THE EFFECT OF VITAMINS AND PROPOLIS ON THE LARVAE OF *ACIPENSER RUTHENUS*

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Abstract

Although the sterlet (Acipenser ruthenus) is a sturgeon that has adapted perfectly to life in freshwater and has characteristics of interest for intensive aquaculture in RAS, growth is limited by the high price of natural food (tubifex used until larvae reach 5 cm) and the high mortality rate that occurs when switching to another feed. The aim of this paper is to evaluate the growth and survival rate of sterlet larvae from 3 weeks post-hatching to 15 weeks, in relation to commercial and improved feed diets with vitamins and propolis. Bioproductive indices such as body weight gain (BW), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated at the end of an experimental period of 84 days.

Key words: *Acipenser ruthenus*, recirculating system, survival rate, growth parameters

INTRODUCTION

Sturgeons in the Danube basin, like populations in other areas, are threatened by environmental pressures, such as water pollution and hydrographic works on the river [3]. Sturgeon aquaculture is a solution for conserving wild populations.

Sterlet (*Acipenser ruthenus*) is a sturgeon that has adapted perfectly to life in freshwater and has characteristics of interest for intensive aquaculture in RAS.

However, rearing in farms is limited by the high price of natural food (the *Tubifex* used until larvae reach 5 cm) and the high mortality rate that occurs when switching to a different feed [3].

The aim of this paper is to evaluate the growth and survival rate of sterlet larvae from 3 weeks post-hatching to 15 weeks, in relation to commercial and improved feed diets with vitamins and propolis.

Vitamins are essential nutrients for fish. Vitamin deficiency in fish reared in aquaculture can produce morphological and functional abnormalities [10]. Propolis is considered one of the best natural antibiotics [9].

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Up to 20 days post-hatching, the larvae were fed tubifex. Between 20-27 days after hatching, fodder diets were gradually introduced and continued until the end of the experiment.

MATERIAL AND METHOD

The subject of the experiment was sterlet (*Acipenser ruthenus*), aged 20 days, obtained in the pilot station of ICDEAPA Galati.

The experiment was performed in the pilot recirculating system belonging to the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture of Galati, for a period of 12 weeks (May-July 2021).

The experiment was performed using 1125 specimens of *Acipenser ruthenus*, with an average mass of 2.00 g / specimen, divided into three growth tanks: Control tank (TC), Experimental tank 1 (T1) and Experimental tank 2 (T2), each with a water volume of 560 litres.

Assessment of growth performance and feed efficiency

Individual Weight Gained (WG_i, g) and total Weight Gained (WG_t, kg), Food Conversion Ratio (FCR, kg/kg), Specific growth rate (SGR, %/day) were determined as follows:

WG_i =Final weight - Initial weight (g/fish);

WG_t =Final lot weight - Initial lot weight (kg/total fish);

FCR = feed fed(kg) / weight gain(kg);

SGR =100 × [(ln Final fish weight) - (ln Initial fish weight)]/ experimental days;

IFC = kg weight gain / kg feed.

Statistical analysis

Statistical analysis was carried out by means of Excel tools. The average values are reported together with standard deviations. The statistical interpretation of the considered data shows a variation within the allowable threshold of $P<0.05$.

RESULTS AND DISCUSSIONS

For the control lot, standard feed (SF) with a protein content of 45%, [8] a lipid content of 15%, ash 6.9%, fibre 3.3% was used, depending on the relatively limited information on the nutrient needs of this species. Studies by Dong - Hoon Lee et al., 2021, revealed an optimal protein level of 45.9%. The grain size of the feed increased from 0.5 mm to 2 mm in proportion to the increase in larval size [5].

For the experimental lots, experimental feed diets were made, supplemented as follows:

Diet 1 (Dv) - Standard feed plus 2% vitamin complex (83.2% vitamin A, 16.5% vitamin D3, 0.1% vitamin K3 and 0.2% vitamin C) and Diet 2 (Dp) - Standard feed plus 0.2 % propolis.

The feed ration ranged from 1.5% / day of biomass weight to 3% / day of biomass weight, depending on water temperature [1]. The total amount of food calculated for one day was portioned and administered every four hours.

