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The Effects of probiotic *Saccharomyces cerevisiae* and *Lactobacillus rhamnosus* on the growth factors and serum biochemistry of *Oreochromis niloticus*

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Abstract

In this study, the effects of a feeding combination of *Saccharomyces cerevisiae* and *Lacticaseibacillus rhamnosus* on the growth and serum biochemical indices of Nile tilapia (*Oreochromis niloticus*) was investigated. For this purpose, a total of 300 Nile tilapia were divided into 10 groups (average weight: 20±7 g) and fed diets containing varying levels of yeast and bacteria for 60 days (30 fish in each treatment group, 10 fish in three replicates). Bacterial strain identification was performed using phenotypic, biochemical, and genetic characteristics. The control group was fed a commercial diet without the selected probiotic strains. Group 1 received only the bacterial strain, while groups 2 to 5 were fed diets containing different doses of yeast supplementation. Groups 6 to 9 received a combination of bacteria and yeast (commercial diet + 0.25%, 0.5%, 1%, and 1.5% yeast + bacteria). At the end of the trial, growth performance and serum biochemical parameters were analyzed. The results showed that the combination of these two probiotics improved the growth indices and the increased survival rates in fish. Additionally, significant changes were observed in some biochemical parameters, including an increase in HDL levels and glucose in yeast-fed groups that reflects the stress response. Instead of changes in biochemical results, based on the growth factors at the end of the study, a combination of *S.cerevisiae* and probiotic *L.rhamnosus* is a nutritional strategy for enhancing aquatic health and performance.

Key words: Probiotics, *Lacticaseibacillus rhamnosus*, *Saccharomyces cerevisiae*, Fish growth, Serum biochemical indices, *Oreochromis niloticus*

Introduction

Probiotics are defined as live microorganisms that, when consumed in adequate amounts, can have some positive effects on host health by improving the microbial community of the digestive system. These microorganisms include various types of beneficial microorganisms including bacteria, yeasts, and algae, each

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playing an important role in promoting fish health. Some of probiotic species, such as *Lactobacillus spp* and *Bifidobacterium* spp, are among the most important probiotic organisms. Probiotics help maintain an optimal pH in the digestive tract by producing lactic acid and preventing the growth of harmful bacteria. Probiotic yeasts (such as *Saccharomyces spp)* and probiotic algae (such as *Spirulina spp*) are rich sources of nutrients and bioactive compounds. They are contributing to immune system enhancement and overall health improvement (Mendonça et al, 2023).

Lacticaseibacillus rhamnosus (*L. rhamnosus*) is a well-known probiotic that has gained attention for its ability to improve gut microbiota composition and protect against antibiotic-induced disruptions. Studies have shown that consuming this probiotic can enhance the production of short-chain fatty acids and aid in the restoration of microbiota function after antibiotic use. Additionally, *L. rhamnosus* has positive effects on digestive health, reducing the incidence of infections and inflammation (Mathipa-Mdakane & Thantsha, 2022).

Saccharomyces cerevisiae (*S. cerevisiae*), also known as baker's yeast, is used as a probiotic because it can enhance the flavor of fermented products and boost the survival and activity of other probiotics. Studies have shown that this yeast can improve the survival and performance of *L. rhamnosus* in acidic conditions, thus increasing the efficacy of probiotic blends. Furthermore, research has indicated that *S. cerevisiae* aids in maintaining a healthy balance of gut microbiota and supports gut health in individuals with digestive disorders (Siesto et al, 2022).

The use of probiotics in aquaculture has been studied extensively in various research studies. Probiotics have been shown to improve gut microbiota balance, enhance nutritional efficiency, and strengthen the immune system, ultimately leading to better growth and reduced disease in aquatic organisms. This underscores the importance of incorporating a diverse range of probiotics, such as bacteria, yeasts, and algae, into aquaculture diets (Wang et al, 2022, Aramoon et al, 2024).

In a study, the effects of the probiotic Lactobacillus rhamnosus GCC-3 on gut and liver health and resistance to bacterial infection were investigated in genetically improved farmed tilapia (GIFT, Oreochromis niloticus) fed a high-fat diet. The results showed that adding this probiotic to the diet improved gut and liver health, enhanced nonspecific immunity, and increased disease resistance in tilapia. In a study, the researchers investigated the effect of incorporating varying levels of Saccharomyces cerevisiae yeast in the diet of rainbow trout with the goal of reducing fishmeal consumption. The results indicated that including Saccharomyces cerevisiae at levels of 28% and 29% had a beneficial impact on the growth indices, survival rates, and body composition of rainbow trout. Based on these findings, yeast could serve as a suitable protein substitute for reducing fishmeal consumption in rainbow trout diets (Zhou et al, 2022; Rafiee & Vafadar, 2021).

