



Research Article

Effect of Dietary Inclusion of Commercial Probiotics and Vitamin C on Growth Performance, Nutrient Utilization and Haematological Profile of *Clarias gariepinus* Fingerlings

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ABSTRACT

This study evaluated the effects of dietary inclusion of commercial probiotics (Aqua Pro) and Vitamin C on growth performance, nutrient utilization, haematological profile of *Clarias gariepinus* fingerlings. A total of 120 fingerlings were randomly distributed into four treatment group (T0 – T3) with varying inclusion levels (0,1,2, and 3g/kg) of probiotics and Vitamin C over 18 weeks feeding trial. Fish were fed a 45% crude protein diet, and growth indices, feed utilization parameters and haematological variables were assessed. Result showed significant improvement ($p < 0.005$) in final weight, weight gain, specific growth rate, and protein efficiency ratio with increasing supplementation levels, with the best performance recorded in T3. Feed conversion ratio decreased significantly, indicating enhanced feed efficiency. Survival rate improved from 80% in control to 93.3% in the highest inclusion group. Haematological parameters (RBC, WBC, PVC, and haemoglobin) were significantly enhanced in supplemental groups, reflecting improved physiological and immune status. Water quality parameters remained within acceptable limits and were not significantly affected by dietary treatments. The study concludes that combined dietary supplementation of probiotics and Vitamin C, particularly at 2 – 3g/kg, enhanced growth performance, nutrient utilization and health status of *Clarias gariepinus* fingerlings.

Keywords: Aqua Pro; *Clarias gariepinus*; Growth performance; Nutrient utilization; Haematology; Probiotics; Vitamin C

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INTRODUCTION

Aquaculture has emerged as one of the fastest-growing food production sectors globally, providing a sustainable source of high-quality animal protein to meet the rising global demand (FAO, 2023). The practice involves the controlled cultivation of aquatic organisms, primarily fish, for commercial or subsistence purposes. One of the most widely cultivated species in Nigeria is *Clarias gariepinus* (African catfish), a hardy and fast-growing specie with

high adaptability to diverse environmental conditions (Akinwale & Faturoti, 2007). Despite the advantages of farming *C. gariepinus*, profitability and productivity are often limited by the high cost of conventional feed ingredients, which account for about 60–70% of production expenses (Gabriel *et al.*, 2007). Thus, there is an urgent need to explore alternative feed additives and supplements to enhance growth performance, reduce feed costs, and improve fish health. Effective feeding practices are also essential

not only for enhancing production efficiency but also for minimizing environmental impacts, as poorly managed feeding can lead to nutrient loss and water quality deterioration (Eyo, 2019). Therefore, understanding the feeding dynamics of *Clarias gariepinus* is critical for the sustainable development of aquaculture in Nigeria, particularly in addressing the challenges of food security and economic development.

Probiotics is importance in gut health, immune system modulation, growth performance; improve water quality by degrading organic waste and reduction of antibiotics uses. It produces several enzymes that can break down the indigestible compounds potentially present in the supplementary feed; thus, it leads to higher feed efficiency in the aquaculture (EL-Haroun *et al.*, 2006).

Aqua Pro is a commercial probiotic formulation commonly used in aquaculture, consisting mainly of beneficial bacterial strains such as *Bacillus* spp. and *Lactobacillus* spp. These bacteria are well documented for their multifunctional roles in fish nutrition, health management, and water quality improvement. *Bacillus* species are spore-forming bacteria capable of surviving extreme environmental conditions and feed processing temperatures. They produce a wide range of extracellular digestive enzymes, including proteases, amylases, cellulases, and lipases, which enhance the breakdown of complex feed components and improve nutrient digestibility and feed conversion efficiency (Wang and Gu, 2010; Mohapatra *et al.*, 2011). *Lactobacillus* species, which belong to the group of lactic acid bacteria, play a vital role in maintaining gut microbial homeostasis. They enhance intestinal health by producing lactic acid and bacteriocins that suppress pathogenic microorganisms, strengthen the intestinal barrier, and stimulate immune responses. Studies have shown that dietary inclusion of *Lactobacillus* spp. improves growth performance, feed utilization, haematological stability, and disease resistance in various fish species (Soliman *et al.*, 2022). In Nigeria, research on the use of probiotics in *Clarias gariepinus* diets has demonstrated promising results, particularly in terms of improved weight gain, feed conversion ratios, and survival rates. This aligns with the need for sustainable aquaculture practices that not only boost productivity but also minimize the environmental footprint of aquaculture operations. Understanding the role of probiotics in fish feeding, its nutritional composition, and its effects on the performance of *Clarias gariepinus* is therefore critical for the advancement of aquaculture in Nigeria.

