View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

Croatian Journal of Fisheries, 2020, 78, 11-20
S.E. Olusola et al. (2020): Efficacy of tamarind and mango leaves on catfish

DOI: 10.2478/cjf-2020-0002

CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

sciendo

EFFICACY OF TAMARIND *Tamarindus indica* LEAVES AND MANGO *Mangifera indica* LEAVES AS FEED ADDITIVES ON GROWTH, BLOOD STATUS AND RESISTANCE TO *Aeromonas hydrophila* IN JUVENILE AFRICAN CATFISH *Clarias gariepinus*

Sunday Emmanuel Olusola[1*], Igbekele Joshua Ajiwoju[1], Benjamin Obukowho Emikpe[2]

[1] *Department of Biological Sciences, (Fisheries and Aquaculture Programme), Ondo State University of Science and Technology, Okitipupa, Nigeria*
[2] *Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria*

*Corresponding Author, Email: se.olusola@osustech.edu.ng

ARTICLE INFO

Received: 20 June 2019
Accepted: 27 November 2019

Keywords:
Clarias gariepinus
Mango leaves
Tamarind leaves
Aeromonas hydrophila
Haematology
Blood serum

ABSTRACT

This study investigated the efficacy of Tamarind Leaves (TL) and Mango Leaves (ML) with Oxytetracycline (OXY) on growth performance, haemato-immunological and disease resistance of *Clarias gariepinus* juveniles against *Aeromonas hydrophila*. Experimental diets consist of control (0%), TL2 (1%), TL3 (2%), ML4 (1%), ML5 (2%), OXY6 (30mg/kg diet), (TL+ML) 7, (TL+OXY) 8, (ML+OXY) 9 and (TL+ML+OXY) 10. The fish (3.02±0.01g) were replicated twice with 20 fish per replicate and were fed twice daily at 3% body weight of 40% crude protein for twelve weeks (8 weeks for feeding trial and 4 weeks for challenge test). Mean Weight Gain (MWG), Specific Growth Rate (SGR), Packed Cell Volume (PCV), Haemoglobin (Hb), Lymphocytes (LYM), Globulin (GLO), Amino Alanine Transferase (ALT) and Aspertate Amino Transferase (AST) contents were ascertained using standard technique. The fish were infected with *A. hydrophila* at 5.94 $\log_{10}$ CFU/ml interperitoneally and fed different diets to evaluate their Relative Percent of Survival (RPS). Data was subjected to descriptive statistics and one-way analysis of variance at P=0.05. *Clarias gariepinus* juveniles fed treated diets had higher growth rates than the control diet but *C. gariepinus* fed (TL+ML+OXY) 10 had a significantly higher MWG and SGR of 7.74±0.69 g and 0.97±0.01 g, respectively. The PCV (44.0±2.00%), Hb (14.7±2.00 g/dl), LYM (37.0±2.00), GLO (42.0 ±2.00 g/dl) were higher in the *C. gariepinus* fed (TL+ML+OXY) 10 than the control diet. The AST and ALT values among the treated groups were lower than the values in the control at the post-challenge test. The RPS against *A. hydrophila* was higher in the treated groups (100%) than in the control (0%). Fish fed tamarind and mango leaves had enhanced mean weight gain and were more resistant to *A. hydrophila* infection.

How to Cite

Olusola, S.E., Ajiwoju, I.J., Emikpe, B.O. (2020): Efficacy of tamarind *Tamarindus indica* leaves and mango *Mangifera indica* leaves as feed additives on growth, blood status and resistance to *Aeromonas hydrophila* in juvenile african catfish *Clarias gariepinus*. Croatian Journal of Fisheries, 78, 11-20. DOI: 10.2478/cjf-2020-0002.

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

INTRODUCTION

Fish constitutes the fastest growing source of animal protein in the developing world as well as the major and cheapest source of protein for the teeming population of the world, being a commodity with no social taboo (Omitoyin, 2007). Fish consumption will have a significant impact on the food security, nutrition, diets and income of poor people in developing countries during the next two decades (Omitoyin, 2007). Fish, as any living organism, require and depend on nutrient for survival to fight against disease, for body maintenance and reproduction.

Fish disease is a problem challenging the growth of fisheries and aquaculture production, especially culture fisheries with consequent adverse effect on the industry's economic development (Ibrahim et al., 2010). There are different means to overcome this problem through the use of drugs such as antibiotics. Attempt to control or prevent such devastating problem using conventional antimicrobial agents and other chemotherapeutants has been generally unsuccessful (Jadhav et al., 2006). The emergence of multidrug resistant strains of many microorganisms due to extensive use of antibiotics has revealed exploration of natural alternative antimicrobial agents such as tamarind and mango leaves (Doughari and Manzara, 2008; Gupta et al., 2014).

Tamarind (*Tamarindus indica*) is a leguminous tree in the family Fabaceae that is indigenous to tropical Africa. The tamarind is a long-lived, medium-growing shrub which attains a maximum crown height of 12 to 18 meters (39 to 59 ft). The crown has an irregular, vase-shaped outline of dense foliage. The evergreen leaves are alternately arranged and pinnately lobed (Boukary et al., 2007). The leaflets are bright green, elliptic-ovular, pinnately veined and less than 5 cm in length. The branches droop from a single, central trunk as the tree matures, and are often pruned in agriculture to optimize tree density and ease of fruit harvest. *Tamarindus indica* is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorders, diarrhea and jaundice, and as skin cleanser (Doughari, 2006).

Mango, the genus Mangifera, belongs to the order Sapindales, Anacardiaceae family. Hundreds of *Mangifera indica* cultivars are distributed throughout the world. Mangos are long-lived evergreen trees that can reach heights of 15–30 m. Most cultivated mango trees are between 3 and 10 m when fully mature, depending on the variety and the amount of pruning. The trees can live for over 100 years and develop trunk girths of over 4 m. Mango trees are usually grow between 3 and 10 m but can reach up to 30 m in some forest situations (Lalisa, 2017). This plant has parts such as the stem, bark, leaves and fruit pulp, which are known for various biomedical applications including anti-inflammatory (Hernandez et al., 2007) and anticancer (Percival et al., 2006). The fruit is rich in antioxidants and reduces the risk of cardiac disease, anticancer and antiviral activities (Abbasi et al., 2011).

Natural alternatives such as the inclusion of plant materials in fish feed formulation do not only have antimicrobial potential but also have been found to have other properties such as digestive stimulant, anti-inflammatory, antioxidant and anti-carcinogenic that can benefit humans (Zheng et al., 2001). These are attributed to the predominant polyphenol compounds in the plant materials. The ability of medicinal plants/herbs to inhibit the activity of bacteria having potential interest as fish pathogens has been documented (Bansemir et al., 2006; Dubber and Harder, 2008), but there is little information on utilization of *T. indica* and *M. indica* in fish farming. The aim of the present study was therefore carried out to assess the efficacy of *T. indica* leaves and *M. indica* leaves as a feed additive on growth, immunity and disease resistance in the farming of *C. gariepinus* juveniles against an experimental challenge infection using *A. Hydrophila*.