An experiment was conducted to determine the effects of different levels of Saccharomyces cerevisiae yeast as a replacement for fishmeal on the growth performance and body composition of Nile tilapia (*Oreochromis niloticus*). The results showed that the daily growth coefficient (DGC), which is the percentage of body weight gained per day, decreased with increasing yeast levels in the diet. In this context, voluntary feed intake (VFI) as a percentage of body weight per day, and protein or fat digestibility did not show significant differences. However, there was a significant reduction in protein retention rate, protein maintenance, and an increase in nitrogen. Studies have shown that the use of probiotics in aquaculture can help improve gut health, strengthen the immune

system, and increase resistance to harmful bacteria, while reducing the use of antibiotics in aquatic farming as much as possible. Probiotics work by attaching to the gut surface, limiting the available space for pathogen attachment, and by increasing mucus production and strengthening the mucosal barrier. This prevents harmful bacteria from penetrating underlying tissues and aids in maintaining gut integrity. Some probiotics also produce bacteriocins and enzymes, creating an unfavorable environment for harmful bacteria, thus preventing their growth and proliferation. Probiotics have been shown to be effective in preventing and treating liver diseases such as non-alcoholic fatty liver disease (NAFLD) and hepatic encephalopathy. They can help improve liver health by reducing inflammatory factors such as TNF-α and IL-6, as well as lowering levels of aminotransferases (Ozório et al, 2012; del Valle et al, 2023; El-Bab et al, 2022; Xiao et al, 2024).

Given the importance of immunization and strengthening the immunity of farmed fish, this study was conducted to investigate the effects of oral administration of the lactic acid bacterium *Lacticaseibacillus rhamnosus* and *Saccharomyces cerevisiae* yeast on growth performance and serum biochemical factors in Nile tilapia (*Oreochromis niloticus*).

Material and Methods

Molecular and Biochemical Identification of Lactobacillus rhamnosus and Bacterial Addition to the Diet

In this study, *Lacticaseibacillus rhamnosus* bacteria isolated from fish in the Shirbet were used as a probiotic dietary supplement due to their good performance. The probiotic *L. rhamnosus* was sourced from a certified stock at the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. This strain had previously been molecularly identified and sequenced (Mohammadian et al, 2016). For confirmation, bacterial strain identification was performed using phenotypic, biochemical, and genetic characteristics (16S rRNA gene sequencing). To confirm the identity of the tested bacteria, they were removed from the -70°C freezer and cultured in MRS broth medium. The lactobacilli were then incubated in an anaerobic jar for 24-48 hours. A loop of the bacteria grown in this medium was then cultured in MRS agar medium under the same conditions. After 48 hours, once the purity of the grown bacteria was ensured, gram staining and biochemical tests were performed, and their appearance was observed under a light microscope.

Following the examination of the amplified product, PCR thermal cycles were carried out in a thermocycler (Eppendorf, Germany) with the following parameters: initial annealing at 95°C for 7 minutes, 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. The amplified products were electrophoresed and analyzed in a 1.5% agarose gel containing safe dye (Cynagen, Iran).

Subsequently, 15 µl of the PCR product was sent to Tekapozyst for sequencing. Upon receiving the sequencing results, the corresponding sequence was examined using BioEdit software, and finally, the nucleotide sequence of the obtained sequences was compared with the nucleotide sequence available in the NCBI gene bank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Chu et al, 2011).

Yeast Extract Preparation

Commercial yeast was obtained from a reputable Iranian company (Kavoshgar Sepehr Javan, Tehran, Iran).

Fish Grouping

A total of 300 Nile tilapia fish (25±5 g) were obtained from the fish breeding centers and transferred to the aquarium hall of the aquatic health department at the faculty of veterinary medicine, Shahid

Chamran University of Ahvaz, under normal condition. The fish were divided into 10 groups of ten each, with three replications in each group. Before the start of the study, the fish were kept in special tanks containing de-chlorinated municipal water for one week for adaptation. The water (50-70%) was replaced with isothermal water daily. The fish were fed commercial feed for 60 days during the experimental period, equivalent to 2% of their body weight daily. The control group was fed commercial feed without probiotics throughout the experimental period. the selected bacterial strain, 10^9 CFU/g was added 60 days to the treatment diet (Chu et al., 2011). Composition of the commercial diet (Biomar) was: Crude proteins %54, Crude lipids: %18, %1 Ashes.

Grouping of treatments was performed according to Table 1.

Table 1: Treatment groups and the control based on different diet composition

Group Name	Food Type	Number of fish
Control Group	Commercial Food	10 pieces (in three replicates)
Treatment 1	Commercial Food + Bacteria	10 pieces (in three replicates)
Treatment 2	*Commercial Food + 0.25% Yeast	10 pieces (in three replicates)
Treatment 3	Commercial Food + 0.5% Yeast	10 pieces (in three replicates)
Treatment 4	Commercial Food + 1% Yeast	10 pieces (in three replicates)
Treatment 5	Commercial Food + 1.5% Yeast	10 pieces (in three replicates)
Treatment 6	Commercial Food + 0.25% Yeast + Bacteria	10 pieces (in three replicates)
Treatment 7	Commercial Food + 0.5% Yeast + Bacteria	10 pieces (in three replicates)
Treatment 8	Commercial Food + 1% Yeast + Bacteria	10 pieces (in three replicates)
Treatment 9	Commercial Food + 1.5% Yeast + Bacteria	10 pieces (in three replicates)

Yeast: *Saccharomyces cerevisiae*; Bacteria: *Lacticaseibacillus rhamnosus*; Commercial food: Based on commercial product catalog

Measurement of Growth Indices

On day 60, various growth indices were measured, including specific growth rate, feed conversion ratio, condition factor, weight gain percentage, protein efficiency ratio and survival percentage (Jahanbani et al, 2023).

