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INFLUENCE OF PROBIOTIC DIETS ON THE GROWTH AND WELL-BEING OF *Acipenser baerii* SPECIES IN A RECIRCULATING AQUACULTURE SYSTEM

Viorica SAVIN[1, 2], Magdalena TENCIU[1], Floricel Maricel DIMA[1], Neculai PATRICHE[1], Marilena Florentina LACATUS[1], Elena Ioana COMAN[1], Victor CRISTEA[2]

[1]Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, 54 Portului Street, Galați, 800211, Romania
[2]"Dunărea de Jos", University of Galați, Food Science and Engineering Faculty, 47 Domnească Street, Galați, 800008, Romania

Corresponding author emails: vio_savin@yahoo.com, magdatenciu@yahoo.com

Abstract

In this study, the impact of a commercial probiotic (PC) on the growth and well-being of siberian sturgeon (Acipenser baerii, Brandt, 1869) in a recirculating aquaculture system was investigated over 63 days. Four experimental groups were created: a control group (A1) received a commercial feed with 45% protein and 15% lipid, while three others (A2, A3, A4) had the commercial feed supplemented with 0.2 g PC kg^{-1}, 0.4 g PC kg^{-1}, and 0.6 g PC kg^{-1}, respectively. Biometric measurements and blood analyses were conducted to assess growth and well-being of the biological material. Probiotic addition improved the growth performance in all experimental groups compared to the control. Erythrocytes, hemoglobin, and leukocytes showed higher mean values in the experimental groups. Serum glucose significantly decreased in the experimental groups ($p<0.05$), while total serum proteins increased with probiotic diets. Protein and lipid accumulation in muscle tissue was observed in all groups fed probiotic diets, indicating a positive influence on the growth and well-being of Acipenser baerii in recirculating aquaculture systems.

Key words*: aquaculture, growth, sturgeon, well-being.*

INTRODUCTION

Sturgeons are valued in aquaculture due to their high-quality meat and caviar production (Fontagné et al., 2006; Zarantoniello et al., 2021).
The Siberian sturgeon (*Acipenser baerii*) is a species with good adaptability to different growing conditions, has a shorter reproduction cycle compared to other sturgeons and is quite resistant to the stress factors that appear in the culture environment. These characteristics make it a good candidate for aquaculture (Bronzi et al., 2011; Dettlaff et al., 2012; Kolman et al., 2018).
Various sturgeon rearing systems are used in aquaculture. In 2016 estimates showed that 21% of all rearing systems were recirculating aquaculture systems (EUMOFA, 2020).
The disadvantage of these rearing systems, however, is that they can favor the occurrence and spread of certain fish diseases. The fact that fish are concentrated in relatively small spaces increases the risk of disease transmission between individuals high (Cristea et al., 2002). Recirculating aquaculture systems face challenges such as oxidative stress, digestive disorders and increased risk of bacterial or fungal infections.
The introduction of probiotics into the feed of fish reared in recirculating aquaculture systems is an important research aspect in the field of sustainable fish rearing in captivity and a promising strategy for improving growth performance and fish health. This is considering the benefits that these beneficial microorganisms can bring to the fish's digestive system (Verschuere et al., 2000; Ringø et al., 2006).
Probiotics, through their ability to colonize and modulate intestinal flora (Nayak, 2010), can help reduce these problems. They can secrete antimicrobial substances and stimulate the fish's immune system, making them more resistant to diseases (Li & Gu, 2018).
In addition to the health benefits of fish, introducing probiotics into their diet can also help improve feed efficiency (Hoseinifar et al., 2018; Neepa et al., 2022) and reduce negative

environmental impacts. By optimizing the digestion and absorption of nutrients, the amount of nutrients removed into the water from the recirculation system can be reduced, thereby reducing the risk of pollution and reducing the costs associated with water treatment.

Also, the application of probiotics in aquaculture can provide an alternative to the utilisation of antibiotics in disease treatment and prevention, thus contributing to the reduction of bacterial resistance and promoting a more sustainable and ecological practice in fish farming (Mondal et al., 2022).

In this context, this work aims to investigate the effects of supplementing the diet of the Siberian sturgeon (*Acipenser baerii*), reared in a recirculating aquaculture system, with a mix of probiotics (Probiotic Complex - PC), focusing on fish growth, welfare, and quality.