However, the specific effects of Aqua Pro and vitamin c supplementation on the performance indices nutrient utilization and haematological profile of *Clarias gariepinus* fingerlings remain underexplored, particularly in the context of Nigeria's aquaculture sector.

Ascorbic acid (vitamin C) is an essential micronutrient in fish nutrition, with critical roles in growth, immunity, stress response, and overall health. Ascorbic acid is vital for collagen synthesis, which supports proper bone formation, tissue repair, and overall growth. Deficiency can lead to skeletal deformities and poor growth rates Abedi *et al.*, (2022). Ascorbic acid acts as a powerful antioxidant that protects fish tissues from oxidative stress caused by metabolic activity, environmental stress, or pollutants. It stabilizes membranes and reduces lipid peroxidation (Ai *et al.*, 2021).

Haematological parameters such as packed cell volume (PCV), haemoglobin concentration, red blood cell (RBC) count, and white blood cell (WBC) count are widely used as indicators of fish health, immune status, and physiological condition. Changes in these parameters reflect the effects of nutrition, stress, disease, and environmental conditions on fish (Gabriel & George, 2023). Dietary probiotics have been shown to positively influence haematological profiles by enhancing immune competence and physiological stability under intensive culture conditions (Adeyemo *et al.*, 2021).

One of the most persistent problems in catfish aquaculture is nutritional deficiency and imbalance, especially under cost-cutting feed regimes where fish are deprived of essential micronutrients and functional additives (Adeyemo *et al.*, 2021). This often results in poor feed conversion, slow growth, compromised immune function, and increased mortality, especially among fingerlings during the critical early stages of development. In response, farmers often resort to frequent antibiotic use, which poses risks such as antibiotic resistance, environmental pollution, and food safety concerns. Vitamin C, is often unstable in feed due to heat sensitivity and oxidation, leading to variable results in performance and haematological stability (Zhou *et al.*, 2023). These have led the aqua-culturists to search for alternative feed formulation with probiotics to improve the feed efficiency through higher digestibility and nutrients utilization.

Aqua Pro probiotics containing *Bacillus* and *Lactobacillus* spp. have demonstrated potential to enhance digestion, nutrient utilization, immune response, and water quality. Vitamin C complements

these effects through its antioxidant and immunomodulatory roles. Understanding the combined effects of these additives will provide valuable scientific information for optimizing feed formulations, reducing production costs, and improving fish health. Several studies have shown that dietary supplementation of Vitamin C can enhance growth performance, haematological profiles, and survival rates in various fish species (Yousefi *et al.*, 2022). Potential synergistic or antagonistic interactions between these additives remain unclear, especially concerning growth indices, haematological responses, and carcass yield. Understanding these interactions is vital for optimizing feed formulations aimed at improving fish health and productivity in cost-effective and sustainable ways. The findings of this study will benefit fish farmers, nutritionists, researchers, and policymakers by providing evidence-based recommendations for the use of Aqua Pro probiotics and vitamin C in African catfish diets, thereby contributing to sustainable aquaculture development in Nigeria. Hence, the study aims to investigate the impact of varying dietary inclusion levels of commercial probiotics and vitamin C on the growth performance, haematological parameters, carcass and mineral composition of *Clarias gariepinus* fingerlings.

MATERIAL AND METHODS

Study Area

The study was conducted at the fish farm/research unit located latitude 12.459°N and longitude 7.498°E approximately, Department of Fisheries and Aquaculture, Federal University Dutsin-Ma, located in Katsina State, Nigeria. Dutsin-Ma Local Government area is situated in the Sudan Savanna Zone in central Katsina State, bordered to the west by Safana and Dan Musa Local Governments, to the north by Kurfi and Charanchi Local Governments, to the east by Kankia, and to the south by Matazu Local Government Areas.

Sample Collection and Preparation

A total of 120 *Clarias gariepinus* fingerlings were obtained from Kaduna State Nigeria. The fish was transported in a twenty-five liters (25) half-filled plastic Jeri can with water. The fishes were acclimatized for two weeks in three plastic tanks at the rate of 40 fish per pond, afterward, the fishes were fed a diet containing 45% crude protein.

