# EFFECT OF TAMARINDUS INDICA (LINN, 1753) PULP AND LEAF- FORTIFIED DIETS ON EXPERIMENTAL AEROMONAS HYDROPHILA INFECTION IN CLARIAS GARIEPINUS (BURCHELL, 1822)

*Olarinke Victoria Adeniyi[1], Flora E. Olaifa[2] and Emikpe B O[3]
[1]Department of Animal Production, Fisheries and Aquaculture, Kwara State University, Malete, Nigeria
[2]Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria
[3]Department of Veterinary Pathology, University of Ibadan, Nigeria

## Abstract

Intensification of aquaculture is associated with infectious diseases with consequent increase in the use of synthetic antibiotics. The rise in antibiotic-resistant bacteria is of global criticism. The role of Tamarindus indica as antimicrobial agents has been reported. This study evaluated the utilization of tamarind pulp and Leaves in the diets of Clarias gariepinus as antimicrobial agent against Aeromonas hydrophila infection. Ten experimental diets consisting of 0.5, 1.0, 1.5, 2.0% of basal diets each of tamarind pulp or leaf meal and 0.0% (untreated control) and 0.2% oxytetracycline (treated control) were fed to *C. gariepinus* for 12 weeks. After the feeding trial, fish were subjected to bath challenge with Aeromonas hydrophila and observed for 28 days to determine mortality and relative percentage survival. The surviving fish after 28 days post-challenge were sampled for haematological studies. Data were analyzed using one-way analysis of variance at P = 0.05. All experimental groups of the fish on tamarind-treated diets exhibited significantly lower (P □ 0.05) mortality compared to the groups fed with the control diets. 100% relative percentage survival was exhibited by the groups of fish on 1.5-2.0% pulp and 2.0% leaf diets. Higher white blood cell counts were obtained from tamarind-treated groups compared to the untreated control diets. The heterophil of the challenged fish fed diet with oxytetracycline was significantly lower (P □ 0.05) than fish fed 2.0% tamarind leaf diet. Inclusion of tamarind in the diets of the challenged *C. gariepinus* did not alter the monocytes, eosinophil and basophil significantly (P > 0.05) compared to those on the control diets. Fortifying the diets of Clarias gariepinus with 2.0% tamarind pulp and leaf meal significantly enhanced protection against Aeromonas hydrophila infection, hence these inclusion levels are recommended as alternatives to oxytetracycline in the control of motile aeromonas septicaemia.

**Keywords:** Clarias gariepinus, tamarind, synthetic antibiotics, Aeromonas hydrophila infection, relative percentage survival

# EFFET DES REGIMES ENRICHIS DE PULPES ET FEUILLES DE TAMARINDUS INDICA (LINN, 1753) SUR L'INFECTION EXPERIMENTALE A AEROMONAS HYDROPHILA CHEZ LES CLARIAS GARIEPINUS (BURCHELL, 1822)

## Résumé

L'intensification de l'aquaculture est associée à des maladies infectieuses qui ont pour conséquence une augmentation de l'utilisation d'antibiotiques de synthèse. L'augmentation des bactéries résistantes aux antibiotiques fait l'objet de critiques au niveau mondial. Le rôle de Tamarindus indica en tant qu'agent antimicrobien a été rapporté. La présente étude a évalué l'utilisation de pulpes et feuilles de tamarin dans les régimes de Clarias gariepinus comme agent antimicrobien contre l'infection à Aeromonas hydrophila. On a administré à *C. gariepinus* pendant 12 semaines dix régimes expérimentaux constitués de 0,5, 1,0, 1,5 et 2,0% de régimes basaux de farine de pulpes ou de feuilles de tamarin et 0,0% (témoin non traité) et 0,2% d'oxytétracycline (témoin traité). Après l'essai alimentaire, les poissons ont été soumis à une infection en bain à Aeromonas hydrophila et observés pendant 28 jours pour déterminer la mortalité et le pourcentage

*Corresponding author email: adeniyiovic@yahoo.com

de survie relatif. Les poissons ayant survécu après 28 jours post-infection ont été échantillonnés pour des études hématologiques. Les données ont été analysées en utilisant une analyse de variance à un facteur à P = 0,05. Tous les groupes expérimentaux de poissons soumis aux régimes traités avec le tamarin ont présenté une mortalité significativement plus faible (P <0,05) par rapport aux groupes nourris aux régimes témoins. Un pourcentage de survie relatif de 100% a été observé pour les groupes de poissons recevant des régimes de 1,5-2,0% de pulpe et 2,0% de feuilles. Un nombre plus élevé de globules blancs a été obtenu chez les groupes traités au tamarin par rapport aux régimes témoins non traités. L'hétérophile du poisson infecté soumis à un régime alimentaire contenant de l'oxytétracycline était significativement plus faible (P <0,05) que celui du poisson soumis au régime à 2,0% de feuilles de tamarin. L'inclusion du tamarin dans les régimes de *C. gariepinus* infectés n'a pas modifié significativement les monocytes, les éosinophiles et les basophiles (P> 0,05) par rapport à ceux soumis aux régimes témoins. L'enrichissement des régimes alimentaires de Clarias gariepinus avec 2,0% de farine de pulpes et de feuilles de tamarin a significativement amélioré la protection contre l'infection à Aeromonas hydrophila et, par conséquent, ces niveaux d'inclusion sont recommandés comme solution de rechange à l'oxytétracycline dans le contrôle de la septicémie à aeromonas mobile.

