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Effects of yeast-derived nucleotides and RNA on growth, nutrient digestibility, hematology and immune response to LPS challenge in Nile tilapia (*Oreochromis niloticus*)

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Dietary nucleotides are functional feed additives that may improve fish health and physiological performance, although their efficacy can vary according to the nucleotide source. This study evaluated the effects of dietary supplementation with yeast (*Saccharomyces cerevisiae*) RNA (YR) and yeast-derived free nucleotides (YFN) on growth performance, immune response, digestive enzyme activity, and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*). Juveniles (100 ± 5 g) were assigned to three dietary treatments (control, YR, and YFN), with four replicates of 15 fish per tank, and fed the experimental diets for 60 days before being challenged with lipopolysaccharide (LPS). Growth performance and survival were not affected by dietary treatments ($p > 0.05$). Whole-body composition was also largely unchanged; however, crude protein content was higher in fish fed YR compared to YFN ($p < 0.05$), while lipid and ash contents remained unaffected. Hematological and immune parameters were modulated by nucleotide supplementation. YFN-fed fish showed increased hematocrit and total serum protein after the LPS challenge, while both YR and YFN groups exhibited reduced red blood cell counts ($p < 0.05$). Additionally, YFN increased serum lysozyme activity and total immunoglobulin levels following the challenge, and all treatments showed enhanced mucus antiprotease activity. Digestive enzyme activity was influenced by dietary treatments, with YR increasing amylase activity in all intestinal sections ($p < 0.05$), while protease activity varied according to intestinal region and nucleotide source. However, apparent digestibility coefficients of dry matter, crude protein, and phosphorus were not affected ($p > 0.05$). *In vitro*, YFN increased superoxide anion production in head kidney leukocytes after LPS stimulation. These findings indicate that dietary nucleotide supplementation modulates physiological responses without affecting growth performance or nutrient digestibility, with YR favoring protein deposition and YFN enhancing immune responses.

KEYWORDS
aquaculture, digestive enzymes, immunity, *in vitro*, saccharomyces cerevisiae by-products

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed fish species worldwide, and its production has become increasingly intensive to meet the growing demand for aquatic protein. However, intensive rearing conditions, characterized by high stocking densities and fluctuations in water quality, can compromise immune function, intestinal integrity, and overall fish health, increasing susceptibility to stress and disease (Yaseen et al., 2020). Consequently, sustainable nutritional strategies capable of improving animal welfare, feed utilization, and disease resistance while minimizing environmental impacts have become increasingly important in modern aquaculture (FAO, 2026; European Market Observatory for Fisheries and Aquaculture Products (EUMOFA), 2026).

Among the nutritional additives investigated for this purpose, yeast-derived products, including free nucleotides and yeast ribonucleic acid (RNA), have received considerable attention due to their potential to support growth, health, and physiological performance in aquatic animals. Nucleotides are bioactive molecules composed of a nitrogenous base, a pentose sugar, and one or more phosphate groups, and they play fundamental roles in nucleic acid synthesis, cellular signaling, and energy metabolism (Bezerra et al., 2024). Although fish can synthesize nucleotides endogenously, they are considered conditionally essential during periods of rapid growth, physiological stress, or immune activation, when endogenous production may not fully meet metabolic demands (Ding et al., 2021).

Several studies have demonstrated beneficial effects of dietary nucleotide supplementation in fish, including improvements in growth performance, immune competence, intestinal function, and resistance to infectious challenges (Kader et al., 2018; Liu et al., 2020; Barducci et al., 2022; Pelusio et al., 2023; Zhang et al., 2025). In Nile tilapia, dietary inclusion levels ranging from 0.05 to 0.20% have been reported to enhance immune responses and intestinal health (Holen et al., 2021). However, the magnitude of these effects appears to vary according to the nucleotide source, supplementation level, species, and rearing conditions. Among the commercially available sources, products derived from *Saccharomyces cerevisiae* are widely used because they provide nucleotides in different forms, including purified yeast RNA and yeast-derived free nucleotides (Xu et al., 2015; Reda et al., 2018; Barducci et al., 2022; Pelusio et al., 2023). While studies evaluating purified yeast RNA remain limited, available evidence suggests positive effects on immune responses and disease resistance in fish and shrimp (Rairat et al., 2022). Likewise, supplementation with free nucleotides has been associated with enhanced resistance against bacterial, viral, and parasitic infections, improved intestinal morphology, and modulation of inflammatory responses (Li and Gatlin, 2006; Barducci et al., 2022; Luo et al., 2024). These findings indicate that different nucleotide sources may influence fish physiology through distinct mechanisms, highlighting the need for comparative evaluations.

Despite the growing body of evidence supporting the benefits of dietary nucleotide supplementation, the mechanisms underlying their effects on growth, feed utilization, immune function, and physiological responses remain incompletely understood. Previous studies suggest that nucleotides may influence digestive processes, nutrient utilization, and intestinal function, potentially contributing to improved metabolic efficiency and health status (Yaseen et al., 2020; Peng et al., 2024; Zhang et al., 2025; Shi et al., 2026; Taklu et al., 2025; Wang et al., 2025). However, information regarding the comparative effects of different nucleotide sources on digestive enzyme activity, systemic physiology, immune responses, and resistance to immune challenges is still limited, particularly in Nile tilapia. Addressing these knowledge gaps is essential for improving the scientific basis for the use of nucleotide-based nutritional strategies in aquaculture.

Therefore, the objective of the present study was to evaluate the effects of two dietary nucleotide sources, yeast RNA and yeast-derived free nucleotides, on growth performance, feed utilization, immune responses, physiological parameters, and resistance to lipopolysaccharide (LPS) challenge in Nile tilapia.

Materials and methods

Three experimental trials were conducted at the Aquaculture Research Laboratory (LAPAQ), Universidade Federal de Jataí (UFJ), to evaluate the *in vivo* effects of different dietary nucleotide sources on growth performance, resistance to lipopolysaccharide (LPS) challenge, physiological responses, and immune response of Nile tilapia. In addition, the *in vitro* effects of yeast-derived free nucleotides (YFN) on head-kidney leukocyte responses to LPS were assessed.

All experimental procedures involving animals were performed in accordance with the guidelines for the care and use of animals established by the National Council for the Control of Animal Experimentation (CONCEA). The study was approved by the Ethics Committee for the Use of Animals of the Universidade Federal de Jataí (CEUA-UFJ; protocol no. 010/23, approved on 16 June 2023).

Experimental diets

A plant-based diet was formulated as a control and supplemented with two types of nucleotides sources: YR (Yeast RNA) and YFN (13% free nucleotides), following the manufacturer's recommendations. YR is a commercial product containing 15% RNA, while YFN is the same matrix used to obtain YR that has been enzymatically hydrolyzed to obtain a product rich in natural free 5-nucleotides, nucleosides and nucleobases (13%). The experimental diets were designed to be isoenergetic, isofibrous, and isonitrogenous, meeting the nutritional requirements of the species (Furuya, 2010; IAFFD, 2023) (Table 1).

The ingredients were ground to particles smaller than 0.5 mm and thoroughly mixed with water (25%). Then, each diet was extruded at 120 °C using a single screw laboratory extruder (Exteec®, Ribeirão Preto-SP) to obtain 1.5 mm diameter floating pellets. Pellets were then dried in a forced-air oven at 65°C for 24h and stored at -20 °C until further use.

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TABLE 1 Ingredient and proximal composition of experimental diets (%).

Ingredients (%)	Control	YR (Yeast RNA)	YFN (15% free Nuc)
Soy protein concentrate	23.30	23.30	23.30
Yeast RNA		0.20	
Yeast-derived free nucleotide			0.20
Soybean meal	25.20	25.20	25.20
Corn Gluten meal	6.00	6.00	6.00
Ground Corn	17.38	17.38	17.38
Poultry by-product meal	8.00	8.00	8.00
Broken rice	14.10	14.10	14.10
Kaolin	0.63	0.43	0.43
DL – Methionine	0.12	0.12	0.12
Dicalcium phosphate	2.40	2.40	2.40
Soybean oil	1.70	1.70	1.70
Choline HCl	0.10	0.10	0.10
Vit C	0.05	0.05	0.05
NaCl	0.40	0.40	0.40
Vit./min. mix[a]	0.60	0.60	0.60
Antioxidant[b]	0.02	0.02	0.02
Chemical composition (%DM)			
Dig Energy (Kcal kg-^{1})	3260.07	3265.59	3266.26
Dig Protein (%)	32.13	32.22	32.23
Crude Protein (%)	34.98	35.08	35.08
Crude Fiber (%)	3.01	3.01	3.01
Ether Extract (%)	4.04	4.04	4.05

[ab]Mineral and vitamin mix supplied the following per kg diet^{-1}: vitamin A, 8000 II; vitamin D3, 2250 IU; vitamin E, 112 mg;vitamin K, 15 mg; vitamin B1, 16 mg; vitamin B2, 16 mg; pantothenic acid, 40 mg; niacin, 85 mg; biotine, 5 mg; folic acid, 5 mg; vitamin B12, 16 mg; vitamin B6, 16 mg. Cobalt, 0.5 mg; copper, 10 mg; iron, 60 mg; iodine, 1.5 mg; manganese, 50 mg; selenium, 0.35 mg; zinc, 100 mg. [b]Antioxidant Banox®. For the digestibility trial, diets were supplemented with 0.2% chromic oxide (Cr_2O_3) as an inert marker, replacing an equivalent proportion of kaolin.

