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Effect of dietary nucleotide supplementation on growth performance and intestinal morphology in Nile tilapia (*Oreochromis niloticus*) fingerlings

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Received: September 2026 Revised:October 2026 Accepted: November 2026

Abstract

This study evaluated the effects of dietary nucleotide supplementation on growth performance, feed utilization, intestinal morphology, and survival of Nile tilapia (*Oreochromis niloticus*) fingerlings fed plant-based diets. Fish were fed diets containing different levels of nucleotide supplementation for 40 days. Growth performance and feed utilization parameters were significantly affected by dietary treatments ($p<0.05$), with estimated optimal inclusion levels varying according to the evaluated parameter. Supplementation levels of approximately 2.3 g kg^{-1} of diet were associated with higher final weight, specific growth rate, weight gain, and protein efficiency ratio. The lowest feed conversion ratio was estimated at 2.8 g kg^{-1}, while maximum protein retention was estimated at 2.5 g kg^{-1}. The lowest estimated mortality occurred at a supplementation level of approximately 2.6 g kg^{-1}. Feed intake was higher in fish receiving 1.0 and 4.0 g kg^{-1} than in fish receiving the other supplementation levels. In contrast, dietary nucleotide supplementation did not significantly affect intestinal fold height, fold-to-crypt ratio, crypt depth, or enterocyte height. Overall, the results indicate that dietary nucleotide supplementation at levels of approximately 2.0–3.0 g kg^{-1} can improve growth performance and feed utilization in Nile tilapia fingerlings fed plant-based diets. Further studies are needed to clarify the effects of nucleotide supplementation on survival and resistance to adverse conditions.

Keywords: Feed conversion ratio, Protein retention, Nutrient utilization, Feed intake, Survival, Plant-based diets

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[DOI: 10.22034/ANFS.1.2.71]

Introduction

Aquaculture plays an important role in global food production, contributing to the supply of animal protein and economic development. In Brazil, fish farming has expanded considerably, with Nile tilapia (*Oreochromis niloticus*) standing out as the leading farmed fish species in the country. In 2025, Brazilian farmed fish production exceeded 1 million tonnes, highlighting the expansion and economic importance of the sector (PEIXE BR, 2026). In this context, improving nutritional strategies is an important approach to promoting fish development and enhancing production efficiency.

Nucleotides are biological compounds involved in essential processes, including DNA and RNA synthesis, energy metabolism, and cell signaling. Although fish can synthesize nucleotides through endogenous metabolic pathways, dietary availability of these compounds may be important during periods of rapid growth or increased physiological demand. For this reason, dietary nucleotides have been investigated as functional ingredients in aquatic animal feeds because of their potential to influence growth, feed utilization, intestinal function, and immune responses (Li and Gatlin, 2006; Hossain *et al*., 2020).

However, the effects of nucleotide supplementation may vary according to fish species, developmental stage, nucleotide source, and experimental conditions. In Nile tilapia, Leal *et al*. (2025) evaluated supplementation with nucleotides and other functional additives and demonstrated that productive responses may vary according to fish size and trial duration. Similarly, Godinho *et al*. (2026) found that dietary supplementation with yeast RNA and yeast-derived free nucleotides did not significantly affect the growth performance of Nile tilapia juveniles, although some physiological and immune responses were modified. In addition to productive performance, intestinal morphology deserves attention because it provides information about the structural characteristics of tissues involved in digestion and nutrient absorption (Hossain *et al*., 2020).

Despite advances in research on dietary nucleotides in aquaculture, their effects at different developmental stages of Nile tilapia remain important to investigate, particularly in recently sex-reversed fingerlings. During this stage, growth and tissue development are relevant to production, making it appropriate to evaluate both productive performance indicators and intestinal morphological characteristics (Leal *et al*., 2025) . The combined assessment of these variables may improve understanding of fish responses to dietary supplementation (Barducci *et al*., 2022) . Therefore, this study aimed to evaluate the effects of dietary nucleotide supplementation on the productive performance and intestinal morphology of recently sex-reversed Nile tilapia fingerlings (*Oreochromis niloticus*).

