

## ENHANCING GROWTH PERFORMANCE, ANTIOXIDANT DEFENSE, AND IMMUNE RESPONSE IN STRIPED CATFISH (*Pangasius hypophthalmus*) THROUGH DIETARY SUPPLEMENTATION WITH *Aloe vera*: A SUSTAINABLE AQUACULTURE APPROACH

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### ABSTRACT

A 60-day feeding trial was conducted to investigate the effects of dietary *Aloe vera* leaf powder supplementation on growth performance, hematological parameters, antioxidant status, and immune responses in *Pangasius hypophthalmus*. A total of 300 juvenile striped catfish with an initial weight of  $14.2 \pm 0.13$  g was randomly allocated to five dietary treatments containing 0% (control), 0.5%, 1%, 2%, and 4% *Aloe vera* leaf powder, with three replicates per group. Results reveal significant dose-dependent enhancements in growth parameters, with the 2% *Aloe vera* group exhibiting the highest final weight  $72.21 \pm 1.54$  g, weight gain  $371.1 \pm 4.5\%$ , and specific growth rate ( $3.21 \pm 0.03\%/day$ ), alongside the lowest feed conversion ratio ( $1.54 \pm 0.01$ ) ( $p < 0.05$ ). Hematological analysis revealed enhanced red blood cell count ( $2.32 \pm 0.06 \times 10^6/\mu L$ ), hemoglobin levels ( $9.0 \pm 0.3$  g/dL), and white blood cell count ( $66.5 \pm 2.1 \times 10^3/\mu L$ ) in the 2% group. Antioxidant enzyme activities (Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx)) increased by 52.1%, 36.2%, and 27.8%, respectively, while malondialdehyde levels decreased by 31.8% related to the (control;  $p < 0.05$ ). Immune parameters were also significantly elevated, including lysozyme activity ( $14.3 \pm 0.5$  U/mL) and phagocytic index ( $33.87 \pm 1.3\%$ ). These findings indicate that 2% *Aloe vera* supplementation optimally enhances growth, physiological resilience, and disease resistance in *P. hypophthalmus*, offering a sustainable alternative to synthetic additives in aquaculture.

**KEYWORDS:** *Pangasius hypophthalmus*, *Aloe vera*, growth performance, antioxidant activity, immune response

### 1. INTRODUCTION

Fish Aquaculture has become essential globally for meeting the world's food requirements because it now provides fish to more than half of the people worldwide (Ahmadu *et al.*, 2024; Ceccotti *et al.*, 2019; Fred-). The global protein requirement is fully provided by this fast-growing industry (fish aquaculture), and this industry is now also creating jobs for workers, particularly in developing countries where many family-run businesses and small fish farms are present (Hassan *et al.*, 2025a; Ragasa *et al.*, 2022; Verdegem *et al.*, 2023;). This developing industry has also introduced serious problems such as environmental damage, dependence on artificial growth enhancers, and more frequent disease outbreaks (Abdelkarim *et al.*, 2025; Fachri *et al.*, 2024; Hassan *et al.*, 2025b).

Furthermore, these emerging problems have urged scientists to discover ecological solutions that improve yields without harming the environment or fish health (Owais *et al.*, 2025; Singh *et al.*, 2024). One species at the forefront is *Pangasius hypophthalmus*, which has become a major global commodity -

Vietnam's annual exports exceed 1.4 million tons of this freshwater fish (Lingam *et al.*, 2025). Farmers favor this species because it grows remarkably fast (achieving 1-1.5 kg in half a year), makes efficient use of feed, and adapts well to different water environments (Mechaly *et al.*, 2025; Tynchenko *et al.*, 2024). Yet crowded farming conditions have increased its susceptibility to dangerous bacteria (including *Edwardsiella ictaluri* and *Aeromonas hydrophila*) and environmental pressures that stunt growth and increase mortality (Das *et al.*, 2024; Haenen *et al.*, 2023). The conventional approach of using antibiotics to control diseases has grown ineffective and controversial, as drug-resistant bacteria emerge and governments tighten regulations. This situation has forced the industry to seek safer, more sustainable health management solutions (Muteeb *et al.*, 2023).

