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Influence of Dietary Probiotic on Welfare and Feed Conversion Ratio on Common Carp (*Cyprinus carpio* Linnaeus, 1758*)* (Cypriniformes: Cyprinidae) Reared in an Intensive Cage System

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Abstract: This experiment assessed the influence of the probiotic *Paenibacillus alvei*, added to carp food, on welfare and production characteristics (FCR). Fulton factor and water parameters were evaluated for welfare status assessment health parameters. FCR was calculated during measuring periods of the two experimental phases. Two experimental groups (A and B) were set up. Group C was the control group, with no added probiotic in the food. Probiotic concentrations for groups A and B were 1 ml/kg and 2 ml/kg feed, respectively. The experiment was conducted during one production season (180 days). Probiotic influence in group A fish had noticeably lower values of FCR (1.4) compared with control group C (1.7). Compared to fish from the control group C, decreased values of FCR were presented in group B (1.5). At the end of the experiment, the welfare status assessment showed the "excellent" status of experimental groups, indicating good rearing practice.

Key words: aquaculture, influence, welfare, FCR, probiotic, feed.

Introduction

Aquaculture development has inevitably caused significant changes in water biotic and abiotic factors, disrupting all their complex relationships. Intensive production is based on increasing the number of individuals per unit of water volume and applying intensive nutrition. The high density of fish with intensive feeding conditions causes the formation of large amounts of metabolic products and unutilised food, the decomposition of which significantly alters the water quality and hygienic conditions in the ponds/cages. Inadequate stocking, excessive density and failures in the transfer of some technological and zoo hygienic measures can contribute to the deterioration of water quality, which can cause health issues and fish mortality.

Implementing the classical and already established methods of treatment, such as applying medicines (primarily antibiotics) and disinfectants, is a common producer practice for preventing fish diseases and treating fish when a particular disease appears.

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It must be emphasised that some of the mentioned substances are already prohibited from being used in aquaculture. (Chen et al. 2014, Allameh et al. 2015).

Consequently, a new alternative approach was developed to enhance the fish's resistance to diseases, optimise the immune system, and stimulate growth, mainly by administering probiotics instead of antibiotics. According to Lazado & Caipang (2014a) and Lazado et al. (2015), probiotic beneficial microorganisms were identified, characterised and applied in aquaculture to improve the aquaculture industry while relying less on antibiotics on application (Lazado & Caipang 2014a, 2014b, Akhter et al. 2015). Faturrahman et al. (2015) stated that probiotic applications improved aquatic animal growth rates, food utilisation and survival rates (Hossain et al. 2017).

According to Merriefield et al. (2010), probiotics improve fish appetite, growth, and feed utilisation, enhance the quality of meat and composition, and reduce malformations. Probiotics represent a new era in modern aquaculture, increasing both scientific and commercial interest (Mohapatra et al. 2013, De et al. 2014, Lauzon et al. 2014, Carnevali et al. 2014, Hosefiniar et al. 2014, Sandeva et al. 2016, Huynh et al. 2017, Suguna 2020).

Welfare is defined as "The state of the individual as he copes with the environment" (Broom 1996, Broom 2007). In aquaculture, the term "health" is often interpreted as the "absence of apparent disease", and thus, emphasis is placed on the prevention and eradication of disease (Broom 2007). Health, from this perspective, means the ability of the animal to perform normal physiological functions and maintain homeostasis, thereby supporting its ability to withstand infectious and non-infectious stressors. Good health is essential (but not sufficient) for good welfare (Duncan 2005, Ashley 2007). The increased trendline of intensive fish production in the Republic of North Macedonia, coupled with the necessity for new alternative technological solutions to improve aquaculture results and increased yields of quality and healthy fish meat, presents a significant challenge to the industry. The objective of this experiment was to ascertain the impact of enriched probiotic food on production characteristics (FCR) and carp welfare status.

Materials and Methods

The experiment was performed in a registered production facility, i.e., a cage farm, in the "Kozjak" reservoir, with the following Google Earth coordinates: 41°49'08.78" N and 21°09'23.40" E. To perform the research, three compartments/cages (A, B, and C) were separated with dimensions of 5x5x5 m, i.e., 3x125 m^3 volume.

As a basis for the carp diet of the experiment, a commercial pelleted food from a manufacturer, "Aqua" from Austria, was used. The pellet size was 4-6 mm, and the following declared values were used: protein 30%, fat 10%, crude fibre 4.5%, crude ash 6.5%, calcium (Ca) 0.90%, sodium (Na) 0.25%, and phosphorus (P) 1.10%.

The food was controlled, and its chemical composition was examined in the UKIM Institute of Animal Sciences and Fisheries chemical laboratory.