**Mots-clés :** Clarias gariepinus, tamarin, antibiotiques de synthèse, infection à Aeromonas hydrophila, pourcentage de survie relatif

## Introduction

Intensification of aquaculture has led to remarkable improvement in productivity (FAO (Food and Agriculture Organization), 2014, 2016), but it is also associated with diseases, considered to be the major source of mortality in aquaculture, and consequently increase in the use of antimicrobials (Defoirdt *et al.,* 2011). Bacterial diseases (including aeromonad septicaemia) are reported to be an important economic limiting factor in intensive aquaculture, responsible for mass losses (Claudia and Jettrey, 2009; Hossain *et al.,* 2011; Pridgeon and Klesius, 2012; Oladosu *et al.,* 2013) leading to therapeutic use of synthetic antibiotics. The use of synthetic antibiotics and other drugs in aquaculture is complicated because of the need to administer them directly into the water where the fish are reared. Hence, safety of the fish products for consumption and environmental impacts are crucial factors for consideration (FAO, 2005).

Aeromonas hydrophila is a bacterial pathogen with a wide geographical distribution in both the terrestrial and aquatic environments. It has been isolated from a variety of sources including soil, lakes, rivers, water reservoirs, ground water, drinking water leaving treatment plant, wound infections and human stools (United State Environmental Protection Agency, 2005; Subashkumar *et al.,* 2006; Newaj-Fyzul *et al.,* 2008, Rogo *et al.,* 2009; Anuradha *et al.,* 2010; Shayo *et al.,* 2012; Saidi *et al.,* 2013). A. hydrophila is the aetiological agents of motile aeromonas septicaemia which have infected a wide variety of fish species such as carp, tilapia, catfish, perch, salmon and gold fish (Janda and Abbott, 2010; Parker and Shaw, 2011). Motile aeromonas septicaemia is a devastating disease of cultured freshwater fish with acute to chronic clinical signs and causing significant mortality (Plumb and Hanson, 2011; Edward, 2012). The incidence of motile aeromonas septicaemia resulting to mass fish mortality that threatened livelihood of people has been reported (Shayo *et al.,* 2012) and great economic losses globally (Pridgeon *et al.,* 2012, Oladosu *et al.,* 2013).

To control infectious diseases in aquaculture, the same antibiotics used in other areas of animal production are employed. The intensive use of antibiotics for prevention and control of disease has contributed to increased antibiotic-resistant bacteria, immune suppression and environmental pollution (FAO, 2011; Sajid *et al.,* 2011; Gent *et al.,* 2012). Limited number of the antibiotics was approved for aquaculture use. The three antibiotics authorized for use in aquaculture by Food and Drug Administration in United States of America are oxytetracycline, sulfadimethoxine /ormetoprim and aquaflor / florfenicol (Pridgeon *et al.,* 2011; Romero

*et al.,* 2012). Motile aeromonas septicaemia is usually treated with oxytetracycline in the food of the fish at the rate of 55 mg per kg fish/ day for 10 days (George, 1987; Edward, 2012, Ferrandez *et al.,* 2014). Oxytetracycline is one of the most commonly used antibiotics in fish farming, both in the hatcheries and grow-out ponds. It is however poorly absorbed through the intestinal tracts of fish, hence it has to be administered at a high rate of 100-150mg/kg fish/day for 10-15 days (Romero *et al.,* 2012).

The role of herbal and medicinal plants as antimicrobial agents for fish pathogens and as immunostimulants in fish has been reported (Aly and Mohamed, 2010; Pakravan *et al.,* 2011; Sajid et. al., 2011, Amirkhani and Firouzbakhsh, 2013). The efficacy of any prophylactic and therapeutic agents for aquaculture use is usually tested through in vivo experimental challenge tests. Some researchers have reported higher level of protection of experimentally challenged fish and shrimp fed herbal products (Logambal *et al.,* 2000; Immanuel *et al.,* 2004; Praseetha, 2005; Aly and Mohamed, 2010; Pakravan *et al.,* 2011; Bello *et al.,* 2012; Yilmaz *et al.,* 2013; Pujarini *et al.,* 2014; Manaf *et al.,* 2016). Tamarind, Tamarindus indica (Linn, 1753), is a medicinal tree species which grows widely in tropical and sub- tropical regions of the world (El-Siddig *et al.,* 2006; Kumar and Bhattacharya, 2008). In vitro antimicrobial activity of tamarind has been reported (Dhoughari, 2006; Daniyan and Muhammad, 2008; Adeniyi *et al.,* 2017). Our previous study demonstrated the in vitro antimicrobial activity of tamarind pulp and leaf on Aeromonas hydrophila (Adeniyi *et al.,* 2017). There is paucity of information on the effects of dietary tamarind on disease resistance in fish. Therefore, the aim of this study was to evaluate the effect of dietary tamarind pulp and leaf meal as feed additives on protection of Clarias gariepinus against experimental Aeromonas hydrophila infection.

## Materials and methods

*Experimental diets*

Experimental diets (Table 1) consisted of ten groups (eight tamarind-treated and two control diets). Air-dried tamarind pulp and leaves were processed into meals and included singly at 0.5. 1.0, 1.5 and 2.0% each to fortify the basal diets while the controls were included at 0.0% (untreated control) and 0.2% oxytetracycline (treated control).

*Pathogen*

Pathogenic Aeromonas hydrophila from a stock isolated from moribund Clarias gariepinus was used for the challenge test. The isolate from the frozen ampules was sub-cultured for 24 hour at 37°C on nutrient agar. The 24-hour bacteria were suspended in physiological saline solution and standardized to 0.5 McFarland standard (1 x 108CFU/ml) (CLSI, 2012). The inoculum was mixed with the water in the challenge tank at 0.2ml/litre of water.