MATERIALS AND METHODS

Plant materials, identification and preparation

Tamarind leaves were obtained in New Bussa, Niger State, Nigeria. Mango leaves were collected in Igodan-Lisa, Okitipupa, Ondo State, Nigeria and both plants were identified by Dr D. O. Aworinde of Department of Biological Sciences (Botany Programme), Ondo State University of Science and Technology (OSUSTECH), Okitipupa, Nigeria. The leaves were plucked and air-dried at an ambient temperature (25° C) for four weeks (3 November- 3 December 2016), after which they were grinded to fine powder and stored until required.

Culture of pathogen

Aeromonas hydrophila was isolated from *Oreochromis niloticus* and *Clarias gariepinus* juveniles. The bacterium was identified in advance by morphological and biochemical characteristics, including the following reactions: Gram stain, Shape, Motility, Catalase, Oxidase, Coagulase, Urease, Indoje, Methyl Red, Voger proker, Gelatin hydrolysis, Starch Hydrolysis, Pigmentation, Oxygen Reduction, H_2S Productivity, Fructose, Lactose, Mannitol, Arabinose, xylose, Dulcitol, Raffinose, Glucose, Maltose and Adonitol (Isaac et al., 2014, Deng et al., 2009) and by PCR for confirmation of genus and species, using the methods described by Bergey's manual. The pure cultures were sub-cultured on nutrient slants and preserved in refrigerator at 4° C until required for the study.

Media preparation

Media such as Nutrient agar (Oxoid, Germany), Potato Dextrose agar (Oxoid, Germany), MacConkey agar (Oxoid, Germany) and Nutrient broth (Oxoid, Germany) were prepared according to manufacturer's instructions. All these media were allowed to cool after sterilization to about 45° C before pouring them into Petri dishes.

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Fish source

The fish were purchased from the Ministry of Agriculture Fish Farm, Alagbaka, Ondo State, Nigeria. *Clarias gariepinus* juveniles (480) were transported in oxygenated bags from point of purchase to the Fisheries and Aquaculture Laboratory, Ondo State University of Science and Technology, Okitipupa, Nigeria. Fish were subjected to a preventive bath of formaldehyde (37%, 30 min) and quarantined as described by Olusola and Nwokike (2018). All experimental protocols were approved by the Bioethical Committee of Ondo State University of Science and Technology, Okitipupa, Nigeria.

Experimental system and feeding experiment

The experiment was carried out in twenty plastic experimental tanks for 12 weeks (8 weeks for feeding trial and 4 weeks for challenge test) in the Fisheries and Aquaculture Laboratory of Ondo State University of Science and Technology, Okitipupa, Ondo State. The fish were acclimated for two weeks in experimental bowls before the experiment. Four hundred (400) *C. gariepinus* (3.02±0.01g) were randomly selected from 480 uniform-sized juvenile fish and then divided into ten (10) treatments. Each treatment had two replicates, with each replicate containing 20 fish. The fish were hand-fed twice daily at 3% body weight for eight-week feeding trials and four-week challenge test. The diet per day was divided into two: 1.5% given in the morning between 8.00 - 9.00 a.m. and 1.5% given in the evening by 5 p.m. The water of the aquaria was changed every three days. Measurement of the weight changes was performed weekly and the feeding rate adjusted weekly according to the new body weight.

Feed formulation

Feed ingredients were purchased from a re-known feed mill industry in Akure, Ondo State, Nigeria. Feed ingredients such as fishmeal, soybean, yellow maize, millet, starch, Di-calcium phosphate, vitamin-mineral premix, dry powder of tamarind and mango leaves were mixed together to formulate 40% crude protein diet. Each diet mixture treated separately was extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine to form a noodle-like strand which was mechanically broken into suitable sizes for *C. gariepinus* juveniles. The pelleted diets were sun dried, packed in labelled polythene bags and stored in a cool dry place to prevent mycotoxin formation (Table 1).

Biological evaluation

Fish were evaluated as follows: weight gain = final body weight - initial body weight; weight gain (%) = 100 (final body weight - initial body weight)/initial body weight; specific growth rate (SGR) = 100 (loge final body weight - loge initial body weight)/time (days) protein efficiency ratio (PER) = wet body weight gain (g)/crude protein fed and protein productive value (PPV) = 100 (final fish body protein - initial body protein)/crude protein intake.

Analytical methods

The sample (50 g) of fish diets from each treatment were taken and five (5) fish from each treatment were collected before and after the experiment and analyzed for their proximate composition according to the methods of Association of Official Analytical Chemists [AOAC] (2005).

Blood analysis

Before and after the challenge test, 5 ml of blood were collected from the caudal vein with 1 ml plastic syringe ringed with heparin, and the blood was then transferred immediately into a heparinize bottle containing heparin solution and shaken gently. The heamatological profile was performed following the methods of Blaxhall and Daisley (1973), using modified hyme's dilution fluid. Blood samples were also collected without heparin, allowed to clot and centrifuged at 7000 rpm for the collection of serum and biochemical analysis, and refrigerated. Serum samples were analyzed as described by Blaxhall and Daisley (1973).

Challenge test

Four hundred *C. gariepinus* (20 from each treatment) were induced by intraperitoneal route with 0.2 ml of 5.94 $\log_{10}$ CFU/ml *A. hydrophila* of 24 hours old culture. The challenged fish were kept under observation for 4 weeks. The clinical signs, skin lesions and mortalities were recorded and the Relative Percent Survival (RPS) among the induced fish was determined as described by Olusola and Nwokike (2018):

$$RPS = 1 - [\text{percentage of mortality in treated group}] / [\text{percentage of mortality in control group}] \times 100$$

Experimental design and statistical analysis

Completely randomized design was employed and data obtained for biological evaluation, proximate composition of fish before and after the experiment. Heamatological parameters, biochemical analysis and blood serum were analyzed by one-way analysis of variance, using Statistical Package for Social Sciences (SPSS 20.0) (SPSS Inc., Chicago, IL, U.S.A.). Duncan's new multiple range test was used to separate means of significant treatment at P=0.05.

