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PROBIOTIC-BASED BIOTECHNOLOGICAL INTERVENTION FOR ENHANCED GROWTH AND HEALTH IN LOCALLY CULTURED TILAPIA

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ABSTRACT

This study investigates the efficacy of a multi-strain probiotic formulation on growth performance, health status, and water quality in intensive tilapia (Oreochromis niloticus) culture. A 60-day feeding trial was conducted with four treatments: control (no probiotics), and three probiotic supplementation levels (0.5%, 1.0%, and 1.5% of feed weight). Results demonstrated significant improvement ($p < 0.05$) in weight gain (28.7% higher), specific growth rate (1.85% day $^{-1}$), and feed conversion ratio (1.23) in the 1.0% probiotic group compared to control. Immunological parameters including lysozyme activity and respiratory burst activity showed marked enhancement in probiotic-treated groups. Water quality analysis revealed reduced ammonia and nitrite levels in probiotic-treated tanks. Histological examination showed improved intestinal villi structure in treated fish. These findings suggest that probiotic supplementation at 1.0% of feed weight represents an effective biotechnological intervention for sustainable tilapia aquaculture.

KEY WORDS

efficacy of a multi-strain, probiotic formulation on growth performance.

1.INTRODUCTION

Global aquaculture faces the critical challenge of meeting rising food demand while adhering to principles of environmental and biological sustainability. Among cultivated species, tilapia (*Oreochromis spp.*) stands as one of the most significant, with global production exceeding 6 million tons annually, providing an affordable and nutritious protein source worldwide [FAO (2014)]. In India, and particularly in the state of Andhra Pradesh, aquaculture is a vital economic sector. The availability of diverse water resources, from the freshwater ponds of inland districts like Chittoor to the extensive brackishwater systems along the coast, has positioned the region as an aquaculture hub. Within these systems, the hardy and fast-growing tilapia has become a species of increasing importance for local farmers due to its resilience and marketability.

However, the intensification of tilapia culture brings forth significant challenges, including increased disease incidence, pressure from antibiotic overuse leading to antimicrobial resistance, and the environmental degradation of water bodies from accumulated waste products. These issues threaten the long-term viability and sustainability of local aquaculture operations, directly impacting the economic well-being of farmers in regions like Andhra Pradesh.

Motivated by the need for sustainable solutions, this study investigates the application of probiotic biotechnology as a viable intervention. Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, have emerged as a promising alternative to antibiotics and chemicals in aquaculture [Merrifield and Ring (2014)]. Their potential to enhance growth performance, improve feed utilization, stimulate immune responses, and concurrently improve water quality aligns perfectly with the needs of modern, sustainable aquaculture practices.

While the benefits of probiotics are recognized globally, their efficacy is highly dependent on the specific environmental conditions, the native microbial communities, and the host species. Therefore, a one-size-fits-all approach is ineffective. There is a pressing need to develop and validate optimized probiotic formulations tailored to local species and regional conditions.

This study is designed to address this gap by developing and evaluating a multi-strain probiotic formulation specifically for the context of locally cultured tilapia. We aim to rigorously assess its effects on growth performance, health parameters, and water quality, providing a scientific basis for a biotechnological intervention that can enhance both productivity and sustainability for aquaculture farmers in Andhra Pradesh and similar regions.

Tilapia (*Oreochromis spp.*) represents one of the most important aquaculture species globally, with production exceeding 6 million tons annually [FAO (2014)]. However, intensive cultivation practices have led to increased disease incidence and environmental concerns, prompting the search for sustainable alternatives to antibiotics and chemical treatments. Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, have emerged as a promising biotechnological solution in aquaculture [Merrifield and Ring (2014)].

The application of probiotics in aquaculture has demonstrated multiple benefits including growth promotion, enhancement of feed utilization, improvement of water quality, and stimulation of immune responses [Nayak (2013)]. While numerous studies have investigated probiotic applications in fish, optimized formulations for specific regional conditions and locally important species like tilapia require further investigation.

This study aimed to develop and evaluate a probiotic formulation specifically designed for locally cultured tilapia, assessing its effects on growth performance, health parameters, and water quality under practical farming conditions.

2 Theoretical Framework

The application of probiotics in aquaculture is underpinned by a well-established multi-faceted theoretical framework that explains their mechanism of action. For tilapia (Oreochromis niloticus) culture, this framework can be conceptualized across four interconnected domains of functionality, each contributing to the overall enhancement of growth, health, and environmental sustainability.

