Assessment of the impact of pond aquaculture on water resources against the background of the use of biogenic feed additive Akwa-Biot-Norm

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Abstract. A biogenic feed additive based on protein components and immunotropic preparations intended for the fulfillment of the bio-resource potential of fish by activating non-specific and specific factors of organism protection against eco-technological pressing and for prevention of infectious and invasive diseases improving competitiveness and the effective functioning of fish farms was developed and suggested for use on fish farms. It was established that upon the use of Akwa-Biot-Norm biogenic feed additive based on a polysaccharide complex of Saccharomyces cerevisiae (S. cerevisiae) yeast cells; the average weight of carps at the end of the experiment period was 2.19% higher than the control index in comparison with 2.71% for the live weight gain. With the maintenance of the pond aquaculture we observed moderate impact on water resources, but the use of Akwa-Biot-Norm biogenic feed additive in the carp growing technology reduces the degree of impact of the pond aquaculture on water resources. For example, the locality of the experimental group pond in relation to the locality of the control group pond; the quantity of readily oxidizable organic matter was 2.52% higher compared to 1.28% in the case of labile organic matter. The amount and biomass of phytoplankton increased on 4.08 and 9.86%, respectively.

1. Introduction
The exponential growth of the population adversely affects the natural resources, including the fish reserves in lakes, rivers, and oceans. Therefore, aquaculture production must be expanded without significant use of the essential natural resources to promote sustainable farming [1].

For decades, the ‘land-locked’ central European countries have been relying mostly on carp culture for fisheries production. Common carp (Cyprinus carpio L.) farming in fishponds has remained the mainstay, both traditionally and commercially. About 80-88% of the aquaculture production in these countries come from carp farming in fishponds [2].

Aquaculture is one of the most vibrant sectors of the global food production system, providing roughly 50% of the worldwide fish consumption (Food and Agriculture Organization of the United...
Nations, 2013). Its impressive rise during the last two decades has generated both optimism and trepidation particularly, among aquaculturists and policy experts, concerned with global aqua food security. Globally, cultivation of finfish and shellfish in different aquaculture systems grew at an annual rate of 7.8% during 1990 to 2010. This rate is substantially higher than that of poultry (4.6%), pork (2.2%), dairy (1.4%), beef (1.0%), and even food grains (1.4%) over the same period [3].

All human activity that exploits natural aquatic ecosystems poses risks of altering their physicochemical and biological quality. This applies especially to intensive aquaculture since it requires a constant supply of water of sufficient quality to provide environments conducive to fish development and survival. To understand the mechanisms of fish farm water pollution, it is necessary to take into account that both fish metabolites and unconsumed feed are burdens for the aquatic environment in which the breeding is conducted. Aquaculture methods are changing constantly, which follows the general tendency in animal breeding towards mass production and shortening production cycles using modern technical and technological solutions. This aim is achieved in intensive aquaculture by high density stocking per unit area or water volume, providing fish with high-protein, high-energy feed, and observing proper health standards. However, this is not easy, since excessively high stocking densities can stress fish, which, in turn, has a negative effect on survival rates and growth. Breeding fish in densely stocked ponds also increases risks of spreading infectious and parasitic diseases [4].

Determining the suitability of aquaculture ponds is one of a valuable factor in sustainable aquaculture. One of the factors to consider in establishing a successful aquaculture is aquatic environment, i.e.: the primary productivity of waters and physical-chemical factors. The value of primary productivity describes aquatic trophic and natural food availability [5].

The aquaculture water in ponds is not only the source of aquatic breeding and cultivation, but also the decomposition site of feces, bait, etc. The decomposition of these substances not only increases the load of organic matter in water, but also accumulates in the sediment of the pond, aggravating the endogenous pollution of the water. Different systems or combinations of pond aquaculture resulting from a variety of cultured species, density, and breeding, have divergent effects on the water environment [6].

