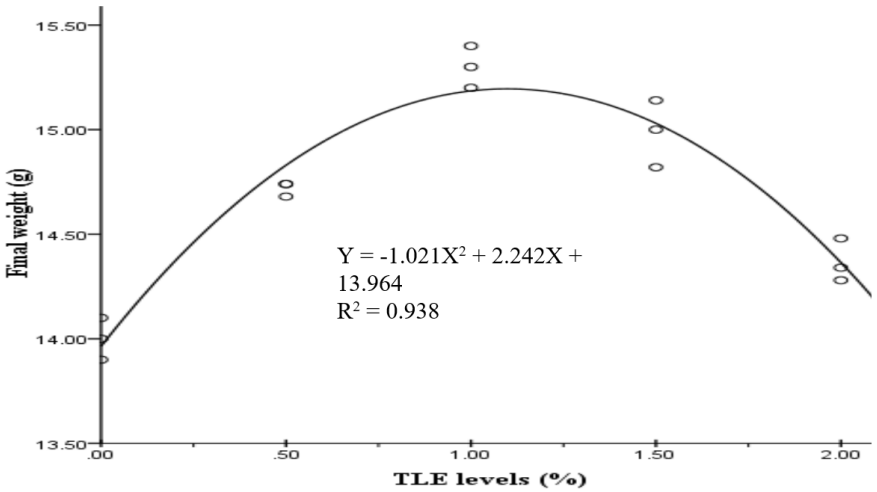

Fig. 4 The relationship between dietary tamarind leaves extract (TLE) and final weight of *Oreochromis niloticus*

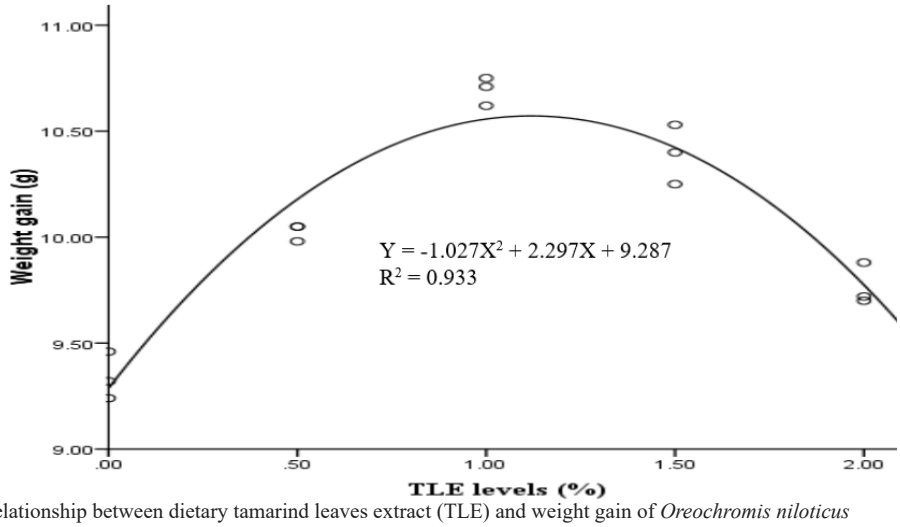

Fig. 5 The relationship between dietary tamarind leaves extract (TLE) and weight gain of *Oreochromis niloticus*

