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Growth performance and feed efficiency of Nile tilapia (*Oreochromis niloticus*) supplemented with β-glucan, mannan oligosaccharides, and nucleotides under different trial periods and sizes of fish

Desempenho zootécnico e eficiência alimentar de tilápias-do-Nilo (*Oreochromis niloticus*) suplementadas com β-glucano, mananoligossacarídeos e nucleotídeos sob diferentes períodos experimentais e tamanhos de peixes

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Highlights

Yeast nucleotides improved growth of Nile tilapia in a 46-day trial vs control.
Larger fish ate less but grew at a satisfactory rate with MOS/β-glucan+nucleotides.
No differences in zootechnical data in the 30-day trial.
Additives showed positive results for aquaculture use.
Findings serve as a basis for future research on additives.

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Abstract

Nile tilapia (*Oreochromis niloticus*) is the most commercially available species of freshwater fish in Brazil. As producers intensify their operations to meet market demand, there is a growing need for strategies to accelerate growth and optimize feed use. Functional feed additives such as β-glucan, mannan oligosaccharides (MOS), and dietary nucleotides have been researched individually, with the goal of enhancing growth performance; however, studies on their combined use in tilapia remain limited. The aim of this study was to compile and analyze growth performance data from three trials evaluating these additives, applied individually and in combination, in Nile tilapia under different initial weights, feeding durations, and rearing conditions. A total of 492 fish were used in the three experiments. In the first 46-day trial with fingerlings (~8 g), six treatments were tested: 1) control diet, 2) β-glucan/MOS (2 g/kg), 3) Nucleotides 15% (2.1 g/kg), 4) β-glucan/MOS combined with Nucleotides 15% (1.05 g/kg each), 5) Free nucleotides (2.1 g/kg), and 6) Free nucleotides+β-glucan/MOS (1.05 g/kg each). The results showed that the diet containing nucleotides extracted from Saccharomyces cerevisiae cell walls (nucleotides 15%) improved the final weight, length, and specific growth rate compared to the corresponding values in the control group, emphasizing the benefits of polynucleotide structures in nutrient absorption. In the second trial (30 days) fish weighing ~25 g were used to specifically evaluate the combined additive (β-glucan/MOS + nucleotides 15% at 1.05 g/kg each) in comparison to the control group. The fish receiving this diet consumed significantly less feed daily while maintaining a similar weight gain, possibly indicating improved feed efficiency and potential metabolic synergy of the additives. The third trial, which replicated the same six treatments as the first experiment but over only 30 days, showed no significant differences among groups in terms of either growth or feed parameters. Notably, none of the additives adversely affected performance in any trial, confirming their safety. These findings suggest that combining β-glucan, MOS, and nucleotides from two different sources, particularly yeast-derived nucleotides, can support enhanced growth over longer feeding periods and may (when combined with β-glucan/MOS) reduce feed intake in larger fish without compromising gains. In future studies, the feeding period should be extended beyond 46 days, and responses to health or environmental stress should be analyzed to better understand the additional benefits of the proposed diet. In summary, this study demonstrates the potential of integrated nutritional strategies to improve the efficiency and sustainability of Nile tilapia production.

Key words: Aquafeed. Feeding trials. Polysaccharides. Prebiotic. Zootechnical parameters.

Resumo

A tilápia do Nilo (*Oreochromis niloticus*) é a espécie de peixe de água doce mais disponível comercialmente no Brasil. À medida que os produtores intensificam suas operações para atender à demanda do mercado, há uma necessidade crescente de estratégias para acelerar o crescimento e otimizar o uso da ração. Aditivos funcionais na alimentação como β-glucano, manano-oligossacarídeos (MOS) e nucleotídeos dietéticos têm sido pesquisados individualmente com o objetivo de melhorar o desempenho de crescimento; no entanto, os estudos sobre seu uso combinado em tilápias permanecem limitados. O objetivo deste estudo foi compilar e analisar dados de desempenho de crescimento de três ensaios que avaliaram esses aditivos, aplicados individualmente e em combinação, em tilápias do Nilo sob diferentes pesos iniciais, durações de alimentação e condições de criação. Um total de 492 peixes foi