Trial 1 – *In vivo* effects of dietary supplementation with yeast RNA(YR) and yeast-derived free nucleotides (YFN) in Nile tilapia

This study was performed to evaluate the *in vivo* effects of the dietary administration of YR and YFN on growth, feed utilization, intestinal enzyme activity, and resistance to LPS challenge of Nile tilapia. The study was conducted using a completely randomized design with three treatments and four replicates, resulting in a total of 12 experimental units. Each experimental unit consisted of 15 juvenile tilapias, totaling 180 fish, with an initial average weight of 100 ± 5 g. A 1000 L-polyethylene tank was considered the experimental unit. All tanks were connected to a water recirculation system equipped with temperature control, an ultraviolet filter, and physical and biological filters. Fish were fed three times daily (8:00 a.m., 12:00, and 4:00 p.m.) throughout a 60-day experimental period. Exceptions of the feeding procedure described previously were taken on the sampling day where fish were fed just once a day after the samples were collected. During each feeding, the diet was offered in three successive rounds to ensure the animals reached satiation. Water quality analyses were performed weekly to measure dissolved oxygen, nitrogen compounds, temperature, and pH. During the experimental period, dissolved oxygen levels remained above 8 mg L-1, total ammonia was 0.25 mg L-1, nitrite levels ranged from 0.0 to 0.25 mg L-1, pH values ranged from 6.8 to 7.2, and the temperature was maintained between 26.1 °C and 27.8 °C. All parameters were kept within the optimal range for the species. The diets were weighed daily, and the fish were individually weighed at 30 and 60 days in order to calculate the performance parameters. Individual weight gain was determined as the difference between final and initial weight (WG = Wf – Wi). Specific growth rate was expressed as the percentage increase in body weight per day, calculated as (SGR = [(ln Wf – ln Wi)/t] × 100), where t is the experimental period in days. Average feed intake was estimated by dividing the total feed consumed by the number of fish and experimental days (AFI = total feed/(N × t)). Feed conversion ratio was obtained as the ratio between feed intake and biomass gain (FCR = feed intake/biomass gain). The survival rate was calculated at the end of the experimental period according to the following formula: SR (%)=(Nf/Ni)×100 where Nf is the number of fish alive at the end of the experiment, and Ni is the initial number of fish (Boyd, 1990; El-Sayed, 2006).

Biomass gain was calculated considering the actual number of surviving fish in each experimental unit, according to the formula: Final biomass = final mean body weight × number of live fish. Similarly, feed conversion ratio (FCR) was calculated using the real biomass gain, thereby avoiding bias from mortality. At the end of the experimental period, fish were randomly sampled across tanks to ensure proportional representation of all experimental units. A total of 10 fish per treatment were used for baseline analyses, of which six were allocated for digestive enzyme activity and whole-body composition. An additional 24 fish per treatment were used for the LPS challenge, resulting in a total of 34 fish sampled per treatment, which was consistent with the number of fish available after accounting for survival.

Samplings

Biological samples were collected at two time points. At the end of the 60-day feeding period, ten fish per treatment were randomly selected from the experimental tanks for baseline hematological, biochemical, and immunological analyses. Prior to handling and sample collection, fish were anesthetized with an alcoholic benzocaine solution (0.1 g L^{-1}). Blood and mucus samples were collected from these fish. Subsequently, six individuals per treatment were euthanized by deepening the anesthetic plane and used for digestive enzyme activity and whole-body composition analyses.

Following baseline sampling, 24 fish per treatment were randomly allocated to the lipopolysaccharide (LPS) challenge assay. Fish were maintained under the same experimental conditions for an additional four days and, on day 64, were again anesthetized with an alcoholic benzocaine solution (0.1 g L-1) prior to blood and mucus collection for hematological, biochemical, and immunological analyses. Blood was collected by caudal vessel puncture using

either heparinized syringes or syringes without anticoagulant. Non-heparinized blood was maintained at room temperature for 1 h and centrifuged at 5,000 rpm for 5 min to obtain serum for biochemical and immunological analyses. Serum samples were stored at −80 °C until analysis. Blood collected with heparinized syringes was used for hematological analyses.

Mucus samples were collected according to Guardiola et al. (2014). Fish were immersed in a 0.5% saline solution for 30 s to stimulate mucus secretion. The mucus was gently scraped, transferred to 15-mL Falcon tubes, diluted 1:1 with sterile PBS, and centrifuged at 2,500 rpm for 10 min. The supernatant was collected, transferred to microtubes, and stored at −80 °C until analysis.

LPS challenge

After 60 days of feeding with the experimental diets, a subgroup of fish (12 per treatment) was subjected to an immunological challenge through intraperitoneal injection of lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* (Sigma-Aldrich, L7018) at a dose of 0.7 mg kg^{-1} body weight. A second group of fish (12 per treatment) received an intraperitoneal injection of phosphate-buffered saline (PBS) and served as the negative control. Six 1000L tanks were used for the assay, with each fish considered an experimental unit. Fish were maintained under the same experimental conditions previously described for an additional four days, after which blood and mucus samples were collected for hematological, biochemical, and immunological analyses. This procedure was adapted from Demers and Bayne (1997) to evaluate the immune response while minimizing animal use and reducing stress associated with experimental pathogen exposure.

Hematological parameters

Erythrocyte count was performed using the hemocytometer method in a Neubauer chamber. A 0.01% toluidine blue solution, diluted in 0.9% Ringer's solution, was used at a 2:400 ratio. Hemoglobin concentration was determined using the cyanmethemoglobin method with a commercial colorimetric kit (Labtest®), following the method described by Collier (1944). Hematocrit percentage was measured using a microhematocrit centrifuge operated at 8000 rpm for five minutes, following the methodology adapted from Anderson and Siwicki (1995). Differential leukocyte count was performed by placing a drop of blood on a glass slides and staining with May-Grunwald-Giemsa dyes, then 200 leukocytes were differentially counted, and relative percentage was calculated.

Serum biochemistry

Total protein and albumin concentrations were determined using a commercial colorimetric kit (Labtest®) with the biuret and bromocresol methods, respectively. The protocols were adapted for use with microplates, and readings were conducted on a microplate reader at 545 nm for total protein and 630 nm for albumin.

Immunological parameters

Lysozyme levels in serum and mucus were determined using the turbidimetric assay based on the lysis of *Micrococcus lysodeikticus*, as originally described by Litwack (1955) and adapted for fish by Sankaran and Gurnani (1972). The assay was based on the lysis of a *Micrococcus lysodeikticus* suspension, measured as a reduction in absorbance at 450 nm. Hen egg-white lysozyme (Sigma-Aldrich, L6876) was used to generate a standard calibration curve, and lysozyme concentrations in serum and mucus samples were estimated from this curve and expressed as lysozyme equivalents (μg mL^{-1}). For each sample, 5 μL of serum or mucus was analyzed in triplicate and mixed with 250 μL of *M. lysodeikticus* suspension (Sigma-Aldrich, M3770) in a 96-well microplate. Absorbance was recorded at 450 nm immediately after mixing (time 0), followed by incubation at 37 °C for 20 min and a second absorbance measurement. The difference in absorbance between readings was used to estimate lysozyme concentration based on the standard curve.

Protease activity was measured following the azocasein hydrolysis assay as described by Guardiola et al. (2014), with some modifications to optimize the procedure for different sample types. 100 μL of mucus extract was mixed with 100 μL of 100 mM ammonium bicarbonate buffer containing 0.7% azocasein and incubated for 19 h at 30 °C. The reaction was stopped by adding 4.6% trichloroacetic acid (TCA), and the mixture was centrifuged at 10,000 × g for 10 min. 100 μL of the supernatants were carefully transferred to a 96-well microplate in triplicate and mixed with 100 μL 0.5 N NaOH. Absorbance was recorded at 450 nm using a microplate reader. Trypsin (5 μg mL^{-1}, Sigma) served as a positive control (100% protease activity), whereas the buffer was used as the blank (0% activity).

Total antiprotease activity was assessed based on the ability of cutaneous mucus to inhibit trypsin activity, as described by Guardiola et al. (2014). Briefly, 10 μL aliquots of mucus extract were mixed with an equal volume of a standard trypsin solution (5 mg mL^{-1}, Sigma) in a 96-well plate and incubated at 22 °C for 10 minutes. Then, 100 μL of ammonium bicarbonate buffer (100 mM) were added, and the samples were subjected to the previously described protocol for the determination of protease activity. The positive control consisted of replacing cutaneous mucus with buffer, representing 100% protease activity and 0% antiprotease activity. In the negative control, trypsin was replaced by buffer, indicating 0% protease activity and 100% antiprotease activity. The percentage inhibition of trypsin activity was calculated for each sample based on the values obtained in the controls. All the procedure was performed in triplicate.

Myeloperoxidase (MPO) activity was measured in fish serum according to the method described by Quade and Roth (1997). Briefly, 20 μL of serum samples were mixed with 100 μL of Ca- and Mg-free Hank's Balanced Salt Solution (HBSS), 35 μL of 20 mM TMB (tetramethylbenzidine – Sigma T2885), and 35 μL of 5 mM H_2O_2 solution in 96-well microplates. The mixture was incubated at room temperature for 2 minutes and the reaction stopped with 50 μL of 2 M H_2SO_4. Absorbance was measured at 450 nm in a INNO LTEK microplate reader. Sample-free wells were used as blanks.

TABLE 2 Initial weight (IW), final weight (FW), weight gain (WG), specific growth rate (SGR), survival rate (SR), biomass gain (BG), feed intake (FI), and feed conversion ratio (FCR) of juvenile Nile tilapia fed diets supplemented with different nucleotide sources for 60 days.

Variables	Treatments		
	Control	YFN (15% free Nuc)	YR (Yeast RNA)
Initial weight (g)	107.8 ± 12.6	104.1 ± 9.5	111.5 ± 10.1
Final weight (g)	260.5 ± 12.7	271.3 ± 18.7	255.0 ± 12.9
Weight gain (g)	159.9 ± 3.0	159.3 ± 14.5	151.8 ± 6.9
Specific growth rate (% day^{-1})	1.47 ± 0.08	1.60 ± 0.10	1.38 ± 0.07
Survival (%)	91.7 ± 6.7	95.0 ± 6.1	86.7 ± 5.4
Biomass gain (g $tank^{-1}$)	2200 ± 126	2273 ± 285	1922 ± 245
Feed intake (kg $tank^{-1}$)	1.727 ± 0.094	1.704 ± 0.108	1.748 ± 0.051
Feed conversion ratio (FCR)	0.79 ± 0.07	0.75 ± 0.09	0.91 ± 0.10

Values are expressed as mean ± standard deviation (n = 4 tanks). Biomass gain and feed conversion ratio were calculated based on the actual number of surviving fish in each experimental unit. Fish mortality during the initial acclimation period was compensated by replacement to maintain stocking density. No significant differences were observed among treatments ($P > 0.05$).

