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RESEARCH

Synergistic effects of synbiotic and hydrolyzed yeast extract in the diet on growth performance, hemato-immunological responses, and digestive enzyme activities of Nile tilapia (*Oreochromis niloticus*) fry

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Abstract

This research aimed to assess the impact of integrating synbiotic (A-Pro Aqua®, SYN; 0.05 and 0.1%) and hydrolyzed yeast extract (Nutri Yeast-Aq®, HYE; 0.05 and 0.1%) as a factorial experiment 2×2 in a completel randomized design in the diet on growth performance, hemato-immunulogical responses, and digestive enzyme activities of Nile tilapia (*Oreochromis niloticus*) fry with intial weight 4 ± 0.2 g for 63 days. Three hundred fish were stocked in fifteen 120-L glass containers (20 fish per each unit) and fed with five diets containing control diet, 0.05% synbiotic + 0.1% yeast, 0.05% synbiotic + 0.05% yeast, 0.1% synbiotic + 0.1% yeast, and 0.1% synbiotic + 0.05% yeast. The findings indicated that elevated SYN and HYE levels significantly ($P < 0.05$) increased final weight, specific growth rate, volountary feed intake, survival rate, protein productive value, and protein efficiency ratio. Additionally, higher SYN and HYE levels resulted in a significant decrease in feed conversion ratio and hepatosomatic index ($P < 0.05$), while the activities of digestive enzymes such as protease, lipase, and amylase were considerably increased ($P < 0.05$). The synergistic application of SYN and HYE in the diet markedly enhanced alternative complement activity and triiodothyronine, immunoglubolin, and lysozyme concentrations in the plasma of test fish ($P < 0.05$). Moreover, the dietary supplementation SYN and HYE significantly elevated high-density lipoprotein concentation in the plasma of test fish while concurrently reducing low-density lipoprotein, very low-density lipoprotein, and triglyceride concentrations ($P < 0.05$). In summary, based on the growth parameters and hemato-immunological responses, dietary supplementation with 0.1% SYN in conjunction with 0.1% HYE was recommended.

Keywords Hydrolyzed yeast · Synbiotic · Nile tilapia · Growth performance · Blood parameters

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Introduction

The expansion of Nile tilapia (*Oreochromis niloticus*) aquaculture, the third most productive finfish species in the world, is crucial for addressing food security but also presents significant environmental and socioeconomic challenges that need careful analysis (FAO 2022). With both benefits and hurdles, tilapia production stands out as a strong candidate for sustainable aquaculture to meet future food demands (Thomas et al. 2021; Geletu and Zhao 2023). To ensure its viability, rigorous research must focus on improving farming practices, managing resources effectively, and addressing disease management challenges that may arise from intensive farming (Mengistu et al. 2020; Naylor et al. 2023; Subasinghe et al. 2009).

Nutritional supplements represent critical instruments in aquaculture feeding paradigms that enhance growth efficacy, bolster resistance to pathogenic threats, optimize gastrointestinal health, and confer additional nutritional advantages to the aquatics (e.g., finfish and shellfish species). These supplements operate through diverse mechanisms and encompass probiotics, prebiotics, and synbiotics (Bhujel 2000; Hossain et al. 2022; Stefanska et al. 2021). Probiotics are defined as live microorganisms that transit into the intestines and aid in the amelioration of the microbial equilibrium within the gasterointestincal tract. These microorganisms can facilitate enhancements in growth performance and nutrient assimilation efficiency, thereby contributing to the overall improvement of gut health and the digestive processes in fish (Maas et al. 2021; El-Saadony et al. 2021a,b). Prebiotics are characterized as indigestible food substances that act as substrates for beneficial intestinal microorganisms. By providing sustenance to these advantageous microbes, prebiotics promote their proliferation and metabolic functions, which in turn exerts a favorable impact on digestive efficiency and overall fish health (Deng et al. 2021; Eissa et al. 2023, 2024a,b; Standen et al. 2013).

Synbiotics denote synergistic combinations that concurrently exhibit the functional attributes of both probiotics and prebiotics. They enhance the viability of beneficial gut microorganisms while simultaneously improving their nutritional profiles. These supplements encompass constituents that can exert beneficial effects on growth perfomane, well-being, and hemato-immunological parameters in fish (Wang et al. 2023). The positive effects of using dietary supplementation of probiotic species including *Bacillus* spp. (*B. coagulans*, *B. subtilis*, *B. licheniformis*, *B. pumilus*, and *B. firmus*), *Lactobacillus* spp. (*Lb. plantarum*, *Lb. rhamnosus*, *Lb. acidophilus*, *Lb. brevis*, *Enterococcus faecium*, *Pediococcus acidilactici*, and *Lc. lactis*), other Gram-positive species (*Micrococcus luteus* and *Clostridium butyricum*), Gram-negative species (*Pseudomonas* spp., *Rhodopseudomonas palustris*, and *Citrobacter freundii*), and yeast (*Saccharomyces cerevisiae*) (Carnevali et al. 2014) and dietary prebiotics such as mannan-oligosaccharide (MOS), inulin, fructo-oligosaccharide (FOS), and short chain FOS (Ringø et al. 2014) on growth performance, immune responses, and resistance against bacterial pathogens have been reported in tilapia feeding trials. Using dietary synbiotic and biofloc meal containing probiotic species such as *Lactobacillus plantarum*, *L. acidophilus*, and *Saccharomyces boulardi* and mannan oligosaccharide as a prebiotic in the diet of Nile tilapia (*O. niloticus*) with initial weight 21.52 g for 83 days reduced the effect of salinity increment (0, 6, and 18 ppt) on growth performance, gut microflora, and histological responses (Hersi et al. 2023).

S. cerevisiae yeast is considered as a microbial product and used in different roles (e.g., pro-, pre-, syn-, post-, and parabiotic) (de Valle et al. 2023). Different nutritional supplements derived from yeasts including cell wall, cytoplasm content (nucleotide), and hydrolyzed yeast extract exhibit a beneficial impact on the feed intake of livestock, poultry, and aquatics, consequently facilitating enhanced and expedited growth as well as augmented productivity in animal husbandry (de Valle et al. 2023). The inclusion of yeast within the dietary regimens of aquatic organisms bolsters the proliferation of advantageous bacterial communities in the gastrointestinal tract while simultaneously inhibiting the proliferation of pathogenic microorganisms. Furthermore, the yeast inclusion in aquafeeds results in an increase in the bioavailability of nutrients, thus yielding more and superior digestible nutrients (Rafiee and Vafadar 2021). The use of nutritional supplements in aquatics feeding is considered an advanced and productive approach that requires detailed scientific investigation.

Based on the available resources, there is currently no information regarding the combined use of synbiotics and hydrolyzed yeast extract in the diets of Nile tilapia fry. Moreover, the selection and use of suitable supplements that align with the needs of fish are among the issues that aquaculture experts should address to ensure optimal performance and the health of the fish. Therefore, the objective of the present study was to investigate the impact of the combined use of synbiotics and hydrolyzed yeast extract in the diet on the biological indices of Nile tilapia fry.

Materials and methods

Diet preparation

In the present study, a fully randomized factorial design (2 × 2) was utilized to assess the influence of hydrolyzed yeast extract and synbiotics on Nile tilapia. The experimental framework comprised five distinct treatments, which included two doses of hydrolyzed yeast extract (HYE; Nutri Yeast-Aq® by Kimia Zim Co., Tehran, Iran) at the advised levels of 0.05% and 0.1%, as well as two doses of synbiotic (SYN; A-Pro Aqua® by Fartak Additives Sepehr Saman Co., Mashhad, Iran) at the prescribed levels of 0.05% and 0.1%. The synbiotics were composed of various bacterial strains, namely *Pediococcus acidilactici*, *Enterococcus faecium*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Saccharomyces cerevisiae*, each at a concentration of $10^{\wedge 9}$ cfu/g, supplemented with fructooligosaccharides.