utilizado nos três experimentos. No primeiro ensaio, com duração de 46 dias, utilizando alevinos (~8 g), foram testados seis tratamentos: 1) dieta controle, 2) β-glucano/MOS (2 g/kg), 3) Nucleotídeos 15% (2,1 g/kg), 4) β-glucano/MOS combinado com Nucleotídeos 15% (1,05 g/kg cada), 5) Nucleotídeos livres (2,1 g/kg) e 6) Nucleotídeos livres + β-glucano/MOS (1,05 g/kg cada). Os resultados mostraram que a dieta contendo nucleotídeos extraídos das paredes celulares de Saccharomyces cerevisiae (nucleotídeos 15%) melhorou o peso final, o comprimento e a taxa de crescimento específico em comparação com os valores correspondentes do grupo controle, enfatizando os benefícios das estruturas polinucleotídicas na absorção de nutrientes. No segundo ensaio (30 dias), foram utilizados peixes com peso de ~25 g para avaliar especificamente o aditivo combinado (β-glucano/MOS + nucleotídeos 15% a 1,05 g/kg cada) em comparação ao grupo controle. Os peixes que receberam essa dieta consumiram significativamente menos ração diariamente, mantendo ganho de peso semelhante, possivelmente indicando melhor eficiência alimentar e uma sinergia metabólica potencial dos aditivos. O terceiro ensaio, que replicou os mesmos seis tratamentos do primeiro experimento, mas por apenas 30 dias, não mostrou diferenças significativas entre os grupos em termos de crescimento ou parâmetros de alimentação. Notavelmente, nenhum dos aditivos afetou negativamente o desempenho em qualquer ensaio, confirmando sua segurança. Esses achados sugerem que a combinação de β-glucano, MOS e nucleotídeos de duas fontes diferentes, particularmente nucleotídeos derivados de levedura, pode favorecer um crescimento aprimorado ao longo de períodos mais longos de alimentação e pode (quando combinada com β-glucano/MOS) reduzir o consumo de ração em peixes maiores sem comprometer os ganhos. Em estudos futuros, o período de alimentação deve ser estendido para além de 46 dias, e as respostas a estresses de saúde ou ambientais devem ser analisadas para melhor compreender os benefícios adicionais da dieta proposta. Em resumo, este estudo demonstra o potencial de estratégias nutricionais integradas para melhorar a eficiência e a sustentabilidade da produção de tilápia do Nilo.

Palavras-chave: Aquafeed. Experimentos de alimentação. Polissacarídeos. Prebiótico. Parâmetros zootécnicos.

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important fish species in global aquaculture and is particularly prominent in Brazil, where it ranks as the foremost freshwater species. According to the Brazilian Fish Farming Association, the farmed tilapia industry has achieved record outputs exceeding 662,230 metric tons annually, which represents nearly 70% of the total farmed fish production in Brazil (Associação Brasileira da Piscicultura [Peixe BR], 2025). This species exhibits great adaptability to a variety of production systems and water quality, making it suitable for regions with limited water resources. Thus, Nile tilapia is a foundational species for sustainable aquaculture growth, with the overall goal of meeting rising protein demands while supporting economic development (Prabut et al., 2019). As the industry scales up to meet the demand, producers face increasing challenges related to optimizing growth under conditions that often involve crowding and environmental stress, and there is growing emphasis on higher growth rates, in order to deliver adequately sized

fish to market in shorter production cycles; accelerating growth rates also allows more efficient use of farming areas (Rodríguez-Hernández et al., 2025).

Driven by the rapid advancement of animal nutrition science and the global shift toward more precise and functional feeding practices, the aquaculture industry has increasingly embraced new dietary strategies (Sonea et al., 2023). Among these nutritional approaches, the use of functional feed additives, such as prebiotics, stands out, reflecting broader efforts to align aquaculture practices with scientific advances (Brum et al., 2025; Leal et al., 2024). β-glucan, a natural polysaccharide primarily derived from yeast cell walls, is widely recognized for enhancing non-specific immune responses in fish (Machuca et al., 2022). Mannan oligosaccharides (MOS) promote gut health and nutrient utilization (Wang et al., 2022a). Dietary nucleotides serve as essential building blocks for nucleic acids and are particularly important for rapidly dividing cells, thereby supporting immune function and resilience under stress (Madhulika et al., 2025).

Although the individual effects of these supplements are well-documented, studies evaluating the combined inclusion of these additives in Nile tilapia diets remain scarce. Exploring such combinations could offer synergistic benefits and enhance productivity beyond what can be achieved with single additives (Amillano-Cisneros et al., 2023). In this context, we aimed to compile and analyze growth performance data from three independent trials involving Nile tilapia that were fed diets supplemented with β-glucan, MOS, and two different types of dietary nucleotides. By comparing results across different cultivation periods, initial fish sizes, and hypoxic conditions, this study offers new insights into how these factors and their unique additive combinations influence tilapia production, potentially contributing to shorter and more efficient production cycles.