Specific Growth Rate (SGR)

It was measured based on the following formula

$$SGR = \frac{\ln(\mathrm{WE}) - \ln(\mathrm{WB})}{t} \times 100$$

Feed Conversion Ratio (FCR)

It was measured based on the following formula

FCR = Total feed consumed / Total weight of product produced

Condition Factor (CF)

To calculate the Condition Factor, divide the fish weight by the fish length cubed, then multiply the result by 100.

$$K = \frac{W}{L^3} \times 100$$

Weight Gain (WG)

This parameter was calculated based on the following formula:

$$WG\% = \frac{Wf}{Wi} \times 100$$

Protein Efficiency Ratio (PER)

$$PER = \frac{Protein\ Intake\ (g)}{Weight\ Gain\ (g)}$$

Survival Rate (SR)

$$SR = \frac{Nf}{Ni} \times 100$$

Biochemical Indices

On day 60, blood samples were obtained from the fish by drawing it through the caudal vein using a 2cc syringe after they were anesthetized with clove powder. The blood was collected in sterile test tubes without anticoagulant and then centrifuged for 10 minutes at 3000 rpm. Subsequently, serum samples were separated and tested immediately.

Serum biochemical parameters such as total protein, albumin, glucose, triglyceride, total cholesterol, HDL-c, LDL-c, creatinine, and the activity of liver enzymes ALP, ALT,

AST, and LDH were measured using a calorimetric method. Commercial kits from Pars Azmoun (Tehran, Iran) were utilized, along with an autoanalyzer (Bio tecnica model BT-1500). The test results are done by an experienced clinical pathologist, according to international reference values and biomedical instruments (Jahanbani et al, 2023).

Statistical Analysis

The data obtained were reported as mean ± standard deviation. Statistical analysis was conducted in completely randomized design using IBM-SPSS Statistics 27.0.1 software. One-way and 2-way analysis of variance (ANOVA) and Tukey's post hoc test were utilized to compare the overall results obtained in different variables between groups. A p-value of less than 0.05 was considered statistically significant.

Results

Results from bacteria confirmation

The stained lactobacilli were observed as Gram-positive rods under the microscope. They were catalase-negative, oxidase-negative, and non-motile. They did not grow in MacConkey's medium. The results of the diagnostic tests for these bacteria are provided in Table 2. The results were obtained from microbial tests referenced in the previous studies (Liu et al, 2023; Nasr and Abd-Alhalim, 2024).

Table 2: Results from *Lacticaseibacillus rhamnosus* confirmation

Test	Catalase	Oxidase	Glucose	Fructose	Saccharose	Raffinose	Arabinose	Xylose	Trehalose	Maltose	Lactose	Galactose	Mannitol
Result	-	-	+	+	+	+	-	-	-	-	+	+	+

Results of growth parameters

Feed conversion ratio

The feed conversion ratio in the groups that consumed 1 and 1.5% yeast and bacteria simultaneously (groups 8 and 9) was statistically and significantly lower than the control group (Table 3).

Specific growth rate

The specific growth rate in the groups that received yeast and bacteria simultaneously was significantly higher than the control group (Table 3).

Weight gain percentage

The weight gain percentage in the groups that consumed yeast and bacteria simultaneously was significantly different from the control group (Table 3).

Survival percentage

No losses were recorded in the groups that received the combination of bacteria and yeast, and the survival of these groups was 100%. In the groups that received 1% and 1.5% yeast alone, survival was high and had a significant difference with the control group (Table 3).

Condition factor

The condition factor showed a statistically significant difference in the groups consuming probiotic bacteria and yeast compared to the control group (Table 3).

Protein efficiency

Protein efficiency in the groups that consumed probiotic yeast and bacteria had a statistically and significantly increase compared to the control group (Table 3).

Table 3: Results of Nile tilapia growth indices in different groups (mean ± standard deviation)

Prameters	Control a	Group1 b	Group2 c	Group3 d	Group4 e	Group5 f	Group6 g	Group7 h	Group8 i	Group9 j	Sig *
Feed Conversation Ratio (FCR)	4.72±0.23 e,i,j	4.03±0.4 e,j	5.31±0.6 d,e,f,g,h,i,j	3.51±0.16 c,e,f,j	5.95±0.27 b,d,g,h,i,j	5.22±1.25 d,e,g,h,i,j	3.8±0.66 c,e,f,j	3.74±0.23 a,b,c,e,f,j	2.84±0.3 a,c,e,f	1.95±1.13 a,b,c,d,e,f,g,h	>0.001
Specific Growth Rate (SGR)	0.25±0.02 d,g,h,i,j	0.3±0.03 d,g,h,i,j	0.31±0.04 d,h,i,g,j	0.45±0.03 a,b,c,e,f,i,j	0.28±0.01 d,g,h,i,j	0.29±0.06 d,g,h,i,j	0.45±0.09 a,b.c.e.f.i.j	0.39±0.02 a,d,g,i,j	0.53±0.06 a,b,c,e,f,h,j	0.76±0.04 a,b,c,d,e,f,g,h,i,j	>0.001
Weight Gain (WG)	42.5±2.5 b,d,g,h,i,j	50±5 e,d,i,j	37.7±4.61 b,d,g,h,i,j	57±2.65 c,e,f,i,j	33.6±1.53 c,e,f,i,j	39.7±8.39 b,d,g,h,i,j	53.6±10 d,e,i,j	53.67±3.2 d,e,i,j	71±7.94 a,b,c,d,e,f,g,h	102±6.8 a,b,c,d,e,f,g,h	>0.001
Survival Rate (SR)	93±1.93 e,f,g,h,i,j	95±1.1	97±1.11	96±1.93	100±0 a	100±0 a	100±0 a	100±0 a	100±0 a	100±0 a	>0.001
Condition Factor (CF)	0.57±0.3 b,c,d,f,g,h,i,j	0.37±0.04 a,d	0.45±0.06 a,e,f,h,j	0.49±0.02 e,f,h,i,j,b	0.33±0.02 a,c,d,	0.33±0.02 a,c,d,	0.4±0.03 a	0.33±0.03 a,c,d,	0.33±0.03 a	0.33±0.02 a,c,d	>0.001
Protein Efficiency Rate (PER)	0.66±0.04 i,j	0.78±0.08 e,i,j	0.59±0.07 d,i,j	0.89±0.04 c,e,f,i,j	0.53±0.02 b,d,g,h,i,j	0.62±0.13 d,i,j	0.84±0.16 e,i,j	0.84±0.05 e,i,j	1.11±0.12 a,b,c,d,e,f,g,h,j	1.6±0.11 a,b,c,d,e,f,g,h,i,j	>0.001