MATERIALS AND METHODS

The experiment was conducted at the Institute of Research-Development for Aquatic Ecology, Fisheries, and Aquaculture Galati, over a period of 63 days, aiming to evaluate the effects of a commercial probiotic (PC) composed of a mixture of bacteria (*Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus casei*) as a supplement in the diet of Siberian sturgeon (*Acipenser baerii*). Accordingly, 120 specimens of Siberian sturgeon, with an average weight of 5.18±0.44 g, obtained through artificial reproduction at the Galati Institute, were randomly distributed into 4 growth units (A1-A4) of a recirculating aquarium system, as described by Savin et al. (2023). Water quality parameters were maintained at optimal levels using Tetra EX 1200 Plus external filters, supplemented with aeration stones. The water volume used was 125 liters, and it was refreshed daily at a rate of 50%. Temperature and dissolved oxygen were monitored daily using the portable analyser HQ40d by Hach Lange, while nitrogen compounds were measured weekly using the portable spectrophotometer DR1900 by Hach Lange and the LCK kits from Hach Lange.

Fish were manually fed twice a day with a basic feed, Aller Bronze type, with a granulation of 2 mm (Dynavit Impex, Brasov), containing 45% protein and 15% lipids. Three experimental variants and one control were tested, in which different amounts of probiotic were added to the basic feed; A1 (0 g PC), A2 (0.2 g PC / 5 x 10^9 CFU), A3 (0.4 g PC / 10 x 10^9 CFU), and A4 (0.6 g PC / 15 x 10^9 CFU). The powdered probiotic was dissolved in distilled water, mixed with a 2% gelatine solution, sprayed onto the feed, and dried at room temperature.

For assessing the efficiency of the probiotics used on growth performance, biometric measurements of the fish were conducted at the beginning and end of the experiment, calculating parameters according to established formulas:

Weight gain (WG) = final weight – initial weight (g)

Daily growth rate (DGR) = (final weight – initial weight) / day (g/day)

Specific growth rate (SGR) =100 x [ln (final weight) – ln (initial weight)] / days (%/day)

Feed conversion ratio (FCR) = feed intake / weight gain (g/g)

Protein efficiency ratio (PER) = weight gain / feed intake x crude protein of the feed

Blood samples were collected from the caudal vein at the end of the experiment. Prior to sampling, the fish were anesthetized by immersion in a solution composed of 2.5 ml of clove oil per 10 liters of water. For haematological analyses, blood was collected in heparinized Eppendorf tubes, while non-heparinized tubes were used for obtaining serum, which were left at room temperature for 30 minutes and then centrifuged at 4000 rpm for 5 minutes.

Red and white blood cells (RBC and WBC) were counted using the Neubauer counting chamber and the Potain pipette, employing Vulpian and Turk solutions as dilution liquids, respectively. The Kern OBN135 microscope, equipped with the Kern ODC 832 camera, was utilized for this purpose (Baker & Silverton, 2014).

The haematocrit (Ht) was determined by introducing blood into heparinized microcapillaries sealed with wax at one end, which were then centrifuged for 2 minutes at 10,000 rpm. Following centrifugation the haematocrit percentage was read directly on the haematocrit counter (Davison et al., 2023).

Haemoglobin (Hb) was determined using the cyanmethemoglobin method, in which the Drabkin reagent lyses the blood, forming a solution of cyanmethemoglobin. The absorbance was read on the UV-VIS spectrophotometer SPEKOL 1300 Analytikjena, at a wavelength of 540 nm (Kondi, 1981).
Serum glucose was measured using the Ortho toluidine method, with absorbance read on a spectrophotometer at 660 nm, while total serum proteins were determined using the biuret method (Kondi, 1981).
The total protein content was quantified using the Kjeldahl method, employing a Gerhardt-type system. Moisture content was measured by drying the sample in an oven at 130°C until a constant mass was achieved. Lipid content was determined using the Soxhlet method, which involves extracting fats with petroleum ether as a solvent, using a VELP-type extraction system. Ash content was estimated by calcining the sample in an oven at 600°C, followed by weighing the resulting residue.

The statistical analysis of the data was conducted using Excel 2019 and SPSS Statistics 17.0 software. To compare more than two groups, a single-factor ANOVA was employed, while a paired t-test was used for comparing two groups. Results were considered statistically different at $P < 0.05$.

RESULTS AND DISCUSSIONS

The addition of probiotics to the diet of the Siberian sturgeon did not significantly influence the water quality parameters (Table 1). Thus, the average dissolved oxygen values were within normal limits for fish growth (6.93-7.32 mg/l), with no significant differences between treatments ($p = 0.14$). The average temperature ranged between 21.05°C (A4) and 21.18°C (A1). Also, the values of nitrogen compounds showed non-significant differences between the experimental treatments, ranging from 0.09-0.1 mg/l (nitrites), and 4.46-4.77 mg/l (nitrates).

