EFFICIENCY OF PROBIOTICS IN CARP (*CYPRINUS CARPIO*) GROWTH IN THE AQUACULTURE RECIRCULATION SYSTEM

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Abstract

This study evaluated the effects of probiotics on feed intake, growth performance, and the biochemical composition of carp meat. For this purpose, 200 carp specimens with an average weight of 20 ± 0.03 g were randomly divided into 4 groups: 3 experimental and one control. The fish were fed twice a day with 0; 80; 160 and 200 mg probiotic / kg feed for 50 days. The probiotic used in this experiment consisted of a complex of bacteria (Bifidobacterium and Lactobacillus). The results indicated an improvement in growth factors (individual growth rate, feed conversion factor, specific growth rate, daily growth rate) in the case of probiotic administration, compared to the control (P <0.05). The highest body weight gained was obtained in variant V2 (26.12 g), 8% higher than in V1, 5% than in V3 and 30% higher than in the control variant. The content of crude protein, lipids, moisture and ash in fish meat had significant differences (P <0.05) at the end of the experiment, compared to the initial values. The proteins in the feed were much better recovered in the case of groups fed probiotic diets, the protein efficiency coefficient (PER) and the protein utilization efficiency (PUE) having higher values than in the control variant (P <0.05). Analyzing the results obtained, it can be stated that probiotics are an effective alternative to growth promoters used in many fish farms.

Key words: probiotics, growth performance, carp, body composition

INTRODUCTION

In Europe, carp is a very popular fish, its farming being considered a real industry. Romania is the country where the common carp (Cyprinus carpio) is one of the most important species of farmed fish, with a great appreciation from consumers. In 2017, carp farming accounted for 79% of all species of farmed fish. Consumption per capita also increased by 9.7% compared to the previous year [5].

The intensification of aquaculture involves high growth densities of biological material, which puts the fish under intense stress, favoring the appearance of bacterial diseases. As an alternative to antibiotics in treating disease, or in order to increase disease resistance, the last few decades have brought probiotic bacteria to the attention of fish farmers. Given in certain doses, probiotics develop in the host body colonizing the intestine and restoring the intestinal microflora, thus ensuring the health of organs and tissues [3].

MATERIAL AND METHOD

Design, biological material and experimental diets

The study was performed in 2020, within the pilot recirculating system of the Research-Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galati. The growth system consisted of 4 rectangular growth units, made of glass, with dimensions of 120 x 40 x 45 cm and a volume of 150 l. Each growth unit was equipped with a technological water treatment system, and a water supply and evacuation installation.

The biological material, represented by 200 one-year old carp specimens (Cyprinus

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carpio), obtained from the Brates Galati farm, with an average initial mass of 20 ± 0.03g, was evenly distributed in the 4 breeding units and fed with experimental diets. (V1 - V3) and a control diet (M) twice a day for 50 days. The feeding ratio was 2.5% of body weight.

The experimental diets were prepared by adding to a commercial carp feed, with a grain size of 2 mm, a commercial probiotic (PC), consisting of a complex of bacteria (Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus casei). The experimental diets were performed as follows: 0 mg PC*kg-1 (M), 80 mg PC *kg-1 (V1), 160 mg PC*kg-1 (V2), 200 mg PC*kg-1 (V3), as presented in table 1.

Physico-chemical parameters of water

Temperature, pH and dissolved oxygen (OD) were monitored daily, using the HQ40d Hach Lange portable analyzer with 2 probe connectors. Nitrogen compounds were determined weekly with the Hach Lange DR1900 portable spectrophotometer, using Hach Lange LCK kits, according to the working protocols established in the standard analysis methods for surface waters [8]. Every 2 days, the water in the system was 50% refreshed to keep nitrogen compounds within optimal limits.

Growth parameters

The fish were weighed at the beginning of the experiment and at the end of the experiment. **Individual weight gain** was calculated as the difference between the final and initial individual mass. **The daily growth rate (GR)** was calculated as the ratio between the difference of the final and initial biomass and the number of days of growth. **The specific growth rate (SGR)** was calculated according to the formula: SGR = [(ln final weight–ln initial weight) x 100 / time in days. **The feed conversion ratio (FCR)** was calculated as the ratio between the amount of feed distributed and the difference between the final and initial biomass of the lot. **Protein efficiency coefficient (PER)** was calculated as the ratio of body weight gain to ingested protein. **Protein utilization efficiency (PUE)** was calculated according to the formula: PUE = 100 x (final weight (g) x final body protein (%)– initial weight (g) x inițial body protein (%)) / diet given (g) x crude feed protein.

Biochemical composition of muscle tissue

At the beginning and end of the experiment, muscle tissue samples were taken to determine the total protein content, moisture, fat content and ash. The analyzes were performed using standard methods of AOAC (2006) [1].

