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QUANTITATIVE AND QUALITATIVE EFFECTS OF 010K – LACTOBACILLUS PARACASEI AND THE "ECOPROBIOTIC" PROBIOTIC PREPARATION ON THE GROWTH AND INTESTINAL MICROFLORA OF FISH

The most important result of fish rearing in closed water systems is to achieve the highest possible growth rate while creating optimal aqueous environment factors. The growth of fish is influenced by many factors, including probiotics in artificial diets. In this sense, functional food supplements, including pro-, pre- and synbiotics, are gaining increasing attention as an environmentally sound strategy to improve fish health. The aim of this study was to investigate the effects of the 010K – Lactobacillus paracasei strain that isolated from Kazakhstan koumiss and the "Ecoprobiotic" probiotic preparation on the growth of intestinal microflora in Nile tilapia. The study outcomes displayed that live weight of fish in the experimental groups (010K – Lactobacillus paracasei and probiotic preparation "Ecoprobiotic") was higher than in the control group. Moreover, fish fed with the "Ecoprobiotic " probiotic and 010K – Lactobacillus paracasei strain had a significantly higher number of yeast cells compared to the control group. In summary, the 010K – Lactobacillus paracasei and probiotic preparation "Ecoprobiotic" may be a promising candidate for the improving growth and intestinal microbiota of Nile tilapia.

Key words: aquaculture, tilapia (Oreochromis niloticus), Lactobacillus paracasei, probiotic, immunity, microbiological indicators.

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010K – Lactobacillus paracasei және «Экопробиотик» пробиотикалық препаратының балықтың өсуі мен ішек микрофлорасына сандық және сапалық әсері

Жабық су жүйелерінде балық өсірудің ең маңызды нәтижесі су ортасының оңтайлы факторларын қалыптастыру кезінде мүмкін болатын ең жоғары өсу қарқынына қол жеткізу. Балықтың өсуіне көптеген факторлар әсер етеді, әсіресе жасанды диетадағы пробиотиктер. Осы тұрғыдан алғанда, функционалды тағамдық қоспалар, соның ішінде про-, пре- және синбиотиктер балық денсаулығын жақсартудың экологиялық қауіпсіз стратегиясы ретінде назар аудартып отыр. Бұл зерттеудің мақсаты – Қазақстан қымызынан бөлініп алынған 010K – Lactobacillus paracasei штамының және «Экопробиотикалық» пробиотикалық препараттың Ніл тілапиясы ішек микрофлорасының өсуіне әсерін зерттеу. Зерттеу нәтижелері эксперименттік топтардағы (010K – Lactobacillus paracasei және «Экопробиотик» пробиотикалық препараты) балықтардың тірі салмағы бақылау тобына қарағанда жоғары екенін көрсетті. Сонымен қатар «Экопробиотик» пробиотигі және 010K – Lactobacillus paracasei штамымен қоректенетін балықтарда бақылау тобымен

салыстырғанда ашытқы жасушаларының саны айтарлықтай жоғары болды. Қорытындылай келе, 010K – Lactobacillus paracasei және «Экопробиотик» пробиотикалық препараты Ніл тілапиясының өсуі мен ішек микробиотасының жақсаруы үшін перспективалы үміткер болуы мүмкін.
Түйін сөздер: аквамәдениет, тілапия (Oreochromis niloticus), Lactobacillus paracasei, пробиотикалық, иммунитет, микробиологиялық көрсеткіштер.

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Количественное и качественное влияние 010K – Lactobacillus paracasei и пробиотического препарата «Экопробиотик» на рост и микрофлору кишечника рыб

Важнейшим результатом выращивания рыб в замкнутых водных системах является достижение максимально возможной скорости роста при создании оптимальных факторов водной среды. На рост рыб влияет множество факторов, в том числе пробиотики в искусственных рационах. В этом смысле функциональные пищевые добавки, в том числе про-, пре- и синбиотики, привлекают все большее внимание как экологически чистая стратегия улучшения здоровья рыб. Целью настоящего исследования явилось изучение влияния штамма 010K – Lactobacillus paracasei, выделенного из казахстанского кумыса, и пробиотического препарата «Экопробиотик» на рост кишечной микрофлоры нильской тиляпии. Результаты исследований показали, что живая масса рыб в опытных группах (010K – Lactobacillus paracasei и пробиотический препарат «Экопробиотик») была выше, чем в контрольной группе. Кроме того, у рыб, скармливаемых пробиотиком «Экопробиотик» и штаммом 010K – Lactobacillus paracasei, было значительно большее количество дрожжевых клеток по сравнению с контрольной группой. Таким образом, 010K – Lactobacillus paracasei и пробиотический препарат «Экопробиотик» могут быть многообещающими кандидатами для улучшения роста и кишечной микробиоты нильской тиляпии.
Ключевые слова: аквакультура, тиляпия (Oreochromis niloticus), Lactobacillus paracasei, пробиотик, иммунитет, микробиологические показатели.