Physicochemical analyses of water

An important condition for the feed to achieve its purpose is to be rationally staggered in terms of quantity and quality. The fodder distributed in excess, degrades very easily, having as final effect the alteration of water quality.

The evaluation of physico-chemical parameters of the aquatic environment was carried out in order to maintain a technological water quality, within the optimal limits for the species *Acipenser ruthenus*, which lives in clear, clean and well oxygenated waters.

The main physico-chemical parameters with implications on the biological material were monitored: pH, temperature, oxygen, organic matter and nitrogen compounds (nitrates, nitrites, ammonia, ammonium).

Water samples were taken daily to monitor pH, temperature and oxygen, the data being presented as averages and weekly to monitor the other parameters.

Table 1. Water chemical parameters in the recirculating system, for *Acipenser ruthenus* rearing

Analysed parameters	U.M.	Nr. of samples	Tank Control	Tank 1	Tank 2
			Average ± SD*		
Temperature	°C	84	21.22±1.55	20.45±2.23	20.95±2.51
Dissolved oxygen	mg/l	84	8.54±0.55	7.44±2.55	7.16±1.15
Ph	uPh	84	8.55±0.14	8.34±0.12	8.25±0.26
Organic matter	mg $KMnO_4$/l	12	51.30±1.65	48.30±2.15	47.46±1.15
Nitrates, (NO_3^-)	mg/l	12	0.52±0.10	0.32±0.20	0.41±0.18
Nitrites, (NO_2^-)	mg/l	12	0.05±0.002	0.06±0.002	0.07±0.001
Ammonia (NH_3)	mg/l	12	0.21±0.01	0.15±0.02	0.13±0.01
Ammonium (NH_4^+)	mg/l	12	0.75±0.17	0.82±0.10	0.78±0.15

The water quality in the system was within the allowed and recommended limits for the waters used in fish farming, being in the second and third quality class, in accordance with the provisions of MMGA Order no. 161/2006. The values of the monitored parameters characterize the technological water as corresponding to the growth and development of *Acipenser ruthenus*. Feeds enhanced with vitamin complex or propolis used in feeding diets do not change the quality of the aquatic environment.

Analysis of the biologic material involved in the experiment

Technological indicators are absolutely necessary in order to obtain information on the performance of applied technology and the rearing system used.

Monitoring the growth dynamics of *Acipenser ruthenus* larvae, reared under controlled conditions is very important for establishing and adjusting the technological parameters that directly influence the growth performance.

Weighing the fish material was performed at the beginning and at the end of the experiment, in order to follow the growth dynamics of sterlet in the three groups.

At the beginning of the experimental period, the individuals from the three lots had very close weights in value, the coefficient of variability being in the range 0-15%, which highlights a very small data spread and a representative average that derives from the homogeneity of the three lots (Table 2).

After 84 days of the experiment, the mean mass of the control lot increased to 10.13 ± 1.60 g / specimen, the mean mass of the lot fed with the addition of vitamins increased to 10.52 ± 1.58 g / specimen and the mean mass of the lot fed with the addition of propolis progressed to 7.48 ± 1.53 g / specimen.

The highest accumulation of biomass was accomplished by feed enriched with a vitamin complex. The propolis added to the feed led to a much lower accumulation compared to the control lot which was fed standard feed.

Table 2 Bioproductive indicators obtained for *Acipenser ruthenus* rearing in a recirculating system, fed with vitamin and propolis supplements

Growth parameters	**UM**	**Lot Control**	**Lot 1**	**Lot 2**
Initial Parameters		standard feed SF	vitamin feed Dv	propolis feed Dp
Number of Specimens	-	375	375	375
Mean individual weight	(g/specimen) mass±SD*	1.90±0.11	1.99±0.15	2.10±0.18
Initial Biomass	Kg	712.50	746.25	787.50
Density of the initial population	kg/m^3	1.27	1.33	1.41
Final Parameters				
Number of Specimens	-	266	299	249
Mean individual weight	(g/specimen) mass±SD*	10.13±1.60	10.52±1.58	7.48±1.53
Final Biomass	kg	2694.58	3145.48	1862.52
Density of the final population	kg/m^3	4.81	5.62	3.33
Growth parameters				
Number of days	days	84.00	84.00	84.00
Weight growth individual (WG_i)	g	8.23	8.53	5.38
Weight growth total (WG_t)	kg	1.98	2.40	1.08
Total Shared Food	kg	2.37	2.46	1.88
Feed Conversion Rate (FCR)	kg/kg	1.20	1.03	1.75
Daily growth rate (DGR)	g/day	0.098	0.102	0.064
Specific growth rate (SGR)	%/ day	1.58	1.71	1.32
Index of feed conversion (IFC)	Kg/kg	0.84	0.98	0.57