Materials and methods

Experimental conditions

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This research was conducted at the Universidade Federal de Goiás, in the Laboratório de pesquisa em Aquicultura – LAPAQ. Animal care and experimental procedures were approved by of animal care and use committee of Universidade Federal de Goiás (protocol 137/11). The animals were randomly assigned into each 30, 7-L rectangular aquarium, connected as a closed recirculation system, constant aeration, porous stones and water flow rate set to 1 L / min in each aquarium. The system was provided with physical and biological filters that ensured the maintenance of water quality. The water temperature was thermostatically controlled.

Experimental diets

The basal diet was formulated to contain 32% of crude protein and estimated digestible energy level of 3.2 kcal g^{-1}. The experimental diets were increasing levels of nucleotides on basal diet, at the rate of 0.5, 1.0, 2.0 e 4.0 g kg^{-1} (Table 1).

Table 1: Composition proximate of experimental diets.

	Nucleotide levels (g kg^{-1})				
Ingredients (g kg^{-1})	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**
Soybean meal	510,5	510,5	510,5	510,5	510,5
Fish meal	50	50	50	50	50
Corn meal	200,5	200,5	200,5	200,5	200,5
Wheat meal	100	100	100	100	100
Rice meal	56,85	56,35	55,85	54,85	52,85
Cassava starch	54,85	54,85	54,85	54,85	54,85
Nucleotide	0	0,5	1	2	4
Dicalcium phosphate	20,3	20,3	20,3	20,3	20,3
Ascorbic acid	0,8	0,8	0,8	0,8	0,8
NaCl	1	1	1	1	1
Vit/min mix[1]	5	5	5	5	5
BHT	0,2	0,2	0,2	0,2	0,2
Calculated nutritional composition (g kg^{-1})					
y matter	881.7	881.7	881.8	881.9	882.0
Moisture	118.3	118.3	118.2	118.1	118.0
Crude protein	292.2	292.1	292.1	292.0	292.0
Ether extract	25.3	25.3	25.3	25.3	25.2
Ash	77.0	77.0	77.0	77.0	77.0
Crude fiber*	39.6	39.6	39.6	39.5	39.4
Nitrogen-free extract	447.6	447.7	447.8	448.1	449.0

1Vitamin and mineral mix (IU or mg kg−1premix): Folic acid 600 mg, Biotine 24 mg, coline 54 g, niacine 12000 mg, Calcium 6000 mg, vit.A 600000 UI, vitB1 2400 mg, vitB12 2400 mg, vitB2 2400 mg, viB6 2400 mg, vit C 24 g, vitD3 100000 UI, vitE 6000 mg, vitK3 1200 mg. Co 1 mg Cu 300 mg, Fe 5000 mg, iodine 10 mg, Mg 2000 mg, Se 10 mg, Zn 3000 mg

For the manufacture of animal feed, all ingredients were ground until sieve in a mesh diameter of 0,5 mm. Diets were mechanically mixed with water at 55ºC (25% of dry weight) and the moist mixture was extruded in a 4.0 mm die of a meat grinder. Diets were oven dried until present moisture <100 g/kg, and stored at -18ºC until further use. At the beginning of the experiment diets were

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ground and sieved in a mesh diameter according to fish size.

Animals and experimental design

Sex reversal of Nile tilapia (n=200, weight=0.88±0.02 g) arising from commercial fish farms were randomly assigned into 30 aquariums (containing six fish each) for 40 days. The experimental design was completely randomized, with five treatments and six replicates. For initial chemical evaluation of whole body, 20 sex reversal of Nile tilapia were collected before the assign, euthanized and stored in a freezer at -20°C. The animals were fed three times a day and weighed at the beginning at 0 and 40 days of experiment to determine the performance parameters: weight gain (GP); feed conversion (FC); Feed intake (FI)=Feed consumption (g)/((FW + IW)/2)×t); specific growth rate in weight (SRW)=100 x [log final weight (g) - initial weight log (g)] / t, protein efficiency ratio (PER)=weight gain (g) / crude $protein_{consumed}$ (g), protein retention rate (PRR)=(final weight x final body protein) - (initial weight x protein initial body) / protein intake x 100. Survival values were calculated as follows (%)=100×(amount of death fish)/(initial amount of fish).