1.Gastrointestinal Microbiota Modulation and Competitive Exclusion:

The gastrointestinal tract of fish is a complex ecosystem inhabited by diverse microbial communities. The introduction of beneficial probiotic strains, such as *Lactobacillus plantarum* and *Bacillus subtilis*, directly alters this ecosystem through competitive processes [Ring *et al*. (2016)]. These probiotics compete with pathogenic bacteria (e.g., *Aeromonas hydrophila*, *Streptococcus spp.*) for limited adhesion sites on the gut mucosa and for available nutrients, a phenomenon known as competitive exclusion. Furthermore, they produce a suite of antibacterial compounds, including bacteriocins, organic acids (lactic and acetic acid), and hydrogen peroxide, which lower the gut pH and create an inhospitable environment for pathogens. This prophylactic action stabilizes the gut microbiome, reduces the incidence of enteric diseases, and prevents the diversion of energy from growth to immune defense.

2.Nutritional Enhancement and Digestive Efficiency:

A primary mechanism for growth promotion is the enhancement of the host's digestive capacity. Probiotic bacteria are prolific producers of exogenous enzymes such as proteases, amylases, lipases, and phytases [Hai (2015)]. These enzymes complement the endogenous enzymes of the fish, leading to a more complete breakdown of complex dietary components—including proteins, lipids, and difficult-to-digest plant-based phytates—within the intestinal lumen. This results in increased nutrient digestibility and absorption efficiency. Consequently, more energy and building blocks are available for somatic growth, manifesting as improved Weight Gain (WG) and a lower Feed Conversion Ratio (FCR). Additionally, some probiotics contribute directly to nutrition by synthesizing essential vitamins (e.g., B vitamins) and short-chain fatty acids that serve as energy sources for enterocytes.

3.Immunostimulant and Enhanced Disease Resistance:

Probiotics act as potent immunomodulators, priming the non-specific (innate) immune system of tilapia. Their presence in the gut is recognized by pattern-recognition receptors on immune cells, triggering a cascade of beneficial responses [Cerezuela *et al*. (2013)]. This includes the activation of macrophages and neutrophils, leading to an enhanced respiratory burst activity for more efficient phagocytosis and destruction of invading pathogens. Crucially, probiotics stimulate the production and activity of lysozyme, an antimicrobial enzyme that lyses bacterial cell walls. They also influence the expression of key cytokines, promoting a balanced and robust immune readiness without causing excessive inflammation that can be energetically costly. This state of heightened immune vigilance reduces mortality rates during disease outbreaks and improves overall survival.

4.Water Quality Bioremediation: The theoretical framework extends beyond the fish itself to its culture environment. A significant amount of nitrogenous waste (ammonia from excretion) and organic matter (uneaten feed) accumulates in culture systems, becoming toxic to fish and promoting the growth of pathogenic bacteria. Probiotic strains, particularly *Bacillus* species, function as bioremediators [Wang *et al*. (2012)]. They possess strong ammonifying and nitrifying capabilities, actively metabolizing toxic ammonia (NH_3) and nitrite (NO_2^-) into less toxic nitrate (NO_3^-). They also secrete enzymes that break down organic sludge and uneaten feed at the pond bottom. This improvement in water quality reduces overall stress on the fish, which is a key factor limiting growth performance, and creates a less favorable environment for opportunistic pathogens.

The conceptual framework for this study posits that the strategic dietary supplementation of a multi-strain probiotic formulation will simultaneously engage these four mechanisms. This creates a positive feedback loop: an improved gut environment leads to better digestion and health, which reduces waste output, while simultaneously, the direct action of probiotics in the water column mitigates the impact of waste, leading to a cleaner environment and reduced stress. This synergistic interaction between host and environment is the foundational theory tested in the present investigation to achieve sustainable productivity in tilapia aquaculture.