Table 1. Water quality parameters during the experimental period (mean ± SD)

Parameters	A1	A2	A3	A4
T (°C)	21.18 ± 2.25	21.13 ± 2.22	21.11 ± 2.13	21.05 ± 2.16
DO (mg/l)	7.32 ± 0.94	6.93 ± 1.01	6.93 ± 1.19	6.99 ± 1.11
NO_2 (mg/l)	0.1 ± 0.08	0.09 ± 0.08	0.1 ± 0.08	0.09 ± 0.08
NO_3 (mg/l)	4.77 ± 1.41	4.46 ± 1.15	4.47 ± 1.16	4.50 ± 1.20

The introduction of probiotics, in different concentrations, generally resulted in improved growth performance of Siberian sturgeon (Table 2). Enhancing the diet with 0.2 g PC per kilogram of feed led to a significant increase in growth rate in treatment A2 ($p<0.05$) compared to the control, while growth in treatments A3 and A4 was non-significant ($p>0.05$).

Table 2. Growth performance and feed utilization in Siberian sturgeon (*Acipenser baerii*) fed with different concentrations of PC

Parameters	A1	A2	A3	A4
Initial weight (g)	5.24	5.19	5.08	5.21
Final weight (g)	35.47	39.48	36.07	35.50
WG (g)	30.23	34.29	30.99	30.29
DGR (g/day)	0.48	0.54	0.49	0.48
SGR (%/day)	2.98	3.22	3.11	2.99
FCR (g/g)	0.64	0.59	0.62	0.65
PER (g)	0.70	0.76	0.72	0.69

At the end of the experiment, fish in groups A2, A3 and A4 had better daily growth rate and specific growth rate compared to the control. Also, the feed conversion factor (FCR) was improved in groups A2 and A3. However, in group A4 the FCR value was higher than that of the control group, suggesting that a higher concentration of probiotic could negatively affect the feed efficiency. Diet supplemented with probiotics had a positive impact on protein efficiency ratio (PER). There was a direct proportional relationship between the amount of probiotic added and FCR and an inverse proportional relationship with PER (Figure 1).

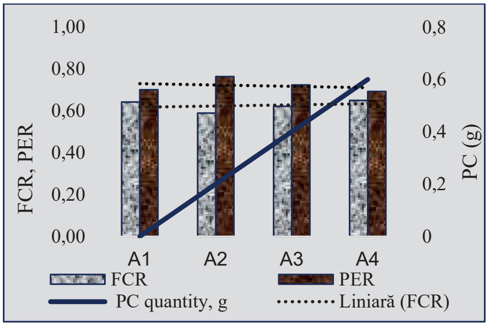

Figure 1. The evolution of FCR and PER during the experimental period

It is demonstrated that a healthy diet is very important for the healthy growth of an animal (Owens et al., 1993). Improved growth efficiency is associated with better nutrient digestibility (Al-Shawi et al., 2020).

In this study, the addition of probiotics to the feed at various quantities/concentrations positively influenced the efficiency of feed consumption. These results are consistent with those of other authors, who have demonstrated the beneficial effects of probiotics on growth performance (Perez-Sanchez et al., 2014; Tarkhani et al., 2020).

Proteins are among the most expensive nutrients required for the growth of organisms, and probiotics supplemented in the diet can help maximize their utilization. Probiotics can improve protein utilization efficiency by increasing the biological value of feed (Ringø & Gatesoupe, 1998).

According to Lara Flores and her colleagues (2003), supplementing diets with probiotics could have a significant effect in improving protein efficiency ratio (PER) (Lara-Flores et al., 2003).

In 2020, Hassani et al. supplemented the diet of Siberian sturgeon (*A. baerii*) juveniles with a mix of bacteria consisting of *Lactobacillus* spp., *Bacillus subtilis*, *and Bifidobacterium bifidum*. After 8 weeks of treatment, they observed the same effect of improving growth and feed efficiency (Hassani et al., 2020).

Improved growth performance has also been observed in other animals (Cakir et al., 2008; Kraimi et al., 2019).

The analysis of the haematological indices of the fish after the addition of feed with probiotics is important in establishing their health status (Fazio, 2019).

The effect of dietary probiotics on the blood indices of Siberian sturgeon (*Acipenser baerii*) juveniles is presented in the Figure 2. The use of the probiotic mix, named Probiotic Complex (PC), significantly improved the haematological parameters of the Siberian sturgeon ($p<0.05$). The experimental group A4, in which 0.6 g PC/kg feed (15×10^9 CFU) was added, showed the highest values of Hb, Ht, RBC and WBC, showing increases of 32.4%, 8.29%, 21.43%, and 13.85% respectively for these indices, compared to the control.