Introduction

The most important result of fish rearing in closed water systems (CAFs) is to achieve the highest possible growth rate. The growth of fish is influenced by many factors, including probiotics in artificial diets (El-Saadony et al., 2021). The role of probiotics is extremely important, because current understanding of their role shows that microbial communities of probiotic organisms and the host organism can enter to a symbiotic relationship (Cristofori et al., 2021). The host organism creates living and feeding conditions for probiotic microbial societies, the latter, in their turn, provide the host with various essential substances, including those that increase the host's immunity, fight against pathogenic fauna, and improve growth performance (Hai, 2015).

The term "probiotic" is mainly used in relation to bacteria that are able to promote the health of other organisms. The list of probiotic strains is quite limited. Lactic acid bacteria (*Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus, Saccharomyces*, etc.) are a diverse group of microorganisms that exist both as natural inhabitants of the gastrointestinal tract and as fermentative lactic acid bacteria in products (Mathur et al., 2020). Most bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* are recognized as safe and *Bifidobacterium*beneficial for humans and animals. Moreover, they have a positive influence on the formation of some enzymes and vitamins that support digestion, as well as antibacterial substances, contribute to the recovery of the normal intestinal microflora after disorders related to diarrhoea, antibiotics and radiotherapy, reduce the pH of meat and blood cholesterol levels, stimulate immune functions, suppress bacterial infections and enhance the absorption of fatty acids (Slattery et al., 2019; Plaza-Diaz et al., 2019).

The various effects of *Lactobacillus* have been reported in humans (Moal & Servin, 2014; Berni et al., 2017). Moreover, it has been revealed that *Lactobacillus paracasei* improved growth performance and intestinal microflora in chicken (Xu et al., 2019). Besides, previous research has investigated the probiotic effects of *Lactobacillus paracasei* and *Bifidobacterium longum in shrimps* (Huang et al., 2022).

In our previous work, we isolated 10K – *Lactobacillus paracasei* strain from Kazakhstan koumiss. However, there is no study on its effect on fish gut microbiota and growth rate (live weight etc.). Considering the importance of the lactic acid bacteria, we hypothesized that 010K – *Lactobacillus paracasei* and probiotic preparation “Ecoprobiotic” may influence growth performance and gut microbiota in Nile tilapia.

Materials and methods

Experimental design and samples The object of research was juvenile Nile tilapia (*Oreochromis niloticus*), grown using different feeds and technologies on the basis of TENGRY FISH LLP (Almaty region, Kazakhstan). The selection of analytical material was carried out every 10 days, during 30 days of cultivation. Samples taken at the beginning of the experiment were used as controls.

The experiment involved two pools with a volume of 100 liters with a circular water exchange with external filtration (biofilter). Each pool was planted with 50 pieces of tilapia weighing no more than 23 g each – the control and experimental groups. During the experiment, the size and weight characteristics of tilapia were measured in the experimental and control groups (Pyrsikov et al., 2017).

Bacteria *Lactobacillus paracasei* was isolated from koumiss, represents sticks with blunt ends measuring 2.7-3.1 x 0.9 microns, and tends to form chains. Gram-positive, catalase-negative, asporogenic, immobile. The macrocolony on agar is convex with a solid edge, the consistency is oily, and the surface of the colony is smooth, shiny, white in color, and opaque. Deep colonies in the form of pieces of cotton wool, matte. On hydrolyzed milk within pH 4.0–5.5, it grows very well giving turbidity with sediment, as well as on milk–whey, MRS, wort medium and their agarized media. Microaerophile, facultative anaerobe. The minimum growth is at 20 ^{o}C, the optimal is 39 ^{o}C, and the maximum is 45 ^{o}C. Milk acidifies to form a dense clot without gas with a pleasant taste and smell. It coagulates after 16-18 hours at an optimal temperature. The active acidity is 136T. The maximum acidity is 220T.

Pond fertilization and fish feeding The prepared diets were dried at room temperature, packed in plastic bags, and cooled at 4 °C to maintain the viability of microorganisms before feeding experimental fish. New diets were prepared every two weeks to ensure that high levels of probiotics were maintained in the diet during the experimental period. The fish were fed by hand 5 times a day in the amount of 5% of the total fish biomass. Technical water change with the removal of faeces and food residues was carried out 2 times a week. The fish were fed by hand. For feeding tilapia, high-protein domestic compound feed brand ALLER PERFORMA 2 mm was used.

