microbiology research

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Medicinal Plant Feed Additives Enhanced Survivability and Growth Performance of *Clarias gariepinus* (African Catfish) against Bacterial Infection

Adebisi Musefiu Tiamiyu [1,*], Isaac Olufemi Olatoye [2,3], Okunlade Akinsola Olayemi [2], Temitope Cyrus Ekundayo [1], Olufemi Bolarinwa Adedeji [2] and Reuben Chukwuka Okocha [4]

1 Department of Biological Sciences, University of Medical Sciences, Ondo City 351104, Nigeria; cyruscyrusthem@gmail.com
2 Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria; olatoyevet@yahoo.com (I.O.O.); yemioklad@yahoo.com (O.A.O.); oluadedeji2001@yahoo.com (O.B.A.)
3 School for Global Animal Health, Washington State University, Pullman, WA, USA
4 Department of Agricultural Sciences, Landmark University, Omu-Aran 251104, Nigeria; okocha.reuben@lmu.edu.ng
* Correspondence: tiaadebisi2014@yahoo.com or atiamiyu@unimed.edu.ng

Abstract: The growth performance and survivability enhancement potential of some medicinal plant feed additives for combatting Pseudomonad infections in *Clarias gariepinus* were evaluated. Three medicinal plants (5 g/kg *Allium sativum*, 10 g/kg *Chromolaena odorata* and 10 g/kg *Talinum triangulare*) were incorporated separately into a basic diet. Juvenile *Clarias gariepinus* (n = 150, 53.05 ± 0.23 g), randomised into four groups, were fed for 42 days. The control group was fed with a non-supplemented diet. Growth parameters were determined and thereafter ten fish from each group, randomly selected, were inoculated intraperitoneally with pathogenic *Pseudomonas aeruginosa* (0.2 mL culture containing 1.4 × 10^6 cfu/mL). Their survivability was observed for 7 days based on mortality rate and relative level of protection (RLP). Mean weight gains were higher in all treated groups and significantly higher ($p < 0.05$) in the group of fish fed with 5 g/kg *Allium sativum* diet compared with the control. The lowest mortality rate (20%) and highest RLP (75) was recorded in the group fed with10 g/kg *Chromolaena odorata*. The results suggest that medicinal plant feed additives enhanced growth and survival of the cultured *Clarias gariepinus*. The study recommends 5 g/kg *Allium sativum* and 10 g/kg *Chromolaena odorata* diet supplementations as an effective growth promoter and anti-*Pseudomonas aeruginosa* agent, respectively, for *Clarias gariepinus* production.

Keywords: African catfish; feed additive; growth performance; *Pseudomonas aeruginosa*; herbs

Citation: Tiamiyu, A.M.; Olatoye, I.O.; Olayemi, O.A.; Ekundayo, T.C.; Adedeji, O.B.; Okocha, R.C. Medicinal Plant Feed Additives Enhanced Survivability and Growth Performance of *Clarias gariepinus* (African Catfish) against Bacterial Infection. *Microbiol. Res.* **2021**, *12*, 744–752. https://doi.org/10.3390/microbiolres12040054

Academic Editor: Yiannis Kourkoutas

Received: 17 July 2021
Accepted: 19 September 2021
Published: 29 September 2021

1. Introduction

Aquatic farming is a developing, exciting and essential segment for the production of highly proteinaceous food and there is intensive cultivation of fish and shellfish globally.