*Challenge protocols*

Clarias gariepinus (30 – 45g) previously fed the experimental diets (Table 1) for 12 weeks were transferred from the culture tanks to the challenge tanks. A bath challenge was conducted with twenty *C. gariepinus* in each tank in triplicate per treatment. The fish were immersed in the culture suspensions for 1 hour (Adelmann *et al.,* 2008; Emmenegger *et al.,* 2013) and then returned to the culture tanks. The challenged fish were offered the experimental diets (Table 1) and observed for 28 days for external signs, erratic swimming, behavioural and clinical signs, response to feed and mortality rates were recorded. The Cumulative Mortality Index (CMI) and Relative Percentage Survival (RPS) were also calculated using these formulae (Amend, 1981);

i. $CMI = Dt_1 + Dt_2 + Dt_3 + \text{---------- } Dtn$ (D is the number of dead fish at the given time (days), t1, t2, t3. ---------- tn)
ii. $RPS = 1 - \frac{\%\text{ mortality in treatment}}{\%\text{ mortality in untreated control}} \times 100$ (Amend, 1981; Thorborn *et al.,* 1987)

*Haematological studies*

The survived fish after 28 days post-challenge were sampled for haematological studies. Blood samples were collected from

**Table 1:** Gross composition (g/100g dry matter) of experimental diets at varying inclusion levels of tamarind pulp and leaves fed to Clarias gariepinus for 16 weeks

| **Ingredients** | **Diets (% inclusion levels)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **+ C** | **- C** | **0.5P** | **1.0P** | **1.5P** | **2.0P** | **0.5L** | **1.0L** | **1.5L** | **2.0L** |
| Fish meal | 24.11 | 24.11 | 24.11 | 24.11 | 24.11 | 24.11 | 24.11 | 24.11 | 24.11 | 24.11 |
| SBM | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| GNC | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 |
| Maize | 10.89 | 10.89 | 10.89 | 10.89 | 10.89 | 10.89 | 10.89 | 10.89 | 10.89 | 10.89 |
| Soy oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 1.50 | 0.50 | 0.50 | 0.50 |
| Bone meal | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| O. shell | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Premix* | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Cr2O3 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Starch | 3.50 | 3.30 | 3.00 | 2.50 | 2.00 | 1.50 | 3.00 | 2.50 | 2.00 | 1.50 |
| Tamarind | - | - | 0.50 | 1.00 | 1.50 | 2.00 | 0.50 | 1.00 | 1.50 | 2.00 |
| OTC | - | 0.2 | - | - | - | - | - | - | - | - |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **100** |

**Vitamin A = 20,500.00IU, Vitamin B1 = 20,000.00mg, Vitamin B2 =15,000.00, Vitamin B3 = 90, 000mg, Vitamin B4 =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, Antioxidant = 125, 000.00mg, Lysine = 50, 000.00mg, Cobalt = 750.00mg, Sodium Selenite 200.00 mcg, Zinc oxide = 40,000.00 mg. +*

*C = untreated Control - C = OTC - treated Control T = Pulp L= Leaves SBM = Soybean Meal GNC= Groundnut Cake O. = Oyster OTC = Oxytetracycline*

the caudal vein of fish in each treatment from the caudal vein hypodermic syringe (Ejraei *et al.,* 2015; Erfan *et al.,* 2015) into heparinised tubes. Haemoglobin (Hb: g/dl) was determined spectrophotometrically (SM23A, England) by measuring the formation of cyanomethaemoglobin using Drabkin's solution. The absorbance of the solution was measured at wave length 540 nm against Drabkin's solution as a blank. Packed Cell Volume (PCV: %) was determined by placing fresh blood in glass capillary tubes and centrifuging at 10,500 rpm in a microhaematocrit centrifuge for five minutes and the height of the tubes measured using haematocrit reader (Hawksley, SH120). Red Blood Cell (RBC), White Blood Cell (WBC) and platelets were determined (Dacie and Lewis, 1991) using a light microscope (Olympus, Olympus Corporation, China) with improved Neubauer bright-line haemocytometer (Marienfeld, Agoda Company Pte. Ltd., Germany).

Leucocytes differential cells were analyzed from blood smears were made on slides and left to air-dry. The slides were stained with Giemsa stain (Giemsa Laboratories Ltd. Molbase, Shanghai) using differential stains. Slides were examined using a light microscope (Olympus, Olympus Corporation, USA) with 1000x magnification. Each slide was moved in one direction while the number of neutrophil, heterophil, basophil, monocytes and *lymphocytes* were counted using blood cell differential counter (Durga, Miniscience, Inc., USA) until a total of 100 leucocytes were counted. The differential cells were expressed in percentage (Harikrishnan *et al.,* 2010). Sera liver enzymes (Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase) were determined by using commercial (Randox) kits, following the procedure described by the manufacturer.

*Statistical analysis*

Data were analyzed using One-way Analysis of Variance (ANOVA). Duncan multiple range tests was used to compare differences among means at 5% probability level using statistical software SAS (Statistical Analysis System, 2010).

## Results

*Fish survival and nutrient utilization*

After 28 days post-challenge, all experimental groups of the *C. gariepinus* on tamarind-treated diets exhibited significantly lower (P ☐ 0.05) cumulative mortality index compared to the groups fed with the control diets (Figure 1). Fish on the diet containing oxytetracycline had significantly lower (P ☐ 0.05) percentage mortality compared to the fish on the untreated control diet. 100% relative percentage survival was exhibited by the groups of fish on 1.5-2.0% TP and 2.0% TL diets. Also groups of fish fed tamarind-treated diets exhibited better (P ☐ 0.05) level of protection against Aeromonas hydrophila than oxytetracycline-treated groups. The feed utilization (Figure 2) was significantly enhanced (P ☐ 0.05) in tamarind-treated groups than in the control groups.