One unit of MPO activity was defined as the amount of enzyme required to produce a change of 1 OD unit.

Total immunoglobulin concentration was indirectly estimated according to Anderson and Siwicki (1995). Briefly, total serum protein concentration was determined before and after precipitation of immunoglobulins with 12% polyethylene glycol (PEG). Immunoglobulin concentration was calculated as the difference between total protein concentration prior to and after PEG precipitation and expressed as mg mL^{-1}.

The activity of the alternative complement pathway was assessed following the method described by Sunyer and Tort (1995). Briefly, sheep erythrocytes suspended in Alsever's solution were added to tilapia serum serially diluted in chilled phosphate-buffered saline (PBS+) containing 0.85% PBS, 0.1% gelatin, 0.15 mM $CaCl_2$, and 0.5 mM $MgCl_2$. The mixtures were transferred to round-bottom microplates and incubated at room temperature for 1 h. Following incubation, the plates were centrifuged at 200 × g, and the supernatants were transferred to a new microplate. Hemolysis was measured spectrophotometrically at 450 nm and converted to percentage hemolysis using distilled water as the 100% lysis control. The serum dilution producing 50% hemolysis (ACH50) was determined by linear regression, and the results were expressed as the base-10 logarithm (log10) of the reciprocal serum dilution.

Digestive enzyme activities

Tilapia (n= 6 per treatment) were euthanized by deepening the anesthetic plane using benzocaine at a concentration of 250 mg L^{-1}, followed by spinal cord sectioning for the analysis of digestive enzymes and carcass composition. Immediately after euthanasia, intestinal segments were collected for digestive enzyme analysis. The remaining carcass was frozen for subsequent composition determination. The intestines of the fish were dissected and divided into three parts of equal size (proximal, medial, and distal) to measure digestive enzyme activities. Intestinal samples were processed individually by homogenizing them in 1 mL of phosphate buffer (pH 7). The homogenates were centrifuged at 12,000 × g for 15 minutes at 4 °C. The resulting crude enzyme extracts (supernatants) were stored at -20 °C until analysis of amylase and protease activities.

Amylase activity was determined based on the detection of reducing carbohydrate groups using 3,5-dinitrosalicylic acid (DNS) (Miller, 1959). A 2% starch solution prepared in 100 mM sodium acetate buffer (pH 6.5) served as the substrate (Bernfeld, 1955). Briefly, 25 µL of enzyme extract was mixed with 25 µL of substrate (1:1, v/v) and incubated at 30 °C. Aliquots were collected every 15 min, and the reaction was stopped by the addition of DNS reagent. The samples were heated at 100 °C for 5 min, diluted with distilled water, and absorbance was measured at 540 nm. Amylase activity was expressed as U mg^{-1} protein.

Protease activity was determined according to Munilla-Morán and Saborido-Rey (1996). Briefly, 10 µL of enzyme extract was added to 50 µL of 1% casein solution prepared in phosphate buffer (pH 7.0) and incubated at 30 °C. The reaction was terminated by the addition of trichloroacetic acid (TCA), followed by sodium carbonate (Na_2CO_3) and Folin's reagent. Absorbance was measured at 650 nm, and protease activity was expressed as U mg^{-1} protein.

Trial 2 - digestibility

A digestibility trial was conducted to evaluate the apparent digestibility coefficient (ADC) of nutrients in the experimental diets. A total of 72 fish were randomly distributed among nine 500-L feeding tanks connected to a recirculating aquaculture system equipped with mechanical and biological filtration. Each dietary treatment was represented by three feeding tanks containing eight fish each (n = 24 fish per treatment). The fish were acclimated to the experimental conditions for seven days and fed the respective experimental diets three times daily (08:00, 12:00, and 17:00 h) to apparent satiation. Following the adaptation period, feces were collected using a sedimentation system consisting of three 300-L conical-bottom collection tanks, as previously described by Cho et al. (1985). During the collection period, the same fish were repeatedly transferred between feeding and collection tanks. Thirty minutes after the last daily feeding, fish from each feeding tank were transferred to the collection tanks and remained overnight (17:30 to 07:00 h). The following morning, the fish were returned to their respective feeding tanks. External aeration was provided continuously to the collection tanks using air stones connected to an air blower.

Feces produced by fish originating from the same feeding tank were collected, pooled across collection days, and considered a single sample for chemical analyses. This procedure was repeated over nine collection rounds to obtain sufficient fecal material for nutrient and marker determinations. Therefore, the feeding tank was considered the experimental unit for digestibility analyses (n = 3 tanks per treatment). After each collection, the tanks were drained, washed thoroughly, and refilled with clean water to avoid contamination among sampling periods. Water quality parameters, including dissolved oxygen, total ammonia, nitrite, pH,

and temperature, were monitored weekly and maintained within the recommended range for Nile tilapia culture. After collection, fecal samples were centrifuged, dehydrated in a forced-air oven at 55 °C for 48 h, ground, and stored at −20 °C until analysis. The apparent digestibility coefficient (ADC) was calculated according to the following equation (Cho and Slinger, 1979): ADC (%) = 100 − [100 × (%Cr_2O_3feed/%Cr_2O_3feces) × (%Nfeces/%Nfeed)], where ADC = apparent digestibility coefficient; Cr_2O_3feed = percentage of chromium(III) oxide in the diet; Cr_2O_3feces = percentage of chromium(III) oxide in the feces; Nfeed = nutrient concentration in the diet; and Nfeces = nutrient concentration in the feces.

Chemical analysis

The macronutrient composition of diets and carcasses was analyzed following standard AOAC methods (AOAC, 1995). Gross energy (GE) of the diets was determined using an adiabatic bomb calorimeter. Digestible energy (DE) values were calculated based on GE and the apparent digestibility coefficients obtained in the digestibility trial. Samples of diets and carcasses were dried in an oven-dryer at 105 °C for 16 hours to determine dry matter (DM) and then ashed in a muffle furnace at 600 °C for 4 hours to determine mineral matter (MM). Lipid content (ether extract, EE) was analyzed using a Soxhlet extractor with petroleum ether as the solvent. Crude protein (CP) of the diets, feces, and carcasses was determined by quantifying the nitrogen content (N × 6.25) using the Kjeldahl method. Phosphorus concentration in the diets and feces was determined colorimetrically using the vanado-molybdate reagent. Chromic oxide content was determined according to the method described by Bremer Neto et al. (2005).

Trial 3 - *In vitro* effects of nucleotide exposure on tilapia head-kidney leukocytes

To evaluate the *in vitro* response of Nile tilapia leukocytes, 12 fish from the control treatment were used. Head-kidney leukocytes (HKL) were isolated and prepared following the protocol described by Secombes (1990) with minor modifications. After euthanasia by anesthetic overdose, the head kidney of each fish was aseptically dissected and processed individually. Thus, each fish represented one biological replicate throughout the experiment. Head-kidney samples were homogenized in RPMI medium supplemented with 2% fetal bovine serum (FBS). The homogenate was passed through a sterile 100-µm filter, and the resulting cell suspension was diluted with ice-cold phosphate-buffered saline (PBS). The suspension was centrifuged at 200 × g for 10 min at 4 °C. Residual erythrocytes were removed using 2 mL of a lysis solution containing 2.01 g ammonium chloride, 0.25 g potassium bicarbonate, and 0.01 g disodium EDTA. The mixture was incubated for 5 min, centrifuged at 200 × g for 10 min at 4 °C, and washed with ice-cold PBS.

Leukocytes were further purified using a 51% (v/v) Percoll gradient and centrifuged at 400 × g for 25 min at 4 °C. The leukocyte layer was collected and washed twice with ice-cold PBS at 200 × g for 10 min. Cell counts were performed using a hemocytometer, and viability was assessed by trypan blue exclusion. Only samples with viability greater than 95% were used in the experiment.

The leukocyte suspension from each fish was adjusted to a concentration of 2×10^7 cells in RPMI medium containing 0.1% FBS. A volume of 100 µL of cell suspension was dispensed into four wells corresponding to the experimental treatments (PBS, PBS + LPS, YFN, and YFN + LPS). Therefore, each fish contributed one observation to each treatment, resulting in 12 biological replicates per treatment and a total of 48 wells. The microplates were incubated at 27 °C for 2 h to allow cell adhesion. Subsequently, the culture medium was removed and replaced with either 100 µL of PBS or 100 µL of a 2 g/L YFN solution diluted in PBS. The microplates were then incubated at 27 °C for 24 h.

LPS challenge

Following the 24-hour incubation period, the cells were challenged with 100 µg mL^{-1} lipopolysaccharide (LPS). One group received 100 µL of LPS solution, whereas the control group received 100 µL of PBS. A total of 48 wells containing isolated leukocytes were distributed among four experimental groups: YFN, YFN + LPS, PBS, and PBS + LPS, with 12 wells per treatment. YFN was selected for the *in vitro* assay because, among the nucleotide sources evaluated in the *in vivo* trial, it induced the most pronounced modulation of immune parameters, including increases in serum lysozyme activity, total immunoglobulin levels, and superoxide anion production following the LPS challenge. Therefore, this nucleotide source was chosen to further investigate whether the immunostimulatory effects observed *in vivo* could be associated with a direct action on head-kidney leukocytes under controlled *in vitro* conditions. The microplates were incubated at 27 °C for an additional 24 hours, after which samples were collected for the determination of myeloperoxidase (MPO) activity and Nitroblue Tetrazolium (NBT) reduction.

Statistics

Data were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene tests, respectively. For growth performance variables (final weight, weight gain, biomass gain, feed intake, feed conversion ratio, specific growth rate, and survival), the tank was considered the experimental unit (n = 4 tanks per treatment). Growth performance, carcass composition, and apparent digestibility coefficient (ADC) data were analyzed by one-way analysis of variance (ANOVA), considering dietary treatment as the fixed factor.