Bacterial culture – probiotic *Paenibacillus alvei* DZ-3, in defined quantities (1 ml/kg food and 2 ml/kg food), was added to the basic food as pellets. The food with added probiotic was prepared through spraying, applied on the pelleted food in a mixer for 3 minutes, then mixed with a mixer for 5 minutes. The mixed food with the added probiotic was placed evenly in a layer of 2 cm in a dry, ventilated place to dry for 2 hours. The sorted and separated carp individuals had an average initial body mass of 170 g and an average total length of 234 mm. The exact number of carp individuals (n = 323) were stocked in each cage. After stocking the fish into separate groups (A, B and C), the total ichthyomass in each group was determined, representing the initial weight of the groups. Experimental group "A" had an average body mass of 172.8 g and total ichthyomass of 55 800 g and was treated with a probiotic with a lower concentration (1 ml/kg), $1.5^{*}10^{8}$ CFU/ml (Colony forming Units/ml). Experimental group "B", with an average body mass of 172.4 g and a total ichthyomass of 55 700 g, was treated with a probiotic with a higher concentration of 2 ml/kg food, with a concentration of $1.5^{*}10^{8}$ CFU/ml (Colony forming Units/ml). The control group "C", with an average body mass of 172.1 g and a total ichthyomas of 55 600 g, was fed with commercial feed, without probiotic addition. The fish in all three groups were fed for 24 hours with automatic feeders, and the diet was according to a table (percentage of body mass and water temperature), recommended and defined by the food manufacturer. Control measurements were performed on equal numbers of carp individuals for a certain period (11 to 16 days). The experiment was conducted in the first phase (juvenile rearing) and the second phase (commercial phase).

In the first phase, five consecutive control measurements of fish were carried out at 14 to 16 days. The control measurements were performed to calculate the FCR and assess the welfare status of the fish.

In the second phase, four control measurements of the total ichthyomass in each cage of the groups were performed, based on which the FCR was calculated.

Among production characteristics such as growth rate (GR), specific growth rate and individual growth rate, during control measurements in the first phase, the following parameters were also determined:

Control measurements

According to the principle of random selection, 30 individuals from each experimental group were selected and measured during the first phase.

The following measurements were taken:

a) W – fish mass (g);

b) L – body length from the mouth to the end of the tail (cm);

c) Ls – standard body length from the mouth to the caudal fin notch (cm);

d) fl – body length from the mouth to the root of the tail (cm);

e) h – body height, which is measured from the abdominal fin to the beginning of the dorsal fin (cm);

f) total mass of fish per group, conducted in four measurements during the second phase (g).

Feed conversion ratio (FCR). The feed conversion ratio presents the amount of feed per kilogram consumed for one kilogram of fish mass and was determined according to the formula: (FCR) = (feed consumed (g))/ (fish growth (g));

Fish condition– Fulton's condition factor – calculated by determining the condition factor (Fulton, 1904) according to the formula:

$$F = W(g) / L(cm) \times 100,$$

where W is the mass of the fish in grams, and L is the length of the fish in centimetres.

Parameters for welfare assessment included health parameters and were recorded as all changes in the fish body, monitored during the observations early in the morning (7 am) before feeding. Each observation was performed by three persons on equal numbers of carp individuals. In particular, body injuries in fish caused by erythrodermatitis and changes caused by mechanical injuries were determined. The percentage of injured fish (or percentage of sick ones), as well as the percentage of recovered individuals, was determined. The rate of survival and the percentage of mortality in each of the rearing stages were also recorded.

1. Health parameters (skin injuries, fin injuries, survival rate)

2. Percentage of survival (%) = (number of dead individuals/ total number of individuals) *100

3. Percentage of injured fish (%) = (number of injured fish/ total number of individuals) *100

During the experiment, with seasonal dynamics, the basic physicochemical parameters important for determining the welfare of the fish were monitored. The temperature was measured daily at a depth of one and two meters with digital thermometers placed in the water and the cages where the experiment was performed. Dissolved oxygen and pH of the water were measured on the days when control catches were made. Oxygen was measured with a YSI DO200 mobile oximeter, and water pH was measured with a YSI pH100 mobile pH meter from China. The concentration of ammonia in the water and other physicochemical parameters were measured spectrophotometrically in the UKIM Institute of Animal Sciences and Fishery laboratory. A spectrophotometer "Novaspec II" and kits for water analysis "Palintest – Novaspec II – Water Analysis System" and "Amersham Pharmacia Biotech AB" from Sweden were used. From the physical parameters, in addition to temperature, the following were investigated: transparency of water (cm Secchi disc), visible and measured colour (Pt-Co) and smell of water. From the chemical parameters, in addition to oxygen and electrochemical reaction, the following were monitored: free CO_2 (mg/l), alkalinity (mg/l $CaCO_3$), 5 days biological oxygen demand (BOD_5), chemical oxygen demand, such as $KMnO_4$, suspended substances (mg/l), dry evaporated residue at 105°C (mg/l), ammonium (mg/l), nitrites (mg/l), nitrates (mg/l), phosphates (mg/l PO_4) and dissolved carbon dioxide CO_2 (mg/l).