*Standard deviation

The population density of the biological material at the beginning of the experiment had very close values in the three tanks (1.27 kg/m^3 in Lot C, 1.33 kg/m^3 in Lot 1, 1.41 kg/m^3 in Lot 2).

At the end of the experiment, there were differences between the quantities of biomass in relation to the volume of water (4.81 kg/m^3 in Lot C, 5.62 kg/m^3 in Lot 1, 3.33 kg/m^3 in Lot 2).

Improved vitamin complex feed has a positive effect on the accumulation of biomass by the *Acipenser ruthenus* species compared to standard feed (SF) and feed in which propolis (Fp) has been incorporated.

The total weight gained was highest in lot 1 (2.40 kg), where supplemental feed with a vitamin complex was administered. In lot 2, where feed with incorporated propolis was used, the weight gained was 45.45% lower (1.08 - Lot 2; 1.98 - Lot C) compared to Lot C fed with standard feed.

The same situation was encountered when calculating the daily growth rate of 0.102 g/day in Lot 1, compared to 0.098 g/day in Lot C and 0.064 g/day in Lot 2 and the specific growth rate of 1.71% /day in Lot 1, of 1.58% /day in Lot C and 1.32% /day in Lot 2.

SGR in the present experiment for all lots has higher values compared to those obtained by Nicula M. et al., 2015 [6], and Andras Ronyai & Tibor Feledi, 2012 [4], studying the growth performance in juveniles of *Acipenser ruthenus* in RAS and comparable to those identified by Prokeš M., et al., 2011 [7], at 120-day *Acipenser ruthenus*.

The feed conversion index registered the highest coefficient (0.98) in Lot 1, the value being determined by the accentuated weight gain of the biological material.

FCR is similar to the results obtained by Nicula M. et al., 2015 [6] studying the growth performance of *Acipenser ruthenus* larvae in RAS, but higher than that calculated by Prokeš M., et al., 2011 [7] in *Acipenser ruthenus* aged between 30-120 days.

The addition of vitamins led to an improvement in the feed conversion coefficient, in lot 1 compared to the control lot fed with standard feed and lot 2 fed with propolis and feed, confirming the positive effect of the addition of vitamins, on this index, which is also demonstrated in other fish species by Wen H. Et al., 2008 [11].

The values obtained show that the nutrition of *Acipenser ruthenus* larvae with diets containing vitamin supplements leads to better growth parameters and the addition of propolis in food should be avoided.

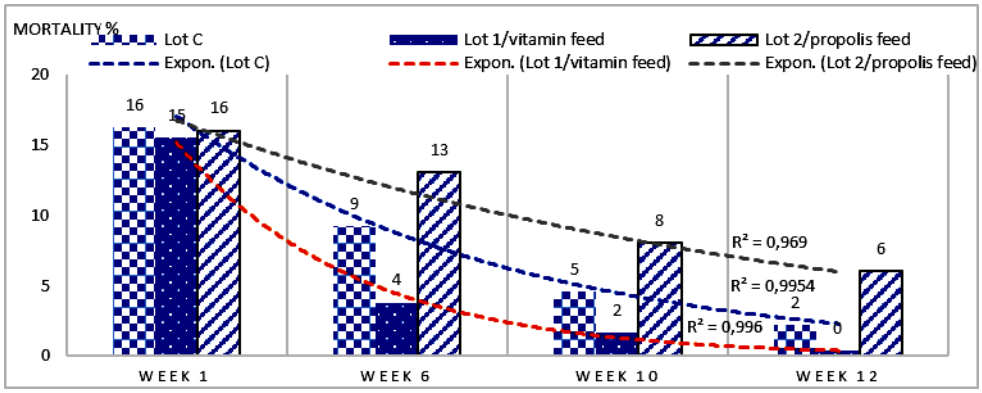

Fig. 1 Evolution of the mortality rate (%) recorded in the three lots during the experiment

