ELABORATION OF ECOLOGICALLY SAFE NEW FEED ADDITIVES FOR INDUSTRIAL FISH FARMING BASED ON NATURAL MATERIALS

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INTRODUCTION

The increased impact of mankind civilization on environmental, persistent environmental pollution, constant growth of humanity population world around and the increasing catch of aquatic biological resources will eventually exhaust the possibilities of nature. Currently, up to 40% of the world's fish consumed is artificially bred and farmed [1]. Available freshwater resources and favorable climatic conditions in the Republic of Armenia have led to the presence of a developed industrial fish farming. Obviously, the search for possible sources of local origin in order to create new components and additives for the production of highly effective feed for fish farming is urgent and important. Another one important problem is the non-competitiveness of domestic feed for valuable fish species compared to foreign ones in terms of shelf life. So, natural mineral sorbents compounds, such as zeolite, marls, bentonites, diatomite, tripoli, etc., are used in the production of feed additives for farm animals. That natural mineral sorbents from different deposits differ in their chemical composition, color, properties, content of various impurities. Therefore, they have different efficiency of use in feeding animals. It should be noted that all natural mineral sorbents have unique properties of adsorbents and ion exchangers. This is determined by the structural features of their crystal lattice, in which up to 50% of the volume is voids and channels [2].

The use of natural mineral additives in world practice shows that their inclusion in the diet of animals strengthens the immune system, increases the digestibility of feed, normalizes metabolism, and removes toxic and harmful metabolic products from the body [3]. The results which were carried out on tripoli by specialists of Institute of Fish Industry of the National Academy of Sciences of Belarus have demonstrated the positive influence of it on fish growing [4].The use of tripoli in feeding carp revealed that the optimal dose of input for carp fingerlings is from 1.5 to 4%, and for the older age group - up to 5%.

Armenia is rich in deposits of natural sorbents, such as like bentonite clays and diatomites. Diatomites (or Diatomaceous earth) arose during the sedimentation of siliceous valves of diatoms (algae Diatomeae or Bacillariophyceae) and are of freshwater or marine origin. They are widely known: Noyemberyan region's clinoptilolite, Shirak region's mordenite, bentonite clays of Ijevan region and diatomites from Jradzor and Sisian regions of Armenia. They are formed from diatomaceous silt accumulated in the seas and lakes. In the stratigraphic section, they are found starting from the Cretaceous system, and are widely distributed in the Cenozoic deposits. Diatomite and tripoli are chemically similar and have the similar properties. The typical chemical composition of oven-dried diatomaceous earth is 80–90% silica, with 2–4% alumina (attributed mostly to clay minerals), and 0.5–2% iron oxide, but tripoli is geologically older, compared to diatomite. For both marine or lacustrine origin, the content of organogenic amorphous silica in them is high (70...95%) [5].

Available freshwater resources and favorable climatic conditions in the Republic of Armenia have led to the presence of developed industrial fish farming. According to the data of the Ministry of Agriculture of Armenia, at present the total water surface of operating fish farms in the republic is more than 3,500 ha; about 14 thousand tons of fish are produced, of which 65...70% are valuable species (salmon, sturgeon). Obviously, the search for possible sources of local origin in order to create new components and additives for the production of highly effective feed for fish farming is urgent and important.

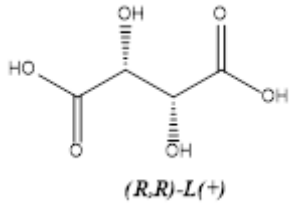
Feed additive based on wine production waste is currently being developed, which will be a natural antioxidant and preservative to extend the shelf life of animal feed, reduce bacterial contamination and the development of pathogenic microorganisms. Thus, the combined feeds used in fish farming include nutritional and bioactive compounds (BAA: unsaturated fatty acids, amino acids, carbohydrates, hormones,

vitamins, carotenoids, etc.) and that are exposed to oxidation during storage [6]. One of the prospective compounds groups, which are harmless and simultaneously active against the spoilage factors and pathogen microorganisms, are tartaric acid and their derivatives [7].

The main purpose of the current research was to study the effectiveness of usage of different doses of diatomite, as component the composition of feed for carp fingerlings with possibilities for various bioactive target compounds addition. In particular study the effects of tartaric acids new antibacterial derivatives on carp fingerlings were described.