Experimental Design

A complete randomized block design was employed for the feeding trial. One hundred and twenty *Clarias*

gariepinus fingerlings of uniform size and weight were distributed randomly into twelve separate tanks, and each tank was considered a block. Water was changed partially every two days. Fish was fed to satiation two times a day (9am, and 8pm) for 18 weeks. Borehole was the water source for the experiment, and physicochemical water parameters (Dissolved oxygen, Alkalinity, Ammonia, PH, and Temperature) were measured.

Feed Preparation

The feed ingredients in the feed formulation includes Fish meal, Soybean meal, Maize meal, guinea corn, Vitamin and Mineral premixes which was purchased from the railway station market Kaduna state, they were processed and grinded into meal for storage. The probiotics Aqua Pro and vitamin C (ascorbic acid) were procured from rail way station market Kaduna State. Following processing and milling of the feed ingredients, a 45% crude protein CP basal diet was formulated via the Person square method. Subsequent experimental diets were formulated by supplementing this basal diet with probiotics at 0g (T0), 1g (T1), 2g (T2), and 3g (T3) and vitamin C (ascorbic acid) at 0g(T0), 1g(T1), 2g(T2) and 3g(T3) supplementation levels (Table 1). The dough of the diets formed was extruded using an extruder machine after weighing appropriately and thorough mixing of the ingredients. Ten *Clarias gariepinus* fingerlings were evenly distributed in each of the plastic tanks. The daily feeding was done by hand which was feeding to satiation. Daily ration time was divided into two feedings per day (09:00am and 08:00pm) and the fingerlings were weighed weekly so as to adjust the feed by virtue of weight gained. An electronic digital scale was used to measure weights of fish per week till the end of the experiment (18weeks).

Data Collection

Growth parameters and nutrient utilization

Growth parameters were calculated based on Tekinay and Davis (2001) as shown below;

Weight Gain: Weight gain was determined the difference between the final weight and initial weight of the experimental.

- i. Weight gain = Final weight - Initial weight.
- ii. **Daily Weight Increase (DWI)** =
$$\frac{\text{Weight Gain}}{\text{Experimental Period}}$$
- iii. **Specific Growth Rate:** It is the percentage rate of change in the logarithmic body weight

$$\text{Specific Growth Rate (\%/day)} = \frac{\log(wt_2) - \log(wt_1)}{t_2 - t_1}$$

Where Wt1= Initial weight gain, Wt2= Final weight gain
 $T_2 - T_1$ = Duration (in days) considered between Wt2 and Wt1

- iv. **Percentage Weight Gain:** The % weight gain was determined as the difference between the final weight and initial weight of experiment fish

$$\% \text{ weight gain} = \frac{\text{Finalweight} - \text{Initialweight}}{\text{Initialweight}} \times 100$$

- v. **Feed Conversion Ratio:** This was calculate using the formula

$$\text{FCR} = \frac{\text{Feedfed}}{\text{Fishweightgain}}$$

- vi. **Protein Efficiency Ratio:** It was calculated from the relationship between the

increment in the weight of fish (i.e weight gain of fish) and protein consumed.

$$\text{PER} = \frac{\text{Meanweightgain}}{\text{Proteinintake}}$$

- vii. **Protein Intake:** PI = Feed intake × % of protein in diet

$$\text{viii. Survival Rate} = \frac{\text{Number of Harvested Fishes}}{\text{Number of Stocked Fishes}} \times 100 (\%)$$

$$\text{ix. Mortality} = \frac{N_0 \times N_i}{N_0} \times 100 (\%)$$

Therefore, N_i and N_0 denote the number of the fishes at the beginning and the end of the experiment respectively.

Table 1: Gross Composition of Experimental Diets with Aqua Pro and Vitamin C supplementation

S/N	Ingredients	T1	T2	T3	T4
1	Fish meal	26.68	26.68	26.68	26.68
2	Soya bean meal	26.68	26.68	26.68	26.68
3	Groundnut cake	26.68	26.68	26.68	26.68
4	Maize	7.23	7.23	7.23	7.23
5	Guinea corn	7.23	7.23	7.23	7.23
6	Salt	0.50	0.50	0.50	0.50
7	Vitamin premix	1.00	1.00	1.00	1.00
8	Lysine	0.5	0.5	0.5	0.5
9	Methionine	0.5	0.5	0.5	0.5
10	Palm oil	1	1	1	1
11	Bone meal	2	2	2	2
	Total	100	100	100	100
	Aqua Pro inclusion	0.0g	1.0g	2.0g	3.0g
	Vitamin c inclusion	0.0g	1.0g	2.0g	3.0g