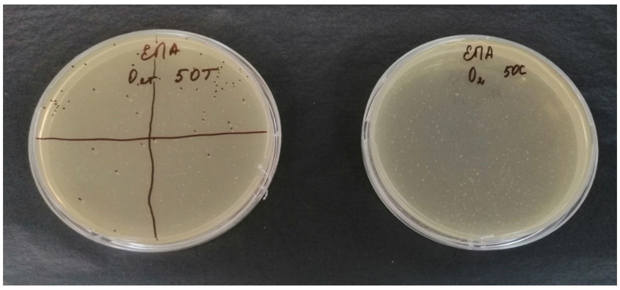

Figure 1 – Lactic acid bacteria were grown in culture media and the process of counting

010K – Lactobacillus paracasei strain and probiotic preparation “Ecoprobiotic” quantitative and study of the impact on the dynamics of qualitative changes: 1st control group; 2nd 010K – group treated with a strain of Lactobacillus paracasei; 3rd group was given the probiotic preparation “Ecoprobiotic”.

Table 1 – Experimental design

№	Experience variant	Features of feeding	Amount of fish
I	Control	100% basic diet	50
	Experience 1	95% basic diet + 5% 010K – *Lactobacillus paracasei*	50
	Experience 2	95% basic diet + 5% probiotic «Ecoprobiotic»	50

Analysis and identification of gut microbiota At the end of the feeding period, the fish were starved for 24 hours to ensure bowel movements, and a random sample of 3 fish was selected after each treatment. The fish was sacrificed, dissected and opened longitudinally. The entire intestine of the fish was aseptically removed. The finished suspension was coarsely sieved using a sterile nylon mesh (100 µm). The homogenates were serially diluted to 10-4 in volumes of 9 ml of sterile 0.85% saline. A total plate count was performed by spreading 0.1 ml of each homogenate onto Tryptone Soy Agar (TSA) and incubating at 37°C for 16 hours.

Yeast cell counts were performed by applying 0.1 ml of the homogenate to Sabouraud agar. The plates were incubated at 25 °C. for 5 days and yeast cells were counted using a colony counter. Dominant colonies were purified and identified based on morphological characteristics and growth parameters using biochemical tests and standard *Lactobac* isolation methods. The number of bacteria and yeast cells was expressed as CFU g−1 of the intestine.

Figure 2 – Dissected Nile talapia

Statistical Analysis All data were expressed as mean ± standard error of the mean (SEM). Bacterial and yeast cell counts in the gut were logarithmically transformed before analysis. A one-way ANOVA test was used to test for significant differences between groups at P <0.05. When overall differences were found, Tukey's HSD test was used for pairwise comparisons between groups at $P < 0.05$. All analyses were performed using Statistical Products and Service Solutions (SPSS version 20) software (Mary et al., 2019).

Results and Discussion

At the end of the experiment, to assess the effectiveness of the effect of strain *010K – Lactobacillus paracasei* and the probiotic "Ecoprobiotic" on the body of the tilapia in artificial conditions, the fish breeding results were obtained, as shown in Table 1.

The total increase in live weight of fish 2nd (*010K – Lactobacillus paracasei*) and 3rd (probiotic preparation "Ecoprobiotic") in the experimental groups was higher than in the 1st (control) group. Among them, the 3rd group gained the largest live weight, that is, the group that used the probiotic preparation "Ecoprobiotic" (P<0.05).

Table 2 – Effect of *10-K Lactobacillus paracasei* and probiotic preparation "Ecoprobiotic" on live weight size of fish (M±m)

Indicators	Experimental groups		
	Control 100% basic diet	Experience 1 95% basic diet + 5% 010K – Lactobacillus paracasei	Experience 2 95% basic diet + 5% probiotic «Ecoprobiotic»
Planting density pcs/100 l	50	50	50
The initial individual weight of fish, g	23	23	23
Final individual weight of fish, g	45±0,2	55±0,2	64±0,2
Individual weight gain of fish, g	22±0,2	33±0,2	41±0,2
Survival, %	90.0	98,0	100

*Differences are significant at $p < 0.05$

An increase in the mass accumulation coefficient indicates an improvement in the digestibility of food by fish. The positive effect of probiotics on the viability of fish, their growth rate and fish productivity is shown in Table 2. The outcomes displayed that the growth rate of fish treated with "Ecoprobiotic" and the *010K – Lactobacillus paracasei* strain is higher than in the control group.