Materials and Methods

All experimental procedures involving animals were reviewed and approved by the Ethics Committee on Animal Use of the State University of Londrina (CEUA UEL, protocol no. 053.2021) and the trials were carried out at the Laboratory of the Aquaculture and Genetics Research Center (NEPAG), also affiliated with UEL.

A total of 492 Nile tilapia were used in all experiments. They were acclimated to the experimental conditions for 15 days before being subjected to the different treatments. After acclimation, the fish were anesthetized with benzocaine at 0.1 $g \cdot L^{-1}$ (prepared by diluting 0.1 $g \cdot mL^{-1}$ in 96% ethanol and adding to 10 L of water), placed on wet towels to minimize stress, weighed (g), measured (length, in cm), and then redistributed into the experimental tanks.

The first experiment was conducted in a recirculating aquarium system equipped with mechanical filtration using polyester fiber as well as sponge filters that had been previously colonized with the aid of a commercial biological starter (Delta Z Biotec, São Paulo, SP, Brazil) to support ammonia control. The second and third experiments were conducted in static aquariums, where similarly colonized sponge filters were used

to maintain water quality. All aquariums in the three trials were additionally supplied with porous air stones to enhance the dissolved oxygen levels in conjunction with the sponge filters. The trials were standardized with respect to key husbandry variables, including water temperature, feeding rate, feeding frequency, photoperiod, and aeration. Additionally, the biological filtration strategy was equivalent across the systems, and the water quality parameters were monitored and maintained to minimize any system-related bias.

Daily water renewal was conducted by replacing 50% of the aquarium volume. During the final third of the experiment, the exchange rate was increased to 70% and was performed twice daily, aiming to maintain optimal conditions throughout the trial. Water quality parameters were monitored periodically using multiparameter instruments (Hanna Instruments, Barueri, SP, Brazil), a pH meter (Akso, São Leopoldo, RS, Brazil), and a commercial colorimetric test kit (Labcon Test Amônia Tóxica Água Doce, Alcon Pet, Camboriú, SC, Brazil). Water temperature was maintained by controlling the laboratory environment using an air-conditioning system combined with thermal insulation of the room to prevent external temperature fluctuations. Throughout the experimental period, the mean water quality parameters compiled from the three experiments were maintained at 25.53 ± 1.36 °C for temperature, 4.53 ± 0.97 $mg \cdot L^{-1}$ for dissolved oxygen, 6.24 ± 0.38 for pH, and 1.5 ± 1.22 $mg \cdot L^{-1}$ for total ammonia nitrogen (TAN). At the recorded mean pH and temperature, less than 0.3% of TAN is converted to NH_3, resulting in an estimated NH_3 concentration of approximately 0.004–0.006 $mg \cdot L^{-1}$.

Preparation of feed

A commercial extruded feed for juvenile fish was used throughout the study (SUPRA®), containing 46% crude protein, 8% ether extract, 3% crude fiber, and 14% mineral matter. The pellet size was 1.7 mm. The additives were measured for each treatment (g/kg) and then incorporated into the feed by first diluting them in distilled water at a ratio of 40 mL per 1 kg of feed. To promote adherence of the additives to the pellets, the same ratio was used, with 40 mL of a binding solution containing carboxymethylcellulose, methylparaben, and propylparaben (Universal Vehicle, Vansil, Descalvado, SP, Brazil) added to the solution and thoroughly mixed with the feed to ensure even distribution. After mixing, the feed was dried in a laboratory oven at 60 °C for 24 h until it reached its original pellet hardness and moisture content. The control treatment did not include any additives but only a binding solution.

Biorigin® supplied the additives employed in this experiment. The first additive, containing β-glucan and MOS (MOS/β-glucan), was derived from yeast cell walls. The MOS component in the product is solubilized to enhance dispersion along the intestinal tract, while this process also partially exposes the β-glucans, enabling them to exert their effects within the gut. The second additive (Nucleotides 15%) consists of 15% yeast RNA (Saccharomyces cerevisiae), offering a high concentration and bioavailability of nucleotides naturally found in cellular structures. The inclusion of nucleotides in the form of polynucleotides may also facilitate a more gradual release and absorption by the organism (Xu et al., 2015).