For hematological parameters, serum biochemistry, immunological variables, and digestive enzyme activities, individual fish were considered the experimental unit. These data were analyzed using a two-way ANOVA in a 3 × 3 factorial arrangement, in which dietary treatment (Control, YR, and YFN) was considered factor 1 and either sampling condition (pre-challenge, LPS-challenged, and saline-injected fish) or intestinal segment (proximal, middle, and distal intestine) was considered factor 2, depending on the variable analyzed.

For the *in vitro* assay, a 2 × 2 factorial arrangement was used, with YFN supplementation (presence or absence) as factor 1 and LPS stimulation (presence or absence) as factor 2. Head-kidney leukocytes obtained from each fish were cultured separately, and each fish was considered one biological replicate and the experimental unit for statistical analyses. When significant effects were detected, treatment means were compared using Tukey's multiple comparisons *post hoc* test. Statistical significance was established at $P < 0.05$. All statistical analyses were performed using the Rbio package in R software (R Core Team, Vienna, Austria), and graphical representations were prepared using GraphPad Prism version 11 (GraphPad Software, San Diego, CA, USA).

Results

Effect of supplementation with different nucleotide sources on growth, physiology, immune response, and carcass composition of Nile tilapia juveniles

Dietary nucleotide supplementation did not affect the growth performance of Nile tilapia juveniles ($p > 0.05$; Table 2). Whole-body chemical composition was generally not affected by dietary treatments; however, crude protein content was higher in fish fed the YR diet compared to those fed YFN ($p < 0.05$), while lipid and ash contents remained unchanged ($p > 0.05$). ($p > 0.05$; Table 3). Survival rates also remained statistically similar among treatments ($p > 0.05$), suggesting that mortality was evenly distributed and, consequently, did not bias biomass gain or feed conversion ratio calculations.

A decrease in hemoglobin concentration was observed in the YR-fed groups compared to the control ($p<0.05$; Figure 1a) after 60 days of feeding. However, the YFN-fed groups showed a significant reduction in the hemoglobin concentration when injected with PBS. Additionally, the YFN-fed groups were the only treatment showing a significant reduction in the hemoglobin concentration when fish were injected with PBS ($p<0.05$). The YFN-fed group induced an increase in the hematocrit after 60 days of feeding ($p<0.05$; Figure 1b). A significant decrease in the red blood cell counts was observed in fish fed the different nucleotides sources irrespective of the challenge ($p<0.05$; Figure 1c).

Regardless of the neutrophil percentage, dietary nucleotide sources and the experimental LPS challenge did not affect the differential leukocyte profile of Nile tilapia (Figure 2). YFN-fed tilapia showed a reduction in neutrophil percentage after 60 days of feeding ($p<0.05$; Figure 2b). Although the experimental LPS challenge tended to decrease the neutrophil percentage in the control and YR groups, this was only significant in the YR-fed fish ($P<0.05$).

Serum albumin and total protein content increased after the LPS challenge only in the YFN-fed group ($p<0.05$; Figure 3). Additionally, the YFN-fed group induced a significant increase in the total protein content after the LPS challenge (Figure 3b).

Serum lysozyme activity tended to decrease after the LPS challenge in the control group with only a significant decrease in activity in the PBS injected group (Figure 4a). On the other hand, an increase in serum lysozyme activity after the LPS challenge was observed in fish fed YFN, however, this was only significant compared to the PBS injected group. The YR-fed group maintained similar lysozyme activity across the different sampling points ($P>0.05$). PBS-injected fish of the YFN-fed group showed the highest MPO (myeloperoxidase) activity which was significantly different only from the YR-fed group ($p<0.05$; Figure 4b). A similar trend was observed for the total immunoglobulin (Ig) level in the LPS-challenged fish (Figure 4d). Additionally, only the LPS-challenged fish of the YFN group showed the highest serum Ig levels compared to the other sampling points. Dietary nucleotides sources nor the LPS challenge affected the complement activity ($p>0.05$; Figure 4c).

The mucus protease activity was not affected by the dietary treatments nor the challenges ($p>0.05$; Figure 5a), however, the mucus antiprotease activity significantly increased after the challenge in all dietary treatment groups ($p<0.05$; Figure 5b). A statically difference between the dietary treatments of the mucus antiprotease activity was only observed between the YFN and YR groups of fish injected with PBS ($p<0.05$). Mucus lysozyme activity decreased in the LPS-challenged fish of the control group, while in the YFN-fed group the activity tended to increase after the LPS challenge ($p<0.05$; Figure 5c). Generally, the dietary nucleotide sources tended to decrease the mucus lysozyme activity of Nile tilapia after 60 days of feeding ($p<0.05$), while a significant decrease was only observed in PBS-injected fish fed YFN compared to the control group ($p<0.05$). The dietary nucleotide sources did not affect mucus lysozyme activity of the LPS-challenged fish ($p>0.05$).

Effect of dietary nucleotide sources on nutrient digestibility and digestive enzyme activity in Nile tilapia juveniles s

Generally, intestinal amylase and protease activity was significantly affected by the dietary nucleotide source ($p<0.05$; Figure 6).

TABLE 3 Whole-body composition of juvenile Nile tilapia fed different dietary nucleotide sources for 60 days.

Treatments	Dry matter (%)	Crude protein (%)	Ash (%)	Lipids (%)
Control	17.59 ± 1.48	73.68 ± 6.80ab	10.64 ± 2.11	24.27 ± 3.38
YR	20.49 ± 1.71	79.37 ± 5.48a	8.36 ± 1.94	17.94 ± 5.02
YFN	20.22 ± 2.92	68.39 ± 3.70b	9.79 ± 1.91	17.97 ± 5.54
P value	0.095	0.042	0.26	0.125

Values are expressed as mean ± standard deviation (SD) on a dry matter basis. Different superscript letters within the same column indicate significant differences among treatments according to Tukey's multiple range test ($p < 0.05$). n = 4 experimental units (tanks) per treatment.

YR-fed fish showed a significantly increased amylase activity in all gut sections ($p<0.001$; Figure 6a). Protease activity seems to be differently modulated by the dietary nucleotide sources according to the specific gut section ($p<0.05$; Figure 6b). YR-fed fish showed a decrease in protease activity in the proximal and distal segments of the intestine of Nile tilapia ($P<0.05$), while YFN induced a drastically decrease in the protease activity in the medium segment ($P<0.001$). Differences in protease activity among intestinal segments were only observed in the fish fed dietary nucleotides sources with the YR-fed fish showing increased activities in the medium segment ($p<0.05$) while YFN-fed fish showed increased activities in the proximal and distal segments ($P<0.001$). Conversely, dietary nucleotide sources did not affect the ADC of dry matter, protein and phosphorus ($p>0.05$; Figure 7).

Effect of in vitro supplementation of commercial free nucleotides on head kidney leukocyte function

Head kidney leukocytes of Nile tilapia showed an increased superoxide anion production measured by the NBT assay when the media culture was supplemented with YFN (Figure 8a). Additionally, the superoxide anion production was augmented when the cells were challenged with LPS. On the other hand, no effect of the YFN supplementation to culture media or the LPS challenge was observed on the MPO activity of the head kidney leukocytes (Figure 8b).

Discussion

Dietary nucleotides are feed additives that have been extensively studied in aquaculture nutrition since the 70s (Kader et al., 2018; Ding et al., 2021). Although a significant understanding of the use and metabolism of these conditionally essential compounds has been made in the last decade (Reda et al., 2018; Huang et al., 2025; Kader et al., 2018), some gaps still exist, i.e. how the different aquatic animal species utilize different sources of dietary nucleotides and how they use these nucleotides in their metabolism. In this paper, our findings suggest that free nucleotides derived from yeast are more effective in modulating the immune response of Nile tilapia compared to the intact yeast RNA. On the other hand, these dietary nucleotide sources seem to have a negative effect on some hematological parameters of tilapia.

The inclusion of either YFN or YR did not affect growth performance of Nile tilapia after 60 days of feeding. Whole-body composition was largely unaffected by dietary treatments, except for crude protein, which was higher in fish fed the YR diet compared to YFN. This difference suggests that the two nucleotide sources may not have exerted identical physiological effects; however, the mechanisms responsible for this response remain unclear.

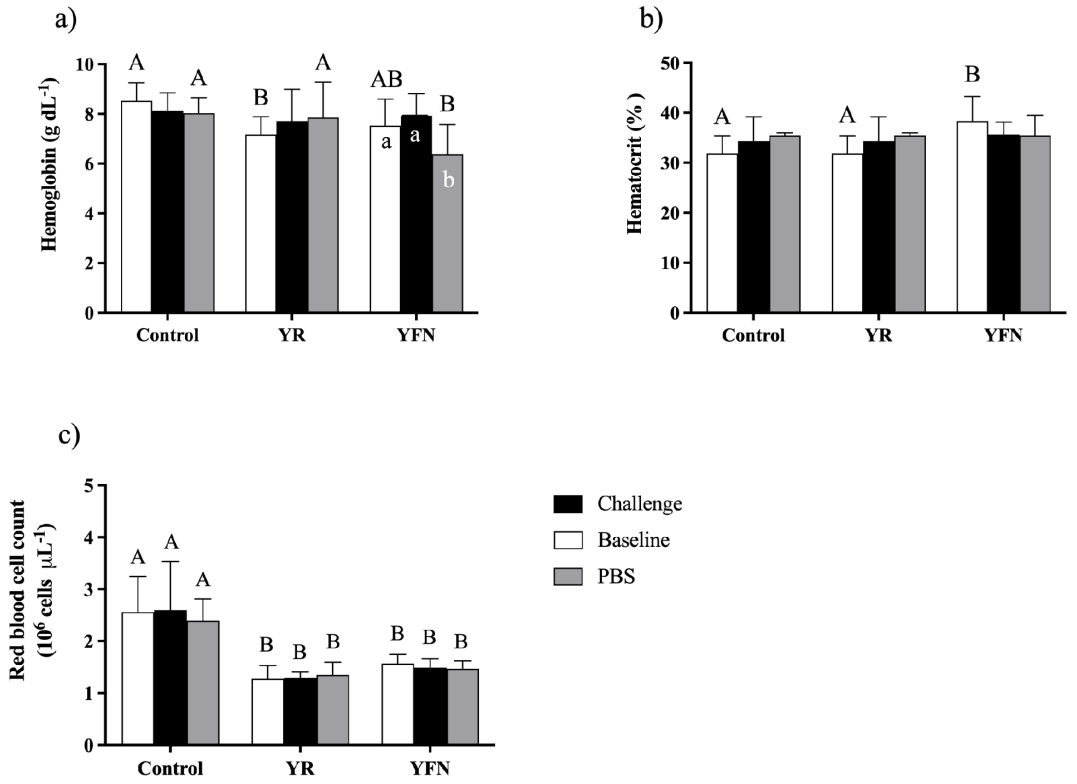

FIGURE 1
Hemoglobin level **(a)**, hematocrit **(b)** and red blood cell count **(c)** of juvenile Nile tilapia fed diets supplemented with two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet for 60 days and subjected to experimental LPS challenge. Capital letters compare the treatments in each sampling point (Baseline, LPS challenge and PBS), while small letters inside the bars evaluate the effect of the sampling points in each dietary treatment using Tukey's multiple range test ($p<0.05$). Bars correspond to means ± SD of two determinations per fish and ten fish per treatment.