METHODS

During the series of *in vitro* and *in vivo* experiments that was evaluated the antimicrobial activity and efficiency of new additive of natural tartaric acid derivatives as feed component. These compounds were synthesized in basic research laboratory of "Agrarian Pesticides Creation and The Quality Control" of National Polytechnic University of Armenia (NPUA) (fig.1) [8].



A **B**

Fig 1. Structures of Tartaric acid and BI.
A: Natural L-tartaric acid; B: Benzylimide of tartaric acid
(BI or 1-benzyl-3,4-dihydroxy-3l3-pyrrolidine-2,5-dione).

As a natural sorbent-carrier in additive was diatomite (1,5% -5% to feed mass) and tartaric acid derivatives adsorbed on diatomite (0.5-1.0% to sorbent mass) were used. The application efficiency evaluation of the mentioned substances was conducted according methodology developed by specialists of Institute of Fish Industry of the National Academy of Sciences of Belarus [5]. They were carried out in aquariums with a volume of 60 L each. In each of the aquariums, 10 specimens of carp under yearlings with an average weight of 20...25 g was planted. The aquariums are equipped with life support equipment with the maintenance of optimal temperature and hydrochemical regimes. Conditions of fish growing: water temperature - 18...19 °C, pH - 7.7...7.8, concentration of oxygen dissolved in water - 6...7 mg/l. The experiments were repeated three times. Daily feeding rates were 3.0% of the fish weight and were determined by the degree of feed consumption. Food without diatomite served as control. The fish were fed three times a day for 15 days. Feed consumption was determined by taking into account the given feed and feed residues. The growth rate of live weight was determined by individual weighing of the fish at the beginning and end of the experiment.

For the evaluation of comparative antimicrobial effect of additives: tartaric acid, its alkali metal salts and benzylamide (BI) *in vitro* (on microbes) testing experiments was conducted. BI was dissolved in DMSO (dimethylsulfoxide). Tartaric acid and alkali metal salts were dissolved in water. Antimicrobial effect of tartaric acid andBIwas tested in "Armbiotechnology" Scientific and Production Center (SPC) of National Academy of Sciences of Republic of Armenia (NAS RA). For the particular research there were used the strains of National Cultural Collection of Microbe Depository Center of "Armbiotechnology" SPC NAS RA. There were used antibiotic-resistant representatives of Salmonella, Staphylococcus, Pseudomonas, Klebsiella, Stenotrophomonas. The cultivation of all bacterial strains was carried out at 37 °C, under aerobic conditions, on selective-differential media with 50μg/ml content of tested compounds [9].

RESULTS

The results of conducted *in vivo* experiments are comparable to previous experiments with tripoli, the fish as a whole grew well, however, the difference between the samples was noticeable. The use of granular feed for fish breeding requires a scientifically based approach to balancing not only organic components, but also minerals. These substances are known to play an important role in the regulation of metabolism. The

natural mineral supplement of tripoli and diatomite is interesting because it contains elements such as calcium, magnesium, manganese, cobalt, zinc, and copper. In addition, diatomite is more promising due to its developed porous structure. The role of the mentioned microelements is great, since they are involved in many physiological and biochemical processes of any organism. At the same time, many mineral salts exhibit antagonistic properties with respect to each other, while a negative effect of the predominant salt on fish growth is observed. Therefore, the criteria for assessing the effect of mineral salts on the body of fish are the indicators of weight and growth. According to the collected data, the usage of new component and sorbent-additive to feeds are affecting positively on growth parameters of carp.

According to *in vitro*experiments and collected data (table 1 and figure 2), new synthetic derivative BI of tartaric acid is effective against the wide range of bacteria which are harmful for fish.

Table 1

Pathogenic microbe growth inhibition by tartaric acid and the derivatives of it. Strains of microorganisms: 1 - *E. coli, 2 - Pseudomonas aeruginosa 9059,* 3-*Klebsiella pneumonia*, 4 - *Staphylococcus aureus 5302*, 5 -*Salmonella enteritidis 5244,* 6 – *Stenotrophomonas maltophilia 9289; "C+"* - positive control,the growth of microbe on nutrient agar cultural media; "C-" – negative control on 50 mcg/ml antibiotic, in correspondence to resistance of strain; K/Na-TA – tartrate of K/Na; TA – tartaric acid, "+" – growth of microbe; "-" – bacterial growth inhibition.

