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Growth and survival of Clarias catfish (*Clarias gariepnus* B., 1822) at different stages of cultivation with the addition to the fodder of "Chiktonik"

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Abstract. Stressful situations decrease the growth rate and survival rate of fish, thus, the search for ways to reduce their adverse impact is relevant. The purpose of the research – to evaluate in aquaculture conditions the effect of different concentrations of vitamin-amino acid complex "Chiktonik" on the growth and survival of larvae and fry of African clarius catfish (*Clarias gariepinus* B*., 1822*) after stressful situations. The stressful situation for the fish arose from significant fluctuations in the content of ammonia, nitrites and nitrates in the water environment of the closed recirculation aqua system during the period of start-up of the biological filter, until the equilibrium was established. A series of experiments were performed, during which it was established that the addition of the drug at the rate of 1 ml per 1 kg of feed accelerates the growth of fish in experimental variants compared to the control. Experimental use of high doses of the drug (5, 15, 30 and 45 ml/kg of fodder) initially inhibited the growth of fish body weight, but 10-30 days after the experiment, the growth rate of the experimental material was equal to that of the control group of fish and even exceeded the control values in the future. The positive effect of the vitamin-amino acid complex "Chiktonik" on the survival of young clarius catfish at the stage of completion of the larval period of life and in the early stages of the fry period was established. In the experiment with older fish, which were fully developed fry, such an effect of the drug was not observed: the survival rate of fish was at the same level both in the experiment and in the control. Therewith, it was established that the fry reacted worse to higher doses of the drug compared to the grown larvae. The growth rate of fry after using high doses of the drug did not equal that of fish from the control group within a month after the experiment, unlike younger fish. In general,

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the expediency and safety of the practical use of the drug “Chiktonik” for young clarius catfish as a fodder additive at a dose of 1 ml/kg of fish fodder have been proved

Keywords: vitamin-amino acid complex, the establishment of biological balance, feeding, stress, aquaculture

Relevance

At the current level of technical and technological support of aquaculture, the conditions for growing fish in the vast majority of fish farming enterprises cannot be considered optimal. Various stress factors in the form of significant fluctuations in hydrochemical, temperature and oxygen regimes, and the impact of various infectious and nutritional diseases cause growth retardation and fish mortality (Bahareva & Grozesku, 2000).

A big problem for industrial-type fish farms is the purification of used water from nitrogenous compounds ($NH_{3\backslash4}$, NO_2 and NO_3), which enter the fish farming system in the process of decomposition of organic matter of fodder and waste products of cultivation objects. To remove nitrogenous compounds from the water, a biofilter is used, in which, under the influence of bacteria, these compounds are converted into substances less toxic to fish. In case of malfunctions or during the start-up phase, before the biological equilibrium is established, when the biofilter is not yet able to effectively remove nitrogenous compounds, the latter accumulate in the water and reach concentrations that are lethal to fish (Bregnballe, 2015; Sharylo *et al.*, 2019). In English literature, the period of establishing biological equilibrium is called the “new tank syndrome” (Alderton, 2019; Ebeling & Timmons, 2010).

Fish poisoning by nitrogenous compounds causes damage to the nervous system and muscular apparatus. Outwardly, this is manifested in the form of convulsions. In addition, the gill apparatus is affected, the liver and spleen abnormally increase in size, and the haemoglobin content in the blood decreases (Potrohov *et al.*, 2006; Krasyuk, 2009). In the absence of timely reaction of technologists, fish can die within a few days or even hours (Tilak *et al.*, 2002; Kofonov, 2017)

With a timely response to the problem, the fish can be saved, but it will still get poisoned, and if its body is not supported at the rehabilitation stage, the death of fish, for this reason, may continue.

The relevance of this scientific research is conditioned upon the necessity to increase the survival rate and maintain the rate of mass accumulation in fish after stressful situations, in particular, by using the vitamin-amino acid preparation “Chiktonik” at different stages of the technological process.

Analysis of Recent Studies and Publications

According to the results of the analysis of foreign and domestic sources of scientific and technical information, it was established that using vitamin-amino acid complexes in aquaculture has great prospects.

During embryogenesis and the transition to external nutrition, fish go through stages called “critical periods” (Martseniuk &

Martseniuk, 2021). During such periods, the highest fish mortality is observed, and it is connected with the fact that the organism enters into new ecological relations with the environment. To prevent the death of fish in the embryonic period, scientists (Lyubomirova *et al.*, 2021) recommend keeping eggs in a solution of vitamins, which positively affects the process of embryogenesis and increases the yield of larvae from eggs. Such larvae are characterised by increased viability and growth rate.