The total protein substances were determined by the Kjeldahl method, according to STAS 6514-75, using a Gerhardt type system. By multiplying the value of the nitrogen content by 6.25, a coefficient characteristic of fish meat, the crude protein content was determined.

Moisture was determined according to STAS 6508-73 by heating in oven at 130 ° C for one hour to constant mass. **The content of lipids** was determined by the Soxhlet method, using the VELP type extraction system, according to STAS 6512-73.

The ash content was determined by calcining the sample at 600°C and weighing the remaining residue.

Statistical analysis

The statistical processing of the obtained data was performed through the Microsoft Excel program (Office 2010) for Windows. Statistical differences between variables were tested by the t test (comparisons between means, significance $p < 0.05$) and the ANOVA One Way test

Table 1 Biochemical composition of experimental diets

Parameters	**Average ± SD**
Crude protein (g/100g)	30
Crude fat (g/100g)	7
Ash (g/100g)	6,5
Fiber (g/100g)	5,0
Carbohydrates (g/100g)	43,5
Digestible energy MJ	12,6
Probiotic (mg/kg feed)	
Diet 1 (V1)	80
Diet 2 (V2)	160
Diet 3 (V3)	200

RESULTS AND DISCUSSIONS

Physico-chemical parameters of water

The results obtained from the physico-chemical analyzes of the water, presented as the mean ± standard deviation, are presented in table 2. All parameters were within the limits allowed for fish waters, according to Order no. 161/2006 for the approval of the Norm regarding the classification of surface water quality in order to establish the ecological status of water bodies [7]. During the study, the temperature values did not indicate statistical differences between the experimental variants (P = 0.92), registering the average value between 23.3°C and 23.5°C.

Dissolved oxygen concentrations were between 4.6 - 8.9 mg L−1, lower values being recorded in periods of higher temperature, values that were corrected by additional aeration. The minimum values of nitrites were 0.01 mg L−1, the maximum 0.2 mg L−1 and the pH varied between 7 and 8.2 during the study period, the differences being significant (P <0.05) between the control and the 3 experimental variants.

Table 2 Values of the physico-chemical parameters of the water (mean ± SD)

Parameter analyzed	UM	M	V1	V2	V3
		mean ± st. dev	mean ± st. dev	mean ± st. dev	mean ± st. dev
Temperature	°C	23.3 ± 1.7	23.5 ± 1.6	23.4 ± 1.8	23.5 ± 1.6
pH	upH	8.0 ± 0.1	7.8 ± 0.2	7.8 ± 0.2	7.9 ± 0.2
Dissolved oxygen, OD	mg/l	7,6 ± 1,0	7,9 ± 1,0	7,7 ± 1,0	7,7 ± 0.9
NO3	mg/l	4.95 ± 0.45	3.88 ± 1,08	3.50 ± 1.41	3.89 ± 1.42
NO2	mg/l	0.12 ± 0.06	0.11 ± 0.05	0.11 ± 0.06	0.11 ± 0.05
NH4	mg/l	1.12 ± 0.24	1.19 ± 0.22	0.21 ± 0.08	1.21 ± 0.10

Growth performance

The results show that probiotics can increase growth performance. Weight gain, daily growth rate (GR) and specific growth rate (SGR) are shown in Table 3. The highest body weight gained was obtained in variant V2 (26.12 g), 8% higher than in V1, by 5% than in V3 and by 30% than in the control variant.

Table 3 Growth performance of carp fed with experimental diets with probiotic

Growth parameters	M (0 mg PC*kg $^{-1}$)	V1 (80 mg PC*kg $^{-1}$)	V2 (160 mg PC*kg^{-1})	V3 (200 mg PC*kg^{-1})
Initial weight (g)	20.12±0.03	20.09 ±0.02	20.16 ± 0.03	20.21 ±0.028
Final weight (g)	40.25 ± 0.51	44.20 ± 0.60	46.28±0.39	44.98 ± 0.57
Body weight gain (g)	20.13	24.11	26.12	24.77
Daily growth rate (GR)	0.40	0.48	0.52	0.50
Specific growth rate (SGR)	1.35	1.54	1.66	1.60
Food conversion ratio (FCR)	1.59	1.37	1.28	1.31

The specific growth rate (SGR) was higher in the case of experimental diets with probiotics (1.66 in V2, 1.60 in V3, 1.54 in V1), compared to the control (1.34). The food conversion ratio (FCR) was influenced by the addition of probiotics in the food, having better values in the case of lots with experimental diets (1.37 for V1, 1.28 for V2, respectively 1.31 for V3), compared with the control group (1.59) (fig. 1). The results obtained regarding the wight gain and the specific growth rate are similar to those obtained by Yang et al., 2019 in the case of goldfish (Carassius auratus), fed with feed with the addition of Bacillus cereus in concentration 1 x 10^9 CFU / kg feed [9].

