Effects of Dietary *Pseudomonas fluorescens* Supplementation on Growth Performance and Immune Response of African Catfish (*Clarias gariepinus*) Juveniles Challenged with *Clostridium botulinum*

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Abstract
A 70-day feeding trial was conducted to evaluate the effects of dietary *Pseudomonas fluorescens* supplementation on the growth performance and immune response of *Clarias gariepinus* juveniles. Five diets containing graded probiotic inclusion levels (T1, control; T2, 10^3 cfu g−1; T3, 10^5 cfu g−1; T4, 10^7 cfu g−1; T5, 10^9 cfu g−1) were fed to fish in triplicate tanks (15 fish per tank). After the feeding period, subgroups were challenged intraperitoneally with *C. botulinum* (0.2 ml of 10^7 cells ml−1) and observed for 10 days. *P. fluorescens*. was confirmed by morphological and biochemical tests and shown to produce in vitro antagonism against *C. botulinum* (mean inhibition zone ≈ 3.7 mm). Fish fed probiotic diets showed significantly ($p < 0.05$) higher final weight, weight gain, specific growth rate (SGR), feed efficiency (FER) and lower feed conversion ratio (FCR) than controls; the best performance occurred at 10^7 cfu g−1. Haematological indices showed elevated total white blood cell (WBC) counts in probiotic-fed fish, with the highest WBC at 10^7 cfu g−1. Following challenge, survival was highest in T4 (≈80%) and lowest in control (0%). Histopathology of gill, intestine and liver revealed fewer lesions in probiotic-fed fish. Results indicate that dietary *P. fluorescens* at 10^7 cfu g−1 improves growth and non-specific immune parameters and increases resistance of *C. gariepinus* to *C. botulinum* under experimental conditions.
Keywords: *Clarias gariepinus*; probiotic; *Pseudomonas fluorescens*; *Clostridium botulinum*; growth; immunity; aquaculture

1. Introduction
Aquaculture's intensification increases susceptibility of cultured fish to infectious diseases, undermining production and trade (Subasinghe *et al.*, 2003; FAO, 2015). Overuse of antibiotics promotes antimicrobial resistance and residues, motivating interest in biological disease-control strategies such as probiotics (Amabile-Cuevas *et al.*, 1995; Balcázar *et al.*, 2006). Probiotics live microbial supplements that benefit the host have been applied in aquaculture to enhance growth, feed utilisation, water quality and host defence (Fuller, 1989; Verschuere *et al.*, 2000; Gatesoupe, 2000). The African catfish (*Clarias gariepinus*) is an important cultured species in Africa due to rapid growth and environmental tolerance (Viveen *et al*., 2005). However, bacterial pathogens including *Clostridium species* remain a threat (Wise *et al.*, 2004). *Pseudomonas species* (e.g., *P. fluorescens*) are indigenous to fish-associated microhabitats and can act as biocontrol agents through siderophore production and antibiotic-like metabolites (González-Palacios *et al.*, 2020; Liu *et al.*, 2015; Nader *et al.*, 2024). Probiotics in aquaculture are applied via feed or water to establish beneficial microbiota or to outcompete pathogens; mechanisms include competitive exclusion, production of inhibitory compounds, digestive enzyme contribution, and immune stimulation (Verschuere *et al*., 2000; Balcázar *et al.*, 2006; Kesarcodi-Watson *et al.*, 2008). Strain selection, dose and delivery method are critical to efficacy (Mohapatra *et al.*, 2012). *Pseudomonads* have been used as probiotic/biocontrol agents in aquatic and terrestrial systems. They produce siderophores, bacteriocins and enzymes that may inhibit pathogens and improve host health (Loper and Buyer, 1991; Klopper *et al.*, 1980). Nevertheless, safety evaluation is essential because some strains have opportunistic pathogenic potential (Austin et al., 1995; Charteris *et al.*, 1998). Gram-positive *Bacillus spp*. is widely used as aquaculture probiotics due to spore-forming resilience and enzyme production; comparative studies show variable performance among probiotics and dose-dependent effects (Moriarty, 1996; Rajesh *et al.*, 2006; Nwanna *et al.*, 2012). *C. botulinum* is an anaerobic spore-former producing potent neurotoxins and is implicated in animal botulism associated with decomposing carcasses and sediments; reducing pathogen load and enhancing host resistance are important to mitigate disease risk and trade restrictions (Peck, 2009; Getchell *et al.*, 2006; Wise *et al.*, 2004). Despite the increasing application of probiotics in aquaculture, most studies have focused on *Bacillus*, *Lactobacillus*, and commercial probiotic formulations, whereas the probiotic potential of indigenous *P. fluorescens* against *C. botulinum* infection in *C. gariepinus* remains largely unexplored. Also, limited information is available regarding the dose-dependent effects of *P. fluorescens* on growth performance, feed utilization, haematological responses, histopathological alterations, and disease resistance following *C. botulinum* challenge. However, the optimum dietary inclusion level of *P. fluorescens* for maximizing growth and enhancing innate immunity in *C. gariepinus* has not been clearly established. The present study addresses this knowledge gap by