Haematology

Blood samples were collected from the fish using a 2ml plastic syringe. Ethylene diamine tetraacetate (EDTA) was used as anticoagulant. (Santiago *et al.*, 2007). Blood, 2.0 ml, was decanted in heparinized bottles for determination of blood parameters. A Sysmex KX – 21 haematology analyser was used to analyse the blood samples in both whole blood mode and pre –diluted mode. The Haematological parameters to be analysed include;

Red blood cell (RBC) count

This is measured using a haemocytometer or an automated blood cell counter (Herbert *et al.*, 2010). The RBC count is an indicator of the fish's oxygen-carrying capacity.

White blood cell (WBC) count

WBC counts are critical for assessing the immune response. This can be done using the same methods as RBC counting (Wright *et al.*, 2012). An increase in WBC count may indicate infection or inflammation.

Haemoglobin (Hb) concentration

Haemoglobin levels are typically measured by a colorimetric method using spectrophotometers (Herbert *et al.*, 2010). The concentration of haemoglobin reflects the oxygen-carrying capacity of the blood.

Haematocrit (Hct) or pack cell volume (PVC)

Haematocrit levels indicate the proportion of blood volume occupied by RBCs. This can be measured using a micro-haematocrit centrifuge or automated analysers (Haro *et al.*, 2013).

RESULTS

This chapter presents the result on effect of varying dietary inclusion of aqua pro and vitamin c on some performance indices, Haematological profile, carcass composition and mineral composition of *Clarias gariepinus* fingerlings. Analysis was carried out on performance indices, Haematological parameters, carcass and mineral composition of different inclusion level of probiotics.

Growth Performance Indices and Nutrient Utilization

The data presented in Table 3 illustrates the growth performance and nutrient utilization of *Clarias gariepinus* fingerlings fed diets with varying inclusions of Aqua Pro and Vitamin C over a specific experimental period. The treatments are designated as T0 (control, 0% inclusion), T1, T2, and T3, representing increasing dietary levels of the additives.

Initial mean weight (g/kg)

The initial mean weight of fish across all treatment groups ranged from 10.09 to 10.13g/kg, showing no significant difference ($p < 0.05$) across the treatment.

Final mean weight (g/kg)

The highest final mean weight was recorded in T3 followed by T2, and T1, whereas the lowest value final mean weight was recorded in T0.

Mean weight gain

Significant difference ($p < 0.05$) existed across all treatment where the highest value recorded in T3 followed by T2 and T1. The lowest value was found in control group which differed ($p < 0.05$) with other treatments group.

Daily weight gain

The highest daily weight gain was recorded in T3 followed by T2 with no significant different ($p < 0.05$). Significant different ($p < 0.05$) existed between T1 and control (T0) group.

Specific growth rate (SGR)

The specific growth rate improved from control group T0 to T3. The highest value was recorded in T3 followed by T2, and T1. The lowest value was found in T0. The pattern shows a stepwise increase from T0, T1, T2 and T3.

Feed conversion ratio

Feed conversion ratio showed a remarkable improvement with supplementation where the highest value was recorded in control group (T0) followed by T1 and T2 whereas the lowest value was recorded in T3 with statistically different ($p < 0.05$) across all treatment groups.

Protein efficiency ratio (PER)

The protein efficiency ratio increased progressively with higher inclusion level of probiotics and vitamin C. the highest was recorded in T3, followed T2, and T1 whereas lowest protein efficiency ratio was found in control group (T0) with significant difference ($p < 0.05$) across all treatment groups.

Survival rates

The survival and mortality rates were significantly differed across all the treatment. The highest survival rates were recorded in T3, followed by T2, and T1 with significant different ($p < 0.05$). The lowest value of survival rates was reported in control group (T0). Mortality rate: The highest mortality rates were recorded in control group (T0) followed by T1, and T2 with significant different ($p < 0.05$). The lowest mortality rates were reported in T3.

