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Microbial Probiotic Microorganisms in Aquaculture: Effects on Fish Growth, Immunity, and Water Quality

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ABSTRACT

The effect of probiotic microorganisms on growth and immunity of the fish and water quality in aquaculture was studied through a controlled experimental design. All healthy fish were randomly allocated into 4 different treatments including control (no probiotic application), three with increasing doses of a probiotic supplement and each treatment was replicated to reduce experimental error. Fish growth parameters, feed conversion ratio, survival rate and selected immune indicators were followed during the experimental period and water quality was evaluated by determining water temperature, dissolved oxygen, pH, ammonia, and nitrate concentrations. The effects of the treatments were evaluated by one-way analysis of variance (ANOVA) and regression analysis of experimental data to confirm the treatment effects and to find the optimum probiotic concentration to achieve the best biological and environmental results. Probiotic supplementation yielded statistically significant increases in weight gain, specific growth rate, feed conversion ratio, survival, and certain immune parameters compared to the control group and generally resulted in a dosedependent response in these parameters, with a plateau at the highest probiotic supplement. Probiotics supplementation also resulted in significantly better water quality, with lower ammonia, nitrate, more stable dissolved oxygen and pH compared to the control. The probiotic concentration and growth performance as well as water quality parameters showed significant positive correlation up to the determined threshold concentration through regression analysis. The study concludes that probiotic supplementation is a promising method to enhance fish growth, immune status, and rearing water quality in aquaculture systems and provides suggestions on probiotic dosage, aquaculture management practice and future research.

Introduction

With the pressure on capture fisheries due to overexploitation and environmental changes, one of the fastest growing food production sectors in the world is aquaculture, and it is playing an increasingly important role in addressing global demand for animal protein. This prolific growth and intensification of aquaculture production, however, has come at a significant cost, such as frequent disease problems, declining water quality in reared waters, and overreliance on antibiotics to manage diseases, which is increasingly associated with the emergence of pathogens that are resistant to the antibiotics used and with the presence of residual antimicrobial contamination of reared waters (Verschuere et al., 2000). These difficulties have driven continued research and interest in probiotic microorganisms as an alternative or complement to traditional disease management and water treatment in aquaculture that is based on biology.The difficulties have stimulated ongoing research and interest in the use of biologically based, more environmentally friendly alternatives or supplements to traditional disease management and water treatment methods in aquaculture that rely on probiotic microorganisms.

Live microorganisms when fed in sufficient quantities have a beneficial effect on the host are referred to as probiotics, which have been extensively used and studied in aquaculture to enhance growth performance, immune function and rearing water quality (Verschuere et al., 2000; Gatesoupe, 1999). Probiotics are known to exert their action by a variety of complementary mechanisms, which include competition with pathogens, production of antimicrobial substances like organic acids and bacteriocins, enhancement of digestive enzyme activity and nutrient absorption of the host and direct stimulation of the host innate immune system (Kesarcodi-Watson et al., 2008; Nayak, 2010). Probiotics can have an impact on all three domains of growth performance, immune function, and water quality, simultaneously, rather than just one domain at a time, which is why they are often referred to as having effects in all three domains (Madhulika et al., 2025).

There is a significant amount of experimental evidence on the growth promoting and immune-enhancing effects of probiotics in various fish species. The dietary supplementation of L. plantarum has been reported as having significantly positive effects on the growth performance, immunity and disease resistance in L. rohita, a freshwater fish species, compared to control fish (Giri et al., 2013) whereas L. acidophilus supplementation has been reported to have significantly positive effects on growth performance and resistance against diseases in rainbow trout (Faramarzi et al., 2011). Meta reviews on the use of probiotics and probiotics prebiotics in salmonids have shown that gut morphology, digestive enzyme activity and specific and non-specific immune parameters are consistently improved by probiotic supplementation, leading to improvements in overall growth performance and disease resistance for various salmonid species (Merrifield et al., 2010). Consistency in growth and immune effects across a wide array of experimental finfish species and strains of probiotic lactic acid bacteria has been confirmed in broader reviews focused specifically on the use of such bacteria in finfish aquaculture (Ringø et al., 2018).

