PRELIMINARY RESULTS ON THE USE OF ECAPSULATED CYSTS, NAUPLII AND ENRICHED ARTEMIA NAUPLII IN STARRY STURGEON (*ACIPENSER STELLATUS*) FEEDING

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Abstract

Fish with high economic value require an intake of live natural food to supplement the deficiency of enzymes in the digestive tract and ensure the nutritional requirements characteristic of the early life stages of fish. Internationally, numerous studies have been carried out on the topic of feeding fish with decapsulated Artemia cysts which recorded very good results and aimed to reduce the material costs required for hatching. Many other studies highlight the advantages of bioencapsulation with nutrients of Artemia nauplii, but there are also studies that have shown that feeding fish with enriched nauplii does not bring significant differences in growth results and survivability of fish in the first stages of life. The present experiment aims to establish the advantages and disadvantages of feeding starry sturgeon in three variants, with decapsulated cysts (V1), nauplii (V2), and enriched Artemia nauplii (V3). The final results for the average body mass values of the fish were higher in the V3 variant compared to V2 and V1, as well as the survival rate (with values of 69.47%, 67.18%, 63.36%), but in the V1 variant water pollution with nitrogen compounds and feeding costs were lower than in the experimental variants V2 and V3, being beneficial for recirculating aquaculture systems.

Key words: *starry sturgeon, feeding, decapsulated cysts, Artemia nauplii, enriched Artemia nauplii*

INTRODUCTION

The food necessity of a population in a continuous demographical expansion imposes on a global scale, the discovery of new food resources (Bostock et al. 2010; Sudakova, 2019). In recent years aquaculture has registered an accelerated growing trend (Rosamond et al. 2021; Handisyde et al. 2010). Thanks to technological development, large scale fish farming systems with outdoor ponds are being replaced by indoor units that effectively monitor living conditions, allowing the use of higher stocking densities and increased fish production (Talpeș et al. 2005).

Sturgeons are ancient fish with a cartilaginous skeleton and high economic value, sought after for the delicacy of the meat and caviar (Lopez et al. 2020), which require a long period of time (between 5 and 12 years) to reach reproductive maturity (Patriche, 2001), for this reason they are reared within recirculating aquatic systems in controlled environmental conditions (Ahmed et al. 2021).

The starry sturgeon (*Acipenser stellatus*, Palls, 1771) is a medium-sized anadromous sturgeon with increased adaptability for fodder consumption and good bioproduction, which is becoming increasingly important among cultured sturgeon species used for production in recirculating systems, or for the repopulation of natural waters, within sturgeon conservation programs (Chandra et al. 2022).

The early life stages of fish are characterized by an insufficiency of the digestive enzymes, because of some unfinished ontogenetic development stages, mainly of the hepato-pancreas, characteristic of the pre and post larval stages (Cristea et al. 2012; Lazu et al. 2008). Because of this reason in the post larvae and juvenile stages the intake of live food is strictly necessary

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(Chebanov, 2013). The starry sturgeon has a prolonged development period from larva to fingerling stage in which it requires quality food that can fulfill its nutritional needs and provide disease resistance.

The brine shrimp *Artemia salina* (Linnaeus, 1758) is the most used live food for feeding fish and crustaceans in the early stages of life. The advantages of this organism for usage in large scale larviculture are its high protein content, the possibility of long-term storage of the cysts and the possibility of enriching the nauplii (Tamaru, 2003).

The hatching of the cysts is done based on necessity, in a short timeframe varying between 12-24 hours, in standard conditions, obtaining freshly hatched nauplii with a high energetic value in the first hours of life (Millan-Alamaraz et al. 2021). Artemia shows a particularity, specific for filtering organisms, to accumulate substances from the environment that allow the use of this crustacean as a transfer vector of nutrients (e.g., unsaturated fatty acids), or vitamins, hormones, antibiotics, probiotics, in fish by enrichment method (Akbarali et at. 2014). This represents the process called bioencapsulation that is used with the aim of increasing the nutritional value of the Artemia nauplii intended for feeding fish in the early stages of development for securing the nutritional needs and the development of the immune system, for obtaining growth increases and a better survival rate in fish (Domingues et al. 2002).