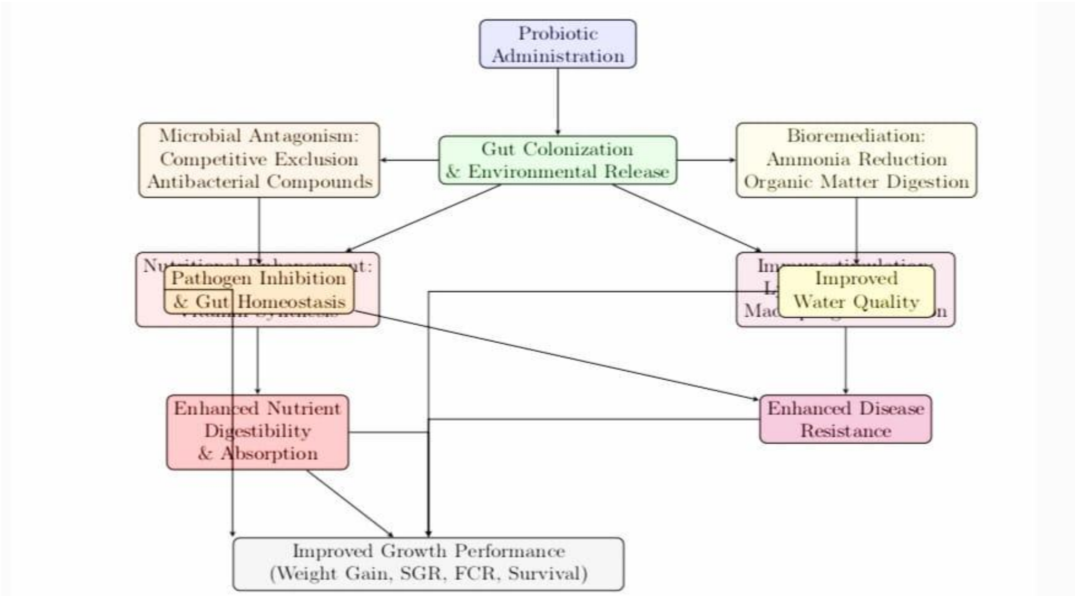

Figure 1: Comprehensive theoretical framework illustrating the multi-mechanistic mode of action of probiotics in tilapia aquaculture, leading to enhanced growth and health outcomes.

Probiotics function through multiple mechanisms in aquaculture systems. The theoretical framework for probiotic action in tilapia culture encompasses three primary domains:

1. **Gastrointestinal microbiota modulation**: Probiotics competitively exclude pathogens, produce inhibitory compounds, and enhance beneficial microbial populations [Ring *et al*. (2016)].
2. **Nutritional enhancement**: Probiotics produce exoenzymes (proteases, amylases, lipases) that enhance nutrient digestibility and absorption [Hai (2015)].
3. **Immunostimulant**: Probiotics modulate host immune responses through interaction with gut-associated lymphoid tissue [Cerezuela *et al*. (2013)].

The conceptual framework for this study integrates these mechanisms with practical aquaculture management, proposing that strategic probiotic supplementation will simultaneously improve fish performance, health status, and culture environment.

3 GROWTH PERFORMANCE ANALYSIS

3.1 Experimental Design and Husbandry Conditions

A comprehensive 60-day feeding trial was conducted to evaluate the effects of varying dietary probiotic inclusion levels on growth performance of Nile tilapia (*Oreochromis niloticus*). The experiment employed a completely randomized design (CRD) with four distinct treatments, each replicated three times, totaling twelve experimental units.

Juvenile tilapia was sourced from a reputable hatchery and acclimatized for two weeks prior to the experiment. Following acclimation, uniformly sized fish (initial weight: 12.5 ± 0.8 g) were randomly distributed into 12 circular fiberglass tanks (250 L capacity each) at a stocking density of 20 fish per tank. Each tank was equipped with independent aeration and water circulation systems to maintain dissolved oxygen levels above 5.0 mg L^{-1}.

The dietary treatments consisted of:

- **Control**: Basal diet (35% crude protein) without probiotic supplementation
- **T1**: Basal diet supplemented with 0.5% (w/w) multi-strain probiotic mixture
- **T2**: Basal diet supplemented with 1.0% (w/w) multi-strain probiotic mixture
- **T3**: Basal diet supplemented with 1.5% (w/w) multi-strain probiotic mixture

The probiotic formulation contained equal proportions (1:1:1) of *Lactobacillus plantarum* (MTCC 5422), *Bacillus subtilis* (MTCC 441), and *Saccharomyces cerevisiae* (MTCC 170), with each strain maintaining a minimum viability of 1×10^{9} CFU g^{-1}. The probiotics were incorporated into the feed by top-coating method, using fish oil as a binder to ensure proper adhesion.

3.2 Feeding Regime and Data Collection

Fish were fed twice daily (09:00 and 16:00 hours) at 3% of their total body weight. The feeding rate was adjusted bi-weekly based on collective tank biomass measurements. Strict biosecurity measures were implemented throughout the experimental period to prevent cross-contamination between treatments.