A control treatment was produced in accordance with the nutritional requiremnets for Nile tilapia (NRC 1996), utilizing commonly accessible feed ingredients within the commercial market (Table 1). The feedstuffs were ground using a hammer mill (Nikoopack® Co., Isfahan, Iran). To prepare pellets, water was added to the feed mixture by 25% by weight (w/w), and finally, oil was added to the dough. Then, it was passed through a homemade meat mincer (Moulinex®, France) with a mesh diameter 2 mm. The produced diets were labelled and stored in the refrigerator 4 °C until use. It should be noted that the amount of synbiotic and hydrolyzed yeast extract required for each treatment was calculated and replaced by the weight of carboxymethyl cellulose (CMC) in control diet. Totally, five treatments were denoted as control, 0.05% SYN + 0.1% HYE, 0.05% SYN + 0.05% HYE, 0.1% SYN + 0.1% HYE, and 0.1% SYN + 0.05% HYE from here onwards.

Table 1 Diet formulation and proximate chemical composition (% dry matter basis) of control diet

Ingredient	Inclusion level
Fishmeal[a, α]	29
Soybean meal[a, β]	22
Wheat gluten[a, γ]	14
Wheat flour[a, δ]	10
Corn flour[a, ε]	14
Fish oil[a]	3.8
Soybean oil[a]	3.8
Mineral premix[b*]	1
Vitamin premix[b**]	1
Caboxymethyl cellulose (CMC)[c]	1
NaCl[a]	0.4
Proximate chemical composition (% dry matter basis)[d]	
Dry matter	90.52
Crude protein	37.3
Crude fat	9.3
Crude fiber	7.8
Nitrogen-free extract	5.38
Ash	6.7

[a]Saramad Fish Aquafeed Co, Iran

[b]Kimia Roshd Co. Iran

[c]Sigma, Germany

[d]All proximate chemical composition of feed ingredients and control diet was measured according to AOAC (2005) protocols with the exception of NFE. NFE = dry matter − (crude protein + crude fat + crude fiber + ash)

[α]Fish meal (55% crude protein; 5% ether extract)

[β]Soybean meal (35% crude protein; 4% ether extract)

[γ]Wheat gluten (82% crude protein)

[δ]Wheat flour (9% crude protein)

[ε]Corn flour (11% crude protein)

[*]Mineral premix contains (mg Kg^{-1}) Mg, 100; Zn, 60; Fe, 40; Cu, 5; Co, 0.1; I, 0.1; antioxidant, 100

[**]Vitamin premix contains (mg Kg^{-1}) E, 30; K, 3; thiamine, 2; riboflavin, 7; pyridoxine, 3; pantothenic acid, 18; niacin, 40; folate, 1.5; choline, 600; biotin, 0.7; cyanocobalamin, 0.02

Fish rearing and experimental conditions

All methodologies employed in the present investigation adhered rigorously to the ethical standards established by Ferdowsi University of Mashhad (IR.UM.REC.1402.200). Three hundred mixed-sex Nile tilapia fries with an average weight of 4 ± 0.2 g were prepared from ornamental fish breeding facilities located in Mashhad, Khorasan Razavi Province, Iran. The fish were transported to the aquatic laboratory through 50-L double-walled plastic bags containing two-thirds of water and one-third of pure oxygen, and after water was combined, they were stored at three 500-L quarantine tanks for 14 days.

Experimental fish were outwardly healthy and did not require any treatment. After adaptation, the specimens were stocked at 15 120-L glass tank (with stocking density 20 fish per a tank). The phtoperiod (L: D) was regulated 12:12 h. The aquatic environment in the rearing systems was subjected to a daily water exchange rate of 30%. Physicochemical factors of the water in the rearing tanks were measured weekly. The mean (± standard deviation) of temperature, pH value, and NH_3 concentration were 28.3 ± 0.5 °C, 7.34 ± 0.12, and < 0.01 mg L^{-1}, respectively. The fish was fed at a rate equivalent to 6% body weight per day at six time intervals (8:00; 10:00: 12:00; 14:00, 16:00, and 18:00) for 63 days. Biometry of weight and length of experimental fish was done in 21-day intervals to adjust the amount of diet for the next stage.

Chemical analysis

Analysis of dry matter (oven drying, 105 °C), crude protein (N × 6.25, Kjeldahl system: Buchi Labortechnik AG, Flawil, Switzerland), crude fat (Soxtec System HT 1043: Foss Tecator, AB), crude fiber (Fibertec™ 8000, Foss, USA), ash (muffle furnace, 550 °C) contents of feed ingredients, test diets, and whole body composition of experimental fish were measured according to protocols described in AOAC (2005). Nitrogen-free extract (NFE) content was calculated by subtraction dry matter minus crude protein, crude fat, crude fiber, and ash contents.

Growth performance and nutritional efficiency indices

Growth performance of test fish fed the experimental diets was assessed using metrics such as specific growth rate (SGR; % body weight (BW) day^{-1}), daily growth index (DGI; % BW day^{-1}), feed conversion ratio (FCR), voluntary feed intake (VFI, % BW day^{-1}), survival rate (%), condition factor (%), protein productive value (PPV, %), and protein efficiency ratio (PER). The specific formulas used were as follows:

$$\text{SGR} = \frac{LnW_f(g) - LnW_i(g)}{time(day)} \times 100$$

$$\text{DGI} = \frac{W_f^{0.33}(g) - W_i^{0.33}(g)}{time(day)} \times 100$$

$$\text{FCR} = \frac{Feed_{consumed}(g)}{W_{gain}(g)}$$

$$\text{VFI} = \frac{Feed_{consumed}(g)}{W_{mean}(g) \times time(day)} \times 100$$

$$\text{SR} = \frac{Finalnumber}{Initialnumber} \times 100$$

$$\text{CF} = \frac{W_f(g)}{L_f^3(cm)} \times 100$$

$$\text{PPV} = \frac{\left(W_f(g) \times N_f \times Crudeprotein_{Finalcarcass}(gKg^{-1})\right) - \left(W_i(g) \times N_f \times Crudeprotein_{Initialcarcass}(gKg^{-1})\right)}{Consumedprotein(g)} \times 100$$

$$\text{PER} = \frac{W_{gain}}{Consumedprotein(g)} \times 100$$

In the above equations, W_i, W_f, W_{mean}, W_{gain}, t, and $Feed_{consumed}$ are initial weight, final weight, mean weight, weight increment (g), time period (day), and consumed feed (g), respectively.

Biochemical indices evaluation

At the end of the feeding trial, fish were deprived of feed for 24 h prior to blood sampling. Blood collection was performed using 25-G syringes after anesthetizing the fish with clove oil (Clove Aqua®, Imad Khorasani aquaculture cooperative, Khorasan Razavi Province, Mashhad, Iran) at a recommended concentration of 0.5 mg L^{-1}. Blood samples ($n = 9$ fish per a treatment) were collected from the caudal vein using heparinized syringes without pooling the samples. After centrifugation (10 min at 3000 rpm), the resulting plasma was frozen at − 20 °C. Plasma glucose levels were measured using an ELISA kit (Cusabio Biotech Co., China) at final reading 450 nm with ELISA reader (NBReader®, Ringbio Co., China). Total cholesterol, triglycerides, and high-density lipoprotein concentrations were measured using fish ELISA kits (MY BioSource Co., USA) at final reading 450 nm with ELISA reader (NBReader®, Ringbio Co., China). Thyroxine (T_4), triiodothyronine (T_3), alternative complement activity (ACH_{50}), lysozyme, and total immunoglobulin concentrations were assessed using fish ELISA kits (MY BioSource Co., USA) with ELISA reader (NBReader®, Ringbio Co., China) at final readings 600, 450, 414, 450, and 450 nm, respectively.