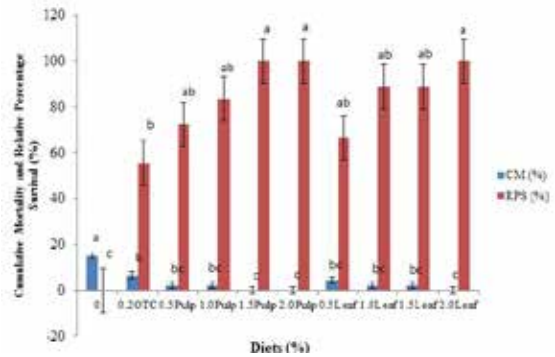



**Figure 1:** Effect of tamarind-fortified diets on cumulative mortality and nutrient utilization of Clarias gariepinus challenged with Aeromonas hydrophila after 28 days post-challenge

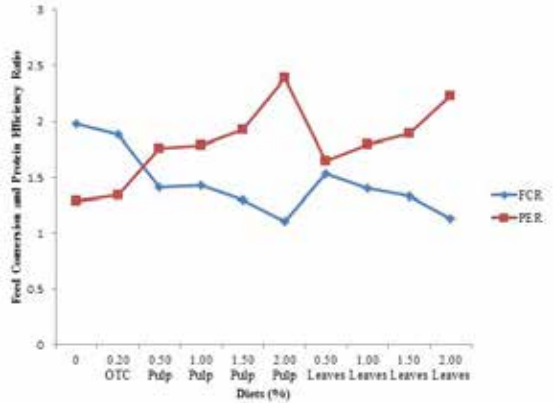



**Figure 2:** Effect of tamarind-fortified diets on feed utilization of *C. gariepinus* challenged with Aeromonas hydrophila after 28 days post-challenge

*Haematology*

Heamatological profiles of the groups of *C. gariepinus* challenged with Aeromonas hydrophila are shown in Table 2. The pack cell volume and haemoglobin of the group of fish fed with diets containing TP did not differ significantly (P > 0.05) from those fed the control diets. The RBC counts of *C. gariepinus* on 1.5% TP and 1.0% TL were significantly higher than in the control groups. The platelet counts of the fish on diet containing 1.0% TP and TL were significantly lower (P ☐ 0.05) than the values obtained from those on other diets Treating the diets with tamarind did not alter the WBC counts of the challenged fish significantly (P > 0.05). However, higher values of WBC counts were obtained from tamarind-treated groups compared to the untreated control diets. At 2.0% TP and TL inclusion levels, WBC counts were higher than in the control diets. The lymphocyte count of the fish fed 1.5% TP diet was significantly higher (P ☐ 0.05) than those fed other TP groups, 1.0 and 2.0% TL and untreated control diets. The neutrophil of the challenged fish fed with OTC-treated diet was significantly lower (P ☐ 0.05) than fish fed 2.0% TL diet. Inclusion of tamarind in the diets of the challenged *C. gariepinus* did not alter the monocytes, eosinophil and basophil significantly (P > 0.05) compared to those on the control diets. Sera aspartate aminotransferase and alkaline phosphatase were significantly (P ☐ 0.05) altered among the treatment groups with

**Table 2:** Effect of tamarind-fortified diets on haematological profiles of Clarias gariepinus challenged with Aeromonas hydrophila after 28 days post-challenge

| Diets (%) | PCV (%) | Hb (g/dl) | RBC ($x10^6$µl) | PLA ($x10^6$µl) | WBC ($x10^6$µl) | LYM (%) | HET (%) | MON (%) | EO (%) | BA (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.00 | 25.50±1.29$^{abc}$ | 8.68±0.09$^{abc}$ | 2.18±0.45$^{cd}$ | 242.50±92.40$^{ab}$ | 14.19±4.42 | 52.75±3.61$^{bc}$ | 41.00±5.57$^{ab}$ | 3.25±0.97 | 3.00±0.82 | 0.00±0.00 |
| 0.20OTC | 25.75±1.71$^{abc}$ | 8.60±0.22$^{abc}$ | 2.11±0.51$^{cd}$ | 195.75±15.00$^{abc}$ | 16.83±1.45 | 59.25±1.71$^{ab}$ | 33.50±1.29$^{bc}$ | 4.25±0.50 | 2.75±0.50 | 0.25±0.50 |
| 0.50P | 30.25±4.73$^{ab}$ | 10.28±3.46$^{a}$ | 2.74±1.34$^{abc}$ | 250.00±71.02$^{a}$ | 15.41±1.55 | 56.75±2.99$^{bc}$ | 33.75±3.11$^{ab}$ | 2.50±0.58 | 2.00±1.15 | 0.25±0.50 |
| 1.00P | 27.75±1.26$^{abc}$ | 8.78±0.09$^{abc}$ | 2.46±0.95$^{bcd}$ | 158.75±34.36$^{c}$ | 15.56±0.25 | 59.25±2.36$^{ab}$ | 37.50±2.22$^{bc}$ | 3.50±0.58 | 4.00±2.00 | 0.5±0.58 |
| 1.50P | 31.25±1.26$^{a}$ | 10.65±0.39$^{a}$ | 3.51±0.08$^{a}$ | 179.00±48.51$^{abc}$ | 15.41±2.99 | 57.00±4.73$^{bc}$ | 37.50±4.73$^{ab}$ | 2.75±1.50 | 2.50±1.29 | 0.25±0.50 |
| 2.00P | 24.50±4.20$^{bc}$ | 8.05±1.45$^{bc}$ | 2.45±1.04$^{bcd}$ | 200.50±64.66$^{abc}$ | 18.54±4.63 | 54.50±2.64$^{ab}$ | 39.75±2.09$^{ab}$ | 2.50±1.00 | 3.25±0.50 | 0.00±0.00 |
| 0.50L | 30.25±1.71$^{ab}$ | 9.73±0.63$^{ab}$ | 3.32±0.07$^{ab}$ | 194.00±40.43$^{abc}$ | 14.96±5.33 | 61.50±1.91$^{ab}$ | 32.25±2.63$^{bc}$ | 3.00±1.82 | 3.25±1.26 | 0.00±0.00 |
| 1.00L | 30.25±1.71$^{ab}$ | 9.90±0.65$^{ab}$ | 3.49±0.29$^{a}$ | 154.25±50.56$^{c}$ | 14.89±1.29 | 56.00±4.58$^{bc}$ | 37.00±5.03$^{ab}$ | 2.75±1.71 | 4.25±0.96 | 0.00±0.00 |
| 1.50L | 23.75±1.71$^{c}$ | 7.53±0.66$^{c}$ | 1.72±0.03$^{d}$ | 171.00±35.81$^{bc}$ | 17.14±1.05 | 66.25±4.99$^{a}$ | 27.00±4.97$^{c}$ | 2.75±0.96 | 3.75±1.50 | 0.25±0.50 |
| 2.00L | 29.75±4.99$^{abc}$ | 9.80±1.27$^{ab}$ | 2.95±0.52$^{abc}$ | 211.50±34.11$^{abc}$ | 18.30±1.39 | 50.00±3.63$^{c}$ | 43.50±5.97$^{a}$ | 3.75±0.96 | 2.75±0.50 | 0.00±0.00 |