At the end of the experiment, after 84 days, the survival rate was 80% in Lot 1, 71% in Lot C and 66% in Lot 2. In the first 7 days of the experiment when artificial food was introduced, the mortality rate of was about 16% in all three lots, after which mortality decreased exponentially, but differently (Fig. 1). Survival rate values explain that the addition of vitamins in feed diets favourably influences the survival rate, while the addition of propolis resulted in a higher mortality rate for larvae of *Acipenser ruthenus*. The survival rate in Lot 1 is comparable to that obtained by Beata L., et al., 2020 [2].

The decrease in mortality rate in the lot fed with vitamin supplements can be explained by stimulating compensatory responses to stressors. Studies need to be thoroughly researched to gain a better

understanding of the nutritional needs of sturgeon larvae.

CONCLUSIONS

The results of our experiment in terms of growth performance and survival rate, using a feed enriched with a vitamin complex indicate that *Acipenser ruthenus* could be considered a potential candidate for species diversification in the Romanian aquaculture sector.

The addition of propolis in feed diets has resulted in low utilization of feed, reduced biomass accumulation and a lower survival rate.

REFERENCES

[1] Akbulut B., Feledi T., Rónyai A., Effect of feeding rate on growth performance, food utilization and meat yield of sterlet (Acipenser ruthenus Linne, 1758). Published 2013, Biology Journal of Fisheries sciences.com

[2] Beata L., Mohammad A. M. S., Elzbieta Z. et al., 2020, Early Weaning Effects on Survival, Growth, and Histopathology of Larval Sterlet Acipenser ruthenus, North American Journal of Aquaculture 82(2).

[3] Gisbert E. and Williot P., 2002. Advances in the larval rearing of Siberian sturgeon, Journal of Fish Biology 60: 1071-1092.

[4] Andras Ronyai & Tibor Feledi, 2012, Short Communication, Co-feeding as a weaning procedure in sterlet (Acipenser rutheus L.) larvae, Aquaculture Research, 1-3, https://www.researchgate.net/publication/263077299_Co-feeding_as_a_weaning_procedure_in_sterlet_Acipenser_ruthenus_L_larvae

[5] Dong-Hoon Lee, Seongryul Lim, Seunghyung Lee, 2021, Dietary protein requirement of fingerling sterlet sturgeon (Acipenser ruthenus), Journal of Applied Ichthyology, volume 37, Issue 5, pg. 687-696.

[6] Nicula M, Zoltan T, Păcală N., Bura M.,Simis E.,Erina S., et al., Scientific Papers: Animal Science and Biotechnologies, 2015, 48 (2), 108-114

[7] Prokeš M., V. Baruš, J. Mareš, M. Peňáz, V. Baráne, Growth of sterlet Acipenser ruthenus under experimental and farm conditions of the Czech Republic, with remarks on other sturgeons, Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2011, vol LIX, 36, 281-291

[8] Sion C., P. Călin, G. M. Bacanu, 2011, The influence of pellets quality on the growth of sterlet, in recirculating aquaculture system, Biology, Aacl Bioflux.

[9] Sara E. Shahin, Wafaa A. Eleraky, Mahmoud F.A. Elgamal, Elsayed I. Hassanein and Doaa Ibrahim, 2019, Effect of Olive Leaves and Propolis Extracts on Growth Performance, Immunological Parameters and Economic Efficiency using Nile Tilapia (Oreochromis niloticus), Zagazig Veterinary Journal, Volume 47, Number 4, p. 447-458

[10] Tatina M., M. Bahmani, M. Soltani, B. Abtahi și M. Gharibkhani, 2010. Efectele diferitelor niveluri de vitamine dietetice C și E asupra unora dintre parametrii hematologici și biochimici ai sterletului (Acipenser ruthenus). Journal of Fisheries and Aquatic Science, 5: 1-11.

[11] Wen H., AS Yan, Î. Gao, M. Jiang, QW Wei, 2008, Dietary vitamin A requirement of juvenile Amur sturgeon (Acipenser schrenckii), Journal of Applied Ichthyology, 24(5):534-538