Hepatosomatic and viscerosomatic indices

Hepatosomatic index (HSI) and viscerosomatic index (VSI) of test fish ($n = 9$ fish per a treatment) were calculated as the ratio of hepatopancreas weight (g) or visceral weight (g) to the total body weight (g), multiplied by 100, respectively.

Digestive enzyme activities

In order to measure the activities of digestive enzymes (protease, lipase and, amylase), three fish from each replicate ($n = 9$ fish per a treatment) were selected at the end of the experiment, subsequently weighed, fainted deeply in clove oil (0.5 mg L^{-1}), and dissected. Finally, the midgut of the experimental fish was removed, assigned a code prior to being encased in aluminum foil, and preserved at − 80 °C (Kong et al. 2021; Safari et al. 2016). The assessment of protease activity was conducted utilizing the substrate benzoyl-arginine-p-nitroanilide alongside a standardized calibration curve derived from bovine trypsin solution. One unit of protease is operationally defined as the release of 1 μmol tyrosine per min per g protein. The evaluation of amylase activity was performed employing starch as the substrate through colorimetric analysis, while the measurement of lipase activity was executed using an emulsion composed of olive oil and arabic gum as the substrate via titration methodology. One unit of amylase is characterized as the release of 1 μmol maltose per min per g of starch. One unit of lipase is similarly defined as the release of 1 μmol of fatty acid per min per g Arabic gum.

Statistical analysis

To assess the normality of the data distribution, the Smirnov-Kolmogorov statistical test was utilized. In cases where the data demonstrated a normal distribution, both one-way and two-way ANOVA analyses were deemed appropriate for the purpose of data investigation. The criterion for statistical significance was set at 95% ($P < 0.05$). Specifically, one-way and two-way ANOVA were executed to compare the treatments across three replicates. The Duncan' multiple range test was applied to identify significant differences among the treatments ($P < 0.05$) utilizing SPSS™ version 27. All results were presented as mean ± SEM.

Results

Growth performance

As shown in Table 2, with an increment in the levels of SYN and HYE from 0.05 to 0.1% in the diets, the highest values of final weight, specific growth rate (SGR), voluntary feed intake (VFI), survial rate, and daily growth index (DGI) and the lowest values of feed conversion ratio (FCR) and hepatosomatic index (HSI) were observed ($P < 0.05$) (Table 2). The findings revealed that Nile tilapia fed the diet supplemented with 0.1% SYN + 0.1% HYE exhibited the highest ($P < 0.05$) final weight, SGR, VFI, DGI, and survival rate (Table 2). In contrast, the fish fed the control group exhibited the lowest growth performance parameters ($P < 0.05$). Conversely, FCR values were significantly lower in the fish fed the treatments containing different levels of SYN and HYE compared to those of fed the control diet ($P < 0.05$) (Table 2).

Biochemical indices

Increasing in the dietary levels of SYN and HYE from 0.05 to 0.1% led to increase the concentrations of insulin, T_3, and HDL in the blood plasma of Nile tilapia fish ($P < 0.05$) (Table 3). Conversely, with increment in the SYN and HYE contents in the diet, the total cholesterol, LDL (Table 3), and TG (Fig. 1a, b) concentrations and ALT activity (Fig. 2a, b) decreased ($P < 0.05$) but the concentration of T_4 and glucose did not change ($P > 0.05$) (Table 3). The findings presented in Table 3 and Figs. 1c and 2c demonstrated that the plasma concentrations of total cholesterol, LDL, and TG and also ALT activity were markedly diminished in the Nile tilapia fish fed the diets containing different levels of SYN and HYE compared to those of fed the control diet ($P < 0.05$). Conversely, the the highest levels of insulin, T_3, T_4, and HDL were observed in the blood plasma of the test fish fed the diet contaning 0.1% SYN + 0.1% HYE ($P < 0.05$) (Table 3).

The concentrations of total plasma protein, total immunoglobulin, and lysozyme and alternative complement activity (ACH_{50}) in the blood plasma of Nile tilapia fish enhanced with an increment in the dietary levels of SYN and HYE from 0.05 to 0.1% ($P < 0.05$) (Table 4). The highest concentration of total plasma protein was observed in the fish fed the 0.1 SYN + 0.1 HYE- diet ($P < 0.05$) (Table 4); however, this did not show any significant difference ($P > 0.05$) with those of fed the 0.05 SYN + 0.1 HYE and 0.1 SYN + 0.05 HYE diets (Table 4). Nile tilapia fish fed the 0.1 SYN + 0.05 HYE and 0.1 SYN + 0.1 HYE diets indicated higher concentrations of total immunoglobulin and lysozyme and alternative complement activity (ACH_{50}), compared to those of fed the other test diets ($P < 0.05$) (Table 4).

Table 2 The mean (± SEM) effects of different levels of synbiotics (0.05% and 0.1%) and hydrolyzed yeast extract (0.05% and 0.1%) on initial weight (g), final weight (g), specific growth rate (SGR; % BW day^{-1}), survival rate (%), daily growth index (DGI; % BW day^{-1}), feed conversion ratio (FCR), and hepatic somatic index (HSI; %) of Nile tilapia fed the experimental diets for 63 days at three replicates[1]

		Initial weight (g)	Final weight (g)	Specific growth rate (% BW day^{-1})	Voluntary feed intake (% BW day^{-1})	Feed conversion ratio	Survival rate (%)	Daily growth index (% BW day^{-1})	Hepatic somatic index (%)
One-way ANOVA comparison									
Control		4.12^{a}	21.5^{a}	2.62^{a}	1.5^{a}	1.7^{e}	66.67^{a}	1.83^{a}	3.01^{d}
0.05 SYN + 0.05 HYE		4.12^{a}	24.8^{b}	2.85^{b}	1.7^{b}	1.6^{d}	76.67^{b}	2.04^{b}	2.87^{bc}
0.05 SYN + 0.1 HYE		4.12^{a}	27.02^{c}	2.99^{c}	1.9^{c}	1.4^{c}	81.67^{c}	2.17^{c}	2.56^{b}
0.1 SYN + 0.05 HYE		4.12^{a}	28.20^{d}	3.05^{d}	2^{d}	1.3^{b}	91.67^{d}	2.24^{d}	2.43^{b}
0.1 SYN + 0.1 HYE		4.12^{a}	36.22^{e}	3.45^{e}	2.4^{e}	1.1^{a}	98.33^{e}	2.65^{e}	2.24^{a}
SEM[2]		–	0.07	0.06	0.003	0.003	1.29	0.003	0.031
***P*-value**		–	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Two-way ANOVA comparison									
SYN (%)	**0.05**	–	25.91^{a}	2.91^{a}	1.86^{a}	1.52^{b}	79.16^{a}	2.11^{a}	2.96^{b}
	0.1	–	32.12^{b}	3.25^{b}	2.25^{b}	1.22^{a}	95^{b}	2.45^{b}	2.74^{a}
SEM		–	–	0.04	0.006	0.007	1.17	0.004	0.007
HYE (%)	**0.05**	–	26.5^{a}	2.95^{a}	1.90^{a}	1.48^{b}	84.16^{a}	2.14^{a}	2.91^{b}
	0.1	–	31.62^{b}	3.21^{b}	2.21^{b}	1.27^{a}	90^{b}	2.41^{b}	2.78^{a}
SEM		–	–	0.04	0.006	0.007	1.17	0.004	0.007
***P*-value**	**SYN**	–	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	HYE	–	< 0.001	< 0.001	< 0.001	< 0.001	0.008	< 0.001	< 0.001
	SYN*HYE	–	< 0.001	< 0.001	< 0.001	0.5	0.631	< 0.001	< 0.001