The obtained data and results of the first phase of the research were processed and analysed through ANOVA. The descriptive statistical presentation of the results was performed through the measures of dispersion (minimum and maximum values), standard deviation (SD) and coefficient of variance (CV). For the determination of statistically significant differences ($p<0.05$), a subsequent Tukey test (post-hoc analysis) was performed. The measured values of tendency and dispersion of the fish mass for all three experimental groups, A, B and C, were determined with descriptive statistical analysis.

Results

The physicochemical characteristics of Kozjak reservoir water are presented in Table 1.

The average temperature of the water from the "Kozjak" reservoir, measured in the cages (at 2.0-

Table 1. Basic physicochemical characteristic of "Kozjak" reservoir water.

Parameter	April	May	June	September
Colour	None	None	None	None
Smell	None	None	None	None
Transparency (cm)	280	650	720	810
Conductivity [uS/cm]	368	305	315	299
Alcality [mg/l $CaCO_3$]	230	205	201	143
Dissolved oxygen [mg O_2/l]	10.8	8.9	8.4	7.8
Dissolved CO_2 [mg O_2/l]	1.6	2.1	2.3	1.8
BOD_5 [mg O_2/l]	3.50	1.28	1.43	2.76
COD $KMnO_4$ [mg O_2/l]	1.29	1.63	1.69	1.41
NH_4 [mg N/l]	0.0016	0.0200	0.0300	0.0048
NO_2 [mg N/l]	0.0055	0.0070	0.0060	0.0070
NO_3 [mg N/l]	0.6826	0.2100	0.2800	0.0070
Phosphates PO_4 [mg PO_4/l]	0.0300	0.0170	0.0150	0.0160

Table 2. Descriptive statistics of central tendency values and dispersion of fish weight (g) of 30 individuals of groups A, B and C from I-Vth control measurement.

Group	measurement	min	max	$\bar{x}$	variance (n-1)	(SD) (n-1)	CV
A	I	111	263	174.2	1372.7	37.05	0.2091
	II	159	383	222.67	1947.5	44.13	0.1949
	III	198	425	289.6	4140.4	64.346	0.2185
	IV	295	578	408.57	7234.7	85.057	0.2047
	V	309	727	483.47	9872.9	99.363	0.2021
B	I	132	240	179.13	979.22	31.293	0.1718
	II	122	273	197.7	1728.6	41.576	0.2068
	III	186	322	266.53	1514.7	38.92	0.1436
	IV	242	518	372.3	4533.5	67.331	0.1778
	V	312	662	500.0	7733.9	87.942	0.1729
C	I	134	306	194.57	1355.5	36.817	0.186
	II	155	322	219.37	1490	38.601	0.173
	III	165	341	254.43	2242.7	47.358	0.183
	IV	216	466	335.33	3369.6	58.048	0.1702
	V	280	541	370.77	3481.8	59.007	0.1565

Table 3. Variance analysis (ANOVA) of weight (g) mean values of 30 individuals of groups A, B and C from I-Vth control measurement in the first experimental phase.

Group	I measurement	II measurement	III measurement	IV measurement	V measurement
	$\bar{x}$(SD)	$\bar{x}$(SD)	$\bar{x}$(SD)	$\bar{x}$(SD)	$\bar{x}$(SD)
A	174.20 (37.05)^{a}	222.67 (44.13)^{a}	289.60 (64.35)^{a}	408.57 (85.06)^{a}	483.47 (99.36)^{a}
B	179.13 (31.29)^{a}	197.70 (41.58)^{a}	266.53 (38.92)^{ab}	372.30 (67.33)^{ab}	500.00 (87.94)^{a}
C	194.57 (36.82)^{a}	219.37 (38.60)^{a}	254.43 (47.36)^{b}	335.33 (58.05)^{b}	370.77 (59.01)^{b}

*Different superscript letters in one column indicate statistically significant values ($p<0.05$).

and 0.5-meters depth), during the period from April to September, increased from 15.1° C in April to 26.5° C in July and August. The oxygen regime presented the lowest values of dissolved oxygen concentrations in July and August (7.9 and 7.3 mg/l O_2) and the highest values in April (10.8 mg/l O_2).

