View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by AJOL - African Journals Online

Growth Performance and Innate Immune Response of *Clarias gariepinus* Infected with *Aeromonas hydrophila* fed diets fortified with *Curcuma longa* leaf

I. Adeshina[1,*], Y. A. Adewale[2], and L. O. Tiamiyu[1]

[1] *Department of Aquaculture and Fisheries, University of Ilorin, Nigeria,*

[2] *School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Australia,*

*Corresponding author; Email: *adesina.i@unilorin.edu.ng*

Abstract

The use of antibiotics as diseases control agents in aquaculture has become cantankerous due to rise in drug-resistant bacteria such as *Aeromonas hydrophila* which has been reported to cause huge biological and economic losses. Studies have revealed antibacterial potential of some botanicals such as *Curcuma longa* as alternative. However, there is rarity of information on the use of *Curcuma longa* as growth promoter and disease control agents in *Clarias gariepinus*. Hence, effects of *Curcuma longa* on growth performance and innate immune response of *Clarias gariepinus* infected with *Aeromonas hydrophila* were evaluated. Fish (10.30 ± 0.15 g) were fed seven isonitrogenous (40% crude protein) diets (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) twice daily to satiation for 12 weeks. Growth performance and innate immune responses were measured and evaluated using standard procedures. Fish at 12 weeks were challenged intra-peritoneally with *Aeromonas hydrophila* (5 10^5 mL) and observed for 14 days. Survival rate and relative protection were monitored. The results revealed that *Clarias gariepinus* fed supplemented diets had better growth and immunity against *Aeromonas hydrophila* at 2.5% inclusion level and therefore could be used as immunodulation against *Aeromonas* infection.

Introduction

The current aquaculture practices has though yield high fish production and filling the huge gap between demand and supply for fish but, the sector has been challenged with disease outbreak questioning the profitability of the venture. Recently, total annual losses in aquaculture as a result of disease outbreak has been estimated to have worth reached billions of dollars worldwide (Pridgeon & Kleius, 2011). One of the major common fish disease is bacteria (RASFF, 2013). Bacteria is of two major categories which are gram-positive and gram-negative bacteria. However, majority of gram-negative bacteria are pathogenic and one of commonest fish pathogen is *Aeromonas hydrophila.*

Aeromonas hydrophila is a gram-negative bacterium in the family of Aeromonadaceae. It is biochemically characterised as facultative anaerobe, motile, rod-shaped and sugar fermented organism. Studies have reported that *Aeromonas hydrophila* infection in fin fish has resulted into fin rot, haemorrhagic, speticemia, furunculosis, red sore disease and high mortality among others (Rahman *et al.,* 1997; Li *et al*., 2006). Unless

adequate treatment and control measures are applied, *Aeromonas hydrophila* infection in fish could lead to a significant biological and economical loss. One of the common methods of its control is the application of synthetic drugs.

Antibiotics as disease control agent in aquaculture has become contentious due to rise in drug resistant bacteria. The chemicals and antibiotics used in the control of bacteria load are expensive, somehow leave residue and cause side effects. More so, residual effect caused by continuous use of these chemicals raises a serious concern and pose a potential risk to the consumers (Adeshina *et al.*, 2017). Therefore, there is need for cheaper, natural and eco-friendly alternative to chemicals such as the use of botanicals. Studies have shown the antibacterial potential of some botanicals such as *Curcuma longa* (Citarasu, 2010; Abdel-Tawwab and Abbass, 2017; Adeshina *et al*., 2017) as alternative.

Curcuma longa (Tumeric) also known as "Ataile pupa" in Yoruba language belonging to the family Zingiberaceae is one of the common medicinal plants used as antimicrobial, wound-healing agent, anti-inflammatory in traditional medicine in Nigeria (Ahilan *et al.*, 2010; Bayoub *et al*., 2010). The presence of active ingredients in *Curcuma longa* such as turmerone, curcuminoids, zingiberene and curcumin among others suggest it usage as antibacterial in fish farm especially in the farming *Clarias gariepinus*. *Clarias gariepinus* belongs to the family Clariidae, is one of the widely distributed fish species in tropical Africa which has become the most cultured fish species in Nigeria. This is because of its high quality flesh, high tolerance level of water characteristics, production performance and high market values (Adeshina *et al*., 2016a). Therefore, this study evaluates the effect of *Curcuma longa* leaf meal on growth performance and innate immune response of *Clarias gariepinus* juveniles infected with *Aeromonas hydrophila*.

Materials and methods

Curcuma longa fresh leaves were obtained from "Oja-Oba" market, Ibadan. The plant was authenticated at herbarium unit Ibadan of Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves were rinsed with sterile water, air-dried at room temperature and grinded to fine powder in an electro-motored hammer mill. Seven isonitrogenous diets (40% crude protein) were prepared at 0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% of *Curcuma longa* leaf. The diets were packed in a labelled polythene bags and stored until use (Table 1).