Another use of the complex of vitamins and amino acids is to eliminate the effects of poisoning in fish, long-term fasting, strengthen immunity and increase resistance to bacterial diseases. Scientists (Lyubomirova *et al.*, 2021; Eleev *et al.*, 2019; Osipova *et al.*, 2005; Metallov *et al.*, 2013) believe that the positive effect of using such substances can be both improved health and increased survival and body weight gain of fish.

Chiktonik is a feed additive for animals that contains a balanced amount of vitamins and amino acids, including essential ones. Using the drug is intended to compensate for the deficiency of biologically active substances in the body of animals, regulate metabolism, and promote nonspecific resistance to adverse environmental factors. In the case of unbalanced feeding and stress, during the period of intensive growth and high productivity, using this additive in the composition of fodder increases the safety of livestock, especially young animals, and increases productivity and duration of use of animals.

Chiktonik has been used in agricultural animal husbandry for more than 10 years. This drug was developed initially for poultry farming, but later it was used in other livestock sectors (Gorchakova, 2013; Adullina, 2014), and more recent research on the effects of Chiktonik on fish has started (Kuznecova & Mosyagina, 2015).

It should be noted that the improper use of vitamin preparations may have adverse effects, namely hypervitaminosis (Ksenofontova, 2019).

The purpose of the study – to evaluate the effect of the drug "Chiktonik" at different concentrations on the growth and survival of juvenile catfish (*Clarias gariepinus* B., 1822).

Materials and Methods of Research

The material for the research was larvae and fry of clarius catfish. Research methods – generally accepted in fish farming (Martseniuk & Martseniuk, 2020).

To estimate the growth rate, the total index of masonry accumulation in the group of fish in the experiment and the percentage of this index to that in the control were used. The survival of fish in each group was determined by counting the number of juveniles and calculating the percentage of survival. Data collection was performed during control catches, with a frequency of once every 10-15 days. All fish were counted and weighed in groups.

The research was conducted in 4 stages, which were distinguished by various experimental conditions and various concentrations of the drug: 1, 5, 15, 30 and 45 ml/kg of fish fodder. The first, second and third stages of the experiments were conducted in the aquarium laboratory of the Department of Aquaculture of the Center for Aquatic Bioresources and Aquaculture of NUBIP of Ukraine. The fourth stage of research was conducted in the production conditions of a private fish farm for the cultivation of clarius catfish, located in the village of Yushky, Obukhov district, Kyiv region.

The stress factor for fish, the consequences of which were planned to be overcome using

the drug "Chiktonik", were increased concentrations in water of $NH_{3\backslash4}$ (0.5-1 mg N/l), NO_2 (0.25-0.5 mg N/l) and NO_3 (30-60 mg N/l). Fish were planted in the growing tanks of a closed aquaculture system until the beginning of the period of biological equilibrium in the water. During the first 10 days, when the fish were feeding the preparation with the addition of artificial fodder, an increase in the content of nitrogen compounds in the water was observed. Part of the ammonia and nitrites were removed using a biofilter, part – by replacing water in the volume of 10% daily. Later, after the completion of the biofilter start-up period, the main hydrochemical parameters were stabilised.

For the first three stages of research, 6 autonomous mini-fish farms with closed water supply were installed. Each unit included a 100-litre glass fish tank (aquarium) and a water regeneration unit (mechanical and biological filters). For water circulation in the system, a pump "MinJang NS F801" with a capacity of 1200 l/h was used. Porous foam sponges connected to the water pump served as mechanical filters. The mechanical filter was cleaned manually as it became dirty. The biological filter was filled with highly porous filler "Separax" produced by "JBL Micromec" as a substrate for the development of the bacterial film.

The water temperature in the UZV was maintained in the optimal range for the cultivation of clarius catfish (27-28°C), using thermostats "Resun Sunlike 200".

The concentration of nitrogenous compounds in aquarium water was determined using express tests TM "Ptero".

The fourth stage of the experiment was performed in fish ponds with a working volume of 1 m^3. Biological water treatment in the system was performed using the classic plastic filler Aqua for the biofilter. The fish were reared in a closed-type aquaculture system in a private fish farm.

To prepare the fodder with Chiktonik, the latter was taken from the bottle with a syringe with a needle in the required volume. The solution of the drug for feeding was prepared with the addition of a small amount of distilled water to evenly distribute the solution over the entire volume of fodder and, therewith, minimally moisten the daily portion of fodder. The required amount of solution was evenly applied to the surface of the fodder with a syringe.

