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RESEARCH ON THE IMPACT OF PHYTOBIOTIC CONTAINING COMPOUND FEED ON THE RESISTANCE OF CARP TO BACTERIAL INFECTIONS

Annotation

Bacterial infections are most dangerous for fish and may cause their 100 % death. The most dangerous bacteria are aeromonas, or pathogens of hemorrhagic septicemia or rubella. The Cyprinids are most susceptible to this disease. As a rule, antibiotics, sometimes probiotics are used to fight aeromonosis. Due to the frequent use of antibiotics, fish develop resistance to some of them, thus making the measures to fight bacterial infection completely ineffective. Nowadays phytobiotics are spreading widely in European countries. Phytobiotics are phylogenous or plant-based complexes that do not contain any drugs, so they are simply referred to as feed additives containing biologically active substances that are able to resist bacterial infections, increase appetite, immunity, improve metabolic processes, etc. The following article provides a research on a phytobiotic "Mix-Oil" and its effectiveness against carp aeromonosis. The phytobiotic "Mix-Oil" is a product of a joint Belarusian-Italian production and is based on the essential oils of oregano, cloves and garlic. It was revealed that the phytobiotic "Mix-Oil" does not have acute and chronic toxicity on carps at a dosage of 80 - 600 g / ton of compound feed. Both preventive and therapeutic effects of Mix-Oil as a part of carp compound feed were also established. Based on the results obtained, it was concluded that a dosage of 600 g / ton of compound feed is the optimal dosage of phytobiotic in mixed feed.

Since the phytobiotic "Mix-Oil" is a heat-sensitive product, the modes of moisture and heat treatment as well as a type of granulation were selected, in which the phytobiotic retains its activity. The optimum parameter values of the moisture and heat treatment of mixed feed with phytobiotic "Mix-Oil" at a dosage of 600 g/t were determined, without loss of activity of the phytobiotic, such as: the temperature of the loose mixed feed before the press granulator matrix is not exceeding 67° C and the duration of the moisture and heat treatment process is not exceeding 5,8-6 seconds. On the basis of the conducted research, a new treatment-and-prophylactic compound feed "Mix-Feed" was developed, together with technical specifications for it: TS BY 100035627.020-2018 "Granulated Compound Feed for two and three year old carps" "Mix-Feed" "K-111-LP". It is recommended for feeding carps during spring and autumn within 5 to 10 days to increase the resistance of fish to bacterial infections. Mix-Feed has passed industrial tests and is used by fish farms of the Republic of Belarus.

Keywords: aeromonas, bacterial infection, phytobiotic "Mix-Oil", immunity, moisture and heat treatment, granulation, Mix-Feed.

Introduction

Fish, like any other animals, are susceptible to diseases of various etiologies (parasitic, bacterial, viral, mycotic, non-contagious) [1]. Bacterial infections are most dangerous because they can cause 100% fish death. The most dangerous bacteria, aeromonas, are causative agents of bacterial hemorrhagic septicemia in fish that can cause hemorrhages in warm-blooded animals. It is well known that a decrease in immunity caused by stressful environmental factors contribute to outbreaks of diseases. Antibiotics are the most widely used to treat and prevent such diseases. The course of antibiotic treatment lasts at least 10 days and often leads to immunosuppressive conditions of fish, deterioration of the epizootic situation as well as of consumer qualities of the fish. To top it all, the widespread use of antibiotics and chemotherapy drugs to prevent and control bacterial diseases in fish farms has led to the emergence of problems such as the resistance of bacterial infections to the effects of antibiotics [2].

In recent years, the use of some antibiotics has been banned in a number of countries due to a serious environmental hazard, as well as some carcinogenic effect caused by them in many teleost fishes [3]. Antibiotics can cause the inhibition of beneficial microflora, which is usually present in the digestive tract of fish [4].

That is why probiotics, prebiotics and phytobiotics are increasingly used instead of antibiotics.

Phytobiotics are plant-based complexes with various effects, such as antimicrobial, antiviral, immunomodulatory, antifungal, anti-inflammatory, etc. The composition of phytobiotics consists, as a rule, of natural plant components. Phytobiotics are obtained from aromatic plants and then added to feed. Studies show that they stimulate the production of endogenous enzymes, thereby improving the digestibility of the nutrients of the feed, and the taste qualities of phytobiotics increase the palatability of the feed. The positive effect of phytobiotics is associated with the content of such substances as carotenoids, polypeptides, phytoestrogens, saponins, etc. [5, 6].

