Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EVALUATION OF THE PROBIOTIC SUPPLEMENT *Saccharomyces cerevisiae* BB06 AS A BENEFICIAL GROWTH PROMOTER FOR CARP (*Cyprinus carpio*) IN RECIRCULATING AQUACULTURE

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Abstract

The current study investigates the effect of the probiotic Saccharomyces cerevisiae BB06 on the growth performance of carp (Cyprinus carpio). The experiments were conducted in a laboratory-scale re-circulating system over an eight-week trial period. Ninety healthy carp were randomly divided into three groups and fed with fish feed containing 0% yeast biomass (control), 3% yeast biomass (YBV3%), and 5% yeast biomass (YBV5%). The fish were fed twice daily with a quantity of feed corresponding to 2.5% of their body weight. Growth performance increased significantly (p<0.05) with increasing levels of probiotic S. cerevisiae BB06. The YBV5% group performed the best in terms of the final body weight, weight gain rate of Cyprinus carpio. The mortality rate of the fish fed with the probiotic S. cerevisiae BB06 was 0%. An increase in the yeast content of the feed had a tendency to decrease in the value of the Fulton condition factor. In addition, fish fed diets enriched with S. cerevisiae BB06 showed improvements in feed conversion ratio and protein efficiency ratio. Water quality parameters were maintained at acceptable levels in all tanks during the eight-week trial period, allowing high growth rates and production of the carp. In conclusion, dietary supplementation with S. cerevisiae BB06 can improve the growth performance of carp (Cyprinus carpio).

Key words: *carp, growth performance, Saccharomyces cerevisiae, feed, water quality.*

INTRODUCTION

Considering that per capita fish consumption has increased by 1.5% over the last half-century, expanding aquaculture production is seen as a viable solution to fulfill the world's increasing food demands, as well as an excellent source of income and employment opportunities (Béné et al., 2016; FAO., 2020; Saha et al., 2022). Feed quality and efficacy are critical factors in aquaculture that have an impact on fish growth, survival, and production, as well as water quality (Kong et al., 2020). Therefore, probiotics are considered suitable additives to improve feed production and utilization (Jamal et al., 2020; Tuan et al., 2013; Chauhan and Singh, 2019). Probiotics are viable microorganisms which, when administered in adequate quantities, can provide health benefits to the host (FAO/WHO., 2002; Hill et al., 2014). Lactic bacteria (such as *Bifidobacterium, Lactobacillus,* and *Pediococcus*) and *Bacillus* spp. have been extensively investigated for use as probiotics in aquaculture to improve fish performance and welfare (Coulibaly et al., 2023; Chizhayeva et al., 2022; Kawser et al., 2022). Nonetheless, there is a possible threat of the transfer of antimicrobial resistance genes to pathogenic bacteria via bacterial probiotics

(Daniali et al., 2020; Li et al., 2020). Instead, yeasts are non-pathogenic and due to their resistance to the gastrointestinal environment (including pH, body temperature, the presence of digestive enzymes, and bile salts), and their ability to resist antibiotics, may be a valuable probiotic adjunct to antibiotic therapy (Agbola et al., 2021; Jack et al., 2015; Alkalbani et al., 2022; Fernández-Pacheco et al., 2021; Mahdy et al., 2022; Navarrete & Tovar-Ramírez, 2014). *Saccharomyces cerevisiae,* isolated from various sources, is the most extensively studied probiotic yeast for aquafeeds (Dumitrache et al., 2022; Mogmenga et al., 2023; Del Valle et al., 2023; Diguță et al., 2023; Abass et al., 2018). Increased scientific research has been conducted to identify and characterize novel non-*Saccharomyces* species, such as *Debaryomyces* sp., *Kluyveromyces* sp., *Hanseniaspora* sp., *Rhodotorula* sp., *Wickerhamomyces* sp., and *Yarrowia* sp., which reportedly enhance fish production (El-Feky et al., 2017; Adel et al., 2017; Rekha et al., 2022; Vidakovic et al., 2019; Corbu et al., 2020; Reyes-Becerril & Alamillo, 2021). As part of the current circular economy, new technologies are being promoted to produce large quantities of yeast from cheap inorganic compounds and low-value agricultural and industrial wastes, particularly for use as aquaculture feed (Ma et al., 2013; Bărbulescu et al., 2018).