Investigations have shown that the dearth of active disease control has been the limiting factor for sustainable fish production [1]. Cultivating highly produced fish (fish with a high percentage of production worldwide) and cultured fish that would be highly resistant to diseases are major headaches for fish farmers. The major fish pathogenic bacteria are *Aeromonad*, *Pseudomonad* and *Edwardsiella tarda*, and they are widely distributed in aquatic environments [2–5]

Pseudomonas spp. are extensively spread in aquatic environment and connected with septicaemia in water faunas [6]. They are found in water, soil, as skin flora and other habitable environments. These bacteria have been reported to be specific pathogens, initiating infection when the host is exposed to stress [7]. *Pseudomonas* spp. causes diseases in aquatic animals such as fish, soft-shelled turtles and frogs with moderate to high losses [8]. The commonly isolated and identified species with diverse degrees of virulence of *Pseudomonas*

are *P. putida*, *P. diminuta*, *P. aeruginosa* and *P. fluorescens* [9]. The typical sign of infection produced by these organisms is notable septicaemia resulting in haemorrhages in the opercula, mouth region and on the ventral side of the body [10]. Tail rot and death are major consequences of *P. aeruginosa infection* in fish.

Antibiotics have been drugs of choice in aquaculture for the preclusion and control of bacterial infections [11,12]. Occurrence of antibiotics residue and the development of bacterial strains that are resistant to antibiotics in treated fish food were the consequences of application of antibiotics in aquaculture [13–15]. The cost implication of usage of chemicals and antibiotic in fish culturing reduces the profitability and not all are effective [16]. Vaccines which can also be used for prevention are always specific against pathogens [17,18] and a diverse nature of pathogens in fish farm also curtail vaccine efficacy [19,20]. Therefore, several alternative strategies to the use of antimicrobials and vaccines such as immunotherapy, for example, probiotics and immunostimulants, have been proposed. Others include herbal plants, alginic acid, mannan oligosaccharides B- glucan and live yeast *Saccharomyces cerevisae* which may serve as dietary supplements to improve fish growth and stimulate immune responses [21]. Immunostimulants have been proved to be useful alternatives to chemotherapy and vaccination in the control of fish diseases as they can enhance the non-specific immune response [22], and it was documented that fish rely greatly on non-specific defense apparatus, more than mammals [22]. Immunostimulants produce an effective and intense immune response to contagious agents and issues of residues in the tissue, toxicity and carcinogenicity have not been reported [23]. The growth enhancement and increase in the survival rates of the fish under stress has been reported to add advantages of the application of immunostimulants in fish culturing [24]. Integration of herbs in the foods of the fish enthused their immune system and improved their ability to resist disease [25].

Herbal products have been proved to serve as anti-stressors, appetisers, tonics, antimicrobial and more so as immunostimulants and they might protect against many diseases [26–28]. Lately, in aqua-farming, numbers of plants have been confirmed to have the ability to control bacterial and viral diseases. For example, *Eclipta alba* [29], *Aloe vera* [30,31], *Ocinum sanctum* [32], *Viscum album*, *Urtica dioica* and *Zingiber officinale* [33–35], *Solanum trilobatum* [36] and *Achyranthes aspera* [37] were described to have improved the immunity of fish. Rainbow trout infested with *Aeromonas* were reported to have been treated with inclusion of garlic in the feed [38]. Therefore, phytobiotics in fish malady controlling are gaining success, as they cause minimal side effects and are environmentally friendly and affordable [39]. The effect of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* on the growth and survival of *Clarias gariepinus* challenged with pathogenic *Pseudomonas aeruginosa* was evaluated.

2. Material and Methods

2.1. Feed Ingredients and Milling

The feed ingredients such as maize, wheat bulk, soya bean, fishmeal, calcium carbonate and limestone were obtained locally from the market. All ingredients were pounded into granulated form by means of a motorised crusher, and then mixed with mineral mixture and plant oil to form a basal diet for the experiment. The ingredients and proximate chemical composition of basal feed (Table 1) were assessed by the procedures described by [40] to decide the crude protein content.

Table 1. Feed ingredients and proximate chemical analysis (% on dry matter basis) of the experimental basal diet.