RESULTS

Proximate composition of experimental diet

The proximate composition of the diets revealed the highest moisture content in diet (ML+OXY) 9 and lowest in diet ML3 (1%); the highest value of crude protein was recorded in diet (TL+ML+OXY) 10 and lowest in the control diet as shown in Table 1. Diet 10 (TL+ML+OXY) recorded the highest value of ether extract, ash content and lowest in Diet 3 and the control, respectively, with significant

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Table 1. Gross and proximate composition of experimental diets (g/100 g diet) of tamarind and mango leaves singularly or in combination with oxytetracycline in partial substitution for millet at different inclusion levels for *C. gariepinus*

INGREDIENTS	Control (0%)	TL2 (1%)	TL3 (2%)	ML4 (1%)	ML5 (2%)	OXY6 (30mg/kg)	(TL+ML)7 (2%)	(TL+OXY)8 (2%)	(ML+OXY)9 (2%)	(TL, ML+OXY)10 (3%)
Fish meal	16.79	16.79	16.79	16.79	16.79	16.79	16.79	16.79	16.79	16.79
Soybean	42.60	42.60	42.60	42.60	42.60	42.60	42.60	42.60	42.60	42.60
Yellow maize	16.31	16.31	16.31	16.31	16.31	16.31	16.31	16.31	16.31	16.31
Millet	16.31	15.31	14.31	15.31	14.31	14.31	14.31	14.31	14.31	13.31
Starch	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
*Vit-min premix	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Tamarind leaves	-	1.00	2.00	-	-	-	1.00	1.00	-	1.00
Mango leaves	-	-	-	1.00	2.00	-	1.00	-	1.00	1.00
Oxytetracycline 30mg/kg	-	-	-	-	-	2.00	-	1.00	1.00	1.00
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Moisture	4.63±0.01^{d}	4.62±0.02^{d}	4.31±0.02^{c}	4.07±0.03^{a}	4.77±0.01^{e}	5.66±0.02^{g}	5.79±0.01^{h}	5.89±0.02^{f}	6.99±0.01^{i}	4.25±0.02^{b}
Crude protein	40.61±0.05^{a}	40.11±0.01^{d}	40.17±0.02^{d}	40.03±0.02^{c}	40.25±0.03^{e}	40.95±0.01^{b}	40.15±0.01^{d}	40.95±0.02^{bc}	40.28±0.01^{e}	40.39±0.01^{f}
Ether extract	6.11±0.01^{d}	6.14±0.01^{d}	5.04±0.01^{d}	5.94±0.01^{a}	5.33±0.01^{c}	6.35±0.03^{e}	6.83±0.03^{f}	6.80±0.02^{f}	6.38±0.02^{e}	6.84±0.01^{f}
Ash	4.94±0.02^{a}	5.85±0.02^{b}	6.16±0.01^{bc}	6.72±0.01^{de}	6.51±0.01^{cd}	6.49±0.01^{cd}	6.45±0.44^{cd}	6.14±0.02^{bc}	6.17±0.03^{bc}	7.03±0.02^{e}
Crude Fibre	4.37±0.01^{bc}	4.54±0.02^{e}	4.28±0.01^{a}	4.28±0.01^{a}	4.45±0.02^{d}	4.39±0.01^{c}	4.34±0.03^{b}	4.52±0.02^{e}	4.65±0.01^{f}	4.52±0.01^{e}
NFE	40.39±0.01^{d}	39.68±0.01^{a}	42.70±0.01^{f}	42.45±0.36^{f}	41.76±0.05^{de}	41.58±0.05^{f}	41.81±0.06^{e}	39.82±0.07^{c}	40.73±0.02^{bc}	40.40±0.09^{b}

DCP = Di – calcium phosphate, TL =Tamarind Leaves, ML = Mango Leaves and OXY = Oxytetracycline. The above values are means of duplicate data, mean values in each row with similar superscripts are not significantly different ($p > 0.05$) *Vit-min premix for vitamin and minerals premix. Each 2 kg of premix contain; 12.5 million international unit (MIU); D_3, 2.5 MIU; E, 40 g; K_3 2g; B1,5.5 g; BB6,5 g; Niacin 55 g; Calcium Pantothenate 11.5 g; Chlorine chloride 500 g; Folic acid, Biotin,0.08 g; Manganese, 120 g; Iron, 100 g; Zinc, 80 g, Copper, 8.5 g; Iodine, 1.5 g; Cobalt, 0.3 g; Selenium, 0.12 g; Anti-oxidant, 120 g.

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

difference (P < 0.05) among dietary groups. The Nitrogen free extract (NFE) was highest in TL3 and lowest in TL2, and they were significantly different (P< 0.05) among the dietary groups.

Proximate composition of the fish before and after the experiment

The result of the proximate composition of fish before and after the experiment was presented in Table 2. The value of crude protein recorded was highest (72.57±0.02) in TL3 (2%) and lowest in the control. The control recorded the highest value for ether extract and lowest in diet (TL+ ML) 7, while TL2 (1%) recorded the highest ash content and lowest in TL3 (2%). Moisture content was reported highest in TL2 (1%) and lowest in TL3 (2%). NFE was highest in diet (TL+OXY) 8 and lowest in TL2 (1%). The values obtained after the experiment for the tested parameters were generally higher than the value obtained before the experiment. There were significant differences (P< 0.05) among the dietary groups.

Growth performance and nutrient utilization of C. gariepinus fed the experimental diet for 8 weeks

The result of the experiment revealed that the treated groups showed better performance in all the parameters compared to the control in terms of feed conversion ratio, specific growth rate, nitrogen metabolism, protein efficiency ratio, protein productive value and protein intake (see Table 3).

Challenge test

The result of this study shows that fish fed TL2 (1%), ML4 (1%), ML5 (2%), (TL+ML) 7, (TL+OXY) 8 and (ML+OXY) 9 recorded no mortality; TL 3(2%), OXY6 and (TL+ML+OXY) 10 recorded two mortalities and the control recorded the highest mortalities (15), and they were significantly different (P<0.05) among the dietary groups. Relative percent survival (RPS) was better in the treated groups compared to the control (Table 4).

Mean haematological parameter of C. gariepinus juveniles fed tamarind and mango leaves

There was an increase in the value of haematological parameters of the post-challenge test compared to the pre-challenge value and the control. Fish fed (TL+ML+ OXY) 10 recorded highest value in PCV, Hb, RBC and WBC when compared with pre-challenge and the control. There were no significant differences (P> 0.05) among the dietary groups, while the lymphocytes, neutrophils, Eosinophils, MCV, MCH and platelets were better in the treated groups compared with pre-challenge and the control, and they were significantly different (P< 0.05) among the dietary groups. Also, those treated in combination relatively showed synergistic effect with a relatively higher value than single application (Table 5).