Descriptive statistics, in which the values of the central tendency (minimum and maximum value and mean value), as well as the dispersion values (variance, standard deviation and coefficient of variation), are presented, which refers to the mass of 30 randomly sampled individuals from each group A, B and C. Statistical results of fish weight from the first phase, during five consecutive measurements and the fifth measurement, are presented in Table 2.

Table 3 presents a variance analysis of the fish mass (g) of 30 individuals sampled from all three groups during the five control measurements in the first phase of the experiment.

The variance analysis indicated that the fish mass from the experimental groups from the first and second control measurements did not differ significantly. On the third and fourth measurements, the higher mass (g) of fish from group A differed from the lower fish mass values of group C with statistical significance ($p<0.05$). In the third and fourth measurements, the mass of group B fish does not present a statistically significant difference from group A and control group C. As for the fifth measurement, univariate ANOVA demonstrated that the lower mass of the control group C differed significantly from the larger mass values of experimental groups A and B.

A descriptive analysis of the condition factor (F) values was performed on the control measures. Table 4 represents the descriptive analysis of the Fulton factor values for groups A, B, and C.

In Table 4, the descriptive analysis showed that during the fourth control measurement, there was a difference in the Fulton factor values between all three groups. In contrast, in the last measurement, experimental groups A and B had mean values of 1.89 and 1.86, respectively, compared to control group C, whose mean value was 1.49. Analysis of variance (ANOVA) of the Fulton factor values for 30 carp individuals from all three groups within the first phase is presented in Table 5.

ANOVA of Fulton factor values showed that there was no statistically significant difference between experimental groups A, B, and C between the first and third control measurements. During the fourth control, the Fulton factor values were statistically significantly different ($p<0.05$) between all three groups. The variance analysis of the last measurement showed that the lower value of the Fulton factor of the control group C differed significantly from the experimental groups A and B. In contrast, the groups did not show a statistically significant difference.

The results of FCR values are presented in Table 6. The feed conversion ratio during the second control in experimental groups A and B was already 1.4 and 1.9, respectively, significantly decreased compared to control C, which was 2.6.

According to Table 6, a lower FCR can be observed only in fish from experimental group A at the first observation of the second phase. The decreasing trendline of FCR in experimental groups A and B was also observed in the seventh and eighth control, with values of 1.4 and 1.2 and 1.5 and 1.4, respectively. In fish from the control group C, the FCR in the seventh and eighth control was 1.7.

Given that in our state, there is no protocol for defining parameters for assessing the welfare of carp reared in cages, an attempt was made to design and apply one during the experiment. For this purpose, the so-called a “five-level” Likert scale (Bracke et al. 1999, Vagias 2006) was used to rank welfare status according to the resulting score. The possible ranges of values for a particular parameter were classified. Five categories (from 1 to 5) were introduced for production and health parameters, while six categories (from 0 to 5) were introduced for water quality parameters. In principle, 0 points should be given for the most undesirable water values. If the measured value of any parameter is optimal, 5 points are given. The total score was divided by the number of parameters, and according to the value, the obtained welfare status was quantified from 1 to 5. It was described as unacceptable (NA< 1.80), acceptable (A, 1.81 – 2.60), good (G, 2.61 – 3.40), very good (VG, 3.41 – 4.20) or excellent (E, 4.21 – 5.00).

Table 7 presents the results of the fish observations performed at the end of the rearing season. On the last control, 318, 320, and 298 individuals were examined from groups A, B, and C, respectively. The number of healed individuals and individuals with active body changes were evaluated.

At the end of the rearing period, 29% of Group A’s individuals were healed, 37% in Group B, and 1.7% in the control group. At the end of the experiment, no new or active body changes caused by erythrodermatitis or mechanical injuries were detected in individuals.

Table 9 presents the range values of the surveyed parameters during the experiment. Range values refer to water temperature, dissolved oxygen, pH, NH_3-N (mg/L), Fulton’s condition factor,

Table 4. Descriptive analysis of measured values of central tendency and dispersion of Fulton factor values, for all three experimental groups (A, B and C) during I-V[th] control measurement in the first experimental phase.

Group	Measurement	Min	Max	$\bar{x}$	Variance (n-1)	(SD) (n-1)	CV
A	I	1.18	1.65	1.37	0.01	0.10	0.07
	II	1.29	1.86	1.59	0.02	0.15	0.09
	III	1.57	2.27	1.82	0.03	0.17	0.09
	IV	1.72	2.35	1.98	0.03	0.17	0.08
	V	1.63	2.22	1.89	0.03	0.16	0.09
B	I	1.16	1.70	1.42	0.02	0.13	0.09
	II	1.26	2.49	1.58	0.08	0.27	0.17
	III	1.50	2.02	1.75	0.02	0.14	0.08
	IV	1.68	2.09	1.87	0.01	0.11	0.06
	V	1.39	2.20	1.86	0.03	0.18	0.10
C	I	1.12	1.58	1.41	0.01	0.10	0.07
	II	1.37	2.09	1.66	0.02	0.15	0.09
	III	1.44	2.10	1.78	0.03	0.16	0.09
	IV	1.51	1.93	1.72	0.01	0.10	0.06
	V	1.29	1.72	1.49	0.01	0.11	0.07

Table 5. Variance analysis (ANOVA) of Fulton factor values of groups A, B and C of I-V[th] measurement in the first experimental phase.