The third additive (Free Nucleotide) contains 15% free nucleotides, which may be either synthetic or sourced from other origins and are not bound to a cellular matrix, unlike yeast-extracted nucleotides. This form is potentially more readily bioavailable, allowing for rapid uptake and providing a direct source of nucleotides (Barducci et al., 2022).

First trial

For the first trial, a total of 216 Nile tilapia fingerlings with an initial average weight of 8.1 ± 0.9 g were used. The fish were distributed in a completely randomized design with five treatments (T2: MOS/β-glucan: 2 g/kg, T3: Nucleotides 15%: 2.1 g/kg, T4: MOS/β-glucan + Nucleotides 15%: 1.05 g/kg from each, T5: Free Nucleotides: 2.1 g/kg, and T6: Free Nucleotides + MOS/β-glucan) and a control treatment (T1), each with three replicates. Twelve fish were allocated to 18 aquariums (60 L), amounting to 36 fish per treatment. The experimental period lasted 46 days, and the fish were fed to apparent satiety. At the end of the experimental period and after a 12-h fasting period, all fish were subjected to biometric assessments to measure growth performance parameters. To minimize stress during handling, the animals were anesthetized using the method described above, and the following measurements were taken: final weight (g), total length (cm), and standard length (cm). These results served as the basis for calculating growth and productivity indicators: daily feed intake (DFI, g) [amount of feed consumed by the fish per day on a wet-matter basis], daily weight gain (DWG, g) [final weight – initial weight) / number of days], feed conversion ratio (FCR) [feed intake on a wet-matter basis (g) / weight gain (g)], total length growth (TLG, cm) [final total length - initial total length], standard length growth (SLG, cm) [initial standard length -final standard length], condition factor using total length (CFTL, %, calculated as[(final weight / final total length (cm)^3)× 100], condition factor using standard length (CFSL, %, calculated as [(final weight / final standard length (cm)^3)× 100], and specific growth rate (SGE, %/day, calculated as [((ln final weight (g) – ln initial weight (g)) / experimental period in days) × 100]. All parameters were analyzed using the methods described by Lopera-Barrero et al. (2024). ANOVA was conducted using R Studio (v3.1.4), and significant differences between means were determined using Tukey's test at $P < 0.05$.

Second trial

The second trial was designed as a follow-up experiment to evaluate the effects of the MOS/β-glucan + nucleotides 15% treatment on juvenile Nile tilapia of greater initial size than the first and third one. The Nucleotide 15% treatment (T3 in the previous trial) alone had shown the best performance in key growth parameters, and β-glucan/MOS (T2 in the previous trial) contributed less prominently. Other combinations did not improve the performance or overlap in effect with the control group when considering individual additives and combinations a finding that yields limited additional insight. This allowed us to investigate whether the possible synergistic effects of the immunomodulatory and metabolic/nutritional support components with potentially complementary mechanisms of action were more evident under different experimental

conditions. For this trial, a smaller number of fish were used (60 Nile tilapia). This parallel experiment was conducted using five 40-L aquariums stocked with fish, averaging an initial weight of 25 g. The use of smaller tanks and larger fish required reduced total biomass per aquarium to maintain water quality and ensure the welfare of the fish throughout the experiment. The experimental period was shortened to 30 days to determine whether the effects of the treatment could be observed within a shorter timeframe. Twelve fish were allocated to each aquarium, with two of them designated as the Control group (24 fish total) and three aquariums randomly assigned to receive the treatments corresponding to T4 in the previous one (MOS/β-glucan + nucleotides 15%, 36 fish in total). The experimental period lasted 30 days, during which the fish were fed until apparent satiation. All feeding and analyses followed the same procedure as described in Experiment 1.

Third trial

For the final trial, the same number of fish, initial weight, and experimental design as those described in the first experiment were used. One difference was that the fish were distributed across 18 aquariums of 42 L each operating under a static water system. All treatments received the same commercial feed formulated for Nile tilapia, along with the same feeding preparations, treatments, protocols, and water quality management practices as in Experiment 1. However, the trial was conducted over a period of 30 days. The purpose of this reduced duration was to evaluate whether previously observed growth and feed-utilization patterns could be consistently reproduced within a shorter experimental window, similar to Trial 2, providing complementary evidence of treatment effects while minimizing long-term system variability. Lastly, calculations were performed to determine the final weight, daily feed intake, daily weight gain, and feed conversion ratio. ANOVA was conducted using R Studio (v3.1.4), and significant differences between means were determined using Tukey's test at $P < 0.05$.

