

# Functional biologically active feed additive for breeding stock of fish

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**Abstract.** The work is devoted to the development and evaluation of the complex effect of a biologically active feed additive that meets the requirements of a functional feeding complex that ensures the regulation of physiological processes in the body of female breeding stock. In particular, influence of feed additives and its components on growth rate, quality of sexual products, reproductive process, embryogenesis, level of anomalies in the development of offspring, its growth potential was studied. The biologically active feed additive for the breeding stock included probiotic «Sporotermin», adaptogen «Trekrezan», vitamin-amino acid complex «Chiktonik». The hypothesis was tested that the developed feed additive, due to the peculiarities of its composition, is able to increase the growth rate of fish, optimize gametogenesis, reduce the level of pathology during embryonic development, increase the yield of larvae, increase their viability, reduce the level of developmental anomalies, increase survival and safety. The research results showed that the biologically active feed additive reduced the time of fish rearing, had a positive effect on the quality of female eggs and sperm in males, increased the quality characteristics of eggs, reduced the level of embryonic death during embryo incubation, increased the yield of larvae and their safety.

## 1 Introduction

In the conditions of modern civilization, when the demand for fish products is steadily growing all over the world and the requirements for its quality are becoming stricter, the development of high-tech industrial aquaculture plays an important role. The use of intensive fish growing technologies allows us to successfully regulate parameters of the environment, but excludes the availability of natural fish nutrition, which they receive in natural ecosystems. The right solution of the problems of a full and balanced diet is the key to the successful cultivation of commercial fish in industrial aquaculture [1]. Of particular importance are the issues of full-fledged feeding when raising females of the breeding stock, which are producers of caviar [1-2]. It is well known that the quality of offspring depends on the quality of the caviar.

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When raising broodstock females, as well as when growing commercial fish, the composition and quality of feed become decisive factors in this process [3]. Compound feeds balanced by the main elements of nutrition, for all their effectiveness, do not fully satisfy the physiological needs of the fish body [4]. This is due to the fact that fish food in their natural habitat contains a wider set of biologically active components that are the most important regulators of metabolic processes in their body. Feed additives that can solve the problem of deficiency of a particular nutrient can make up for the deficiency of biologically active substances in the body of fish in the conditions of aquaculture [4].

Our authoring team has developed a biologically active feed additive for functional feeding complexes that correct the work of fish body. The composition of feed additive included probiotic «Sporotermin», adaptogen «Trekrezan», vitamin acid complex «Chiktonik»; the composition of the components was not chosen by chance [5, 6].

The introduction of probiotic "Sporotermin" into the composition of functional biologically active feed additive for fish, which includes bacterial spores of *Bacillus subtilis* and *Bacillus licheniformis* at a dose of 1 g/kg of feed, is due to the need to normalize the intestinal microbiocenosis in fish, increase their nonspecific resistance and provide protection from pathogenic microbiota.

The adaptogen «Trekrezan» is introduced into a functional biologically active feed additive in the amount of 0.04 g/kg of feed, since it is able to have a general healing effect, increase antioxidant and hepatoprotective properties, increase stress resistance, activate immunity and hematopoiesis.

The vitamin-amino acid complex «Chiktonic» is introduced into the composition of a biologically active functional feed additive in the amount of 2 ml/kg of feed to enrich feed with vitamins and essential amino acids. The drug is necessary for the regulation of metabolism, the work of nervous system, hematopoiesis system, increasing growth rate and regenerative processes, to ensure proper development and reduce stress levels [7].

The aim of the work was to evaluate the effect of functional biologically active feed additive on the females of the breeding stock and growth characteristics of their offspring.

## **2 Materials and methods**

The studies were carried out on females and males of African sharptooth catfish and on their offspring, which were raised using mixed feeds «LimKorm», "Som". In the experiment, males and females of the African sharptooth catfish were used, who for 6 months received a biologically active feed additive, which included the probiotic «Sporotermin» - 2 g/kg of feed, the adaptogen «Trekrezan» - 0.04 g/kg, the vitamin-amino acid complex «Chiktonic» from the company «Invesa» 2 ml/kg of feed. Later, reproductive products for artificial insemination were obtained from such females and males. The control group of broodstock was grown on traditional mixed feeds «LimKorm» without biologically active substances. The quality of obtained reproductive products of females of the experimental and control groups was evaluated by a set of characteristic parameters.