evaluating the efficacy of graded dietary levels of an indigenous *P. fluorescens* isolate on growth performance, feed efficiency, haematological indices, tissue histopathology, and resistance to experimental *C. botulinum* infection in juvenile *C. gariepinus*. Unlike previous studies that primarily assessed general probiotic effects, this study integrates in vitro antagonistic activity, dose-response evaluation through polynomial regression, and pathogen challenge testing to identify the optimum probiotic inclusion level and provide evidence supporting the use of *P. fluorescens* as a potential biological alternative to antibiotics in African catfish production. Accordingly, the study tested the hypothesis that dietary supplementation with *P. fluorescens* would produce a dose-dependent improvement in growth performance, feed utilization, innate immune responses, and survival of *Clarias gariepinus* following experimental *C. botulinum* challenge, with an optimum response occurring at an intermediate probiotic inclusion level rather than at the highest dose.

2. Materials and methods

Experimental Design

A completely randomized design with five treatments in three replicates (15 fish per tank) was used. *C. gariepinus* Juveniles were fed experimental diets for 70 days followed by a 10-day post-challenge observation.

Test Organisms and Culture

Probiotic isolates (Pseudomonas *fluorescens*) and pathogenic bacterium (*C. botulinum*) were obtained from gut, gill, skin and pond sediment of adult *C. gariepinus* from commercial farms.

Morphological and Biochemical Characteristics of Bacterial Isolates.

Isolation used standard culture media: EMB agar for *Pseudomonas* and nutrient/anaerobic-adapted media for *Clostridium*. Morphological and biochemical identification included Gram staining, motility (hanging-drop), spore test, catalase, oxidase, indole, citrate, sugar fermentation, MR-VP, TSI (Table 1) and other standard tests (Cowan and Steel, 1993). Pure isolates were stored at 4°C for short-term use. The result confirmed the isolates to be *P. fluorescens and C. butilinum* based on the reaction on biochemical and morphological test carried out as shown in table 1. The result revealed that citrate, methyl-red, glucose and mortility were present on biochemical and morphology test carried out on *P. fluorescens*. However, gram stain, spore, indole, nitrate, hydrogen sulphide production voges proskauer, oxidase test, mannitol, maltose, sucrose and lactose were absent on biochemical and morphology test carried out on *P. fluorescens*. The pigment of organism on nutrient agar was greenish to brown in *P. fluorescens* while *C. butilinum* was creamy

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Table 1: Characterisation test of bacteria sample

Biochemical test	***P. fluorescens***	***C. botulinum***
Gram Stain	-	+
Spore	-	+
Indole	_	+
Nitrate	-	+
Hydrogen Sulphide production	_	+
Citrate	+	_
Methyl – Red	+	_
Voges Proskauer	_	_

Oxidase Test	–	–
Glucose	+	+g
Mannitol	-	–
Maltose	-	+g
Sucrose	-	–
Lactose	–	–
Motility	+	+
Pigment of organism on nutrient agar	Greenish blue to brown	Creamy

Key: + = Present, - =Absent, +g = production of acid and gas.

Diet Preparation

A basal diet approximately 40% crude protein was formulated from fishmeal, soybean meal, groundnut cake, maize and vitamin–mineral premix; cassava starch served as binder. Five diets were prepared: T1 (control, no probiotic), T2 (10^3 cfu g−1), T3 (10^5 cfu g−1), T4 (10^7 cfu g−1) and T5 (10^9 cfu g−1). Probiotic suspensions were incorporated into cooled pelleted feed; pellets were air-dried and stored at −2°C until use.