For aquatic recirculating systems, antibiotic treatments (established based on the microbiological analysis with antibiotic sensitivity testing) that used the bioencapsulation method to transfer the antibiotic in nauplii (Prusińska et al. 2020), represent the best solution for avoiding water pollution with unconsumed medicated fodder (that can generate in time the phenomenon of bacterial bioresistance), but also to prevent the destruction of bacterial filters.

The use of decapsulated Artemia cysts, with a high nutritional content in fish feeding (Mamcarz et al. 2011) has the advantage of reducing the energy cost for hatching and maintaining water quality in recirculating water systems, by avoiding the decomposition of Artemia nauplii (with a limited vital activity in fresh water), but also for increasing the hatching efficiency and avoiding the intestinal obstruction from cyst shell ingestion (Asem, 2011).

This paper has the objective of establishing the most advantageous feeding variant in the early life periods of the starry sturgeon development, using cysts, nauplii and enriched Artemia nauplii, for obtaining the maximum increase in growth performance and survivability.

MATERIAL AND METHOD

The nutrition experiment was carried out between 01.07-16.07.2022 with 191 starry sturgeon specimens (aged 18 days after hatching, with a body mass of 14.967±0.30 g), distributed in varying numbers, with a constant average body mass, in three groups for the experimental variants V1- 5.307 g/68 specimens, V2-4.981 g/64 specimens and V3-4.679g/61 specimens. The fish were chosen, weighed, measured, and distributed in three groups after a mild narcosis with clove oil $0.1ml \cdot L^{-1}$

Subsequent, the fish were distributed in triplicate, for the experimental variants, in nine aquariums with a useful water volume of 10 l for each and reared in identical photoperiod conditions 12L:12D. For the experiment, dechlorinated water was used with an average temperature of 24±0.21 ^{0}C, pH=7.8±0.4, with an oxygen content of 7.4±0.3 mg/l (under continuous additional aeration, and the saturation did not fall below 88% during the experiment) and variations of the NH_4/NH_3 ratio between 1 and 5.5 $mg \cdot L^{-1}$. About a quarter of the total volume of the rearing aquariums was replaced daily.

Experimental feeding variants V1- with decapsulated cysts, for a group of fish with an average initial weight of 0.78±0.016 g and average of total length of 2.56±2.4 cm; V2- with nauplii, for a group of fish with an average initial weight of 0.078±0.015 g and an average length of 2.5±0.04 cm; V3- with enriched Artemia nauplii, for a fish group with an average mass of 0.77±0.012 g and an average length of 2.68 ±0.2 cm. The selection

of fish groups based on uniformity was attempted, but due to size differences found in sturgeon and to increase precision, only average body mass was used to compare groups.

The survival percentage was calculated based on the number of fish remaining at the end of the experiment relative to the initial number of individuals per group and analyzed in correlation with the final average mass of the fish population, obtained in each experimental variant.

The weighing process was done by an electronic balance Kern&Sohn GmbH (MODEL: ABJ 120-4M). The length was recorded on a measuring scale in cm. Sampling was performed to observe growth and health conditions in the morning at 7:30 a.m. prior to feed delivering. Air stone aerators were applied to provide sufficient oxygen, powered by electricity. A single air stone was allocated for each aquarium. The aeration was operated for 24 hours during the experimental period.

Artemia cysts were decapsulated, using standardized techniques (Treece, 2000), deposited in a saline solution with a concentration of 35 ppt. at a temperature of 2-4ºC, being used for the direct feeding of the fish in the experimental variant V1, incubated for obtaining nauplii for fish feeding in V2 variant, or for the enrichment of nauplii used for fish feeding in V3 variant.

The incubation of the cysts was achieved in two cylindroconical containers with bottom aeration, with a volume of 3,5 l and cyst density of 1.5 $g \cdot L^{-1}$, in a saline solution of 15 ppt. with an addition of 2$g \cdot L^{-1}$ potassium bicarbonate, on a constant temperature of 28ºC and an illumination of 2000 lux.