Substance	1			2		3	4	5
	DH5α	*DH5α/VOG16*	*DH5α/pUC18*	*9056*	*9059*			
BI	+	+	+	-	-	+	-	-
K/Na-TA	-	+	-	-	+	+	+	+
TA	+	+	+	+	+	+	+	+
C+	+	+	+	+	+	+	+	+
C-	-	-	-	-	-	-	-	-

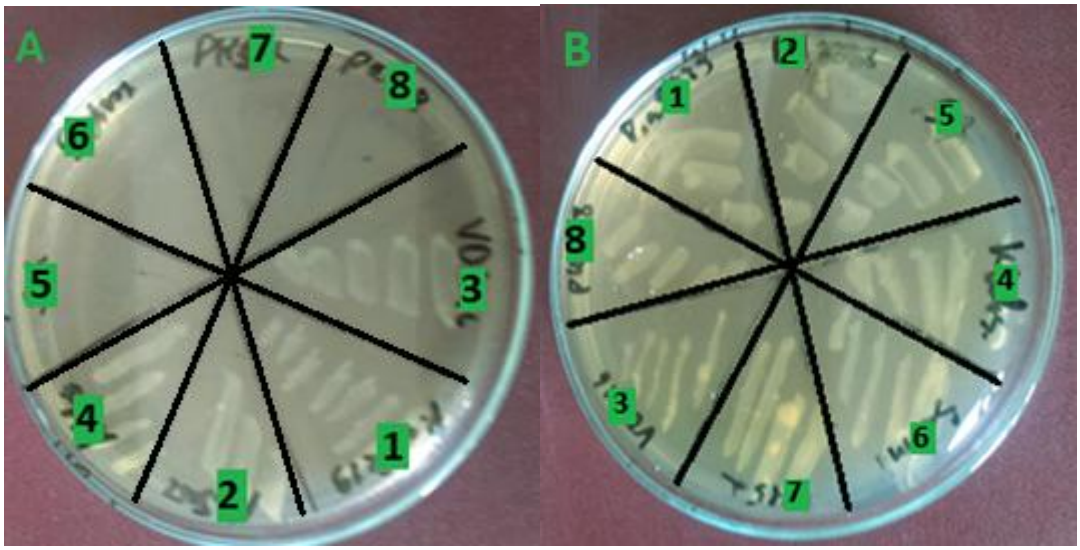


Fig. 2. Bacterial growth inhibition by tartaric acid synthetic derivatives. A – experimental sample, B– positive control sample; 1- E. coli DH5α/VOG16, 2 – P. chlororaphis, subsp. chlororaphis 9189, 3 – P. taetrolens 9248, 4 – Klebsiella pneumonia, 5 – P. aeruginosa 9058, 6 – S.maltophilia 9289, 4 - Staphylococcus aureus 5302, 5 - Salmonella enteritidis.

It must be noted, that *in vitro* laboratory tests of biodegradation potential evaluation have demonstrated that benzyl-, cyclohexyl-, phenyl- and ethamonlamino- complex salts are able to be used by 6 strains of *Pseudomonas taetrolens* and 15 strains of *Pseudomonas chlororaphis*. Imides are able to be degraded in soil by basic-acidic hydrolysis. Thus, they are chemically degradable, and cannot be considered as stabile pollutants of water and soil.

CONCLUSION

A new preservative-antimicrobial agent was developed from wine production waste based natural material: cream of tartar. Tartaric acid benzylamide (BI) have demonstrated high activity against antibiotic-resistant strains of Pseudomonas, Salmonella and Staphylococcus, causing high mortality of fish when using mixed feed contaminated by them. New preparations using some natural mineral sorbents as component of granular fodder for fish and preservative-antimicrobial additive was tested. According to the obtained data, application of these granular fodder positive influence effects on growth are indexed. Thus, these compounds can potentially be considered as carriers for target additives with various functions.

The further laboratory and pond testes, as well as more detailed toxicological analyses are planned for BI of tartaric acid, as new potentially ecologically safe alternative to classical antibiotics.

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