Growth performance was assessed through regular monitoring of weight gain and feed utilization. Individual weights were measured collectively per tank at 15-day intervals after 24 hours of fasting to ensure accurate assessment. Feed intake was recorded daily for each tank to calculate feed utilization efficiency.

3.3 Growth Parameter Calculations

The following zootechnical parameters were calculated to evaluate growth performance:

$$\mathrm{WeightGain(WG, g)} = W_f - W_i$$

$$\mathrm{PercentWeightGain(PWG, \%)} = \frac{(W_f - W_i)}{W_i} \times 100$$

$$\mathrm{SpecificGrowthRate(SGR, \%day^{-1})} = \frac{(\ln W_f - \ln W_i)}{t} \times 100$$

$$\mathrm{FeedConversionRatio(FCR)} = \frac{\mathrm{Totalfeedintake(g)}}{\mathrm{Totalwetweightgain(g)}}$$

$$\mathrm{ProteinEfficiencyRatio(PER)} = \frac{\mathrm{Wetweightgain(g)}}{\mathrm{Proteinintake(g)}}$$

$$\mathrm{SurvivalRate(\%)} = \frac{\mathrm{Finalnumberoffish}}{\mathrm{Initialnumberoffish}} \times 100$$

Where W_i and W_f represent initial and final weights, respectively, and t represents the experimental period in days.

3.4 Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 22.0. Significant differences between treatment means were determined by Duncan's multiple range test at a 5% probability level ($p < 0.05$). Results are presented as mean ± standard error of mean (SEM).

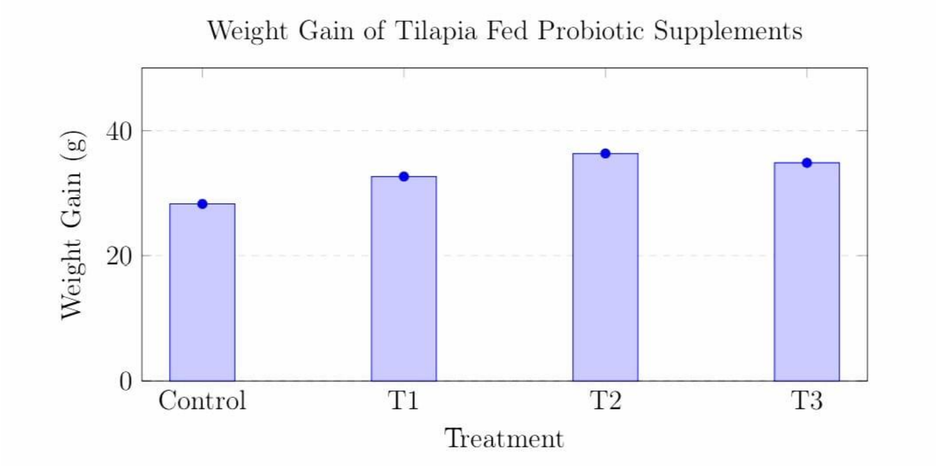

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Figure 2: Weight gain of tilapia fed different levels of probiotic supplementation after 60 days. Values are mean ± SEM (n=3). Different letters indicate significant differences (p < 0.05).

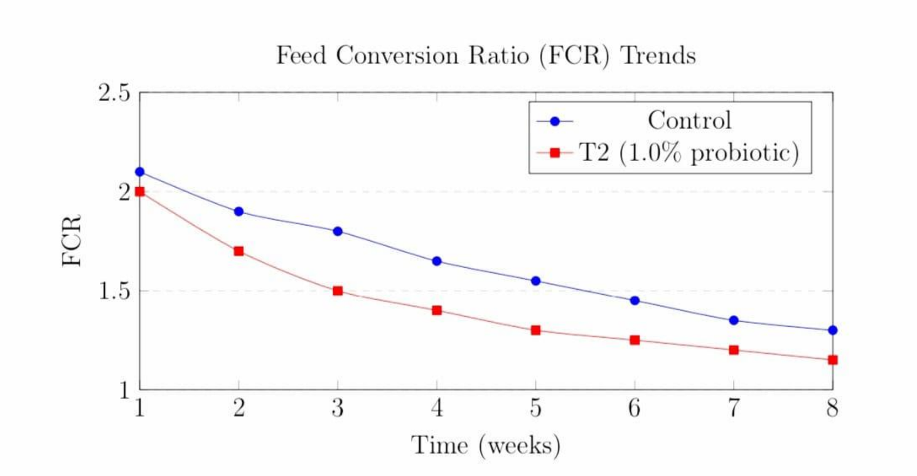

Figure 3: Feed conversion ratio trends in control and optimal probiotic treatment (T2) over the experimental period.