Chemical analysis

The chemical analysis were carried out in the biochemistry laboratory of the Federal University of Goiás - Regional Jataí. The dry matter (method 930.15), crude protein (Nx6.25; method 988.05), lipids (method 920.39) and ash (method 942.05) in diets and whole body were determined according to AOAC Protocols (1995).

Intestinal morphology

At the end of the feeding trial, four fish per treatment were anesthetized with Benzocaine (ethyil-p-aminobenzoate, 100mg L^{-1}), euthanized by medullary section and orally injected formalin fixative solution. The entire gastrointestinal tract was removed and injected with formalin fixative solution and then transferred segments (w0.5 and 7cm length) of proximal, mid and distal intestine were sliced transversely into 5 μm sections and stained with hematoxylin and eosin (H&E). The slides were examined under a light microscope (Olympus, CX-41) equipped with a camera (Olympus SC 30) and VGA 460 Osteomeasure software (Osteometrics, Decatur, GA, for image acquisition) for assessing dimensions folds number (FN), fold height (FH), fold: crypt relation (F:C R), crypt depth (CD), and enterocyte height (EH) in different enteric sections (10 measurements per fish).

Statistical analysis

All data were analyzed for homoscedasticity of variance and the results were considered significant when $p<0.05$. The data were submitted to variance analysis and when significant differences among treatments were observed, it was proceeded the adjustment of different regression models. For the variables of that were significant, the means were compared

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using the Student Newman Keuls test (SNK). All analyzes were performed using the GLM procedure of the computer Statistical Analysis System (SAS 9.0).

Results

Water quality parameters were monitored daily throughout the experimental period. Mean water temperature and dissolved oxygen concentrations were 26.33±1.26°C and 6.95±0.92 mg L^{-1}, respectively, while mean pH and total ammonia concentrations were 6.63±0.24 and 0.67±0.63 mg L^{-1}, respectively.

Dietary nucleotide supplementation significantly affected all productive performance variables analyzed (p<0.05; Table 2).

Table 2: Final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion (FC), feed intake (CRA), mortality (MORT), protein efficiency ratio (PER) and protein retention rate (PRT) of tilapia fingerlings fed diets with graded levels of supplementation by nucleotides (n=6, mean±SD).

Level g/kg^{-1}	FW (g)	WG (g)	SRW (%)	FC	FI (g)	Mort (%)	PER (%)	PRR (%)
0.00	9.21±0.72	3.94±0.65	1.59±0.19	1.56±0.15	2.26±0.22	77.78±8.61	0.99±0.10	18.98±2.18
0.5	11.04±0.45	5.81±0.43	2.14±0.11	1.05±0.16	2.36±0.15	63.9±12.55	1.36±0.16	31.73±1.57
1.0	13.47±0.72	8.17±0.81	2.66±0.20	1.12±0.04	2.36±0.14	44.44±8.61	1.92±0.22	39.14±2.77
2.0	13.06±0.75	7.77±0.79	2.58±0.19	1.07±0.11	2.24±0.14	47.22±1.55	1.92±0.22	41.71±1.87
4.0	11.68±1.01	6.32±0.97	2.22±0.23	0.99±0.08	2.02±0.17	50.00±14.91	1.76±0.37	32.02±2.04
Regression								
Linear P value	ns	ns	ns	p<0.0001	p<0.0001	p<0.0001	ns	ns
Quadratic P value	p<0.0001	p<0.0001	p<0.0001	p<0.0001	ns	p<0.0001	p<0.0001	p<0.0001

ns – Not significant at 5% probability.

Linear and/or quadratic regression models provided the best fit to the data, with coefficients of determination (R^2) greater than 0.60. Derivative analysis of the regression equations estimated a supplementation level of 2.3 g kg^{-1} for maximum final weight, specific growth rate, and weight gain. Supplementation levels above this estimate tended to reduce growth performance. The similar estimated levels for these three variables indicate a consistent response in growth-related parameters. Feed intake and feed conversion ratio showed different response patterns, and linear regression models provided the best fit to the data. The estimated nucleotide supplementation level associated with the lowest feed conversion ratio was 2.8 g kg^{-1}. Fish fed diets containing 1.0 and 4.0 g kg^{-1} of nucleotides showed higher feed intake than fish receiving the other supplementation levels. Dietary nucleotide supplementation also significantly affected protein utilization, as indicated by protein efficiency and protein retention rate (p<0.05). Regression analysis estimated supplementation levels of 2.3 and 2.5 g kg^{-1} for maximum protein efficiency and protein retention, respectively (Fig. 1).