Results and Discussion

In the first experiment (Table 1), no significant differences ($P > 0.05$) were observed among the treatments in terms of initial weight (IW), daily feed intake (DFI), feed conversion ratio (FCR), initial total length (ITL), initial standard length (ISL), or condition factors calculated using total length (CFTL) and standard length (CFSL). The lack of differences in IW, ITL, and ISL validated the initial experimental setup and ensured that any subsequent effects could be objectively attributed to the dietary additives. However, significant differences ($P < 0.05$) were found among the treatments for final weight (FW), daily weight gain (DWG), final total length (FTL), total length growth (TLG), final standard length (FSL), standard length growth (SLG), and specific growth rate (SGR). Treatment T3 (Nucleotides 15%) exhibited superior results compared to the control group (T1), whereas the other treatments showed intermediate values that did not differ statistically. T3 also showed similar performance in DWG and SGR when compared to T2 (MOS/β-glucan) and T5 (Free Nucleotides), respectively, both being better than the control group.

Dietary nucleotides extracted from the cell walls of S. cerevisiae, are essential components of nucleic acids and cellular metabolism; however, endogenous synthesis may be insufficient in rapidly growing fish. They also contribute to intestinal development by enhancing mucosal integrity, cell proliferation, and repair processes, which improve nutrient absorption and overall digestive efficiency (El-Bab et al., 2022). These bound nucleotides may offer more gradual release and sustained availability, potentially enhancing intestinal health and immune modulation more effectively than free nucleotides, which are more rapidly absorbed but less sustained (Xu et al., 2015). Our findings are consistent with previous reports that nucleotides are conditionally essential during periods of rapid growth, facilitating enhanced protein synthesis and cellular proliferation (Ding et al., 2021). The treatment T2, containing MOS and β-glucan, likely contributed by promoting gut integrity and modulating the intestinal microbiota, improving nutrient assimilation and overall health (Mameloco & Traifalgar, 2020), promoting a superior DWG than the control group, similar to the work of Hoang et al. (2024). In addition, T5, which supplies free nucleotides, potentially offers more immediate support to cellular functions owing to the rapid absorption nature of free nucleotides, in contrast with nucleotides bound within yeast cell walls, which may allow a slower, prolonged availability (Xu et al., 2015). Thus, both types of nucleotides seem to help compensate for endogenous limitations and support the key anabolic processes required for growth through different mechanisms. Under conditions without significant health challenges, the immediate availability offered by free nucleotides may have been sufficient to match the SGR observed with nucleotide 15%, explaining why both outperformed the control group. Furthermore, it is reasonable to expect that under prolonged stress or sanitary challenges, the feed supplemented with nucleotides at 15% may offer additional advantages owing to its ability to deliver a continuous supply of these precursors.

Table 1
Performance parameters (mean ± standard deviation) of Nile tilapia fingerlings fed diets supplemented with β-glucan, mannan oligosaccharides, and dietary nucleotide from two different sources for 46 days

Parameters	T1	T2	T3	T4	T5	T6	P-value
IW	8.37±2.35	8.10±1.29	7.88±2.26	8.88±2.44	7.59±1.97	7.82±2.17	0.729
FW	61.39±16.15b	78.87±15.46ab	83.89±18.07a	74.02±19.46ab	75.95±18.07ab	73.84±18.60ab	0.025
DFI	1.06±0.01	1.16±0.07	1.23±0.09	1.20±0.04	1.03±0.26	1.16±0.08	0.530
DWG	1.15±0.01b	1.54±0.05a	1.65±0.19a	1.42±0.08ab	1.49±0.17ab	1.43±0.05ab	0.017
FCR	0.92±0.02	0.75±0.07	0.75±0.04	0.85±0.07	0.71±0.21	0.81±0.03	0.350
ITL	7.49±0.76	7.50±0.41	7.39±0.71	7.80±0.74	7.45±0.64	7.43±0.66	0.533
FTL	14.18±1.32b	15.15±0.94ab	15.57±1.10a	14.92±1.37ab	15.09±1.25ab	15.15±1.40ab	0.048
TLG	6.69±1.33b	7.65±0.89ab	8.18±0.98a	7.12±1.49ab	7.64±1.18ab	7.72±1.30ab	0.011
ISL	6.03±0.65	6.02±0.36	5.97±0.59	6.27±0.62	5.93±0.57	5.97±0.59	0.576
FSL	12.07±1.04b	12.76±0.78ab	13.21±0.95a	12.77±0.92ab	12.66±1.05ab	12.70±1.07ab	0.046
SLG	6.04±1.07b	6.74±0.73ab	7.24±0.89a	6.50±1.12ab	6.73±0.99ab	6.73±1.00ab	0.024
CFTL	2.10±0.18	2.16±0.11	2.18±0.12	2.18±0.20	2.16±0.13	2.09±0.22	0.480
CFSL	3.41±0.39	3.62±0.28	3.58±0.28	3.45±0.35	3.67±0.26	3.53±0.27	0.143
SGR	4.33±0.76b	4.88±0.50ab	5.18±0.59a	4.60±0.77ab	5.01±0.66a	4.89±0.76ab	0.007