Table 2: Proximate composition of formulated diets

Parameters	T ₀	T ₁	T ₂	T ₃
Crude protein	44.32±0.21	44.42±0.22	44.63±0.35	44.71±0.33
Crude lipid	6.35±0.01	6.28±0.22	6.26±0.11	6.25±0.13
Crude fiber	3.12±0.01	3.12±0.02	3.12±0.03	3.12±0.10
Ash	11.51±0.22	11.62±0.31	11.65±0.32	11.68±0.31
Moisture	6.47±0.22	6.42±0.11	6.45±0.22	6.47±0.21
Nitrogen Free Extract (NFE)	33.35±0.41	20.15±0.41	29.35±0.43	29.35±0.35

Table 3: Influenced of Aqua Pro and Vitamin C on growth performance indices and Nutrient Utilization

Parameters	T0	T1	T2	T3
IMW (g/kg)	10.10±0.58 ^a	10.13±0.09 ^a	10.09±0.13 ^a	10.12±0.10 ^a
FMW (g/kg)	551.30±1.76 ^d	623.63±5.33 ^c	698.10±2.65 ^b	701.70±2.52 ^a
MWG (g/kg)	541.20±1.18 ^d	613.50±5.24 ^c	688.01±2.52 ^b	691.58±2.42 ^a
DWI (g/kg)	6.01±0.93 ^c	6.82±0.74 ^b	7.65±0.56 ^a	7.68±0.23 ^a
SGR (%)	3.05±0.03 ^c	3.10±0.07 ^b	3.16±0.39 ^a	3.17±0.79 ^a
FCR (g/kg)	2.00±0.01 ^a	1.45±0.03 ^b	1.15±0.91 ^c	1.13±0.99 ^d
PER	112.75±1.22 ^d	127.81±1.43 ^c	143.34±1.64 ^b	144.06±1.69 ^a
ISR (n)	30.00	30.00	30.00	30.00
FFP (N)	24.00	26.00	27.00	28.00
MR (%)	20.00	14.00	10.00	6.67
SR (%)	80.00	86.00	90.00	93.33

Values within each row not sharing a common superscript letter are significantly different. Data are means ± Standard Error (SE) of triplicate tanks

Haematological Parameters

The result from table 4 showed the red blood cell (RBC) values ranged from $1.273 \pm 0.696 \times 10^6 \mu/L$ to $2.226 \pm 0.002 \times 10^6 \mu/L$. There was a significant difference ($p < 0.05$) among treatments. T1 recorded the highest RBC value, followed by T2 and T3, while T0 had the lowest value. Each treatment differed significantly from the others. Result also showed there was a significant different ($p < 0.05$) in white blood cells component where T1 recorded the highest WBC count, followed by T2 and T3 respectively with T0 recorded with lowest value. The parked cell volume result showed significant difference ($p < 0.05$) among the treatments with T1 has the highest value followed by T2. However, the lowest park cell volume was recorded in T0 followed by T3 from the table of the experiment. The result from the table showed treatments differed significantly in haemoglobin concentration with highest recorded in T3 followed by T2, T1 and lower in T1 respectively.

Water Quality Analysis

Dissolved oxygen result from table 5 showed there was no significant difference ($p < 0.05$) across all treatments. Although T0 and T2 recorded slightly mean values than T1 and T3, the differences were statistically insignificant. The result from table 5 indicates no significant difference ($p < 0.05$) among all treatments. This is an indication that all treatments maintained relatively stable and slightly acidic conditions throughout the experimental period. Ammonia concentration from the table 5 result showed no significant difference ($p < 0.05$) across the treatment with T1 recorded lowest ammonia value, while T0, T2 and T3 recorded similar values. The temperature results from table 5 entails no significant difference ($p < 0.05$) across all the treatments in the experimental units. However, alkalinity mean value showed no significant difference ($p < 0.05$) among all the treatment in the experimental unit.

Table 4: Haematological parameters of *Clarias gariepinus* fingerlings fed with diets of different levels of probiotics and Vitamin C

Blood Parameters	T ₀	T ₁	T ₂	T ₃
RBC ($10^6 \mu/L$)	1.273 ± 0.696^a	2.226 ± 0.002^d	1.898 ± 0.008^c	1.626 ± 0.107^b
WBC ($10^3 \mu/L$)	39.02 ± 0.009^a	83.01 ± 0.003^d	68.22 ± 0.063^c	57.02 ± 0.058^b
PCV (%)	33.60 ± 0.153^a	49.01 ± 0.003^d	43.01 ± 0.000^c	37.90 ± 0.058^b
HGB (g/dL)	10.13 ± 0.067^a	11.72 ± 0.003^b	13.61 ± 0.006^c	16.67 ± 0.032^d

Values within each row not sharing a common superscript letter are significantly different. Data are means ± Standard Error (SE) of triplicate tanks