Technological processes of industrial fish farms cause, in some way or another, pollution of the aquatic environment with products of metabolism of growing fish and remains of feed not eaten by it, which results in an increase in the level of primary producing – eutrophication [7]. A large part of aquaculture waste mostly sinks to the bottom of the water body under ponds or in direct vicinity to them, which causes higher consumption of oxygen by the benthonic ecoculture due to its oversaturation with organic remains [8]. This makes it possible to predict an increase in the organic nitrogen remineralization rate, biomass reduction, and change in the species composition of the bottom community [9]. It can finally lead to the creation of lifeless areas under the fish breeding ponds that lack oxygen and have a high concentration of carbon dioxide, methane, and hydrogen sulfide. Besides, a high density of growing fish predisposes to the development and fast spread of diseases of infectious and other etiology [10].

The water-quality parameters of all ponds remained within ranges allowing high fish growth rates. The main primary factors influencing the abiotic and biotic properties of the pond water were time and sediment resuspension by common carp. The effect of time on water quality, and the abundance of phytoplankton, zooplankton and benthic macro invertebrates in ponds with different densities of common carp are clearly shown in study [11]. Decreasing DO concentrations with increasing common carp density reflects additional respiration by common carp and higher aerobic decomposition rates caused by common carp sediment resuspension during grazing on benthic macro invertebrates. Higher aerobic decomposition can be supported by higher sediment resuspension, which was greater in ponds with 1 common carp in m², followed by 1 common carp, and without common carp [11].

The effects of two benthivorous fishes, common carp (Cyprinus carpio) and calbasu (Labeocalbasu), on bottom soil resuspension, water quality, nutrient accumulations, fish diet and growth of rohu (Labeorohita) were compared. Resuspension of bottom soil, free CO₂, and nitrogen
and phosphorous concentrations in the water column were greater in the tanks with common carp while dissolved oxygen, pH, and total alkalinity were lower. Gut contents of harvested calbasu contained a combination of benthic macroinvertebrates and zooplankton, rohu contained phytoplankton and zooplankton, and common carp contained almost entirely (82%) macroinvertebrates [12].

Microbes, as an important part of aquaculture ponds, can improve the recirculation of nutrients. This nearly 1-year-long study aimed to understand the factors and mechanisms that cause temporal changes in the concentration of microbiological indicators and nutrient concentration in the grass carp pond water of Chongqing Jiguan Aquaculture Limited. Principal component analysis revealed that pathogens (Pseudomonas and Flavobacterium) are a significant negative correlation with water temperature and a positive correlation with ammonium concentration and dissolved oxygen [13].

All the above said makes it impossible to consider aquaculture fields as ecologically safe directions of human activity [14]. In water bodies used by the aquaculture, in conditions of intensive growing of hydrobionts, in particular, fish, a specific ecosystem, with different qualitative indicators, is formed, which can negatively affect natural water sources and ecology, as a whole. In addition, the existing reverse correlation of quality of water in the natural water body and aquaculture objects will finally, lead to a reduction of effectiveness of fish farming processes and quality of products [15].

The aim of the present study is to assess the impact of pond aquaculture on water resources as a result of carrying out of production fish farming processes upon the use of Akwa-Biot-Norm biogenic feed additive [16].

2. Materials and methods

The first part of the work was carried out in conditions of the laboratories of the Department of Morphology, Obstetrics, and Therapy and the Department of Epizootology, Parasitology and Veterinary and Sanitary Inspection” of the Federal State Budgetary Educational Institution of Higher Education “Chuvash State Agricultural Academy” (Russia); namely, a technology of introducing Akwa-Biot-Norm biogenic feed additive in the composition of complete feeds for carps was developed. Akwa-Biot-Norm biogenic feed additives a suspension of 50 weight parts of 1% gelatin solution, 1 weight part of S. cerevisiae purified polysaccharide complex concentrate and 3 weight parts of levomisole.

The second part of the research work was carried out in production conditions of the full-system pond fish farm of LLC Fish Farm “Kirya”. Within the framework of the scientific and production experiment Akwa-Biot-Norm biogenic feed additive was tested on “Petrovsky” cross carps.