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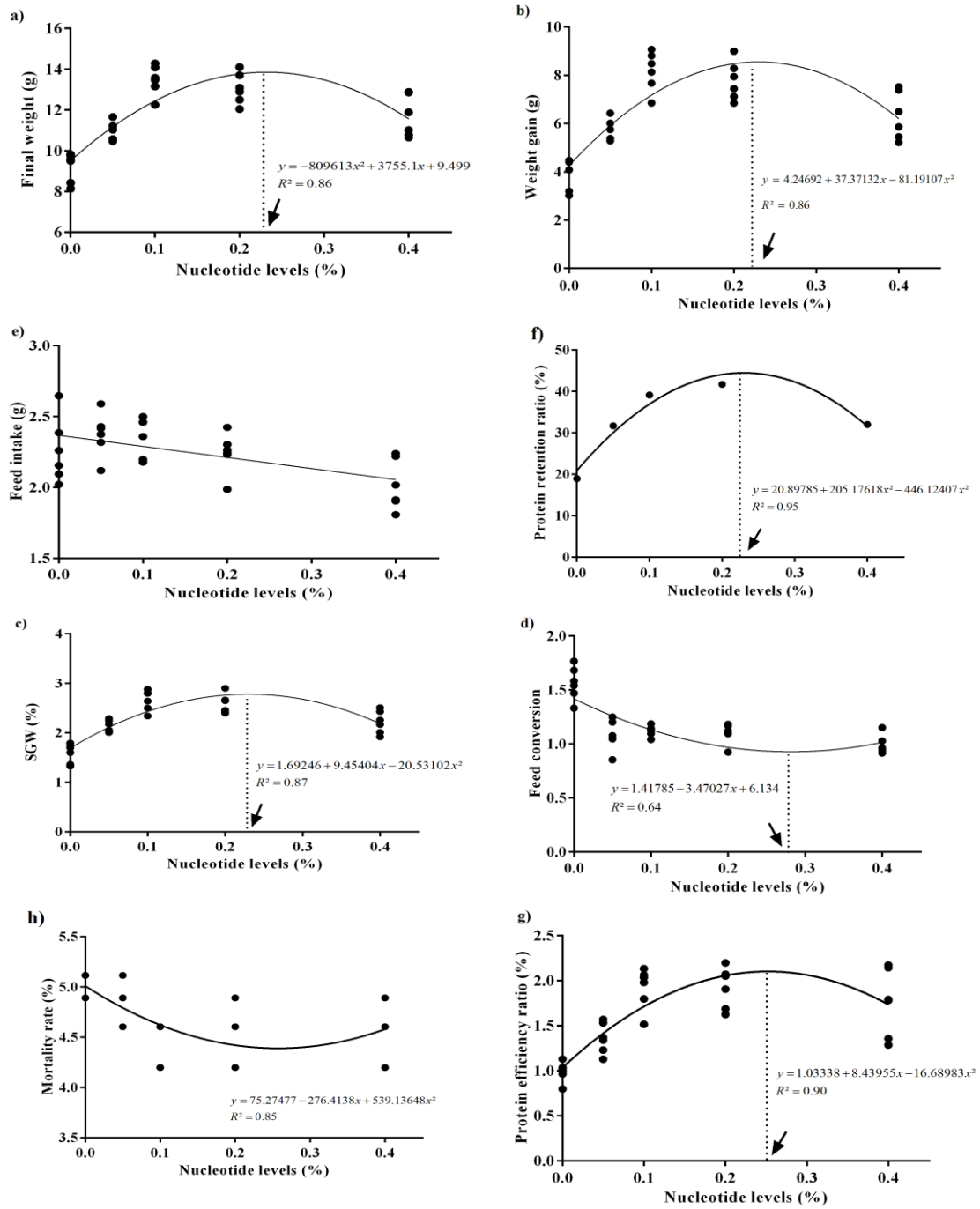


Figure 1: Final weight (a), weight gain (b), specific growth rate (c), feed conversion (d), feed intake (e), protein retention rate (f), protein efficiency ratio (g) and mortality (h) of tilapia fingerlings fed diets with graded levels of supplementation by nucleotides (n = 20, except PRT which represents the mean of 6 replicates, mean±SD). The arrow represents the highest level of supplementation.