abcdefghij in each row indicates statistically significant differences between different groups
*Statistically significant difference between control group and other groups, Tukey post hoc test

Results of the study of biochemical indices

By examining the biochemical indices in groups with different dietary regimens, the results generally showed that the diet with only *Saccharomyces cerevisiae* yeast increased the levels of alkaline phosphatase and triglycerides and decreased the levels of alanine aminotransferase and aspartate aminotransferase. The diet with only *Lactobacillus rhamnosus* bacteria increased the levels of HDL, LDL and decreased the levels of glucose and VLDL. The mixed yeast + bacteria diet increased the levels of LDH, HDL, LDL and VLDL. In other indices, no statistically significant difference was observed between the groups with the yeast and bacteria diet alone and mixed, although these results do not apply to the control group.

Creatinine: No significant difference was observed between the different groups

Glucose: In all groups except group one, which used only the probiotic *Lactobacillus rhamnosus* in the diet, there was a statistically significant increase in glucose levels compared to the control group. On the other hand, glucose levels in group one were significantly lower than those in the other tested groups (Figure 1).

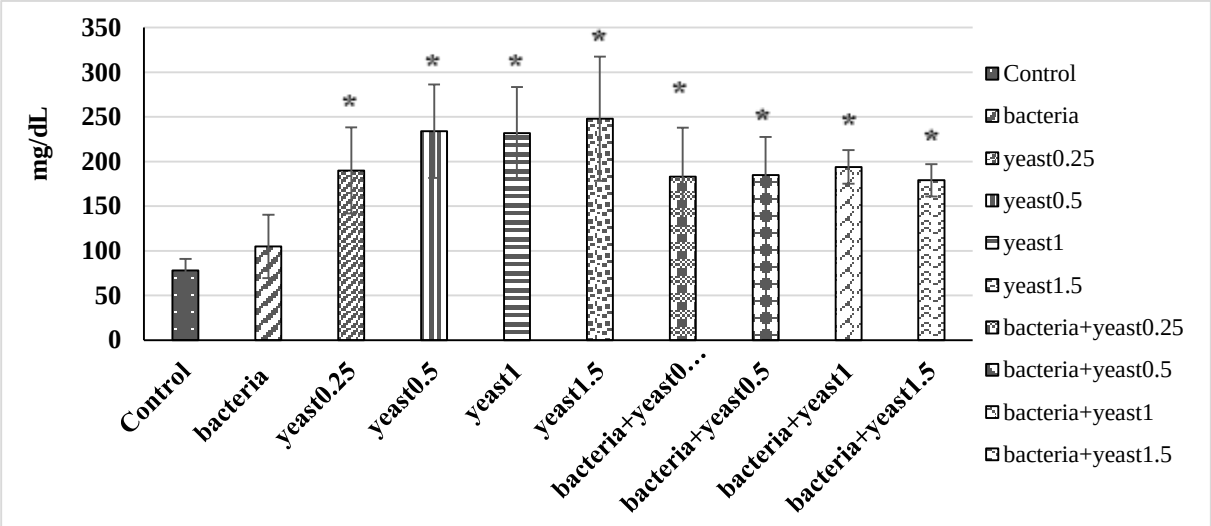

Figure 1: Glucose level of blood serum in control and different experimental groups. (*= significant difference between control and the selected group, P>0.001).

Protein: No significant increase in protein levels was observed in any experimental group compared to the control group.

Albumin: Albumin levels did not differ significantly among the experimental groups compared to the control group.

Cholesterol: Cholesterol levels rose significantly across all groups compared to the control group; however, no significant differences were found between the various food groups (Figure 2).