For feeding juvenile clarius catfish, fodder of the trademark "Aller Aqua" was used with the size of grains or pellets from 0.1 mm to 6 mm, depending on the age of the fish. Fish were fed 5 times during daylight hours. The daily fodder rate was 7% of the body weight of 15- and 30-day-old juveniles and 3% for 65-day-old fry. Fodder with the addition of the drug was given to fish once a day, mainly in the morning.

The dosage of the drug "Chiktonik", age groups and the amount of experimental material by variants in the context of the stages of the experiment are presented in Table 1:

Table 1. *Scheme of the experiment to assess the effect of the drug "Chiktonik" on young clarius catfish*

Stage no.	Variant	Dose of the drug, ml/kg of fodder	Aquatic system No.	Characteristics of the experimental material of fish			Stage duration, days
				age group	average weight, g	quantity, pcs.	
1	Control		1	15-day grown larva	0.08	56	20
			2			56	

Table 1. Continued

Stage no.	Variant	Dose of the drug, ml/kg of fodder	Aquatic system No.	Characteristics of the experimental material of fish			Stage duration, days
				age group	average weight, g	quantity, pcs.	
1	Experiment	1	3	15-day grown larva	0.08	56	20
			4			56	
			5			56	
			6			56	
2	Control	-	1	30-day old fry	0.39	82	35
			2			82	
	Experiment 1	15	3			82	
			4			82	
	Experiment 2	45	5			82	
			6			82	
3	Experiment 1	15	1	65-day old fry	32.08	6	53
	Experiment 2	30	2			6	
	Experiment 3	45	3			6	
	Control	-	4			6	
4	Experiment	5	pool 1	30-day old fry	0.41	1000	8
	Control	-	pool 2			1000	

As the table demonstrates, the research at the first two stages was conducted with repeated variants, from 2 to 4. In the third and fourth stages, the study was conducted without repeated variants.

The duration of the experiment by stages depended mainly on the ability of the water regeneration unit to maintain the biological balance in the aquatic system. The experiment was stopped as soon as the system could no longer cope with the organic load, which grew as the weight of the fish increased. The exception was the fourth stage, which lasted only 10 days since the biological filter in the aquaculture system of the enterprise, where the research was conducted, failed after the second control catch, which resulted in a sharp deterioration of water quality. The fish began to die en masse, prompting the owner of the enterprise to plant the surviving fish in another aquatic system to prevent further losses of biological material.

Research Results and their Discussion

In the first stage of research, the smallest by age and weight experimental material was used, and the minimum, so-called basic dose of the drug "Chiktonik" in fish feed was applied. Three control catches were conducted during the stage: at the beginning, middle and end of the stage. The results of processing the material of control catches are presented in Table 2.

Table 2. *Changes in the total mass and number of experimental materials during the 1st stage of the experiment*

Variant	Aquatic system No.	Date of control catch		
		01.08.21	**10.08.21**	**20.08.21**
Weight of experimental material, g (± average to control, %)				
Control	1	4.25	82.00	296.00
	2	4.25	85.00	304.00
	average	4.25	83.50	300.00
Experiment	3	4.25	94.00	316.00
	4	4.25	87.00	310.00
	5	4.25	92.00	315.00
	6	4.25	82.00	325.00
	average	4.25	88,75 (+6,3)	316,5 (+5,5)
Fish survival, pcs (± experiment average to control, %)				
Control	1	56	49	45
	2	56	51	45
	average	56	50	45
Experiment	3	56	53	51
	4	56	55	52
	5	56	52	50
	6	56	54	51
	average	56	53,5 (+0,9)	51 (+13,3)

As the table demonstrates, the addition of the drug "Chiktonik" to fish fodder at a dose of 1 ml/kg had a positive effect on the growth and survival of juvenile clarius catfish. Thus, in terms of masonry accumulation, the advantage of the experimental variant over the control at the end of the stage was +5.5%, and in terms of survival – +13.3%.

The purpose of the following stages of the experiment was to explore the effect of high doses of vitamin-amino acid complex "Chiktonik" (5, 15, 30 and 45 ml/kg of fodder) on young clarius catfish in the laboratory (2 and 3 stages) and production conditions (4 stage). The results of the second stage of research are presented in Table 3.