*Means with the same letter on the same column are not significantly different at P □ 0.05* *OTC = Oxytetracycline P= Pulp L = Leaves PCV = Packed CellVolume Hb = Haemoglobin RBC = Red Blood Cell PLA = Platelet WBC = White Blood Cell LYM = Lymphocytes HET = Heterophil MON = Monocyte EO = Eosinophil BA = Basophil*

the highest in the untreated control group (Figure 3).

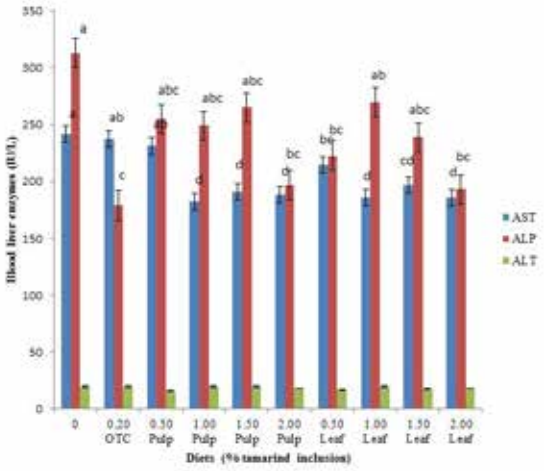



**Figure 3:** Effects of dietary tamarind pulp and leaf on Blood liver enzymes of Clarias gariepinus challenged with Aeromonas hydrophila ALT=Alanine aminotransferase AST = Aspartate aminotransferase ALP = Alkaline phosphatise

## Discussion

Higher level of protection was exhibited by the groups of fish fed tamarind-treated diet and OTC-treated diet compared to the untreated control group. Significantly higher relative percentage survival demonstrated by *C. gariepinus* on dietary tamarind pulp and leaf against Aeromonas infection at 1.5-2.0% inclusion levels than the treated-control group shows the great antimicrobial potentials of these phytogenics as natural alternative to oxytetracycline. Relative percentage survival above 70 is good potency index of aquaculture vaccine (European Pharmacopoeia, 2002). The RPS of fish fed diets containing 1.0-2.0% TP and TL were above 80%. This might be as a result of the antimicrobial activity of TP, TL and OTC (Adeniyi *et al.,* 2017) and enhanced feed utilization. This observation coincided with the reports of other researchers on the effects of phytobiotics on disease resistance of fish. Higher level of protection was obtained in Oreochromis niloticus fed diets containing Echinacea and garlic (Aly and Mohamed, 2010) and green tea (Abdel-Tawwad *et al.,* 2010) against A. hydrophila. Goldfish fed diets supplemented with mixed herbal extracts (Harikrishnan *et al.,* 2010) also exhibited higher protection against A. hydrophila. Also, feeding carps on diets treated with herbal mixture (Sudagar and Hajibeglou, 2010), willow herb (Pakravan *et al.,* 2011), basil (Amirkhani and Fibarouzkhsh, 2013) and Euphorbia hirta leaves extracts (Pratheepa and Sukumaran, 2014) resulted in higher level of protection against A. hydrophila. Furthermore, Mystus montanus fed powdered Ocimum tenniforum, Zingiber offiniale and Allium cepa (Kumar et al. 2014) and rohu fed garlic-based adjuvant (Pujarini *et al.,* 2014) were significantly protected against A. hydrophila infection.

Similarly, shrimps fed diets treated with sea weed (Immanuel *et al.,* 2004) and Withania somnifera (Praseetha, 2005) as well as O. mossambicus fed with herbal additives (Immanuel *et al.,* 2009) against Vibrio species exhibited higher survival. Higher percentage survival was also exhibited by African catfish fed diets containing walnut leaf and onion bulb against *Pseudomonas aeruginosa* (Bello *et al.,* 2012b). O. mossambicus fed diets treated with cumin (Yilmaz *et al.*. 2013), Vitex trifolia, Strobilanthes crispus and Aloe vera mixtures (Manaf *et al.,* 2016) against Streptococcus species also exhibited higher protection against the infection. The observed higher protection of the fish against the pathogens enhanced by the herbal additives compared to synthetic antibiotics further establish their potential as suitable alternative to synthetic antibiotics.