On the market of the Republic of Belarus there is a completely plant-based feed additive based on essential oils or phytobiotic "Mix-Oil" of Belarusian-Italian production. Therefore, the purpose of our research was the study of the phytobiotic and its effect on fish as well as the development of a new therapeutic and prophylactic compound feed Mix-Feed based on it.

Research results and discussion

Considering the goal of the research, the acute and chronic toxicity of phytobiotic "Mix-Oil" on carps was studied at first.



The studies were carried out on carp yearlings with an average weight of 22 g, brought from the breeding section "Isobelino" of the Republican Unitary Enterprise "Institute of Fisheries", 120 pcs in total. At the time of the experiments, all the fish were clinically healthy, well fed, with no signs of parasitic or infectious diseases. When conducting research, the experimental fish were grouped by 10 pcs and placed in 60 liters aquariums with constant aeration. A change of water of 1/3 to 2/3 volume of aquariums was carried out daily. In each aquarium, the total weight of the fish was determined, and, based on this, the daily dose of therapeutic food was measured (5% of the weight of the fish planted). For a better consumption or palatability, the daily dose was introduced with an interval of 4 hours: at 9.00 in the morning, at 13.00 in the afternoon and at 17.00 in the evening. The fish in the control aquariums were fed according to the same calculation, but without the phytobiotic "Mix-Oil".

The results of feeding show that the samples of therapeutic and preventive feed containing the antibacterial component at the rate of 80-600 g/t do not have any acute toxicity for fish. The feed was eaten with more intensity than in the control group. No changes in the behavior and physiological state as well as death of the fish were observed in any variant of the experiment.

To determine the preventive effect of phytobiotics "Mix-Oil" on fish, the fish under test were first fed with mixed feeds with various dosages of "Mix-Oil", then each specimen of carp, including control fish, was injected under the pectoral fin with 0.2 ml of a 24-hour cultured *Aeromonas hydrophyla*, strain 14, as shown in Fig. 1.

During previous stages of work, the specified strain showed high virulence for fish.

To determine the therapeutic effect, the fish were first injected with the bacterial strain mentioned

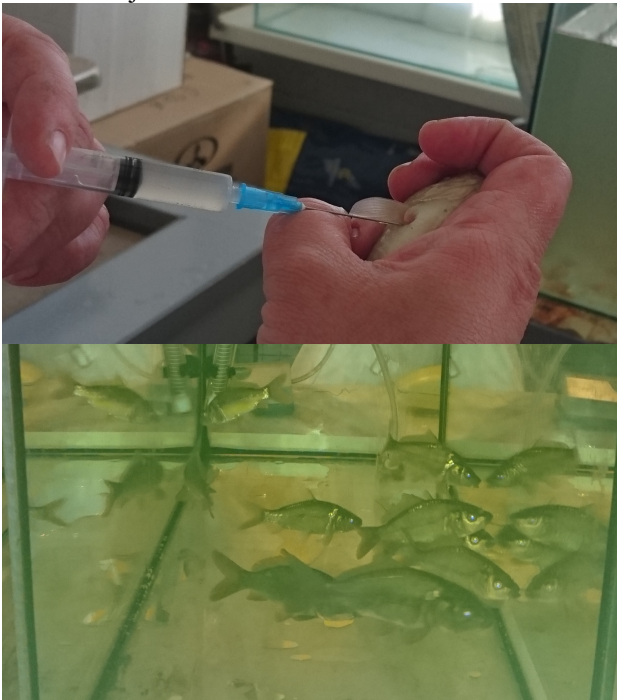


Fig. 1 – Injection of a 24-hour cultured of *Aeromonas hydrophyla*, strain 14 and the state of carps within the first hour after injection

Table 1 – The effectiveness of therapeutic and prophylactic feed containing phytobiotic Mix-Oil in various doses and frequency of use for carp aeromonosis

