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Original article

MICROBIOLOGICAL STUDY OF WOUNDS AND LARGE INTESTINE OF STURGEONS USING β-CYCLODEXTRIN COMPLEX WITH LEVOFLOXACIN

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Abstract

Background. The research laboratory Progressive biotechnologies in aquaculture of the Saratov State University of Genetics, Biotechnology and Engineering named after N.I. Vavilov studied the effect of complexes of ß-cyclodextrin on the healing of skin wounds in hybrid sturgeon fish. The total microbial count of sturgeon cut wounds was determined using the culture method and the composition of the microflora of the fish colon using the qPCR method under the influence of the ß-cyclodextrin complex. This complex was ß- cyclodextrin with levofloxacin included in a chitosan shell. The complex was adsorbed on the surface of the feed that the sturgeons received daily. Changes in the total microbial count demonstrate the nature of the inflammatory process and the effectiveness of the use of therapeutic drugs. The study of the colon microbiota also demonstrates the result of using these complexes in fish. It was found that the use of antibacterial complexes of ß-cyclodextrins with chitosan on sturgeons leads to a significant decrease in the total microbial count of the wound surface. The highest antimicrobial activity was established for the chitosan-ß-cyclodextrin complex with 20 and 15% levofloxacin content. The presence of bacteroides, eubacteria, clostridia, peptostreptocci, enterobacteria, lactobacilli and staphylococci were determined in the composition of the normal flora. At the same time, by the end of the experiment, the microbiome of the large intestine of fish is characterized by the restoration of the number of lactic acid bacteria. The research results can be used in aquaculture in the process of fish cultivation in the treatment of injuries to the outer coverings received during transportation and sorting.

Purpose. Reproduction and cultivation of fish in aquaculture is inevitably associated with trauma to the skin of fish, infection with saprophytic, opportunistic and

pathogenic microorganisms during transportation, sorting, dense planting, and transition to new feed. All these factors reduce the immune status of the fish organism and lead to the occurrence of various diseases that affect all metabolic processes. Currently, antibiotics of various groups with a wide spectrum of action are use to combat infectious diseases, as well as for prevention purposes. Due to the specific lifestyle of aquatic organisms, antibiotics are introduced into the fish organism in aquaculture using medicinal baths, injections, or orally with feed.

Materials and methods. The effect of the chitosan-ß-cyclodextrin complex on the fish organism under aquarium conditions was studied. This complex was synthesized and provided by the Department of Chemical Enzymology of the Lomonosov Moscow State University. The studied complex is a light yellow powder, slowly soluble in water due to the content of chitosan and cyclodextrin and added to fish feed. For the experiment, 5 groups of sturgeons with wounds in the form of dorsal cuts of the skin 2 cm long and 0.5 cm deep were formed using the pair-analogue method. Before the experiment, the fish were fed with compound feed with a peroxide value of 24.68±2.22 for 10 days to form a model digestive disorder and intestinal dysbiosis. The fish received feed with the preparation daily (3 times a day). Individuals of the 1st and 2nd control groups did not receive the studied complex, in addition, individuals of the 2nd control group continued to receive low-quality feed during the experiment. The experimental groups received high-quality feed with the complex in different dosages of levofloxacin (the first - a complex with 20%, the second - with 15% and the third - with 10% of the antibiotic, respectively) for 7 days.

Results. The study of sturgeon wound microflora showed that the studied chitosan-ß-cyclodextrin complex with an antibiotic reduces the TMC of cut wounds. In individuals of the control groups (K1 and K2), the inflammatory process in the wounds continued until the end of the experiment (day 8). On the 8th day, the greatest suppressive effect on the microflora of fish wounds was exerted by complexes with 15 and 20% antibiotic content: in both groups, the decrease in TMC relative to day 1 was 1000 times, below K1 by 1000 times, and K2 by 10000 times. The results of assessing the diversity of sturgeon large intestine microflora indicate that the control group of fish is characterized by normal microflora: bacteroides, eubacteria, clostridia, peptostreptocci, enterobacteria, lactobacilli and staphylococci. In the experimental groups a decrease in the number of lactobacilli, enterobacteria, fusobacteria, eubacteria and clostridia genomes was recorded. Lactate-utilizing bacteria were not detected. Gradual recovery of the fish organism is confirmed by the presence of lactobacilli on the 14th day in the same quantity as before injury and the use of the complex, as well as the absence of mycoplasmas, streptococci and Candida fungi.