Haematological parameters are indicators of the health status of fish (Fagbenro *et al.,* 2013). WBCs are the blood cells mediating the innate and adaptive responses in animals, providing protection against infection caused by chemical and microbial agents (Mak and Saunders, 2006; Aly and Mohammed, 2010; Pakravan *et al.,* 2011).The improved erythrocytes and leukocytes counts of the groups of challenged fish fed with the tamarind-treated diets, although without significant difference, is an indication of the ability of the phytogenic additives to enhance non-specific immune system of the experimental fish. Also,

the higher *lymphocytes* count in the fish fed tamarind-treated diets and the complementary higher values of heterophil in the groups of fish fed diet containing 2.0% tamarind pulp and leaves might have contributed to the significantly higher protection of these groups against Motile Aeromonas Septicaemia (MAS) in the experimental fish compared to the group fed OTC-treated and negative control diets.

Varying haematological observations on the effects of phytogenic additives on experimentally challenged fish have been reported. WBC counts increased while RBC and Hb reduced significantly when infected goldfish were fed diets containing 0.1-0.2g/kg of mixture of some herbal extracts (Harikrishnan *et al.,* 2010). Improvements in haematological parameters of A. hydrophila infected O. niloticus fed diets containing green tea (Abdel-Tawwab *et al.,* 2010) and carp fed varying inclusion levels of herbal extract mixture (Sudagar and Hajibeglou, 2010) were also reported. Pakravan et al. (2011) also reported lower RBC and PCV of A. hydrophila infected carp fed diets containing willow herb extracts while the authors also observed higher WBC counts and insignificant differences in Hb, *lymphocytes*, monocytes and neutroplil. Treating the diets of carp with basil leaves extract also resulted in significant increase in Hb, RBC and WBC counts when challenged with A. hydrophila (Amirkhani and Firouzbakhsh, 2013). Increase in activities of blood liver enzymes (aspartate aminotransferase and alkaline phosphatase) in the blood is an indication of reduction in the functional integrity of cell membrane of the liver, resulting to hepatic necrosis (Modesto *et al.,* 2013). The lower activities of these enzymes in fish on tamarind-fortified diets might be because the level of haemorrhage caused by Aeromonas hydrophila was significantly reduced and consequently enhanced their survival.

In conclusion, feeding Clarias gariepinus with diets fortified with 2.0% tamarind pulp and leaf meal improved some immunity characteristics, reduced blood liver enzymes and consequently gave highest protection against Aeromonas hydrophila infection. Hence, this inclusion level is recommended as alternatives to oxytetracycline in the control of motile aeromonas septicaemia in African catfish.

## Acknowledgement

We are grateful for the laboratory stock of Aeromonas hydrophila obtained from Dr. G.A. Oladosu of the Department of Veterinary Medicine, University of Ibadan, Nigeria.

## References

Abdel-Tawwab M., Ahmad M.H., Medhat E.A. and Saleh F.M. 2010. Use of green tea, Camellia sinensis L. in practical diet for growth and protection of Nile tilapia, Oreochromis niloticus (L) against Aeromonas hydrophila infection. Journal of the World Aquaculture Society, 41.2: 203-213.

Adelmann M., Bernd K., Bergmann S.M., Fischer U., Lange B., Weitschies W., Enzmann P. and Fichtner D. 2008. Development of an oral vaccine of immunization of rainbow trout, Oncorhynchus mykiss against viral Haemorrhagic septicaemia. Vaccine, 26:837-844.

Adeniyi O.V., F. E. Olaifa, B.O. Emikpe and S.T. Ogunbanwo (2017): Phytochemical components and antibacterial activity of Tamarindus indica Linn extracts against some pathogens. Biotechnology Journal International, 17 (2): 1-9

Aly S.M. and Mohamed M.F. 2010. Echinacea purpurea and Allium sativum as immunostimulants in fish culture using Nile tilapia (Orechromis niloticus). Journal of Animal Physiology and Animal Nutrition, 94: e31-e39.

Amend D. F. 1981. Potency testing of fish vaccines. Developments in Biological Standardization, 49: 447-454.

Amirkhani N. and Firouzbakhsh F. 2013. Protective effects of basil (Ocimum bacilicum) ethanolic extract supplementation diets against experimental Aeromonas hydrophila injection in common carp (Cyprinus carpio). Aquaculture Research, 46.3: 716-724.

Anuradha K., Foo H.L., Mariana N.S., Loh T.C., Yusoff K., Hassan M.D., Sasan H. and Raha A.R. 2010. Live recombinant Lactococcus lactis vaccine expressing aerolysin genes D1 and D4 for protection against Aeromonas hydrophila in tilapia (Oreochromis niloticus). Journal of Applied Microbiology, 109: 1632-1642.

Bello O.S., Emikpe B.O. and Olaifa F.E. 2012. The protective effect of walnut (Tetracarpidium conophorum) leaf and onion (Allium cepa) bulb residues on the experimental *Pseudomonas aeruginosa* infection in Clarias gariepinus juveniles. Bulletin of Animal Health and Production in Africa, 60:511-519.

Claudia H. and Jeffrey C. 2009. Morphologic effects of the stress response in fish. Institute of Laboratory Animal Resources (ILAR) Journal, 50.4: 387-396

CLSI (2012): Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute's Twenty-Second Information Supplement, 32.3: 44-160.

Dacie J.V. and Lewis S.M. 1991. Practical Haematology. 6th Edition, New York: Churchill. 22p.

Daniyan S.Y. and Muhammad H.B. 2008. Evaluation of the antimicrobial activities and phytochemical properties of extracts of Tamarindus indica against some diseases causing bacteria. African Journal of Biotechnology, 7.14. 2451-2453.

Defoirdt T., Sorgeloos P. and Bossier P. 2011. Alternatives to antibiotics for the control of bacterial disease in aquaculture. Current Opinion in Microbiology, 14.3: 251-258.