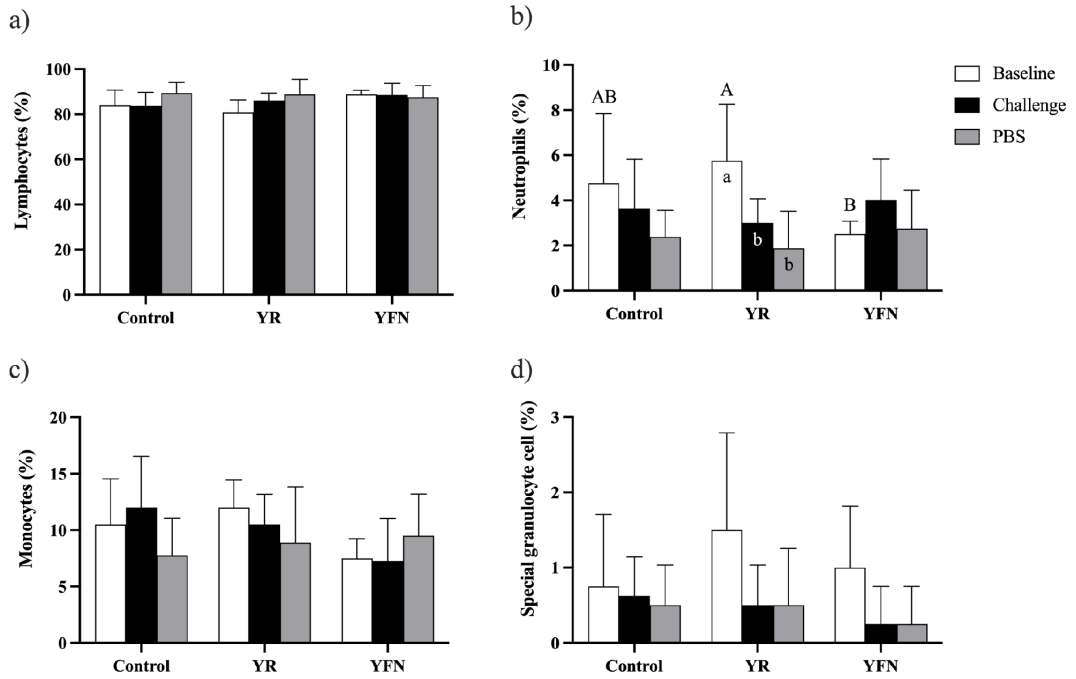

FIGURE 2
Relative proportion of lymphocytes **(a)**, neutrophils **(b)**, monocytes **(c)** special granulocyte cells **(d)** of juvenile Nile tilapia fed diets supplemented with two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet for 60 days and subjected to experimental LPS challenge. Capital letters compare the treatments in each sampling point (Baseline, LPS challenge and PBS), while small letters inside the bars evaluate the effect of the sampling points in each dietary treatment using Tukey's multiple range test ($p<0.05$). Bars correspond to means ± SD of one determination per fish and eight fish per treatment.

The absence of growth-promoting effects observed in the present study was expected, given the fish's initial size (approximately 100 g), which exceeds the developmental stages at which dietary nucleotide supplementation typically enhances growth performance (Barros et al., 2015; Hossain et al., 2020). Dietary nucleotides are particularly important during early life stages because they support DNA and RNA synthesis, cellular proliferation, protein synthesis, and immune function, processes associated with rapid tissue development in muscle, intestinal epithelium, and immune organs (Dawood, 2021; Hossain et al., 2020; Reda et al., 2018; Taklu et al., 2025; Yaseen et al., 2020). Consequently, growth benefits are generally more evident in young fish or under physiological challenges, such as stress or infection, when endogenous nucleotide synthesis may not fully meet metabolic demands (Ding et al., 2021; Guo et al., 2017; Shi et al., 2026). As fish grow, metabolic maturation of the liver and intestine improves the efficiency of the salvage pathway, enabling the recycling of nucleobases and nucleosides from cellular turnover and reducing reliance on exogenous nucleotide sources (Ding et al., 2021; Zhang et al., 2025). At the same time, cellular proliferation rates decline, and a greater proportion of metabolic energy is directed toward physiological maintenance rather than somatic growth, reducing the likelihood of a growth response to dietary nucleotide supplementation (Guo et al., 2017; Hossain et al., 2021). Therefore, the lack of growth enhancement observed in the present study is consistent with the current understanding of nucleotide nutrition in aquaculture. The most

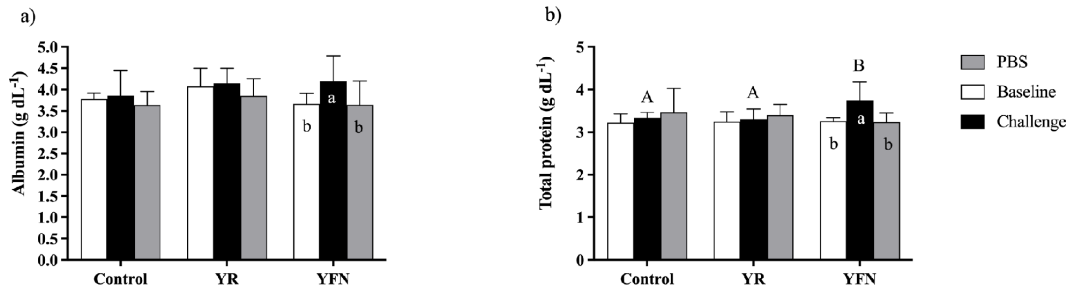

FIGURE 3
Albumin **(a)** and total protein **(b)** levels in juvenile tilapia fed diets supplemented with two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet for 60 days and subjected to experimental LPS challenge. Capital letters compare the treatments in each sampling point (Baseline, LPS challenge and PBS), while small letters inside the bars evaluate the effect of the sampling points in each dietary treatment using Tukey's multiple range test ($p<0.05$). Bars correspond to means ± SD of two determinations per fish and ten fish per treatment.

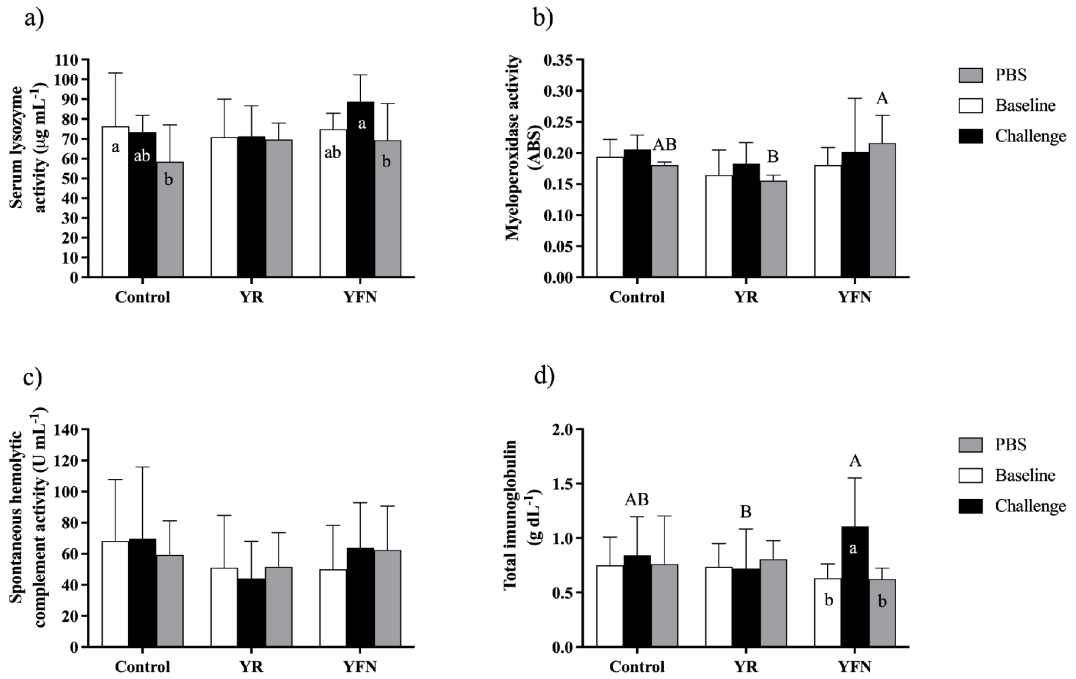

FIGURE 4
Serum lysozyme activity **(a)**, myeloperoxidase activity **(b)**, alternative complement pathway activity **(c)** and total serum immunoglobulin concentration **(d)** of juvenile Nile tilapia fed diets containing two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet for 60 days and subjected to experimental LPS challenge. Capital letters compare the treatments in each sampling point (Baseline, LPS challenge and PBS), while small letters inside the bars evaluate the effect of the sampling points in each dietary treatment using Tukey's multiple range test ($p<0.05$). Bars correspond to means ± SD of two determinations per fish and ten fish per treatment.