Plant-based feed supplements are gaining attention as effective alternatives, providing multiple health benefits without the negative effects of artificial additives (Biswas *et al.*, 2024; Kirubakaran *et al.*, 2025; Yousefi *et al.*, 2025). The natural compounds that are well-designed plant-based feed additives work in numerous ways that can enhance growth, boost fish farm

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by up to 12-18% while reducing the death rates by up to 20-30%, increase immunity, and fight cellular damage (Direito *et al.*, 2024; Fan *et al.*, 2022; Sumana *et al.*, 2025).

Aloe vera's active compounds in the fish farming industry reveal varying benefits depending on dosage levels, such as 1% Aloe vera in Nile tilapia produces important advancement in such it increases 28% stronger immunity and 19% faster growth performance than untreated fish (Abiri *et al.*, 2021; Abdel-Latif *et al.*, 2024; Habib *et al.*, 2025; Tok *et al.*, 2025). In the rainbow trout 2% Aloe vera in their diet produces central advancement, such as it protects the fish from bacterial contaminations and shows less signs of cellular stress (Cordiano *et al.*, 2025; Darzi *et al.*, 2021; Dang *et al.*, 2024). Aloe vera sugar-based compounds work through numerous balancing mechanisms that activate immune sensors in fish digestive tissue and then start natural defense systems (Al-Kattan and Al-Bassam, 2024; Kumaresan *et al.*, 2024; Krupa *et al.*, 2025). It also helps to maintain the fish gut microbiota, that is leading to improved digestion mechanisms and intestinal structure (Gupta *et al.*, 2021; Hafeez *et al.*, 2024; Shen *et al.*, 2024). However, different types of fish respond differently to ideal dosage levels varying between 0.5-3% Aloe vera depending upon various factors such as water quality parameters, fish size, and fish health (Raza *et al.*, 2024). Moreover, it shows potential in various fish species, but its effects on *P. hypophthalmus* remain poorly understood (Thema *et al.*, 2024; Bagheri *et al.*, 2024). Therefore, the research is required to understand how Aloe vera works under various levels and how it works in high-density culture and fluctuating water parameters. The current study was designed to understand these gaps by thoroughly testing different Aloe vera levels in *P. hypophthalmus* feed.

## 2. MATERIALS AND METHODS

### Experimental Fish Rearing and Environmental Conditions:

The present was conducted at the Saline Water Aquaculture Research Center (SWARC) Muzaffargarh, Punjab, Pakistan. 300 catfish fingerlings with an average weight were purchased from the Tawakal Fish Hatchery, Muzaffargarh. After the arrival of the experimental fish to the site, the fish were acclimatized up to two weeks by maintaining the fish in cemented tanks with having water capacity of 1,500 1,500-liter. Before shifting the fish, the tanks were treated with chlorine to remove any pathogens and the tanks were equipped with an aeration system. During the acclimatization period 30% Crude protein diet was given to the

fish at 3% of their total body weight (Al Sulivany &, Hoseini 2024).

After the acclimatization period, the experimental fish were evenly distributed in each fifteen indoor tanks with having capacity water (250-liter, 20 fish per treatment group, each having three replicate groups. All the treatment tanks were equipped with the proper water circulation systems having inlet and outlet pipes to maintain water quality conditions by continuously removing waste and ensuring optimal water quality for fish health. Furthermore, excellent oxygen levels were maintained through air stones connected to the pumping systems, and a fine filter was used at the drainage opening to keep the fish safely inside the tanks. Breathable fabric covers were placed over tank openings to reduce dust and other airborne contaminants. Before the start of the experiment, fish were checked and selected the active fishes for the experiment following the established selection standards (Langi *et al.*, 2024). Whole the period of study, water quality parameters were maintained in optimum conditions for *P. hypophthalmus*. The water quality parameters were maintained, such as temperature 27-29°C, Dissolved oxygen level 7.5-7.8 mg/L and pH stayed within 7.2-7.6.