Table 2. Gross composition of the experimental diets (*P. fluorescens* inclusion) used to feed *C. gariepinus*

Ingredients	**T1**	**T2**	**T3**	**T4**	**T5**
Fish meal (72% CP)	25.4	25.4	25.4	25.4	25.4
Soybean meal (45% CP)	35.4	35.4	35.4	35.4	35.4
Ground nut cake (48% CP)	12.1	12.1	12.1	12.1	12.1
Yellow maize	15.0	15.0	15.0	15.0	15.0
Oyster shell	2.00	2.00	2.00	2.00	2.00
Vegetable oil	5.60	5.60	5.60	5.60	5.60
Vitamin-premix	2.50	2.50	2.50	2.50	2.50
Starch	2.00	2.00	2.00	2.00	2.00
P. fluorescens dose(cfug^{-1})	0.00	10^3	10^5	10^7	10^9

Composition of vitamin-mineral mix (Aquamix) (quantity/kg), Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6

mcg; Calcium; Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450. L- lysine, 10 g; Selenium, 50 ppm.

Key:

T1 - basal diet (without bacterial treatment
T2 - diet with 10^3 level of bacteria inclusion
T3 - diet with 10^5 level of bacteria inclusion
T4 - diet with 10^7 level of bacteria inclusion
T5 - diet with 10^9 level of bacteria inclusion

Experimental Animals and Husbandry

C. *gariepinus* Juveniles (initial mean weights ≈ 3.8–9.8 g depending on phase) were obtained from FUTA fish farm, acclimated two weeks, then stocked at 15 fish per 70-L glass tank. Fish were fed twice daily to apparent satiation; uneaten feed removed after one hour. Daily water management included 75% exchange and monitoring of temperature, pH and dissolved oxygen.

Safety and Antimicrobial Tests

Safety of probiotic isolates was assessed by intraperitoneal injection (0.3 ml of 10^7 cfu ml−1) in 20 juvenile fish; control fish received saline. Mortality and morbidity were monitored for 14 days. In vitro antagonism was assessed by agar disc diffusion using tryptic soy agar seeded with pathogenic strains; inhibition zones were measured (Tadesse *et al.,* 2012).

Growth, Feed utilisation and Proximate Analysis
Fish were weighed biweekly. Growth and feed indices (WG, SGR, FI, FCR, FER, PER) followed Cho and Kaushik (1985). At termination, ten fish per treatment were analysed for proximate composition (moisture, ash, crude protein, crude lipid, crude fibre, NFE) using AOAC methods (1990).

Haematology and Histopathology
Blood was collected by cardiac puncture (heparinized syringes) from five fish per tank for WBC, RBC, Hb, PCV, MCH, MCV, MCHC and leukocyte differentials (Svobodova *et al.,* 2006). Liver, gill and intestine samples were fixed, processed and stained for histological assessment (Bernet *et al.,* 1999).

Challenge Test
Three fish per tank were challenged IP with 0.2 ml of *C. botulinum* (10^7 cells ml−1). Control subgroups received saline. Fish were observed for 10 days for clinical signs and mortality; survival (%) recorded.

Statistical Analysis
Data were analysed by one-way ANOVA and Duncan's multiple range test ($p < 0.05$) using SPSS. Polynomial regression was used to estimate optimal probiotic concentration for selected responses.

3. Results
Identification and Safety Assessment of *Pseudomonas fluorescens*
The probiotic isolate was identified as *Pseudomonas fluorescens* based on its colony morphology, Gram staining characteristics, motility, pigment production, and biochemical properties. Intraperitoneal administration of the isolate (0.3 mL of 10^7 CFU mL^{-1}) produced no clinical signs of disease or mortality throughout the 14-day observation period, indicating that the isolate was non-pathogenic under the experimental conditions.
Antagonistic Activity Against *Clostridium botulinum*
The Pseudomonas isolate exhibited inhibitory activity against *Clostridium botulinum* in the agar disc diffusion assay, producing a mean inhibition zone of approximately 3.7 mm. This result demonstrates the antagonistic potential of *P. fluorescens* against the pathogenic bacterium.
Water Quality
Water quality parameters remained within the recommended ranges for juvenile African catfish throughout the feeding trial. Mean water temperature ranged from 27 to 28°C, pH ranged from 7.0 to 7.3, and dissolved oxygen averaged approximately 7.2 mg L^{-1}. No significant differences ($P > 0.05$) were observed among treatments, indicating that environmental conditions did not influence treatment responses.