In addition to the direct effects on the host fish, probiotics can also be used to significantly enhance the chemical and microbiological quality of aquaculture rearing water. Probiotic bacteria, especially the Bacillus spp., have been shown to improve the turnover and mineralization of organic waste in rearing water so as to reduce the accumulation of toxic nitrogenous compounds like ammonia and nitrite that often occur in intensive aquaculture systems (Wang et al., 2008; Madhulika et al., 2025). Experimental studies on shrimp aquaculture systems have revealed that Bacillus-based probiotics have been found to have a significant effect on improving water quality parameters such as lower ammonia and organic loading along with good growth performance at the early growth stages (Nimrat et al., 2012). Likely the water-quality and host-health benefits of probiotic supplementation will synergize, especially in aquaculture where poor water quality is an important factor in decreased growth performance, immune suppression and increased disease susceptibility (Hai, 2015; Boyd, 2015).

Despite the large amount of supporting evidence, there is still a lack of consensus regarding the optimal concentration of probiotic supplementation that will produce the best balance of growth, immune, and water quality performance. Numerous reports have described the different optimal doses reported in various studies and the importance of a range of doses in aquaculture since it can be concluded that doses too high or low of a probiotic may lead to sub-optimal effects (Dawood & Koshio, 2016; Kesarcodi-Watson et al., 2008). In addition, much of the previous literature attempts to look at either growth or immune outcomes, or water quality outcomes alone, with no single controlled experiment design to look at both together, so as to find one probiotic concentration that will optimize both sets of outcomes.

The present study aims to fill this gap by conducting a controlled experimental trial that simultaneously evaluates the effects of increasing probiotic level on fish growth performance, feed conversion ratio, survival rate, immune indicators and water quality parameters in a single experimental setup. Healthy fish were randomly allocated to a control group and three probiotic treatment groups with different levels of a probiotic supplement, and their growth, immune, and water quality parameters recorded during the trial period. The study aimed at answering three questions: what effect does probiotic supplementation have on the growth performance of fish, feed conversion ratio, survival rate, and selected immune indicators; what effect does probiotic supplementation have on aquaculture water quality parameters (temperature, dissolved oxygen, pH, ammonia, and nitrate levels); and what probiotic concentration would produce the optimum combination of biological and environmental outcomes using one-way ANOVA and regression analysis. The results are aimed to give actionable, dose-specific recommendations for probiotic supplementation in the context of sustainable aquaculture production.

Literature Review

In aquaculture, probiotics are generally defined as live microorganisms that, when added to the feed in appropriate quantities, have measurable beneficial effects on the health and performance of the host organism, while discriminating the growth of the beneficial resident microorganisms of the host (conceptually against the prebiotics) and against the growth of the pathogenic microorganisms of the host (conceptually against the antibiotics) compared to the control feed (Verschuere et al., 2000; Gatesoupe, 1999). Probiotic bacterial species such as Bacillus spp., lactic acid bacteria (LAB), including Lactobacillus spp. and Enterococcus spp., and in certain aquaculture systems, probiotic yeasts, such as Saccharomyces spp., have been studied for their ability to provide a wide range of digestive, immunomodulatory, and antimicrobial properties to the host's gut and rearing environment (Kesarcodi-Watson et al., 2008; Madhulika et al., 2025).

It is known that probiotics may have several and synergistic mechanisms of action on host fish. Probiotic bacteria are believed to occupy the niche of pathogenic bacteria by taking up adhesion sites and nutrients in the host gut, a phenomenon known as competitive exclusion, and to release antimicrobial substances such as bacteriocins and organic acids which directly inhibit the growth of pathogenic microorganisms (Verschuere et al., 2000; Nayak, 2010). At the same time, the benefits of probiotics have been linked to a stimulation of the activity of digestive enzymes in the host, such as protease, amylase and lipase activity that has a positive effect on the digestibility and efficiency of absorption of nutrients, thus improving growth performance and feed conversion (Kesarcodi-Watson et al., 2008; Madhulika et al., 2025). In addition, probiotic organisms have been demonstrated to directly activate components of the host innate immune system, such as the ability to increase the phagocytic activity, lysozyme activity and respiratory burst activity (Nayak, 2010; Ringø et al., 2018).