Conclusion. A study of wound microflora using the culture method showed that chitosan-ß-cyclodextrin complexes with levofloxacin, used in the treatment of sturgeons, have a significant antimicrobial effect on the number of microorganisms compared to the control groups, with the best effect by the end of the experiment being provided by complexes with 15 and 20% levofloxacin.

A study of wound microflora using the culture method showed that chitosan-ß-cyclodextrin complexes with levofloxacin, used in the treatment of sturgeons, have a significant antimicrobial effect on the number of microorganisms compared to the control groups, with the best effect by the end of the experiment being provided by complexes with 15 and 20% levofloxacin. Molecular genetic research using the PCR method in real time determined the presence of bacteroids, eubacteria, clostridia, peptostreptocci, enterobacteria, lactobacilli and staphylococci in the composition of the normal flora. In the 14 days the microflora of the large intestine of fish is characterized by a confident restoration of the number of lactic acid bacteria that regulate immune processes.

Keywords: reproduction; biotechnology; aquaculture; inflammatory process; bacteroides; eubacteria; clostridia; peptostreptocci; enterobacteria

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Научная статья

МИКРОБИОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ РАН И ТОЛСТОГО КИШЕЧНИКА ОСЕТРОВЫХ ПРИ ИСПОЛЬЗОВАНИИ КОМПЛЕКСА В-ЦИКЛОДЕКСТРИНА С ЛЕВОФЛОКСАЦИНОМ

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Аннотация

Обоснование. В научно-исследовательской лаборатории «Прогрессивные биотехнологии в аквакультуре» Саратовского государственного университета генетики, биотехнологии и инженерии имени Н.И. Вавилова было изучено влияние комплексов производных ß-циклодекстрина с левофлоксацином на заживление ран кожных покровов гибрида осетровых рыб. Культуральным методом определяли общее количество микроорганизмов в резаных ранах осетровых, а методом qPCR – состав микрофлоры толстой кишки рыб под воздействием комплекса ß-циклодекстрин. Данный комплекс представлял собой ß-циклодекстрин с левофлоксацином, заключенный в хитозановую оболочку. Комплекс адсорбировался на поверхности корма, который осетры получали ежедневно. Изменения общего микробного числа демонстрируют характер течения воспалительного процесса и эффективность применения лечебных препаратов. Исследование микробиоты толстого кишечника также демонстрирует результат применения данных комплексов у рыб. Обнаружено, что применение антибактериальных комплексов ß-циклодекстринов с хитозаном на примере осетров приводит к существенному уменьшению общего микробного числа раневой поверхности. Наибольшую эффективность продемонстрировали комплексы хитозан-ß-циклодекстрин с 20 и 15 % содержанием левофлоксацина. В составе нормофлоры кишечника определено наличие бактероидов, эубактерий, клостридий, пептострептокков, энтеробактерий, лактобацилл и стафилококков. При этом к концу эксперимента микробиом толстого кишечника рыб характеризуется восстановлением числа молочнокислых бактерий.

Цель. Воспроизводство и выращивание рыбы в аквакультуре неизбежно связано с травмированием кожных покровов рыбы, заражением сапрофитными, условно-патогенными и патогенными микроорганизмами при транспортировке, сортировке, плотной посадке и при переходе на новый корм. Все эти факторы снижают иммунный статус организма рыбы и приводят к возникновению различных заболеваний, затрагивающих все обменные процессы. В настоящее время для борьбы с инфекционными заболеваниями, а также в целях профилактики используются антибиотики различных групп с широким спектром действия.

Материалы и методы. Действие комплекса хитозан-ß-циклодекстрин на организм рыб в условиях аквариумной установки исследовалось в научно-исследовательской лаборатории «Прогрессивные биотехнологии в аквакультуре» кафедры «Генетика, разведение, кормление животных и аквакультура» ФГБОУ ВО Вавиловский университет (г. Саратов). Комплекс разработан и предоставлен кафедрой «Химическая энзимология» МГУ им. М.В. Ломоносова. Исследуемый комплекс – порошок светло-желтого цвета, медленно растворимый в воде за счет содержания хитозана и циклодекстрина и добавляемый в корм рыбам. Для эксперимента методом пар-аналогов были сформированы 5 групп осетров с ранами, которые представляли собой дорсальные надрезы