### Diet Formation and Experimental Arrangement:

Experimental diets of different levels of Aloe vera were prepared to assess its impact on *P. hypophthalmus*. For making uniform feed formulations, all the dry components were measured accurately and blended by using mechanical mixing equipment. Five different feed formulations were formed by adding increasing amounts of Aloe vera powder viz; 0%, 0.5%, 1%, 2%, and 4%.

warm purified water with an average temperature 30-35°C were mixed with the powders to form a uniform dough, which was then shaped into 2mm pellets using specialized equipment. After that, these pellets at room temperature (28-30°C) were dried naturally for about two days to attain appropriate consistency, then stored in wrapped plastic bags under refrigeration to preserve freshness and nutritional value until feeding time (Ghiasi *et al.*, 2025). Experimental fish were given fish twice per day (8 AM and 4 PM) during the 60-day study trial at a 3% of their total weight. All fish groups were maintained under identical conditions during the experiment. Leftover food was removed half an hour after each feeding, and tanks were cleaned frequently to preserve water cleanliness.

Table 1 details the feed ingredients and nutritional content of each diet formulation.

**Table 1:** Diets Composition with graded levels of *Aloe vera* powder.

| Ingredients     | (T1) 0% | (T2) 0.50% | (T3) 1% | (T4) 2% | (T5) 4% |
|-----------------|---------|------------|---------|---------|---------|
| Fish meal       | 190     | 190        | 190     | 190     | 190     |
| Soybean meal    | 80      | 80         | 80      | 80      | 80      |
| Cottonseed cake | 110     | 110        | 110     | 110     | 110     |
| Wheat bran      | 310     | 307        | 304     | 298     | 286     |
| Wheat pollard   | 130     | 130        | 130     | 130     | 130     |
| Maize germ      | 100     | 100        | 100     | 100     | 100     |
| Soybean oil     | 18      | 18         | 18      | 18      | 18      |

|                              |    |    |    |    |    |
|------------------------------|----|----|----|----|----|
| Vitamin-mineral premix*      | 12 | 12 | 12 | 12 | 12 |
| <i>Aloe vera</i> leaf powder | 0  | 5  | 10 | 20 | 40 |

**Note:** \*The vitamin-mineral premix provided per kg of feed: Vitamin A – 7200 IU, Vitamin D<sub>3</sub> – 1600 IU, Vitamin E – 200 IU, Vitamin C – 90 mg, Vitamin B<sub>1</sub> – 1.5 mg, Vitamin B<sub>2</sub> – 10 mg, Vitamin B<sub>6</sub> – 8 mg, Vitamin B<sub>12</sub> – 0.02 mg, Niacin – 55 mg, Pantothenic acid – 40 mg, Folic acid – 0.08 mg, Biotin – 1.8 mg, Copper – 6 mg, Iron – 110 mg, Zinc – 30 mg, Manganese – 18 mg, Iodine – 0.6 mg, Selenium – 0.2 mg, and Cobalt – 0.12 mg.

#### Ethical Approval and Consent:

All experimental protocols involving live fish were conducted in strict accordance with the ethical guidelines for the care and use of laboratory animals. The study procedures were reviewed and approved by the Animal Ethics Committee of the Institutional Research Committee, Department of Fisheries, Government of Punjab, Pakistan (Approval Code: APF-188; 2023).

#### Growth Performance Calculation:

At the end of the 60-day feeding trial, all *P. hypophthalmus* fingerlings were subjected to a 24-hour fasting period to ensure complete evacuation of the digestive tract. Each fish was weighed using a digital balance with 0.01 g precision to record final body weights.

Growth performance and feed utilization efficiency were determined using the following standard formulas (Abdulrahman and Al Sulivany, 2025).

$$\text{Weight Gain (\%)} = ((\text{Final Weight} - \text{Initial Weight}) / \text{Initial Weight}) \times 100$$

$$\text{FCR} = \text{Feed intake (g)} / \text{WG (g)}.$$

$$\text{SGR (\%)} = [(\ln \text{FW}) - \ln \text{IW}) / t] \times 100.$$

$$\text{Survival Rate (\%)} = (\text{Fish Final Number} / \text{Fish Initial Number}) \times 100.$$

#### Hematological and Biochemical Assessments:

For blood analysis, researchers collected five fish randomly from each test group. To reduce stress during the procedure, fish were sedated using a 0.1 g/L MS-222 solution (tricaine methanesulfonate) following established protocols (Reverter *et al.*, 2021). Blood was carefully drawn from the tail vein using sterile 3 mL syringes with thin needles (23-gauge). Each sample was immediately separated - some placed in EDTA tubes to stop clotting, others left to naturally coagulate for serum collection. The plain tubes sat at room temperature until the blood clotted, then were spun in a centrifuge (3500 rpm for 10 minutes) to separate the clear serum, which was frozen at -20°C for later testing (García-Márquez *et al.*, 2023).