Concentration, g/t	Feeding frequency			
	1	2	3	5
Prevention				
1	2	3	4	5
200	9/4	7/4	5/3	5/2
400	8/4	7/4	5/2	5/1
600	9/2	5/2	4/0	0/0
800	7/2	6/4	4/0	0/0
1000	6/3	4/1	3/0	0/0
C	9/6	8/6	9/5	10/6
Treatment				
200	10/3	8/6	7/5	6/4
400	10/4	6/5	7/5	6/4
600	7/4	6/2	5/0	0/0
800	6/2	5/4	5/1	0/0
1000	5/1	5/3	2/0	0/0
C	10/7	7/6	9/7	8/5

Note: the numerator is the number of diseased fish; the denominator is the number of the fish that died

above. Then, after the first signs of an infectious process (hyperemia in the area of the pectoral fins, ruffled scales), the fish were fed with treatment food containing phytobiotic "Mix-Oil". The observation continued for 14 days after the experiments were over. The results of the research are stated in Tabl. 1.

As one can see (Table 1), one-and two-time feeding of carp is not enough neither for prevention nor for the treatment of bacterial infections: in almost all variants of the experience, the number of dead and diseased fish practically does not differ from the control one. A different result was observed with a triple feeding: with at a dose of 600 g/t and more, the number of diseased fish is reduced by half on average, and the number of dead fish tends to zero. With five times feeding, the disease and the death of fish was observed only in groups where the preparation was added to the feed at the rate of 200 and 400 g/ton. Based on the results obtained, the optimal dosage of phytobiotic in mixed feed is a dosage of 600 g/ton of mixed feed.

When using heat-sensitive products (milk powder, sugar, phytopreparations, probiotics, etc.), a small amount of steam should be added during granulation. This will determine the low humidity of the mixture during conditioning. The process of pressing such products proceeds with certain difficulties, therefore it is necessary to maintain a small capacity of the press-pelleting machine. Water should be used instead of steam or a small amount of steam [7].

To determine the parameters of moisture and heat treatment of compound feed without loss of efficiency of the phytobiotic "Mix-Oil", experimental design was used, namely SPE 2² with a star. The time of moisture thermal treatment and the temperature of the process were chosen as independent factors affecting the efficiency of the process. The following output parameters



a) – conditioner mixer;
 б) – flat die press-pelleting machine
Fig. 2 – Laboratory conditioner mixer and press-pelleting machine

characterizing the final quality of the feed were selected: feed moisture, swelling and pellet density [8].

Moisture and heat treatment of compound feed (MHT) was carried out in a laboratory conditioner mixer, and the granulation of feed was carried out with a laboratory press granulator (Fig. 2).

A full factorial experiment 2² with a stellar arm (PFE 2²) was carried out. The duration and temperature of moisture and heat treatment were determined as independent factors. After processing the results of the experiment, an empirical relationship was obtained describing

the effect of the duration and temperature of the moisture and heat treatment on the final moisture of the compound feed with a phytobiotic dosage of 600 g / t, which has the following form:

$$W_K = 12,94 + 0,152 \cdot t - 0,234 \cdot T + 0,00032 \cdot t^2 + 0,00025 \cdot t \cdot T + 0,002 \cdot T^2 \quad (1)$$

with W_K – final moisture content of feed after moisture and heat treatment, %;

t – Duration of moisture and heat treatment, c;

T – Temperature of moisture and heat treatment, °C.

The density of the granules is one of the most important indicators of the structural and mechanical properties of the compound feed: the higher the density of the granules, the higher their water resistance. Empirical dependence describing the dependence of the density of granules on the parameters of moisture and heat treatment at a phytobiotic dosage of 600 g / t has the following form:

$$\rho_r = -23,74 - 0,076 \cdot t + 0,84 \cdot T + 0,0027 \cdot t^2 - 0,0012 \cdot t \cdot T - 0,006 \cdot T^2 \quad (2)$$

with ρ_r – granule density, g / cm³;

t – Duration of moisture and heat treatment, c;

T – Temperature of moisture and heat treatment, °C.

The optimum parameter values of the moisture and heat treatment of mixed feed with phytobiotic “Mix-Oil” at a dosage of 600 g/t were determined, without loss of activity of the phytobiotic, such as: the temperature of the loose mixed feed before the press granulator matrix is not exceeding 67° C and the duration of the moisture and heat treatment process is not exceeding 5,8-6 seconds.