LLC Fish Farm “Kirya” is located in 0.5 km to the north-west of the settlement of Kudeikha, Poretsk Region of the Chuvash Republic (Russia). The fish farm was established in 1979 as a subdivision of the feeding state-owned farm “Zasursky”, Poretsk Region. In 1982 the fish farm started to operate as a standalone enterprise.

LLC Fish Farm “Kirya” is a full-system pond fish farm. The total area is 310 ha. The total number of ponds is 24, having the area of 293.7 ha, including: 6 spawning ponds – 1.2 ha, 4 summer stocking ponds – 2.9 ha, 3 wintering ponds – 2.7 ha, 3 winter stocking ponds – 0.3 ha, 5 nursery ponds – 66.6 ha, 3 finishing ponds – 219.0 ha. The water is supplied to the ponds from the Kirya river on a forced basis. The design capacity with regard to production of stocking material is 30 t, with regard to the commercial fish - 290 t.

Two carp breeds are grown on the fish farm “Kirya”– “Chuvashsky scalp carp” and “Anishsky mirror carp”, “Petrovsky” cross, created in the Chuvash republic as well as herbivorous fish brought from the southern regions of the country. Apart from the carp, the silver carp and pike are grown and earlier the pélyad fish was grown on the fish farm “Kirya”.

A complex of fish farming and a melioration measures with the account for the season, age of fish, water source quality, and presence of animals being intermediate hosts of fish was developed for each category of pond.

For the purpose of the experiment, two groups of carps with average weight 66.8±0.73 and
66.4±1.08 g (500 heads in each), respectively, were isolated to breeding ponds 1 ha each. In the experience, the carp of the breeds “Anishsky mirror carp” was used. Cultivation was carried out under optimal conditions for this fish. The same physical and chemical parameters of the aquatic environment (physical and chemical parameters of the aqueous medium are given in table 2) as well as fish feeding conditions were adjusted in both fish groups. The temperature of the pond was monitored daily at 7 am, 1 and 7 pm; the water content of oxygen dissolved therein and the hydrogen index were determined once a week. Feeding of fish was carried out two times in the daylight using feed tables. All individuals of the control and test carp groups received a high-nutritional granular combination feed consisting of wheat (20.5%), barley (22.5%), wheat bran (13%), sunflower cake (34%), full-fat soybean (3%), protein feed mixture (4%), meat and bone flour (1.5%), and limestone flour (3%). These feedstuffs were prepared at the Saratov Feedstuff Plant open joint-stock company. The fish of the experimental group received Akwa-Biot-Norm biogenic feed additive in the amount of 25 ml/kg of feed for 14 days. The observation period lasted 20 weeks.

For the duration of the experiment there was a control catch of carps every 7 days for diagnostic examination and control weighing using a VN-series floor balance for live fish with the aim of revealing the growth dynamics.

In order to assess the impact of the pond aquaculture on water resources, at the end of the experiment period water samples were taken directly from the place of experimental groups ponds. And from the control area as close to the ponds as possible, but with no influence of the fish farming processes. In the water samples the content of readily oxidizable organic matter [17], labile organic matter [18], total and mineral phosphorus (Ptot and Pmin) [19], nitrogen compounds (NH4+, NO2-, NO3-) [20], quantity of phytoplankton, zooplankton and benthos were measured in Bogorov and Naumann counting chambers and their biomass were determined by the geometric method.

3. Results and discussion

The dynamics of the weight of carps and their average daily gains of the studied groups upon the use of Akwa-Biot-Norm biogenic feed additive is shown in table 1.

Table 1. Dynamics of carp weight and its average daily gains upon use of Akwa-Biot-Norm biogenic feed additive.

| Experiment period, weeks | Average weight of carps | Average daily weight gains of carps |
|--------------------------|-------------------------|-----------------------------------|
|                          | Control | Experimental | Control | Experimental |
| Start                    | 66.8±0.73 | 66.4±1.08    | –       | –            |
| 1                        | 91.8±1.13 | 91.6±1.26    | 3.57±0.25 | 3.60±0.22 |
| 2                        | 114.1±1.17 | 114.8±1.12 | 3.19±0.13 | 3.31±0.20 |
| 5                        | 174.7±1.22 | 177.3±1.67 | 2.77±0.14 | 2.93±0.13 |
| 10                       | 291.5±2.64 | 297.1±2.64 | 3.44±0.13 | 3.69±0.18 |
| 15                       | 367.5±3.12 | 375.3±4.56 | 1.90±0.14 | 2.21±0.17 |
| 20                       | 420.6±6.11 | 429.8±7.02 | 1.69±0.11 | 1.51±0.13 |