Furthermore, with rapidly expanding aquaculture and limited fishmeal availability, it is critical to identify sustainable protein replacements. In aquafeed production, the substitution of fishmeal for plant protein sources (mainly soybean meal or concentrate, and grains glutens) has become an important standard practice (Øverland & Skrede, 2016). However, certain fish species are unable to efficiently assimilate plant-based feed due to nutritional deficiencies, an imbalanced amino acid composition, and the presence of antinutritional compounds (Hardy, 2010; Zhou et al., 2018). Single-cell ingredients derived from bacteria, yeast, and microalgae, are used as supplements in food or feed Bratosin et al., 2021; Jach et al., 2022; Ferreira et al., 2010; Shah et al., 2017; Glencross et al., 2020; Banerjee end Ray, 2017; Balcazar et al., 2006). In particular, due to its high content of protein, carbohydrates (alpha-glucan, beta-glucan, alpha-mannan), nucleic acids, vitamins, antioxidants, and minerals, the yeast *S. cerevisiae* has been widely used as a valuable source of functional ingredients in the production of feed and food (Agboola et al., 2021; Navarrete & Tovar-Ramírez, 2014; Jach et al., 2022; Ferreira et al., 2010).

Due to their probiotic abilities and high protein content, yeast supplementation improves growth performance (Abass et al., 2018; El-Fely et al., 2017; Adel et al., 2017; Vidakovic et al., 2019; Korkmaz et al., 2011; Zhanga et al., 2020; Banu et al., 2020), feed digestibility (Rekka et al., 2022; Vidakovic et al., 2019; Korkmaz & Cakirogullari, 2011), intestinal microbiota (Adel et al., 2017; Vidakovic et al., 2019; Islam et al., 2021), stress tolerance (Abass et al., 2018), immune system (Adel et al., 2017; Ma et al., 2013; Li et al., 2003; Øverland et al., 2013; Tewary and Patra, 2011; Saini et al., 2014) and disease control (Abass et al., 2018; Reyes-Becerril and Alamillo, 2021; Ma et al., 2013; Chiu et al., 2010; Zhanga et al., 2020) in aquaculture species.

Therefore, the aim of this study was to investigate whether the partial replacement of fishmeal with probiotic *Saccharomyces cerevisiae* BB06 has a beneficial effect on growth performance, survival of carp (*Cyprinus carpio*), as well as water quality.

MATERIALS AND METHODS

Yeast strains and feed preparation

S. cerevisiae strain BB06 was isolated from grapes and identified by 5.8S rRNA gene sequencing (NCBI GenBank accession no. OL757483). According to a previous study, the strain was found to possess valuable probiotic properties (Diguță et al., 2023). The strain was preserved at -20°C in the Microbial Collection of the Faculty of Land Reclamation and Environmental Engineering (USAMV Bucharest, Romania), as suspensions in 25% (v/v) glycerol. The yeast strain was cultivated in a yeast-malt medium consisting of yeast extract 0.5%, malt extract 2%, sucrose 2%, peptone Hy-Soy 0.5%, and agar 2% (abbreviated as YMSP) and kept at 30°C for 18h (Frîncu et al., 2022). Cells were harvested by centrifugation at 3500 - 4000 rpm for 5 min at 4°C, as described in (Bărbulescu et al., 2018). The yeast biomass was stored by freeze-drying as described in (Diguță et al., 2023). Cellular viability was confirmed by plate counting prior to the use of yeast biomass as a functional supplement.

Diet formulation

Three experimental diets were formulated according to nutrient requirements. A yeast-free product (YBV0%) was used as the control diet and two other diets were supplemented with 3% (YBV3%) and 5% (YBV5%) freeze-dried *S. cerevisiae* BB06, respectively. The composition of the ingredients is given in Table 1. To prepare the desired diets (YBV3% and YBV5%) (Table 1), a dough was prepared with 30% water and mixed for 15 min. The dough was then pelleted to a thickness of 6 mm using a feed mill, dried overnight at 30°C, crushed and stored at 4°C until use. All experimental diets, including the control, were prepared according to the manufacturer's instructions for Carp Aller Classic (https://www.aller-aqua.com/species/warm-freshwater-species/carp) using the same size and pellet extrusion parameters and 6 mm pellet size.