Group	I measurement	II measurement	III measurement	IV measurement	V measurement
A	1.36 (0.01)[a]	1.58 (0.15)[a]	1.82 (0.17)[a]	1.97 (0.17)[a]	1.89 (0.16)[a]
B	1.42 (0.13)[a]	1.58 (0.27)[a]	1.75 (0.14)[a]	1.87 (0.11)[b]	1.85 (0.18)[a]
C	1.41 (0.10)[a]	1.65 (0.15)[a]	1.77 (0.16)[a]	1.71 (0.10)[c]	1.48 (0.11)[b]

*Different superscript letters in one column indicate statistically significant values ($p<0.05$).

Table 6. The feed conversion ratio for A, B and C groups of controls from the first and second phases.

First phase					
Group	**I control**	**II control**	**III control**	**IV control**	**average**
A	1.7	1.4	1.4	1.7	1.6
B	1.9	1.9	1.6	1.9	1.9
C	1.8	2.6	2.1	2.6	2.3
Second phase					
Group	**V control**	**VI control**	**VII control**	**VIII control**	**average**
A	1.5	1.5	1.4	1.2	1.4
B	1.7	1.6	1.5	1.4	1.5
C	1.7	1.9	1.7	1.7	1.7

FCR, and the percentage of the fish survival rate and illness/injuries, according to the scale of the parameters' values, which are scored from 1 to 5.

The welfare status assessment at the end of the experiment indicated that all groups had "excellent" status. Carp individuals that received feed supplemented with probiotics at a concentration of 2 ml/kg proved to be slightly better at maintaining fish welfare than feed supplemented with 1 ml/kg and, by far, had a better effect on commercial food without added probiotics.

The best ranking of the welfare status was found in group B, according to Table 10. After the end of the experiment, this group's welfare status was described as "excellent", with the highest rating of 4.88. Groups A and C also had an "excel-

Table 7. Quantification of welfare status.

Mark	Welfare status description	International mark
1 (<1,80)	Not acceptable	Not Acceptable (NA)
2 (1.81 – 2,60)	Acceptable	Acceptable (A)
3 (2.61 – 3,40)	Good	Good (G)
4 (3.41 – 4,20)	Very Good	Very Good (VG)
5 (4.21 – 5.00)	Excellent	Excellent (E)

Table 8. Number and percentage of healed and active changes in fish at the end of the experiment.

Group	Number of examined individuals	Number of healed individuals	Percentage of healed individuals (%)	Number of individuals with active changes	Percentage of individuals with active changes (%)
A	318	93	29	0	0
B	320	119	37	0	0
C	298	5	1.7	0	0

Table 9. Range values of the parameters for welfare status grading.

Parameter	Range values	Points
Temperature (°C)	22.00-25.99	5
	19.00-21.99	4
	17.00-18.99	3
	15.00-16.99 or >27	2
	13.00-14.99 or >29	1
	<13.00 or >30	0
DO (mg/L)	>= 8.00	5
	7.00-7.99	4
	6.00-6.99	3
	5.00-5.99	2
	4.00-4.99	1
	<4.00	0
pH	6.8 – 7.3	5
	6 – 6.8 or 7.3 – 8	4
	5.6 – 6.7 or 8 – 8.5	3
	4.5-5.5 or 8.5 - 8.7	2
	4-4.5 or 8.7-9	1
	< 4 и >9	0
NH_3-N (mg/L)	<0,05	5
	0.05-0.10	4
	0.11-0.15	3
	0.16-0.20	2
	0.20-0.25	1
	>0.25	0

Parameter	Range values	Points
Fulton's condition factor	>= 1.80	5
	1.60-1.79	4
	1.40-1.59	3
	1.20-1.39	2
	<1.20	1
Feed conversion ratio	<1.40	5
	1.40-1.59	4
	1.60-1.79	3
	1.80-1.99	2
	>=2.00	1
% survival rate	> 99.00	5
	95.00 – 99.00	4
	90.00 – 94.99	3
	80.00 – 89.99	2
	< 80.00	1
% illness/ injuries	< 5	5
	5 – 9.99	4
	10 – 19.99	3
	20 – 29.99	2
	≥ 30	1

Table 10. Welfare status quantification by parameters per group at the end of the experiment.