Regarding intestinal morphology, dietary nucleotide supplementation did not significantly affect intestinal fold height, the fold height-to-crypt depth ratio, crypt depth, or enterocyte height (Table 3). Nevertheless, numerical differences were observed among treatments. Fish fed the diet containing 2.0 g kg^{-1} of nucleotides had a higher number of intestinal folds than the

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control group. Fish receiving 1.0 and 2.0 g kg^{-1} of nucleotides also showed numerically greater intestinal fold height than the control group. Crypt depth was numerically greater in fish fed 2.0 and 4.0 g kg^{-1} than in the other supplementation groups. However, these differences were not statistically significant. The high coefficient of variation observed in this experiment may have contributed to the lack of significant differences in intestinal morphology, although this possibility cannot be confirmed from the data alone. Figure 2 illustrates the histological structures evaluated in the proximal intestine of Nile tilapia fingerlings fed the basal diet. Regression analysis estimated a nucleotide supplementation level of 2.6 g kg^{-1} associated with the lowest mortality.

Table 3: Folds number (FN), fold height (FH), fold: crypt relation (F:C R), crypt depth (CD), and enterocyte height (EH) in the proximal intestine of tilapia fingerlings fed diets with graded levels of supplementation by nucleotides (n = 4 mean±SD).

Nucleotide g/kg-1	FN (Unity)	FH (µm)	F:C R	CD (µm)	EH (µm)
0.00	10.00±2.16	64.78±5.70	3.58±0.34	18.12±0.67	15.65±2.90
0.5	14.25±3.86	61.54±2.87	3.03±0.41	20.62±3.00	18.76±2.38
1.0	13.50±5.00	81.41±11.20	4.32±0.68	18.94±1.38	15.84±2.86
2.0	15.50±1.73	75.27±21.33	3.70±1.08	20.50±1.90	20.27±3.29
4.0	15.00±2.58	62.05±24.60	3.56±1.10	17.09±1.70	17.20±5.87
Linear	ns	ns	ns	ns	ns
Quadratic[1]	ns	ns	ns	ns	ns
P value	>0.05	>0.05	>0.05	>0.05	>0.05

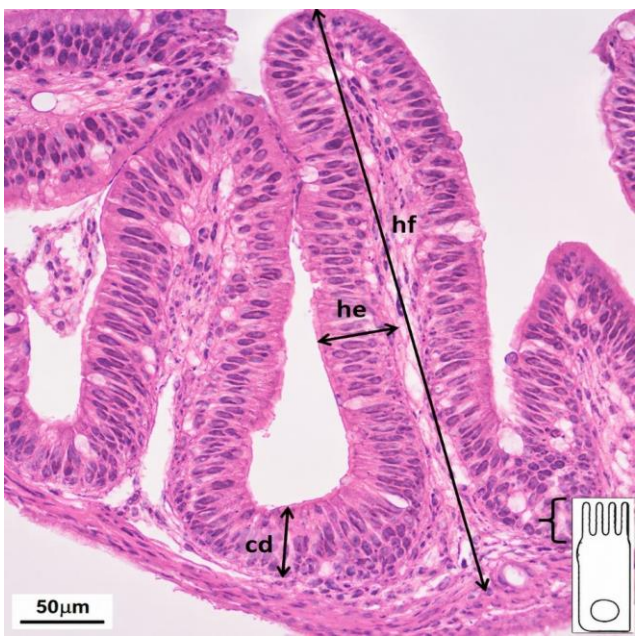


Figure 2: Transversal section photomicrographs of Nile tilapia fingerlings proximal intestine. Enteric section from fish fed the basal diet, small arrow 'he' indicates enterocyte height, 'hf' fold height and 'cd' crypt depth (hematoxylin and eosin, 50 µm).