Keys: RBC is red blood Cells, WBC is White Blood Cells, PCV is Parked Cell Volume and HGB is Haemoglobin concentration

Table 6: Mean Value of Physicochemical of Water during Experiment

Parameters	T ₀	T ₁	T ₂	T ₃
D.O (mg/l)	5.533 ± 0.667^a	4.867 ± 0.667^a	5.533 ± 0.667^a	4.867 ± 0.667^a
PH (mg/l)	6.567 ± 0.067^a	6.567 ± 0.120^a	6.700 ± 0.058^a	6.567 ± 0.120^a
NH ₃ (mg/l)	8.230 ± 0.590^a	7.640 ± 0.590^a	8.230 ± 0.590^a	8.230 ± 0.590^a
Temp (°C)	26.00 ± 0.577^a	25.67 ± 1.202^a	26.00 ± 0.577^a	26.33 ± 0.333^a
Alkalinity (mg/l)	23.33 ± 3.333^a	23.33 ± 3.333^a	23.33 ± 3.333^a	23.33 ± 3.333^a

Values within each row not sharing a common superscript letter are significantly different. Data are means ± Standard Error (SE) of triplicate tanks

Keys: D.O is Dissolved oxygen, NH₃ is Ammonia, and Temp is Temperature

DISCUSSION

The results of this study clearly demonstrate the beneficial effects of dietary inclusion of Aqua Pro and Vitamin C on the growth performance, nutrient utilization, and survival of *Clarias gariepinus* fingerlings. The enhanced growth performance, particularly the significant increase in final weight and

SGR in treatments T2 and T3, can be attributed to the synergistic roles of the two additives. Aqua Pro, a commercial probiotic, is known to improve the intestinal microbial balance. By populating the gut with beneficial bacteria, it can enhance digestive enzyme activity, improve feed digestibility, and increase the absorption of nutrients (Ige, 2013). This

leads to more nutrients being available for growth rather than being lost in faeces.

Concurrently, Vitamin C (ascorbic acid) is an essential micronutrient for fish, crucial for numerous metabolic functions, including collagen synthesis, which is vital for skeletal development and tissue repair (NRC, 2011). The improved length gain observed in this study directly supports this role. Vitamin C also acts as a powerful antioxidant, protecting cells from oxidative stress and enhancing the immune response, thereby allowing the fish to channel more energy into growth (Lim & Lovell, 1978). The combination of better nutrient absorption from the probiotic action and the metabolic support from Vitamin C likely created an optimal physiological condition for rapid growth. The marginal difference between T2 and T3 suggests that an optimum inclusion level was reached, beyond which additional supplementation may not yield further significant growth benefits. This finding aligns with the law of diminishing returns often observed in nutrient supplementation studies (Gatlin *et al.*, 2007).

The dramatic improvement in FCR from 2.00 in the control to as low as 1.13 in T3 is a key finding. A lower FCR indicates that fish utilized feed more efficiently to gain weight. Probiotics are well-documented for improving feed conversion by optimizing gut microflora and enhancing the digestibility of nutrients. This study aligns with Zhoe *et al.*, (2009) reported that *Bacillus* species produces extracellular enzymes such as proteases, amylases, and lipases thereby improving digestion and nutrient absorption (feed conversion efficiency) in fish. Vitamin C also contributes to better feed utilization by reducing stress and improving metabolic efficiency. Probiotics have been shown to produce exogenous enzymes that aid in the breakdown of complex nutrients, including proteins, into more absorbable forms (El-Haroun *et al.*, 2006). The combined probiotics with Vitamin C's role in maintaining the structural integrity of the gut lining for optimal absorption (Dabrowski, 2001), synergistically improves the overall efficiency of nutrient utilization. Studies have consistently shown that both probiotics and vitamin C supplementation lead to significantly better FCR compared to unsupplemented controls. The superior FCR in T2 and T3 suggests that a threshold level of supplementation is needed to achieve maximal feed efficiency.

The increasing PER values demonstrates that fish in supplemented groups could convert dietary protein into body mass more effectively. This is a direct consequence of improved digestive function and

nutrient absorption facilitated by the probiotic component of Aqua Pro. Similar enhancements in PER have been reported for *C. gariepinus* fed probiotic or vitamin C-supplemented diets (Dabrowski, 2001). The high PER values (up to 144.06) recorded in this study indicate excellent protein utilization, which has economic implications for reducing feed costs in aquaculture operations.