To assess the quality and fertility of germ cells, approximately 100 oocytes were selected from freshly obtained caviar, to which 6% solution of sodium chloride was poured, then measurements and observations were carried out under a microscope using an eyepiece micrometer. When evaluating the quality, attention was paid to such characteristics of caviar as polarization of nucleus, presence of vacuoles, their size and location, presence of fat droplets and yolk inclusions.

Fertilization of ovulated freshly obtained eggs was carried out by the «dry» method. Immediately after fertilization, mature eggs were washed with an aqueous solution of adaptogen «Trekrezan» 40 mg/l for 15-20 minutes. When processing caviar of the control group of fish, «Trekrezan» was not used.

Further incubation of washed fertilized eggs of experimental and control groups took place in the Weiss apparatus. During the incubation of eggs of the experimental group, an aqueous medium was used, in which a probiotic, an adaptogen, vitamins and amino acids were present. In our studies, we used the same composition for the incubation of fertilized eggs as for feed additive, but all calculations were made for 1 liter of incubation medium. The medium for incubation of caviar contained all the above ingredients in the following ratios: probiotic «Sporotermin» 1g/l, adaptogen «Trekrezan» 0.04 g/l, vitamin-amino acid complex «Chiktonic» 1ml/l of the incubation medium.

The eggs of control group were incubated in an aqueous medium without the addition of functional ingredients.

The fertility of eggs in females of experimental and control groups was determined immediately by the field of fertilization, and then at different stages of embryonic development.

To detect developmental anomalies, developing eggs at different stages of embryogenesis during incubation were selected from the total portion, and then fixed for two minutes with a mixture consisting of 96% alcohol with glacial acetic acid in a ratio of 3:1. The quality of the embryos was evaluated under a microscope. The proportion of viable embryos that did not stop developing, anomalies of embryo development, and the yield of pre-larvae were determined.

To evaluate the research results, we used the following scale, according to which the pre-larval age fits in three days, the larval age lasts no more than 4 weeks, the fry stage covers 6 weeks, the juvenile stage - no more than 7, the commercial fish stage fits in the next 7 weeks.

### **3 Results**

At the first stage of research, reproductive potential of the broodstock females raised using functional feed additive was evaluated. The results of the studies are shown in the Table 1.

African sharp-tooth catfish, which was the object of research, is one of the fish with multi-portioned spawning [5]. All the characteristics given in table 1 are based on the indicators of the first portion of caviar.

The analysis of growth characteristics of females of experimental and control groups showed that the use of biologically active feed additive for six months allowed to accelerate the growth processes of females. At the beginning of the experiment, the weight of broodstock females in experimental and control groups did not significantly differ. At the end of the six-month period, the difference in the weight of the experimental and control groups was 530 g, and the difference in length exceeded 10 cm.

Further studies have shown that the ingredients of functional dietary supplement developed by us had a positive effect on the reproductive potential of females of breeding stock, increasing their working fertility by 22.5%. At the same time, the proportion of abnormal oocytes in ovulated caviar decreased by 6%, and the proportion of unripe caviar significantly decreased. No dead caviar was detected in females who received the feed additive and did not receive it. The calculation of the maturity coefficient showed that it is higher in females raised using a functional biologically active feed additive.

**Table 1.** Comparative assessment of reproductive potential of females when using biologically active feed additive.

Characteristics	Experiment	Control
Body length, cm	78,7±8,3	68,3±9,1
Body weight, g	2950±180,2	2420±160,4
Weight of caviar portion, g	356,0±46,5	253,0±39,5
Working fertility, thousands of eggs	158,1±17,3	129,2±15,6
The proportion of abnormal oocytes, %	to 10	to 16
Unripe caviar, %	8-12	15-20
Dead caviar	-	-
Maturity coefficient, %	12,0	10,4

Incubation of fertilized eggs is the most vulnerable link in reproduction [8]. During artificial breeding, fish eggs during the entire incubation period are the main factor in transmission of pathogenic bacteria to offspring. One of the reasons for the death of fertilized eggs during incubation is a high level of pathogenic microbiota.

Therefore, in order to reduce technological losses during embryo incubation, we tested and introduced a complex of biologically active substances into the incubation medium, which is the basis of a functional feed additive. This complex included a probiotic, an adaptogen, vitamins and amino acids. We used it for the proembryonic and embryonic processing of caviar. According to our hypothesis, a probiotic was necessary to increase protection against pathogenic microbiota, an adaptogen to activate the antioxidant defense system, vitamins and amino acids are necessary to correct the metabolism of embryos during critical stages of development.