According to table 1, the average weight of carps of the control and the experimental groups, with no statistically significant difference at the beginning of the experiment, steadily increased up to the end of the observation period – week 20 of the experiment. Starting with the second week and up to the end of the observation period, the weight of carps of the experimental group was higher than that of the Control Group. For example, at the end of week 5, 10, 15 and 20 of the experimental period, the weight of carps of the experimental group was 2.6 g, 5.6 g, 7.8 g and 9.2 g, or 1.49 %, 1.92 %, 2.12 % and 2.19% higher than the weight of the carps of the control group. It should be noted that the difference of the average weight of carps between the groups had no statistical significance. The absolute weight gain of carps of the experimental group was also higher than that in the control group: at the end of week 5 – by 3.0 g, or 2.78 %, week 10 – by 6.0 g, or 2.67 %, week 15 – 8.2 g, or 2.73 %,
and the end of the experiment– by 9.6 g, or 2.71%.

The value of the average daily weight gain of carps of the studied groups had similar dynamics. In certain periods the given index was higher in the control group, in other periods – in the experimental group, but, nevertheless, during the whole period of the experiment the average daily weight gain was 0.07 g higher in carps of the experimental group.

The dynamics of the absolute weight gain of carps of the groups by weeks throughout the study upon the use of Akwa-Biot-Norm biogenic feed additives shown in figure 1. According to figure 1, the values of the absolute weight gain of carps of the control and experimental groups had similar dynamics, but, nevertheless, in the experimental group, upon the use of the tested biogenic feed additive, the average absolute weight gain for the whole period of the experiment was 0.48 g higher per week than that of the control group.

Figure 1. Absolute weight gain of carps during the experiment.

Thus, the results of weighing fish demonstrate that at the end of the experiment the average weight of fish of the experimental group was 2.19% higher than in the control group and made up 429.8±7.02 g (average weight in the control group was 420.6±6.11 g). The gain in the live weight of the experimental group fish made up 363.4±5.96 g, which is 9.6 g, or 2.71% higher than in the control group (figure 1). As a result, Akwa-Biot-Norm biogenic feed additive, when included in the composition of feed for carps, has a growth-stimulating effect.

A similar result was to evaluate the effects of Chinese herbal medicines mixture (CHMM: consisted of asafoetida Ferula sinkiangensis K. M. Shen, Medicago falcata L. and Allium sativum) on feeding attraction activity (FAA), growth performance, nonspecific immunity and digestive enzyme activity of juvenile Japanese seabass (Lateolabrax japonicus). The 28-day feeding experimental results indicated that dietary CHMM significantly influenced weight gain (WG) and specific growth rate (SGR) of fish (P < 0.05), with the highest WG and SGR observed in fish fed 20 g/kg CHMM diet [21].

The sanitary and hygienic conditions of the waters, adherence to technical processes, and quality of feed influence the effectiveness of aquaculture. It is not always possible to consider these factors in production. Fish is constantly subjected to stress factors related to disruptions in the oxygen and temperature conditions, and the use of low-quality feeds. As a result, this leads to the development of infectious processes caused by various microorganisms.

One of such diseases is saprolegniosis, a disease affecting fish and spawn spreading both in natural and industrial water bodies. The causative agents of this disease areoomycetes mold fungi of Saprolegniales order: Saprolegnia, Leptolegnia, Achlya, Dictyuches, Aphanomyces and others. The most pathogenic of them are A. flagellata, D. monosporus, S. Mixtra, A. laevis, and others. They have the ability to pass from saprophyte to necrophyte. Being saprophytes, they are present in water and ground. All artificially bred fish are prone to the disease. Saprolegniosis is considered to be a
Secondary disease arising in damaged places, in conditions of resistance level reduction and also in case of other infectious and invasive diseases.