Parameter	Points	Measured values			Grade		
		A	B	C	A	B	C
Temperature ^{0}C	5	21.5	21.5	21.5	5	5	5
DO (mg/L)	5	8.4	8.4	8.4	5	5	5
pH	5	7.4	7.4	7.4	5	5	5
NH_3-N (mg/L)	5	0.0048	0.0048	0.0048	5	5	5
Fulton's condition factor	5	1.91	1.92		5	5	
	3			1.54			3
Feed conversion ratio	4	1.42	1.51		4	4	
	3			1.72			3
% survival rate	5		99			5	
	4	98			4		
	3			92			3
% illness/injuries	5	0	0	0	5	5	5
Welfare status quantification					4.77	4.88	4.33
Description of welfare status					E	E	E

lent" status, with values of 4.77 and 4.33, respectively. The percentage of injured fish demonstrates that individuals were handled carefully during the experiment. The scores for the parameters presented in the table, Fulton's condition factor values and FCR indicated satisfactory production results, except for those of the control group. The fish in all three groups had a 100% survival rate. Probiotic addition, good environmental conditions, good water quality and well-implemented ichthyotechnical measures at the facility led to excellent fish welfare.

Discussion

Motivated by the knowledge about the positive effect of probiotics on production performance and fish welfare, this experiment aimed to test the impact of the probiotic *Paenibacillus alvei* applied in the technological process. Simultaneously, by achieving increased fish growth with reduced food consumption (resulting in a decreased FCR), it is possible to obtain a higher yield of fish per fishpond. This leads to reduced costs associated with the cultivation and production technology, which is a crucial factor for economic profitability for fish producers.

In the experiment's frame, the best FCR values were achieved in experimental group A. Regardless of fish species and developmental stage, research data showed a positive effect of the application of probiotics in the diet, ensuring a high percentage of survival, reducing the loss of juveniles (during critical stages of their growth), increasing growth rate, decreasing FCR, and of course, of great importance is the achievement of significant economic effects (Dawood et al. 2015, Munir et al. 2016, Nathanailides C. et al. 2021). The positive influence of adding the probiotic to the carp diet was determined during the experiment, both on fish welfare and FCR. Increased fish body mass, one of the basic parameters that quantifies the effect of the applied probiotic in the diet, was the first indicator, showing a positive and continuous increase throughout the experimental period. The different concentrations of probiotics used in experimental groups A and B do not seem to contribute to the difference in their weight, as the results of body mass evaluation between groups A and B presented no statistically significant difference.

Dietary Primalac® probiotic included in common carp and Persian sturgeon fry feed resulted in significantly improved body weight and survival rate compared to the control group (Salaghi et al. 2013). The results obtained regarding efficiency in food utilisation showed the same trend as in Renuka et al. (2013) and Munir et al. (2016). They noted an improvement in feed and protein utilisation, reflecting an increase in growth due to higher protein and energy deposits in the tissues of the fish. Khalil et al. (2012) and Totewad et al. (2021) reported an increase in total growth rate, food intake and utilisation of nutrients, as well as in the parameters of the chemical composition of the fish body and reduced mortality.

Fulton coefficient parameters can be influenced by various factors, including season, sex, differences among the samples, population density, quality and quantity of food, health status of fish, and environmental conditions (Lemma et al. 2015, Opiyo M.A. et al. 2019, Mihai C. et al. 2023). In the first phase, the analysis of variance revealed a significant difference between the control group C and the experimental groups A and B in terms of the F coefficient value. The obtained results correspond with the research of Asadian et al. (2015) on experimental individual carp treated with a combined commercial probiotic. Significant positive effects were recorded in weight, length, growth, and Fulton factor, which was increased by 58% on day 45 of the experiment.

The fish in all three groups presented a 100% survival rate, and at the last control of the experiment, the fish had already recovered. All three groups had no individuals with new body changes caused by erythrodermatitis or mechanical injuries. According to the welfare indicators, group B had the highest grade when evaluating the three groups based on welfare status, although all groups were rated “excellent”. Opiyo et al. (2019) reported that the decreased mortality rate indicates improved health status and immunity in fish fed with probiotic-enriched feed. It was reported that probiotics positively influenced the immune system, disease resistance, and both. As a current strategy for a bio-control method in intensive aquaculture, probiotic application is believed to have a positive influence on water quality (Sandeva et al. 2016), fish health modulation, modification of the microbial community about the aquatic environment, and promotion of a non-specific immune response and resistance against pathogens, resulting in an improvement of production characteristics and nutrient utilisation (Rajikkannu et al. 2015, Mohammad et al. 2016, Van Doan et al. 2018). Feed supplemented with probiotics at a concentration of 2 ml/kg presented better results for fish welfare than feed supplemented with 1 ml/kg feed in terms of the number of new lesions in the fish body, but also in the healing process, as well as the prolonged effect in terms of protecting the fish from the reappearance of new lesions on the body. This experiment provided an initial framework to help conduct further studies on the welfare assessment of carp cultures in the Republic of North Macedonia, providing an indication of the positive influence of probiotic application in the carp diet within a cage system in a reservoir, improving health condition, production performance characteristic (FCR) and welfare.

