Field application of a synergistic blend of organic acids to promote growth performance of fish and shrimp

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nfectious pathogens such as bacteria, viruses, parasites, and fungi threaten production in aquaculture. These threats manifest with various symptoms, from asymptomatic to reduced growth performance and severe infections. Even individuals without symptoms can spread diseases among cultured ponds, slowing growth and increasing production costs. These pathogens often go unnoticed but can further lead to significant outbreaks when multiple contributory factors come together. For example, tilapia lake virus (TiLV) has been reported in Thailand, affecting both healthy adult tilapia and fingerlings, but does not display any clinical signs or cause mortality (Senapin et al., 2018). Similarly, the white spot syndrome virus can remain asymptomatic in shrimp for an extended period but trigger outbreaks under specific stressors such as spawning or environmental changes like temperature and salinity (Lo and Kou, 1998; Hsu et al., 1999).

In the past, antimicrobial agents such as disinfectants and antibiotics to control the growth of pathogens have been commonly used during disease outbreaks. However, the disease had already spread by the time farmers noticed the symptoms or mortalities. This has led to the emergence of antibiotic-resistant bacteria due to the overuse of antibiotics. As regulations become stricter, the use of antibiotics is being reduced. Instead, various strategies, such as improving pond management and reducing pathogen loads through nutritional strategies, are gaining popularity and becoming the preferred methods to prevent disease and improve farm efficiency.

Evidence supporting the benefits of organic acids

Dietary supplementation of organic acids has shown promising potential in enhancing disease resistance and growth performance in aquatic animals. Synergistic effects in disrupting bacterial cell walls and altering cytoplasmic pH, have been shown to effectively inhibit the growth of both gram-positive and gram-negative bacteria (Batovska et al., 2009; Defoirdt et al., 2009). Furthermore, the combinations of organic acids have contributed to energy generation in multiple metabolic pathways, including ATP production (Lim et al., 2015). The beneficial effects of including organic acids in the diet have been documented in several commercially important farmed aquatic species, including tilapia and shrimp (Ng et al., 2009; Nuez-Ortín et al., 2020)

Bacti-Nil®Aqua is a blend of organic acids designed specifically for aquatic species. It effectively combats the clinical and subclinical presence of pathogenic bacteria, which in turn promotes growth, particularly in high-density farming environments. Research under controlled infection conditions has demonstrated the efficacy of the health additive in reducing the impact of

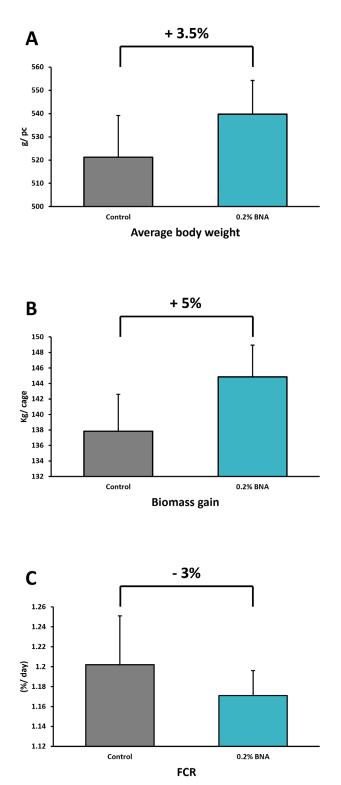


Figure 1. Effects of 0.2% Bacti-Nil®Aqua on average body weight (A), biomass gain (B), and FCR (C) of tilapia after 12 weeks of feeding.

francisellosis and streptococcosis in tilapia (da Silva et al., 2023), or vibriosis in shrimp (Morales-Covarrubias et al., 2022; Eissa et al., 2022).

This article presents two studies, one in tilapia and the other in whiteleg shrimp, that further corroborate the efficacy of Bacti-Nil[®]Aqua to control subclinical infections and promote growth in farm conditions.

Promoting health and growth performance of tilapia

The tilapia experiment conducted in the central region of Thailand, aimed to assess the impact of Bacti-Nil[®] Aqua on health and feed performance of tilapia in a farm trial setting. The study evaluated a control feed and a treatment 0% fishmeal feed both formulated to contain 32% crude protein and 4% fat. This blend of organic acids was supplemented at 0.2% in the treatment feed.

Tilapia were housed in cages within a reservoir pond, with each cage measuring $4 \times 3 \times 1.2m$. A total of four replicates were used for each treatment, with 300 red tilapia fingerlings in each cage. The average initial weight of the fish was 56.5g, and the culture period spanned 12 weeks, involving two daily feedings.

At the beginning of the trial, a comprehensive health assessment was conducted, including general necropsy

and pathology examinations, to ensure that the fish were healthy and free of specific pathogens. Regular sampling occurred every four weeks, involving weighing and counting the fish to adjust feeding and monitor mortality and abnormal behaviours.