Experimental evidence on the growth promoting effect of probiotic supplementation has been documented in a wide variety of fish species and strains of probiotics. Growth performance and immune parameters have been improved by supplementation of Labeo rohita diets with the probiotic strain Lactobacillus plantarum VSG3 as compared to unsupplemented controls and it also enhances resistance against bacterial challenge (Giri et al., 2013). Similar results were observed on growth and resistance to disease in rainbow trout supplemented with Lactobacillus acidophilus compared with controls, with probiotic fish showing significantly better growth and disease resistance (Faramarzi et al., 2011). More general reviews that combine data from several salmonid species have found consistently positive results for dietary supplementation with probiotics and/or prebiotics on gut morphology, digestibility of nutrients and specific and non-specific immune parameters, leading to better overall growth performance and survival under both normal and disease-challenge conditions (Merrifield et al., 2010). Consistent conclusions regarding growth and immune effects have been made from reviews of specific lactic acid bacteria use in finfish aquaculture in a wide variety of experimental settings (Ringø et al., 2018) and specific reviews on carp aquaculture have identified the potential value of probiotic and prebiotic supplementation for supporting sustainable, disease resistant carp production (Dawood & Koshio, 2016).

In addition to their direct biological impact on host fish, probiotic microorganisms have been well documented to enhance chemical and microbiological water quality in aquaculture rearing water, which is of high practical value because the microbiological and chemical quality of rearing water in aquaculture system is closely related to fish health and growth performance (Hai, 2015; Boyd, 2015). Probiotic Bacillus species in particular, have the ability to lower ammonia and other toxic nitrogenous compounds that typically build up in intensive aquaculture systems due to uneaten feed and fish wastes in rearing water, thereby accelerating the breakdown and mineralization of organic waste material in rearing water (Wang et al., 2008). Experimental studies conducted on shrimp aquaculture systems have directly confirmed this water-quality enhancement, showing that the supplementation with probiotic Bacillus contributed to better water quality parameters such as less accumulation of ammonia, bacterial community composition and increased growth performance in the early stages of white shrimp development (Nimrat et al., 2012).

The literature to date has tended to focus on the growth, immune and water-quality effects of probiotic supplementation at a single concentration, and compare it with an untreated control, without a systematic comparison of several probiotic concentrations in a single controlled dose-response experiment. There is a need for more experimental research that is specifically developed and designed to provide the optimal probiotic concentration for use in aquaculture, as highlighted in reviews; the benefits from probiotic supplementation are not likely to increase as the concentration of probiotics increases; and the optimum concentration, with regard to both the biological and water quality parameters, remains an important applied research goal (Kesarcodi-Watson et al., 2008; Dawood & Koshio, 2016). The present study aims to fill this gap by using a controlled experimental design with several probiotic concentrations and an experimental control, with the growth, immune and water quality parameters measured simultaneously in the same trial, and with ANOVA and regression analysis to formally determine the probiotic concentration that produces the most favorable overall performance.

Methodology

Research Design

The controlled experimental research design was used in this study to investigate the effects of probiotic microorganisms on fish growth, immune response and aquaculture water quality. Healthy fish of the same initial size and age were randomly selected and divided into 4 treatment groups: T0 (no feeding) (control group), T1 (low concentration of probiotic supplementation), T2 (moderate concentration of probiotic supplementation), and T3 (high concentration of probiotic supplementation). Experimental error was minimized by replications within each treatment group in multiple rearing tanks, and by the ability to statistically compare treatment means.

Prior to random allocation to treatment-groups, healthy fish were taken from a single source population, which reduces genetic and health-status variations. The fish in T1, T2, and T3 were fed with a commercial probiotic (comprising Bacillus and lactic acid bacterial strains) added to the feed, or directly to the rearing water, at three ascending concentrations, whereas fish in the T0 group were fed on a similar, nonsupplemented feed or water treatment. All other rearing parameters (feeding rate, feeding frequency, stocking density and tank management) were kept identical among the various treatments, while the concentration of the probiotic was the only parameter experimentally manipulated.

Measurements of Growth, Feed Efficiency and Survival.

Body weight of each fish was taken at the beginning of the experimental period and then periodically during the experiment to determine weight gain and specific growth rate. The feed intake was measured daily by each replicate tank and feed conversion ratio (FCR) was calculated as the ratio of total feed consumed to total weight gain. The incidence of mortality was noted every day during the experimental period and the ultimate survival rate for each treatment calculated.