References

Akhter N., Wu B., Memon AM. & Mohsin M. 2015. Probiotics and prebiotics associated with aquaculture: A Review. Fish and Shellfish Immunology 45(2): 733-741.

Allameh S.K., Noaman V. & Nahavandi R. 2017. Effects of probiotic bacteria on fish performance. Advanced Techniques in Clinical Microbiology 1(2): 11.

Asadian M., Shahsavani D. & Kazerani H.R. 2015. Growth promoting effects of a multi-strain probiotic on common carp (*Cyprinus carpio*) fingerlings. Iranian Journal of Veterinary Science and Technology 7(2): 63-74.

Ashley P.J. 2007. Fish welfare: current issues in aquaculture. Applied Animal Behavioral Science 104: 199-235.

Banerjee G. & Ray A.K. 2017. The advancement of probiotics research and its application in fish farming industries. Research in Veterinary Science 115: 66-77.

Bracke M., Metz M. & Spruijt B.M. 1999. Overall animal welfare reviewed. Part 2: Assessment tables and schemes. Netherlands Journal of Agricultural Science 47: 293-305.

Broom D.M. 1996. Animal welfare defined in term of attempts to cope with environment. Acta Agriculturae Scandinavica, Section Animal Science Suppl. 27: 22-28.

Broom D.M. 2007. Welfare in relation to feelings, stress and health. Revista electronica de Veterinaria 8: 1697-704.

Carnevali O., Sun Y.Z., Merrifield D.L., Zhou Z. & Picchietti S. 2014. Probiotic application in temperate and warm water fish species. In: Merrifield D.L.& Ringø E. (Eds.): Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics. Oxford, UK: Wiley-Blackwell Publishing, 253-289.

Chen Y., Zhu X., Yang Y., Han D., Jin J. & Xie S. 2014. Effects of dietary chitosan on growth performance, haematology, immune response, intestine morphology, intestine microbiota and disease resistance of gibel carp *Carassius auratus gibelio.* Aquatic Nutrition 20: 532-546.

Dawood M.A.O. & Koshio S. 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A Review. Aquaculture 454: 243-251.

De B.C., Meena D.K., Behera B.K., Das P., Das Mohapatra P.K. & Sharma A.P. 2014. Probiotics in fish and shellfish culture: Immunomodulatory and ecophysiological responses. Fish Physiology and Biochemistry 40: 921-971.

Duncan I.J. 2005. Science-based assessment of animal welfare: farm animals. Review Science Technology 24: 483-492.

Faturrahman Rohyati I.S. & Sukiman D. 2015. Improved of growth rate of abalone (*Haliotis asinine)* fed pudding probiotic-enriched protein. Procedia Environmental Sciences 23: 315-322.

Hossain M.I., Sadekuzzaman M. & Ha S.D. 2017. Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. Food Research International 100: 63-73.

Hoseinifar S.H., Sun Y.Z., Wang A. & Zhou Z. 2018**.** Probiotics as Means of Diseases Control in Aquaculture, A Review of Current Knowledge and Future perspectives. Frontiers in Microbiology 9: 2429.

Hussein M.S., Zaghlol A., El Hakim N.F.A. & El Nawsany M. 2016. Effect of Different Growth Promoters on Growth Performance, Feed Utilization and Body Composition of Common Carp (*Cyprinus carpio*). Journal of Fisheries and Aquatic Sciences 11: 370-377.

Huynh T.G., Shiu Y.L., Nguyen T.P., Truong Q.P., Chen J.

C. & Liu C.H. 2017. Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. Fish Shellfish Immunology 64: 367-382.

Khalil F.F., Mehrim A.I. & Hassan M.E.M. 2012. Effect of Hydroyeast Aquaculture® as a growth promoter for adult Nile tilapia (*Oreochromis niloticus)*. Journal of Animal and Poultry Production 3(6): 305-317.

Lazado C.C. & Caipang C.M. 2014a. Bacterial viability differentially influences the immunomodulatory capabilities of potential host-derived probiotics in the intestinal epithelial cells of Atlantic cod Gadus morhua. Journal of Applied Microbiology 116(4): 990-998.