Experimental fish and design

Clarias gariepinus juveniles (mean weight = 10.30 ± 0.15 g) were obtained from a reputable farm in Ibadan. The fish were acclimatised in plastic tanks (60 38 27 cm) before the experiment and fed commercial feed for 2 weeks. Fish were weighed and distributed into twenty-one rectangular plastic tanks (60 38 27 cm) in a completely randomised design. Each tank contained twenty fish. The experimental diets were fed to the fish to satiation twice daily for 12 weeks. The water in the tanks were replaced on three days interval throughout the period of the experiment. Measurement of the weight

TABLE 1
Gross and proximate composition of experimental diets fortified with Curcuma longa

Ingredients (%)	*Curcuma longa inclusion levels (%)*						
	T0 (0.0)	*T1 (0.5)*	*T2 (1.0)*	*T3 (1.5)*	*T4 (2.0)*	*T5 (2.5)*	*T6 (3.0)*
GNC	27.0	27.0	27.0	27.0	27.0	27.0	27.0
Soybean meal	46.3	46.3	46.3	46.3	46.3	46.3	46.3
Fish Meal	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Yellow maize	8.0	7.5	7.0	6.5	6.0	5.5	5.0
DCP	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Starch	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Veg. Oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix*	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Curcuma longa	0.0	0.5	1.0	1.5	2.0	2.5	3.0
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition (%)							
Crude protein	40.4	40.1	40.2	40.6	40.2	40.8	40.6
Dry Matter	89.4	89.8	90.2	90	89.9	89.6	89.9
Ether extract	11.5	11.6	12.3	12.5	13.5	15.4	16.3
Fibre	7.6	8.4	7.8	9.0	8.6	8.8	9.3
Ash	10.2	11.1	11.6	10.4	10.8	10.3	11.0
NFE	19.7	18.6	18.3	17.5	16.8	14.3	12.7
GE (kcal/100g)	417.9	412.6	418.6	419.4	423.8	434.8	435.6

* Premixes = HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A, 4,000,000 International Unit (IU); vitamin D3, 8,00,000 IU; vitamin E, 40, 000 IU; vitamin K3, 1,600 mg; vitamin B1, 4,000 mg; vitamin B2, 3,000 mg; vitamin B6, 3,800 mg; vitamin B12, 3 mcg; Nicotinic acid 18000 mg; Pantothenic acid, 8000 mg; Folic acid, 800 mg; Biotin, 100 mcg; Choline chloride 120,000 mg; Iron, 8000 mg; Copper, 800 mg; Manganese, 6000 mg; Zinc, 20,000 mg; Iodine, 400 mg; Selenium, 40 mg; Vitamin C C(coated), 60,000 mg; Inositol, 10,000 mg; Colbat, 150 mg; Lysine, 10,000 mg; Methionine, 10,000 mg; Antioxidant, 25,000 mg.
GNC = Groundnut cake meal; DCP = Di-calcium phosphate; NFE = Nitrogen free extract; GE= Gross energy (Calculated according to NRC (1993): protein = 5.65 kcal/g; lipid = 9.45 kcal/g; carbohydrate = 4.11 kcal/g)

changes were performed fortnightly and the feeding rate was adjusted accordingly with respect to the new body (Tiamiyu *et al.*, 2014; Akpoilih *et al*., 2017).

D*etermination of water quality Parameters*

Water samples were collected from each tank fortnightly. Dissolved oxygen were measured using digital D.O. meter [LABTECH (R)] Model AVI-660 (Power: 220V, AC: 50 Hz: Sr./No. 376), pH was measured with the aid of a digital pH meter [LABTECH (R)] Model Photoic 20 (Power: 230V AC: 50 Hz: Sr./No. 1223) and temperature was measured with the aid of mercury-in-glass thermometer (Boyd, 1984; Adeshina *et al.*, 2016b). The D.O., pH and temperature were between 4.57 ± 0.73 to 6.558 ± 0.38 mg/l, 7.21 ±

0.31 to 7.82 ± 0.12 and 25.33 ± 1.07 to 26.82 ± 1.19 °C respectively throughout the experimental period.

Determination of proximate composition of experimental diets and fish

Experimental diets and fish samples were subjected to proximate analysis. Moisture, ash, crude protein, crude lipid, crude fibre and nitrogen-free extracts contents were recorded using various analytical methods (AOAC, 2005). The moisture contents were determined by pre-weighing the samples and air-drying in a hot air-oven. The final weight was subtracted from the initial weight. Crude protein was determined using micro-Kjeldahl distillation method. The percentage protein was calculated by multiplying the nitrogen content of the sample by a factor of 6.25. Ash content was determined by burning the samples in a muffle furnace at 550 °C for three hours, the samples were allowed to cool, weighed and expressed as percentage as content. Ether extract was determined in a soxhlet extractor using petroleum ether (40–60 °C) for three hours. The solvents were evaporated and the ether extract was determined as the residue obtained. Crude fibre was achieved by subjecting the residual sample from the ether extraction to a successive treatment with boiling acid (0.25N sulphuric acid) and alkali of defined concentration (0.313N sodium hydroxide) under controlled condition. Also, nitrogen free extract was determined using the following expression as described by AOAC (2005).