[1]Different superscripts within a column indicated significant differences at $P>0.05$

[2]Standard error of mean

Table 3 The mean (± SEM) effects of different levels of synbiotics (SYN; 0.05% and 0.1%) and hydrolyzed yeast extract (HYE; 0.05% and 0.1%) on insulin (ng mL^{-1}), T_3 (ng dL^{-1}), T_4 (ng dL^{-1}), total closterol (mg dL^{-1}), high-density lipoprotein (HDL; mg dL^{-1}), low-density lipoprotein (LDL; mg dL^{-1}), and glucose (mg dL^{-1}) concentrations in the blood plasma of Nile tilapia fed the experimental diets for 63 days at three replicates[1]

		Insulin (ng mL^{-1})	T_3 (ng dL^{-1})	T_4 (ng dL^{-1})	Total cholesterol (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	Glucose (mg dL^{-1})
One-way ANOVA comparison								
Control		9.21^{a}	1.13^{a}	1.7^{a}	275.16^{d}	79.9^{a}	177.36^{e}	62.31^{a}
0.05 SYN + 0.05 HYE		9.71^{b}	1.28^{b}	1.8^{b}	275.03^{d}	103.1^{b}	154.4^{d}	62.18^{a}
0.05 SYN + 0.1 HYE		10.37^{c}	1.36^{c}	1.5^{c}	264.43^{c}	109.8^{c}	139.15^{c}	62.31^{a}
0.1 SYN + 0.05 HYE		11.46^{d}	1.42^{d}	1.8^{d}	259.76^{b}	122.4^{d}	122.95^{b}	62.28^{a}
0.1 SYN + 0.1 HYE		14.18^{e}	1.44^{e}	1.7^{e}	239.26^{a}	124.5^{e}	104.03^{a}	62.31^{a}
SEM[2]		0.019	0.008	0.09	0.34	0.29	0.45	0.045
***P*-value**		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Two-way ANOVA comparison								
SYN (%)	**0.05**	10.04^{a}	1.32^{a}	1.69^{a}	269.73^{b}	106.40^{a}	146.78^{b}	62.25^{a}
	0.1	12.82^{b}	1.43^{b}	1.76^{a}	249.50^{a}	123.46^{b}	113.49^{a}	62.30^{a}
SEM		0.018	0.005	0.07	0.26	0.13	0.33	0.031
HYE (%)	**0.05**	10.59^{a}	1.35^{a}	1.80^{a}	267.4^{b}	112.76^{a}	138.68^{b}	62.23^{a}
	0.1	12.27^{b}	1.40^{b}	1.68^{a}	251.8^{a}	117.16^{b}	121.59^{a}	62.31^{a}
SEM		0.018	0.005	0.07	0.26	0.13	0.33	0.031
***P*-value**	**SYN**	< 0.001	< 0.001	0.35	< 0.001	< 0.001	< 0.001	0.29
	HYE	< 0.001	< 0.001	0.28	< 0.001	< 0.001	< 0.001	0.095
	SYN*HYE	< 0.001	< 0.001	0.37	< 0.001	< 0.001	< 0.001	0.26

[1]Different superscripts within a column indicated significant differences at $P>0.05$

[2]Standard error of mean

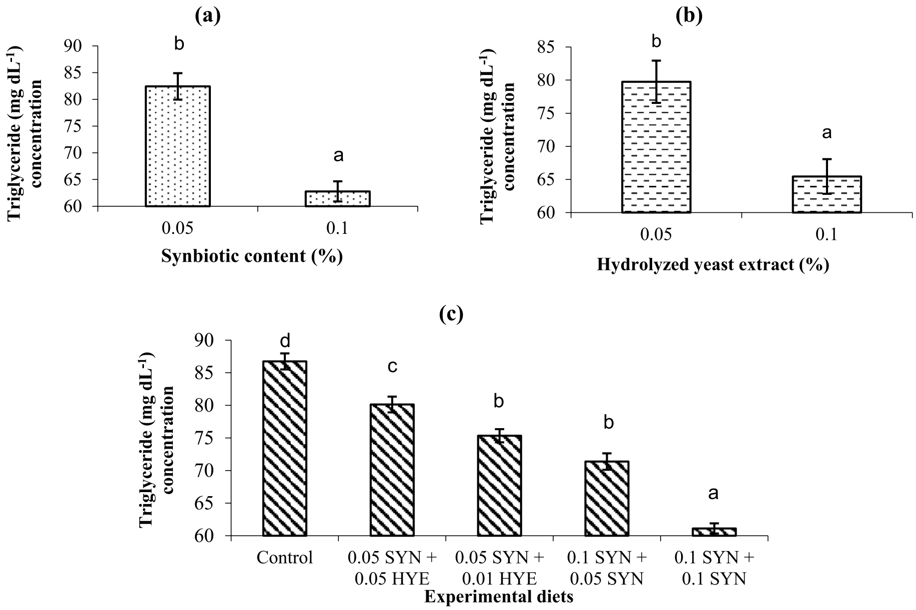

Fig. 1 The mean (±SEM) effects of using different levels of synbiotics (SYN; 0.05% and 0.1%) and hydrolyzed yeast extract (HYE; 0.05% and 0.1%) in the diet on triglyceride (mg dL$^{-.1}$) concentration in the blood plasma of Nile tilapia fed the experimental diets for 63 days at three replicates. The letters a and b showed two-way ANOVA comparisons, and c showed one-way ANOVA comparison at $P<0.05$

Body composition and nutritional efficiency indices

The findings of present study revealed that the inclusion of SYN (0.05–0.1%) and HYE (0.05–0.1%) led to increase crude protein, crude fat, and ash contents in the whole body composition and nutritional efficiency indices including PER and PPV values, while causing a decrease in nitrogen-free extract content (Table 5). The carcass of Nile tilapia fish fed the 0.1 SYN+0.1 HYE- diet had the highest crude protein, crude fat, and ash contents and PER and PPV values ($P<0.05$) (Table 5).

Digestive enzyme activities

Increasing in the dietary levels of SYN and HYE from 0.05 to 0.1% resulted in a significant increase in the enzymatic activities of protease, lipase, and amylase within the midgut of Nile tilapia fish ($P<0.05$) (Table 6). The findings elucidated in Table 6 demonstrated that the activities of protease, lipase and, amylase showed a markedly enhancement in the Nile tilapia fish fed the 0.1 SYN+0.1 HYE- diet ($P<0.05$).