The marked improvement in survival rate (from 80% in control to 93.33% in T3) and the corresponding decline in mortality are noteworthy. Probiotics are known to enhance immune responses and disease resistance by promoting beneficial gut microbiota that compete with pathogens. Vitamin C is a well-documented immunostimulant that enhances leukocyte activity and antibody production, making fish more resistant to pathogens and stressors (Verlhac *et al.*, 1998). The study agrees with finding that reported the lactobacillus supplemented fish show lower mortality rates when challenged with pathogens such as *Aeromonas hydrophila* and *Vibrio* species, indicating improved disease resilience (Nayak, 2010). When combined, *Bacillus* and *Lactobacillus* offer complementary effects: The combination suppresses both Gram-negative and Gram-positive pathogens more effectively than single strains (Merrifield *et al.*, 2010).

Probiotics can also boost immunity by modulating the gut-associated lymphoid tissue (GALT) and potentially excluding pathogenic bacteria from colonizing the gut (Nayak, 2010). The combined effect would be a more robust and resilient fish population, better equipped to withstand the inherent stresses of culture conditions, such as handling and crowding, leading to higher survival rates.

Haematological indices are reliable indicators of physiological condition, nutritional adequacy, stress response, and immune competence in fish. Blood parameters respond rapidly to dietary manipulation and environmental changes; therefore, they are widely used in aquaculture nutrition trials to evaluate health status. Significant difference ($p < 0.05$) was observed across treatments for all measured Haematological parameter (RBC, WBC, PCV and HGB), indicating that dietary inclusion of Aqua Pro (probiotics) and Vitamin C exerted measurable physiological effects on the experimental fish.

Red blood cells are responsible for oxygen transportation from the gill to body tissues. Increases RBC concentration enhances aerobic metabolism and support higher growth performance. The significantly higher RBC counts are recorded in T1, followed by T2 and T3 compared to control T0 which was the lowest

value. This is an indication of erythropoiesis in fish supplement diets. Normal RBC range for African catfish has been reported approximately $1.0 - 3.0 \times 10^6 \mu/L$ (Svobodove *et al.*, 1992, indicating that all treatments remained within physiological limits. However, the control group T0 recorded values closer to the lower threshold, which may indicate relatively reduced oxygen carrying capacity.

White blood cells play a major role in non-specific immune defence, including phagocytosis and antibody production. White blood cell counts increased significantly $39.02 \times 10^3 \mu/L$ (T0) to $83.01 \times 10^3 \mu/L$ (T1). It was observed the T1, T2 and T3 are significantly higher WBC compared to control (T0) WBC. This marked elevation suggests immune stimulation due to dietary supplementation. Normal WBC range in catfish varies widely but typically falls within $20-150 \times 10^3 \mu/L$ depending on physiological state (Hrubec *et al.*, 2000). All treatments fall within this acceptable range, indicating immune enhancement rather than pathological stress.

Packed cell volume represents the proportion of blood volume occupied by erythrocytes. It is a critical indicator of anaemia or improved oxygen transport capacity. Normal PCV range for *Clarias gariepinus* is approximately 20–50% (Svobodova *et al.*, 1991). Thus, all treatments fall within acceptable physiological limits. The significantly higher PCV in supplemented groups indicates: Improved red cell mass, enhanced erythropoiesis, better oxygen transport efficiency, and **improved** nutritional status. Low PCV values are typically associated with: nutritional deficiencies, chronic stress and poor water quality. Given that water quality parameters remained stable during the experiment, the improvement in PCV can be confidently attributed to dietary supplementation rather than environmental effects. Similar improvements in PCV have been reported in probiotic-fed catfish (Adewolu *et al.*, 2008).

Haemoglobin concentration directly determines oxygen-carrying capacity of blood. A progressive increase was observed: Normal haemoglobin values in African catfish range from 8–17 g/dL (Hrubec *et al.*, 2000). All treatments were within normal limits, but T3 approached the upper physiological boundary. The progressive increase suggests a dose-dependent enhancement effect of dietary supplementation. Probiotic Significance: Higher haemoglobin enhances oxygen delivery to tissues, supports higher metabolic rate, improves feed conversion efficiency, enhances growth performance and improves stress resistance. Vitamin C plays a direct role in haem synthesis and

iron metabolism. Its antioxidant properties also protect haemoglobin molecules from oxidative degradation. The highest HGB observed in T3 suggests that higher inclusion levels may enhance haem synthesis more strongly than moderate levels, although RBC and PCV peaked at T1. This indicates a possible variation in erythrocyte size or haemoglobin concentration per cell. Therefore, any improved growth performance observed in this study is physiologically supported by the enhanced blood profile.

