Original Research Article

Efficacy of *Terminalia catappa* leaf as an alternative to synthetic antibiotics in the diet of *Oreochromis niloticus* challenged with *salmonella typhi*

Abstract

Terminalia catappa leaf meal was incorporated into 30% crude protein basal diets of *Oreochromis niloticus* at different inclusion levels of 0 (control), 25, 50, 75 and 100% and diets were designated as TCD1, TCD2, TCD3, TCD4 and TCD5 respectively. Fish (10.38 ± 0.35) g were fed at 4% body weight for 70 days and later immersed in water with 2 ml / l of pathogenic strain of *Salmonella typhi* for 10 days. At the expiration of the feeding trials, growth performance indices of *O. niloticus* were significantly different ($P < 0.05$), with fish fed *T. catappa* having higher weight gain, specific growth rate and better feed conversion ratio. Crude protein of whole fish body was higher in fish fed *T. catappa* leaf supplemented diets. Challenge test with pathogenic strain of *S. typhi*, indicated that survival of *O. niloticus* can be improved by 50% when *T. catappa* is incorporated into fish diets. Therefore, incorporating *T. catappa* into the diets of *O. niloticus* between 25 – 100% from this study is sufficient to boost growth and improve survival against *S. typhi*.

Keywords: *Terminalia catappa*, Bacteria, Antibiotics, *Oreochromis niloticus*

Introduction

In Nigeria, tilapia production encompasses a diverse range of systems and methods, ranging from backyard residential ponds to small-scale industrial systems. Despite the benefits of tilapia farming around the world, it is fraught with dangers such as a lack of good and viable fish seeds, a lack of sufficient and balanced aqua feeds, and disease outbreaks (Adewumi and Olaleye, 2011). *Salmonella typhi* is one of the most common bacteria linked to disease outbreaks in freshwater fish. (Citarasu, 2001). Disease outbreaks, which have been managed with antibiotic growth promoters (AGPs), are probably the biggest limiting factor to successful fish farming. However, in the last two decades, government policymakers and consumers in affluent countries have frowned upon the use of AGPs in animal and fish diets.

This is due to the fact that animals build tolerance to these compounds over time, potentially causing harm to human health (Botsoglou and Fletouris, 2001).

Due to a growing awareness of healthy living, the general population is clamouring for organic products, necessitating the need for natural antibiotic alternatives. The majority of researchers have effectively included ethnobotanicals (medicinal plants) into fish diets as natural feed additives that have been shown to increase growth performance and immunological response. (Abdel-Tawwab *et al.,* 2010; Anusha *et al.,* 2014; Anjusha *et al.,* 2019). Plants have been documented to offer antibacterial, growth-promoting, appetite-stimulating, and immune-stimulating properties in aquaculture techniques, and are regarded natural sources of safer and cheaper additions in aqua diets. (Citarasu *et al.,* 2001 and 2002; Sivaram *et al.,* 2004). In addition, following controversial debates over the use of human antimicrobials as growth promoters in fish farming, natural plant extractives have been researched as an alternate strategy for removing chemical residues that could harm consumers' health. (Olorunsanya *et al.,* 2010). Antibacterial effects have been discovered in phytochemicals found in most therapeutic plants, such as tannins, alkaloids, and flavonoids. Medicinal herbs are more successful than synthetic antibiotics at treating infectious diseases, and they have also been proven to reduce many of the negative effects associated with them. (Essawi and Srour, 2000; Punitha *et al.*, 2008).

In Nigeria, Terminalia catappa is a well-known herb with nutritional and therapeutic properties. This plant contains medically important phyto-constituents such phenols, flavonoids, tannins, quinones, betacyanins, steroids, terpenoids, coumarins, and carotenoids, and pharmacological studies have verified its antibacterial properties. (Nikoskelainen *et al.,* 2005). The importance of *Terminalia catappa* leaf meal as an alternative to antibiotics in the diet of *Oreochromis niloticus* challenged with *Samonella typhi* is justified by the aforementioned. As a result, this experiment was conducted to evaluate the efficacy of *Terminalia catappa* leaf meal as an alternative to antibiotics in the diet of *Oreochromis niloticus* challenged with *Samonella typhi*.