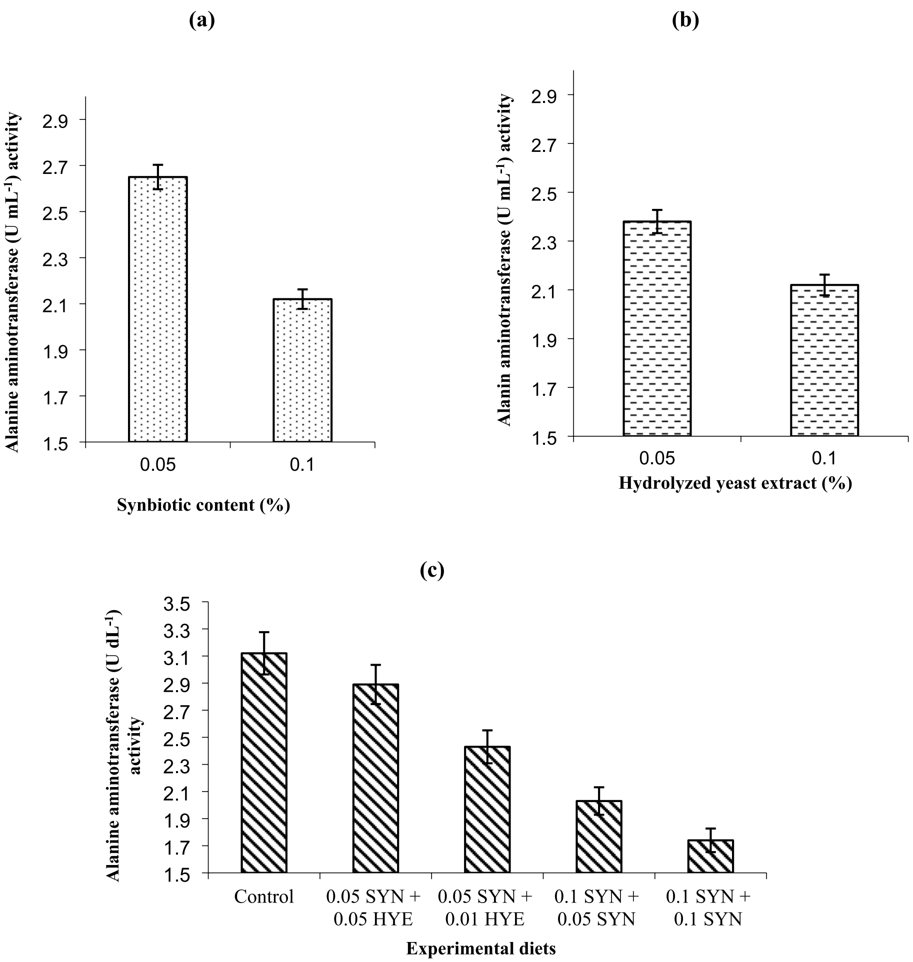

Fig. 2 The mean (±SEM) effects of using different levels of synbiotics (SYN; 0.05% and 0.1%) and hydrolyzed yeast extract (HYE; 0.05% and 0.1%) in the diet on alanine aminotransferase activity (U dL^{-}.^{1}) in the blood plasma of Nile tilapia fed the experimental diets for 63 days at three replicates. The letters a and b showed two-way ANOVA comparisons, and c showed one-way ANOVA comparison at $P<0.05$

Discussion

Growth performance

The findings derived from the present study elucidated that the administration of SYN and HYE facilitated an enhancement in both growth metrics, survival rate, and HSI among tilapia fish. Final weight (24.8–36.22 vs 21.5 g), SGR (2.85–3.45% vs 2.62 BW day^{-1}), and DGI (2.04–2.65 vs 1.83 BW day^{-1}) values of test fish fed the diets containing different levels of SYN and HYE increased compared to the those of fed the control diet. Against, the values of FCR (1.1–1.6 vs 1.7) and HSI (2.24–2.87 vs 3.10%) in the test fish fed the diet SYN and HYE decreased compared to the those of fed the control diet. Supplementation

Table 4 The mean (± SEM) effects of different levels of synbiotics (SYN; 0.05% and 0.1%) and hydrolyzed yeast extract (HYE; 0.05% and 0.1%) on total plasma protein (mg mL^{-1}), total immunoglobulin (mg mL^{-1}), alternative complement (ACH_{50}; U mL^{-1}), and lysozyme (mg mL^{-1}) concentrations in the blood plasma of Nile tilapia fed the experimental diets for 63 days at three replicates[1]

		Total plasma protein (mg mL^{-1})	Immunoglobulin (mg mL^{-1})	Alternative complement (U mL^{-1})	Lysozyme (mg mL^{-1})
One-way ANOVA comparison					
Control		4.38^{a}	1.89^{a}	2.18^{a}	2.68^{a}
0.05 SYN + 0.05 HYE		4.39^{a}	2.41^{abc}	2.76^{c}	3.24^{c}
0.05 SYN + 0.1 HYE		4.43^{ab}	2.03^{ab}	2.36^{b}	2.91^{b}
0.1 SYN + 0.05 HYE		4.45^{ab}	2.55^{bc}	2.51^{bc}	3.06^{bc}
0.1 SYN + 0.1 HYE		4.56^{b}	2.85^{c}	2.63^{c}	3.12^{c}
SEM[2]		0.17	0.16	0.15	0.16
***P*-value**		< 0.001	< 0.001	< 0.001	< 0.001
Two-way ANOVA comparison					
SYN (%)	**0.05**	4.32^{a}	2.22^{a}	2.54^{a}	2.87^{a}
	0.1	4.43^{b}	2.70^{b}	2.57^{b}	3.09^{b}
SEM		0.18	0.15	0.14	0.012
HYE (%)	**0.05**	4.34^{a}	2.31^{a}	2.41^{a}	3.01^{a}
	0.1	4.41^{b}	2.44^{b}	2.69^{b}	3.15^{b}
SEM		0.19	0.15	0.14	0.17
***P*-value**	**SYN**	< 0.001	< 0.001	< 0.001	< 0.001
	HYE	< 0.001	< 0.001	< 0.001	< 0.001
	SYN*HYE	0.54	0.41	0.59	0.72

[1]Different superscripts within a column indicated significant differences at P>0.05

[2]Standard error of mean

of *Bacillus infantis*, *B. subtilis*, *Exiguobacterium profundum*, and *B. megaterium* isolated from biofloc systems in the diet of genetically improved farmed tilapia with initial weight 10 g for 42 day led to augment the growth performance and immune capabilities (Menaga et al. 2020). Moreover, the potential implications of utilizing either single or multi-strain probiotics on Nile tilapia were documented over a span of 112 days, thereby substantiating that the incorporation of probiotics considerably influences the growth performance of tilapia (Mohammadi et al. 2021), which is in concordance with the outcomes of the current study. The application of probiotics, which encompasses *Lysinibacillus fusiformis*, *Bacillus amyloliquefaciens*, and *Enterococcus hirae*, alongside a commercial probiotic and a mixed probiotic has been demonstrated to significantly enhance the growth rate of tilapia while concurrently improving the feed conversion ratio (Zabidi et al. 2021). Additionally, the researchers indicated that the incorporation of these specific probiotics into tilapia diets led to a marked increase in survival rates and a reduction in mortality within the assessed ponds. The outcomes of this investigation regarding the profound influence of probiotics on the enhancement of growth parameters and the augmentation of tilapia survival are in alignment with the results of the current study. To elucidate the role of probiotics in promoting survival among fish, it is pertinent to highlight that opportunistic probiotics mitigate the presence of pathogenic microorganisms by appropriating host resources

Table 5 The mean (± SEM) effects of different levels of synbiotics (SYN; 0.05% and 0.1%) and hydrolyzed yeast extract (HYE; 0.05% and 0.1%) on whole body composition (% dry matter basis), protein efficiency ratio (PER), and protein productive value (PPV; %) of Nile tilapia fed the experimental diets for 63 days at three replicates[1]