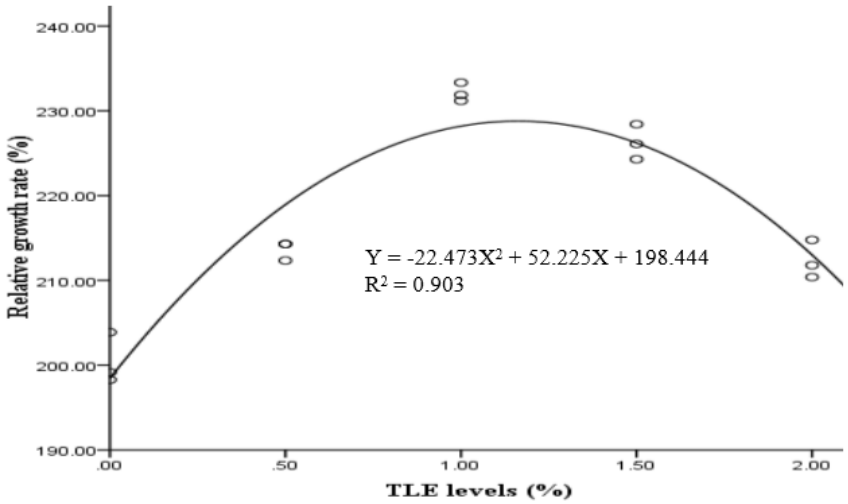

Fig. 6 The relationship between dietary tamarind leaves extract (TLE) and relative growth rate of *Oreochromis niloticus*

showed 1.10, 1.12, and 1.16%, respectively as the optimum dietary inclusion levels of TLE in the present study.

Table 4 Apparent digestibility coefficient (ADC) in *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks

TLE (%)	ADC protein (%)	ADC Lipid (%)	ADC Energy (%)
0.0	64.64 ± 1.15^{d}	63.39 ± 2.14^{b}	63.39 ± 1.64^{c}
0.5	67.74 ± 0.34^{c}	67.26 ± 3.26^{b}	65.91 ± 4.76^{c}
1.0	82.83 ± 1.11^{a}	85.48 ± 3.12^{a}	77.13 ± 0.21^{a}
1.5	77.14 ± 1.49^{b}	84.10 ± 4.11^{a}	74.04 ± 0.86^{ab}
2.0	77.90 ± 1.07^{b}	80.40 ± 1.81^{a}	71.79 ± 0.24^{b}

Means with different superscripts in the same column are significantly different at $P < 0.05$.

Table 5 Whole-body proximate compositions (% dry weight) of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) after 12 weeks

TLE (%)	Moisture	Crude protein	Crude fat	Ash
0.0	10.76 ± 0.09^{a}	63.36 ± 0.20^{bc}	7.60 ± 0.13^{a}	18.28 ± 0.11^{ab}
0.5	10.61 ± 0.03^{b}	63.40 ± 0.12^{abc}	7.65 ± 0.07^{a}	18.34 ± 0.06^{a}
1.0	10.63 ± 0.03^{b}	63.63 ± 0.08^{a}	7.42 ± 0.03^{b}	18.32 ± 0.06^{a}
1.5	10.65 ± 0.09^{ab}	63.57 ± 0.08^{ab}	7.61 ± 0.09^{a}	18.17 ± 0.08^{b}
2.0	10.65 ± 0.02^{ab}	63.31 ± 0.10^{c}	7.67 ± 0.08^{a}	18.37 ± 0.06^{a}

Means with different superscripts in the same column are significantly different at $P < 0.05$

Table 6 The morphometrics of villi and number of goblet cells in *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks

TLE (%)	Villi width (µm)	Crypt depth (µm)	Villi height (µm)	VAA (µm^2)	MMT (µm)	Number of goblet cells
0.0	156.17 ± 14.14^{b}	96.40 ± 4.55^{a}	322.87 ± 15.35^{c}	50156.68 ± 343.57^{c}	52.57 ± 1.22^{a}	2.00 ± 0.00^{d}
0.5	157.60 ± 6.22^{b}	54.50 ± 9.96^{d}	361.33 ± 12.48^{bc}	56993.91 ± 288.73^{bc}	53.50 ± 3.46^{a}	6.40 ± 0.21^{c}
1.0	204.06 ± 7.40^{a}	59.40 ± 3.67^{cd}	475.67 ± 7.70^{a}	96344.87 ± 1301.06^{a}	44.70 ± 2.23^{b}	8.58 ± 0.52^{b}
1.5	215.80 ± 13.35^{a}	68.13 ± 7.26^{bc}	432.00 ± 25.59^{ab}	93316.67 ± 2751.70^{a}	45.50 ± 2.00^{ab}	9.43 ± 0.81^{b}
2.0	171.57 ± 10.87^{b}	72.80 ± 5.54^{b}	365.40 ± 14.88^{bc}	62779.18 ± 403.62^{b}	41.47 ± 1.17^{b}	11.00 ± 0.50^{a}