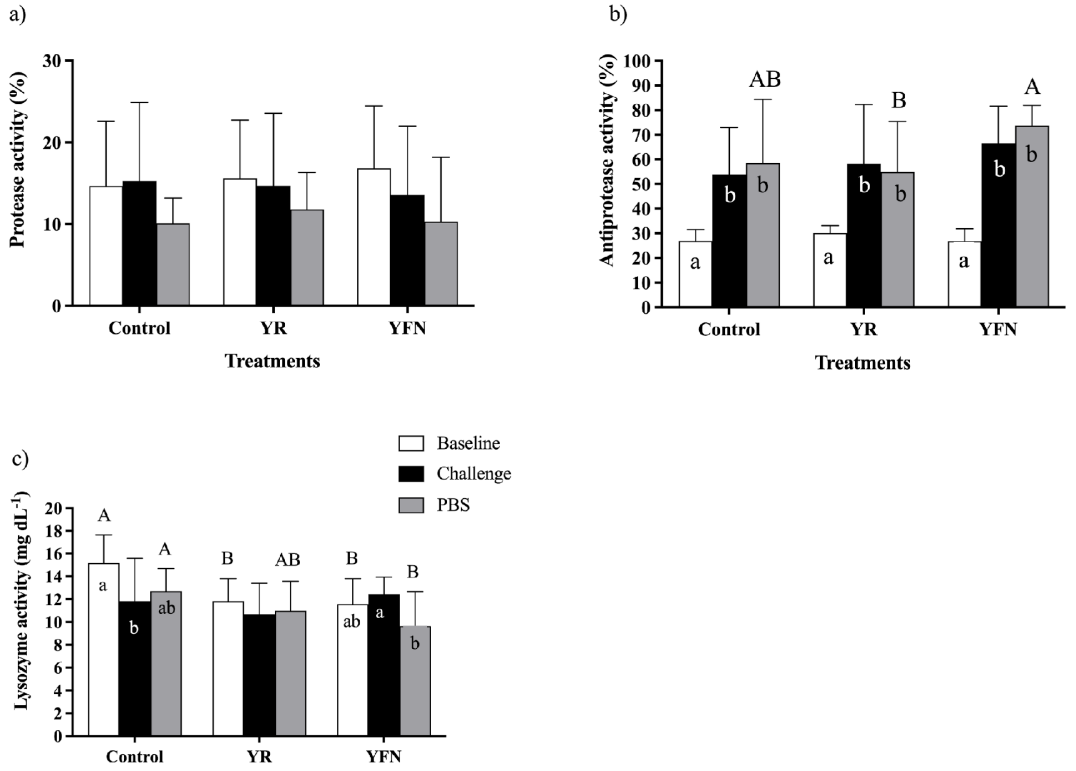

FIGURE 5
Mucus immune parameters (protease **(a)**, antiprotease **(b)** and lysozyme **(c)** activities) of juvenile Nile tilapia fed diets containing two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet for 60 days and subjected to experimental LPS challenge. Capital letters compare the treatments in each sampling point (Baseline, LPS challenge and PBS), while small letters inside the bars evaluate the effect of the sampling points in each dietary treatment using Tukey's multiple range test ($p<0.05$). Bars correspond to means ± SD of three determinations per fish and ten fish per treatment.

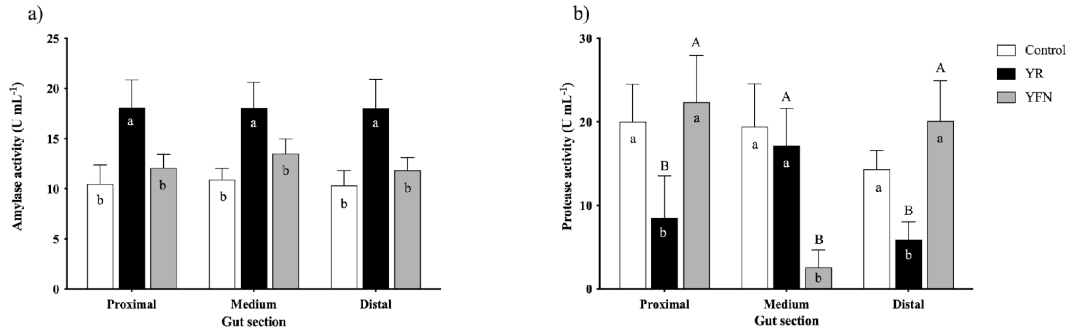

FIGURE 6
Intestinal enzyme activity (amylase **(a)** and protease **(b)**) of juvenile Nile tilapia fed diets containing two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet for 60 days. Capital letters compare the enzyme activity of a single treatment in each gut section, while small letters inside the bars evaluate the effect of the dietary treatments in each gut section using Tukey's multiple range test ($p<0.05$). Bars correspond to means ± SD of two determinations per fish and six fish per treatment.

common effect of dietary nucleotide supplementation in fish is their ability to improve intestinal health by assisting in the cell turnover of the enterocytes (Barducci et al., 2022; Xu et al., 2015; Carver, 1994).

However, few studies have evaluated the effect of different nucleotide sources on intestinal enzyme activity and whether these changes in intestinal health can be translated into increased digestibility and feed efficiency (Huang et al., 2025; Wang et al., 2025; Hossain et al., 2021). Our findings indicate that different nucleotide sources differently modulated intestinal protease activity according to the intestinal section. Additionally, yeast RNA could significantly increase the amylase activity of all gut segments of Nile tilapia. However, these changes did not translate into improved nutrient digestibility or feed utilization. To our knowledge, this could be the first report on the effect of different nucleotide sources on intestinal enzyme activity of Nile tilapia.

Studies in poultry have reported an increase on intestinal alkaline phosphatase and aminopeptidase activity in broilers fed purified pyrimidine and purine nucleosides (Daneshmand et al., 2017). Nucleotides and nucleosides are essential for the rapid proliferation and differentiation of intestinal epithelial cells, particularly in the crypt-villus axis. Because the intestinal epithelium has a high turnover rate, enterocytes require a continuous supply of nucleotides for DNA replication and RNA synthesis to maintain epithelial integrity and function (Hossain et al., 2021). The increased alkaline phosphatase and aminopeptidase activities observed by Daneshmand et al. (2017) suggest that dietary nucleosides may promote enterocyte maturation. Although the enzymes evaluated in the present study differ from those assessed by Daneshmand et al. (2017), both findings support the hypothesis that dietary nucleotide-related compounds can modulate intestinal enzymatic activity. Collectively, these results suggest that enhanced digestive enzyme activity may reflect improved intestinal absorptive capacity, although further studies are needed to confirm this relationship in teleost fish.

Free nucleotide mixture reduced protease activity primarily in the medium intestinal section. One possible explanation is that dietary nucleotides influence intestinal inflammatory status, which may indirectly affect digestive enzyme regulation, although these mechanisms were not evaluated in the present study. Previous studies have shown that dietary nucleotides can reduce the expression of pro-inflammatory cytokines (Huang et al., 2025; Krogdahl et al., 2023), potentially decreasing enterocyte stress and altering protease secretion. However, the mechanisms underlying the

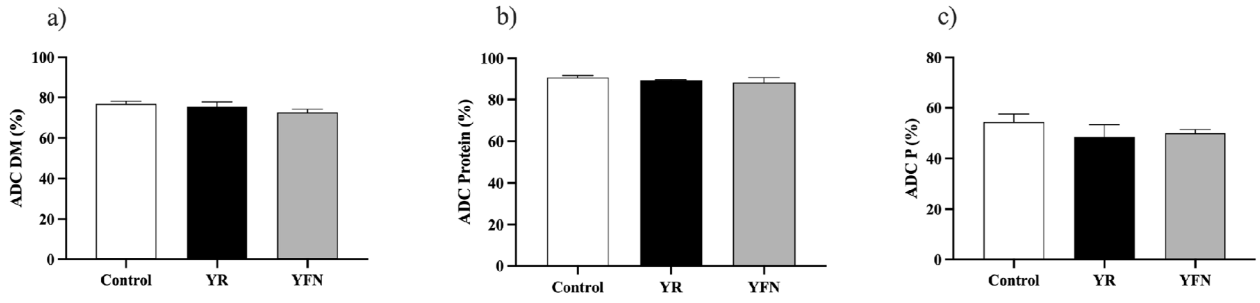

FIGURE 7
Apparent digestibility coefficients (ADC) of dry matter (DM) **(a)**, crude protein **(b)** and phosphorus (P) **(c)** of juvenile Nile tilapia fed diets containing two nucleotide sources (YR: yeast RNA and YFN: yeast-derived free nucleotides) and a control unsupplemented diet. Different letters above the bars indicate significant differences between the two nucleotides according to the Tukey's multiple range test ($P<0.05$). Bars correspond to means ± SD of three aquaria per treatment.

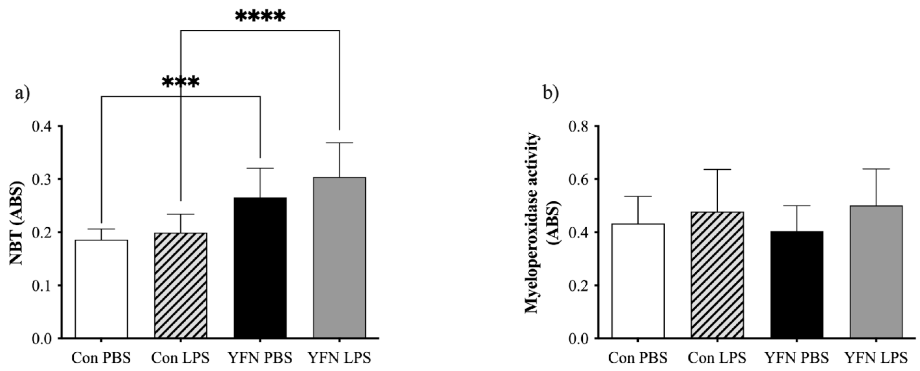

FIGURE 8
NBT **(a)** and myeloperoxidase activity **(b)** of head kidney tilapia leukocytes supplemented with a commercial nucleotide mixture (Yeast Free Nucleotides – 15%) and challenged with LPS. *** and **** indicates significant differences at p< 0.0002 and < 0.0001 by the Dunnet test with the respective controls, respectively. Bars correspond to means ± SD.

observed reduction in protease activity remain unclear, and further studies are needed to determine how dietary nucleotides influence digestive enzyme regulation across different intestinal tissues.