T1: Control, T2: β-glucan/MOS , T3: Nucleotide 15%, T4: β-glucan/MOS + Nucleotide 15%, T5: Free Nucleotide, T6: β-glucan/MOS + Free Nucleotide. Initial weight (IW, g), final weight (FW, g), daily feed intake (DFI, g), daily weight gain (DWG, g), feed conversion ratio (FCR), initial total length (ITL, cm), final total length (FTL, cm), total length growth (TLG, cm), initial standard length (ISL, cm), final standard length (FSL, cm), standard length growth (SLG, cm), condition factor using total length (CFTL, %), condition factor using standard length (CFSL, %), specific growth rate (SGR, % day^{-1}). Lowercase letters in the row indicate statistically significant differences ($P \leq 0.05$).

Although most of the other treatments did not differ between treatment T3 and the control group, the results showed that the supplementation with β-glucan/MOS as well as the feed containing free nucleotides, alone and in combination, has no adverse effects detected, supporting the additives safety. Fish fed these diets maintained growth within expected biological standards; thus, these feed types would be expected to provide resilience under other, more challenging farming scenarios (e.g., disease or environmental stress), in which immune-enhancing effects become more pronounced.

In the second trial, which involved fish with a higher initial body weight (Table 2), significant differences were detected only DFI, where the control group exhibited increased consumption compared with the other group ($P > 0.05$). None of the other evaluated parameters were influenced by the selected additive, indicating that under these specific conditions, the additive did not affect growth performance or feed efficiency. Similarly, the third experiment, conducted under comparable initial conditions and management to the first trial, with a shorter duration (30 d), revealed no significant differences in zootechnical parameters between the treatments.

Table 2
Performance parameters (mean ± standard deviation) of Nile tilapia ranging from 25 to 69 g, fed diets supplemented with β-glucan/MOS + Nucleotide 15% for 30 days

Parameters	Control	β-glucan/MOS + Nucleotide 15%	P-value
IW	25.66±3.62	23.64±5.08	0.274
FW	65.87±10.49	69.23±10.70	0.444
DFI	0.855±0.005a	0.805±0.026b	0.001
DWG	1.489±0.432	1.688±0.403	0.255
FCR	0.613±0.154	0.503±0.129	0.071
ITL	11.26±0.505	10.93±0.866	0.273
FTL	14.85±0.689	15.21±0.706	0.222
TLG	3.591±0.944	4.275±0.899	0.083
ISL	3.050±0.848	3.717±0.720	0.059
FSL	9.28±0.389	8.97±0.693	0.193
SLG	12.32±0.703	12.68±0.704	0.225
CFTL	1.997±0.075	1.985±0.397	0.920
CFSL	3.501±0.198	3.441±0.800	0.804
SGR	2.046±0.489	2.355±0.511	0.144

Initial weight (IW, g), final weight (FW, g), daily feed intake (DFI, g), daily weight gain (DWG, g), feed conversion ratio (FCR), initial total length (ITL, cm), final total length (FTL, cm), total length growth (TLG, cm), initial standard length (ISL, cm), final standard length (FSL, cm), standard length growth (SLG, cm), condition factor using total length (CFTL, %), condition factor using standard length (CFSL, %), specific growth rate (SGR, % day^{-1}). Lowercase letters in the row indicate statistically significant differences ($P \leq 0.05$).