		Dry matter (%)	Crude protein (%)	Crude fat (%)	Nitrogen-free extract (%)	Ash (%)	Protein efficiency ratio	Protein productive value (%)
One-way ANOVA comparison								
Control		92.21^{a}	67.63^{a}	5.67^{a}	24.35^{e}	2.34^{a}	1.34^{a}	19.09^{a}
0.05 SYN + 0.05 HYE		92.34^{a}	69.81^{b}	5.70^{a}	19.84^{d}	4.64^{b}	1.43^{a}	21.35^{b}
0.05 SYN + 0.1 HYE		92.37^{a}	69.78^{b}	5.87^{b}	19.48^{c}	4.86^{c}	1.56^{b}	24.56^{bc}
0.1 SYN + 0.05 HYE		92.38^{a}	69.92^{b}	5.83^{ab}	18.99^{b}	5.25^{d}	1.98^{c}	28.94^{c}
0.1 SYN + 0.1 HYE		92.31^{a}	70.73^{c}	6.08^{c}	16.79^{a}	6.38^{e}	2.32^{d}	32.46^{d}
SEM[2]		1.18	1.28	1.09	1.39	1.21	2.56	35.85
***P*-value**		0.538	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Two-way ANOVA comparison								
SYN (%)	**0.05**	92.32^{a}	69.8^{a}	5.78^{a}	19.66^{b}	4.75^{a}	1.82^{a}	23.08^{a}
	0.1	92.35^{a}	70.32^{b}	5.96^{b}	17.89^{a}	5.82^{b}	2.46^{b}	36.29^{b}
SEM		0.038	0.042	0.038	0.071	0.04	0.011	0.11
HYE (%)	**0.05**	92.34^{a}	69.84^{a}	5.76^{a}	19.41^{b}	4.95^{a}	1.94^{a}	24.81^{a}
	0.1	92.33^{a}	70.26^{b}	5.98^{b}	18.13^{a}	5.62^{b}	2.34^{b}	34.57^{b}
SEM		0.052	0.042	0.038	0.071	0.04	0.011	0.13
***P*-value**	**SYN**	0.076	< 0.001	0.032	< 0.001	< 0.001	< 0.001	< 0.001
	HYE	0.058	< 0.001	0.004	< 0.001	< 0.001	< 0.001	< 0.001
	SYN*HYE	0.062	< 0.001	0.48	< 0.001	< 0.001	< 0.001	< 0.001

[1]Different superscripts within a column indicated significant differences at $P > 0.05$

[2]Standard error of mean

Table 6 The mean (± SEM) effects of different levels of synbiotics (SYN; 0.05% and 0.1%) and hydrolyzed yeast extract (HYE; 0.05% and 0.1%) on total protein content of the midgut tissue (g mL^{-1}) and digestive enzyme activities of protesase (U mg^{-1}), lipase (U mg^{-1}), and amylase (U mg^{-1}) in Nile tilapia fed the experimental diets for 63 days at three replicates[1]

		Total protein content (g mL^{-1})	Protesase (U mg^{-1})	Lipase (U mg^{-1})	Amylase (U mg^{-1})
One-way ANOVA comparison					
Control		3.09^a	2.12^a	1.54^a	0.21^a
0.05 SYN + 0.05 HYE		3.21^b	2.35^b	1.79^b	0.34^b
0.05 SYN + 0.1 HYE		3.23^b	2.43^c	1.86^c	0.41^c
0.1 SYN + 0.05 HYE		3.43^c	2.78^d	1.92^d	0.54^d
0.1 SYN + 0.1 HYE		3.59^d	2.98^e	2.19^e	0.89^e
SEM[2]		0.018	0.019	0.017	0.021
***P*-value**		< 0.001	< 0.001	< 0.001	< 0.001
Two-way ANOVA comparison					
SYN (%)	**0.05**	3.21^a	2.41^a	1.61^a	0.36^a
	0.1	3.43^b	3.13^b	2.33^b	0.90^b
SEM		0.019	0.014	0.14	0.012
HYE (%)	**0.05**	3.41^a	2.57^a	1.77^a	0.52^a
	0.1	3.56^b	2.97^b	2.17^b	0.74^b
SEM		0.032	0.014	0.014	0.012
***P*-value**	**SYN**	< 0.001	< 0.001	< 0.001	< 0.001
	HYE	< 0.001	< 0.001	< 0.001	< 0.001
	SYN*HYE	< 0.001	< 0.001	< 0.001	0.93

[1]Different superscripts within a column indicated significant differences at $P > 0.05$

[2]Standard Error of Mean

and eradicating pathogens, thereby diminishing the likelihood of infections and subsequent mortality in fish administered various probiotic strains. Prior research has documented the antagonistic properties of probiotics against other pathogenic organisms, particularly those belonging to the genera *Vibrio* sp. and *Streptococcus* sp. (Masduki et al. 2020). An examination of the mechanisms underlying increased survival in fish treated with diverse probiotics has revealed that these probiotics may synthesize inhibitory compounds, which facilitate competitive exclusion between probiotics and pathogens, a critical factor in diminishing microbial load in the gastrointestinal tract and reducing the incidence of various diseases (Elewasy et al. 2024; Hassanin et al. 2024; Sahu et al. 2008). The conclusions drawn from the current study bolster this hypothesis. However, further investigations need to show metabolic pathways.

The recommendations regarding the incorporation of probiotics into the aquatic ecosystems of juvenile Nile tilapia across both biofloc and traditional aquaculture frameworks were meticulously examined. The results revealed that the amalgamation of biofloc technology with probiotic supplementation significantly improved the survival rates, growth metrics, and overall feed conversion efficiency of the fish, while simultaneously augmenting the total bacterial population within both the aquatic environment and the intestinal microbiota (Waiyamitra et al. 2020). The results of this investigation are additionally congruent with the advantageous effects of probiotics on survival rates, weight gain, and growth rate in fish as documented in the present study. The strategies of designing and inclusion of probiotics as an efficient feed additive in aquafeed production industry are preventive or therapeutic applications (Xia et al. 2020). In a more comprehensive context, probiotics within aquaculture refer to any microbial agents that provide advantages to the fish, aquaculturists, or end consumers, primarily by facilitating microbial equilibrium within the physiological systems of the fish (Torres-Maravilla et al. 2024; Zhang et al. 2025). The efficacy of probiotics transcends the gastrointestinal tract, significantly contributing to the overall health of the host by promoting growth performance, enhancing immunological responses, and optimizing fish productivity (Chauhan and Singh 2019). The positive effects of using probiotics in tilapia rearing systems on growth parameters, water quality, and disease prevention are reported in the literature review (Abdel-Tawwab et al. 2021; Hassaan et al. 2021), thereby validating the conclusions of the current study; the utilization of synbiotics correlates with improved survival rates in fish. The primary rationale for this enhancement in survival rates, along with the inclination for augmented growth performance in fish, is ascribed to the beneficial dietary influences and the establishment of resilience against pathogenic challenges (Diwan et al. 2024; Eissa et al. 2023, 2024a, b; Torres-Maravilla et al. 2024; Zhang et al. 2025). In this regard, dietary supplementation of Nile tilapia with *B. subtilis* and nano- *B. amyloliquefaciens* increased disease resistance against *Vibrio chilerae* and *Aeromonas hydrophila*, respectively (Elewasy et al. 2024; Hassanin et al. 2024). The increment in growth parameters originated from using microbial products, especially synbiotics, which can be related to improved benign gut microflora, increased absorption capacity of microvilli via secretion of exo-enzymes, reduced pH value of digesta, and, as a result, decreased in the population of pathogenic bacteria in the digestive system. The competition for nutrient absorption and the contest for attachment to binding sites on intestinal villi further diminish the presence of pathogenic entities. Furthermore, an increased predominance of beneficial microbiota within the gastrointestinal tract stimulates the synthesis of peroxides, which also antagonistically affects the populations of pathogenic microorganisms (Diwan et al. 2024; Merrifield and Ringø 2014; Torres-Maravilla et al. 2024; Zhang et al. 2025).