Evaluation of growth performance and nutrients utilisation of experimental fish

Growth parameters were monitored biweekly. Initial weight, weight on every two weeks, and final weight were recorded using weighing scale (Model: M1207) and performance was calculated using the following formula:

$$\text{Weight gain (\%)} = \frac{\text{Mean weight gained}}{\text{Initial mean weight}} \times 100$$

$$\text{Specific Growth Rate (SGR)} = \frac{\text{LogFinal Weight -Log Initial Weight}}{\text{Length of culture period}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Dry weight of feed fed (g)}}{\text{Fish weight gain (g)}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Wet body weight gain (g)}}{\text{Crude protein fed}}$$

$$\text{Energy Intake} \left(\frac{\text{kcal}}{\text{fish}}\right) = \text{Feed intake (g)} \times \text{Dry matter of feed} \times \text{Gross energy of feed (kcal)}$$

$$\text{Survival Rate (\%)} = \frac{\text{Initial number of fish stocked-Mortality}}{\text{Initial number of fish stocked}} \times 100$$

Determination of innate immune response parameters

Superioxide dismutase (SOD) activity was determined using assay kit (Randox Kit SOD Kit 0223). The Solution comprises of 65 µmole phosphate buffer, 1 µmole hydrochloric hydroxylamine, 0.75 µmole xanthine and 2.3 10^{-3} IU xanthine at pH 7.8. Fifty (50) uL of the supernatant was incubated in the system for 40 minutes at 37 °C. Two (2) mL of 3.3 g/L p-aminobenzene sulfonic acid was added followed by addition of 10 g/L of naphthylamine ((Ellis, 1990; Hermes-Lima *et al*., 1998).

Nitroblue tetazolium (NBT) counts. The NBT was measured by determining the respiratory burst activity (Anderson *et al.* 1992). The blood samples were incubated at 25 °C for 30 mins and washed with 0.067 mM sodium phosphate buffer (pH 6.4). A single drop of 0.2% of NBT solution placed on a slide and incubated for 30 mins at 25 °C. The NBT cells were counted under the microscope (x 100 objective lens) in triplicates.

Immunoglobulin M (IgM). The total immunoglobulin M (IgM) were determined by enzyme-linked immunosorbent assay (ELISA) using immunoglobulin M (IgM) ELISA kit for fish following the manufacturer's instruction.

Lysozyme activity. Lysozyme activity was determined using lysoplate technique (Grinde *et al.*, 1989). In brief, 0.60mg/mL *Micrococcus luteus* was cast in 1% agarose gel (Difco, USA) with 50mM phosphate buffer (pH 6.2). Wells (6mm) were created nutrient agar plates. The wells were filled 25 µL of serum samples and incubated for 20 hours at 25°C. The zones of inhibition were measured. The data determined from semilogarithmic graph paper.

Challenge test

At 12 weeks fish fed diets fortified *Curcuma longa* were challenged with 0.1 mL of *A. Hydrophila* (intraperitoneally except the control group which were injected 0.1 mL of saline water (Schaperclaus *et al.*, 1992). The fish were allotted to twenty-one rectangular plastic tanks (60 38 27 cm) in a completely randomised design (each tank contained 10 fish) for 14 days. Survival rate was determined and level of protection was estimated as

$$(1 - \frac{\%\text{ mortality in experimental group}}{\%\text{ mortality in control group}} \quad 100)$$

Azza and Abd-El-Rahman (2009)

Statistical analysis

The data obtained were analysed using one-way analysis of variance to examine the effect of *Curcuma longa* on *Clarias gariepinus* juveniles and means were separated using Duncan Multiple Range Test. The optimum inclusion level of *Curcuma longa* was estimated using quadratic regression with the aid of Statistical Package for Social Science (IBM version 20).

Results

Table 2 revealed that final weight (g), weight gain (g), percentage weight gain (%), specific growth rate (%) and protein efficiency ratio were significantly increased with increase in the level of *Curcum longa* meanwhile, there was significantly decreased in feed conversion

TABLE 2

Growth performance and nutrient utilisation of Clarias gariepinus fed diets fortified with various concentrations of Curcuma longa for 12 weeks