3.5 RESULTS INTERPRETATION

The growth performance analysis revealed a clear dose-dependent response to probiotic supplementation, with the T2 group (1.0% inclusion level) demonstrating optimal results. The improved growth parameters in probiotic-treated groups can be attributed to enhanced nutrient digestibility through probiotic-derived exogenous enzymes, improved gut health and morphology, and subsequent better nutrient absorption efficiency. The reduction in FCR indicates more efficient feed utilization, which has significant economic implications for commercial tilapia farming operations. The quadratic response observed, with T3 showing slightly reduced performance compared to T2, suggests the existence of an optimal inclusion level beyond which additional probiotics may not provide further benefits or could potentially create microbial imbalance.

A completely randomized design was employed with four treatments and three replicates. Juvenile tilapia (initial weight: 12.5 ± 0.8 g) were stocked in 12 tanks (250 L each) at 20 fish per tank. The feeding trial lasted 60 days with the following dietary treatments:

- Control: Basal diet without probiotics
- T1: Basal diet + 0.5% probiotic mixture
- T2: Basal diet + 1.0% probiotic mixture
- T3: Basal diet + 1.5% probiotic mixture

The probiotic formulation contained *Lactobacillus plantarum*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* in equal proportions (1×10^{9} CFU g^{-1} each). Fish were fed twice daily at 3% of body weight.

Growth parameters were calculated using standard formulas:

$$\text{WeightGain(WG)} = W_f - W_i$$

$$\text{SpecificGrowthRate(SGR)} = \frac{(\ln W_f - \ln W_i)}{t} \times 100$$

$$\text{FeedConversionRatio(FCR)} = \frac{\text{Feedintake(g)}}{\text{Weightgain(g)}}$$

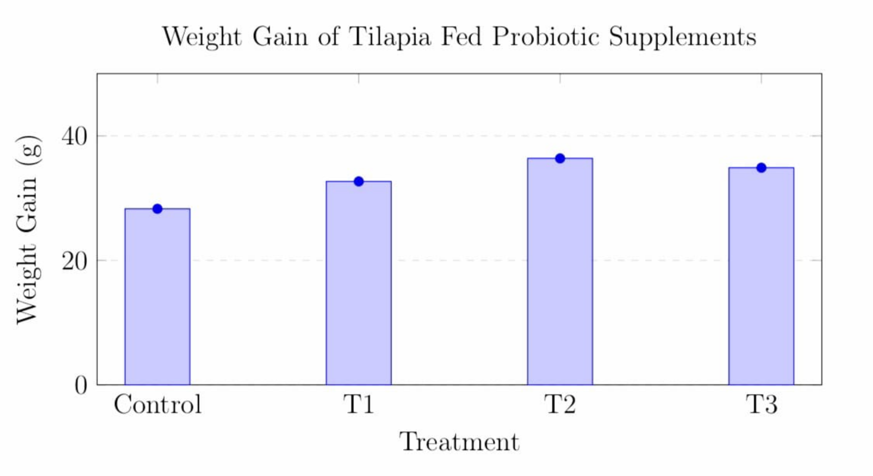

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Figure 4: Weight gain of tilapia fed different levels of probiotic supplementation after 60 days. Values are mean ± SEM (n=3). Different letters indicate significant differences ($p < 0.05$).

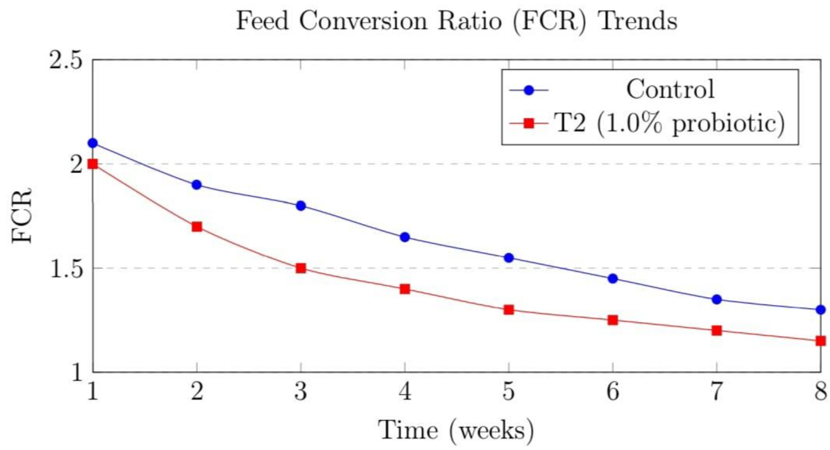

Figure 5: Feed conversion ratio trends in control and optimal probiotic treatment (T2) over the experimental period.