Table 1. Composition of experimental feeds (% freeze-dried weight)

Ingredients (%)	Diet 1	Diet 2	Diet 3
Formulation			
Fish meal	20.30	17.30	15.30
Cereals	20.60	20.60	20.60
Wheat bran	15.10	15.10	15.10
Vegetable proteins	14.00	14.00	14.00
Protein concentrate from fish	18.10	18.10	18.10
Algae	7.40	7.40	7.40
Oils and fats	1.40	1.40	1.40
freeze-dried *S. cerevisiae* BB06	0	3	5
Proximate chemical composition of feeds			
Ash	8.6	7.1	7.5
Crude protein	40.00	42.49	45.07
Crude lipid	4.32	4.2	4.3
Carbohydrates	6.74	6.93	6.81
Moisture	8.1	8.6	9.2

Trial and experimental design

The present study was carried out under laboratory conditions in a recirculating aquaculture system (RAS), during an experimental period of eight weeks. The aquaculture system consists of 9 rearing units of water (120 L) where the fishes were distributed randomly. Aquaculture system include several components such as a fish tank, a biofilter, water pumps, and air pumps to ensure proper circulation. Maintained under standardized illumination, all tanks experienced identical natural photoperiods with 12 to 13 hours of light. To support the growth of bacteria involved in the nitrification process, expanded clay aggregates were used for biofiltration (Figure 1), resulting in a reduction of ammonia and nitrite concentrations (Estim et al., 2019). A total number of 90 fish Cyprinus carpio, provided by the aquaculture farm of SCDP Nucet (Dambovita, Romania) were used. Ten carp fish stocked in each of the aquariums containing 120 L of treated water fitted with air stones to provide an adequate mixture of oxygen and water in the aquarium.

Figure 1. Recirculation aquaculture system (RAS) used in the study and compartments with expanded clay aggregates as biofiltration substrates. The water circulated through an overflow in the biofiltration compartment and returned through a submersible pump

The mean initial weight of the fish was 81.88 ± 0.2 g. The fish were acclimated for 2 weeks with a commercial fish pellet feed containing

40% crude protein and 8% crude fat (JBL GranoMix, JBL GmbH & Co. KG, Germany). Subsequently, the fish were fed on experimental diets twice daily for eight weeks, which were equal to 2.5% of their body weight to diminish feed wastage and prevent the fish from becoming overfed and vulnerable to diseases (https://www.fao.org/fishery/docs/CDrom/FAO_Training/FAO_Training/General/x6709e/x6709e10.htm). The schedule of biometric measurements was as follows: one at the beginning of the experiment, one at the end of it, and every 7 days interval, between.

Growth performance

The length and body weight of each fish were recorded in both the control and experimental aquariums at the start and end of the experiment, as well as after 7-day intervals. The number of fish was also noted to calculate the survival rate. The average values of the measured parameters of all fish in each aquarium were used for the interpretation of the data.

Various parameters have been approximated using different formulas, such as:

- Survival Rate (SR%) = (Initial fish number-Final fish number/Initial fish num-ber) × 100);
- Weight gain (WG%) = [(Final weight (Wf), g–Initial weight (Wi), g)/Initial weight (Wi), g] × 100;
- Specific growth rate (SGR%) = [(Final weight, g–Initial weight, g)/No. of day] × 100;
- Relative growth rate (RGR%) = WG/days of experiment/Wi(g/g day−1) x 100;
- Feed Conversion Ratio (FCR%) = Feed consumed (g)/(Wf-Wi) (g);
- Fulton`s condition factor (K) = (Tw/TL3) x 100; Tw-total weight, TL-total length;
- Protein efficiency ratio (PER) = weight gain of fish (g) / total protein(g).

Chemical Analysis

The study followed the procedures for the handling and use of the aquatic animals, in line with the Romanian legislation (Low 43 of 11 April 2014) and approved by the Ethical Commission of UASMV Bucharest (Decision No. 26 of 23 June 2022). The fish were sacrificed with a lethal dose of 2-phenoxyethanol 2 ml/L^{-1} (Sigma, Germany) in a bucket for 3 minutes to obtain data on moisture, ash, protein, lipid, and carbohydrate content. Samples of fish meat were refrigerated at 8°C until chemical analysis was performed. The head, the viscera, the skin, and the bones were removed manually to obtain the meat and then freeze-dried. For the freeze-dried process, the fish meat was frozen at -80°C for 24 hours, and under the vacuum applied by the freeze-dried machine the water goes directly into the gaseous state and condenses on the cooled coil of the device. At the end of the process, the samples were bagged in polyethylene bags and kept at 4°C until the analyses.