Mean plasma biochemistry and blood serum parameters of C. gariepinus juveniles fed tamarind and mango leaves

Table 2. Proximate composition of the fish before and after the experiment

Parameter	Before	Control	TL2 (1%)	TL3 (2%)	ML4 (1%)	ML5 (2%)	OXY6	TL+ML7	TL+OXY8	ML+OXY9	TL+ML+OXY10
Moisture	12.33±0.01^{c}	13.64±0.02^{f}	20.61±0.01^{b}	8.23±0.01^{g}	16.13±0.01^{h}	16.32±0.01^{d}	16.12±0.01^{e}	13.34±0.01^{i}	13.67±0.0^{k}	16.73±0.01^{j}	13.15±0.01^{a}
Crude protein	63.39±0.01^{a}	64.58±0.0^{b}	65.79±0.01^{d}	72.57±0.02^{j}	66.46±0.01^{e}	67.51±0.01^{f}	66.47±0.02^{e}	68.44±0.01^{g}	70.89±0.0^{h}	65.56±0.01^{c}	71.69±0.01^{i}
Ether extract	4.22±0.01^{d}	5.38±0.02^{i}	4.52±0.02^{g}	4.21±0.01^{d}	4.48±0.03^{fg}	4.59±0.01^{h}	4.42±0.02^{f}	3.53±0.02^{a}	4.31±0.01^{e}	3.95±2.1^{c}	3.87±0.01^{b}
Ash	12.33±0.01^{b}	13.64±0.0^{e}	20.61±0.01^{i}	8.23±0.01^{a}	16.13±0.01^{f}	16.32±0.01^{g}	16.12±0.01^{f}	13.34±0.01^{d}	13.67±0.0^{e}	16.73±0.01^{h}	13.15±0.01^{c}
NFE	2.81±0.05^{c}	5.97±0.06^{e}	0.31±0.025^{a}	4.31±0.015^{d}	2.18±0.06^{b}	1.93±0.02^{b}	3.20±0.04^{c}	3.82±0.05^{d}	6.67±0.01^{f}	1.91±0.03^{b}	3.05±0.04^{c}

TL = Tamarind leaves, ML = Mango leaves, OXY = Oxytetracycline, NFE = Nitrogen free extract. The above values are means of duplicate data; mean values in each row with the same superscripts are not significantly different (p>0.05).

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Table 3. Growth performance and nutrient utilization of *C. gariepinus* fed the experimental diet for 8 weeks

Parameter	Control	TL2 (1%)	TL3 (2%)	ML4 (1%)	ML5 (%)	OXY6	TL+ML7	TL+OXY8	ML+OXY9	TL+ML+OXY10
Initial body weight (g)	3.02±0.10^{a}	3.02±0.25^{a}	3.03±0.05^{a}	3.02±0.00^{a}	3.03±0.50^{a}	3.02±0.10^{a}	3.02±0.05^{a}	3.02±0.15^{a}	3.02±0.05^{a}	3.02±0,05^{a}
Final body weight (g)	10.20±10.20^{a}	10.08±10.80^{a}	10.55±10.55^{a}	10.00±9.91^{a}	10.13±10.1^{a}	10.18±10.18^{a}	10.11±10.1^{a}	10.10±10.1^{a}	10.09±10.09^{a}	10.50±10.49^{a}
Body weight gain (g)	7.18±0.05^{a}	7.06±0.01^{a}	7.53±0.58^{a}	6.98±0.09^{a}	7.11±0.05^{a}	7.16±0.11^{a}	7.09±0.09^{a}	7.08±0.05^{a}	7.07±0.10^{a}	7.74±0.69^{a}
Body weight gain (%)	237.19±1.88^{a}	233.78±1.11^{a}	248.73±0.60^{b}	230.96±2.81^{a}	234.88±0.3^{a}	236.54±3.87^{a}	234.27±0.7^{a}	234.27±0.2^{a}	234.11±3.32^{a}	247.35±2.85^{b}
Feed conversion ratio	2.01±0.01^{e}	1.01±0.01^{a}	1.06±0.06^{ab}	1.71±0.02^{c}	2.11±0.01^{f}	1.96±0.03^{e}	1.84±0.03^{d}	1.65±0.01^{c}	1.14±0.02^{b}	2.66±0.05^{g}
Survival rate percent (%)	57.50±2.50^{a}	72.50±2.50^{bc}	72.50±2.50^{dc}	75.00±5.00^{cd}	72.50±2.50^{c}	65.00±0.00^{ab}	67.50±2.50^{bc}	72.50±2.50^{bc}	72.50±2.50^{bc}	82.50±2.50^{d}
Specific growth rate	0.95±0.01^{a}	0.93±0.00^{a}	0.97±0.04^{a}	0.93±0.01^{a}	0.94±0.01^{a}	0.94±0.01^{a}	0.94±0.01^{a}	0.94±0.01^{a}	0.82±0.14^{a}	0.97±0.01^{a}
Nitrogen metabolism	203.30±0.54^{a}	201.38±0.16^{a}	208.68±1.01^{b}	200.07±1.31^{a}	202.22±1.1^{a}	202.99±1.46^{a}	201.84±1.3^{a}	201.60±1.0^{a}	201.53±1.54^{a}	207.68±0.61^{b}
Protein efficiency ratio	0.16±0.00^{a}	0.16±0.00^{a}	0.17±0.01^{a}	0.16±0.01^{a}	0.16±0.00^{a}	0.16±0.00^{a}	0.16±0.00^{a}	0.16±0.00^{a}	0.16±0.00^{a}	0.16±0.00^{a}
Protein productive value	0.03±0.00^{a}	0.05±0.00^{a}	0.20±0.00^{c}	0.07±0.00^{ab}	0.09±0.00^{abc}	0.07±0.00^{a}b	0.11±0.00^{abc}	0.17±0.00^{bc}	0.05±0.00^{a}	0.18±0.00^{bc}
Protein intake	6.43±0.01^{h}	3.20±0.00^{a}	3.48±0.00^{b}	5.37±0.00^{e}	6.79±0.01^{i}	6.30±0.00^{g}	5.87±0.00^{f}	5.25±0.00^{d}	3.64±0.00^{c}	8.90±0.00^{j}

TL = Tamarind leaves, ML = Mango leaves, OXY = Oxytetracycline, the above values are means of duplicate data; mean values in each row with the same superscripts are not significantly different (p>0.05).