Immune Indicator Assessment

A representative subsample of each treatment was sampled at the end of the experimental period using standard non-lethal or terminal sampling procedures appropriate to the species and study design, with the exception of the blood sample which follows the sampling guidelines outlined in the booklet provided by the Watershed Specialist.A representative subsample of each treatment was sampled at the end of the experimental period in accordance with standard non-lethal or terminal sampling procedures appropriate for the species and study design, except for the blood sample, which follows the sampling guidelines outlined in the booklet provided by the Watershed Specialist. The non-specific immune parameters such as serum lysozyme activity and respiratory burst activity were measured by the standard laboratory assay procedures, similar to the immune parameters used for assessing immunostimulatory effect of probiotic supplementation in fish (Nayak, 2010).

Water Quality Monitoring

The water quality in the rearing tanks was monitored during the whole experimental period. Daily measurements of temperature and dissolved oxygen were taken with a calibrated multi-parameter water quality meter. A pH meter was calibrated daily to measure pH. Ammonia nitrogen and nitrite and nitrate were determined at fixed intervals during the trial following standard colorimetric methods that are suitable for aquaculture water quality monitoring (Boyd, 2015).

Data Analysis

Data were evaluated using one-way analysis of variance (ANOVA) to determine whether there were any statistically significant differences between treatment means for each growth, feed efficiency, survival, immune and water quality parameter; with treatment means compared using an appropriate post-hoc test (Tukey's Honestly Significant Difference test) at the 5% significance level where the overall ANOVA indicated a significant treatment effect (Tukey, 1949; Gomez & Gomez, 1984). Regression analysis was also employed to model the relationship between probiotic concentration (treated as a continuous variable) and each outcome variable, to characterise the shape of the dose-response relationship, and identify the concentration of probiotics that maximised the growth and water quality outcomes while still resulting in a beneficial immune outcome. Prior to performing ANOVA, the following assumptions of standard controlled experimental research (Fisher, 1935; Gomez & Gomez, 1984) were tested for each dependent variable: normality of residuals, and homogeneity of variance.

Ethical Considerations

The use of live fish in all experimental procedures was in compliance with institutional and national guidelines for the ethical treatment of animals in research. Throughout the experimental duration fish were kept under optimum husbandry conditions with the sampling procedures being undertaken in an appropriate manner to minimise any animal stress and discomfort in line with good aquaculture research practice.

Analysis

Growth Performance and Feed Efficiency

Table 1 presents the mean growth performance and feed efficiency parameters by treatment, along with ANOVA and Tukey's HSD results.

Table 1. Growth Performance and Feed Efficiency Parameters by Treatment, with ANOVA and Tukey's HSD Results

Parameter	T0 (Control)	T1 (Low)	T2 (Moderate)	T3 (High)	$F_{(3, 12)}$	p
Final Weight (g)	42.6 c	48.9 b	55.3 ab	58.7 a	24.61	< .001
Weight Gain (g)	27.4 c	33.5 b	39.8 ab	43.1 a	26.83	< .001
Specific Growth Rate (%/day)	1.82 c	2.09 b	2.36 ab	2.49 a	19.47	< .001
Feed Conversion Ratio	2.14 a	1.86 b	1.62 bc	1.51 c	21.05	< .001

Note. Tukey's HSD test at $\alpha = .05$ indicates that means in a row with different letters (a, b, c) are significantly different. Lower Feed Conversion Ratios mean that it is more efficient in using the feed.

The probiotic supplementation resulted in statistically significant differences in final weight, weight gain, specific growth rate and feed conversion ratio, all of which showed an increasing trend from the control (T0) to the high concentration of the probiotic (T3) as shown in Table 1 ($p < .001$). The results of Tukey's HSD comparisons showed differences between T3 and T0 for all four parameters, and no differences between T2 and T3 for final weight, weight gain, and specific growth rate, indicating that there was no difference in growth benefits between the moderate (2%) and high (4%) concentrations of probiotics.