Table 3. *Changes in the total mass and number of experimental materials during the 2nd stage of the experiment*

Variant	Aquatic system No.	Date of control catch			
		24.08.21	**04.09.21**	**14.09.21**	**29.09.21**
Weight of experimental material, g (± average to control, %)					
Control	1	32.00	116.00	482.60	930.00

Table 3. Continued

Variant	Aquatic system No.	Date of control catch			
		24.08.21	04.09.21	14.09.21	29.09.21
Control	2	32.00	115.00	529.00	1041.00
	average	32.00	115.50	505.8	985.5
Experiment 1	3	32.00	108.00	531.00	1320.00
	4	32.00	88.00	523.40	1207.00
	average	32.00	98,00 (–15,2)	527,2(+4,2)	1263,5 (+28,2)
Experiment 2	5	32.00	108.00	622.00	1406.00
	6	32.00	108.00	582.00	1352.00
	average	32.00	108,00 (–6,5)	602,0 (+19,0)	1379 (+39,9)
Fish survival, pcs (± experiment average to control, %)					
Control	1	82	72	62	50
	2	82	68	63	50
	average	82	70	62.5	50
Experiment 1	3	82	78	77	76
	4	82	74	70	70
	average	82	76 (+8,6)	73,5 (+17,6)	73 (+46,0)
Experiment 2	5	82	71	70	70
	6	82	65	61	60
	average	82	68 (–2,8)	65,5 (+4,8)	65 (+30,0)

According to the data of Table 3, a significant increase in the content of the drug "Chiktonik" in fodder resulted in the following effects: ten days after the start of the stage, according to the control catch, in experimental variants 1 and 2 there was a lag behind the control in terms of masonry accumulation by 15.2 and 6.5%, respectively. In variant Experiment 2, the lowest survival rate was recorded compared to the control and variant Experiment 1, in which, on the contrary, this indicator was the highest.

During the second "ten days" fish from experimental variants both reached and overtook fish from the control group in terms of masonry accumulation: Experiment 1 – by 28.2, experiment 2 – by 39.9% more than in the control. In terms of fish survival rate from the beginning of the experiment, the experimental variants demonstrated better results compared to the control: Experiment 1 – by 46.0, experiment 2 – by 30.0% more.

The task of the third stage of the experiments was to test high doses of the drug "Chiktonik" (15, 30 and 45 ml/kg of fodder) on catfish fry, twice as old as the experimental material of the 2nd stage and almost ten times larger. Due to the limited number of juvenile clarius catfish in this age group, the study was conducted without repeating the variants. The results of the third stage of the experiment are presented in Table 4.

Table 4. *Changes in the total mass and number of experimental materials during the 3rd stage of the experiment*

Variant	Aqua system No.	Date of control catch					
		01.10.21	**12.10.21**	**22.10.21**	**03.11.21**	**13.11.21**	**23.11.21**
Weight of experimental material, g (± average to control, %)							
Control	1	775	1080	1456	1906	2458	3050
Experiment 1	2	760	1031 (–4,5)	1364 (–6,3)	1863 (–2,3)	2502 (+1,8)	3572 (+17,1)
Experiment 2	3	770	1035 (–4,2)	1383 (–5,0)	1891 (–0,8)	2570 (+4,6)	3467 (+13,6)
Experiment 3	4	775	1040 (–3,7)	1380 (–5,2)	1896 (–0,5)	2050 (–16,5)	2560 (–16,0)
Fish survival, pcs (± experiment average to control, %)							
Control	1	6	6	6	6	6	6
Experiment 1	2	6	6	6	6	6	6
Experiment 2	3	6	6	6	6	6	6
Experiment 3	4	6	6	6	6	6	6

As the table demonstrates, the best result in terms of masonry accumulation was obtained in Experiment 1 (concentration of "Chiktonik – 15 ml/kg of fodder), the worst – in Experiment 3 (concentration of the drug – 45 ml/kg of fodder). The variant Experiment 2 had the second result, and Control – the third. During 34 days from the beginning of the experiment, research variants 1 and 2 lagged behind the control in terms of the rate of body weight accumulation but then reached and overtook the latter, and variant Experiment 3, on the contrary, increased the gap in this indicator. The difference between the results of the third stage and the second one can be explained by the fact that the metabolism of fish decreases with age, as the body's reaction to overcome the adverse effects of adverse factors, in this case – excessive concentration of vitamins, which could probably result in hypervitaminosis.

The third stage of the experiment demonstrated no advantages for any variant in terms of survival rate, as there was no fish death at all.

The fourth stage of the experiment was performed in the production conditions of an existing enterprise for the cultivation of clarius catfish in a recirculating aquaculture system. In this stage, there were only two options: control and experimental, with a concentration of "Chiktonik" in fodder of 5 ml/kg of fodder. The short duration of the experiment at this stage is explained by force majeure circumstances that arose at the enterprise – the failure of the biological filter a few days after the second control catch, which resulted in mass mortality of fish in the aquatic system pools and termination of the experiment. The results of the fourth stage, according to the indicators of two control catches, are presented in Table 5.