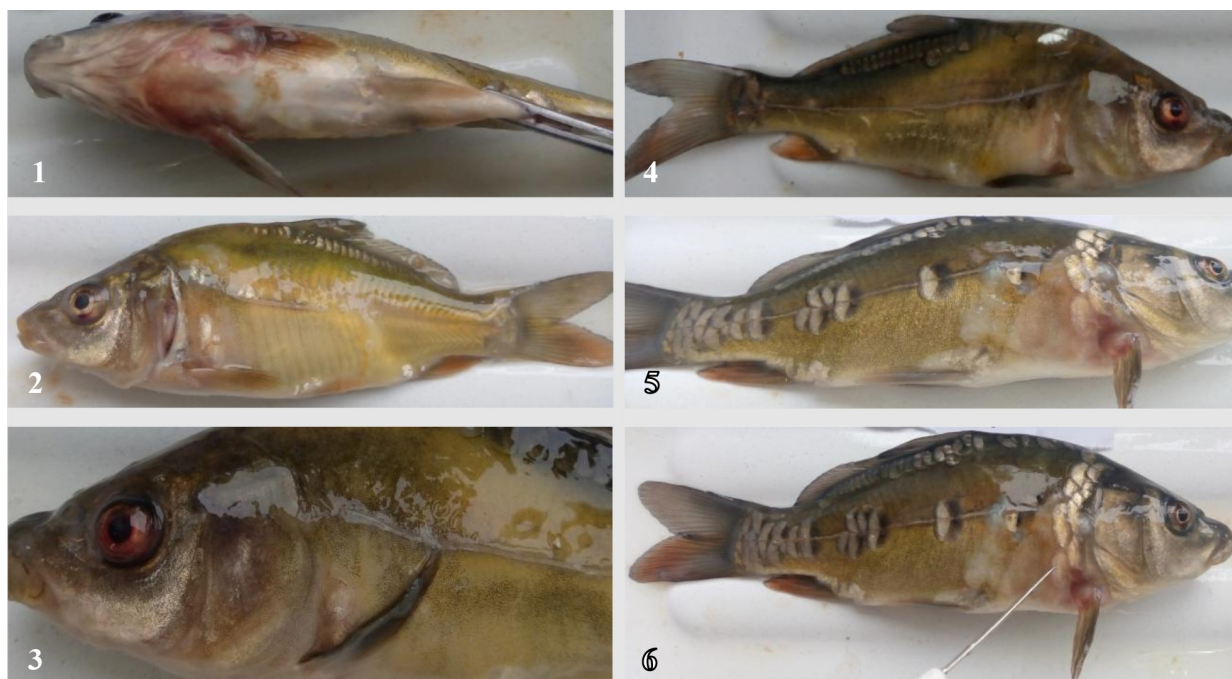
At the next stage of the research, experimental samples of treatment-and-prophylactic compound feed with the given parameters of moisture and heat treatment were prepared. The effectiveness of these samples in the prevention of carp aeromonosis was determined. To do so, carp yearlings were fed for 6 days; after that the fish from both experimental and control groups were injected intraperitoneally with 0.2-0.3 ml of a 24-hour cultured bacterial suspension of the aggressive *Aeromonas hydrophyla* strain. After that, carp survival was monitored for 7 days after injection.

The results of carp survival fed with the moisture and thermal processed feed and when this process was not carried out, at the dosage of the phytobiotic “Mix-Oil” 600 g/t are stated in Table. 2.

The development of clinical signs of aeromonosis (first of all, a strongly observed exophthalmia in 100% of specimen, ruffled scales and hyperemia in the thoracic part of the body) in fish of the control group were observed during the 1st day already. The process developed rapidly. The death of the carp in the control aquarium began on the first day. 6 specimina died

Table 2 - Dynamics of death of fish infected with *Aeromonas hydrophyla*: experimental and control groups

Mix-Oil Dosage	1 day and night	2 day and night	3 day and night	4 day and night	5 day and night	6 day and night	7 day and night	% death
600 w/o MHT	-	1	-	1	-	-	-	20
600 with BTO	-	1	-	1	-	-	-	20
Control	2	6	1	1	-	-	-	100



1 – hyperemia in the pectoral fins and exophthalmos; 2 – weak scales; 3 u 4 – hemorrhage in the eyes and exophthalmos; 5 u 6 – exophthalmos and ulcer at base of fin

Fig. 3 – Signs of aeromonosis in fish

on the 2nd day, on the 3rd and 4th days – 1 specimen. At the autopsy in the body cavity of the dead fish exudate of straw-yellow or bloody color was revealed, the kidneys were flabby, of smearing consistency. Thus, within 4 days a 100% death of fish from the control aquarium took place, accompanied by the rapid development of clinical signs of aeromonosis. The appearance of carp with signs of aeromonosis is shown in Figure 3.