The enrichment of Artemia nauplii was done using a modified nutritional recipe, by substituting the unsaturated fatty acids of animal origin (cod oil), with unsaturated fatty acids of vegetal origin, black cumin (Nigella sativa) oil (Ali et al. 2014; Latif et al. 2020; Maqbool et al. 2021) and cannabis (Cannabis sativa) oil (Saoud et al. 2017) administered 8 hours after hatching in quantitie of 0.15 gL^{-1}, well emulsified before administering to the nauplii. Collecting and washing the nauplii was done on a sieve of 120 micron.

The feeding was carried out at least 2-4 hours after the enrichment process, and the excess of enriched nauplii were kept in a nutritious environment in the dark and at refrigeration temperature levels, to reduce metabolic activity and slow down the growth process of the nauplii (so that the dimensions of the nauplii to be compatible with the dimensions of the oral cavity of the starry sturgeon larvae).

The enrichment recipe contains krill oil-Coril =500mg; Ascorbic acid (vitamin C)-700g/kg fodder; thiamine hydrochloride/ pyridoxine hydrochloride (vitamins B1 and B6-Milgamma)=100mg/50mg/ml; Tocopherol (vitamin E)=200 mg; Cholecalciferol (vitamin D3)=0,25 ml= 45000 IU/ml; Retinol (vitamin A)=20mg/ml; 400 ml distilled water; 2 egg yolks; 15 g gelatine; Magnesium 75 mg; Potassium chloride=5mg; 60 ml cannabis oil; 20 ml black cumin oil.

The fish were fed at 8:00 a.m. and 12:00 a.m. with natural food (cysts V1, nauplii V2, enriched nauplii V3) and at 16:00 with fodder (which was administered uniformly for each of the three experimental variants, not to influence the final results, and to cover the nutritional needs of the fish from 5:00 p.m. until the next day at 8:00 a.m. when the fish were not fed, but also to ensure the food fasting necessary to obtain a maximum consumption of natural food the next morning).

The quantity of natural food administered was 0,20 g/aquarium in all variants with a determined number of 87.000 decapsulated cysts/g and of 58.000 hatched nauplii/g of decapsulated cyst (Sellami et al. 2020) that after the results of the experiments of individual consumption of the fish, can be assimilated with ''ad libitum'' feeding.

The fodder was calculated at 20% of the sturgeon body mass, with a daily ration of aprox. 0.03 g/aquarium and was composed of: Crude protein 45%; Crude lipids=155; Carbohydrates=22,3%; Fiber=3,2%; Ash=6,5%; Ptotal=1,1%; total N =0,2%; total Ca=0,9%; Vit A=10.000 IU/kg; Vit D_3=1.250 IU/Kg, anhydrous Calcium iodide=3mg/kg; Chelated magnesium E5=12 mg/kg; Chelated zinc E6=50 mg/kg; BHT antioxidant (E321)=70mg/kg; BHA (E3200=45mg/kg).

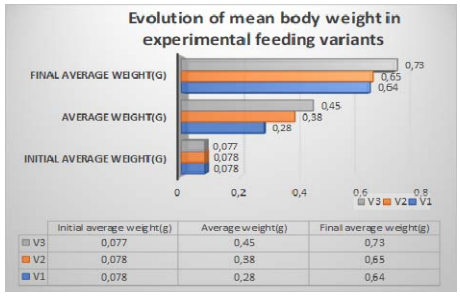
The processing of the final data was done graphically and statistically with the EXCEL software measurements and statistical models, all clearly and briefly described, in accordance with the reference literature conventions.

RESULTS AND DISCUSSIONS

The results obtained from the experiment indicates and increase of the average body mass in variant V3 of feeding the starry sturgeons with enriched Artemia nauplii, in comparison with variants V2 of feeding with plain nauplii and V1 of feeding with cysts (statistically significant with P<0.05; Graph 1).