Table 4. Challenge test of *Aeromonas hydrophila* injected by intraperitoneal route and relative level of protection among *C. gariepinus* (n = 20) treated with tamarind and mango leaves

Parameter	Control	TL2 (1%)	TL3 (2%)	ML4 (1%)	ML5 (2%)	(OXY) 6	(TL+ML) 7	(TL+OXY)8	(ML+OXY)9	(TL+ML+OXY)10
Mortality (N)	15^{c}	0^{a}	2^{ab}	0^{a}	0^{a}	2^{ab}	0^{a}	0^{a}	0^{a}	2^{ab}
Mortality (%)	75^{c}	0^{a}	10^{b}	0^{a}	0^{a}	10^{b}	0^{a}	0^{a}	0^{a}	10^{b}
RPS	0^{a}	100^{c}	90^{bc}	100^{c}	100^{c}	90^{bc}	100^{c}	100^{c}	100^{c}	90^{bc}

TL = Tamarind leaves, ML = Mango leaves, OXY = Oxytetracycline, RPS = Relative percent survival. The above values are means of duplicate data; mean values in each row with the same superscripts are not significantly different (P> 0.05).

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Table 5. Mean values of some haematological, blood serum and plasma biochemistry parameters of *C. gariepinus* juveniles before and after the challenged experiment

Parameter	Pre-Challenge	Control	TL2 (1%)	TL3 (2%)	ML4 (1%)	ML5 (2%)	(OXY) 6	(TL+ML) 7	(TL+OXY) 8	(ML+OXY)9	(TL+ML+OXY)10
PCV (%)	39.00±0.00^{a}	40.00±0.01^{a}	41.00±0.00^{a}	42.00±0.02^{a}	38.00±0.03^{a}	43.00±0.00^{a}	42.00±0.01^{a}	40.00±0.02^{a}	41.00±0.04^{a}	42.00±0.01^{a}	44.00±0.01^{a}
Hb (g/dl)	12.70±0.05^{a}	13.30±0.02^{a}	13.70±0.00^{a}	13.00±0.03^{a}	14.00±0.05^{a}	14.30±0.01^{a}	14.00±0.01^{a}	13.30±0.01^{a}	13.70±0.01^{a}	14.40±0.00^{a}	14.70±0.06^{a}
RBCx1012/l	4.00±0.01^{a}	4.90±0.04^{a}	4.70±0.06^{a}	4.80±0.01^{a}	4.90±0.04^{a}	5.10±0.01^{a}	5.00±0.06^{a}	4.60±0.00^{a}	4.80±0.07^{a}	4.70±0.04^{a}	4.90±0.02^{a}
WBCx109/l	8.70±0.06^{a}	6.80±0.02^{a}	9.40±0.01^{a}	9.30±0.03^{a}	10.10±0.02^{a}	10.40±0.01^{a}	10.60±0.03^{a}	8.90±0.01^{a}	11.40±0.03^{a}	10.10±0.01^{a}	10.30±0.03^{a}
Platelet (m/µl)	21.40±0.00^{a}	19.70±0.05^{a}	20.30±0.02^{a}	20.40±0.03^{a}	18.70±0.01^{a}	29.40±0.01^{c}	28.00±0.03^{bc}	26.00±0.00^{abc}	21.40±0.05^{ab}	20.10±0.01^{a}	24.70±0.02^{abc}
MCV (Fl)	85.70±0.04b^{c}	87.00±0.01^{bc}	87.20±0.03^{bc}	81.30±0.01^{ab}	77.60±0.00^{a}	84.30±0.02^{bc}	84.00±2.00^{bc}	100.00±0.01^{d}	85.40±0.00^{bc}	89.40±0.04^{c}	89.80±0.01^{c}
MCH (Pg)	28.60±0.01^{ab}	28.90±0.00^{ab}	29.20±0.06^{ab}	27.10±0.02^{ab}	25.90±0.05^{a}	28.00±0.01^{ab}	28.00±0.02^{ab}	33.30±0.03^{b}	33.40±0.04^{ab}	33.30±0.02^{ab}	33.40±0.01^{ab}
MCHC (g/dl)	33.30±0.01^{a}	33.30±0.01^{a}	33.40±0.03^{a}	33.30±0.01^{a}	33.40±0.05^{a}	33.30±0.03^{a}	33.30±0.04^{a}	33.30±0.01^{a}	33.40±0.01^{a}	33.30±0.02^{a}	33.40±0.03^{a}
Nutrophil	62.00±0.08^{ab}	63.00±0.06^{ab}	60.30±0.01^{ab}	64.00±0.01^{ab}	66.00±0.01^{ab}	68.00±0.01^{b}	67.00±0.03^{ab}	60.00±0.00^{a}	64.00±0.07^{ab}	65.00±0.05^{ab}	64.00±0.04^{ab}
Lym x109/l	32.00±0.00^{ab}	29.00±0.02^{a}	33.00±0.01^{ab}	34.00±0.01^{ab}	33.00±0.04^{b}	34.00±0.01^{ab}	33.00±0.05^{ab}	37.00±0.03^{b}	34.00±0.02^{ab}	36.00±0.04^{b}	37.00±0.01^{b}
Mono x109/l	3.00±0.02^{a}	2.00±0.01^{a}	4.00±0.01^{a}	2.00±0.03^{a}	2.00±0.04^{a}	2.00±0.01^{a}	2.00±0.01^{a}	3.00±0.04^{a}	3.00±0.00^{a}	2.00±0.02^{a}	3.00±0.00^{a}
Eos x109/l	1.00±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{b}	1.00±0.01^{b}	1.00±0.00^{b}	1.00±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.00±0.01^{b}	1.00±0.00^{b}	1.00±0.00^{b}
Total protein (g/dl)	69.00±0.05^{a}	70.00±0.02^{a}	71.00±0.03^{a}	71.00±0.01^{a}	73.00±0.02^{a}	72.00±0.01^{a}	70.00±0.01^{a}	71.00±0.04^{a}	76.00±0.00^{a}	75.00±0.06^{a}	74.00±0.01^{a}
Albumin (g/dl)	36.00±0.02^{a}	37.00±0.06^{a}	38.00±0.04^{a}	37.00±0.02^{a}	40.00±0.03^{a}	40.00±0.04^{a}	41.00±0.02^{a}	39.00±0.06^{a}	38.00±0.07^{a}	39.00±0.03^{a}	42.00±0.04^{a}
Globulin (g/dl)	31.00±0.00^{a}	30.00±0.05^{a}	35.00±0.01^{a}	34.00±0.00^{a}	33.00±0.01a	35.00±0.03^{a}	29.00±0.01^{a}	32.00±0.01^{a}	39.00±0.02^{a}	36.00±0.08^{a}	32.00±0.03^{a}
A. G Ratio	1.16±0.00^{b}	1.26±0.00^{ab}	1.09±0.01^{ab}	1.09±0.00^{ab}	1.21±0.03^{ab}	1.14±0.01^{ab}	1.41±0.02^{b}	1.22±0.02^{ab}	1.00±0.00^{ab}	1.08±0.01^{a}	1.31±0.02^{b}
AST (IU/l)	10.00±0.02^{a}	13.00±0.01^{a}	12.00±0.03^{a}	12.00±0.03^{a}	11.00±0.02^{a}	12.00±0.02^{a}	10.00±0.01^{a}	11.00±0.04^{a}	9.00±0.01^{a}	10.00±0.00^{a}	12.00±0.06^{a}
ALT (IU/l)	12.00±0.03^{a}	15.00±0.01^{a}	13.00±0.01^{a}	14.00±0.01^{a}	13.00±0.01^{a}	14.00±0.01^{a}	13..00±0.01^{a}	13.00±0.01^{a}	12.00±0.06^{a}	11.00±0.02^{a}	13.00±0.03^{a}