The results of the first performance experiment (Table 1) indicated that the experimental period (46 days) was sufficient to elicit the individual effects of each additive and their combinations. On the other hand, the results from the second performance trial (Table 2) showed that even with the use of one additive (in this case MOS/β-glucan + Nucleotides 15%) in larger fish (25 g initial weight) and over a shorter feeding period (30 days), there was evidence of effects on zootechnical parameters only for daily feed intake (DFI). There was lower feed consumption in the supplemented group, and the treated fish showed similar weight gain to the control, suggesting that the additive mix may have enhanced feed efficiency or nutrient utilization, allowing fish to achieve comparable growth with reduced feed intake. This result is compatible with studies utilizing β-glucan (Hoang et al., 2018), MOS (Wang et al., 2022b), and nucleotide (Liu et al., 2020).

The reduced feed intake observed in the supplemented fish could also be related to improved satiety or altered feeding behavior owing to enhanced nutrient absorption (Bowyer et al., 2019; Jami et al., 2019; Hoang et al., 2024). Importantly, despite consuming less feed, these fish did not show a reduction in growth performance, which is advantageous for aquaculture systems aimed at optimizing feed costs and reducing waste. There is a possibility that if the second trial had lasted 46 days or more, other parameters might have been influenced, possibly similar to those observed in the first performance experiment. Notably, the FCR observed in all three experiments could be considered low (e.g., 0.503 ± 0.129 in Trial 2) and was not significantly influenced by treatment ($P > 0.05$). Such FCRs are plausible under laboratory conditions because controlled environmental factors, such as stable temperature, oxygenation, ideal photoperiod, and ad libitum feeding, minimize stress and maximize feed utilization efficiency, as reported in other studies on tilapia and other fish under similar laboratory conditions and initial weights (Trejchel et al., 2014; De Verdal et al., 2018; Rodde et al., 2021).

The third experiment (Table 3), unlike the first one, which demonstrated clear benefits of dietary supplementation over a longer duration, showed that the shorter duration than Experiment 1 and smaller fish size than Experiment 3 may have limited the observable effects of the additives. The lack of significant results in the third trial contrasts with the increased feed intake observed in the second experiment involving larger fish and highlights that fish size and feeding period are key factors influencing the response to dietary additives. It is possible that the 30-day feeding period was too short for the additives to exert measurable effects in smaller fingerlings, or that their physiological demands were not sufficient to reveal benefits. Importantly, the absence of negative effects on growth performance, feed intake, or feed conversion indicates that supplementation with β-glucan, MOS, and dietary nucleotides did not impair fish health or productivity in this case.

Table 3
Performance parameters (mean ± standard deviation) of Nile tilapia fingerlings fed diets supplemented with β-glucan, MOS, and dietary nucleotide from two different sources for 30 days

Parameters	T1	T2	T3	T4	T5	T6	P-value
IW	7.91±1.74	7.70±1.96	7.12±1.62	7.70±1.78	7.65±1.87	7.02±1.61	0.117
FW	31.25± 2.15	34.50±2.79	30.69±1.50	33.20±1.17	33.50±1.07	30.75±1.06	0.203
DFI	0.66±0.03	0.68±0.05	0.68±0.09	0.67±0.01	0.71±0.07	0.68±0.04	0.969
DWG	0.77±0.08	0.89±0.09	0.78±0.05	0.84±0.04	0.86±0.04	0.79±0.04	0.378
FCR	0.86±0.12	0.76±0.03	0.86±0.07	0.79±0.06	0.82±0.05	0.86±0.06	0.605

T1: Control, T2: β-glucan/MOS , T3: Nucleotide 15%, T4: β-glucan/MOS + Nucleotide 15%, T5: Free Nucleotide, T6: β-glucan/MOS + Free Nucleotide. IW: Initial weight, FW: Final weight, DFI: Daily feed intake, DWG: Daily weight gain, and FCR: Feed conversion ratio.

Given these findings, future studies should involve an extension of the feeding period beyond 46 days to explore the long-term benefits and evaluate these additives under environmental or health challenge conditions. Additional research into the optimal inclusion levels and combinations of additives could further increase the knowledge about their use across different production stages.

Conclusions

The results of this study indicated that certain feed additives, especially those including yeast-derived nucleotides (nucleotide 15%), can enhance growth performance of Nile tilapia over longer feeding periods, as evidenced by the positive outcomes observed in the 46-day trial. In larger fish, supplementation reduced the daily feed intake while maintaining growth, suggesting improved feed efficiency.

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