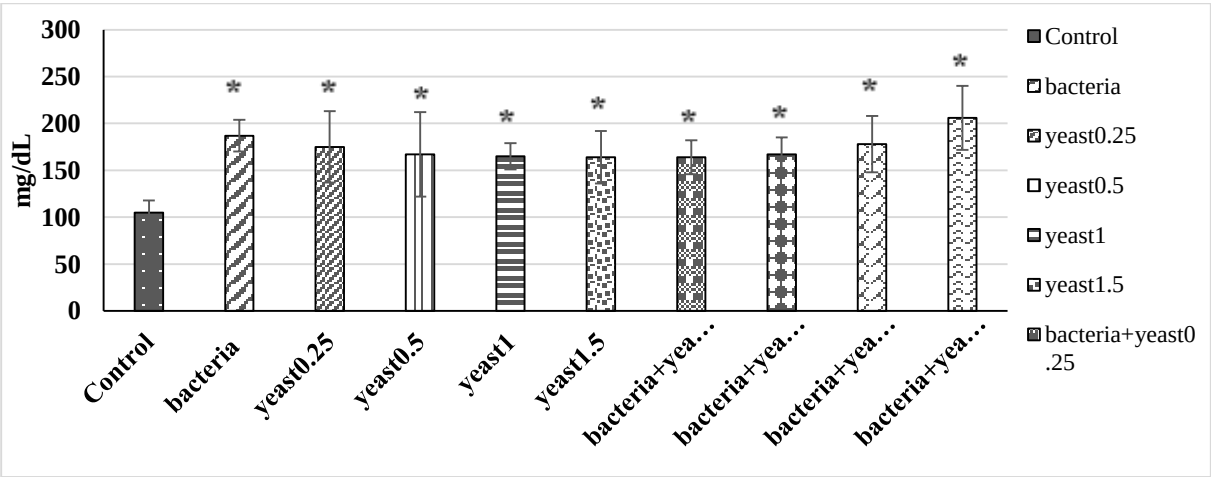

Figure 2: Cholesterol level of blood serum in control and different experimental groups. (*= significant difference between control and the selected group, P>0.001).

HDL: Groups 1, 8, and 9, which included bacteria in their diets, showed a significant increase in HDL levels compared to the control group, while the yeast-only diet group did not differ significantly.

LDL: LDL levels also increased significantly in groups 1, 8, and 9 with bacteria in their diets, whereas the yeast-only diet group exhibited no significant difference compared to the control group.

VLDL: Groups 2, 3, 4, and 5, which had only yeast diets, showed a significant increase in VLDL levels compared to the control group, while group 1 (only bacteria) had a significant decrease in VLDL compared to groups 2 to 5.

Triglycerides: The yeast-only groups (2, 3, 4, and 5) experienced a significant increase in triglycerides compared to the control, whereas the other groups showed no significant differences. Moreover, triglyceride levels in the groups incorporating bacteria (1, 6, 7, 8, and 9) were significantly lower than those in the yeast-only groups (2, 3, 4, and 5) (figure 3).

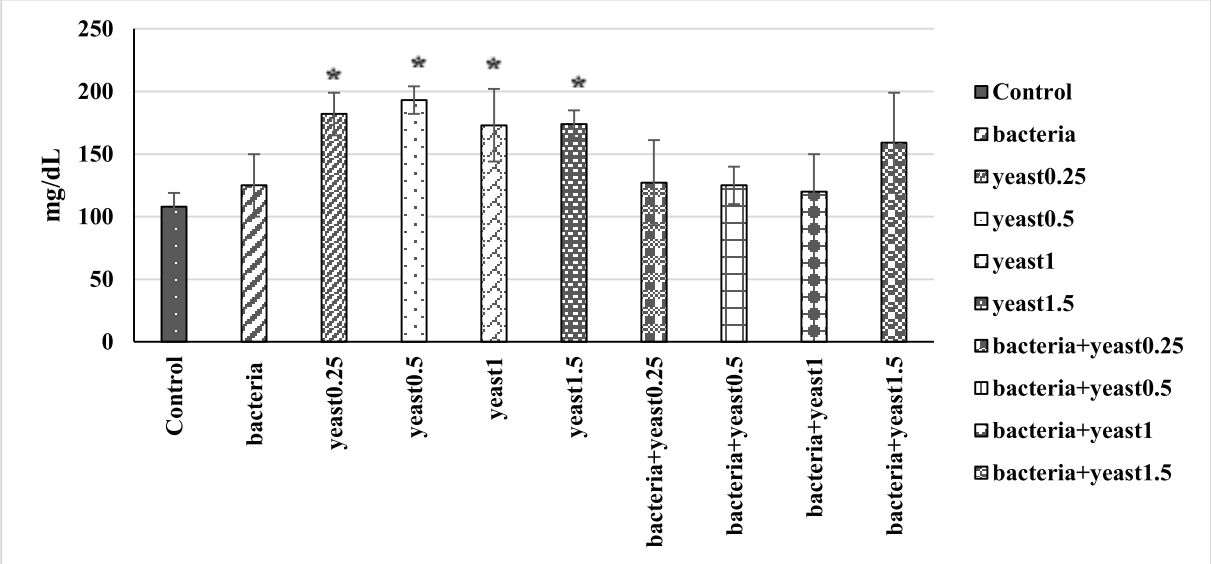

Figure 3: Triglyceride level of blood serum in control and different experimental groups. (*= significant difference between control and the selected group, P>0.001).

Alkaline phosphatase: In group 3, alkaline phosphatase levels significantly increased compared to other groups, though no statistically significant differences were observed among the other groups when compared to the control. Groups that used only yeast exhibited higher alkaline phosphatase levels than those using yeast + bacteria, with significant differences in groups 2 vs. 7, 3 vs. 6, 7, and 8, and 5 vs. 6, 7, and 8.

Alanine aminotransferase: This liver enzyme's levels did not differ significantly from the control across groups, with all remaining within the normal range. However, significant differences were noted among the food groups. Groups 4 and 2, which utilized only yeast, showed a significant decrease in enzyme levels compared to yeast + bacteria groups 8 and 9. Furthermore, increased yeast levels in yeast-only groups led to a significant rise in enzyme levels compared to those with less yeast.