Parameters	*Curcuma longa concentrations (%)*					
	0.0 (control)	*0.5*	*1.0*	*1.5*	*2.0*	*2.5*	*3.0*
Initial weight (g)	10.23 ± 0.20^{a}	9.92 ± 0.80^{a}	9.98 ± 0.64^{a}	10.18 ± 0.39^{a}	9.97 ± 0.33^{a}	9.98 ± 0.64^{a}	10.18 ± 0.39^{a}
Final weight (g)	35.52 ± 5.10^{d}	41.87 ± 6.37^{e}	43.02 ± 2.74^{e}	46.20 ± 2.99^{b}	52.12 ± 4.24^{b}	59.13 ± 4.90^{a}	64.71 ± 4.69^{a}
Weight gain (g)	25.29 ± 5.24^{c}	31.95 ± 6.20^{c}	33.04 ± 3.09^{c}	36.02 ± 3.18^{b}	42.16 ± 3.92^{b}	49.15 ± 5.20^{a}	54.54 ± 4.69^{a}
Weight gain (%)	247.79 ± 54.72^{c}	323.19 ± 62.47^{bc}	332.74 ± 46.02^{bc}	354.77 ± 40.55^{b}	422.43 ± 25.74^{b}	494.93 ± 71.22^{a}	536.48 ± 50.72^{a}
SGR (%g/day)	0.64 ± 0.08^{b}	0.74 ± 0.08^{b}	0.76 ± 0.06^{ab}	0.78 ± 0.05^{ab}	0.85 ± 0.03^{ab}	0.92 ± 0.06^{a}	0.96 ± 0.04^{a}
Feed Intake (g)	61.38 ± 15.18^{a}	62.73 ± 10.15^{a}	61.91 ± 6.24^{a}	58.25 ± 1.75^{a}	59.78 ± 9.23^{a}	60.41 ± 4.61^{a}	68.98 ± 7.02^{a}
FCR	2.41 ± 0.16^{a}	1.98 ± 0.22^{a}	1.87 ± 0.03^{a}	1.62 ± 0.11^{a}	1.41 ± 0.09^{ab}	1.44 ± 0.13^{ab}	1.26 ± 0.02^{b}
PER	0.63 ± 0.13^{b}	0.80 ± 0.16^{ab}	0.82 ± 0.08^{ab}	0.90 ± 0.07^{ab}	1.05 ± 0.10^{a}	1.23 ± 0.13^{a}	1.36 ± 0.12^{a}
Energy intake (kcal/fish)	256.48 ± 63.43^{a}	$258.82, \pm 41.88^{a}$	259.16 ± 26.08^{a}	244.32 ± 7.36^{a}	253.36 ± 39.10^{a}	306.17 ± 20.04^{a}	300.40 ± 30.55^{a}

SGR = Specific Growth Rate; FCR = Feed Conversion Ratio; PER = Protein Efficiency Ratio;
Mean with different superscripts in the same row are significantly different ($P < 0.05$), while mean without superscript are not significantly different ($P > 0.05$).

ratio of the *Clarias gariepinus* fed diets fortified with *Curcuma longa* ($P < 0.05$). Also, there were increase in the feed intake (g) and energy intake (kcal/fish) but these were not statistically significantly different ($P > 0.05$) among the fish. However, fish fed diet containing 3.0% *Curcuma longa* had highest final weight (64.71 ± 4.69 g), weight gain (54.54 ± 4.69 g), SGR (0.96 ± 0.04% g/day), feed intake (68.98 ± 7.02g), PER (1.36 ± 0.12) and energy intake (300.40 ± 30.55 kcal/fish) while the lowest were observed in fish fed control diet except feed intake which was lowest in fish fed 1.5% *Curcuma longa* based diet (Table 2). On the contrary, the lowest FCR (1.26 ± 0.02) was obtained in fish fed 3.0% *Curcuma longa* based diet and highest was recorded in fish fed basal diet (Table 2).

Also, there were no significantly difference in the proximate composition of the fish fed experimental diets ($P > 0.05$). However, highest crude protein (18.40 ± 0.23%), ether extract (5.40 ± 0.02 %), moisture content (73.94 ± 0.65%) and ash content (3.13 ± 0.15 %) were obtained in fish fed 3.0%, 0.5%, 1.5% and 0.0% *Curcuma longa* based diets respectively (Table 3).

Table 4 depicts that fish fed experimental diets had higher survival rate and well protected against pathogenic *Aeromonas hydrophila* than fish fed basal diet.

TABLE 3

Proximate composition of body carcass of *Clarias gariepinus* fed diets fortified with various concentrations of *Curcuma longa* for 12 weeks

Curcuma longa *inclusion levels*	*Crude protein (%)*	*Ether extract (%)*	*Moisture (%)*	*Ash(%)*
0.0	17.96 ± 0.13	5.13 ± 0.13	73.78 ± 0.75	3.13 ± 0.15
0.5	18.03 ± 0.02	5.40 ± 0.02	73.80 ± 1.04	2.77 ± 0.16
1.0	18.50 ± 0.22	5.03 ± 0.01	73.48 ± 1.13	2.99 ± 0.12
1.5	18.21 ± 0.35	5.12 ± 0.12	73.94 ± 0.65	2.73 ± 0.06
2.0	18.31 ± 0.21	5.01 ± 0.01	73.56 ± 2.16	3.12 ± 0.05
2.5	18.42 ± 0.12	5.11 ± 0.12	73.83 ± 1.38	2.64 ± 0.10
3.0	18.40 ± 0.23	5.20 ± 0.13	73.75 ± 1.53	2.65 ± 0.19

TABLE 4

S*urvival rate and level of protection of Clarias gariepinus fed diets fortified with various concentrations of Curcuma longa for 12 weeks and infected with Aeromonas hydrophila*

Curcuma longa *inclusion levels*	*Survival rate (%)*	*Level of protection (%)*
0.0	23.33	0.00
0.5	40.00	21.74
1.0	56.67	43.48
1.5	80.00	73.91
2.0	93.33	91.30
2.5	96.67	95.65
3.0	96.67	95.65

Highest and least survival rate were recorded in fish fed 2.5% and 0.0% *Curcuma longa* based diets. In the same vein, highest level of protection (95.65%) was obtained in fish fed 2.5% *Curcuma longa* diet and lowest (21.74%) was obtained in group treated 0.5% *Curcuma longa* fortified diet with no protection at all in fish fed control diet.