Growth Performance and Feed Utilisation

Dietary supplementation with *Pseudomonas fluorescens* significantly improved growth performance and feed utilization of *Clarias gariepinus* ($P < 0.05$). Fish fed the diet containing 10^7 CFU g^{-1} (T4) recorded the highest final body weight, weight gain, specific growth rate (SGR), feed efficiency ratio (FER), and protein efficiency ratio (PER). While control group exhibited the lowest growth performance and the highest feed conversion ratio (FCR), indicating poorer feed utilization.

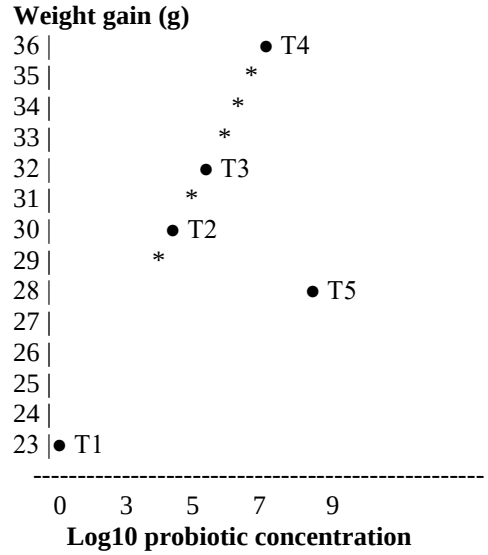
Table 3. Growth Performance and Nutrient Utilisation

Parammeter	T1 (Control)	T2 (10^3)	T3 (10^5)	T4 (10^7)	T5 (10^9)
Initial weight (g)	5.27 ± 0.00^a	5.27 ± 0.01^a	5.26 ± 0.01^a	5.27 ± 0.01^a	5.26 ± 0.00^a
Weight gain (g)	23.1 ± 2.74^a	30.8 ± 2.52^bc	31.9 ± 2.99^bc	35.4 ± 1.00^c	28.0 ± 0.48^b
SGR (% day−1)	2.40 ± 0.14^a	2.74 ± 0.09^b	2.78 ± 0.11^b	2.92 ± 0.03^b	2.63 ± 0.02^ab
Feed intake (g)	34.7 ± 5.76^a	41.6 ± 1.19^b	42.3 ± 2.40^b	45.2 ± 2.30^c	39.5 ± 0.86^ab
FCR	1.50 ± 0.11^d	1.35 ± 0.05^b	1.33 ± 0.16^b	1.28 ± 0.04^a	1.41 ± 0.14^c
FER	0.66 ± 0.07^a	0.74 ± 0.03^b	0.75 ± 0.04^b	0.78 ± 0.03^c	0.71 ± 0.04^ab
PER	1.66 ± 0.20^a	1.86 ± 0.10^c	1.89 ± 0.09^c	1.96 ± 0.08^d	1.77 ± 0.09^b

Key: IW = initial weight; FW = final weight; WG = weight gain; SGR = specific growth rate; FI = feed intake; FCR = feed conversion ratio; FER = feed efficiency ratio; PER = protein efficiency ratio.

Polynomial regression analysis:

Polynomial regression analysis suggested that probiotic supplementation at approximately 10^7 CFU g^{-1} represented the optimum inclusion level for maximizing growth performance.



Polynomial regression fitted curve analysis revealed a significant quadratic relationship between dietary probiotic concentration and weight gain. Weight gain increased progressively with increasing *Pseudomonas fluorescens* supplementation, reaching a maximum at approximately **10^7 CFU g^{-1} diet**, after which performance declined at **10^9 CFU g^{-1}**. The negative quadratic coefficient indicates the existence of an optimum probiotic inclusion level beyond which additional supplementation does not further improve growth performance.

Carcass Composition

Whole-body crude protein was significantly higher in probiotic-fed fish (notably T3 and T4) compared with control. Lipid content and moisture varied among treatments but changes were generally modest.

Table 4. Proximate composition (whole-body) of *C. gariepinus.*

Table 4. Proximate composition (whole-body) of Clarias gariepinus.