Hematology is widely used as biomarkers to evaluate the nutritional status, toxicity and the impact of some compounds on overall physiology of fish. The effects of dietary nucleotides on hematology remain poorly investigated in fish, including Nile tilapia. The available literature on tilapia has shown conflicting results. Some studies have shown no effect of dietary nucleotide supplementation on RBC, Htc and Hb (Koshio et al., 2016), while others have shown a positive effect of dietary nucleotides supplementation on these parameters (Hossain et al., 2021). Surprisingly, we observed an overall decreasing effect of different nucleotide sources on Nile tilapia hematology, except for the YFN-fed group, which showed an increase in hematocrit before the LPS challenge. Despite this effect, the hematology values in all groups are within the normal range of healthy tilapia (Hossain et al., 2021).

The discrepancies among studies evaluating dietary nucleotides are likely related to differences in the composition and source of the supplements used. For example, Barros et al. (2015) evaluated a commercial nucleotide mixture formulated for poultry, whereas Koshio et al. (2016) used a yeast-derived product containing 15% free nucleotides. Similarly, Hossain et al. (2021) compared a single nucleotide salt (inosine monophosphate) with a yeast-derived mixture containing 34% free nucleotides (80% pyrimidines and 20% purines). In the present study, intact yeast RNA and a yeast-derived product containing 15% free nucleotides were evaluated. We hypothesize that the prolonged use of these ingredients, particularly intact yeast RNA, may have contributed to a reduction in iron bioavailability. Dietary RNA contains phosphate groups capable of binding metal cations such as iron, potentially forming complexes that limit intestinal absorption (Ding et al., 2021). Although this mechanism has not been extensively documented for dietary RNA, it is analogous to the well-established chelating effects of phytates, which reduce iron availability by forming insoluble complexes in the gastrointestinal tract (Călinoiu and Vodnar, 2018). Overall, these findings suggest that the hematological effects of dietary nucleotide supplementation may depend not only on nucleotide source and composition but also on their interactions with mineral metabolism, warranting further investigation into the mechanisms involved.

Innate and adaptive immune parameters have been extensively used as proxies of disease resistance in studies evaluating dietary nucleotides in fish (Kader et al., 2018; Barducci et al., 2022; Huang et al., 2025; Yaseen et al., 2020). Although dietary nucleotide supplementation had limited effects on serum and mucus immune parameters in the present study, the responses were generally positive and most consistent in fish fed the YFN diet. While the control and YR groups tended to maintain or decrease lysozyme activity and immunoglobulin levels following the LPS challenge, fish fed YFN exhibited increases in these parameters, suggesting enhanced innate and humoral immune responsiveness. A similar trend was observed for mucus immunity, although significant differences among treatments were limited. The relatively modest effects observed may be related to the challenge model employed, as most studies reporting significant improvements in similar immune parameters in Nile tilapia used virulent pathogen challenges rather than LPS-induced immune stimulation (Barros et al., 2015; Barducci et al., 2022).

Nucleotide supplementation is generally considered beneficial for tissues with high cellular turnover and metabolic activity, such as immune cells and intestinal enterocytes (Carver, 1994). These cells have a high demand for nucleotides to support proliferation, cellular signaling, and functional responses, which may exceed their capacity for *de novo* synthesis under certain conditions (Carver, 1994; Hossain et al., 2021). In addition, nucleotides can serve as alternative energy substrates, helping sustain cellular metabolism when energy availability is limited.

This rationale is consistent with the results of the *in vitro* assay, in which supplementation with YFN significantly increased superoxide anion production by head kidney leukocytes. In contrast, no effects were observed on myeloperoxidase (MPO) activity, suggesting that nucleotide supplementation may selectively modulate specific leukocyte functions and that the response may depend on the type of immune stimulus. These findings highlight the need for further studies investigating the effects of individual nucleotides and nucleotide mixtures on fish immune cell function, as information regarding their specific mechanisms of action remains limited.

Conclusions

In sum, this study demonstrated that dietary supplementation with yeast RNA and yeast-derived free nucleotides did not significantly affect growth performance in Nile tilapia with an initial body weight of approximately 100 g. Additionally, these nucleotide sources differently modulated intestinal enzyme activity according to the gut section, however, this was not translated in improved nutrient digestibility. In contrast, dietary inclusion of 0.2% yeast-derived free nucleotides promoted more consistent immunomodulatory effects, particularly through increased serum and mucosal immune-related parameters following LPS stimulation in both *in vivo* and *in vitro* assays.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All experimental procedures involving animals were performed in accordance with the guidelines for the care and use of animals established by the National Council for the Control of Animal Experimentation (CONCEA). The study was approved by the Ethics Committee for the Use of Animals of the Universidade Federal de Jataí (CEUA-UFJ; protocol no. 010/23, approved on 16 June 2023).

Author contributions

MG: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. MF: Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. BA: Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. DO: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing, Methodology, Supervision, Validation. CS: Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Data curation, Resources, Software. IG: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Project administration.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author IG declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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References

Anderson, D. P., and Siwicki, A. K. (1995). "Basic hematology and serology for fish health programs," in *Diseases in Asian Aquaculture II*. Eds. M. Shariff, J. R. Arthur and R. P. Subasinghe (Asian Fisheries Society, Manila, Philippines), 185–202.

AOAC (1995). *Official Methods of Analysis. 16th Ed* (Arlington: Association of Official Analytical Chemists).

Barducci, R. S., Abreu, V., Santos, A. A. D., Pacheco, L. G., Koch, J. F. A., Florencio, M., et al. (2022). Natural feed additive containing Saccharomyces cerevisiae-originated free nucleotides improves innate immunity, gut histology, and disease resistance in Nile tilapia. *Anim. Feed Sci. Technol.* 289, 115337. doi: 10.1016/j.anifeedsci.2022.115337

Barros, M. M., Guimarães, I. G., Pezzato, L. E., Oliveira Orsi, R., Fernandes Junior, A. C., Teixeira, C. P., et al (2015). The effects of dietary nucleotide mixture on growth performance, haematological and immunological parameters of Nile tilapia. *Aquacult. Res.* 46 (4). doi: 10.1111/are.12229

Bernfeld, P. (1955). "Amylases, alpha and beta," in *Methods in Enzymology*. Eds. S. P. Colowick and N. O. Kaplan (Academic Press, New York), 149–158.

Bezerra, F. W. F., Moraes, V. S. V., Pantoja, G. V., Salazar, M. D. R., Silva, M. P., Ferreira, B. F. Z., et al. (2024). "Nucleosides and nucleotides as classes of bioactive food compounds," in *Bioactive Compounds From Food*. Eds. L. M. L. Nollet and J. Ahmad (CRC Press, Boca Raton, FL), 205–219. doi: 10.1201/9781003386247-15

Boyd, C. E. (1990). *Water Quality in Ponds for Aquaculture* (Alabama: Auburn University).

Bremer Neto, H., Graner, C. A. F., Pezzato, L. E., and Padovani, C. R. (2005). Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado da 1,5-difenilcarbazida. *Ciênc. Rural* 35 (3), 691–697. doi: 10.1590/S0103-84782005000300033

Călinoiu, L. F., and Vodnar, D. C. (2018). Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients* 10, 1615. doi: 10.3390/nu10111615

Carver, J. D. (1999). Dietary nucleotides: effects on the immune and gastrointestinal systems. *Acta Paediatr.* 88, 83–88. doi: 10.1111/j.1651-2227.1999.tb01306.x

Cho, C. Y., and Slinger, S. J. (1979). "Apparent digestibility measurement in feedstuffs for rainbow trout," in *Finfish Nutrition and Fishfeed Technology*, vol. 2 . Eds. J. E. Halver and K. Tiews (Heinemann, Berlin, Germany), 239–247.

Cho, C. Y., Slinger, S. J., and Bayley, H. S. (1985). "Bioenergetics of salmonid fishes. I. A bioenergetic model for estimating the digestibility of food in fish," in *Nutrition and Feeding in Fish*. Eds. C. B. Cowey, A. M. Mackie and J. G. Bell (Academic Press, London), 81–99.

Collier, H. B. (1944). Standardization of blood haemoglobin determinations. *Can. Med. Assoc. J.* 50, 550.

Daneshmand, A., Kermanshahi, H., Danesh Mesgaran, M., King, A. J., and Ibrahim, S. A. (2017). Effect of purine nucleosides on growth performance, gut morphology, digestive enzymes, serum profile, and immune response in broiler chickens. *Br. Poult. Sci.* 58, 536–543. doi: 10.1080/00071668.2017.1335859

Dawood, M. A. O. (2021). Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. *Rev. Aquacult.* 13, 642–663. doi: 10.1111/raq.12492

Demers, N. E., and Bayne, C. J. (1997). The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. *Dev. Comp. Immunol.* 21 (4), 363–373. doi: 10.1016/S0145-305X(97)00009-8

Ding, T., Ge, S., Liu, X., Xu, M., and Li, Y. (2021). Nucleotides as optimal candidates for essential nutrients in living organisms: A review. *J. Funct. Foods* 82, 104498. doi: 10.1016/j.jff.2021.104498

El-Sayed, A. F. M. (2006). *Tilapia Culture* (Wallingford, UK: CABI Publishing).

European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) (2026). *EUMOFA Talk at European Maritime Day 2026: Aquaculture's Market Potential in the EU: Ensuring Food Security and Trade Balance in a Sustainable Blue Economy*. Available online at: https://fishery-aquaculture-market-observatory.ec.europa.eu/en/news-%26-events/events/eumofa-emd-2026 (Accessed May 15, 2026).