Materials and Methods

Experimental area

The experiment took place at the Federal University of Technology, Akure (FUTA) in Nigeria's Ondo State. The feeding trials were carried out in the wet laboratory of the

Department of Fisheries and Aquaculture Technology, while the preparation of experimental diets, proximate analyses of diets, and fish samples were done in the Nutrition laboratory of the Department of Fisheries and Aquaculture Technology.

Preparation of test ingredient (*Terminalia catappa* Leaves)

Matured green leaves of *T. catapp*a were collected from The Federal University of Technology, Akure Environment and authenticated in the Department of Soil, Crop and Pest Management of the Institution. Leaves were properly washed under running tap water and then rinsed in distilled water. The rinsed leaves were shade-dried at room temperature (27°C) for 5 days. Dried leaves were later milled into powder with the aid of an electric kitchen blender and powder was stored in air-tight glass container and stored prior to use.

Preparation of experimental diets

All feed ingredients were purchased in a local market in Akure, Ondo State, Nigeria. Five iso-nitrogenous (30% crude protein) diets of same basal ingredients (Table 1) were formulated with different proportions *of T. catappa* (0, 25, 50, 75 and 100%) supplementing yellow maize in diets. All ingredients were milled to fine powder and thoroughly mixed. One litre of boiled water was added to gelatinize starch to obtain homogenous dough. Compounded mixtures were pelletized using a Hobart A-200 pelleting machine (London, UK) through 2 mm die diameter. Feeds were oven-dried at 60°C for 24 hours, allowed to cool down under room temperature, packed in air tight containers and refrigerated prior to use. The *T. catappa*-deficient (0%) diet that served as the reference / control, was designated as TCD1, while others are TCD2, TCD3, TCD4 and TCD5.

Experimental design and set-up

The experiment was a complete randomized design with inclusion levels of *T. catappa* serving as the only source of variation. The experiment was set up in an indoor system with aerators attached to each experimental unit for constant supply of dissolved oxygen. Fifteen (15) rectangular glass tanks (75 × 45 × 30 cm^3) 0f 70 litres water carrying capacity were used with water filled up to 50 litres level during the feeding trial.

Experimental fish and feeding trial

One hundred and fifty apparently healthy juveniles of *Oreochromis niloticus* (10.38 ± 0.35) g were obtained from the Teaching and Research Farm of the University, acclimated for 14

days and fed with a 30% crude protein farm-made feed during acclimation. Fish were randomly distributed into culture tanks at a stocking density of 10 fish / tank with each treatment in triplicate. Fish were fed at 4% body weight twice daily in equal portions at 08:00 – 09:00 GMT and 16:00 – 17:00 GMT for 70 days. Unconsumed feed and faeces were siphoned from aquaria daily before feeding while culture water was changed on alternate days to avoid deterioration of water quality as a result of unconsumed feed and faeces. Water quality parameters such as temperature, dissolved oxygen and pH of the experimental tanks were determined using water quality kit (Pro. Kit, Florida).

Experimental analyses

At the expiration of feeding trial, three fish were randomly selected 24 hours after the lasting feeding from each aquarium for whole body proximate analysis prior to the challenge test.

Proximate analyses

Proximate compositions of *T. catappa* (test ingredient), experimental diets and whole body of *O. niloticus* and experimental diets were determined in triplicate following standard methods by AOAC (2010). All samples collected were oven-dried at 105°C to test for moisture, Kjeldahl procedure to test crude protein, crude lipid was done by ether extraction using the Soxhlet System while crude ash was examined by combustion at 550°C for 5 hours in a furnace.

Growth performance evaluation

At the end of the feeding trial, all fish were batch-weighed and growth performance was evaluated using following equations described by Brown (1957) and Winberg (1956).