Thus, analyzing the dynamics of the pathological process in infected fish when fed with mixed feed with moisture and heat treatment and without it, we note that when moisture and heat treatment is carried out according to selected optimal process parameters, the activity of Mix-Oil does not decrease. At the same time, it was experimentally confirmed that at higher temperatures of loose feed before pressing, the loss of activity of phytobiotic “Mix-Oil” is directly proportional to the temperature rise (for every 5° C - 30% of phytobiotic activity).

To determine the state of the internal organs and their microbiological contamination during the feeding of therapeutic and prophylactic feed, a plating was carried out before feeding. In plating taken out from the parenchymal organs of fish and carried out prior to the start of the experiment, an intensive growth of bacteria was observed in almost all sectors of the Petri dishes, Gram-negative oxid-positive microorganisms (potential pathogens of fish) *Hafnia alvei*, *Ochrobacter anthropi*, *Aeromonas hydrophyla* as well as gram-positive bacilli and cocci were isolated. Microorganisms were plated from the liver, kidneys and spleen with 90% of the examined fish, and from blood - with 40% of the examined fish.

The study of the bacterioflora of the control group fish after feeding revealed that its qualitative and quantitative composition remained almost unchanged: *Aeromonas hydrophyla*, bacteria p. *Hafnia* and

Ochrobacter were detected in plating, as well as representatives of saprophytic microflora (gram-positive bacilli and gram-negative cocci). Bacteria were isolated with 100% of the control fish, while abundant growth of microbial cultures was observed in almost all sectors of the dishes.

When studying the growth of bacterial cultures isolated from parenchymal organs and carp blood of the experimental group, the following results were observed: the cocci were isolated with the liver of 30% of fish and with blood of 7% of the fish; with the spleen and kidneys of 13% of fish single colonies of *Aeromonas hydrophyla* were isolated; 1 colony of *Hafnia alvei* is also isolated from the spleen. Thus, the bacterial contamination of the internal organs is higher, and the species composition of the microflora is more diverse in carps from the control group who did not receive compound feed with phytobiotic. In fish fed with phytobiotic feed, the indicated indices are sharply reduced both in comparison with the initial level and in comparison with the control group.

Based on the conducted research, a new treatment-and-prophylactic compound feed “Mix-Feed” was developed, together with technical specifications for it: TS BY 100035627.020-2018 “Granulated Compound Feed for two and three year old carps“ Mix-Feed ”K-111-LP”. Physicochemical parameter of the feed Mix-Feed are presented in Chart 3.

According to the results of 10-day carp feeding at the fish farm “Volma”, carried out at the beginning of the season, when the fish were fed with the developed feed, no outbreaks of bacterial infections were observed, all fish are completely healthy. “Mix-Feed” has the odor of garlic, which is an attractant for carp and the feed palatability is increased (40% on average) compared to the feed in which an antibiotic or probiotic is added. Due to eating this compound feed, the metabolic processes in the



Table 3- Requirements for the physicochemical parameters of the granulated mixed feed "Mix-Feed" for two and three years old carp (K-111-LP)

Specification	Characteristic and value of the indicator
1. Appearance	Homogeneous in composition granules of cylindrical shape with a glossy or matte surface
2. Color	From gray to brown according to the color of the ingredients in the compound feed or darker
3. Odor	Without musty, moldy and other foreign smells
4. Moisture, %, no more	13,5
5. Mass fraction of crude protein,%, not less	21,0
6. Mass fraction of crude fat,%, not less	2,0
7. Size of granules, mm, not more - diameter - length	4,7 two diameters
8. Mass fraction of metallomagnetic impurity, mg / kg: - particles up to 0.2 mm inclusive, not more than - particles larger than 0.2 mm and sharp edges	10,0 Not allowed
9. Crumbleness of granuled, % no more	8,0
10. Swollenness of granules, min. not less	10,0
11. Mass fraction of crude fiber, % no more	8,0
12. Mass fraction of calcium, %, not less	0,7
13. Mass fraction of phosphorus, %, not less	0,7



Fig. 4 – Treatment and prophylactic mixed feed "Mix-Feed", produced by CJSC "Ecomol-Agro" for fish farm "Volma"

carp body after wintering become faster, with observed resistance increases, and after 10 days of feeding the fish switch to compound feed for different age carp K-111.