Means with different superscripts in the same column are significantly different at $P < 0.05$.
MMT = *Mucosa muscularis* thickness VAA = Villi absorption area

Nutrient digestibility

The apparent digestibility of nutrients of Nile tilapia fed dietary TLE is shown in Table 4. The ADCs of protein, lipid and energy of fish fed 1.0 – 2.0% TLE were higher than those fed the control diet. The highest nutrients digestibility was obtained at 1.0%; however, there were no significant differences ($P > 0.05$) in the ADC of lipid, as well as between the ADCs of crude protein, lipids, and energy in the fish at 1.5 and 2.0% TLE dietary levels.

Fish carcass proximate composition

The moisture content of the whole-body of Nile tilapia fed TLE-enriched diets did not differ ($P > 0.05$) at 1.5-2.0% inclusion levels, when compared to the control and lower TLE-enriched diets (Table 5). Also, the crude protein content of the fish fed diet containing 1.0% TLE was higher ($P < 0.05$) than the fish fed the control and 2.0 TLE-enriched diets. On the other hand, the crude fat did not differ among the fish fed control and TLE-enriched diets, except in fish fed 1.0% TLE-enriched diet, which had lower fat content. The ash content of the fish fed TLE-enriched diets appeared not to differ ($P > 0.05$) from the fish fed the control diet.

Gut morphometrics and microflora

The villi width and height of *O. niloticus* fed 1.0 -1.5% TLE-fortified diets were higher ($P < 0.05$) than the fish fed the control diet, while the higher ($P < 0.05$) crypt depth were observed in fish fed the control diet, compared to those fed TLE-fortified diets (Table 6). The number of goblet cells increased with TLE supplementation in the experimental fish. Higher ($P < 0.05$) villi absorption area were observed in fish fed

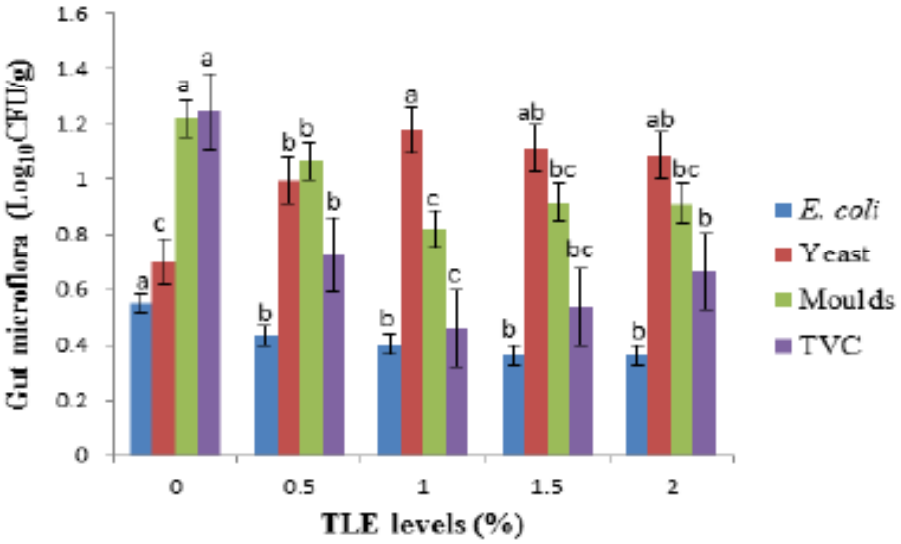

Fig. 7 The gut microflora of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks. TVC = Total viable count