Challenge Test with *Salmonella typhi*

A challenge test was conducted for 10 days at the end of the feeding trial. The pathogenic strain of *S. typhi* (1.7×10^9 *cfu / ml*) was obtained from the Department of Microbiology, FUTA. This strain was re-isolated and cultured on agar plates at 37°C for 24 hours in the laboratory. Isolates were stored in 30% glycerol in Broth heart infusion (BHI) chamber at -70°C prior to use. Three fish samples were randomly selected from each of the treatments and introduced into 50 litres of water containing 2 ml / l of the *S. typhi* culture strain. Fish were not fed nor during the 10-day challenge period but were observed for clinical signs.

Statistical analysis

All data generated were subjected to one-way ANOVA using statistical package for social sciences SPSS version 22.0 (SPSS, Inc. USA) to determine any significant relationship between varying inclusion levels of *T. catappa* in *O. niloticus*. Where significant differences ($P < 0.05$) were observed among treatments, evaluation was done using Turkey's Multiple Range Test and data are presented as mean ± SEM.

Results

Proximate compositions of experimental diets and *T. catappa*

Proximate compositions of experimental diets are as shown on Table 1. There were no significant differences ($P > 0.05$) in dry matter and crude ash contents of diets with *T. catappa* but there were significant differences ($P < 0.05$) when compared with the control diet. However, crude protein and lipid contents of experimental diets on the other hand showed no significant differences ($P > 0.05$) among treatments. Tables 2 show the proximate composition of dried green leaves of *T. catappa*. The amount of NFE (carbohydrate), crude lipid and protein were found to be 52.6, 4.2 and 8.2% respectively. The percentages of crude fibre, ash and moisture contents were also 13.5, 13.1 and 8.4% sequentially.

Growth performance and carcass composition of *O. niloticus*

The addition of *T. catappa* in *O. niloticus* diets showed significant differences ($P < 0.05$) in growth performance indices evaluated (Table 3). There were no significant differences ($P > 0.05$) in the initial stocking weight of experimental fish, thus, no differential weight biasness. *T. catappa* at 25 and 50% inclusion levels in *O. niloticus* diets showed increase in growth, higher SGR and better FCR while at other inclusion levels; results were not different from the control. Also, higher survival was recorded in *O. niloticus* fed *T. catappa* supplemented diets with 100% in fish fed diet CTD5. Furthermore, results revealed that feed intake was not proportional to weight gain, as *O. niloticus* fed diets CTD2 and CDT5 had same feed intake but different weight gain.

Water quality parameters

Results of culture water parameter were not significantly different ($P > 0.05$) in all experimental units and are as shown in Fig. 1. Water temperature was (27.40 – 28.20°C), dissolved oxygen (5.56 – 5.73 mg / l) and pH (7.65–7.76).

Challenge Test with *Salmonella typhi*

Mortality and percentage survival of *O. niloticus* after the challenge test is as reflected in Fig. 2. There was a decrease in fish mortality as the quantity of *T. catappa* increased denoting a linear relationship. *O. nilotcus* fed diet TCD5 had 100% survival while the control recorded 50%.

Discussion

Nitrogen free extract (NFE) in *T. catappa* as revealed by this study, was high (52.6%), this corroborates the report by Okpako *et al.,* (2017) of 57.9% in leaves of same plant. Crude fibre, ash, and moisture contents were within the range as reported Vijaya *et al.,* (2012). The leaves of *T. catappa* in this experiment were not as rich in crude protein like the seeds as reported by Muhammad and Oloyede (2004). This low crude protein value of 8.2 ± 0.64%, made it suitable as a partial or total supplement for maize in the experiment. The components of *T. catappa* as whole have been reported very useful; leaves, bark, fruits, roots or seeds in the treatment of different ailments (Kritikar and Basu, 1991; Corner 1997; Nagappa *et al.,* 2003). Also, researches have shown that they have strong antimicrobial properties (Osagie, 1998; Pawar and Pal, 2002 and Moody *et al.,* 2003).