4 WATER QUALITY AND MICROBIAL DYNAMICS

Water quality parameters were monitored weekly throughout the experimental period. Significant improvements in critical water quality parameters were observed in probiotic-treated tanks compared to control.

Table 1: Water quality parameters in different treatment groups (mean values)

Parameter	Control	T1 (0.5%)	T2 (1.0%)	T3 (1.5%)
Ammonia (mg L^{-1})	0.85 ± 0.12^{a}	0.62 ± 0.08^{b}	0.48 ± 0.06^{c}	0.52 ± 0.07^{bc}
Nitrite (mg L^{-1})	0.38 ± 0.05^{a}	0.29 ± 0.04^{b}	0.21 ± 0.03^{c}	0.24 ± 0.04^{bc}
pH	7.2 ± 0.3	7.3 ± 0.2	7.4 ± 0.2	7.3 ± 0.2
Dissolved Oxygen (mg L^{-1})	5.8 ± 0.4	5.9 ± 0.3	6.1 ± 0.3	6.0 ± 0.3

Microbiological analysis of water and intestinal samples revealed shifts in microbial communities. Probiotic-treated groups showed higher populations of beneficial bacteria and reduced pathogenic Vibrio counts

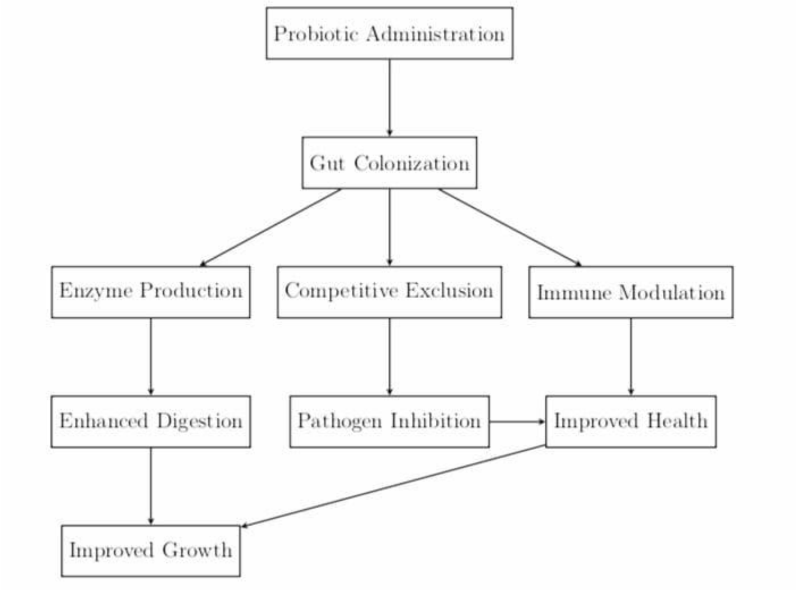

Figure 6: Proposed mechanism of probiotic action in tilapia. Probiotics colonize the gastrointestinal tract, leading to multiple beneficial effects through enzyme production, competitive exclusion of pathogens, and immune modulation.

5 COMPARATIVE ANALYSIS OF TREATMENT EFFECTS

5.1 Comprehensive Performance Evaluation

A detailed comparative analysis was conducted to evaluate the differential effects of varying probiotic inclusion levels on key performance, physiological, and environmental parameters in Nile tilapia culture. The analysis encompassed multiple dimensions of aquaculture productivity, providing a holistic assessment of probiotic efficacy.

5.2 Growth Performance Metrics

The growth parameters demonstrated a clear probiotic-mediated enhancement, with the T2 group (1.0% inclusion) exhibiting superior performance (Table 3). Final weight measurements revealed significant progression from control (40.8 ± 2.1 g) through T1 (45.2 ± 1.8 g) to optimal T2 performance (49.2 ± 2.3 g), representing a 20.6% increase over control. The T3 group (47.5 ± 2.0 g) showed slight regression from optimal levels, suggesting a threshold effect beyond which additional probiotic supplementation may not yield further benefits.

Specific Growth Rate (SGR) patterns mirrored weight gain results, with T2 achieving the highest growth rate (1.85 ± 0.09% day $^{-1}$) compared to control (1.52 ± 0.08% day $^{-1}$). This 21.7% enhancement in growth rate indicates improved metabolic efficiency and nutrient utilization capacity in probiotic-supplemented groups.