Dhoughari J.H. 2006. Antimicrobial activity of Tamarindus indica Linn. Tropical Journal of Pharmaceutical Research, 5.2:597-603.

Edward J.N. 2012. Fish Diseases Diagnosis and Treatment. Wiley - Blackwell Publisher, Second Edition, pp 184 – 268.

El-Siddig K., Gunasena H.P.M., Prasad B.A., Pushpakumara D.K.N.G., Ramana K.V.R., Vijayanand P. and William J.T. 2006. Fruit for the Future: Tamarind, Tamarindus indica L. Revised Edition, pp 11-34.

Ejraei F., Ghiasi M., Khara H. 2015. Evaluation of hematological and plasma indices in grass carp, Ctenopharyngodon idella, with reference to age, sex, and hormonal treatment. Archives of Polish Fisheries, 23: 163-170.

Emmenegger E. J., Chang H.M., Paul K. H. and Gael K. 2013. Virulence of viral hemorrhagic septicemia virus (VHSV) genotypes Ia, IVa, IVb, and IVc in five fish species. Diseases of Aquatic Organisms, 107: 99–111, doi: 10.3354/dao02671

Erfan S., Gunhyun P., Dong-Mok L., Sunggun K. and Sungchul C.B. 2015. Effects of dietary Macsumsuk® supplementation on growth performance, haematological parameters, disease resistance and body composition of juvenile Nile tilapia, Oreochromis niloticus L. Journal of Applied Animal Research, 43:2, 125-130, DOI: 10.1080/09712119.2014.928626

European Pharmacopoeia. (2002). Furunculosis Vaccine (Inactivated, Oil-Adjuvanted, Injectable) for Salmonids, 4th Edition. European Pharmacopoeia, Strasbourg, France.

Fagbenro O.A., Adeparusi E.O. and Jimoh W.A. 2013. Haematological profile of blood of African catfish (Clarias gariepinus, Burchell 1822) fed sunflower and sesame-based diets. Journal of Fisheries and Aquatic Science, 8 (1): 80-86.

FAO 2005. Responsible use of antibiotics in aquaculture. FAO Fisheries Technical Papers, by P.H. Serrano, pp1- 43.

FAO 2014. The State of World Fisheries and Aquaculture: Opportunities and challenges, Rome, 243p.

FAO 2016. The state of the world Fisheries and Aquaculture. Contributing to food security and nutrition for all. Rome. 200pp.

Fernandez J.B., Yambot A.V. and Almana O. 2014. Vaccination of Nile tilapia (Oreochromis niloticus) using Lipopolysaccharide (LPS) prepared from Aeromonas hydrophila. International Journal of Fauna and Biological Sciences, 1.4: 1-3.

George P. 1987. Revised and expanded textbook of fish health. Chapter IV: Bacterial diseases of fishes, 30-80.

Gent Y., Wang K.Y., Huang X.L., Chen D.F., Li C.W., Ren S.Y., Liao Y.T. Zhou CZ.Y., Liu Q.F., Du Z.J., and Lai W.M. 2012. Streptococcus agalactiae, an emerging pathogen for cultured Ya-fish, Schizothorax prenanti in China. Transboundary and Emerging Diseases, 59:369-375.

Harikrishnan C., Balasundaram C. and Heo M. 2010. Herbal supplementation diets on hematology and innate immunity in goldfish against Aeromonas hydrophila. Fish and Shellfish Immunology, 28.2: 354-361.

Hossain M.K., Islam K.T., Hossain M.D. and Rahman M.H. 2011. Environmental impact assessment of fish diseases on fish production. Journal of Science Foundation, 9.1&2: 125-131.

Immanuel G., Vincy Bai V.C., Palavesam A. and Peter M.M. 2004. Effect of Butanolic extracts from terrestinal herbs and seed weeds on the survival, growth and pathogen (Vibrio parahaemolyticus) load on Shrimp, Penaueus indicus, juveniles. Aquaculture, 236: 53-65.

Immanuel G., Uma R.P., Iyapparaj P., Citarasu T., Peter S.M.P., Babu M.M. and Palavesam A. 2009. Dietary medicinal extracts improve growth, immune activity and survival of tilapia, Oreochromis mossambicus. Journal of Fish Biology, 74: 1462-1475.

Janda J.M. and Abbott S.L. 2010. Aeromonas: Taxonomy, pathogenicity and infection. Clinical Microbiology Review, 23: 35-73.

Kumar C.S. and Bhattacharya S. 2008. Tamarind seed: Properties, processing and utilization. Food Science and Nutrition, 48: 1-20.

Kumar I.V., Chelladurai G., Thangapandi V., Peeran S.S.H. and Mohanraj J. 2014. Medicinal plants as immunostimulant for health management in Indian catfish. Journal of Coastal Life Science, 2.6: 426-430.

Logambal S.M., Ventajalakshmi S. and Micheal R. D. 2000. Immunostimmmulatory effects of leaf extract of Ocimum sativum Linn in Oreochromis mosambians (Peters). Hydrobiologia, 430:113-120.

Mak T.W. and Saunders M.E. 2006. The cells and tissues of the immune response. In The immune response: Basic and clinical principles. Elsevier Inc., 35-67.

Manaf S.R., Daud H.M., Alimon A.R., Mustapha N.M., Hamdan R.H., Muniand K.G., Mohamed N.F.A., Razak R. and Hamid N.H. 2016. The Effects of Vitex trifolia, Strobilanthes crispus and Aloe vera herbal-mixed dietary supplementation on growth performance and disease resistance in red hybrid tilapia (Oreochromis sp.). Journal of Aquatic Research and Development, 7.4: 425. doi:10.4172/2155-9546.1000425

Modesto Dos Santos, V., Da Costa Arruda Jr., Z., De Farias Polcheira, M., Da Silva De Souza, D.W., Rodrigues Oliveira Santos, A.M. and Santos Correa Da Costa, M. 2013. Acute hepatitis due to infectious mononucleosis in a 21-year old man. Revista Medica de chile, 141: 917-921.