The innate immune response parameters measured were improved in fish fed *Curcuma longa* fortified diets. The NBT, immunoglobulin, lysozymes activity, and SOD values were inspired in fish fed experimental diets. The highest NBT, immunoglobulin and lysozyme activity were obtained at 2.5% *Curcuma longa* based diet and highest SOD was obtained at 2.0% *Curcuma longa* based diet. However, the lowest NBT, immunoglobulin, lysozyme activity and SOD values were obtained in fish fed control based diet (Figs. 1–4). The relationships between dietary *Curcuma longa* concentrations and NBT (Fig. 1), immunoglobulin (Fig. 2), lysozyme activity (Fig. 3) and SOD (Fig. 4) values were best expressed by the following regression equations: and respectively (Fig. 1–4).

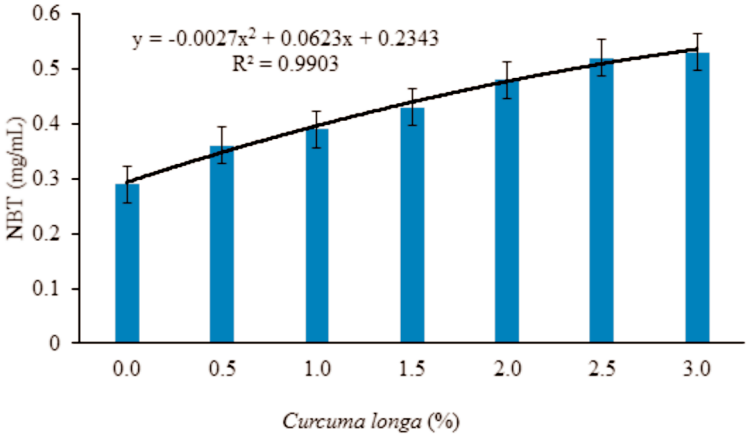

Fig. 1. The nitroblue tetrazolium (NBT, mg/mL) in blood of *Clarias gariepinus* fed diets fortified with various concentrations of *Curcuma longa* for 12 weeks

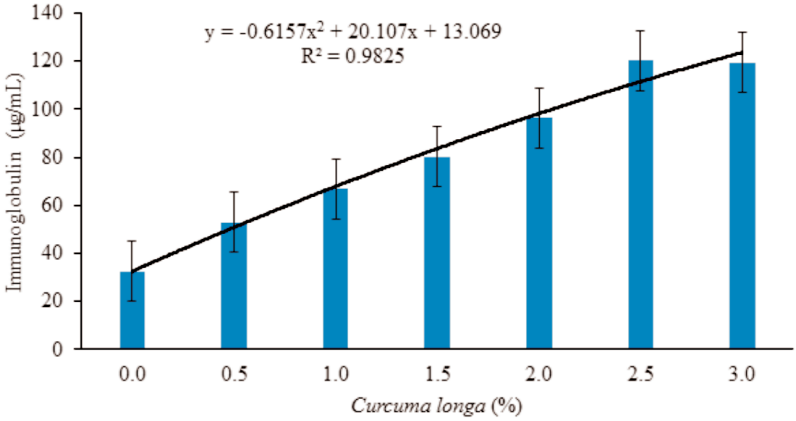

Fig. 2. The immunoglobulin M (IgM, µg/mL) in blood of *Clarias gariepinus* fed diets fortified with various concentrations of *Curcuma longa* for 12 weeks

Discussion

The results indicated that inclusion of *Curcuma longa* in the diet improved growth performance and nutrients utilization of *Clarias gariepinus* juveniles better than the basal diet. Although, feed intake was not significantly affected but utilization were better in group treated experimental diets which suggest enhancement and modification of gut flora with beneficial microbiota and microbial enzymatic activities resulting in better digestion and absorption cumulating into the significantly higher growth recorded. This finding is in line with the report of Prassad and Aggarwal (2011) which revealed that *Curmuma longa* is a good digestive stimulant that promotes the activities of enzymes like amylase, chrmotrypsin and lipase. Also, this study