Table 5. *Changes in the total mass and number of experimental materials during the 4th stage of the experiment*

Variant	Pool No.	Date of control catch	
		01.10.21	11.10.21
Weight of experimental material, g (± average to control, %)			
Control	1	775	1080
Experiment	2	760	1031 (–4,5)
Fish survival, pcs (± experiment average to control, %)			
Control	1	1000	965
Experiment	2	1000	890 (–7,8)

As the table demonstrates, the results of the fourth, so-called production, stage of the experiment, in general, repeated the results of the second and third stages conducted in laboratory conditions. In addition, during the first ten days from the beginning of the experiment, fish in the experimental pool, which received fodder with vitamin-mineral additives, lagged behind the rate of body weight gain from fish in the control pool by 4.5%, and the mortality rate of juveniles was 7.8% higher than that in the control. It can be assumed that, in the case of continuation of the experiment, the indicators of fish in the experimental variant after a while would be equal to those in the control and possibly exceed them. To check this assumption, it is advisable to conduct a repeated experiment in production conditions.

Conclusions and Perspectives

According to the results of the research, it was established:

1. Vitamin-amino acid complex "Chiktonik" has a biologically active effect on larvae and fry of clarius catfish. It was established that at a concentration of 1 ml per 1 kg of fodder the drug has a positive effect on the growth and survival of clarius catfish under stressful situations.

2. At high concentrations (5, 15, 30 and 45 ml/kg of fodder) "Chiktonik" for some time (in research conditions – from 10 to 30 days from the beginning of the experiment) initially caused a slowdown in the growth of fish in experimental variants compared to fish in the control, which did not receive the drug supplement. Subsequently, fish from the experimental variants reached and overtook fish from the control group in terms of weight gain rate. Therewith, in younger fish, this process was faster: in a 30-day fry – during the next ten days, in older fish – in 20-30 days.

3. The survival rate of fish under the influence of vitamin-amino acid supplementation in the experiment, in general, significantly increased: by 13.3-46.0%, depending on the variant of the experiment.

4. It is considered promising to continue research to clarify the doses of the drug for different age groups of clarius catfish. In addition, it is advisable to evaluate the effect of the drug "Chiktonik" on the fertility of females, the quality of caviar and the offspring of clarius catfish.

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Ріст та виживаність кларієвого сома(*Clarias gariepnus* B., 1822) на різних стадіях вирощування з додаванням в корм препарату «Чиктонік»

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Анотація. Стресові ситуації призводять зниження темпу росту та виживаність риб, тому пошук шляхів зменшення їх негативного впливу є актуальним. Мета досліджень – оцінити в умовах аквакультури вплив різних концентрацій вітамінно-амінокислотного комплексу «Чиктонік» на ріст та виживаність личинок і мальків африканського кларієвого сома (*Clarias gariepinus* B., 1822) після стресових ситуацій. Стресова ситуація для риб виникала внаслідок значних коливань вмісту аміаку, нітритів та нітратів у водному середовищі замкнутої рециркуляційної аквасистеми протягом періоду запуску біологічного фільтра, до встановлення рівноваги. Було проведено серію дослідів, в ході яких встановлено, що додавання препарату з розрахунку 1 мл на 1 кг корму прискорює ріст риб у дослідних варіантах, у порівнянні з контролем. Експериментальне використання високих доз препарату (5, 15, 30 і 45 мл/кг корму) спочатку призвело до гальмування приросту маси тіла риб, але через 10-30 днів після завершення експерименту темп росту дослідного матеріалу зрівнявся з таким у контрольної групи риб і навіть перевищив показники контролю надалі. Встановлено позитивний вплив вітамінно-амінокислотного комплексу «Чиктонік» на виживаність молоді кларієвого сома на етапі завершення личинкового періоду життя і на перших стадіях малькового періоду. В експерименті з рибами старшого віку, які були повністю сформованими мальками, такого ефекту від використання препарату не спостерігалося: виживаність риб була на одному рівні як в досліді, так і в контролі. Одночасно було встановлено, що мальки гірше реагують на підвищені дози препарату, у порівнянні з підрощеними личинками. Темп росту мальків після використання високих доз препарату так і не зрівнявся з таким у риб з групи контролю протягом місяця після завершення експерименту на відміну від молодших за віком риб. Загалом доведено доцільність і безпечність практичного використання препарату «Чиктонік» для молоді кларієвого сома як кормової добавки в дозі 1 мл/кг рибного корму

Ключові слова: вітамінно-амінокислотний комплекс, встановлення біологічної рівноваги, годівля, стрес, аквакультура