Haematological parameters viz; Red blood cells, white blood cell counts, were measured by specialized counting chambers after sample preparation with a staining solution, and investigated under high-powered microscopes. Hemoglobin examination involved chemical conversion followed by precise optical measurements at specific wavelengths while for PCV small blood-filled tubes were rotated speedily to distinct cells from plasma, with results calculated as the percentage of stable cells relative to total blood volume (Ahmed *et al.*, 2023; Ahmed *et al.*, 2024).

The study analyzed two key blood components: glucose and total protein levels. Glucose measurements used an enzymatic colorimetric method (GOD-POD), while proteins were assessed through copper-based color change reactions (Biuret method). Both tests employed standardized commercial kits (Randox Laboratories, UK) according to established protocols, and all the tests were repeated three times to attain accurate results (Adewale *et al.*, 2024; Banaee *et al.*, 2024).

#### Innate Immune and Assays Antioxidant:

The fish were anaesthetised and blood samples were collected to determine the antioxidant enzymes and innate immune parameters.

#### Antioxidant Enzyme Activities:

The SOD (activity of superoxide dismutase) was measured following the protocol drawn by Marklund and Marklund (1974), with minor modifications adapted for aquatic species by Taysi *et al.* (2024). Absorbance was verified at 560 nm. Catalase (CAT) activity was measured by the modified method of Aebi (1984). The decline in absorbance was used to quantify enzyme activity (Li *et al.*, 2025). Glutathione peroxidase (GPx) activity was measured following procedures validated for fish tissues (Verdi *et al.*, 2024). The thiobarbituric acid reactive substances (TBARS) assay was used to measure Malondialdehyde (MDA) levels. Serum was reacted with the trichloroacetic acid and thiobarbituric acid in a boiling water bath for 15 minutes then, after cooling, absorbance was measured at 532 nm MDA concentrations were expressed as nmol/mg protein (Moussa *et al.*, 2022). The total protein concentration in each supernatant was estimated using the Bradford assay (Kielkopf *et al.*, 2020), which utilizes Coomassie Brilliant Blue G-250 dye and provides a rapid estimation of protein content. This was used to normalize enzymatic activities.

#### Innate Immune Parameters:

Serum lysozyme activity was measured by the bacterial breakdown test. The measured amount of *Micrococcus* bacteria (0.2 mg/mL in buffer) was mixed with the small serum samples (25 µL), and the solution's cleared at 450 nm for five minutes. Based on the rate of clearing activity, levels were calculated in standardized units (Hukić *et al.*, 2018). By using a specialized density gradient solution, Immune cells were separated and mixed with stained, inactive yeast cells at a precise ratio (1 immune cell:10 yeast cells). After incubating at 28°C, the samples were prepared on slides and examined under a microscope to calculate immune activity by determining what percentage of cells absorbed yeast particles (Lulijwa *et al.*, 2019).

#### Statistical Analysis:

ANOVA (One-way analysis of variance) was performed by using SPSS software (version 26.0) to identify significant differences among treatments, followed by Tukey's Honestly Significant Difference post hoc test and data were also tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively.

### 3. RESULTS

The present 60-day feeding trial was conducted to check the effects of dietary *Aloe vera* supplementation on growth performance, hematological and biochemical parameters, as well as antioxidant and immune responses in *Pangasius hypophthalmus*. Statistical significance ( $p < 0.05$ ) was observed for most parameters, indicating a clear dose-dependent influence of *Aloe vera* inclusion.