	%
Maize	35
Soybean (44%)	28.5
Fish meal (65%)	17
Wheat bran	9.5
Calcium Carbonate	0.3
Ground lime stone	0.7
Vegetable Oil	6.5
Mineral mixture	1.7
Vitamin mixture	1
Nutrients composition	%
Dry matter (DM)	90.4
Crude protein (CP)	30.65
Ether extract (EE)	11.73
Ash	2.7
Crude fiber	10.11
Nitrogen-free extract (NFE)	44.81
Gross energy (kcal/100 g DM) (GE) *	467.77
Protein/energy (P/E) ratio (mg CP/kcal GE) *	65.52

NFE (Nitrogen–free extract) = 100 − (protein + lipid + ash + crude fibre). GE (kcal/100 g DM) = CP × 5.64 + EE × 9.44 + NFE × 4.11 calculated according to NRC (1993), * not in percentage.

Healthy, fresh leaves of *Chromolaena odorata* and *Talinum triangulare* were harvested from the wild, and the bulbs of *Allium sativum* were sourced from local markets in South-western parts of Nigeria. The flowers were washed thoroughly with clean water and then with sterilized water. The flowers were air dried in the shade for some weeks at room temperature of 25 ± 2 °C in the laboratory and then ground to powder with a mechanical grinder. Pieces of garden-fresh garlic rhizome were skinned and cut into smaller pieces and oven dried at 70 °C until a perpetual weight was obtained.

Four dissimilar diets with or without additives, signifying 4 nutritional variants (Table 2), were then prepared by incorporating *Allium sativum*, *Chromoleana odorata* and *Talinum triangulare* powder at levels of 5, 10 and 10 g/kg into the basal diet, referred to as dietary D2, D3 and D4, respectively. Dietary D1 was the basal diet containing no medicinal plant, and it served as control. The grounded ingredients were thoroughly mixed. The mixed materials were homogenised using warm water to make a dough-like paste. The diets were then pelletised into 2 mm size. The diets were sun dried for 4 days and kept in sealed containers throughout the trial period.

Table 2. Details of the experimental diets (different inclusion rates).

Diet Variants	Details
D1	Basal Diet (BD) + 0 g/kg (as a control)
D2	Basal Diet (BD) + 5 g/kg *A. sativum* powder
D3	Basal Diet (BD) + 10 g/kg *C. odorata* powder
D4	Basal Diet (BD) + 10 g/kg) *T. triangulare* powder

A = Allium, *C* = Chromolaena, *T* = Talinum.

2.2. Experimental Procedure

The trial was carried out in fifteen experimental plastic tanks, measuring 40 cm × 27 cm × 27 cm with water volume sustained at least 2/3 level of the aquarium. One hundred and fifty African catfish juveniles (*Clarias gariepinus*) with mean weight 53.05 ± 0.23 g and initial length of 18.79 ± 0.03 cm were randomly allocated at the rate of 30 fish per treatment set up in triplicate. The fish were permitted to adapt to the environment for 15 days preceding the beginning of the experiment. During the acclimatisation period, fish were fed a basal diet

without herbal plants ad libitum. The fish were primarily divided into four experimental groups (A–D). The group A (n = 60) was kept as control group and fish were fed with control diet (D1). The group B (n = 30) was fed with 5 g/kg *Allium sativum* incorporated diet (D2), group C (n = 30) was fed with 10 g/kg *Chromolaena odorata* incorporated diet (D3) and group D (n = 30) was fed with 10 g/kg *Talinum triangulare* incorporated diet (D4). The first group was sub divided into two, A1 and A2, to serve as positive and negative control groups, respectively, during the challenge test, while groups B, C and D served as treated groups. Each group had 30 experimental fish, subdivided into 3 groups with 10 fish each. Feed was provided at 5% of body weight and fed in two portions, one in the morning and another in the afternoon The ration was adjusted every week when new average weights of fish for the different groups were calculated. Leftover feed and faeces in each tank were drained out daily. Each group of the trial fish was fed for 42 days with their corresponding diet variants. Growth parameters were evaluated post 42 days feeding using the following standard formulae.