Moisture and Ash

The moisture content of fish was determined by calcination for 24 h. After the samples had been burned in a muffle oven at a temperature of 600°C for 2 hours, the total ash content was determined.

Crude Lipid, Carbohydrates, and Crude Protein Analysis

The total protein content was determined by CHNS elemental analysis using the Dumas method with the EA 3100 elemental Analyzer (manual operating v1.3/2019). Cystine (B2131-1, elemental Microanalysis) has been used as a calibration standard according to SR EN ISO 16634-1:2009. The measured total nitrogen was converted to equivalent crude protein (%) using a conversion factor of 6.25. CHNS elemental analysis is the most widely used method of determining nitrogen and protein content in food and feed due to its high level of accuracy and reproducibility. The total fat content was determined with the Soxhlet automatic extractor (model R254, Behr Labor-Technik) and the extraction was carried out using a well-determined volume of solvent (petroleum ether). The total soluble carbohydrates was determined after the method described by Scott and Melvin, 1953.

Water Quality Parameters

Key physicochemical parameters were monitored to check water quality. Dissolved oxygen, electrical conductivity, water pH, and temperature were measured using a HI-9811-5 pH/EC/TDS/°C portable meter (Hanna Company, Cluj-Napoca, Romania). The nitrate concentration was measured using the phenol disulphonic acid method. Nitrate is typically converted to nitrite, which is then quantified by the standard Griess reaction (the sample was treated with sulphanilic acid and naphthyl-1-amine in an acidic medium) (Stavrescu-Bedivan et al, 2015). Ammonia nitrogen concentration was determined using the Nessler reagent spectrophotometric method at 420 nm wavelength. Phosphate concentration was determined using the molybdenum blue method at 700 nm wavelength. The measurements were performed using an SP-830 Plus spectrophotometer (Metertech Inc, Taipei, Taiwan). All determinations were performed according to ASRO Standard Methods (SR EN ISO 10523:2012, 5814:2013, 7150:2001). All water parameters were measured weekly.

Statistical analysis

The experiment was performed in triplicate and results were expressed as arithmetic mean ± standard deviation (SD). Quantitative data were statistically analyzed using repeated measures ANOVA followed by a post hoc test ($p < 0.05$). The analysis was performed using Jasp version 0.16.3.0 (an open-source statistical analysis program supported by the University of Amsterdam).

RESULTS AND DISCUSSIONS

Growth performance

In our study, we used freeze-dried *S. cerevisiae* BB06 with a cell viability of 10^9 CFU/g. Growth performance and feed utilization of *Cyprinus carpio* treated with and without different *S. cerevisiae* BB06 levels (YBV0%, YBV3%, and YBV5%) are shown in Table 2.

Table 2. Growth performance of common carp for each of the experimental diets

Indicators	Control (YBV0%)	YBV3%	YBV5%
Initial weight (IW-g)	81.63±0.50	82.50±0.70	81.88±0.62
Final weight (FW-g)	130.58±1.22	161.28±0.94	181.29±0.85
Weight gain (WG %)	59.96±2.29	96.41±2.13	108.04±2.17
Initial length (cm)	17.71±0.10	17.70±0.26	17.97±010
Final length (cm)	23.50±0.10	28.08±0.36	29.72±0.10
Feed conversion ratio (FCR %)	5.07±0.10	3.39±0.05	3.11±0.08
Condition factor (K)	1.41±0.01	1.02±0.01	1.16±0.01
Specific growth rate (SGR %/day)	0.84±0.02	1.20±0.02	1.31±0.02
Relative growth rate (RGR %/day)	0.01±0.00	0.02±0.00	0.03±0.00
Survival rate (SR %)	90.00±0.00	100±0.00	100±0.00
Protein efficiency ratio (PER g/g)	1.42±0.05	2.38±0.02	2.54±0.05