Digestive enzyme activities

According to the results of present study, diet supplementation with different levels of SYN and HYE compared to control diet considerably increased the digestive enzyme activities including protease (2.35–2.98 vs 2.12 U mg^{-1}), lipase (1.79–2.19 vs 1.54 U mg^{-1}), and amylase (0.34–0.89 vs 0.21 U mg^{-1}) of Nile tilapia. Similar outcomes have been reported from a wide range of studies on the effects of microbioal products on the digestive system capacity. Feeding Nile tilapia with the diet containing probiotics increased their digestive enzymes (Lou et al. 2014), which is consistent with the results of this investigation. Microbial products such as synbiotics have been shown to increase the activities of digestive enzymes, which benefits tilapia's ability to digest diet and absorb macro- and micronutrients. The beneficial bacterial load, increment in digestive enzyme activities, and efficient intestine digestion and nutritional absorption in experimental tilapia may be related to improved gut histomorphology, which enhances the structural integrity of the intestinal lining and promotes optimal nutrient uptake (Abdel-Aziz et al. 2020; De Marco et al. 2023).

The results of the current study are in line with the synergistic combination of amylase, protease, cellulase, xylanase, and probiotics, which showed a significant positive effect on the digestion of feed organisms produced in biofloc systems and the absorption of digested nutrients (Asha et al. 2024). Interestingly, probiotics have important nutritional benefits on growth performance of aquatics by producing organic acids like lactic acid, vitamins, and enzymes like amylase and protease. Fish with lower gut pH have more active digestive enzymes, as seen by the higher enzyme activity reported in the present study. It is confirmed in literature that with a reduction in pH value in the gasterointestinal tract, bioavailability of minerals as cofactors will increase, and consequently, this led to enhance the enzyme activity in the intestine tract (Amenyogbe et al. 2024; De Marco et al. 2023). However, it needs further research.

The findings derived from the present investigation are concordant with the synergistic amalgamation of various enzymes, specifically amylase, protease, cellulase, xylanase, and the incorporation of probiotics, all of which have demonstrated a markedly significant positive influence on the digestive processes of feed organisms cultivated within biofloc systems, as well as on the subsequent absorption of the nutrients that have been digested (Asha et al. 2024). In addition, probiotics are well recommended, as they offer exceptional nutritional benefits to fish or the aquaculture organism. In particular, the production of these metabolites like organic acids (e.g., lactic acid), some essential vitamins, and digestive enzymes such as amylase and protease plays significant roles in the metabolism of these animal species. Furthermore, empirical observations suggest that fish exhibiting a lower pH level within their gastrointestinal tracts tend to possess a more pronounced activity of digestive enzymes, a phenomenon that is corroborated by the elevated enzyme activity reported in the current study. In addition to this, it appears that a reduction in pH levels not only facilitates but also enhances the bioavailability of essential minerals, some of which may act as crucial cofactors that significantly improve the efficacy and operational capabilities of various digestive enzymes (Merrifield and Ringø 2014). But this should be noted that this area is yet to be researched for a more thorough understanding of all dynamics involved in it. These findings could be crucial in the establishment of aquaculture practices in considering feed efficiency and growth metrics. Thus, this research in continuing should try to expand on their understanding of these relationships and develop a greater knowledge of the complicated interactions in biofloc systems.

The results obtained from the current study corroborate findings on the synergistic combination of the various enzymes, amylase, protease, cellulase, xylanase, and also by incorporating probiotics, which have been discovered to have a statistically significant positive influence on the digestion processes of the feed organisms raised in biofloc systems and subsequent absorption of nutrients as digested (Asha et al. 2024). Apart from these, probiotics provide a positive nutritional impact within which they enhance the growth performance of aquatic organisms through the production of organic acids, lactic acid, important vitamins, and digestive enzymes like amylase and protease—very much vital in the metabolic processes of these organisms. Furthermore, empirical observations indicate that fish having less acidity within their digestive system would exhibit more pronounced activities of digestive enzymes. This has also been observed, corroborating the current study where high enzyme activity is reported. Along with this, it seems that reduced pH levels can further improve an organism's bioavailability with essential minerals that, in part, might function as major cofactors in establishing the increased effectiveness and functioning of various digestive enzymes (Bielik and Kolisek 2021). This area of research, however, requires much more complete study to clarify the complex interactions involved with this. However, further studies must be done to display the effect of using microbial product in the diet on physiological pathways related to growth parameters.

Moreover, probiotics have different inhibitory roles against pathogenic bacteria such as producing organic acids (e.g., lactic acid and acetic acids) and bacteriocins (e.g., pediocins). This trait increases fish survival rates and aids in the fight against infections. According to a study looking into how probiotics affect tilapia's digestive enzymes, amylase and protease enzymes are crucial for breaking down proteins and carbohydrates (starch), respectively, which helps fish diets use nutrients more effectively (Adineh et al. 2023). According to the results of present study, some researchers confirmed that dietary supplementation with various strains of probiotics led to increase digestive enzymes (Abdel-Aziz et al. 2020; Amenyogbe et al. 2024; Zhang et al. 2025). It is crucial to emphasize that the augmentation of amylase activity may facilitate enhanced digestion and nutrient assimilation, ultimately contributing to increased body mass and growth rates. The findings of this investigation, in conjunction with a plethora of other scholarly inquiries, underscore a substantial influence of probiotics on the enhancement of protease enzyme concentrations. Protease enzymes have been classified as one of the specific kinds of antimicrobial proteins. Some of their roles are in the regulation of the more complex metabolic processes engaged in the synthesis of various polymicrobial antimicrobial molecules and, consequently, in installing base defense mechanisms for organisms against pathogenic microorganisms. Serine and cysteine proteases work as a defense mechanism. They fight bacteria protozoa and parasites and neutralize pathogenic entities effectively. Indeed, proteases possess the capability to directly proteolyze harmful factors (Song et al. 2016; Abdel-Aziz et al. 2020; Zhang et al. 2025). In the present investigation, elevated concentrations of digestive enzymes were noted in Nile tilapia fish subjects administered probiotic interventions in comparison to the control diet. This observation not only emphasizes the influence of probiotics on the augmentation of immune functionality in Nile tilapia but also accentuates their significance in the enhancement of growth performance and overall well-being.