TL = Tamarind leaves, ML = Mango leaves, OXY = Oxytetracycline, PCV = Packed cell volume, Hb = Haemoglobin, RBC = Red blood cell, WBC = White blood cell, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, Lym = Lymphocytes, Mono = Monocytes, Eos = Eosinophils, ALT = Amino alanine transferase, AST = Aspertate amino transferase. N = 4 fish from each tank were pooled for blood analysis. The above values are means of duplicate data; mean values in each row with the same superscripts are not significantly different (P >0.05).

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

Result of plasma biochemistry and blood serum obtained in this study were presented in Table 5. Pre-challenge fish recorded the lowest numerical value (69±2.00) in total protein, and fish fed (TL+OXY) 8 recorded the highest value (76±2.00). Also the value obtained in the control was lower than in the treated groups but higher than the pre-challenge value. There were no significant differences (P>0.05) in total protein, globulin and albumin among the dietary groups. The value of globulin recorded in pre-challenge test had a higher numerical value than the control but lower when compared to the treated groups. There were significant differences (P<0.05) in albumin-globulin ratio except in pre-challenge test (OXY6), (ML+OXY9) and (TL+ML+OXY) 10. The blood serum value recorded for both AST and ALT in the treated groups showed reduced value when compared to the pre-challenge and control. No significant differences (P > 0.05) were obtained in both parameters assayed among the dietary groups.

DISCUSSION

The proximate composition of experimental diet of this study supports the growth of *C. gariepinus* juveniles. This observation was supported by the findings of Olusola and Olorunfemi (2017) who reported 40% crude protein in the diet of *C. gariepinus* fed guava and drumstick leaf extracts. It also aligns with the report of Eyo (1995) that for maximum growth rate, fry and juveniles must have a diet in which nearly half of the digestible ingredients consist of balanced protein.

The treatments with tamarind leaves obtained significantly higher value (P < 0.05) of crude protein compared with the control and before the experiment. Inclusion of tamarind and mango leaves recorded a higher value of crude protein probably because the free amino acid was better utilized and growth stimulant constituent was present in tamarind and mango leaves. Fish fed diet of the combined group showed a relatively higher value compared to those of individual treatment. It is suggested that this plant and antibiotic has a combination effect. These results support the report of Lehar et al. (2009) who reported that synergistic drug combinations showed to be highly efficacious and therapeutically more specific. Also fish fed diet including tamarind leaves recorded a higher value compared to diet including mango leaves. From this observation, it could be deduced that *C. gariepinus* juvenile utilized tamarind leaves better than mango leaves. However, the result revealed that the diet supported the growth of fish as increased crude protein and body weight gain were recorded, and this showed that for African catfish the protein requirement was met for body maintenance and growth. Generally, groups treated with tamarind and mango leaves showed higher performance compared to the control and before the experiment. The present study is similar to the report of Fafiolu et al. (2006) who reported a higher value of crude protein among treated groups who were fed growing rabbit with wheat offal diet substituted with different graded levels of mango leaves. The present study also aligns with the earlier report of Jokthan et al. (2003) who reported a higher value in crude protein when fed *Mangifera indica* and *Ficus thonningii* leaves to rabbit.

The growth performance and nutrient utilization of *C. gariepinus* fed experimental diet revealed that the treated group (TL+ML+OXY) 10 recorded the highest value (7.74±0.69 g) for body weight gain, followed by TL3 (2%) (7.53±0.58 g). The highest value recorded by diet (TL+ML+OXY) 10 could be due to the synergistic effect of tamarind and mango leaves and oxytetracycline. There were no significant differences (P> 0.05) in body weight gain among the treatments.

The results showed that feed conversion ratio was best in TL2 (1%), (1.01±0.01) when compared among the treated groups and control. The treated groups recorded the highest value in survival rate, specific growth rate, nitrogen metabolism, protein efficiency ratio and protein productive value, (82.50±2.50), (0.97±0.04), (208.68±1.01), (0.17±0.01) and (0.20±0.00). Following this observation, it can be deduced that fish fed diet including tamarind performed better than those fed mango leaves, and that *C. gariepinus* juvenile better utilizes tamarind leaves when compared to mango leaves. Generally, fish fed treated tamarind and mango leaves when compared to the control had a better performance in the parameters assayed. The result of the present study was similar to the report of Zhang et al. (2014). However the report of the present study is not in agreement with Fafiolu et al. (2006) who reported decrease in body weight gain in the treated groups compared to the control.

Results of the challenge test showed that the mortality rate following the challenge test with *A. hydrophila* (5.94 $\log_{10}$ CFU/ml) was reduced in the treated groups compared to the control. Relative percent of survival was 100% in TL2 (1%), ML4 (1%), ML5 (2%), (TL+ML) 7, (TL+OXY) 8 and (ML+OXY) 9 and 0% in the control. There were significant differences (P <0.05) in mortality rate, percentage mortality and RPS among the dietary groups. This result aligned with the report of Shalaby et al. (2006) who recorded that, compared to the control, diet including *Allium sativum* and chloramphenicol showed decrease in the mortality rate of *O. niloticus* challenged intraperitoneally with *A. hydrophila*. Also, Sharma et al. (2010) reported that the challenge test with *A. hydrophila* proves that increased percent survival rate was highest in the treated groups compared to the control, which was in support of this study.

This is an indicator that these plants have non-specific immunostimulants that enhance the immunity of *C. gariepinus* juvenile against pathogen *Aeromonas hydrophila* and it can be inferred from the challenge test study that the increase RPS of the treated groups could be due to the enhancement in the defence system emanating

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

from the increase in value of immune indicators such as lymphocytes, neutrophils and white blood cells recorded in Table 5. Clinical signs such as abnormality in swimming, ulcerative lesion on the skin, oedema (swollen belly near the heart) were observed. This present study agrees with some of the identified signs reported by Mamnur et al. (2013).