The appearance of the new treatment-and-

prophylactic compound feed Mix-Feed is presented in Fig. 4.

Conclusion

This research presents the results of a study to determine the effectiveness of the phytobiotic Mix-Oil for the prevention and treatment of bacterial infections of carp, *Aeromonas hydrophyla* in particular. It was revealed that the phytobiotic "Mix-Oil" is not toxic for fish and its optimal dosage in the composition of feed for carp is 600 g/ton. The optimum parameter values of the moisture and heat treatment of mixed feed with phytobiotic "Mix-Oil" at a dosage of 600 g/t were determined, without loss of activity of the phytobiotic, such as: the temperature of the loose mixed feed before the press granulator matrix is not exceeding 67° C and the duration of the moisture and heat treatment process is not exceeding 5,8-6 seconds. A new treatment-and-prophylactic compound feed "Mix-Feed" has been developed using phytobiotics, its tests have been carried out and its effectiveness has been established. At present, the treatment-and-prophylactic compound feed "Mix-Feed" is used by the fish farms of the Republic of Belarus instead of antibiotics, which makes it possible to obtain environmentally friendly products.

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ КОМБИКОРМА С ФИТОБИОТИКОМ НА УСТОЙЧИВОСТЬ КАРПА К БАКТЕРИАЛЬНЫМ ИНФЕКЦИЯМ

Аннотация

Бактериальные инфекции наиболее опасны для рыб и могут вызывать их 100 % гибель. Наиболее опасные бактерии – это аэромонады или возбудители геморрагической септицемии или краснухи. Рыбы семейства карповых наиболее подвержены данному заболеванию. Для борьбы с аэромонадом используют антибиотики, иногда пробиотики. Из-за частого применения антибиотиков у рыб вырабатывается устойчивость к некоторым из них и меры борьбы с бактериальной инфекцией оказываются совершенно неэффективными. В настоящее время в странах Европы получают распространение фитобиотики. Это комплексы растительного происхождения, не содержащие лекарственных препаратов, поэтому их относят просто к кормовым добавкам, содержащим биологически активные вещества способные противостоять бактериальным инфекциям, повышать аппетит, иммунитет, улучшать обменные процессы и т.п. В статье представлены исследования фитобиотика «Микс-Ойл» и его эффективности в борьбе с аэромонадом карпа. Фитобиотик «Микс-Ойл» это продукт совместного белорусско-итальянского производства, основу которого составляют эфирные масла орегано, гвоздики и чеснока. Было установлено, что фитобиотик «Микс-Ойл» не обладает острой и хронической токсичностью на организм карпа при дозировке 80 – 600 г/т комбикорма. Был установлен профилактический и лечебный эффект фитобиотика «Микс-Ойл» в составе комбикорма для карпа. На основании полученных результатов оптимальной дозировкой фитобиотика в комбикорм является дозировка 600 г/т комбикорма.

Фитобиотик «Микс-Ойл» является термочувствительным продуктом, поэтому были подобраны режимы влаготепловой обработки и гранулирования, при которых фитобиотик сохраняет свою активность. Определены оптимальные значения параметров влаготепловой обработки комбикорма с фитобиотиком «Микс-Ойл» при дозировке 600 г/т, без потери активности фитобиотика, а именно температура рассыпного комбикорма перед матрицей пресс-гранулятора не выше 67 °С и продолжительности процесса влаготепловой обработки не более 5,8 - 6 секунд. На основании проведенных исследований был разработан новый лечебно-профилактический комбикорм «Микс-Корм» и технические условия на него ТУ ВУ 100035627.020-2018 «Комбикорм гранулированный для двух- и трехлеток карпа «Микс-Корм» К-111-ЛП». Данный комбикорм рекомендуется скармливать карпу в весенний и осенний период в течение 5 – 10 суток для повышения резистентности его организма к бактериальным инфекциям. Микс-Корм прошел промышленные испытания и используется рыбхозами Республики Беларусь.

Ключевые слова: аэромонады, бактериальная инфекция, фитобиотик «Микс-Ойл», иммунитет, влаготепловая обработка, гранулирование, Микс-Корм.

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