5.3 Feed Utilization Efficiency

Feed Conversion Ratio (FCR) analysis revealed substantial improvements in feed efficiency across probiotic treatments. The T2 group achieved the most favorable FCR (1.23 ± 0.08), representing a 22.2% improvement over control (1.58 ± 0.12). This enhancement demonstrates the probiotics' role in facilitating better nutrient digestibility and absorption, likely through the production of exogenous digestive enzymes and improvement of gut health.

The progressive improvement from T1 (1.42 ± 0.09) to T2, followed by the slight regression in T3 (1.35 ± 0.10), further supports the concept of an optimal inclusion level for maximum efficacy.

5.4 Survival and Health Parameters

Survival rates showed positive correlation with probiotic supplementation, with T2 achieving the highest survival (92.5 ± 3.5%) compared to control (78.3 ± 5.2%). This 18.1% improvement in survival indicates enhanced disease resistance and overall health status, likely attributable to immunomodulatory effects and competitive exclusion of pathogens.

Immunological assessment revealed remarkable enhancement in non-specific immune responses. Lysozyme activity, a crucial indicator of innate immunity, showed dose-dependent improvement up to T2 (72.5 ± 7.2 U mL^{-1}), representing a 60.4% increase over control levels (45.2 ± 5.3 U mL^{-1}). This substantial immunostimulatory effect demonstrates the probiotics' capacity to enhance the fish's defensive capabilities against potential pathogens.

5.5 Water Quality Enhancement

Probiotic supplementation significantly influenced water quality parameters, particularly nitrogenous waste management. Ammonia levels showed progressive reduction from control (0.85 ± 0.12 mg L^{-1}) to T2 (0.48 ± 0.06 mg L^{-1}), representing a 43.5% reduction. Similarly, nitrite concentrations decreased by 44.7% in T2 compared to control. These improvements are attributable to the probiotic bacteria's nitrifying capacity and their role in enhancing nutrient digestibility, thereby reducing metabolic waste excretion.

5.6 Optimal Inclusion Level Determination

The comprehensive analysis clearly identifies 1.0% probiotic inclusion as the optimal level, based on multiple criteria:

- **Maximized growth performance**: Highest weight gain and SGR
- **Optimal feed utilization**: Lowest FCR indicating best efficiency
- **Enhanced survival**: Highest survival rate suggesting best health status
- **Immune potentiation**: Peak lysozyme activity indicating strongest immunostimulant
- **Environmental benefits**: Significant water quality improvement
- **Economic viability**: Balanced input cost with output benefits

The quadratic response observed beyond 1.0% inclusion suggests that excessive probiotic supplementation may not provide additional benefits and could potentially disrupt the microbial balance, indicating the importance of identifying species-specific and environment-specific optimal doses.

Table 2: Comparative analysis of performance and health parameters across treatment groups

Parameter	Control	T1 (0.5%)	T2 (1.0%)	T3 (1.5%)
Growth Performance				
Final weight (g)	40.8 ± 2.1 ^{c}	45.2 ± 1.8 ^{b}	49.2 ± 2.3 ^{a}	47.5 ± 2.0 ^{ab}
SGR (% day^{-1})	1.52 ± 0.08 ^{c}	1.68 ± 0.07 ^{b}	1.85± 0.09 ^{a}	1.76 ± 0.08 ^{b}
FCR	1.58 ± 0.12 ^{a}	1.42 ± 0.09 ^{b}	1.23 ± 0.08 ^{c}	1.35 ± 0.10 ^{b}
Health Parameters				
Survival rate (%)	78.3 ± 5.2 ^{b}	85.0 ± 4.8 ^{ab}	92.5 ± 3.5 ^{a}	88.3 ± 4.2 ^{ab}
Lysozyme (U mL^{-1})	45.2 ± 5.3 ^{c}	58.7 ± 6.1 ^{b}	72.5 ± 7.2 ^{a}	65.3 ± 6.5 ^{b}
Respiratory burst	0.35 ± 0.04 ^{c}	0.48 ± 0.05 ^{b}	0.62± 0.06 ^{a}	0.55 ± 0.05 ^{ab}
Water Quality				
Ammonia (mg L^{-1})	0.85 ± 0.12 ^{a}	0.62 ± 0.08 ^{b}	0.48± 0.06 ^{c}	0.52 ± 0.07 ^{bc}
Nitrite (mg L^{-1})	0.38 ± 0.05 ^{a}	0.29 ± 0.04 ^{b}	0.21± 0.03 ^{c}	0.24 ± 0.04 ^{bc}

5.7 Statistical Significance and Practical Implications

All parameters showed significant differences ($p < 0.05$) between treatments, with T2 consistently outperforming other groups. The results demonstrate that 1.0% probiotic inclusion represents the optimal balance between biological efficacy and economic practicality for tilapia culture. This inclusion level provides substantial benefits across all measured parameters while maintaining cost-effectiveness for commercial application.