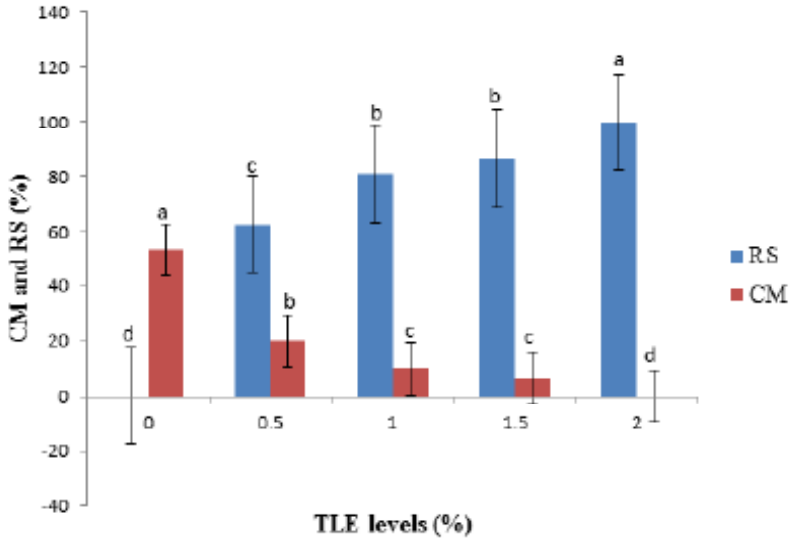

Fig. 8 The cumulative mortality (CM) and relative survival (RS) of *Oreochromis niloticus* fed diets enriched with varying levels of tamarind leaves extract (TLE) for 12 weeks and post-challenged with *A. hydrophila* infection for four weeks

1.0 -2.0% TLE, while on the other hand the thickness of *Mucosa muscularis* reduced ($P < 0.05$) at these inclusion levels of TLE, compared to the control treatment. The population of gut total viable count (TVC), *E. coli*, and moulds (*Aspergillus* sp) in the fish decreased ($P < 0.05$) while the colony forming unit (CFU) of yeast (*Saccharomyces* sp) increased with dietary TLE supplementation in the experimental fish, compared to the group fed with the control diet (Fig. 7).

Fish protection against *A. hydrophila* infection

The behavioral and physical changes observed in the *A. hydrophila* challenged fish included weakened swimming ability, gasping, low response to fish and fin rots. The effects of dietary TLE on the cumulative (CM) mortality and relative survival (RS) of experimentally infected Nile tilapia are presented in Figure8. The fish fed with the TLE-enriched diet showed lower ($P < 0.05$) cumulative mortality (0.0 – 20%), compared to those fed control diet (53.34%) and the mortality reduced with increasing level of TLE in the diets. The relative survival of the fish fed TLE-enriched significantly rose ($P < 0.05$) and the highest (100%) was obtained at 2.0% inclusion level.

Discussion

The rise in global concern on the adverse effects of uncontrolled utilization of synthetic antibiotics in the production of food fish necessitates search for alternative natural products, including herbal additives, in aquaculture. The present study has shown that flavonoids have highest composition in TLE among the three phytochemicals analyzed. The higher growth performance (FW, WG, SGR, RGR) and nutrient utilization (FCR, PER, AEU, APU, nitrogen metabolism) of *O. niloticus* with dietary 0.5 – 1.5% TLE in the present study might be linked with the bioavailable phytochemicals and the antimicrobial activity of TLE which enhanced nutrient digestibility, utilization, and consequently promoted growth in the *O. niloticus*. Flavonoids are the most common type of phenolic compounds found in plants possessing antimicrobial, anti-inflammatory, anti-oxidative, and hepatoprotective properties (Tapas et al. 2008; Kumar and Pandey 2013) and might have contributed to the enhanced growth performance in the present study.