Aspartate aminotransferase: The levels of this enzyme across groups did not significantly differ from the control and remained within the normal range. Notably, groups using only yeast exhibited a significant decrease in enzyme levels compared to the bacteria-only and bacteria + yeast groups.

Lactate dehydrogenase: A significant increase in lactate dehydrogenase levels was observed in groups that consumed a diet containing both bacteria and yeast (6, 7, 8, and 9) compared to the control group. Additionally, these groups showed significantly higher levels compared to both bacteria-only and yeast-only groups (Figure 4).

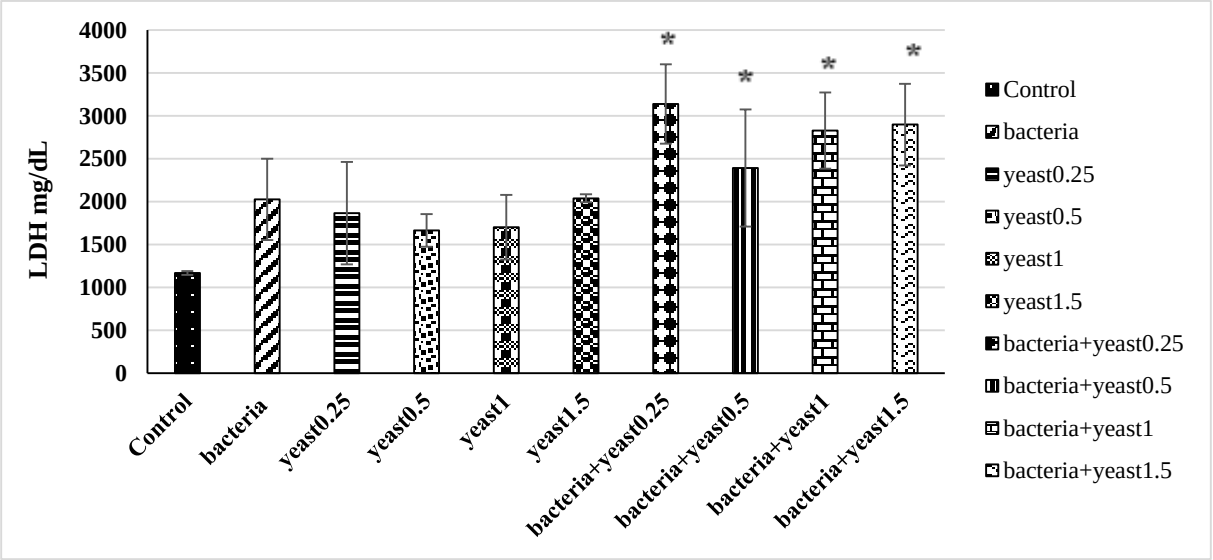

Figure 4: LDH enzyme level of blood serum in control and different experimental groups. (*= significant difference between control and the selected group, P>0.001).

Discussion

In this study, the effect of combined use of S. cerevisiae and *L. rhamnoses* on growth and biochemical indices of Nile tilapia serum was investigated. The results showed that adding these compounds to the fish diet improved the growth performance of the fish. Prebiotics are non-digestible carbohydrates that enhance animal health by modulating beneficial intestinal organisms. Yeast extract can act as a prebiotic containing high concentrations of yeast β-glucan (βG) and mannan-oligosaccharide (MOS). In a study by Nermeen et al. (2018), oral administration of a mixture of β-glucan and MOS reduced mortality after microbial infection with *Lactococcus garvieae* and *Aeromonas hydrophila*. Therefore, including yeast-containing diets in feed is an efective way to increase production rates (Abu-Elala et al, 2018).

The use of *S.cerevisiae* in ruminant diets has been shown to reduce methane emissions from anaerobic fermentation, decrease the number of enteric pathogenic bacteria, and repopulate the intestinal microflora in cases of diarrhea (Ben said et al, 2022). A study by Choi et al, (2022) focused on the effect of probiotics such as *Lactobacillus rhamnosus* on fish growth and health. The study demonstrated that these bacteria could improve fish growth and strengthen the immune system (Choi et al, 2022). The genus *Saccharomyces sp*. is a yeast found in nature; it has been used as a probiotic recently. The Previous studies have shown that this species has anti-inflammatory, immunomodulatory and microbiome regulating effects and is recognized as safe by the Food and Drug Administration. This yeast can inhibit the secretion of microbial inflammatory cytokines such as IL6. This yeast can survive inside the gastrointestinal tract because it can tolerate high temperatures and low pH and is also used to treat diarrhea. The most important feature in a probiotic microorganism is its survival when passing through the stomach and maintaining its function and viability in the intestine. Therefore, methods to improve the survival rate of probiotics are of high priority (Ghorbani-Choboghlo et al, 2019). In the present study, the use of this bacteria together with yeast, similar to this mechanism, increased the infiltration of immune cells and improved disease resistance in tilapia fish.