The dissolved oxygen values recorded in this study (4.867–5.533 mg/L) fall within the acceptable range for the culture of *Clarias gariepinus*. According to Boyd (2020), warm-water fish species perform optimally at dissolved oxygen levels above 5 mg/L, although *C. gariepinus* is highly tolerant of lower concentrations due to its accessory air-breathing organ (Viveen *et al.*, 1985). The absence of significant differences among treatments indicates that varying dietary inclusion of Aqua Pro and Vitamin C did not influence oxygen dynamics in the culture system. Similar DO ranges (4.5–6.0 mg/L) were reported in feeding trials of *C. gariepinus* by Akinwale and Faturoti (2007). Adequate dissolved oxygen likely supported normal metabolic activity, feed utilization, and Haematological stability observed during the study.

The pH values (6.567–6.700) recorded during the experiment was within the recommended range (6.5–8.5) for tropical aquaculture species (Boyd, 2020). Viveen *et al.* (1985) reported that *C. gariepinus* tolerates slightly acidic to neutral water conditions without adverse physiological effects. Stable pH across treatments suggests adequate buffering capacity of the water and minimal metabolic accumulation of acidic wastes. Since extreme pH fluctuations can impair gill function and oxygen transport, the stable pH observed in this study likely contributed to the overall good performance of the fish.

The ammonia values recorded (7.640–8.230 mg/L) appear relatively high compared to recommended safe limits for unionized ammonia in aquaculture systems (<0.05 mg/L) (Boyd, 2020). However, if the values represent total ammonia nitrogen (TAN), the toxic fraction may have been reduced by the slightly acidic pH and moderate temperature recorded in this study. African catfish are known to possess higher tolerance to ammonia compared to many cultured species (Viveen *et al.*, 1985). Nevertheless, prolonged exposure to elevated ammonia can cause stress, reduced growth, and gill damage. The absence of

significant differences among treatments suggests that dietary inclusion of Aqua Pro and Vitamin C did not significantly influence nitrogenous waste accumulation in the culture water. Similar moderate ammonia levels have been reported in intensive catfish production systems (Adewolu *et al.*, 2008).

The temperature range (25.67–26.33°C) recorded during the experiment falls within the optimal range (25–30°C) for the growth and survival of *C. gariepinus* (Boyd, 2020). Temperature directly influences metabolic rate, enzyme activity, immune response, and nutrient utilization. Stable temperature throughout the experimental period suggests that environmental conditions were suitable and did not confound dietary treatment effects. Similar temperature ranges have been reported in catfish feeding experiments conducted in tropical regions (Akinwale and Faturoti, 2007).

The alkalinity value (23.33 mg/L) recorded in this study is at the lower acceptable threshold for aquaculture systems (20–200 mg/L) (Boyd, 2020). Alkalinity plays an important role in buffering water against sudden pH changes. The uniform alkalinity across treatments explains the minimal variation observed in pH values. Low alkalinity may limit primary productivity in pond system.

CONCLUSION

The findings demonstrate that dietary inclusion of Aqua Pro probiotics and Vitamin C at 2-3g/kg each is highly beneficial for optimizing growth, nutrient utilization, Haematological profile, and survival of *Clarias gariepinus* fingerlings. The combination of these additives offers a sustainable, cost-effective strategy for improving catfish aquaculture productivity while reducing reliance on antibiotics and enhancing fish health.

Based on the findings of this study, the following recommendations are made: Dietary inclusion of Aqua Pro and Vitamin C at 2g/kg each (T2) is recommended for optimal growth performance, nutrient utilization, and cost-effectiveness in *Clarias gariepinus* fingerling production. The supplementation strategy should be adopted to reduce mortality rates during the critical fingerling stage, thereby improving farm profitability and sustainability. Commercial fish feed producers should consider incorporating Aqua Pro probiotics and Vitamin C at 2g/kg each into standard catfish feed formulations to enhance product value and farmer satisfaction. Quality control measures should ensure the stability and viability of probiotics during feed processing and storage. Further studies should

investigate the long-term effects of Aqua Pro and Vitamin C supplementation on reproductive performance, broodstock health, and offspring quality. Research should explore potential synergistic effects with other feed additives, prebiotics, or immunostimulants to further optimize catfish nutrition.

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