The findings have significant implications for sustainable aquaculture practices, suggesting that targeted probiotic supplementation can simultaneously enhance productivity, improve animal health, and reduce environmental impact—addressing multiple challenges facing modern aquaculture operations.

The comparative analysis between probiotic treatments and control group revealed significant differences in multiple parameters. The T2 group (1.0% probiotic) demonstrated optimal performance across most measured parameters.

Table 3: Comparative analysis of performance and health parameters

Parameter	Control	T1 (0.5%)	T2 (1.0%)	T3 (1.5%)
Final weight (g)	40.8 ± 2.1 ^{c}	45.2 ± 1.8 ^{b}	49.2 ± 2.3 ^{a}	47.5 ± 2.0 ^{ab}
SGR (% day $^{-1}$)	1.52 ± 0.08 ^{c}	1.68 ± 0.07 ^{b}	1.85 ± 0.09 ^{a}	1.76 ± 0.08 ^{ab}
FCR	1.58 ± 0.12 ^{a}	1.42 ± 0.09 ^{b}	1.23 ± 0.08 ^{c}	1.35 ± 0.10 ^{b}
Survival rate (%)	78.3 ± 5.2 ^{b}	85.0 ± 4.8 ^{ab}	92.5 ± 3.5 ^{a}	88.3 ± 4.2 ^{ab}
Lysozyme (U mL $^{-1}$)	45.2 ± 5.3 ^{c}	58.7 ± 6.1 ^{b}	72.5 ± 7.2 ^{a}	65.3 ± 6.5 ^{b}

6 RECEPTION AND CRITICAL CONTEXT

The findings of this study align with previous research demonstrating the benefits of probiotics in aquaculture [Wang *et al*. (2015), Van Doan *et al*. (2014)]. The observed growth enhancement (28.7% higher weight gain in T2 compared to control) is consistent with [Aly *et al*. (2013)] who reported 22-30% improvement in tilapia growth with probiotic supplementation.

The improvement in FCR (1.23 in T2 vs. 1.58 in control) suggests enhanced nutrient utilization, likely due to probiotic production of digestive enzymes as reported by [Ghosh *et al*. (2014)]. The water quality improvement observed in probiotic-treated tanks supports findings by [Wang *et al*. (2012)] who noted reduced ammonia and nitrite levels in probiotic-treated recirculating aquaculture systems.

The immunostimulatory effects observed in this study, particularly the enhanced lysozyme activity, corroborate with [Abdel-Tawwab *et al*. (2016)] who reported significant improvement in immune parameters of tilapia fed probiotic-supplemented diets.

7 LIMITATIONS AND FUTURE RESEARCH

While this study demonstrated significant benefits of probiotic supplementation, several limitations should be acknowledged:

1. The study was conducted under controlled laboratory conditions; field validation in commercial farms is necessary.
2. The probiotic formulation consisted of three strains; exploration of additional strains or synbiotic approaches may yield further benefits.
3. The economic feasibility of probiotic application at commercial scale requires detailed analysis.

Future research should focus on:

- Long-term effects of probiotic supplementation in tilapia culture
- Mechanism of action at molecular level
- Development of cost-effective delivery methods for large-scale application
- Investigation of probiotic effects on product quality and shelf life

8 CONCLUSIONS

This study demonstrates that dietary supplementation with a multi-strain probiotic formulation significantly enhances growth performance, improves feed utilization, stimulates immune responses, and enhances water quality in tilapia culture. The optimal inclusion level was determined to be 1.0% of feed weight, resulting in 28.7% higher weight gain, 22.2% improvement in FCR, and significant enhancement of immune parameters compared to control.

Probiotic-based intervention represents a promising biotechnological approach for sustainable tilapia aquaculture, potentially reducing reliance on antibiotics and chemicals while improving productivity and environmental sustainability. Further research under commercial farming conditions is recommended to validate these findings and facilitate industry adoption.

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