Fish fed diets containing *Aloe vera* exhibited marked improvements in growth metrics compared to the control group (0%). The FW was highest in the 2% *Aloe vera* group ( $72.21 \pm$

$1.54$  g;  $p = 0.021$ ), representing a 23.8% increase over the control ( $58.30 \pm 1.40$  g). Intermediate FW were recorded for the 1% ( $67.52 \pm 1.68$  g) and 4% ( $67.89 \pm 1.47$  g) groups, while the 0.5% group ( $62.11 \pm 1.41$  g) showed a more modest improvement. The WG (%) mirrored this trend, with the 2% group achieving the highest gain ( $371.1 \pm 4.5\%$ ;  $p = 0.016$ ), significantly surpassing the control ( $288.5 \pm 4.1\%$ ). The SGR was maximized at 2% supplementation ( $3.21 \pm 0.03\%/day$ ;  $p = 0.014$ ), while the FCR was most efficient in the same group ( $1.54 \pm 0.01$ ;  $p = 0.008$ ), reflecting superior nutrient utilization. Survival rates ranged from 90.2% (control) to 94.0% (2% group), but differences were not statistically significant ( $p = 0.146$ ) (Table 2).

**Table 2:** Growth parameters of *P. hypophthalmus* following dietary administration of graded *Aloe vera* concentrations over 60 days.

| Parameters         | (T1) 0%            | (T2) 0.50%            | (T3) 1%            | (T4) 2%            | (T5) 4%            | p- value |
|--------------------|--------------------|-----------------------|--------------------|--------------------|--------------------|----------|
| Initial weight (g) | $14.20 \pm 0.13$   | $14.17 \pm 0.18$      | $14.13 \pm 0.21$   | $14.15 \pm 0.20$   | $14.18 \pm 0.22$   | 0.023    |
| Final weight (g)   | $58.30 \pm 1.40^a$ | $62.11 \pm 1.41^{ab}$ | $67.52 \pm 1.68^b$ | $72.21 \pm 1.54^b$ | $67.89 \pm 1.47^b$ | 0.021    |
| Weight gain (%)    | $288.5 \pm 4.1^a$  | $314.5 \pm 5.2^{ab}$  | $351.1 \pm 5.9^b$  | $371.1 \pm 4.5^b$  | $353.0 \pm 6.1^b$  | 0.016    |
| SGR (%/day)        | $2.81 \pm 0.04^a$  | $2.94 \pm 0.01^{ab}$  | $3.11 \pm 0.03^b$  | $3.21 \pm 0.03^b$  | $3.11 \pm 0.04^b$  | 0.014    |
| FCR                | $1.83 \pm 0.04^a$  | $1.75 \pm 0.03^{ab}$  | $1.58 \pm 0.04^b$  | $1.54 \pm 0.01^b$  | $1.61 \pm 0.03^b$  | 0.008    |
| Survival rate (%)  | $90.2 \pm 2.1$     | $91.5 \pm 1.4$        | $93.5 \pm 1.3$     | $94.0 \pm 1.3$     | $93.5 \pm 1.2$     | 0.146    |

Note: Values are presented as mean  $\pm$  standard error. common superscript letters (a, b, c, d) are significantly different ( $p \leq 0.05$ ) according to Tukey's HSD test. Means within the same row that do not share the same superscript letter.

*Aloe vera* supplementation significantly improved hematological parameters in *P. hypophthalmus*. The 2% group (T4) exhibited the highest RBC count ( $2.32 \pm 0.06 \times 10^6/\mu\text{L}$ ), hemoglobin ( $9.0 \pm 0.3$  g/dL), WBC count ( $66.5 \pm 2.1 \times 10^3/\mu\text{L}$ ), PCV ( $30.2 \pm 1.2\%$ ), and total protein level ( $4.4 \pm 0.2$  g/dL), all

significantly higher ( $P < 0.05$ ) than the control group (T1). In contrast, the highest serum glucose was observed in the control group (T1:  $71.5 \pm 3.1$  mg/dL), while the lowest values were recorded in the 1% and 4% groups (T3, T5:  $\sim 64.1$ – $64.5$  mg/dL), though this difference was not statistically significant ( $p > 0.05$ ).