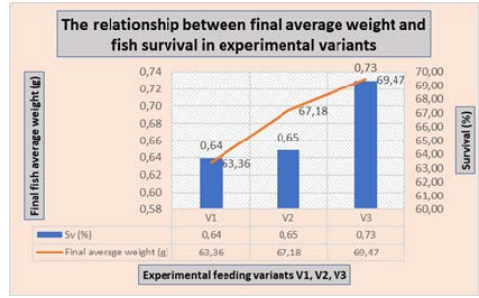
This result demonstrates the beneficial effect on growth and survival in the variant of feeding starry sturgeon with enriched Artemia nauplii, in contradiction with the results of another study (El-Magsodi et al. 2016) on the insignificant differences registered in the administration of enriched nauplii and plain nauplii for the feeding of crustaceans.

The evolution of the average body mass increase recorded a growing trend, from initial average body masses with statistically insignificant differences (P>00.5) to final average body masses according to the direction of growth V3>V2>V2 (with the initial values of V1i=0.78±0.016 g, V3i=0.77±0.012 g to the final values of V1f=0.64±0.031 g V2f=0.65±0.015 g, V3f=0.73±0.040 g).



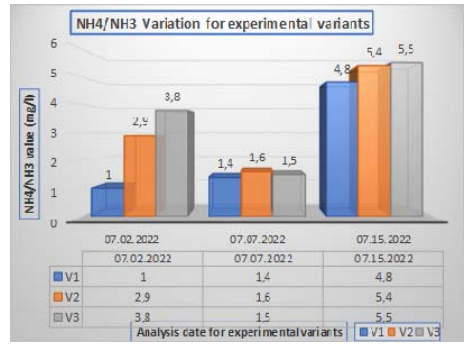
Graph no. 1.

The survivability of the fish in the three experimental variants show the highest value for the experimental variant V3 of feeding with enriched Artemia nauplii in comparison with V2 of feeding with nauplii and V1 of feeding with decapsulated cysts, in the following order: Survivability in variants V3>V2>V1, with statistically significant different values, with P<0.05 (ANOVA-Two Factor Without Replication) shown in Graph 2.



Graph no. 2

The evolution of the water chemical parameters (Graph 3) reveals a variation of NH_4/NH_3 concentration with increasing values V3>V2>V1 which is explained decomposition of enriched nauplii (with an addition of egg yolk with 17% proteins, 30,6% lipids, 0,6% carbohydrates and 1,7% by the minerals-Stadelman & Cotterill, 1995) used in feeding variant V2.



Graph no. 3

The survival time of nauplii in fresh water is approx. 3.5 hours, after which their decomposition causes deterioration of the chemical parameters of the water.

Using the feeding variant with decapsulated Artemia cysts, that do not decompose rapidly, offer a feeding variant that is less polluting and more affordable regarding production costs.

CONCLUSIONS

-Using Artemia nauplii enriched for fish feeding, plays an important role in maintaining a strong immunity and a good growth rate of the starry sturgeon in the first stages of life.

-Feeding starry sturgeons with Artemia cysts in the experimental feeding variant V1, reduces the energy costs for incubation, and protects the water from the rapid increase in NH_4/NH_3 concentrations, which resulted from the decomposition of dead nauplii. A precise economic calculation is recommended to establish the ratio of benefits and losses (survival and growth rate of fish) in the exclusive use of decapsulated Artemia cysts for feeding starry sturgeon alevins, considering the trend of continuous increase in the price of energy, due to the world economic crisis.

-It is recommended to evaluate the enrichment costs of Artemia nauplii used in the V3 nutrition experiment (nutrients, labor, energy costs, etc.) and the risk of water pollution versus fish survival and growth for large-scale sturgeon production in recirculating systems.

-The experimental variant of feeding V2 with Artemia nauplii, under the conditions of optimizing the food ration (in terms of the survival time of the nauplii in fresh water and the nutritional needs of starry sturgeon in different stages of ontogenetic evolution-from larvae to fingerlings, but also the costs of bioencapsulation), represents the optimal option recommended for feeding sturgeons raised in a recirculating system in the first stages of life.

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