After 12 weeks of feeding, all the fish in the control and supplemented groups remained healthy, with no signs of infection as per the health assessments. Negative results were obtained for all tests conducted for *Streptococcus*, parasitology, and the TiLV PCR test. The survival rate exceeded 99% at harvest in all groups, which can be attributed to cooler water temperatures (i.e. around 28°C and on average 3°C lower than the previous year) and the subsequent lower risk of streptococcosis.

Despite the lack of environmental challenge, the use of Bacti-Nil[®]Aqua improved growth performance, evidenced by an average increase of 3.5% in body weight, 5% gain in total biomass, and a 3% reduction in the feed conversion rate (Figure 1). A recent study found that tilapia fed diets supplemented with Bacti-Nil[®]Aqua for 21 days showed a change in the fish's gut microbiota, reducing harmful bacteria like Vibrio spp. and increasing beneficial probiotics like *Cetobacterium* and *Bacillus* spp (da Silva et al., 2023). These are known to produce beneficial metabolites that support fish health and growth.

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The functional additive increased feed cost, but this investment was paid off by the biomass gain and economic returns. The return on investment (ROI) calculation indicated that for each USD invested in the additive application, there was a gain of USD3.5.

In summary, this farm tilapia trial demonstrated that incorporating a preventive dose of 0.2% Bacti-Nil®Aqua in feed has the potential to positively impact farm profitability even when production conditions are more optimal than expected.

Improving the performance quality of extensive shrimp feeds

A shrimp trial was conducted in a farm located in southerncentral Mexico during May-July. The main objective of the research was to improve the performance of an extensive feed to reach that of a semi-extensive feed under a semiextensive culture system. Three feeds were tested: a semiextensive feed as positive control, and extensive feed as negative control, and an extensive feed supplemented with 0.2% Bacti-Nil®Aqua.

The extensive feed was formulated to the same protein and fat levels (35% and 6%, respectively) than those of the semi-extensive feed, but included less fishmeal and lowerquality protein sources. The cost of the extensive feed supplemented with the additive was lower than that of the semi-extensive feed.

Healthy juvenile shrimp of approximately 3g were stocked in submerged cages $(3 \times 3 \times 1m)$ at a density of 25 shrimp/ m². Feed was provided twice daily, and the feeding ratio was adjusted based on feeding tray consumption and weekly biometric measurements.

Results show high survival rates of around 90%. This was attributed to the period during which the trial was conducted; May-July is considered an optimal period in terms of environmental challenges and disease outbreaks in the area. Results also confirmed that 0.2% Bacti-Nil®Aqua improved the performance quality of the extensive feed. In relation to the extensive feed without supplementation, the additive significantly increased survival by 6% and numerically increased biomass gain and conversion efficiency by 7.8% and 8%, respectively (Figure 2). More importantly, biomass gain and conversion efficiency by the additive in the extensive feed were numerically similar to those achieved by the semi-extensive feed.

Such improvements resulted in an ROI of 18.1, indicating that the supplementation strategy, by improving the performance quality of extensive shrimp feed, can potentially bring a noteworthy positive impact on the profitability of semi-extensive farms.

Conclusion

Organic acid-based additives are commonly used in aquafeeds as a preventive strategy to reduce the impact of bacterial infections. Animals that receive Bacti-Nil®Aqua under an optimal culture environment have been shown to have improved survival and growth rates. This is likely attributed to better control of subclinical levels of pathogens and to a better use of energy resources towards growth promoting mechanisms. These studies validate the efficacy of Bacti-Nil® Aqua as an additive to ensure the performance and profitability of functional feeds.

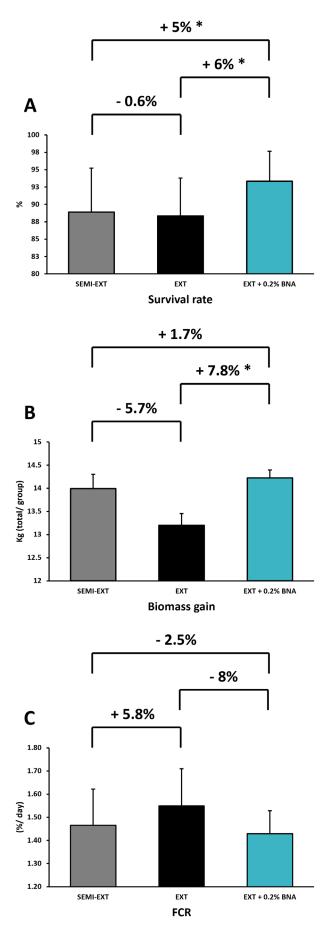


Figure 2. Effects of the extensive feed +0.2% Bacti-Nil[®]Aqua diet on survival (A), biomass gain (B), and FCR (C) on shrimp in comparison to semi-extensive and extensive control diets after 70 days of feeding. The results show significant differences (p<0.05) compared to the control feeds.

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