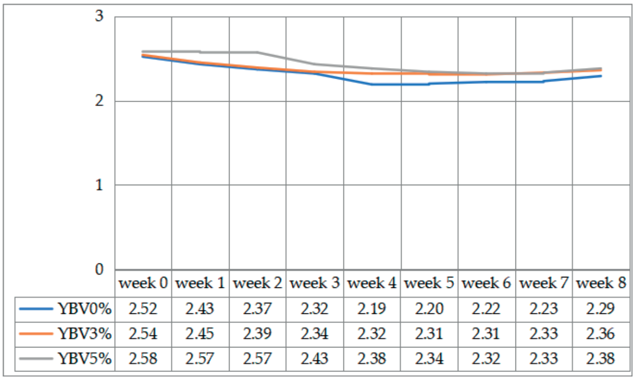
Experimental diet: YBV0% - Control, commercial feed without yeast, YBV3% - diet supplemented with 3% yeast biomass, YBV5% - diet supplemented with 5% yeast biomass. Values are means ± SD (n=10)

Parameters like weight gain, final fish weight, and specific growth rate increased significantly ($p <0.05$) with the increase in yeast biomass addition. The group that received no yeast supplement (YBV 0%) showed the lowest growth. The group that received a 5% yeast biomass supplement had a significantly higher final weight compared to the group that received a 3% yeast biomass supplement.

The YBV5% group had a higher percentage of weight gain (108.04%) compared to YBV3% (96.41%) and almost double that of the control group (59.96%). By statistical analysis, it was found that the length and final weight of *Cyprinus carpio* fish in the three aquariums were significantly different between the control (YBV0%) versus YBV3% and YBV5% ($p < 0.05$). Among the treatments, the SGR % was highest in YBV5%. Nevertheless, the condition factor (K) for the control group (YBV0%) was significantly higher than for the two groups receiving probiotic *S. cerevisiae* BB06. The survival rate of *Cyprinus carpio* was 100% for the groups receiving probiotic *S. cerevisiae* BB06. The feed conversion ratio (FCR) provides an objective metric for quantifying the amount of feed necessary for producing 1 kg of fish. However, practical applications of FCR are subject to variations resulting from diverse conditions such as temperature, water quality, etc. (Barbacariu et al., 2021; Mocanu et al., 2021).The feed conversion ratio (FCR) of

Cyprinus carpio treated with *S. cerevisiae* BB06 was significantly improved compared to the control. The protein efficiency ratio (PER) is the amount of body protein obtained per unit of dietary protein consumed and is used to assess the quality of different dietary proteins. In addition, the protein efficiency ratio was significantly higher ($p < 0.05$) in fish receiving yeast-treated diets than in the receiving control diets. In the current study, diets containing *S. cerevisiae* BB06 significantly increased the protein efficiency ratio (PER) compared to the yeast-free diet (control). An increase in the yeast content of the feed had a slight tendency to decrease the value of the Fulton condition factor.

At the same time, were determined the allometry coefficient to observe if the fish had grown more in mass or length. The allometric coefficient during the entire experiment for each experimental diet is presented in Figure 2.



	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
YBV0%	2.52	2.43	2.37	2.32	2.19	2.20	2.22	2.23	2.29
YBV3%	2.54	2.45	2.39	2.34	2.32	2.31	2.31	2.33	2.36
YBV5%	2.58	2.57	2.57	2.43	2.38	2.34	2.32	2.33	2.38

Figure 2. Allometric coefficient of fish weight *versus* length during the experimental period

In our case, the allometry coefficient obtained had values between 2.29 and 2.58. Weight increases faster than length (about 2.5 times faster).

Meat composition in carp

Meat composition in carp is presented in Table 3. Using the Jasp version 0.16.3.0 Pearson correlations (Pearson's R) were made between crude lipid and crude protein content in fish meat for each experimental diet Figure 3. It is well known that in the case of carp, the fat content is an important marketable parameter and depends very much on the growth conditions. Content over 15% can adversely affect the taste of the meat and, in some countries, allow on the market only carp with a fat content below 10% (Tewary and Patra, 2011; Bauer end Schlott, 2009).