Dietary TLE remarkably enhanced fish growth and improved feed efficiency and conversion ratios, nutrient digestibility and gut physiology in the present study. The observed growth promotion and improved nutrient utilization might be attributed to the beneficial phytochemicals (flavonoids, alkaloids and saponin) in TLE, which enhanced well-balanced microbiota, nutrient digestibility due to enhanced secretion of digestive enzymes. Polyphenolic compounds are common in herbs and have poor bioavailability in animals, and are often transformed by gut flora to beneficial bioavailable compounds with antimicrobial, anti-inflammatory, digestive and anti-oxidative properties (Scalbert and Williamson 2000; Espin et al. 2017; Karl et al. 2018). Our earlier studies have demonstrated the best growth performance in African catfish fed 1.0% tamarind leaf meal (Adeniyi et al. 2018a). Previous studies also showed improved growth performance and nutrient utilization of tilapia fed diets containing caraway seed (Ahmad and Abdel-Tawwab 2011), *Tribulus terrestris* (Yilmaz et al. 2014), *Nelumbo nucifera* leaf (Munglue 2015), *Cinnamomum camphora* bark, *Euphorbia hirta* shoot, *Carica papaya* seed (Kareem et al. 2016), *Aspilla mossambicensis* leaf (Kapinga et al. 2018), T. indica pulp (Adeniyi et al. 2020); and catfish fed diets enriched with Gossypium herbacium leaf (Adeniyi and Lawal 2017), *Ocimum gratissimum* leaf (Abdel-Tawwab et al. 2018), Cymbopogon citratus (Adeniyi 2020). The reduced growth performance at higher inclusion levels might be due to higher dose of phytochemicals which might have impaired growth of the fish; similar observations of reduced growth in fish fed at higher inclusion levels of herbal extracts were also reported in previous research (Dada and Ikuerowo 2009; Yilmaz et al. 2014; Kapinga et al. 2018). Dietary TLE did not affect fish survival in the current study, which could indicate that TLE is safe and did not have significant detrimental effect on the wellbeing of the fish. Our observation on insignificant differences in the survival of tilapia in the current study coincided with previous studies (Ahmad and Abdel-Tawwab 2011; Abdel-Tawwab 2012; Kareem et al. 2016; Adeshina et al. 2021)

Intestinal morphology and microbiota balance are relevant indices of gut health. Significant reduction in villus height and increase in crypt depth have been associated with the presence of toxin and reduction in nutrient absorption (Darabighane et al. 2011). Hence the increased villi height and lower crypt depth observed could indicate benefit of TLE in improving gut health and physiology as reflected by the reduction in total viable count, *E. coli* , mould and increase in yeast population, which might have contributed to the enhanced growth, nutrient digestibility and utilization in the present study. Dietary TLE might have played significant role in enhancing healthy composition of gut microbiota in this study; promoting growth of beneficial microbes and inhibiting colonization of harmful ones. The *in vitro* antimicrobial activity of TLE has been reported in our earlier study against some pathogenic microbes including *E. coli* (Adeniyi et al. 2017b). An et. al (2019) reported that medicinal herbs regulate the composition of gut microbiota by enhancing growth of beneficial and inhibiting the pathogenic microbes while Dawood (2021) opined that beneficial gut microbiota enhanced feed utilization and combat harmful microbes within the gut. Yeast has been identified as normal microbiota of healthy fish, playing significant role in decomposition of plant substrates (Romero et al. 2014; Boonanuntanasarn et al. 2017) and might have contributed to digestion of nutrients in the present study. Similar to our observation in this study, fish fed phytogenic-based diets exhibited enhanced growth and reduction in intestinal enterobacteriaceae (Honghai et al. 2004), anaerobic (Giannenas et al. 2012), total plate (Hardi et al 2016), and *Vibrio* counts (Boonanuntanasarn et al 2017).