It is known that embryonic death often occurs under the influence of pathogenic microbiota. The probiotic «Sporothermin», which is part of the feed additive, is known to be an alternative to antibiotics. The bacteria *Bacillus subtilis* and *Bacillus licheniformis*, on the basis of which this drug is produced, are not elements of normoflora in microbial communities of animals, but are able to indirectly maintain the normocenotic state [9]. Suppression of infectious agents by «sporothermin» can be realized directly by direct antagonism. The drug «Sporothermin» is effective against a wide range of microorganisms that cause infectious diseases of humans and animals.

In the study of embryonic development, which was carried out on fertilized eggs during its incubation, we identified critical stages, including: early gastrula, late gastrula, neurulation, organogenesis and pre-larval hatching. The results of the studies are presented in the table 2.

In general, the safety of embryos and hatched pre-larvae from the fertilized eggs of females who received «LimKorm» mixed feed enriched with a functional biologically active feed additive, and then incubated in the presence of probiotic, adaptogen and vitamin-amino acid complex was 78%. The safety and viability of embryos incubated in water without the addition of biologically active ingredients was 54.5%. The difference is obvious.

In the control group that did not receive a functional dietary supplement, the highest percentage of death was observed at the gastrula stage. During gastrulation, the total death rate was more than 20%, it was high, but less distinct, at the stage of pre – larval hatching-9.7%.

Embryos incubated in an environment with a probiotic, adaptogen, amino acids, vitamins, obtained from an experimental group of females raised against the background of functional dietary supplement, were more viable, so their embryonic death at gastrula stage totaled 13.6%, and at pre - larval hatching stage-5.4%. Less distinct death of embryos in the

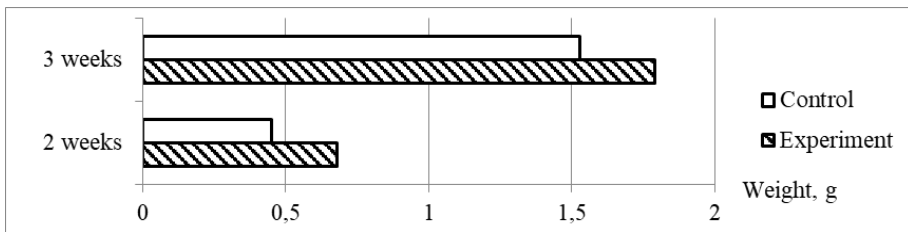
experimental group was observed at the neurula stage and during organogenesis. The results of studies are shown in the Table 2.

**Table 2.** Indicators of death of female embryos raised with and without a feed additive (control).

Stages of embryonic development	Experimental group	Control group
Early gastrula, %	5,5±1,9	9,8±1,5
Late gastrula, %	8,1±1,8	11,5±1,7
Neurula, %	2,0±1,5	6,1±2,5
Organogenesis, %	3,0±1,2	8,4±2,8
Prolarva hatching, %	5,4±1,7	9,7±2,7

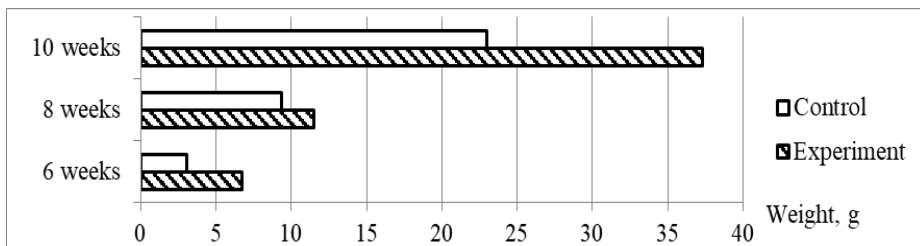
Further, we continued our observations of growth and development of offspring of females of the experimental group who received a functional feed additive at larval stage, at fry stage and at juvenile stage during their cultivation. During the growing period, these offspring, by analogy with their parent individuals, received functional feed additive in the diet, including a probiotic, adaptogen, vitamin-amino acid complex. The results of the research are presented in Figures 1-3.

Fish larvae that received functional feed additive in starting feed at two and three weeks of age showed higher growth rate compared to their peers who did not receive functional feed additive (Figure 1).



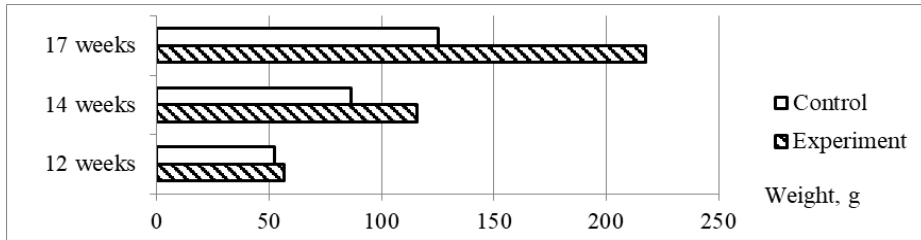
**Fig. 1.** Comparative indicators of biomass growth in larval period when using biologically active additive and without it.