All parameters, except crude protein, were significantly influenced ($p<0.05$) by supplemented yeast biomass. The analysis of meat composition indicates a significant decrease in lipid content alongside increased protein content. YBV5% showed significantly higher carbohydrate and ash content than the control (YBV0%), however, the lipid content was found to be the lowest.

Since the values obtained for Pearson's R correlation are negative, $R = -0.92$ for the YBV3% diet, with a significant difference ($p< 0.05$) and $R = -0.85$ for YBV5% ($p< 0.05$), indicate that large values of one variable involve small values at the other variable. Thus, the higher the percentage of fat the lower the percentage of protein, and vice versa, the lower the percentage of fat the higher the percentage of protein. There was a significant correlation between crude fat content and crude protein in diets supplemented with probiotic *S. cerevisiae* BB06 and the yeast-free diet control group ($p>0.05$).

Table 3. Meat composition of fish fed with different experimental diets (% freeze-dried weight)

Indices	Control (YBV0%)	YBV3%	YBV5%
Ash (%)	3.45±0.12	3.08±0.42	4.24±0.62
Crude protein (%)	79.20±1.44	80.27±1.85	81.35±1.07
Crude lipid (%)	8.11±0.23	8.06±0.77	7.26±0.17
Carbohydrates (%)	4.13±0.03	4.19±0.15	4.22±0.10
Moisture (%)	3.15±0.23	4.41±0.25	2.80±0.48

Experimental diet: YBV0% - Control, commercial feed without yeast, YBV3% - diet supplemented with 3% yeast biomass, YBV5% - diet supplemented with 5% yeast biomass. Values are means ± SD (n=10)

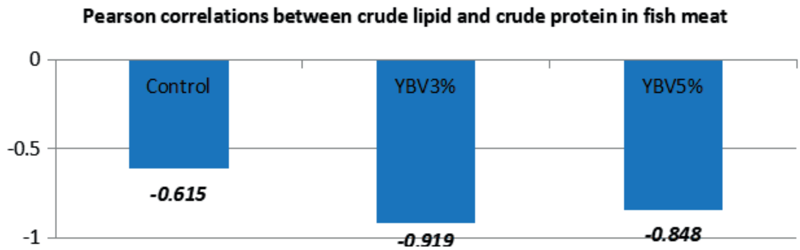


Figure 3. Pearson correlations of meat composition with different experimental diets

The analysis of the meat composition showed a considerable decrease in fat content, from 8.11% at YBV0% to 7.26% at YBV5%. In our study, the fat content was below the maximum recommended on the market, so the fish can be accepted from a qualitative point of view. Also, the addition of probiotic *S. cerevisiae* led to a significantly higher content of carbohydrates and ash content in the fish diet, and this may help the optimal growth of common carp. However, should be emphasized that in some studies was proved that the yeast addition led to a protein and ash content increase, while in others no changes were noticed; in this regard, it is most probable that the results depend on the employed yeast and the fish studied specie (Ljubojević et al., 2013). Crude protein is significantly higher in diet YBV5% compared to other diets, but a high protein content can lead to diseases caused by excessive growth (Guo et al., 2019). Fish require diets containing 30% to 55% crude protein and a supply of amino acids focused on specific requirements for maximum growth (Aragão et al., 2022). If the diet includes easily digestible ingredients, the high demand for protein in fish food can be met.

Water quality parameters

In our study, water quality parameters were regularly monitored in recirculating aquaculture systems (RAS), which conducted in them being maintained at acceptable levels in all tanks over eight weeks. The mean values (±SD) of the water quality parameters during the experimental variants are detailed in Table 4. Statistical analyses showed that the studied parameters: ammonium, nitrite, nitrate, phosphate, pH, electrical conductivity (EC), and dissolved oxygen (DO) were not significantly different ($p>0.05$) between the three experimental sets. Water pollution, caused by uneaten feed, high levels of ammonium and nitrite discharges, and toxic metabolites in fecal waste, is a major factor affecting fish growth and increasing the incidence of disease (Balcazar et al., 2006). In our study, we chose to use an ecological recirculating aquaculture system, which allows fish to be reared in controlled indoor conditions, limiting the likelihood of adverse environmental effects on carp production. The data showed no significant differences ($p> 0.05$) in any of the three aquariums in terms of water pH, temperature, and OD. Ammonia and nitrate concentrations were lowest ($p< 0.05$) in the aquarium with YBV3%, followed by YBV5% and control. Although N-NO_3 is not very toxic to fish, its accumulation in high concentrations for long periods is not suitable for fish growth (Estim et al., 2019). Toxic compounds, including ammonium and nitrogen compounds, are regulated by naturally occurring microorganisms, particularly denitrifying bacteria. However, various studies have demonstrated the effectiveness of certain probiotics, particularly Gram-positive bacteria, in reducing ammonia nitrogen levels, indicating their potential as a sustainable additive to enhance water quality (Mohapatra et al., 2013; Zorriehzahra et al., 2016; Camargo et al., 2005; Verschuere et al., 2000; Jóźwiakowski et al., 2009; Cha et al., 2013). Nonetheless, certain differences may arise depending on the yeast and fish species (Essa et al., 2011).