FAO (2026). *Species Analysis: Tilapia. Fao Globefish*. Available online at: https://openknowledge.fao.org/server/api/core/bitstreams/ef5cfd74-e95e-48b4-a71f-1fa326d7a182/content (Accessed April 17, 2026).

Furuya, W. M., Pezzato, L. E., Barros, M. M., Boscolo, W. R., Cyrino, J. E. P., Furuya, V. R. B., et al (2010). *Tabelas brasileiras para a nutrição de tilápias* (Toledo: GFM), 100.

Guardiola, F. A., Cuesta, A., Abellán, E., Meseguer, J., and Esteban, M. A. (2014). Comparative analysis of the humoral immunity of skin mucus from several marine teleost fish. *Fish Shellfish Immunol.* 40, 24–31. doi: 10.1016/j.fsi.2014.06.018

Guo, X., Ran, C., Zhang, Z., He, S., Jin, M., and Zhou, Z. (2017). The growth-promoting effect of dietary nucleotides in fish is associated with an intestinal microbiota-mediated reduction in energy expenditure. *J. Nutr.* 147, 781–788. doi: 10.3945/jn.116.245506

Holen, E., Austgulen, M. H., and Espe, M. (2021). RNA from baker's yeast cultured with and without lipopolysaccharide (LPS) modulates gene transcription in an intestinal epithelial cell model, RTgutGC from rainbow trout (Oncorhynchus mykiss). *Fish. Shellfish Immunol.* 119, 397–408. doi: 10.1016/j.fsi.2021.10.018

Hossain, M. S., Koshio, S., and Kestemont, P. (2020). Recent advances of nucleotide nutrition research in aquaculture: A review. *Rev. Aquacult.* 12, 1028–1053. doi: 10.1111/raq.12370

Hossain, M. S., Sony, N. M., Koshio, S., Ishikawa, M., Mian, S., and Kumar, V. (2021). Comparative assessment of purine nucleotides adenosine, guanosine and inosine monophosphates as functional supplements on growth and health performances of red sea bream, Pagrus major juvenile. *Aquacult. Nutr.* 27, 187–197. doi: 10.1111/anu.13177

Huang, W., Xu, W., Yao, C., Liu, Y., Xu, N., Yin, Z., et al. (2025). Compound nucleotides as feed attractants improve the survival, growth, digestion, antioxidant capacity, and immunity of large yellow croaker (Larimichthys crocea) larvae. *Fish. Shellfish Immunol.* 165, 110435. doi: 10.1016/j.fsi.2025.110435

International Aquaculture Feed Formulation Database (IAFFD) (2025). *International Aquaculture Feed Formulation Database*. Available online at: https://www.iaffd.com/ (Accessed January 5, 2023).

Kader, M. A., Bulbul, M., Abol-Munafi, A. B., Asaduzzaman, M., Mian, S., Noordin, N. B. M., et al. (2018). Modulation of growth performance, immunological responses and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus*) (Linnaeus 1758) by supplementing dietary inosine monophosphate. *Aquacult. Rep.* 10, 23–31. doi: 10.1016/j.aqrep.2018.03.003

Koshio, S., Hossain, M. S., and Ishikawa, M. (2016). "Nucleotide nutrition in fish and shrimp," in *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*. Eds. E. Ringø and S. H. Hoseinifar (John Wiley & Sons, Chichester), 257–272.

Krogdahl, Å., Chikwati, E. M., Krasnov, A., Dhanasiri, A., Berge, G. M., Aru, V., et al. (2023). Dietary fish meal level and a package of choline, β-glucan, and nucleotides modulate gut function, microbiota, and health in Atlantic salmon (Salmo salar, L.). *Aquacult. Nutr.* 2023 (1), 1–29. doi: 10.1155/2023/5422035

Munilla-Morán, R., and Saborido-Rey, F. (1996). Digestive enzymes in marine species. I. Proteinase activities in gut from redfish (Sebastes mentella), seabream (Sparus aurata) and turbot (Scophthalmus maximus). *Comp. Biochem. Physiol. B* 113 (2), 395–402. doi: 10.1016/0305-0491(95)02057-8

Li, P., and Gatlin, D. I. (2006). Nucleotide nutrition in fish: Current knowledge and future applications. *Aquaculture* 251, 141–152. doi: 10.1016/j.aquaculture.2005.01.009

Litwack, G. (1955). Photometric determination of lysozyme activity. *Proc. Soc Exp. Biol. Med.* 89, 401–403. doi: 10.3181/00379727-89-21824

Liu, L., Fang, J., Liang, X. F., and He, S. (2020). Nucleotide promotes feed intake and protein utilization via regulating the gene expression of feeding and nitrogen metabolism in juvenile Chinese perch (Siniperca chuatsi). *Aquacult. Nutr.* 26, 1702–1712. doi: 10.1111/anu.13121

Luo, W., Zhang, L., Zhu, Y., Tang, Z., Huang, W., Qin, Q., et al. (2024). Effects of combined application of nucleotides and β-glucan on intestinal health and flesh quality in grass carp (Ctenopharyngodon idella). *Aquacult. Rep.* 39, 102497. doi: 10.1016/j.aqrep.2024.102497

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugars. *Anal. Chem.* 31, 426–428. doi: 10.1021/ac60147a030

Pelusio, N. F., Parma, L., Volpe, E., Ciulli, S., Errani, F., Natale, S., et al. (2023). Yeast-extracted nucleotides and nucleic acids as promising feed additives for European sea bass (Dicentrarchus labrax) juveniles. *Front. Mar. Sci.* 10, 1145660. doi: 10.3389/fmars.2023.1145660

Peng, L., Zhang, L., Xiong, S., You, J., Liu, R., Xu, D., et al. (2024). A comprehensive review of the mechanisms on fish stress affecting muscle qualities: Nutrition, physical properties, and flavor. *Compr. Rev. Food Sci. Food Saf.* 23, e13336. doi: 10.1111/1541-4337.13336

Quade, M. J., and Roth, J. A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Veterinary Immunol. Immunopathology* 58, 239–248. doi: 10.1016/S0165-2427(97)00048-2

Rairat, T., Chuchird, N., Keetanon, A., Carcano, P., Comi, M., and Koppe, W. (2022). Effects of dietary yeast-derived nucleotide and RNA on growth performance, survival, immune responses, and resistance to Vibrio parahaemolyticus infection in Pacific white shrimp (Litopenaeus vannamei). *Aquacult. Rep.* 27, 101352. doi: 10.1016/j.aqrep.2022.101352

Reda, R. M., Selim, K. M., Mahmoud, R., and El-Araby, I. E. (2018). Effect of dietary yeast nucleotide on antioxidant activity, non-specific immunity, intestinal cytokines, and disease resistance in Nile tilapia. *Fish Shellfish Immunol.* 80, 281–290. doi: 10.1016/j.fsi.2018.06.016

Sankaran, K., and Gurnani, S. (1972). On the variation in the catalytic activity of lysozyme in fishes. *Indian J. Biochem. Biophys.* 9, 162–165.

Secombes, C. J. (1990). "Isolation of fish leucocytes," in *Techniques in Fish Immunology*. Eds. J. S. Stolen, T. C. Fletcher, D. P. Anderson, B. S. Roberson and W. B. van Muiswinkel (SOS Publications, Fair Haven, NJ), 137–154.

Shi, Y., Zhang, Q., Cheng, G., Zhang, Y., Yang, P., Cai, C., et al (2026). Yeast nucleotide enhances barrier function by regulating the intestinal microbiota and metabolic pathways of fish to alleviate virus-induced intestinal damage. *Mar. Life Sci. Technol.* 8 (2), 580–597. doi: 10.1007/s42995-025-00330-9

Sunyer, J. O., and Tort, L. (1995). Natural hemolytic and bactericidal activities of sea bream Sparus aurata serum are effected by the alternative complement pathway. *Vet. Immunol. Immunopathol.* 45 (3-4), 333–345. doi: 10.1016/0165-2427(94)05430-Z

Taklu, M., Rajabi Islami, H., Hosseini Shekarabi, S. P., Mousavi, S. A., and Yousefi Jourdehi, A. (2025). Supplemental effect of dietary nucleotides on hematological profile, hepatic biomarkers, antioxidant capacity, and digestive functions in sterlet sturgeon, Acipenser ruthenus. *Sci. Rep.* 15, 11408. doi: 10.1038/s41598-025-96116-1

Wang, S., Li, W., Yan, Y., Li, E., Wang, X., Qin, J. G., et al. (2025). Dietary nucleotides improve growth of juvenile Eriocheir sinensis under a low-fish meal diet via enhanced feed intake and gut health. *Aquacult. Nutr.* 2025 (1), 8633112. doi: 10.1155/anu/8633112

Xu, L., Ran, C., He, S., Zhang, J., Hu, J., Yang, Y., et al. (2015). Effects of dietary yeast nucleotides on growth, non-specific immunity, intestine growth and intestinal microbiota of juvenile hybrid tilapia *Oreochromis niloticus* ♀ × Oreochromis aureus ♂. *Anim. Nutr.* 1, 244–251. doi: 10.1016/j.aninu.2015.08.006

Yaseen, M. S., Abdelaziz, M., Abdel-Moneam, D. A., Abd-Elhay, E., Wassif, I. M., and Moustafa, M. (2020). Efficacy of dietary nucleotides (Nucleoforce™) on growth, haemato-immunological response and disease resistance in Pangasianodon hypophthalmus (Sauvage 1878) in Egypt. *Egypt. J. Aquat. Biol. Fish.* 24, 405–424. doi: 10.21608/EJABF.2020.112821

Zhang, Y., Wu, P., Jiang, W. D., Liu, Y., Ma, Y. B., Kuang, S. Y., et al. (2025). A nutritional strategy for improving sub-adult grass carp (Ctenopharyngodon idella) flesh flavor: The role of α-lipoic acid in regulating muscle nucleotide and fatty acid metabolism. *Aquaculture* 603, 742437. doi: 10.1016/j.aquaculture.2025.742437