Parameter (%)	T1 (Control)	T2 (10^3)	T3 (10^5)	T4 (10^7)	T5 (10^9)
Moisture	8.25 ± 0.03a	8.31 ± 0.02a	9.62 ± 0.01b	9.22 ± 0.01b	8.33 ± 0.03ab
Ash	9.65 ± 0.05ab	10.7 ± 0.01b	9.48 ± 0.01a	10.3 ± 0.01b	9.71 ± 0.09ab
Crude protein	57.9 ± 0.02a	61.2 ± 0.04c	62.6 ± 0.05c	61.5 ± 0.01c	60.9 ± 0.01b
Crude fat	11.4 ± 0.02a	13.6 ± 0.02c	13.1 ± 0.01c	12.9 ± 0.03b	12.2 ± 0.02b
NFE	12.8 ± 0.05b	6.19 ± 0.03ab	5.20 ± 0.08a	6.08 ± 0.05ab	8.86 ± 0.04b

Notes: NFE = nitrogen-free extract. Superscripts (a–c) as in thesis ($p < 0.05$).

Proximate composition (whole-body) of Clarias gariepinus fed diets supplemented with Pseudomonas fluorescens. Values are means ± SE. Means in the same row with different superscript letters are significantly different ($p < 0.05$).

Haematology

Total WBC counts increased significantly ($p < 0.05$) in probiotic-fed fish compared with control; the highest WBC occurred at T4. RBC, Hb and PCV displayed moderate increases in some probiotic treatments

Table 5. Haematological parameters of experimental fish

Parameter	T1 (Control)	T2 (10^3)	T3 (10^5)	T4 (10^7)	T5 (10^9)
WBC (×10^3 mm−3)	5.50 ± 0.58a	5.89 ± 0.26ab	6.17 ± 0.07ab	6.78 ± 0.15b	5.70 ± 0.23a
RBC (×10^6 mm−3)	4.05 ± 0.01a	4.08 ± 0.00a	4.05 ± 0.01a	4.15 ± 0.01b	4.96 ± 0.23c
Hb (g/100 ml)	12.1 ± 0.07a	11.9 ± 0.09a	12.1 ± 0.07a	12.6 ± 0.12b	12.7 ± 0.23b
PCV (%)	36.0 ± 0.58a	35.0 ± 0.58a	36.0 ± 0.00a	37.0 ± 1.73a	36.3 ± 2.40b
MCHC (%)	33.7 ± 0.59a	34.1 ± 0.32a	33.7 ± 0.20a	34.2 ± 1.30a	35.4 ± 4.79a
MCH (pg)	30.0 ± 0.20ab	29.3 ± 0.23b	30.0 ± 0.20ab	30.4 ± 0.38b	25.7 ± 0.23a
MCV (fl)	88.9 ± 1.67b	85.8 ± 1.41b	88.9 ± 0.23b	89.2 ± 4.45b	73.4 ± 5.53a
NEU (%)	56.7 ± 4.18a	58.3 ± 1.76ab	61.3 ± 1.86b	58.0 ± 5.51ab	56.7 ± 2.60a
LEU (%)	36.7 ± 3.53a	40.3 ± 0.88a	39.0 ± 1.15b	36.3 ± 3.18a	39.7 ± 2.91b

Notes: WBC = white blood cell count; RBC = red blood cell count; Hb = haemoglobin; PCV = packed cell volume; MCHC = mean corpuscular haemoglobin concentration; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; NEU = neutrophils; LEU = lymphocytes. Superscripts as in thesis ($p < 0.05$).

Haematological parameters of Clarias gariepinus fed diets supplemented with Pseudomonas fluorescens for 70 days. Values are means ± SE (n as in thesis). Means in the same row with different superscript letters are significantly different ($p < 0.05$).

Challenge Test and Survival

After IP challenge with C. botulinum, control fish experienced 100% mortality within the 10-day observation. Probiotic-fed groups showed markedly higher survival; T4 recorded the highest survival (~80%), followed by T3 and T5. Clinical signs (skin lesions, discoloration, abnormal swimming) were evident in control but largely absent in survivors from probiotic groups.

Table 6. Challenge test results

Treatment	Number challenged (n)	Mortality (%)	Mortality (fish, computed)	Survival (%)	Survival (fish, computed)
T1 (Control)	15	100	15	0	0
T2 (10^3)	15	46.7	7 (46.7% of 15 ≈ 7.0)	53.3	8
T3 (10^5)	15	26.7	4 (26.7% of 15 ≈ 4.0)	73.3	11
T4 (10^7)	15	20.0	3 (20% of 15 = 3)	80.0	12
T5 (10^9)	15	46.7	7	53.3	8

Clarias gariepinus fed diets supplemented with Pseudomonas fluorescens and challenged intraperitoneally with Clostridium botulinum (0.2 ml of 10^7 cells ml−1). n = 15 fish challenged per treatment. Values reported in thesis (mortality % and survival %) are reproduced and counts computed from reported percentages (rounded to nearest whole fish).