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Discussion

The results of the present study indicate that dietary nucleotide supplementation influenced the productive performance of recently sex-reversed Nile tilapia (*Oreochromis niloticus*) fingerlings fed plant-based diets. The estimated inclusion level associated with the highest final body weight, specific growth rate, and weight gain was 2.3 g kg^{-1}, whereas the lowest feed conversion ratio was estimated at 2.8 g kg^{-1}. These findings suggest that the response to nucleotide supplementation depended on the inclusion level and the performance parameter evaluated. Nucleotides participate in essential metabolic processes, including nucleic acid synthesis and cellular renewal, and their dietary supply may be relevant during periods of rapid growth. However, the magnitude of these responses may vary according to fish species, developmental stage, dietary composition, nucleotide source, and experimental conditions (Li and Gatlin, 2006; Hossain *et al*., 2020).

The growth responses observed in this study are consistent with previous investigations reporting improvements in fish performance following dietary nucleotide supplementation. Xu *et al*. (2015) found that dietary yeast nucleotides improved growth and feed efficiency in juvenile hybrid tilapia, although the responses varied with the inclusion level. Similarly, Chen *et al*. (2022) reported improvements in growth performance and feed utilization in juvenile largemouth bass fed nucleotide-supplemented diets. Nevertheless, the optimal levels identified in those studies cannot be directly extrapolated to the present experiment because of differences in species, fish size, dietary formulations, and nucleotide sources. Furthermore, although the present diets were formulated using plant-based ingredients, their basal nucleotide content was not established. Therefore, the possibility that dietary nucleotide availability contributed to the observed growth responses remains a hypothesis rather than a demonstrated mechanism (Xu *et al*., 2015; Chen *et al*., 2022; Hossain *et al*., 2020).

Feed intake varied among the dietary treatments, with fish receiving 1.0 and 4.0 g kg^{-1} showing higher intake than fish receiving the other inclusion levels. This pattern does not indicate a consistent linear response to increasing nucleotide supplementation. Nucleotides and related compounds have been investigated as potential feeding stimulants in aquatic animals, but the observed variation in intake cannot be attributed specifically to improved palatability without direct measurements of feed preference or feeding behavior. Moreover, yeast-derived products may contain other compounds that influence feeding responses, making it difficult to isolate the effects of nucleotides when complex ingredients are used (Berto *et al*., 2016; Li and Gatlin, 2006). Berto *et al*. (2016) evaluated yeast extract in Nile tilapia and demonstrated the importance of considering the composition of the supplemented product when interpreting performance responses. Thus, the

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differences in feed intake observed in the present study should be interpreted alongside growth and feed conversion results rather than as direct evidence of a palatability effect.

The estimated inclusion levels associated with the highest protein efficiency ratio (PER) and protein retention rate (PRR) were 2.3 and 2.5 g kg^{-1}, respectively. In addition, the lowest feed conversion ratio was estimated at 2.8 g kg^{-1}. The fact that these estimates differed among parameters suggests that the level associated with the most favorable response was not identical for all aspects of nutrient utilization. Nucleotides are involved in cellular metabolism and nucleic acid synthesis, but their supplementation does not necessarily result in greater dietary protein utilization under all nutritional conditions. Although improvements in protein-related parameters could be consistent with changes in metabolic demands or nutrient utilization, the present experiment did not directly investigate the mechanisms underlying these responses. The effects of dietary nucleotides on growth and nutrient utilization in fish vary according to the experimental conditions (Li and Gatlin, 2006; Hossain *et al*., 2020). Therefore, the estimated levels should be regarded as parameter-specific responses under the conditions tested rather than as a single universal optimum.

Dietary nucleotide supplementation did not significantly affect the intestinal morphological variables evaluated, including intestinal fold height, the fold-height-to-crypt-depth ratio, crypt depth, and enterocyte height. Although some numerical differences were observed among treatments, these differences cannot be interpreted as significant improvements in intestinal structure. In particular, the numerical variation in measurements from fish receiving 2.0 g kg^{-1} does not establish a beneficial effect of this inclusion level. Studies conducted with other fish species have reported changes in intestinal morphology following nucleotide supplementation. Cheng *et al*. (2011) observed improvements in selected intestinal morphological measurements in red drum (*Sciaenops ocellatus*), while Chen *et al*. (2022) reported changes in intestinal fold height and other structural parameters in juvenile largemouth bass. Xu *et al*. (2015) also investigated intestinal development in hybrid tilapia fed dietary yeast nucleotides. Differences between these findings and those obtained in the present study may be related to variations in species, developmental stage, supplementation levels, nucleotide sources, and experimental conditions. In the present study, however, nucleotide supplementation did not result in statistically significant improvements in intestinal morphology in Nile tilapia fingerlings.