Immue responses

It can be concluded from the present findings that the addition of different levels of SYN and HYE beneficially affects the improving traits of the immune response parameters, including total immunoglobulin level (2.41–2.85 vs 1.89 mg mL^{-1}), ACH_{50} (2.36–2.76 vs 2.18 mg mL^{-1}), and lysozyme (3.12–3.24 vs 2.68 mg mL^{-1}) concentrations compared to Nile tilapia fed the control diet. Probiotics have a role in improving metabolisms of nutrients, decreasing oxidative stresses, and reducing the pathogen load; therefore, they improve immunity in ray fins (Sewaka et al. 2019; Geuking et al. 2014). Apart from the above mentioned, probiotics are also responsible for reducing the pathogenic microorganisms found in fishes and also alleviate oxidative stress and modulate expression of genes concerning inflammatory pathways in fishes: These scholars also reported that gut microbiota and specific probiotics can metabolize the amino acid tryptophan to produce metabolites enriched in indole, which modulate the immune responses via activation of the aryl hydrocarbon receptor. It is confirmed that pro-inflamatory cytokine expression was modulated via tryptophan metabolism (Nicolas and Chang 2019). In addition, Ringø et al. (2016) state that probiotics can increase the immune response of fish because of stimulated production of specific antibodies and activation of immune cells, leading to increased resistance against pathogenic organisms. This is in line with the present study, which noted similar increases in immune indices in tilapia given probiotics. The research by El-Saadony et al. (2021a,b) further revealed that viruses have positive influence on general health status and immunity levels in fish, being incorporated into probiotics in aqua feeds. This gave probiotics health preservation within the intestines and systemic immunity boost to give fish a more efficient reaction to pathogenic infections; such an improvement was put between increased performance and a more robust immune system, thus validating the current study's assertions.

Hormonal index

The findings from present investigation indicated that use of SYN and HYE in the diet have a very pronounced beneficial effect on hormone levels in Nile tilapia, showing a greatest increase in insulin level (9.71–14.18 vs 9.21 ng mL^{-1}) compared to those of fed the control diet. Apparently, such enhancement of the carbohydrate metabolism and liver function with more increases in insulin levels and reduction of the blood glucose. The effect of insulin requlation with probiotics species in the aquafeed was reported by Martinez et al. (2004). Besides, such finding according to Badi et al. (2021) indicated that probiotics have positive effects on hormonal responses of fishes and thus improve metabolic efficiency and growth performance. It further adds that dietary inclusion of probiotics in fish could thus improve the hormonal balance, laying a foundation for optimal growth conditions. Also, reference in recent work by Qiang et al. (2020) was placed on probiotics for balancing the endocrine system in aquaculture species, as this paper was founded on findings surrounding administering better insulin sensitivity coupled with improved metabolic health. This is worth noting due to the role played on nutrient utilization, where fish convert feed into tissue mass more efficiently.

The revelation of the present study indicated that probiotics as an application can enhance all the immunological parameters in Nile tilapia namely, total immunoglobulin level, complement, and lysozyme levels. Moreover, probiotics were capable of improving metabolic activity related to nutrients, alleviating oxidative stress-causing organisms, and reducing the pathogenic loads for weight gain on the studied fish species to confer on them an added advantage with respect to immunity (Sewaka et al. 2019; Geuking et al. 2014). Also, probiotics can smooth oxidative upsets and promote changes in gene expressions associated with inflammatory pathways in addition to the removal of pathogenic microorganisms from fishes. Among all these, they elucidated that gut microbiota with some specific probiotics can metabolize amino acid tryptophan with indole-enriched metabolites modulating immunologic responses through activation of aryl hydrocarbon receptor. Besides, research highlighted that metabolism of tryptophan can deprive the pro-inflammatory cytokines from their expression, thus enhancing immune responses (Nicolas and Chang 2019). Similarly, the use of prebiotic mixtures in the diet of *Litopenaeus vannamei*, *O. niloticus*, and red tilapia (*O. niloticus* × O. *mossambicus*) has led to improved growth performance, liver enzyme activity, and expression of genes related to immunity and resistance against biological stressors including *Fusarium solani*, *Streptococcus iniae*, and *Vibrio alginolyticus* (Eissa et al. 2023, 2024a,b). The findings of the current investigation, when considered alongside these supplementary results, emphasize the prospective role of probiotics in augmenting hormonal parameters and metabolic functions in Nile tilapia, thereby facilitating enhanced growth and overall health within aquaculture methodologies.

Biochemical index

This research indicated that the use of SYN and HYE has a clear impact on the biochemical parameters of Nile tilapia, resulting in a significant decrease in total cholesterol levels compared to those of fed the control diet (239.26–264.43 vs 275.16 mg dL^{-1}). Cholesterol, which is classified as a steroid lipid, is present in almost all tissues due to incorporation into the cellular membranes and also exists in blood plasma because of its endogenous production in liver cells. All these findings prove that probiotics have lowered cholesterol levels as compared to the control. Metabolites, such as short-chain fatty acids, which are fermented probiotics, are then transported to the liver through circulation after being produced in the gastrointestinal tract of fishes. Such metabolites cause cholesterol synthesis to be low, which corresponds to the findings reported in this present work. Such studies have also found a similar reduction in triglyceride levels, which relates to increased production of short-chain fatty acids resulting from probiotic-based intervention compared to the control group (Bajelan et al. 2017).

The research conducted by Tovar et al. (2020) reported that adding probiotics in fish feed resulted in considerable lipid reductions, including cholesterol and triglycerides, and in better overall metabolic health, stating that probiotic effects on lipid metabolism would result in improved growth performance and health status in fish. This research also reveals that the beneficial effects of probiotics with regard to lipid metabolism would enable better performance of fish in growth and health conditions. In addition, the study by Muro et al. (2019) proved that dietary probiotics reduced plasma lipid concentrations in fish, suggesting that probiotics play an important role in lipid metabolism regulation and promotion of healthy physiological status in farmed species. Thus, all the previous evidence in these studies illuminates the importance of probiotics in biochemical health indices improvements for growth and welfare for Nile tilapia in aquaculture systems.

Carcass quality

The results of present study showed that diet supplementation with different levels of SYN and HYE compared to control diet considerably increased the crude protein (69–81-70.73 vs 67.63%), crude fat (5.70–6.08 vs 5.67%), and ash (4.64–6.38 vs 2.34%) contents in carcass of test fish. The values of PPV (21.35–35.85 vs 19.09%) and PER (1.43–2.56 vs 1.34) in Nile tilapia fed the diets containing SYN and HYE increased compared to those of fed the control diet. From the research, it was shown that *Lactobacillus plantarum* intervention was able to prove carcass yields through very extensive modifications in the architecture of intestines, resulting in increased dimensions and thickness of intestinal villi and subsequent improvements in growth factors to Nile tilapia (Davood et al. 2019). Such observations agreed with results derived from this study. Similarly, it was observed from the current study that probiotics increased carcass qualities in most cases. Interestingly, research conducted by Ebrahimi et al. (2021) has also shown that the value addition of probiotic feeds into tilapia feed has further gotten improvements in carcass quality characteristics such as more muscle yield and less fat. This study states that probiotics affect growth performance and improve most nutritional quality of the fish, making them an important component of aquaculture feed formulations. All evidence accumulated in these studies emphasizes the role of probiotics in the carcass quality of Nile tilapia, thus furthering the effectiveness and profitability of aquaculture.

Conclusion

These result of the present study has theoretically supported the finding that a diet containing 0.1% synbiotic (A-Pro Aqua®) and 0.1% hydrolyzed yeast (Nutri Yeast-Aq®) significantly supports Nile tilapia survival and growth. In addition to growth support, the corresponding application of synbiotics and hydrolyzed yeasts also improved digestion enzyme activity, biochemical parameters of blood, immune responses, hormonal profiles, and carcass quality. As per the conduct of this study, however, it was advisable to put probiotic supplementation in practice for further growth and immune development of Nile tilapia. Certainly, thus far, very extensive research work is required to be undertaken towards elucidating the whole impact of probiotics in aquaculture species.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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