The proximate compositions of all experimental diets analysed were within the acceptable range for culturing commercial fish species in tropical region. In this study, protein content of experimental diets ranged between 29.50 – 30.28%, which was in line with the recommendations of Faturoti *et al.,* (1989) and Ayinla (1997) that 30 – 35% CP is appropriate for culturing *O. niloticus*. The observed lipid values of 11.04 – 11.92% was also in line with the opinion by (Ross 1985) of 10 – 20% in diets freshwater fish species such as cichlids, clupeids, clariids and cyprinids. Lipid gives optimal energy without producing an excessive fatty carcass. The analysed crude fibre content of all the diets were within the safety dietary limit for fish as reported by (De Silva and Anderson, 1995) that fibre content above 12% is not desirable in fish diets as increase in fibre content would consequently result in decrease in nutrient quality. Moisture content in fish diets were generally low, (less than 15%) which is good keeping quality (Daramola and Osanyinlusi, 2006).

Growth indices are the major parameters used to determine nutrient utilization in most farmed animals, fish inclusive. Results showed that fish accepted all experimental diets as reflected in the weight gain. Higher growth, SGR and better FCR values were recorded in *O. niloticus* fed at 25 and 50% *T. catappa* inclusion levels. Similar report was given by Olapade and Kargbo (2015), who reported higher weight gain in *C. gariepinus* fed *T. catappa* seed meal supplemented diets at 50 and 75%. Weight gain was not proportional to feed intake as *O. niloticus* fed control and TCD5 diets had the highest feed intake but not the highest growth when compared with other treatments. This work also corroborated that of Apata (2010) who recorded higher growth in broiler chickens fed *T. catappa* fruit supplemented diets at more than 40% inclusion levels. All experimental diets with *T. catappa* had higher crude protein in *O. niloticus* body compared with the control and vice-versa for lipids. This finding has buttress the fact that when there was a higher crude protein in *T. catappa* leaves, while the lipid was low. Survival in *O. niloticus* fed *T. catappa* supplemented diets during the experimental period was higher when compared with the control. *T. catappa* leaf has been reported to promote growth, stimulate appetite and boost immunity (Citarasu, 2001; Chakraboty *et al.*, 2012 and 2014).

O. niloticus like other fish species, require optimum levels of water parameters for survival, growth and reproduction. The physical parameters of culture water in this experiment were within the acceptable ranges of 6.5 – 9.5 (pH), 22 – 27 (temperature) and a minimum of 2 mg / l (dissolved oxygen) as recommended for rearing and culture of most tropical fishes by NRC (1996). Dissolved oxygen value below 4 mg / l is stressful to fishes while an acidic pH of 4 is lethal to fish health and reduces the animal's appetite of fish thereby reducing growth (Adesulu, 2001). At a pH of 9, Water becomes unproductive at a pH of 9 because of the unavailability of carbon dioxide while fish dies at pH 11.

The challenge test showed that *T. catappa* in the diets of *O. niloticus* can improve survival by 50%. Fish fed diet CTD5 exhibited the greatest resistance to *Salmonella typhi* when compared with others. This is in line with result of Abdel-Tawwab *et al.,* (2010) who recorded a higher survival in Nile tilapia (*O. niloticus*) challenged with *hydrophila* after 12 weeks of feeding with green tea (*C. sinensis*) diets supplemented. Administration of herbal additive diets in *O. niloticus* diets led to a reduced mortality against *A. hydrophila* (Pachanawan *et al.,* 2008). Higher survival was recorded in ornamental fish (*Betta spp.*) immersed in *T. catappa* leaf extracts (Nugroho *et al.*, 2017). A combination of herbs can enhance both specific and non-specific immune system (Clotfelter and Rodriguez, 2006, Ashraf and Bengston, 2007;

Anjusha *et al.,* 2019). In another research, use of *P. guajava* eliminated *Vibrio* infection in Black tiger shrimp (*P. monodon)* than the antibiotics; oxytetracycline (Direkbusarakom, 2004). Anusha (2014) also affirmed that *Ixora coccinea* in diets of *Carassius auratus* improved survival when challenged with *Aeromonas hydrophila*. Furthermore, *in-vivo* study demonstrated that *Vernonia amygdalina* extracts inhibited the growth of S*aprolegnia* on the body of *O. niloticus* (Ilondu *et al.,* 2009). Administration of *Zataria multiflora* leaf was effective in controlling bacterial contamination in cultured shrimp, *Litopenaeus vannemei* (Rohani *et al.,* 2013).