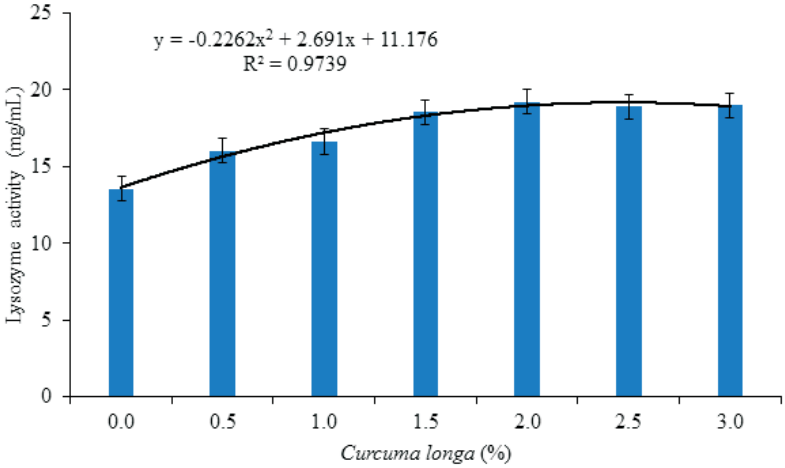

Fig. 3. The lysozyme activity (mg/mL) in blood of *Clarias gariepinus* fed diets fortified with various concentrations of *Curcuma longa* for 12 weeks

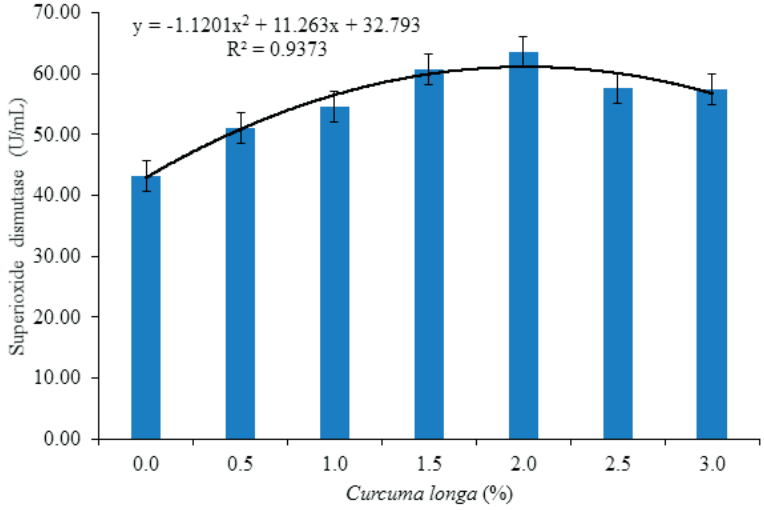

Fig. 4. The superoxide dismutase (SOD, U/mL) in blood of *Clarias gariepinus* fed diets fortified with various concentrations of *Curcuma longa* for 12 weeks

disclosed similar view with Sahu *et al.* (2008), Mahumoud *et al*. (2014) and Abdel-Tawwab and Abbass (2017) who reported that inclusion of *Curcuma longa* improved the growth performance of *labeo rohita, Tilapia niloticus* and *Cyprinus carpio,* respectively. As earlier reported, fish fed phytogenic fortified diets such as garlic (Shalaby *et al*. 2006), *Camellia sinensis* (Abdel-Tawwab *et al*., 2010),

Carumcarvi (Ahmad & Abdel-Tawwab, 2011), onion bulb and walnut leaves (Bello *et al*. 2013) significantly improved growth performance and nutrient utilization in fish.

Presence of *Curcuma longa* in the diets of *Clarias gariepinus* was found to have no significant influence on the proximate composition of the fish. This result was in agreement with the findings of Goda (2008) and Abdel-Tawwab & Abbass (2017). Higher crude protein values were obtained in fish fed experimental diets while lower ether extract and ash contents values were obtained in treated groups. The result obtained in crude protein followed similar trend with the findings of Hwang *et al*. (2013) and Abdel-Tawwab & Abbass (2017) but differ with the work of Mohamoud *et al*. (2014). However, ether extract and ash contents values is in conformity with the reports of Abdel-Tawwab *et al.* (2010) who reported decreased in ether extract and ash contents of *Tilapia niloticus* fed diets fortified with *Curcuma longa*.

Fish fed *Curcuma longa* based diets had higher survival rate and relative protection levels than the fish fed control diet. The higher survival rate obtained in this study may be attributed to antimicrobial properties of *Curcuma longa* which provide better relative protection against pathogenic. The presence of turmerone, curcuminoids, zingiberene and curcumin, an antimicrobial agent in *Curcuma longa* might have serve as bacteriocidal and bacteriostatic agents in the body of *Clarias gariepinus* against *Aeromonas hydrophila* hence increased the survival rate. The results concord with findings of Mahmoud *et al.* (2014) and Abdel-Tawwab & Abbass (2017) who reported protection levels and survival rate of *Tilapia niloticus* and *Cyprinus carpio* infected with *Aeromonas hydrophila* and *Pseudomonas fluorescens,* respectively. Furthermore, higher inhibitory effect of *Curcuma longa* against *Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio parahaemolyticus, Bacillus cereus, Pseudomonas aeruginosa* has been reported (Mahady *et al.,* 2002; Yano *et al.,* 2006; Paramasivam *et al.,* 2007; Mahmoud *et al.,* 2014).