$$G = F - I \tag{1}$$

where

F represents final weight
I represents initial weight
G represents weight gain

$$\text{Relative weight gain (RWG, \%)} = \frac{\text{Weight gain}}{\text{Initial Weight}} \times 100 \tag{2}$$

Specific growth rate, SGR:

$$\text{SGR (\% per day)} = \frac{netLog\ W_2 - netLog\ W_1}{T_2 - T_1} \times 100 \tag{3}$$

where:

W_2 = Final weight
W_1 = Initial weight

2.3. Disease Challenge

After 42 days post feeding, 10 fish from each of diet variant group were randomly selected and then challenged with 0.2 mL culture suspension containing 1.4×10^6 viable cells of *Pseudomonas aeruginosa* injected intraperitoneally. Ten fish from group A1 and 10 fish from group A2 were randomly picked and injected with sterile saline and culture suspension to serve as negative and positive control, respectively. The inclusion of negative control is to assure that mortalities in the system were from the disease and not from stress resulting from the injection or environmental conditions. Mortalities were monitored for 7 days following the injection. The relative level of protection (RLP) and mortality (%) among the challenged fish were determined according to [41].

$$\text{RLP} = 100 - \frac{\text{\%age of fish died in the treated group}}{\text{\%age of fish died in the control group}} \times 100 \tag{4}$$

$$\text{Mortality (\%)} = \frac{\text{\# of fish (death)}}{\text{\# of fish (injected)}} \times 100 \tag{5}$$

2.4. Data Analysis

Quantitative data was analysed using the Graph Pad Prism statistical software Version 5.1 and represented as mean ± standard error of mean and percentile was used to express mortality and relative level of protection. Statistical difference between mean weight gain and specific growth rate of experimental catfish were analysed using one-way ANOVA. Tukey post hoc test was used to verify the differences between the means.

2.5. Ethical Approval

The ethical approval was obtained from the University of Ibadan Animal Care and Use for Research Ethical committee. The approval number is UI-ACUREC/App/2015/066. Experiment was conducted according to ACUREC approved protocol.

3. Result

3.1. Water Quality

Mean values of water quality measured once a week throughout the trial period showed the levels of temperature to be (28.7 ± 0.4 °C), pH (7.1 ± 0.2), DO (5.58 ± 0.6 mg/dL) and ammonia concentrations was less than 0.1 mg/L in all treatment tanks.

3.2. Growth Performance

Growth performance of the African catfish (*Clarias gariepinus)* juvenile fed with 5 g/kg *Allium sativum* (Group B), 10 g/kg *Chromolaena odorata* (Group C) and 10 g/kg *Talinum triangulare* (Group D) as feed additives over a 42-day period is presented in Table 3. Fish fed with 5 g/kg *Allium sativum* additive gave the best mean weight gain of 5.99 ± 0.53 g and was significantly higher compared with the control. Fish fed a basal diet (0.0 g/kg, i.e., without herbal additives (Group A) resulted in the slowest growth performance with mean weight gain of 3.75± 0.34 g. The final weight was significantly higher ($p < 0.0001$) in the group of fish fed with 5 g/kg *Allium sativum* (Group B) compared with the control group. The specific growth rate showed an increasing tendency in all trial groups with significantly higher ($p < 0.05$) values detected in the fish nurtured with 5 g/kg *Allium sativum* (Group B). No death was documented within the first 42 days of the trial.

Table 3. Growth performance of African Catfish juveniles (*C. gariepinus*) fed with feed supplemented with herbal additives for 42 days.