During the study we revealed the diseases of fish having the following clinical signs: white thin lines on fins and skin perpendicular to the fish body surface. In a couple of days, a cottony white-color coat consisting of interwoven hyphae was formed in these regions. This was especially evident during the microscopic examination of a skin scraping. Based on these signs, a preliminary diagnosis was made for saprolegniosis. Pathological material was selected for laboratory analysis.

Throughout the fish examination in the control and experimental groups showed the ratio of carps’ diseases in per cent. According to the examination results, the percentage of infection was 13.6 % in the control group and 9.4% in the experimental group. Fish of both groups were subjected to prevention and treatment procedures in accordance with the “Temporary instruction on measures of fighting saprolegniosis of fish and spawn on fish farms” [22].

When analyzing the pathological material in the laboratory, after obtaining a pure culture and growing on Czapek's medium the saprolegniosis-causing pathogen was revealed. No other diseases of invasive and infectious etiology were revealed. The survival rate of fish during the whole period was 86.2 % in the control group and 89.4 % in the experimental group. Thus, according to the results of the conducted experiment, the use of Akwa-Biot-Norm additive for “Petrovsky” cross carps increases resistance to diseases (such as saprolegniosis) related to negative impact of environmental stress factors. Probiotics have been proposed as one of the alternatives to the chemical treatments currently used in aquaculture (MDPI, Basel, Switzerland). Recently, the possible usefulness of certain microorganisms, mainly bacteria, has been highlighted as a potential biocontrol for saprolegniosis [23].

The content of readily oxidizable and labile organic matter, total and mineral phosphorus (Ptot and Pmin), nitrogen compounds (ammonium – NH4+, nitrous acid salt – NO2-, nitric acid salt – NO3-), quantity and biomass of phytoplankton, zooplankton, and benthos in water samples are given in tables 2 and 3.

### Table 2. Indices of condition of water resources.

| Index                          | Sampling place          |
|-------------------------------|-------------------------|
|                               | control area | control group pond | experimental group pond |
| Readily oxidizable organic matter, mg O/l | 13.2         | 11.9               | 12.2                      |
| Labile organic matter, mgO2/l | 1.73          | 1.56               | 1.58                      |
| ammonium (NH4+), mg/l         | 0.16          | 0.23               | 0.21                      |
| nitrous acid salt (NO2-), mg/l| 0.003         | 0.006              | 0.005                     |
| nitric acid salt (NO3-), mg/l | 0.16          | 0.27               | 0.25                      |
| Pmin, mg/l                   | 0.05          | 0.07               | 0.07                      |
| Ptot, mg/l                   | 0.014         | 0.019              | 0.018                     |
| Phytoplankton                |             |                    |                           |
| - quantity, thousands/l       | 147          | 98                 | 102                       |
| - biomass, g/m³              | 0.309         | 0.213              | 0.234                     |
| Zooplankton                  |             |                    |                           |
| - quantity, thousands/m³     | 81.9         | 104.6              | 101.4                     |
| - biomass, g/m³              | 1.42          | 1.84               | 1.78                      |
| Benthos                      |             |                    |                           |
| - quantity, instances/m²     | 274          | 339                | 321                       |
| - biomass, g/m²              | 1.15          | 1.43               | 1.33                      |
The results of the comprehensive research shown in table 2 are indicative of moderate impact of the pond aquaculture on water resources. For example, in the area of location of ponds the content of readily oxidizable organic matter decreased by 7.58-9.85 %, than that of labile organic matter – by 8.67-9.83 %. The quantity of total and mineral phosphorus increased by 28.57-40.0 %, than that of nitrogen compounds – by 31.25-100.0 %. Mean while the phytoplankton amount decreased by 30.61-33.33 %, while its biomass decreased by 24.27-31.07 %. On the other hand, the quantity of zooplankton and benthos increased by 23.81-27.72 % and by 17.15-23.72 %, while their biomass increased by 25.35-29.58 % and 15.65-24.35 % (table 2).