The observation on the intestinal morphology in the present study also coincided with the earlier studies in catfish (Abdel-Tawwab et al. 2018) and tilapia (Adeniyi et al. 2020) while on the other hand, Daniels

et al. (2010), Reyes-Becerril et al. (2014) and Zahran et al. (2014) observed insignificant differences in villus morphology of fish fed herbal-supplemented diets, compared to the control groups. The number of intestinal goblet cells in TLE-supplemented *O. niloticus* was affected by the experimental diets. Goblet cells synthesize mucins, which protect the intestinal epithelial surface from bacteria, toxins and threats from dietary constituents (Kim and Khan 2013). The significantly higher number of goblet cells in fish fed TLE-supplemented diets might indicate stimulation of non-specific immune response in the fish by the herbal product and could also be associated with the regulation of the composition of gut microbiota observed in the present study. The number of goblet cells was also higher in rainbow trout fed diet containing 5g/kg diet Aquavac Ergosan phytogenic additive (Heidarieh et al. 2012) and tilapia fed diets containing *Helianthus tuberosus* (Boonanuntanasarn et al. 2017), tamarind pulp extracts (Adeniyi et al. 2020) than fish in control treatment.

The body composition of the *O. niloticus* in the present study showed no significant variations, except at 1.0% TLE-supplementation in which lower lipid and higher protein were observed, compared to the fish fed the control diet; which might be due to the enhanced fat-catabolism which released energy for fish activities and higher protein retention, respectively, in fish fed TLE at this inclusion levels. Dietary protein contributes significantly towards meeting the energy requirements of fish and hence utilization of energy from lipids in sparing protein for fish growth (Kaushik and Seiliez 2010). Fast growing fishes often have higher retention of synthesized protein; hence higher protein retention could signify more efficient use of dietary protein (Halver and Hardy 2002) as observed in the lower FCR and higher PER of fish fed TLE-enriched diets in the current study. Wafaa et al. (2014) similarly observed lower body lipid while the crude protein and ash increased. On the other hand, Abdel-Tawwab et al (2010) and Ahmad and Abdel-Tawwab (2011) reported increased body lipid and lower crude protein and ash in *O. niloticus* fed green tea and caraway seed meal, respectively.

The utilization of TLE boosted the resistance and survival of the experimentally challenged *O. niloticus* in the present study. The application of medicinal herbs have been reported to inhibit bacterial growth and boost non-specific immunity contributing to fish resistance and survival during infection and outbreak of diseases (Hardi et al. 2017). The results of the challenge test in the present study coincided with increased survival of *A. hydrophila* challenged- tilapia with dietary supplementation with *Echinacea* and garlic (Aly and Mohamed 2010), green tea (Abdel-Tawwab et al. 2010), *Azadirachta indica* leaves aqueous extract (Thanigaivel et al. 2015), ginger (Sahan et al. 2016); and pulp (Adeniyi et al. 2020). The level of protection against *A. hydrophila* infection similarly increased in common carp fed henna extract (Soltanian and Fereidouni 2016) and African catfish fed supplemented with tamarind powder (Adeniyi et al. 2017a) and clove bud extract (Adeshina et al. 2019). In the same vein, higher relative survival was also reported in experimentally-infected Nile tilapia fed phytogenic-based diets (Manaf et al. 2016; Suphoronski et al. 2019). The behavioral changes and higher survival of the experimentally-infected fish fed TLE-based diets in the present study is similar to the observations found on Nile tilapia challenged with *A. hydrophila* and *Pseudomonas* sp fed with *Boesenbergia pandurata*, *Solanum ferox*, and *Zingiber zerumbet* (Hardi et al. 2016, 2017).

Conclusion

It could be concluded that dietary supplementation with of 1.0% tamarind leaves extract in *Oreochromis niloticus* significantly enhanced nutrients digestibility, villi absorption area, growth performance, and whole-body crude protein as well as increased its protection against *A. hydrophila* infection. Therefore, the application of tamarind leaves extract in fish diets at this level is recommended for aquaculture use.

Competing interest The authors declare that they do not have any competing interest.

Authors' contributions OVA participated in the conceptual design, experimentation, data collection, statistical analysis and drafted the manuscript. FEO participated in the experimental design, general supervision, revised the manuscript. BOE participated in gut histomorphometrics, challenge test and revised the manuscript. STO designed and participated in gut microflora and challenge analysis. All authors read and approved the final manuscript.

Acknowledgment The authors are grateful for the support of Mr Steve Temilade Adeniyi during the study, especially in preparation of tamarind leaves and feed production.

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