Strict regulations have been implemented to ban or reduce the use of preventive

antibiotics in order to minimize their detrimental effects on aquatic life. Probiotics, prebiotics, and symbiotic have emerged as viable alternatives to antibiotics. Research has indicated that these supplements are particularly effective in managing or treating bacterial, viral, and parasitic diseases in shrimp. The mechanisms through which these supplements operate include enhancing immune responses, promoting the development of antibacterial factors, altering gut microflora, competing for nutrients and binding sites, and influencing enzyme activities (Butt et al, 2021). Our study corroborated these findings, demonstrating that the incorporation of yeast and bacteria into the diet of tilapia likely boosted immune cell infiltration, bolstered disease resistance, and ultimately led to enhanced growth performance and survival rates among the fish.

In our study, the effects of yeast and bacterial probiotics alone and in combination did not have an adverse effect on the liver, as determined by the levels of liver enzymes measured. Aini et al. explored the impact of a dual-strain probiotic (*Lactobacillus casei* and *Bacillus subtilis*) on digestive enzymes, liver function and antimicrobial activity in catfish (*Clarias gariepinus*) treated with *Aeromonas hydrophila*. The results indicated that probiotic supplementation increased digestive enzyme activity and improved liver function in catfish (Aini et al, 2024). Adorian et al. studied the effects of different doses of Bacillus species (*Bacillus licheniformis* and *Bacillus subtilis*) on growth performance, digestive enzyme activity and blood parameters in *Asian sea bass* (*Lates calcarifer*). The findings showed that probiotic supplementation led to improved growth performance and increased activity of digestive enzymes, including liver enzymes, in fish (Adorian et al, 2019). The previous studies have proposed that the general mechanisms for the positive effect of probiotics on liver function in fish include modulation of the gut microbiota. Probiotics can alter the composition of the gut microbiota and reduce the population of harmful bacteria that may produce endotoxin, negatively affecting liver function. A healthier gut microbiome can lead to less systemic inflammation and, consequently, better liver health (X. Wang et al, 2021). Another mechanism is the reduction of intestinal permeability. Probiotics can strengthen the intestinal barrier by enhancing mucus production and tight junctions in intestinal cells, which help prevent pathogens and harmful toxins from entering the bloodstream. This reduces the burden on the liver, responsible for detoxifying the body (Gou et al, 2022). Additionally, the anti-inflammatory effect of probiotics also contributes to liver health. Probiotics prevent inflammation in the liver and other organs by reducing inflammatory cytokines such as TNF-α and IL-6, which can improve liver function (Cristofori et al, 2021). Several studies have demonstrated the antioxidant defense enhancement of probiotics, which can significantly impact liver health. Some probiotics may increase the body's antioxidant capacity and help protect the liver from oxidative stress and damage that can affect liver enzymes. In a study by Hosseinifar et al, (2020) investigating the effects of probiotics, prebiotics, and symbiotic on reducing oxidative stress in *Oncorhynchus mykiss* (rainbow trout) and Nile tilapia fish, it was found that the use of probiotics such as *Lactobacillus* spp. and *Bifidobacterium* spp., prebiotics such as fructooligo saccharides (FOS) and mannan-oligosaccharides (MOS), and their combinations (symbiotic) can have positive effects on reducing oxidative stress and enhancing the antioxidant defense of fish. These compounds reduced the level of malondialdehyde (MDA) as an indicator of oxidative damage and increased the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and

glutathione peroxidase (GPX) (Hoseinifar et al, 2020).

In our study, the groups that received yeast alone had the highest glucose levels. It is likely that yeast causes greater glucose absorption from the intestine, which is less effective when combined with bacteria, as this increase is lower in combined treatments. *Saccharomyces cerevisiae* contains minerals such as chromium, which must be effective in regulating blood sugar levels; however, we find elevating sugar level in blood. The chromium in this yeast helps improve insulin function by increasing insulin sensitivity, facilitating glucose entry into cells by increasing the expression of GLUT4 (glucose transporter) proteins in the cell membrane, activating enzymes related to glucose metabolism, and reducing insulin resistance, which can lead to a decrease in blood glucose levels (Hua et al, 2012). In the study by Ringø et al, (2022), glucose homeostasis is investigated as an important process in the regulation of blood glucose levels and energy balance in fish and shrimp. Probiotics have positive effects on glucose homeostasis, which is observed particularly through an increase in the expression of genes related to carbohydrate metabolism, such as AMPKα and PGC-1α. AMPK plays a crucial role in regulating energy consumption and fat storage, helping to regulate glucose levels by increasing glucose consumption in cells and reducing the synthesis of stored fats (Nadar et al, 2024). On the other hand, PGC-1α helps regulate energy production at the mitochondrial level and leads to increased glucose and fat consumption in various tissues, including muscles and liver (Abu Shelbayeh et al, 2023).

In our study, the administration of bacteria and yeast did not cause any changes in plasma albumin and protein levels. The levels of serum protein in fish fed probiotics can be influenced by various factors such as the type and dosage of probiotics, the duration of probiotics administration, the species of fish, environmental conditions (including temperature, water pH, nutrition, and contaminants), physiological stresses, and interference with nutritional or therapeutic factors (Ringø et al, 2022). Shehata et al, (2024) conducted a study investigating the effects of using the probiotics *S.cerevisiae* and *Lactobacillus bulgaricus*, both individually and in combination, on the growth and body composition of mullet fish. Their findings indicated that the groups receiving probiotics experienced significant improvements in growth and feed efficiency compared to the control group. Furthermore, the content of crude protein and crude fat in the groups receiving probiotics, particularly in the combination group, showed a significant increase (Shehata et al, 2024). In a study by Raja et al, (2024), the effects of the probiotic Lactobacillus acidophilus on the biochemical parameters of *Labeo rohita fingerlings* were examined. The results revealed that incorporating *Lactobacillus acidophilus* into the fish's diet led to a notable increase in the level of total serum protein. This rise in total serum protein could suggest an enhancement in the nutritional status and overall health of the fish (Raja et al, 2024).