In addition, we should point out less evident changes in parameters of assessment of water resources with respect to the control area in the area of location of ponds of the experimental group of carps. For example, in the area of location of ponds of the experimental group with respect to the area of location of ponds of the control group the quantity of readily oxidizable organic matter was 2.52 % higher, while that of labile organic matter was 1.28 % higher. The quantity of nitrates was 7.41-16.67 % lower and the quantity of total phosphorus was 5.24 % lower. The quantity and biomass of phytoplankton were 4.08 and 9.86 % higher. Meanwhile in case of zooplankton, they were 3.06 and 3.26 % lower, while those of benthos revealed 5.31 % and 6.99 % lower (table 3).

Table 3. Degree of impact of aquaculture on water resources.

| Parameter                        | Cage of control group / control zone, % | Cage of experimental group / control zone, % | Cage of experimental group / cage of control group, % |
|----------------------------------|----------------------------------------|---------------------------------------------|-----------------------------------------------------|
| Readily oxidizable organic matter | 90.15                                  | 92.42                                       | 102.52                                              |
| Labile organic substances        | 90.17                                  | 91.33                                       | 101.28                                              |
| ammonium (NH₄⁺)                  | 143.75                                 | 131.25                                      | 91.30                                               |
| nitrous acid salt (NO₂⁻)         | 200.00                                 | 166.67                                      | 83.33                                               |
| nitric acid salt (NO₃⁻)          | 168.75                                 | 156.25                                      | 92.59                                               |
| P₉₀     | 140.00                                 | 140.00                                      | 100.00                                              |
| P₉₅     | 135.71                                 | 128.57                                      | 94.74                                               |

Phytoplankton
- quantity                  | 66.67                                  | 69.39                                       | 104.08                                              |
- biomass                   | 68.93                                  | 75.73                                       | 109.86                                              |

Zooplankton
- quantity                  | 127.72                                 | 123.81                                      | 96.94                                               |
- biomass                   | 129.58                                 | 125.35                                      | 96.74                                               |

Benthos
- quantity                  | 123.72                                 | 117.15                                      | 94.69                                               |
- biomass                   | 124.35                                 | 115.65                                      | 93.01                                               |

As a result, upon pond aquaculture maintenance, moderate impact on water resources was observed; however, the use of Akwa-Biot-Norm biogenic feed additive in the carp breeding technology reduces the degree of impact of the pond aquaculture on water resources.

4. Conclusion

Upon use of Akwa-Biot-Norm biogenic feed additive the average weight of carps at the end of the experiment was 2.19 % higher than that of the control group, while the live weight gain was 2.71 % higher. Addition of Akwa-Biot-Norm biogenic feed additive to the feed for carps increases the resistance of fish to diseases and ensures a higher survival rate. For example, there were 1.45 times fewer individual carps affected by saprolegniosis in the experimental group than in the control group, while the survival rate was 3.2 % higher.
The suggested technology of the introduction of Akwa-Biot-Norm biogenic feed additive in composition of feed for carps reduces the negative impact on water resources as a result of undertaking of production fish farming processes. The analysis of physical and chemical conditions of the water body showed that all hydrochemical water indices met requirements for carp breeding and were within the optimal ranges in [24]. As a result, upon pond aquaculture maintenance, moderate impact on water resources was observed; however, the use of Akwa-Biot-Norm biogenic feed additive in the carp breeding technology reduces the degree of impact of the pond aquaculture on water resources.

Disclosed is a unique, unparalleled, method of producing a biogenic feed additive based on yeast cell polysaccharides. Recommendations have been developed for application in world practice to protect water resources and ensure environmental safety in the area of activity of pond aquaculture facilities. It is recommended to feed fish with combined feed enriched with biogenic feed additive Akwa-Biot-Norm at the rate of 25 ml per 1 kg of feed for 14 days. As a result, the degree of impact of pond aquaculture on water resources is reduced, growth stimulation and prevention of fish diseases are achieved.

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