The beneficial effects of probiotics on growth performance have also been reported by Zaineldin et al., 2018, in the case of red sea bream, when adding probiotic bacterium Bacillus subtilis to food in a concentration of 10^8 and 10^{10} CFU kg-1 [10].

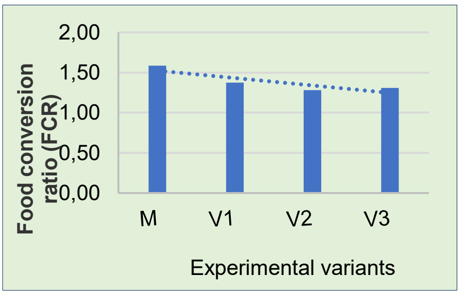

Figure 1. Influence of food probiotic on food conversion ratio

The crude protein, lipid, moisture and ash content of fish meat, presented as mean ± standard deviation, had significant differences (P <0.05) at the end of the experiment compared to initial values. Analyzing the values obtained at the end, significant differences between the experimental variants were obtained for protein content, moisture and ash (P <0.05), while differences in crude lipid content were insignificant (P> 0.05; P = 0.606) (table 4). Compared to the initial time, the meat lipid content increased by 57% in the control, by 59% in V1, 57% in V2, and 54% in V3 (fig. 2), due to the ability of probiotics to increase the activity of digestive enzymes, which increase the digestibility and absorption of feed [6].

Similar to the results obtained by us, Dhanaraj et.al, 2010, observed an increase in the amount of lipids in carp meat to a dietary supplement with 0.5% Lactobacillus acidophilus [4]. On the contrary, after 90 days of feeding the African catfish (Clarias gariepinus) with probiotic-supplemented diets, Ayoola et al., 2012 obtained the highest amount of lipids in the control group [2].

Table 4 Proximate composition of carp meat fed experimental probiotic diets

Proximate composition (%)	Inițial	Experimental variants / Final			
		M	V1	V2	V3
Proteins	14,86 ±0,32	16,98 ± 0,22	17,89 ± 0,07	18,93 ± 0,04	18,13 ± 0,04
Lipids	2,02 ±0,37	3,18 ±0,21	3,22 ± 0,05	3,17 ± 0,18	3,12 ± 0,03
Moisture	81,07 ± 0,73	76,26 ± 0,11	74,98 ± 0,1	74,37 ± 0,28	74,92 ± 0,09
Ash	1,76 ± 0,1	3,26 ±0,04	3,35 ±0,04	3,12 ± 0,01	3,33 ± 0,21

Compared to the control, the protein efficiency coefficient (PER) improved in the probiotic fed variants (P <0.05). However, no significant differences (P>0.05) were reported between the groups fed with different concentrations of probiotic.

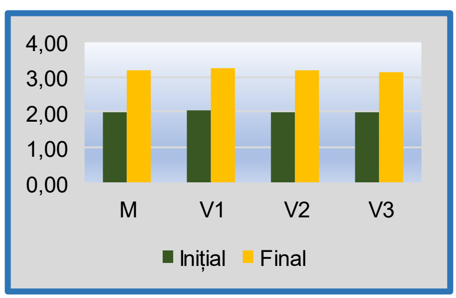

Fig. 2 The variation of lipids in carp meat fed with different concentrations of probiotic

The efficiency of protein utilization (PUE) was also high in the case of experimental diets with probiotics, indicating that probiotics help to make better use of protein in food. (fig. 3).

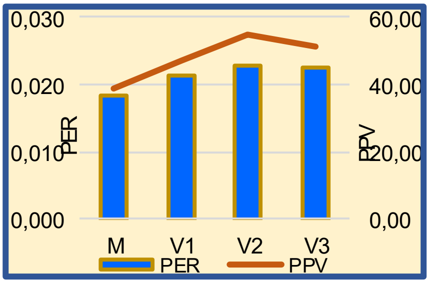

Fig. 3 PER and PPV dynamics in fish fed with different concentrations of probiotic

CONCLUSIONS

In conclusion, this study showed that probiotics incorporated in fish feed did not change the quality of the aquatic environment, the parameters were within the recommended limits for fish waters. They have positive effects on growth performance, feed use and the nutritional quality of carp *(Cyprinus carpio)* due to their ability to improve the activity of digestive enzymes, increasing digestibility and absorption of feed.

Probiotics can be an effective alternative to growth promoters used in many fish farms. However, research in this area needs to be continued in order to better understand the mode of action and doses used for each species.

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