Groups	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Specific Growth Rate (%)
A (control)	53.67 ± 0.10 ^{a}	57.42 ± 0.28 ^{a}	3.75 ± 0.34 ^{a}	6.99 ^{a}	0.07 ± 0.01 ^{a}
B	54.04 ± 0.46 ^{a}	60.03 ± 0.11 ^{c}	5.99 ± 0.53 ^{c}	11.08 ^{c}	0.11 ± 0.01 ^{b}
C	54.67 ± 0.10 ^{a}	58.82 ± 0.26 ^{b}	4.15 ± 0.32 ^{a}	7.59 ^{a}	0.08 ± 0.01 ^{a}
D	53.67 ± 0.40 ^{a}	57.69 ± 0.69 ^{a}	4.69 ± 0.51 ^{b}	8.85 ^{b}	0.09 ± 0.01 ^{a}

The same superscript alphabets in the same column are not significantly different ($p > 0.05$). Key: A = control treatment without herbal feed additive, B = treatment with 5 g/kg *Allium sativum*, C = treatment with 10 g/kg *Chromolaena odorata*, D = treatment with 10 g/kg *Talinum triangulare.*

3.3. Disease Resistance

Fish mortality after intraperitoneal (IP) injection of *P. aeruginosa* occurred post 21 h. and increased in the 1st day after injection and then declined till the 6th day when mortality stopped. The accumulative death was 40%, 20% and 30% in fish fed with 5 g/kg *Allium sativum* (D2), 10 g/kg *Chromoleana odorata* (D3) and 10 g/kg *Talinum triangulare* (D4), respectively, for 42 days against *P. aeruginosa* and they conferred relative protection of 50, 75 and 62.5, respectively (Table 4). The highest mortality of 80% and relative level of protection of 0.0 were detected in the group of fish fed with control diet (D1) as shown in Table 4. Fish nurtured with basic diet (0.0 g/kg), negative control) and injected with normal saline showed a survivability percentage of 100%. No mortality was recorded in the experimental animals during the 42 days before the challenge test.

Table 4. Immunocompetence test (disease resistance).

Fish Group	No. of Fish	Type of Innoculate	Days after Challenge							No of Dead Fish	M (%)	S (%)	RLP
			1	2	3	4	5	6	7				
A (−ve)	10	*NS	0	0	0	0	0	0	0	0	0	100	100.0
A (+ve)	10	*P a*	3	1	2	1	1	0	0	8	80	20	0.0
B	10	*P a*	1	2	1	0	0	0	0	4	40	60	50.0
C	10	*P a*	2	0	0	0	0	0	0	2	20	80	75.0
D	10	*P a*	1	1	1	0	0	0	0	3	30	70	62.5

*NS = normal saline, M (%) = mortality, S (%) = survival, *P a* = *Pseudomonas aeruginosa*, RLP = relative level of protection. Key: A = control treatment without herbal feed additive, B = treatment with 5 g/kg *Allium sativum*, C = treatment with 10 g/kg *Chromolaena odorata*, D = treatment with 10 g/kg *Talinum triangulare.*

4. Discussion

The temperature of 28.7 ± 0.4 °C, pH of 7.1 ± 0.2, dissolved oxygen of 5.58 ± 0.6 mg/dL and ammonia equal or less than 0.1 mg/l documented from the culture vats in this study were within the suitable range for catfish production [42] and have no apparent influences on catfish growth. In a study carried out by [43], it was found that growth rate is related to the water quality. The result of this study showed that water quality was not altered by the inclusion of herbal feed additives to the food of the experimental fish. Regular changing of water carried out in this experiment must have helped maintain good water quality. The interaction of poor water quality or environmental disorder and the presence of harmful microbes and nutritional disorder caused disease in aquatic animals, and this has been a major obstacle to aquaculture worldwide [44]. Nevertheless, in African catfish culturing, harmful effects of low water quality are somewhat rare as fish are tolerant to a wide variety of ecological factors.

The supplementation of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* improved catfish growth following the 42 days feeding trial compared with the basal diet. This could be attributed to the possible growth-enhancing influence of the supplementation on *Clarias gariepinus*. The enhanced fish growth observed in this trial might be due to better nutrient digestibility. Thus, incorporation of *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* in the diet of the experimental fish might have an enhanced result on deliciousness, digestion and nutrient absorption. These agree with the findings presented in [45], which found that *Echinacea purpurea* and *Allium sativum* supplementation improve growth in Nile Tilapia (*Oreochromis niloticus*); Abdel-Tawwab et al. [46] revealed that green tea (*Camellia sinensis*) and amla-fortified diets enhanced Nile Tilapia (*Oreochromis niloticus*) growth yield and production as well as [35], who showed that *Phyllathus emblica*–formulated diets led to improved growth and haematological parameters in *Tilapia mossambicus* challenged with *Pseudomonas aeruginosa*.