Conclusion

The application of ethnobotanicals in aqua feeds as growth promoters, antibacterial, antiviral, antifungal, anti-paratasitic and immuno-stimulatory compounds cannot be over emphasized. Apart from having greater accuracy and application than most chemotherapeutic agents because of their broad spectrum activity, plants are also cost effective and eco-friendly. The positive response of *O. niloticus* to *T. catappa* in this study indicates its potential as alternative to antibiotics through higher survival and growth enhancement. *T. catappa* in this study has been proven to act as immune-stimulant, modulating response to enhance the health status of *O. niloticus*. Therefore, it can be recommended that *T. catappa* leaf meal may be supplemented between 25 and 100% in the diets of *O. niloticus* fingerlings to boost growth and immune responses.

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Table 1: Gross and proximate compositions (%) of experimental diets

Feed ingredients	**Experimental diets (%)**				
	TCD1	**TCD2**	**TCD3**	**TCD4**	**TCD5**
Fish meal	18.00	18.00	18.00	18.00	18.00
T. catappa leaf meal	0.00	13.50	27.00	40.50	54.00
Yellow maize	54.00	40.50	27.00	13.50	0.00
Soybean meal	18.00	18.00	18.00	18.00	18.00
Tapioca starch	4.00	4.00	4.00	4.00	4.00
Fish oil	3.00	3.00	3.00	3.00	3.00
*Min. / Vit. premixes	3.00	3.00	3.00	3.00	3.00
Parameter (%)	**Experimental diets**				
	TCD1	**TCD2**	**TCD3**	**TCD4**	**TCD5**
Crude ash	8.90±0.09[a]	9.02±0.05[b]	9.28±0.03[b]	9.31±0.07[b]	9.37±0.06[b]
Crude lipid	11.04±0.14[a]	10.51±0.11[a]	10.58±0.90[a]	10.71±0.51[a]	10.92±0.30[a]

Crude protein	30.08±0.04[a]	30.93±0.31[a]	30.75±0.67[a]	30.58±0.32[a]	30.50±0.21[a]
Crude fibre	10.50±0.18[a]	10.50±0.76[a]	10.62±0.28[ab]	10.79±0.45[b]	10.77±0.19[b]
NFE	33.13±0.15[a]	33.06±0.01[a]	33.75±0.08[b]	33.35±0.06[ab]	32.80±0.03[a]
Dry matter	93.65±0.03[a]	94.02±0.15[b]	94.98±0.41[b]	94.74±0.63[b]	94.36±0.17[b]

Means with the same superscript in the same row are not significantly different ($P > 0.05$)

*Vitamin and mineral mix (IU or mg/ kg of diet): Vitamin A, 900 000 IU; Vitamin D, 250 000 IU; Vitamin E, 4500 mg; Vitamin K 3, 220 mg; Vitamin B1, 320 mg; Vitamin B2, 1090 mg; Vitamin B5, 2000 mg; Vitamin B6, 5000 mg; Vitamin B12, 116 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60000 mg; Biotin, 50 mg; Niacin acid, 2500 mg

Table 2: Proximate composition (%) of *T. catappa* (dry matter) leaves

Parameter	**Composition (%)**
Moisture content	8.4±0.72
Crude ash	13.1±1.05
Crude lipid	4.2±0.03
Crude protein	8.2±0.64
Crude fibre	13.5±1.37
Nitrogen free extract (NFE)	52.6±0.96

Table 3: Growth performance and nutrients utilization in *Oreochromis niloticus* fed *T. catappa* leaf supplemented diets (±SEM)