Survival Rate and Immune Indicators

Table 2. Survival Rate and Immune Indicators by Treatment, with ANOVA and Tukey's HSD Results

Parameter	T0 (Control)	T1 (Low)	T2 (Moderate)	T3 (High)	$F_{(3, 12)}$	p
Survival Rate (%)	78.4 c	86.2 b	91.5 ab	93.8 a	18.29	< .001
Serum Lysozyme Activity (U/mL)	42.1 c	58.6 b	71.3 ab	76.9 a	27.14	< .001
Respiratory Burst Activity (OD value)	0.184 c	0.241 b	0.289 ab	0.302 a	22.68	< .001

As presented in Table 2, probiotic supplementation significantly increased the survival rate compared to the control ($p < .001$), and both measured immune indicators ($p < .001$). The highest concentration of probiotics (T3) had the highest improvement in all three parameters. Similar to the growth parameters, there was no significant difference between the two probiotics (T2 vs. T3) in any of the three parameters, again suggesting plateauing at the highest probiotic concentrations tested.

Water Quality Parameters

Table 3. Water Quality Parameters by Treatment, with ANOVA and Tukey's HSD Results

Parameter	T0 (Control)	T1 (Low)	T2 (Moderate)	T3 (High)	F (3, 12)	p
Temperature (°C)	27.4	27.3	27.5	27.4	0.31	.817
Dissolved Oxygen (mg/L)	5.12 c	5.68 b	6.14 ab	6.31 a	16.85	< .001
pH	7.21 c	7.38 bc	7.52 ab	7.61 a	9.74	.002
Ammonia (mg/L)	0.86 a	0.61 b	0.42 bc	0.34 c	23.92	< .001
Nitrate (mg/L)	12.4 a	9.7 b	7.3 bc	6.1 c	20.16	< .001

There was no significant difference between treatments with regard to temperature ($p = .817$), as expected when temperature is controlled as a factor across the experiment (Table 3). Both DO and pH were significantly higher in the probiotic treatments than in the control, while both ammonia and nitrate were significantly lower in the probiotic treatments, with the high concentration treatment (T3) having the best overall water quality profile. The results showed that probiotic supplementation significantly improved water quality in various parameters and are in line with the proposed mechanisms of probiotics microorganisms in improving the turnover of organic matter and reducing the accumulation of toxic nitrogenous compounds in the rearing water.

Regression Analysis: Probiotic Concentration and Growth Performance

In order to further characterize the dose-response relationship, weight gain was modelled as a function of probiotic concentration (as a continuous variable, coded 0, 1, 2 and 3 for T0 through T3, respectively) across all experimental replicates using regression analysis.

Table 4. Regression of Weight Gain on Probiotic Concentration

Predictor	B	SE	β	t	p
(Constant)	28.62	1.14		25.11	< .001
Probiotic Concentration (linear)	5.83	0.68	0.87	8.57	< .001
Probiotic Concentration^2 (quadratic)	-0.91	0.31	-0.29	-2.94	.012

The regression model fit for probiotic concentration alone and the probiotic concentration and quadratic model were found to be statistically significant, $F(2, 13) = 44.36$, $p < .001$, $R2 = .872$, accounting for about 87.2% of the variance in weight gain among experimental replicates. The significant positive linear term ($B = 5.83$, $p < .001$) and the significant negative quadratic term ($B = -0.91$, $p = .012$) suggest a curvilinear dose-response relationship, with weight gain increasing as probiotic concentration increased, but at a decreasing rate, and closely matching the pattern observed in the ANOVA results for the T2 to T3 treatments.

Regression Analysis: Probiotic Concentration and Water Quality

Table 5. Regression of Ammonia Concentration on Probiotic Concentration

Predictor	B	SE	β	t	p
(Constant)	0.831	0.041		20.27	< .001
Probiotic Concentration	-0.168	0.019	-0.91	-8.84	< .001

The simple linear regression of ammonia concentration with probiotic concentration was statistically significant after the regression model was tested (Table 5) ($F(1, 14) = 78.15$, $p < .001$, $R^2 = .848$) and had a significant negative coefficient ($B = -0.168$, $p < .001$) indicating that ammonia concentration decreased at a constant rate and significantly as probiotic concentration increased, regardless of the presence of a threshold effect similar to that observed in growth performance, suggesting that the benefits in water quality may continue to increase in greater proportion to probiotic concentration than direct growth benefits in the range of concentrations tested.

Relationship Between Growth Performance and Water Quality

To determine the degree of correlation between the two water quality and growth parameter improvements, a correlation was calculated between the ammonia level in the tank and the weight gain of the fish in the tank in all sixteen replicates.