Histopathology

Control fish exhibited pronounced histopathological lesions in gill (mucosal erosion, stunting of lamellae), intestine (mucosal erosion) and liver (periportal necrosis). Fish in probiotic groups, especially T4, showed reduced lesion severity and more normal histological architecture.

4. Discussion

Dietary inclusion of P. *fluorescens* produced significant improvements in growth performance, nutrient utilisation and carcass protein deposition, consistent with reports for Pseudomonas and other probiotics in fish (Balcázar *et al.*, 2006; El-Haroun *et al.*, 2006; Mohapatra *et al*., 2012). Improved FCR and FER may reflect probiotic-mediated enhancement of digestive enzyme activities and gut microbiota modulation, improving nutrient digestibility (Moriarty, 1996; Marzouk *et al.*, 2008). The Polynomia regression quadratic response observed agrees with previous studies demonstrating that probiotic supplementation exerts dose-dependent effects on fish growth. Moderate concentrations enhance nutrient utilization through improved digestive enzyme activity, modulation of intestinal microbiota, and stimulation of innate immunity, whereas excessive concentrations may reduce these benefits because of microbial competition and increased energetic costs associated with maintaining intestinal homeostasis Merrifield and Ringø, 2022). Therefore, 10^7 CFU g^{-1} diet appears to represent the optimum dietary inclusion level for *P. fluorescens* under the present experimental conditions. Elevated white blood cell (WBC) counts in probiotic-fed fish are indicative of enhanced innate immune responses, reflecting improved leukocyte proliferation and activation of non-specific defense mechanisms. This immunostimulatory effect is associated with increased resistance to bacterial infections and higher survival rates following pathogen challenge. In addition, probiotics exert direct antagonistic effects against pathogenic bacteria through the production of antimicrobial metabolites, including bacteriocins, organic acids, hydrogen peroxide, and siderophores, which inhibit the growth of *C. botulinum* and other fish pathogens. These combined immunomodulatory and antimicrobial mechanisms likely contributed to the improved survival observed in probiotic-treated fish following *C. botulinum* challenge (Simón *et al.*, 2021; Shahriar *et al.*, 2024; Hamad *et al*., 2022)

Dose–response patterns showed optimum effects at 10^7 cfu g−1; higher inclusion (10^9) did not further improve outcomes, consistent with previous observations that probiotic benefits are strain- and dose-dependent and not necessarily linear (Son *et al*., 2009). Safety testing indicated acute innocuity at the tested dose; however, comprehensive molecular identification (16S rRNA sequencing) and screening for virulence and antibiotic resistance genes are necessary before commercial application (Charteris *et al.*, 1998). The antagonistic activity observed in the present study may be attributed to the production of siderophores, antimicrobial proteins, biosurfactants, and other extracellular metabolites by *P. species*, which inhibit the growth of fish pathogens through iron sequestration, competitive exclusion, and direct antimicrobial activity (Liu *et al.*, 2015; González-Palacios *et al.*, 2020; Nader *et al*., 2024).

5. .Conclusion

Dietary *Pseudomonas spp*. supplementation improved growth, feed utilisation and non-specific immune indicators in *Clarias gariepinus* and conferred significant protection against experimental Clostridium botulinum challenge. The most favourable effects were observed at approximately 10^7 cfu g−1 feed. The isolate demonstrated in vitro antagonism against *C. botulinum* and was acutely non-pathogenic in safety testing.

Recommendations

Conduct molecular identification (e.g., 16S rRNA sequencing) of the probiotic isolate and screen for virulence factors and antibiotic resistance genes prior to field or commercial use.

Perform longer-term pond-scale trials across life stages and under commercial conditions to confirm efficacy and environmental safety.

Investigate mechanisms of action (enzyme profiling, gut microbiota analysis, immunological assays such as lysozyme, complement and specific antibody responses).

Assess environmental persistence and impact on native microbial communities and sediments.

Consider formulation studies (encapsulation, synbiotic approaches) to maximise probiotic viability and efficacy in practical feeds.

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