The higher NBT, immunoglobulin, lysozyme activity and SOD obtained in fish fed diets fortified with *Curcuma longa* signify a better innate immune response. Lysozymes has been reported to have ability to destroy the cell wall of bacteria (Ellis, 1990), SOD act as cellular antioxidants (Hermes-Lima *et al*., 1998) while NBT and immunoglobulin also serves as indicator of immunity (Mak & Saunder, 2004). In addition, the presence of some active compound such as curcumin in *Curcuma longa* might be responsible for better innate immune response parameters of *Clarias gariepinus*. The findings further strengthen the protection levels observed in fish challenged with *Aeromonas hydrophila* by improving innate immune system of the fish. Correspondingly, the result of the present study agreed with the observation of Mahmoud *et al*. (2014) and Abdel-Tawwab and Abbass (2017) who reported higher innate immune response parameters in fish fed *Curcuma longa* based diets and protection levels in fish challenged with *Aeromonas hydrophila (*Abdel-Tawwab, 2012).

Conclusion

The study revealed that *Clarias gariepinus* fed diets fortified with *Curcuma longa* had improve growth performance and enhanced immune system. Survival rate, level of protection and innate immune response of *Clarias gariepinus* fed 2.5% *Curcuma longa* based diets performed better and could be used to improve growth and immunity of *Clarias gariepinus* against pathogenic *Aeromonas hydrophila*.

Acknowledgement

The authors thank colleagues and friends at School of Life and Environmental Science, Faculty of Science, Engineering and Built Environment, Deakin University, Australia for the support received during the laboratory analyses.

References

Abdel-Tawwab M. (2012). The use of American ginseng (*Panaxquin quefolium*) in practical diet for Nile tilapia (*Orechromis niloticus*); growth performance and challenge with *Aeromonas hydrophilla*. *J. Appl. Aquac.* **24:** 366–376.

Abdel-Tawwab M. And **Abbass F. E.** (2017). TuUmeric Powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity and challenge against pathogenic *Aeromonas hydrophilla* infection. *J. Wrld. Aquac. Soci.* **48**: 303–312.

Abdel-Tawwab M., Ahmad M. H., Seden M. E. A. and **Sakr S. M. F.** (2010). Use of green tea, *Camellia sinensis* L. in practical diets for growth and protection of Nile tilapia, *Orechromis niloticus* (L.) against *Aeromonas hydrophilla* infection. *J. Wrld. Aquac. Soci.* **41**: 203–213.

Adeshina I., Adewale Y. A. and **Yusuf Y. O.** (2016b). *Eugenia cayrophyllata* Oil as Anesthetic in Cultured African Catfish, (*Clarias gariepinus*, Burchell 1822) Juveniles. *N. J. Fish. Aquac.* **4**: 8-17.

Adeshina I., Jenyo-Oni A., Ajani E. K. and **Adewale Y. A.** (2016a). Natural occurrence of Diplostomum spp. In farm-raised African catfish (*Clarias gariepinus*) from Oyo state, Nigeria. *Intl. J. Vet. Sci. Med.* **4**: 41–45.

Adeshina I., Jenyo-Oni A., Ajani E. K., Emikpe B.O. and **Alao S. O.** (2017).The effect of fresh leaf *Ocimum gratissimum* and dried buds *Eugenia caryophyllata* extracts on the tissues bacteriological changes of *Clarias gariepinus* juveniles. *Bull. Ani. Hlth. Prod. Afri.* **65**: 191–199.

Ahilan B., Nithiyapriyatharshini A. and **Ravaneshwaran K.** (2010). Influence of certain herbal additives on the growth, survival and disease resistance of goldfish, *Carassius auratus* (Linnaeus). *Tam. J. Vet. Ani. Sci.* **6**: 5–11.

Ahmad M. A. and **Abdel-Tawwab M.** (2011). The use of caraway seed meal as a feed additive in fish diets: growth performance, feed utilization, and whole-body composition of Nile tilapia, *Orechromis niloticus*(L.) fingerlings. *Aquac.* **314**:110–114.

Akpoilih B. U., Omitoyin B. O. and **Ajani E. K.** (2017). Phosphorus utilization in juvenile *Clarias gariepinus* fed phytase-supplemented diets based on soya bean (oil-extracted) and full-fat (roasted): A comparison. *J. Appl. Aquac.* DOI: 10.1080/10454438.2016.1276000.

Anderson D. P., Moritomo T. ***and*** **Grooth R. D.** (1992). Neutrophile, glass-adherent, nitroblue tetratzolium assay gives early indication of immunization effectiveness in rainbow trout. *Vet. Immuno. Immunopath*. 30:419–429.

Association of Official Analytical Chemist (AOAC) (2005). Official Methods of Analysis (18th edition) Association of Official Analytical, Chemists International, Maryland, USA.

Azza M. and **Abd-El-Rhman M.** (2009). Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, *Orechromis niloticus*. *Fish. Shellfish. Immun*. 27:242–459

Bayoub K. T., Baibai D., Mountassif A., Retmane A. and **Soukri A.** (2010). Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogenes* and some other pathogenic strains, *Afri J. Biotec.* **9**: 4251–4258.

Bello O. S., Olaifa F. E., Emikpe B. O. And **Ogunbanwo S. T.** (2013). Potentials of walnut (*Tetracarpidium conophorum* Mull. Arg) leaf and onion (*Allium cepa* Linn) bulb extracts as antimicrobial agents for fish. *Afri. J. Micro. Rese.* **7**: 2027–2033.