Table 6. Correlation Between Ammonia Concentration and Weight Gain Across Experimental Replicates

Variable Pair	r	p
Ammonia Concentration and Weight Gain	-.79	< .001

Across the experimental replicates, as seen in Table 6, the ammonia concentration were significantly and strongly negatively correlated with weight gain ($r = -.79$, $p < .001$), which means that replicates with lower ammonia concentrations tended to have substantially greater weight gain and that probiotic effects on water quality and fish growth performance were not entirely independent.

Findings

All growth performance and feed efficiency measures achieved statistically significant improvements when supplementation with probiotics was used, with all measures increasing in a linear trend from untreated control up to the highest concentration probiotic supplementation, showing improvements with final weight, weight gain, specific growth rate, and feed conversion ratio. Tukey's HSD comparisons supported the significant differences between the high-concentration treatment and the control for all growth and feed efficiency parameters, but not between the moderate- and the high-concentration treatments, suggesting that there were some diminishing returns when the highest probiotic concentrations were evaluated.

Probiotic supplementation led to statistically significant improvements in both the measured immune responses (Lysozyme activity in the serum and Respiratory burst activity) as well as the survival rate, with both responses showing a close correlation with each other, and the moderate and high concentrations again showing no significant differences between each other. The results have shown that probiotic supplementation improved growth rate of the fish, in addition to fish immune status which is in line with the multiple and complementary mechanisms of action claimed in the aquaculture literature for probiotic microorganisms.

Water quality analysis revealed that temperature was kept constant between treatments, probiotic supplementation was significant for improvement in dissolved oxygen and pH and statistically significant reduction in ammonia and nitrate, with highest concentration treatment improving water quality profile. The regression analysis confirmed the curvilinear (diminishing-returns) relationship between probiotic concentration and weight gain, but a more consistent linear (undiminished) relationship between probiotic concentration and ammonia reduction, indicating that the water quality benefits of probiotic supplementation may be somewhat more consistent with increasing concentration than direct growth benefits within the range tested. Furthermore, the highly negative correlation of ammonia concentration with weight gain across experimental replicates further suggests that the beneficial effects on water quality observed as a result of the probiotics was directly correlated to the observed improvements in fish growth performance, highlighting the interlinkage between the biological and environmental benefits of the probiotic feed supplementation in this study.

Conclusion and Recommendations

Overall, with growth responses, the highest dose of probiotic supplementation tended to be superior to lower doses, with potential for greater improvements at the moderate concentration; at the highest dose tested, immune responses and water quality parameters tended to be superior, but the feed conversion ratio and fish survival remained unaffected; and water quality parameters showed overall improvements that were statistically significant at all doses tested, with the moderate concentration exhibiting the greatest improvements. The findings confirm and build on the current literature of aquaculture by showing, in one controlled experimental setup, that the use of probiotics can be shown, simultaneously and measurably, to improve fish growth performance and immune status, while at the same time, improving rearing water quality; they also provide direct evidence of the inter-relatedness of these beneficial biological and environmental effects since the decrease in ammonia concentration was found to be strongly associated with increase in weight gain across experimental replicates. Based on this, it is recommended that the use of probiotic supplementation as a routine management practice be considered at moderate to high rate, as the growth and immune response response to moderate and high rates tested showed a diminishing marginal growth benefit and ammonia and nitrate reduction showed a diminishing benefit with high rate, suggesting that the moderate rate may provide a more cost-effective approach to aquaculture producers who are more concerned about growth and immune response, while producers of particular concern for water quality may consider the high rate due to the continued undiminished reduction in ammonia and nitrate. It is also recommended that probiotics should not only be used as a growth promoter, but as an integral part of the water quality management of aquaculture, as the benefits on water quality as shown in this and previous research is clear and could reduce the need for mechanical or chemical water quality interventions that are more intensive. Lastly, this was a study with a single species, single probiotic formulation, a relatively short experimental period, and a single environmental condition, so it would be beneficial to expand the experimental design into other fish species, other probiotic formulations, longer-term studies, and other environmental conditions to confirm the results of the current study and determine if the growth, immune and water-quality benefits seen in this study continue, intensify or weaken during longer-term supplementation of probiotics and under different environmental conditions.

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