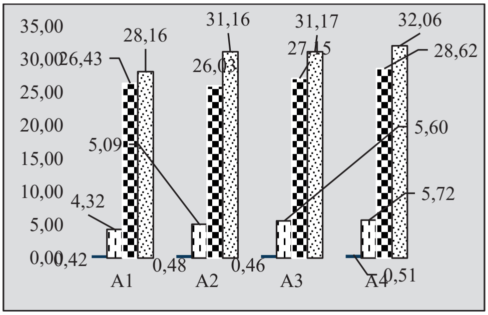

Figure 2. Variations in haematological parameters at the end of the experimental period

These results are consistent with those obtained in a previous study, where the erythrocyte count and haemoglobin level of Persian sturgeon (*Acipenser persicus*) were improved after treatment with *Pediococcus pentosaceus* and *Lactococcus lactis* (Soltani et al., 2016).

Also, Pourgholam et al. (2017) identified higher erythrocyte and haemoglobin values when supplementing the diet of Siberian sturgeon with *Lactobacillus plantarum.*

The biochemical parameters were also significantly improved upon supplementing the diet with PC ($p<0.05$) (Figure 3). The value of total proteins increased with the increase in the amount of PC added to the feed, being higher by 5.9%, 3.9% and 11.8% in A2, A3 and A4, respectively, compared to the control group. Regarding serum glucose, fish fed probiotic-supplemented diets showed lower blood glucose levels compared to the control, indicating an optimal and healthy physiological state. The most difference compared to the control group was observed in A4, where the glucose level was 1.7% lower than in the control.

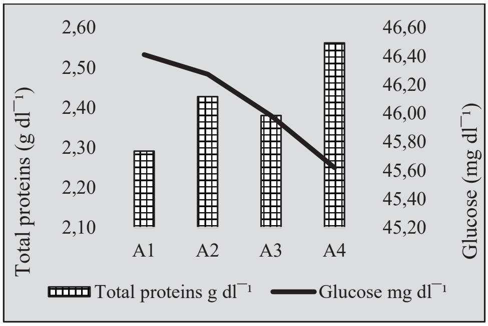

Figure 3. Variation in serum proteins and glucose of the *Acipenser baerii*

Similar results regarding the mean value of total proteins were obtained by Eissa et al. (2022) for sea bass fed diets supplemented with different amounts of *Pediococcus acidilactici*. However, contrary to the results of the present study, in their experiment, the mean glucose values were lower in the groups treated with probiotics than in the control group (Eissa et al., 2022).

Serum glucose also decreased in rainbow trout (*Oncorhynchus mykiss*) when feed was treated with *Bacillus subtilis* (Kamgar et al., 2014).

Regarding the biochemical composition of the meat, significant differences were observed between the control and the experimental groups (table 3). The crude protein content was significantly higher in probiotic treated groups (A2-A4) ($p<0.05$), while the moisture content decreased with the increasing probiotic content. Also, the analysis of the composition of the fish meat indicated higher percentages of lipids in the experimental groups compared to the control, the values increasing with the amount of probiotic included in the feed.

These results confirm those of Eissa et al. (2022).

Table 3. The biochemical composition of the meat of the Siberian sturgeon (*Acipenser baerii*) at the end of the experimental period

Parameter / Experimental group	A1	A2	A3	A4
Crude protein % (mean ± sd)	14.19±0.06	14.50±0.01	14.61±0.02	14.51±0.01
Lipids % (mean ± sd)	7.99±0.07	9.13±0.02	9.56±0.01	11.28±0.03
Moisture % (mean ± sd)	76.29±0.12	75.27±0.06	74.09±0.04	72.99±0.03
Ash % (mean ± sd)	1.20±0.01	1.03±0.02	1.16±0.04	1.16±0.05

Yones et al. (2019) obtained similar results when testing different levels of sorghum supplemented with Lacto cel-con probiotic in Nile tilapia (*Oreochromis niloticus*).

The increase in the protein content of fish meat could be explained by the fact that probiotics, once in the intestine, help to improve digestion and more efficient feed consumption, stimulating the body's growth. This leads to a greater accumulation of feed protein in the meat (Mehrabi et al., 2012; Noshair et al. 2023).

CONCLUSIONS

In conclusion, it can be stated that the inclusion of the bacterial mix from Probiotic Complex has improved the growth, health and quality of Siberian sturgeon meat.

Although the results obtained in this experiment, together with previous studies have demonstrated the beneficial potential of probiotics in aquaculture, it is important to continue research to better understand how they can influence fish and their rearing environment. The results of this study could contribute to the development of sustainable and efficient practices in the farming of Siberian sturgeon and other fish species in similar aquaculture systems.

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