Newaj-Fyzul A., Mutani A., Ramsubbag A. and Adesiyun A. 2008. Prevalence of bacteria pathogens and their antimicrobial resistance in Tilapia and their pond water in Trinidad. Zoonoses Public Health, 55:206-213.

NRC. 2008. Chapter 19: Tamarind. In: Lost crops of Africa. Volume III: Fruits. Washinton, DC, The National Academic Press, 149-164.

Oladosu G.A., Tijani M.O., Akpavie S.O. and Adedeji O.B. 2013. Clinicopathological features of arborescent organ necrosis syndrome in Clarias gariepinus (Burchell, 1822). Tropical Veterinarian, 31.3:113-120.

Pakravan S., Hajimoradloo A. and Ghorbani R. 2011. Effect of dietary willow herb, Epilobium hirsutum, extract on growth performance, body composition, haematological parameters and Aeromonas hydrophila challenge on common carp, Cyprinus carpio. Aquaculture Research, 42: 1-9.

Parker J.L. and Shaw J.G. 2011. Aeromonas species: Clinical microbiology and disease. Journal of Infection, 62: 109-118.

Plumb J.A. and Hanson L.A. 2011. Health maintenance and principal microbial disease of cultured fishes. 3rd Edition, Blackwell Publishing Ltd, 244p.

Praseetha R. 2005. Enrichment of brine shrimp, Artemia franciscana with commercial probiotics and herbal extracts and their resistance against shrimp pathogen Vibro sp (vibrio parahaemolyhous and vibro damsel) M. Phil. Dissertation, Monanmasiam Sundaranar University, India.

Pratheepa V. and Sukumaran N. 2014. Effect of Euphorbia hirta plant leaf extract on immunostimulant response of Aeromonas hydrophila infected Cyprinus carpio. PeerJ, 2: e671.

Pridgeon J.W., Klesims P.H. and Mu X. 2011. Evaluation of an in vitro cell assay to select attenuated bacterial mutants of Aeromonas hydrophila and Edwardsiella tarda to Channel catfish Journal of Applied Microbilogy, 111:1310-1318.

Pridgeon J.W. and Klesius P.H. 2012. Major bacterial diseases in aquaculture and their vaccine development. CAB Reviews, 7.48: 1-16. http://www.cabi.org/cabreviews

Pujarini D., Swati Y, Sahoo P.K. 2014. Immunadjuvant effect of garlic (Allium sativum)-mineral oil suspension on immunity and resistance of Aeromonas injection in rohu, Labeo rohita. International Aquatic Research, 6: 167-173.
Ratanasinlapaphong, P., Thanasukarn, P., Thippila, P., Ratanatriwong, P.A., and Suwansri, S. 2011. Fatty acid compositions of tamarind seed polysaccharides. 12th Asian Food Conference, 699-703.

Rogo L.D., Attah M., Bawa E. and Aishatul A.M. 2009. Antimicrobial susceptibility pattern of Aeromonas hydrophila among patient presented with diarrhoea attending ABUTH Zaria and AKTH Kano, Nigeria. World Journal of Biological Research, 2.1: 1-7.

Romero J., Feijoo C.G. and Navarrete P. 2012. Chapter 6: Antibiotics in Aquaculture – Use, Abuse and Alternatives. In Tech: Health and Environment in Aquaculture, Edmir D.C., Gianmarco S.D. and Reinaldo J.S. (Eds.), pp 159-198. DOI: 10.5772/28157.

Saidi N, Lagha R., Abdallah F.B., Rokbani K.B. and Bakhrouf A. 2013. Slime producing, heavy metals and antibiotics resistance in Aeromonas hydrophila isolated in Tunisia. African Journal of Microbiology Research, 7.50: 5697-5608.

Sajid M., Prabjeet S., Munir H. S. and Khusheeba M. 2011. Emerging role of immunostimulants in combating the disease outbreak in aquaculture. International Aquatic Resources, 3: 147-163.

Shayo S.D., Mwita C.J. and Hosea K. 2012. Ulcerative Aeromonas infections in Tilapia (Cichlidae: Tilapini) from Mtera hydropower Dam, Tanzania, Open Access Scientific Reports, 1:115.

Subashkumar R., Thayunmanavan T., Vivekanandhan G. and Lakshmanaperumasamy P. 2006. Occurrence of Aeromonas hydrophila in acute gastroenteristic among children. Indian Journal of Medical Research, 123: 61-66.

Sudagar M. and Hajibeglou A. 2010. Effect of plant extracts supplemented diets on immunity and resistance to Aeromonas hydrophila in common carp (Cyprinus carpio). Research Journal of Animal Sciences 4.1: 26-34.

Thorburn M.A., Carpenter T.E. and Ljungberg O. 1997. Effects of immersion in live Vibrio anguillarum and simultaneous oxytetracycline treatment on protection of vaccinated and non-vaccinated rainbow trout Salmo gairdneri against vibriosis. Diseases of Aquatic Organisms, 2: 167-171.

United State Environmental Protection Agency (U.S. EPA) 2005. Aeromonas detection what does it means. http/www.Epagor/safe water urenr/dateAeromonas.htmt

Yilmaz S., Sebahattin E. and Nergiz S. 2013. Dietary supplementation of cumin (Cuminum cyminum) preventing streptococcal diseases during first-feeding of Mozambique tilapia (Oreochromis mossambicus). Journal of Biosciences and Biotechnology, 2.2: 117-124.