The result of the study revealed that (TL+ML+OXY) 10 showed highest numerical value in PCV, Hb, MCH, MCHC and Lym among the treated groups in post-challenge test compared with the value recorded in pre-challenge and the control. This present study does not agree with Das et al. 2009 who reported decrease in Hb after 10 days of challenge. Although the values reported in the present study showed no significant difference (P>0.05) in Hb but recorded values higher than the pre-challenge, both in the control and in the treated groups. Generally there were increased values of lymphocytes obtained among the treated groups which recorded higher values than the pre-challenge and control, and the values were significantly different (P < 0.05) among the dietary groups. The present study showed that there was a correlation in the study report of Bello et al. (2014) who recorded that lymphocyte numerical values obtained in post-challenge test were also higher than the ones in pre-challenge and the control. It can be deduced from this study that tamarind and mango leaves could enhance antibacterial response of specific and non-specific metabolites. The result of this study showed that increase in WBC and lymphocytes, following the feeding of tamarind and mango leaves, supports the antimicrobial potentials of tamarind and mango leaves (Dipali et al., 2010).

The result revealed that total protein, globulin, albumin and albumin–globulin ratio values recorded were higher in post-challenge test than the pre–challenge and control. They were not significantly different (P < 0.05) among the dietary groups. This present study was similar to the report of Zhang et al. (2014), Garba and Abubakar (2012). However, Bello et al. (2014) recorded an increase in values of albumin and globulin ratio as compared to the control and pre-challenge on *C. gariepinus juveniles*, which also shows similarity with the present study.

The change in AST and ALT has thus been a focal point, as several activities might change AST and ALT function, chemical, biological and physiological factors or a disturbance in the kreb's cycle. Decreased activities of kreb's cycle cause a disease in its intermediates, thereby letting AST and ALT compensate by providing a-ketoglutarate (Salah El-Deen and Rogers, 1993). This present finding showed decrease in the treated groups when compared with the control and there were no significant differences (P> 0.05) among the dietary groups. This study was similar to the study of Bello et al. (2014) who reported a decrease in AST and ALT value obtained after feeding *C. gariepinus juveniles* with walnut and onion bulb at different graded level.

Tamarind leaves, mango leaves and oxytetracycline appear to provide stimulating effect on the parameters assayed, hence, they can be used as a potential tool for antimicrobial activity, as a growth promoter and to enhance non-specific immunity against *A. hydrophila*.

ACKNOWLEDGEMENTS

We wish to appreciate the efforts of Mr T. A. Fatokun for his technical assistance during the study.

UČINKOVITOST LIŠĆA TAMARINDA *Tamarindus indica* I MANGA *Mangifera indica* KAO DODACI RIBLJOJ HRANI NA RAST, KRVNI STATUS I OTPORNOST NA *Aeromonas hydrophila* KOD MLAĐI AFRIČKOG SOMA *Clarias gariepinus*

U istraživanju se ispitivala djelotvornost lišća tamarinda (TL) i manga (ML) pri usporedbi s oksitetraciklinom (OXY) na učinak rasta, krvni status i otpornost mlađi afričkog soma *Clarias gariepinus* na *Aeromonas hidrofila*. Eksperimentalna hrana sastojala se od kontrole (0%), TL2 (1%), TL3 (2%), ML4 (1%), ML5 (2%), OXY6 (30 mg/kg hrane), (TL + ML) 7, (TL + OXY) 8, (ML + OXY) 9 i (TL + ML + OXY) 10. Ribe (3,02 ± 0,01 g) su smještene u bazene s dva ponavljanja (20 riba u ponavljanju). Hranjene su dva puta dnevno izoproteinskom hranom (40% sirovog proteina), količinom od 3% tjelesne mase u trajanju od dvanaest tjedana (8 tjedana za pokusnu hranidbu i 4 tjedna za bakterijski test). Srednje vrijednosti prirasta mase (MWG), specifične stope rasta (SGR), hematokrita (PCV), hemoglobina (Hb), limfocita (LYM), globulina (GLO), amino-alanin transferaze (ALT) i aspartat amino-transferaze (AST) utvrđene su standardnom tehnikom određivanja. Ribe su zaražene sa 5,94 $\log_{10}$ CFU / ml *A. hydrophila* interperitonealno te su hranjene s eksperimentalnim hranama kako bi se procijenio relativni postotak preživljavanja (RPS). Podaci su obrađeni deskriptivnom statistikom i jednosmjernom analizom varijance pri P = 0,05. Riba hranjena eksperimentalnom hranom imala je više vrijednosti stope rasta s naglaskom na ribu hranjenom hranom (TL + ML + OXY) 10 koja je imala značajno viši MWG i SGR (7,74 ± 0,69 g, odnosno 0,77 ± 0,01 g). Također, vrijednosti PCV (44,0 ±2,00%), Hb (14,7 ±2,00 g / dl), LYM (37,0 ± 2,00), GLO (42,0 +2,00 g / dl) bile su više kod riba skupine (TL + ML + OXY) 10 nego kod riba kontrolne skupine. Vrijednosti AST i ALT kod riba u tretiranim skupinama bile su niže od vrijednosti kontrole. RPS na *A. hidrofila* bio je viši u tretiranim skupinama (100%) od kontrolnih (0%). Riba čija je hrana obogaćena listovima tamarinda i manga, imala je poboljšan prirast te veću otpornost na infekciju uzrokovanu *A. hydrophila*.

Ključne riječi: *Clarias gariepinus*, lišće manga, lišće tamarinda, *Aeromonas hidrophila*, hematologija, krvni serum.

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)

REFERENCE

Abbasi, K., Anjum, N., Sammi, S., Masud, T., Ali, S. (2011): Effect of coatings and packaging material on the keeping quality of mangoes (*Mangifera indica* L.) stored at low temperature. Pakistan J. Nutr., 10,129-138.

AOAC (2005): Association of Official Analytical Chemist Official methods of analysis (W. Horwit zeachtor) 18th edition A.O.A.C Washington, DC, 129-146.

Bansemir, A., Blume, M., Schröder, S., Lindequist, U. (2006): Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252, 79–84.

Bello, O. S., Olaifa, F. E., Emikpe, B. O. (2014): Haematological and biochemical changes in African catfish, *Clarias gariepinus* fed walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues - supplemented diets. American Journal of Experimental Agriculture. 4, 12, 1593 – 1603.

Blaxhall, P. C., Daisley, K. W. (1973): Routine haematological methods for use with fish blood. Journal of Fish Biology, 5, 771.

Boukary, A. R., Chaïbou M., Marichatou H., Vias G. (2007): Caractérisation des systèmes de production laitière et analyse des stratégies de valorisation du lait en milieu rural et périurbain au Niger: cas de la communauté urbaine de Niamey et de la com-mune rurale de Filingué. Rev. Elev. Med. Vet. Pays Trop., 60, 113-120.