Parameter	**TCD1**	**TCD2**	**TCD3**	**TCD4**	**TCD5**
Initial weight (g)	10.71±0.20[a]	10.35±0.05[a]	10.23±0.64[a]	10.12±0.06[a]	10.48±0.80[a]
Final weight (g)	35.88±0.87[a]	40.48±0.14[b]	39.93±3.45[b]	35.04±0.09[a]	34.86±0.14[a]
Weight gain (g)	25.17±0.68[a]	30.13±2.58[b]	29.70±3.49[b]	25.93 ± 0.05[a]	24.40±0.06[a]
SGR (%/day)	0.93±0.04[a]	1.07±0.01[b]	1.07±0.20[b]	0.96±0.00[ab]	0.93±0.00[a]
FCR	1.94±0.09[c]	1.56±0.03[a]	1.63±0.01[a]	1.89±0.09[b]	1.93±0.07[c]
Survival (%)	90.00±0.00[a]	96.66±3.33[b]	96.66±3.33[b]	96.66±3.33[b]	100.00±0.00[c]
Feed Intake	48.74±0.96[ab]	47.17±0.16[a]	48.30±1.25[ab]	49.12±0.44[b]	47.17±0.33[a]

Means with the same superscript in the same row are not significantly different ($P > 0.05$)

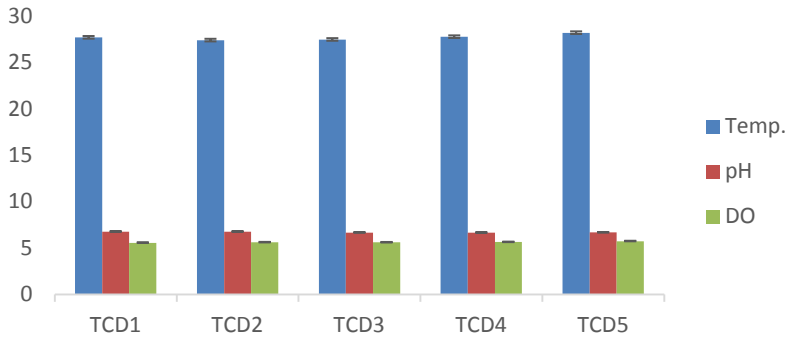

Figure 1: Water quality parameters of culture water

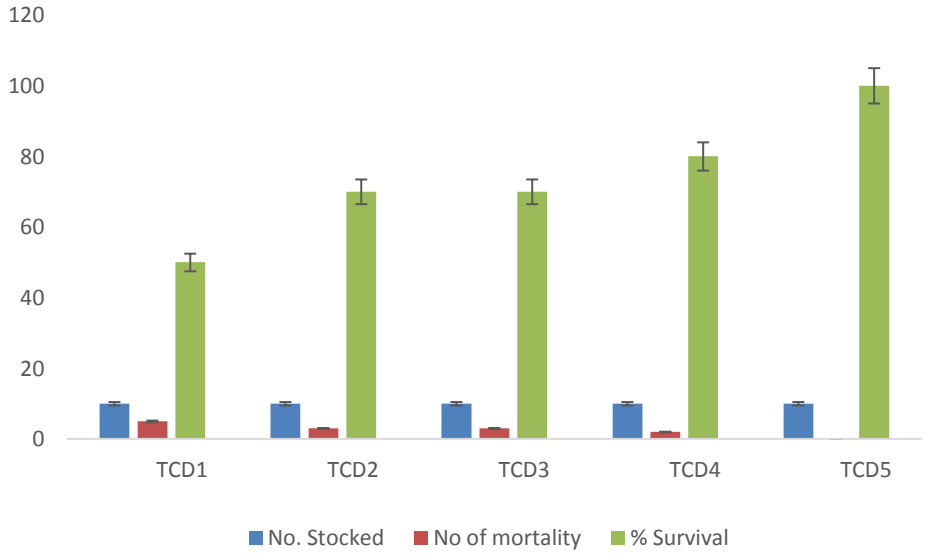

Figure 2: Survival of *O. niloticus* after the challenge test with *S. typhi*