The *P. aeruginosa* challenge infection of *Clarias gariepinus* revealed a low mortality percentage when compared with the control. The relative level of protection (RLP) obtained in 10 g/kg *Chromolaena odorata* (75.0), greater than 10 g/kg *Talinum triangulare* (62.5), greater than 5 g/kg *Allium sativum* (50.0) and greater than positive control group. The survivability of the treated fish increased in this trial compared with untreated fish, as indicated by the RLP. The improved growth and increased RLP observed in this experiment indicated that *Allium sativum*, *Chromolaena odorata* and *Talinum triangulare* could be incorporated as immunostimulants. The medicinal plants might be rich in bioactive compounds which could have immunostimulant effects on fish. The administered herbs might improve the innate and adaptive immune response of the Clarias against pseudomonad infection. These results were in agreement with [47], who reported that the death rate reduced in Nile Tilapia fed with diet incorporated with 10 g/kg and 20 g/kg curcumin and challenged with *Pseudomonas fluorescens* when compared with the control group. One hundred percent and eighty-nine percent survivability were recorded in *Labeo rohita* fish fed with 5.0 and 1.0 g tumeric/kg incoporated in feed for 60 days, respectively, when fish were challenged and fish that were challenged with *Aeromonas hydrophila* were also reported [48].

Fish use a range of specific and nonspecific defense mechanisms against invading pathogens [49]. Since immunostimulants confer overall advantage in terms of survival and resistance to diseases, animals receiving them can be expected to perform better in terms of growth and thereby contribute to production. Immunomodulators increase specific immunity and reduce mortality in immunocompromised carp [50]. The study of disease control in crustacean farming through use of immunostimulants [51] and the demonstrated effect of medicinal plant extracts on rainbow trout [33] has also been established.

5. Conclusions

This study demonstrated that 5 g/kg *A. sativum*, 10 g/kg. *odorata* and 10 g/kg *T. triangulare* added to African Catfish separately acted as growth promoters and could improve resistance of fish to *Pseudomonas aeruginosa* infections. The inclusion of 10 g/kg *C. odorata* as an additive in the feed of African Catfish was shown to improve their immune response. This study has pointed out that further study to determine the effective dose under culture conditions, testing with a refined extract of these medicinal plants, is needed. The degree and duration of the resistance conferred along with doses for different age group of fish and time of application to ensure high yield in culture ponds need to be evaluated. More studies are necessary to determine the potential of these plants in other fish species for growth enhancement, disease prevention and control strategies.

Author Contributions: Conceptualization and Methodology, A.M.T.; Formal analysis, I.O.O.; Investigation, A.M.T. and O.A.O.; Data curation, I.O.O. and O.B.A.; Writing—original draft preparation, A.M.T.; Writing—review and editing, O.B.A., I.O.O., R.C.O. and T.C.E.; Visualization, R.C.O.; Supervision, I.O.O.; Project administration, A.M.T. and I.O.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional Review Board Statement: The ethical approval was acquired from the University of Ibadan Animal Care and Use for Research Ethical Committee. The approval number is UI-ACUREC/App/2015/066. Experiment was conducted according to ACUREC approved protocol.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data on the study are provided in the manuscript. There is no shared data.

Acknowledgments: Our acknowledgements go to (i) management and staff of F. M Sanusi Aqualife Farms, Olodo Ibadan, Oyo State, Nigeria, (ii) the technical staff of the Veterinary Public Health (Bacterial and Parasitic Zoonoses) Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan.

Conflicts of Interest: The authors declare no conflict of interest.

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