Fish fry obtained from females raised on feeds that included functional biologically active feed additive containing probiotic, adaptogen, vitamin-amino acid complex also received this feed additive. The results of the study showed that the most significant increase in biomass, against the background of the feed additive, was given to fry at 6 and 10 weeks of cultivation. In the rest of the time, the difference in growth rate compared to the control group of fry who did not receive feed additive was less pronounced. However, during all growth periods, the rate of biomass growth in the fry of the experimental group was higher than in the control group. The results are shown in Figure 2.



**Fig. 2.** Comparative indicators of biomass growth in fry against biologically active additive and without it.

The fry of experimental group grew up and became young. Next, we investigated the effectiveness of using feed additive at the next stage of fish ontogenesis - at the stage of juveniles. Studies on the use of feed additives in experimental group of African sharptooth catfish juveniles showed that their growth rate during all growing periods was significantly higher than in the control group. With the age of the juveniles, the difference in the growth rate of fish of experimental and control groups progressively increased and reached its maximum values at the 17th week of cultivation (Figure 3).



**Fig. 3.** Comparative indicators of biomass growth in juveniles against biologically active additive and without it.

## 4 Discussion

It should be noted that the problem of fish reproduction is one of the most urgent, and at the same time the least developed in industrial aquaculture [10]. A large percentage of caviar obtained from females as a result of hormonal stimulation and subsequent pumping is characterized by various disorders [11]. Often, these disorders in the conditions of artificial breeding reach the level of 70%. Externally, this is manifested in the deformation of oocytes. Internal factors of such deviation from the norm are protein deficiency in oocytes, which is accompanied by a decrease in the definitive size of germ cells. The embryonic development of such oocytes proceeds with violations of varying degrees of severity [12].

The pre-larvae hatched from such caviar are smaller in size than the standard of the species and they are characterized by developmental anomalies. They are expressed in disorders of vertebral column, digestive system, organs of vision, respiration, etc., of varying degrees of evidence [13-15].

In order to improve the quality of eggs and reduce the level of developmental abnormalities, we used a functional feed additive in the breeding of broodstock females and subsequently used in the reproduction of the offspring of these females, introducing probiotic, adaptogen, vitamins and amino acids into the incubation medium of fertilized eggs to reduce embryonic death.

The analysis of research results also revealed that after artificial fertilization, the eggs of females raised using a functional biologically active feed additive during their individual development at all stages of embryogenesis were more viable and showed higher survival than those of females raised on compound feeds «Limkorm», «SOM».

The study of embryonic death showed that during the cultivation of fertilized eggs, the highest percentage of embryo death was observed during the passage of critical stages of development. According to the results of our research, this was associated with the beginning of gastrulation, the end of gastrulation, the formation of the rudiment of the heart, hatching from egg shells. The "peephole" stage, which came with the appearance of pigmentation of the embryo's eyes, was a landmark for the completion of critical periods of development. The effect of the use of biologically active substances such as probiotic, adaptogen, vitamins and amino acids during incubation of emrions didn't provoke doubt.

## 5 Conclusion

The use of functional feed additive in cultivation of broodstock females has shown that qualitative characteristics of germ cells of broodstock females have significantly improved in all indicators, and their ovulated caviar has a much greater development potential than the caviar of females grown on conventional compound feeds.

As a result of the conducted studies, it was shown that against the adaptogen, probiotic, vitamins and amino acids used in the incubation medium, embryonic death at the stage of gastrulation decreased by 40%, also decreased, although to a lesser extent, at the stage of organogenesis, at the stage of formation of the rudiment of the heart, at the stage of hatching (exit of pre-larvae).

The use of functional biologically active feed additive allowed to increase the survival rate of embryos, depending on the stage of development, by more than 20%.

The conducted studies allowed us to establish that the functional feed additive developed by us, including probiotic «Sporothermin», adaptogen «Trekrezan» and vitamin-amino acid complex «Chiktonic», increased productivity of fish, accelerated the growth of female African sharp-tooth catfish, increased reproductive potential, working fertility, improved the quality of caviar, increased fertility, reduced embryonic death at all stages of development and increased the yield of pre-larvae.

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