Intestinal microbiota At the end of the experiment, the highest level of total bacteria in all Petri dishes was recorded in the second experiment, i.e. fish fed with the probiotic "Ecoprobiotic" (2.05 × 10-4 log Coe G-1), and the lowest indicator was in the control group. In addition, it was significantly higher for all fish fed with the *010K-Lactobacillus paracasei* strain in the intestine compared to control. The average values of lactic acid bacteria from the intestinal microflora of fish were higher in fish belonged to the 2nd experimental group.

As a result of the experiment, fish from in the both groups using the strain *010K-Lactobacillus* *paracasei* and the probiotic drug “Ecoprobiotic” showed good general clinical condition.

Modern fish farming is based on intensive technologies, including in closed water supply installations, the peculiarity of which is the high density of planting in limited areas, which significantly increases the risk of infection of fish with pathogens of dangerous infections (Blandford et al., 2018). Antibiotics of various functional groups are used as preventive and therapeutic agents for infectious diseases (Schmidt et al., 2017). As a result, the virulence of microorganisms increases, fish weights drop significantly and there is a strong decline in fish-breeding indicators (Kibenge et al., 2012). One of the ways to solve this problem is the use of modern probiotic drugs.

Table 3 – Quantitative and qualitative indicators of the intestinal microflora of fish after the application of *010K – Lactobacillus paracasei* and the probiotic drug “Ecoprobiotic”, lg CTB / g. (M±m; n=50)

Indicators	Total plate count (log CFU g−1) (10−4)MRS	Yeast (log CFU g−1) (10−4)	Lacto.paracasei (log CFU g−1 (10−4)
	Mean±S.E.	Mean±S.E.	Mean±S.E.
1	2	3	4
Control	1.69 ± 0.06	1.38 ± 0.02	1.35 ± 0.02
Experience 1	1.75 ± 0.02	1.94 ± 0.02	1.79 ± 0.03
Experience 2	2.05 ± 0.07	2.07 ± 0.12	2.33 ± 0.05

MRS- de Man, Rogossa and Sharpe; CFU- colony-forming unit.

In this study, we observed an increase of yeast cells and in the intestines of fish fed by *010K – Lactobacillus paracasei*. This indicates that the respective probiotic has caused bacteria to multiply in the intestines of the fish. There were fewer pathogenic bacteria in the intestines of fish fed with lactic acid bacteria, which is a sign of increased immunity. The intestinal microbiota often plays an important role in preventing intestinal colonization by pathogens. Based on the results of the study, it confirms the outcomes of our previous studies that showed the antagonistic effect of *010K – Lactobacillus paracasei* that may act against pathogenic bacteria and lead to the stimulation of the immune system and the improvement of the microbial balance of the intestine. Biwas et al., (2013) studied the improvement of cytokine-mediated immunity in Japanese fish by use of the *Lactobacillus paracasei* strain. Lactic acid bacteria isolated from koumiss diminished the harm caused by *E. coli* and increased the expression levels of tight junction proteins in mice (Ren et al., 2022). In addition, *Lactobacillus casei Zhang* affected immune responses in humans (Ya et al., 2008).

Furthermore, the weight and growth rates of the Nile tilapia were greater in both experimental groups than the control group. The efficiency of aquaculture is largely determined by the quality and quantity of feed used. Reducing feed costs is one of the main economic factors that increase the profitability of fish farming (Ringo et al., 2020). Probiotics added to the feed have a significant impact on feed consumption per unit of fish growth because they contribute to their fuller assimilation, neutralization of mycotoxins coming with feed, displace pathogenic microflora and strengthen the general resistance of the fish organism (Aguilar-Toalá et al., 2021). Based on the results obtained during the experiment, we can assume the economic efficiency of probiotics application in fish farming. Previously, Ljubobratovic et al., (2017) reported positive effects of *Lactobacilli* growth, microbiota balance and skeletal development in fish. *L. plantarum* significantly stimulated the growth and protection against infections in fish (Van Doan et al., 2014).

Meanwhile, there are few studies on potential effects of the *Lactobacillus paracasei* on body weight and growth rate in fish, and therefore, this study was conducted to partially fill that gap.

Conclusion

In the course of experimental feeding and rearing of the Tilapia experimental group, a pronounced positive effect of the industrial probiotic preparation “Ecoprobiotic” and *010K-Lactobacillus paracasei* was established. The live weights of experimental groups were increased compared to control group. Thus, the 010K – *Lactobacillus paracasei* and probiotic preparation “Ecoprobiotic” may be a promising candidate for the improving growth and intestinal microbiota of Nile tilapia.

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