Boyd C. E. (1984). Water quality in warm water fish pond. Auburn University Agriculture Experimental Station. Auburn, Alabama, USA.

Citarasu T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquac. Intl.* **18**:403–414.

Ellis A. E. (1990). Techniques in fish immunology. Pages 101–103 *in* J. S. Stolen, T. C. Fletcher, D. P. Anderson, B. S. Robertson, and W. B. Van Muiswinkle, editors. Lysozyme assays. SOS Publications, Fair Haven, Massachusetts, USA.

Food and Agriculture Organisation (FAO) (2016). The State of World Fisheries and Aquaculture: Contributing to Food Security and Nutrition For All, FAO, Rome Italy 24.

Goda A. M. A.-S. (2008). Effect of dietary ginseng herb (Ginsana_ G115) supplementation on growth, feed utilization and haematological indices of Nile tilapia, *Orechromis niloticus* (L.) fingerlings. *J. Wrld. Aquac. Soci.* **39**: 205–214.

Grinde B. (1989). Lysozyme from rainbow trout *Salmo gairdneri* Richardson an anti-bacterial agents against fish pathogens. *J. Fish. Dis.* 12: 207–210.

Hermes-Lima M., Storey J. M. and **Storey K. B.** (1998). Antioxidant defences and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. Comparative Biochemistry and Physiology. Part B, *Bioch. Molec. Biol.* **120**: 437–448.

Hwang J. H. Rha S. J., Han K. H. and **Kim S. J.** (2013). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquac.* **317**: 1–15.

Li A., Yang W., Hu J., Wang W., Cai T. and **Wang J.** (2006). Optimization by orthogonal array design and humoral immunity of the bivalent vaccine against in crucian carp (*Carassius auratus* L.) *Aquac. Res.* **37**: 813–820.

Mahady G. B., Pendland S. L., Yun G. and **Lu Z. Z.** (2002). Turmeric (*Curcum longa*) and curcumin inhibit the growth of *Helicobacter pylori,* a group 1 carcinogen. *Antican. Res.* **37:** 813–820.

Mahmoud M. M. A., El-Lamie M. M. M., Dessouki A. A. and **Yusuf M. S.** (2014). Effect of turmeric (*Curcuma longa*) supplementation on growth performance, feed utilization, and resistance of Nile tilapia (*Orechromis niloticus*) to *Pseudomonas fluorescens* challenge. *Glb. Res. J. Fish. Sci. Aquac.* **1**:26–23.

Mak T. W. and **Saunder, M. E.** (2004). *The immune esponse*. Ontario. Elsevier Academic Press,*r* Burlington, United States of America.

Paramasivam S., Thangaradjou T. and **Kannan L.** (2007). Effect of natural preservatives on the growth of histamine-producin bacteria. *J.Envi. Bio.***28**: 271–274.

Prassad S. and **Aggarwal B. B.** (2011). Tumeric, the golden spice from traditional medicine to modern medicine. In *Herbal Medicice: Biomolecular and Clinical aspect,* 2nd edn. (I. F. F. Bemzie and S. Wachtel-Galor, eds). CRC Press, Taylor and Francis Group, Boca Raton, Florida, USA.

Pridgeon J. W. and**Klesius P. H.** (2011). Development and efficacy of a novobiocin-resistant *Streptococcus iniae* as a novel vaccine in Nile Tilapia (*Orechromis niloticus*). *Vacc.* **29**:5986–5993.

Rahman M. H., Kawai K. And **Kusuda R.** (1997). Virulence of starved *Aeromonas hydrophilla* to cyprinid fish. *Fsh. Path.* 32: 163-168.

Rapid Alert System for Food and Feed (RASFF) (2013). Food and feed importation, Tech Reprt RASFF, **46**: 1–72.

Sahu S., Das B. K., Mishra B. K., Pradhan J., Samal S. K. and **Saragu N.** (2008). Effect of dietary *Curcuma longa* on enzymatic and immunological profiles of rohu, *Labeorohita* (Ham.) infected with *Aeromonas hydrophilla. Aquac. Res.* **39**: 1720–1730.

Schaperclaus W., Kulow H. and **Schreckenbach K. (**1992). Fish disease. A.A. Balkema, Rotterdam, Netherland.

Shalaby A. M., Khattab Y. A. and **Abdel-Rahman A. M.** (2006). Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia. *J. Ven. Ani. Tox. Trop. Dis.* **12**: 172–201.

Tiamiyu L. O., Ayuba V. O., Okomoda V. T. and

Umar S. (2014). Effect of various levels of raw *Citrullus lanatus* seed meal diets on growth performance of *Cyprinus carpio* fingerlings. *Jrdn. J. Biol. Sci.* **7**: 269–274.

Yano Y., Satomi M. and **Oikawa H.** (2006). Anitomicrobial effect of spices and herbs on *Vibrio parahaemolyticus*. *Intl. J. Fd. Micro.* **111**: 6–11.