**Table 3:** Hematological and biochemical parameters of *P. hypophthalmus* following dietary administration of graded *aloe vera* concentrations over 60 days.

| Parameters                        | (T1) 0%           | (T2) 0.50%           | (T3) 1%           | (T4) 2%           | (T5) 4%           | p-value |
|-----------------------------------|-------------------|----------------------|-------------------|-------------------|-------------------|---------|
| RBC ( $\times 10^6/\mu\text{L}$ ) | $1.83 \pm 0.05^a$ | $2.01 \pm 0.04^{ab}$ | $2.24 \pm 0.07^b$ | $2.32 \pm 0.06^b$ | $2.27 \pm 0.05^b$ | 0.012   |
| Hemoglobin (g/dL)                 | $7.2 \pm 0.2^a$   | $7.9 \pm 0.3^{ab}$   | $8.5 \pm 0.3^b$   | $9.0 \pm 0.3^b$   | $8.7 \pm 0.2^b$   | 0.015   |
| WBC ( $\times 10^3/\mu\text{L}$ ) | $55.4 \pm 2.2^a$  | $58.8 \pm 2.2^{ab}$  | $64.1 \pm 2.3^b$  | $66.5 \pm 2.1^b$  | $66.1 \pm 2.3^b$  | 0.019   |
| PCV (%)                           | $23.7 \pm 1.1^a$  | $25.5 \pm 1.2^{ab}$  | $28.3 \pm 1.1^b$  | $30.2 \pm 1.2^b$  | $29.3 \pm 1.2^b$  | 0.017   |
| Glucose (mg/dL)                   | $71.5 \pm 3.1^a$  | $67.2 \pm 2.3^b$     | $64.1 \pm 2.2^c$  | $64.7 \pm 2.3^c$  | $64.5 \pm 2.1^c$  | 0.089   |
| Total Protein (g/dL)              | $3.4 \pm 0.1^a$   | $3.7 \pm 0.1^{ab}$   | $4.2 \pm 0.1^b$   | $4.4 \pm 0.2^b$   | $4.3 \pm 0.1^b$   | 0.013   |

Note: Values are presented as mean  $\pm$  standard error. common superscript letters (a, b, c, d) are significantly different ( $p \leq 0.05$ ) according to Tukey's HSD test. Means within the same row that do not share the same superscript letter.

Antioxidant enzyme activities were significantly upregulated with *Aloe vera* inclusion. The activity of SOD was

highest in the 2% group ( $10.8 \pm 0.5$  U/mg), when compared with the control ( $7.1 \pm 0.4$  U/mg). Similarly, CAT and GPx activities

increased at 2% supplementation ( $6.4 \pm 0.3$  U/mg and  $6.9 \pm 0.3$  U/mg, respectively;  $p < 0.05$  for both). The levels of MDA were decreased in the 2% group ( $1.5 \pm 0.2$  nmol/mg), as compared to the control ( $2.2 \pm 0.1$  nmol/mg). Lysozyme activity peaked in the

2% group ( $14.3 \pm 0.5$  U/mL;  $p = 0.009$ ), when compared with the control ( $10.3 \pm 0.5$  U/mL). Phagocytic activity was highest in the 4% group ( $34.0 \pm 1.4\%$ ;  $p = 0.014$ ), though the 2% group ( $33.87 \pm 1.3\%$ ) showed comparable efficacy (Table 4).

**Table 4:** Antioxidant and innate immune responses of *P. hypophthalmus* following dietary administration of graded aloe vera concentrations over 60 days.