Lazado C.C. & Caipang C.M.A. 2014b. Mucosal immunity and probiotics in fish. Fish Shellfish Immunology 39: 78-89.

Lauzon H.L., Pérez-Sánchez T., Merrifield D.L., Ringø E. & Balcázar J.L. 2014. Probiotic application in cold water fish species. In: Merrifield, D.L. & Ringø E. (Eds.): Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics. Oxford, UK: Wiley-Blackwell Publishing, 223-252.

Lemma A., Getahun A. & Lemma B. 2015. Assessment of length-weight relationship, sex ratio and condition factor of common carp (*Cyprinus carpio* Linnaeus,1758) in Lake Ziway, Ethiopia. Global Journal of Fisheries and Aquaculture 3(1): 192-197.

Mehrabi F., Khalesi M. & Hazaie K. 2018. Effects of pre-and probiotics on growth, survival, body composition, and haematology of common carp (*Cyprinus carpio* L.) fry from the Caspian Sea. Turkish Journal of Fisheries and Aquatic Sciences 18(4): 597-602.

Merriefield D.L., Dimitroglou A., Foey A., Davies S.J., Baker R.M.T., Bogwald J., Castex M. & Ringo E. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302: 1-18.

MohammadJalil Z., SomayehTorabi D., Milad A., Ruchi T., Karthik K.D. & Carlo C.L. 2016. Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: A Review. Veterinary Quarterly 36(4): 228-241.

Mohapatra S., Chakraborty T., Kumar V., De Boeck G. & Mohanta K.N. 2013. Aquaculture and stress management: A review of probiotic intervention. Journal of Animal Physiology. Animal Nutrititon 97: 405-430.

Mihai C., Dumitrache C., Teodorescu I.R., Pomonaci M.C., Hassan A.A.M., Nicolae G.C., Diguta F.C. & Matei F. 2023. Evaluation of the probiotic supplement Saccharomyces cerevisiae BB06 as a beneficial growth promoter for carp (*Cyprinus carpio*) in recirculating aquaculture. Scientific Papers. Series D. Animal Science 2: 66.

Munir M.B., Hashim R., Chai Y.H., Marsh T.L. & Nor S.A.M. 2016. Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striata*) fingerlings. Aquaculture 460: 59-68.

Nathanailides C., Kolygas M., Choremi K., Mavraganis T., Gouva E., Vidalis K. & Athanassopoulou F. 2021. Probiotics Have the Potential to Significantly Mitigate the Environmental Impact of Freshwater Fish Farms. *Fishes* 6(4): 76.

Nathanailides C. 2024. Probiotics for Freshwater Fish Farming. Encyclopedia. Available online: https://encyclopedia.pub/entry/17299 (accessed on 09 February 2024).

Opiyo M.A., Jumbe J., Ngugi C.C. & Charo-Karisa H. 2019. Different levels of probiotics affect growth, survival and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. Scientific African 4, p.e. 00103.

Rajikkannu M., Natarajan N., Santhanam P., Deivasigamani B., Ilamathi J. & Janani S. 2015. Effect of probiotics on the haematological parameters of Indian major carp (*Labeo rohita*). International Journal of Fisheries and Aquatic Studies 2(5): 105-109.

Renuka K.P.M., Venkatershwarlu A. T. R. & Kumara S.M.P. 2013. Influence of probiotics on growth performance and digestive enzyme activity of common carp *(Cyprinus carpio)*. International Journal of Current Research (5): 1696-1700.

Salaghi Z., Imanpuor M. & Taghizadeh V. 2013. Effect of different levels of probiotic Primalac on growth performance and survival rate of Persian sturgeon (*Acipenser persicus*). Global Veterinaria 11: 238-242.

Sandeva G., Atanasoff A., Nikolov G. & Zapryanova D. 2016. A preliminary study on some chemical parameters of water from probiotic-treated common carp (*Cyprinus carpio* L.) enclosures. Conference paper. 2nd International Congress on Applied Ichthyology & Aquatic Environment 10-12 November 2016, Messolonghi, Greece.

Suguna T. 2020. Role of Probiotics in Aquaculture. International Journal of Current Microbiology Applied Science 9(10): 143-149.

Totewad N.D. & Gyananath, G. 2021. Effect of Probiotic *Enterococcus Gallinarum* N3 Supplemented Feed on Growth Performance of Freshwater Fish *Cyprinus Carpio.* Indian Journal of Animal Research 18: 100-102.

Vagias W.M. 2006. Likert-type scale response anchors. Clemson International Institute for Tourism & Research Development, Department of Parks, Recreation and Tourism Management. Clemson University 4(5).

Van Doan H. Hoseinifar S. H., Khanongnuch, C., Kanpiengjai, A., Unban, K. & Srichaiyo S. 2018. Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 491: 94-100.