In our study, the lipid profile was found to fluctuate due to the consumption of yeast and bacterial probiotics, resulting in an increase in lipid indices with yeast consumption. Research has indicated that incorporating probiotics into the diet can impact the lipid profile. Probiotics generate short-chain fatty acids that positively influence lipid metabolism regulation (van der Beek et al, 2017). They have been shown to lower LDL levels and raise HDL (Wang et al, 2021), as well as potentially reduce cholesterol absorption and LDL levels (Song et al, 2023). Probiotics can enhance the lipid profile by boosting enzyme activity linked to lipid metabolism, regulating it through key metabolites like short-chain fatty acids and secondary bile acids. They can also curb cholesterol

synthesis by producing certain enzymes and inhibitors (Song et al, 2023). In a study by Ringo et al, (2022), the impact of intestinal microbiota and probiotics on fat, carbohydrate, protein, and amino acid metabolism in fish and shrimp was explored using the zebrafish (*Danio rerio*) model. The findings revealed that probiotics enhance lipid balance and control fat storage in the body by influencing intestinal microbiota. These effects are tied to an increase in short-chain fatty acid (SCFA) production from microbial food fermentation in the gut. SCFAs function as metabolic signals, aiding in reducing tissue fat storage. Furthermore, probiotics enhance fat burning efficiency and decrease the stored fat accumulation in tissues by regulating the expression of fat metabolism-related genes, particularly those involved in fat synthesis like SREBP1 (Sterol regulatory element-binding protein 1), FAS (Fatty acid synthase), and ACC (Acetyl-CoA carboxylase), while increasing the activity of fat-burning enzymes like CPT1 (Carnitine palmitoyltransferase 1) and ATGL (Adipose triglyceride lipase). This study also indicated that probiotics' fat-reducing effects are linked to alterations in gut microbiota and bacterial equilibrium, which can impact metabolic functions in the body (Ringø et al, 2022).

The addition of *Saccharomyces cerevisiae* and *Lacticaseibacillus rhamnosus* to tilapia diets positively impacted growth, increased survival rates, and improved blood biochemical composition. These results highlight the potential of this combination to enhance aquatic animal performance, suggesting that probiotics (bacteria and yeasts) can effectively serve as an alternative to traditional feed additives.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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تأثیر عصاره مخمر *ساکارومایسس سرویزیه* به همراه *لاکتوباسیلوس رامنوسوس* بر فاکتورهای رشد و بیوشیمیایی سرم ماهی *تیلاپیای نیل*

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چکیده

در این تحقیق اثرات ترکیبی *Saccharomyces cerevisiae* و *Lacticaseibacillus rhamnosus* بر رشد و شاخص‌های بیوشیمیایی سرمی ماهی تیلاپیا نیل (*Oreochromis niloticus*) بررسی گردید. در مجموع ۳۰۰ ماهی تیلاپیا نیل به ۱۰ گروه (متوسط وزن ۷ ± ۲۰ گرم) تقسیم شدند (تیمارهای ۳۰ تایی در سه تکرار ۱۰ تایی) و به مدت ۶۰ روز با جیره‌های حاوی سطوح مختلف مخمر و باکتری تغذیه شدند. گروه کنترل با رژیم غذایی تجاری بدون سویه‌های پروبیوتیک انتخاب شده تغذیه شدند. شناسایی سویه‌های باکتریایی با استفاده از ویژگی‌های فنوتیپی، بیوشیمیایی و ژنتیکی انجام شد، گروه ۱ فقط سویه باکتریایی را دریافت کرد، در حالی که گروه‌های ۲ تا ۵ با جیره‌های حاوی دوزهای مختلف مکمل مخمر تغذیه شدند. گروه‌های ۶ تا ۹ ترکیبی از باکتری و مخمر (رژیم غذایی تجاری + ۰/۲۵ درصد، ۰/۵ درصد، ۱ درصد، ۱/۵ درصد مخمر + باکتری) دریافت کردند. در پایان کارآزمایی، عملکرد رشد و پارامترهای بیوشیمیایی سرم مورد تجزیه و تحلیل قرار گرفت. نتایج نشان داد که استفاده ترکیبی از این دو پروبیوتیک باعث بهبود شاخص‌های رشد و افزایش میزان بقا در ماهی می‌شود. هر چند تغییرات قابل توجهی در برخی از پارامترهای بیوشیمیایی، از جمله افزایش سطح HDL و گلوکز در گروه‌های تغذیه شده با مخمر مشاهده شد که این تغییرات شاخص استرس هستند. بر اساس نتایج فاکتورهای رشد در پایان مطالعه، ترکیبی از *S.cerevisiae* و پروبیوتیک *L.rhamnosus* می‌تواند یک استراتژی تغذیه‌ای برای افزایش سلامت و عملکرد آبزیان باشد.

کلمات کلیدی: عصاره مخمر، ساکارومایسس سرویزیه، لاکتوباسیلوس رامنوسوس، فاکتورهای رشد، بیوشیمیایی سرم، ماهی تیلاپیای نیل

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