Intestinal morphology is commonly investigated because the structure of the intestinal mucosa is associated with digestive and absorptive function. However, morphological measurements alone cannot establish the extent to which nutrients are digested or absorbed (Hossain *et al*., 2020). Likewise,

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numerical differences in fold height or crypt depth should not be interpreted as evidence of improved intestinal function when statistical significance has not been demonstrated (Hossain *et al*., 2020). The absence of significant morphological responses in the present study therefore indicates that the productive improvements were not accompanied by detectable changes in the measured intestinal structures. In a study of Nile tilapia, Godinho *et al*. (2026) found no significant effects of yeast-derived RNA or free nucleotides on the apparent digestibility coefficients of dry matter, protein, and phosphorus. Although the experimental conditions differed from those of the present study, these findings illustrate that supplementation may affect some physiological or productive parameters without producing detectable changes in other indicators. Further investigations combining intestinal histology with digestive enzyme activity, nutrient digestibility, and other functional measurements could help clarify the relationship between nucleotide supplementation and intestinal function in early-stage tilapia

Mortality was another important observation in the present experiment. Regression analysis identified an estimated inclusion level of 2.6 g kg^{-1} associated with the lowest mortality. However, this association alone does not demonstrate that dietary nucleotides reduced mortality or protected the fish against infection. Nucleotide supplementation has been investigated for its potential effects on immune function and disease resistance in aquaculture, but the responses reported in the literature vary according to the species, supplementation protocol, and type of health challenge. Chen *et al*. (2022), for example, reported effects of dietary nucleotides on mortality following an experimental bacterial challenge in largemouth bass. In contrast, Berto *et al*. (2016) did not find significant improvements in survival or the measured innate immune responses in Nile tilapia following an *Aeromonas hydrophila* challenge when fish received yeast extract. These contrasting findings highlight the need to distinguish mortality recorded during a feeding trial from mortality measured following a controlled pathogen challenge. Because the cause of mortality in the present study was not confirmed, the observed losses cannot be attributed to a specific bacterial infection or interpreted as evidence of a nucleotide-mediated protective effect

The results of the present study partially support the beneficial effects of dietary nucleotide supplementation on the productive performance of Nile tilapia (*Oreochromis niloticus*) fingerlings fed plant-based diets. Similar to the findings reported by Xu *et al*. (2015) in hybrid tilapia and Chen *et al*. (2022) in juvenile largemouth bass, supplementation was associated with favorable responses in productive parameters, although the estimated inclusion levels varied among the variables evaluated. In contrast, the absence of significant changes in intestinal morphology indicates that the

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improvements in productive performance were not accompanied by detectable changes in the intestinal structures analyzed, unlike the findings reported in studies involving other fish species (Cheng *et al*., 2011; Chen *et al*., 2022). Furthermore, although regression analysis indicated an inclusion level associated with the lowest estimated mortality, this finding does not support the conclusion that nucleotides increased disease resistance, since the cause of mortality was not determined. Overall, these findings highlight the potential of nucleotide supplementation to improve specific productive parameters, while indicating that its effects on intestinal morphology and survival require further investigation.

Conclusion

In conclusion, the results of this study indicate that dietary nucleotide supplementation at levels ranging from 2.0 to 3.0 g kg^{-1} of diet improved growth performance and feed utilization in Nile tilapia during the early stages of development. The estimated optimal inclusion levels varied among the evaluated parameters, generally falling within this range. However, further studies are needed to determine whether nucleotide supplementation improves resistance to adverse conditions.

Authors' Contributions

Igo Gomes Guimarães: supervision and project guidance. Cristielle Nunes Souto: co-supervision and scientific guidance. Micael Barbosa Godinho, Carolinne Silva da Mota, and Alana Lucena: writing and manuscript formatting. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethics approval

The animal care and experimental procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Goiás (protocol No. 137/11).

Funding

The authors declare that no specific funding was received for this study.

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