However, further research is needed to understand how probiotics work to improve water quality.

Table 4. Average values of the water parameters in recirculation aquaculture system

Water quality parameter	GROUPS			p-Value
	CONTROL[1]	*YBV3%*[1]	*YBV5%*[1]	
N-NH_4 (mg/L)	0.079±0.029	0.048±0.004	0.048±0.004	0.06
N-NO_2 (mg/L)	0.34±0.02	0.01±0.03	0.14±0.01	0.29
N-NO_3 (mg/L)	29.67±3.24	23.57±5.28	25.13±7.85	0.74
PO_4 (mg/L)	1.47±0.51	0.73±0.10	0.43±0.19	0.50
pH	6.70±0.46	7.28±0.19	6.64±0.35	0.14
EC (µS/cm)	524.44±168.53	671.11±276.33	620.0±210.53	0.28
DO (mg/L)	4.77±0.09	4.91±0.36	5.26±0.44	0.94
Temperature (ºC)	24.94±0.58	25.33±0.64	24.86±0.66	0.92

[1] Mean values ± standard deviation of the water parameters during the experiments (56 days) (temperature, pH, salinity, and oxygen: n = 56; ammonium, nitrite, and nitrate: n = 28).

CONCLUSIONS

The current study showed that the addition of freeze-dried probiotic *S. cerevisiae* BB06 at 3% and 5% in the diet significantly improved the body weight, weight gain, specific growth rate, and feed conversion ratio compared to the control group. The results show that diets yeast supplemented represent alternatives worthy of consideration for ensuring sustainable aquaculture. The high content of additionally administered yeasts contributes to the increase in the protein content of the carp meat. Previous research has shown that the *S. cerevisiae* BB06 strain has functional properties such as high resistance to the gastrointestinal environment, hydrophobicity, self-aggregation, antioxidant, and antibacterial activities, as well as safety properties such as γ-hemolysis and resistance to bacterial antibiotics, making it a suitable candidate (Diguță, 2023). It also has high freeze-drying survival, making it optimal for storage purposes. (Diguță, 2023).

The results of the study confirm that the partial replacement of fish meal with 3% or 5% probiotic *S. cerevisiae* BB06 has a beneficial effect on growth parameters, in agreement with previously published literature (Aragão et al., 2022; Essa et al., 2011; Ma et al., 2019; Oliva-Teles and Gonçalves, 2011; Tovar et al., 2002). In our study, including probiotic *S. cerevisiae* BB06 in the fish diet may have contributed to maintaining the water quality by improving feed utilization and reducing the food conversion ratio (FCR), leading to high growth rates and carp production. Substituting fishmeal partially with probiotic *S. cerevisiae* BB06 was shown to promote beneficial growth of carp (*Cyprinus carpio*), while maintaining meat fat content and improving protein retention. In addition, the feed conversion ratio was reduced. Overall, the results indicate that yeast-supplemented diets represent viable alternatives to ensure sustainable aquaculture. The recommendation for future carp feed recipes is to completely replace fishmeal with yeast to ensure better growth performance, viability and maximize sustainability in aquaculture.

Further research is required to explore the role of the probiotic *S. cerevisiae* BB06 in disease resistance, as well as on the host fish's immune system.

ACKNOWLEDGEMENTS

The present work was funded by the University of Agronomic Sciences and Veterinary Medicine of Bucharest – Romania, Research Project 1262/30.07.2021, acronym FEED4FISH in the Competition IPC 2021.

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