Das, B. K., Pradhan, J., Sahu, S. (2009): The effect of *Euglena viridis* on immune response of rohu, *Labeo rohita* (Ham.). Fish and Shellfish Immunology, 26, 871–876.

Deng, G. C, Jiang, X. Y., Ye, X., Liu, M. Z., Xu, S. Y., Liu, L. H. (2009): Isolation, identification and characterization of *Aeromonas hydrophila* from Hemorrhagic Grass carp. Microbiol Chin, 36, 1170-1177.

Dipali, Y, J, Akshaya K. S., Jai S. G., Rahul C. R., Aruna M. M. (2010): Phytochemical detection and in vitro evaluation of tamarind fruit pulp for potential antimicrobial activity. International Journal of Tropical Medicine, 5, 3, 68-72.

Doughari, J. H. (2006): Antimicrobial Activity of *Tamarindus indica* Linn. Tropical Journal of Pharmaceutical Research, 5, 2, 597-603.

Doughari, J. H., Manzara, S. (2008): *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. Afr J Microbiol Res., 2, 067- 072.

Dubber, D., Harder, T. (2008): Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. Aquaculture, 274, 196–200.

Eyo, A. A. (1995): Fish feed and feeding practices in Nigeria In: Report of national Aquaculture Diagnostic Survey; May – July 1994 [Edited by Ayeni, J.S.O] National Institute of Fresh water Fisheries Research, New Bussa, Niger State Nigeria,41 -51.

Fafiolu, A. O., Oso, A. O., Bangbose, A. M., Omodia, R. E., Sediq, R. M. (2006): Performance of weaner rabbits fed cottonseed cake as replacement for soyabean meal. Proceedings of the 31st Annual conference of the Nigerian Society for Animal Production. 12th–15th March, 376–379.

Garba, Y., Abubakar, A. S. (2012): Haematological Response and blood chemistry of Yankasa Rams fed Graded Levels of Tamarind (*Tamarindus indica*) leaves. Nigerian Journals of Basic and Applied Sciences, 20, 1, 44-48.

Gupta, C., Prakash, D., Gupta, S. (2014): Studies on the antimicrobial activity of Tamarind (*Tamarindus indica*) and its potential as food bio-preservative. International Food Research Journal, 21, 6, 2437-2441.

Hernandez, P., Rodriguez, P. C., Delgado, R., Walczak, H. (2007): Protective Effect of *Mangifera indica* L. Polyphenols on Human T Lymphocytes against Activation Induced Cell Death. Pharmacological Res., 55, 167-173.

Ibrahim, M. D, Fthi, M., Mesalhy, S., Abd El-Aty, A. M. (2010): Effect of dietary supplementation of insulin and vitamin C on the growth, haematology, innate immunity and resistance of Nile tilapia (*Oreochromis niloticus*). Fish and Shellfish immunology, 29, 241- 246.

Isaac, A. O., Olusola, S. E., Oyelese, O. A. (2014): Microbial safety and quality of smoked *Clarias gariepinus* Burchell, 1822 on different concentrations of preservatives during ambient storage. International Journal of Aquaculture, 4, 21, 123 – 130.

Jadhav, V. S., Khan, S. I., Girkar, M. M., Gitte, M. J. (2006): The role of immunostimulants in fish and shrimp aquaculture. Aquatic animal health, Aquaculture Asia Magazine, July – September 2006, 24 – 27.

Jokthan, G. E., Afikwu, E. V., Olugbemi, T. S. (2003): The Utilization of Fig (*Ficus thonningii*) and Mango (*Mangifera indica*) Leaves by Rabbits. Pakistan Journal of Nutrition 2, 4, 264-266.

Lalisa, W. G. (2017): Phytochemical Screening and Antioxidant Activity of selected mango (*Mangifera indica*). Journal of Applied Chemistry, 10, 5, 24-28.

Lehar, J., Krueger, A. S., Avery, W., Heilbut, A. M., Johansen, L. M. (2009): Synergistic drug combinations tend to improve therapeutically relevant selectivity. Nature Biotechnology, 27, 659–666.

Mamnur, M. R., Hossain, M. S., Ali, M. F. (2013): Isolation and identification of *Aeromonas hydrophila* from Silver Carp and its culture environment. J. Bangladesh Agril. Univ, 11, 2, 373-376.

Olusola, S. E., Olorunfemi, B. V. (2017): Bioproductive effects of Clarias gariepinus fingerlings fed guava (*Psidium guajava*) leaves and drumstick (*Moringa oleifera*) leaves extracts supplemented diet. Journal of Applied and Tropical Agriculture, 22 (2): 156-165.

Olusola, S. E., Nwokike, C. C. (2018): Effects of dietary leaves extracts of bitter (*Vernonia amygdalina*) and pawpaw (*Carica papaya*) on the growth, feed conversion efficiency and disease resistance on juveniles Clarias gariepinus, Aquaculture Research, 49, 1858–1865.

Omitoyin, B. O. (2007): Introduction to fish farming in Nigeria, Royal bird ventures. 87.

Percival, S. S., Talcott, S. T., Chin, S. T., Mallak, A. C., Lounds-Singleton, A., Pettit-Moore, J. (2006): Neoplastic Transformation of BALB/3T3 Cells and Cell Cycle of HL-60 Cells are inhibited by mango (*Mangifera indica* L.) juice and mango juice extracts. The Journal of Nutr., 136, 1300-1304.

Salah El-Deen, M., Rogers, W. A. (1993): Changes in total protein and transaminase activities of grass carp exposed to Diquat. Journal of Aquatic and Animal Health 5, 280-286.

Shalaby, A. M, Khattab, Y. A., Abdel -Rahman, A. M. (2006): Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia. Journal of Venomous Animal Toxins including Tropical Diseases 12, 2, 172 – 201.

Sharma, A., Deo, A. D., Riteshkumar, S. T., Chanu, T. I., Das, A. (2010): Effect of *Withania somnifera* (L. Dunal) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in *Labeo rohita* (Hamilton) fingerlings. Fish and Shellfish Immunology, 29, 508- 512.

Zhang, G., Gong, S., Yu, D., Yuan, H. (2009): Propolis and *Herbed Epimedii* extracts enhance the non-specific immune response and disease resistance of Chinese sucker, Myxocyprinus asiaticus. Fish and Shellfish Immunology, 26, 3, 467–47.

Zheng, M., Wang, X., Templaton, L. J, Samulski, D. R., Larossa, R. A., Storz, G. (2001): Microarray-Mediated Transcriptional Profiling of the *Escherichia coli* Response to Hydrogen Peroxide. Applied and Environmental Microbiology, 183, 15, 4562-70.

© 2020 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/3.0/)