| Parameters               | (T1) 0%          | (T2) 0.50%          | (T3) 1%          | (T4) 2%           | (T5) 4%          | p-value |
|--------------------------|------------------|---------------------|------------------|-------------------|------------------|---------|
| SOD (U/mg)               | $7.1 \pm 0.4^a$  | $8.1 \pm 0.5^{ab}$  | $9.6 \pm 0.6^b$  | $10.8 \pm 0.5^b$  | $10.7 \pm 0.5^b$ | 0.006   |
| CAT (U/mg)               | $4.7 \pm 0.2^a$  | $5.3 \pm 0.1^{ab}$  | $6.2 \pm 0.2^b$  | $6.4 \pm 0.3^b$   | $6.2 \pm 0.4^b$  | 0.017   |
| GPx (U/mg)               | $5.4 \pm 0.4^a$  | $5.7 \pm 0.4^{ab}$  | $6.7 \pm 0.5^b$  | $6.9 \pm 0.3^b$   | $6.7 \pm 0.4^b$  | 0.019   |
| MDA (nmol/mg)            | $2.2 \pm 0.1^a$  | $1.8 \pm 0.2^{ab}$  | $1.6 \pm 0.2^b$  | $1.5 \pm 0.2^b$   | $1.6 \pm 0.2^b$  | 0.020   |
| Lysozyme Activity (U/mL) | $10.3 \pm 0.5^a$ | $11.7 \pm 0.6^{ab}$ | $13.4 \pm 0.7^b$ | $14.3 \pm 0.5^b$  | $13.7 \pm 0.6^b$ | 0.009   |
| Phagocytic Activity (%)  | $25.4 \pm 1.3^a$ | $29.2 \pm 1.5^{ab}$ | $32.5 \pm 1.6^b$ | $33.87 \pm 1.3^b$ | $34.0 \pm 1.4^b$ | 0.014   |

Note: Values are presented as mean  $\pm$  standard error. common superscript letters (a, b, c, d) are significantly different ( $p \leq 0.05$ ) according to Tukey's HSD test. Means within the same row that do not share the same superscript letter.

#### 4. DISCUSSION

The 2% Aloe vera feed produced the best results, showing superior weight gain, faster growth rates, and more efficient feed conversion compared to other tested formulas. These findings indicate that this specific dosage most effectively helps fish utilize nutrients for growth. These results match findings in other fish species - Nile tilapia and rainbow trout showed better growth and feed efficiency when given 1-2% Aloe vera (Albassam, 2022; Li *et al.*, 2023; Purbomartono *et al.*, 2024). Cherukumudi *et al.* (2024) also describe that Aloe vera enhanced digestive enzyme activity, gut structure and gut microbiota for effective fish growth performance.

The current study indicates notable enhancements in white blood cells, red blood cells, and packed cell volume at 2% fish receiving Aloe vera. These changes show the safe oxygen transport and strong immune defence. Kalaiselvan *et al.* (2024) and Yousaf *et al.* (2025) also studied the active compounds in Aloe vera, such as anthraquinones, polysaccharides, which also caused to boost in blood cell formation. Radwan *et al.* (2024) also studied the effect of Aloe vera in tilapia and African catfish and found increased white blood cells, which indicates stronger immune defences. The present study indicates that all the groups receiving Aloe vera showed increased blood protein levels, especially at the 2% dose level. Similarly, Batoo *et al.* (2024) also conducted a study on the fish receiving Aloe vera and found better nutrient absorption, increased blood protein levels, and lower stress levels. The present study also describes the decreased blood sugar with the different levels of Aloe vera. Eldamrawy *et al.* (2024) also found the same result with the Aloe vera inclusion in fish diet and found blood sugar-lowering properties that enhance insulin function or help regulate stress reactions.

In this study Aloe vera presented the increased activity of SOD, CAT, GPx (antioxidant enzymes) with decreased MDA

levels that indicates the reduced oxidative damage and it clearly shown the protective function of Aloe vera Hu *et al.* (2025) and Ghosh *et al.* (2025) also describe that Aloe vera components such as anthraquinone and flavonoid boost up the antioxidant enzymes activity (fish's natural defense systems). The study found higher lysozyme levels and improved pathogen-clearing ability in treated fish that strengthened immune function. These outcomes confirm previous research that with the Aloe vera's active compounds presented the improvement making of immune enzymes and help identify harmful microbes (Ahmad, 2024; Acar *et al.*, 2025). Furthermore, at 4% Aloe vera indicate the strongest immune activation, antioxidant improvements, which is also noted by Gruber *et al.* (2025). The study revealed that Aloe vera help to improve immunity, growth and survival rates.

#### CONCLUSION

This research confirms Aloe vera's effectiveness as a natural feed additive for striped catfish farming, supporting more sustainable aquaculture methods. Using such plant-based supplements can decrease dependence on antibiotics and artificial growth enhancers while improving both yields and food safety. Future studies should examine extended use impacts, effects on gut bacteria, and performance in real farm settings to encourage broader use of Aloe vera in fish diets.

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