кожных покровов длиной 2 см и глубиной 0,5 см. До начала опыта в течение 10 дней рыбу кормили комбикормом с перекисным числом 24,68±2,22 для формирования модельного нарушения пищеварения и дисбиоза кишечника. Корм с препаратом рыбы получали ежедневно (3 раза в день). Особи 1-й и 2-й контрольных групп изучаемый комплекс не получали, кроме того, особи 2-й контрольной группы в процессе опыта продолжали получать в пищу некачественный корм. Опытные группы получали качественный корм с комплексом в различной дозировке левофлоксацина (первая – комплекс с 20 %, вторая – с 15 % и третья – с 10 % антибиотика соответственно) в течение 7 суток. Определяли общее микробное число (ОМЧ) в смывах с ран рыб культуральным методом и состав микрофлоры толстого кишечника рыб методом qPCR.

Результаты. Исследование раневой микрофлоры осетров показало, что изучаемый комплекса хитозан-ß-циклодекстрин с антибиотиком снижает ОМЧ резаных ран. У особей контрольных групп (К1 и К2) воспалительный процесс в ранах продолжался до конца эксперимента (8 сутки). На 8 сутки наибольшее подавляющее действие на микрофлору ран рыб оказали комплексы с 15 и 20% содержанием антибиотика: в обеих группах снижение ОМЧ относительно 1 суток – в 1000 раз, ниже К1 в 1000 раз, а К2 – в 10000 раз.

Исследование представителей микрофлоры толстого кишечника рыб определило наличие бактероидов, эубактерий, клостридий, пептострептококков, энтеробактерий, лактобацилл и стафилококков. В опытных группах на протяжении всего времени наблюдений зафиксировано снижение количества геномов лактобацилл, энтеробактерий, фузобактерий, эубактерий и клостридий, что указывает на антимикробную эффективность применяемых лечебных препаратов и закономерный дисбаланс (дисбактериоз). Лактат-утилизирующие бактерии не были обнаружены. Постепенное восстановление организма рыб подтверждается наличием на 14 сутки лактобацилл в том же количестве, что до ранения и применения комплекса, а также отсутствием микоплазм, стрептококков и грибов рода кандида.

Заключение. Изучение микрофлоры ран культуральным методом показало, что комплексы хитозан-ß-циклодекстрин с левофлоксацином, примененные в лечении осетров, оказывают значительное антимикробное влияние на количество микроорганизмов по сравнению с контрольными группами, при этом к концу эксперимента наилучший эффект оказывали комплексы с 15 и 20% содержанием левофлоксацина.

Молекулярно-генетическое исследование методом ПЦР в режиме реального времени определило в составе нормофлоры наличие бактероидов, эубактерий, клостридий, пептострептококков, энтеробактерий, лактобацилл и

стафилококков. К концу наблюдений микрофлора толстого кишечника рыб характеризуется уверенным восстановлением числа молочнокислых бактерий, регулирующих иммунные процессы.

Ключевые слова: размножение; биотехнологии; аквакультура; воспалительный процесс; бактероиды; эубактерии; клостридии; пептострептококки; энтеробактерии

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Introduction

The research laboratory Progressive biotechnologies in aquaculture of the Saratov State University of Genetics, Biotechnology and Engineering named after N.I. Vavilov studied the effect of complexes of ß-cyclodextrin derivatives with levofloxacin on the healing of skin wounds in hybrid sturgeon fish. The total microbial count of sturgeon cut wounds was determined using the culture method and the composition of the microflora of the fish colon using the qPCR method under the influence of the ß-cyclodextrin complex. This complex was ß-cyclodextrin with levofloxacin included in a chitosan shell. The complex was adsorbed on the surface of the feed that the sturgeons received daily. Changes in the total microbial count demonstrate the nature of the inflammatory process and the effectiveness of the use of therapeutic drugs. The study of the microbiota of the colon also demonstrates the result of using these complexes in fish. It was found that the use of antibacterial complexes of ß-cyclodextrins with chitosan on the example of sturgeons leads to a significant decrease in the total microbial count of the wound surface. The greatest efficiency was demonstrated by chitosan-ß-cyclodextrin complexes with 20 and 15% levofloxacin content. The presence of bacteroids, eubacteria, clostridia, peptostreptocci, enterobacteria, lactobacilli and staphylococci were determined in the composition of the normal intestinal flora. At the same time, by the end of the experiment, the microbiome of the large intestine of fish is characterized by the restoration of the number of lactic acid bacteria.

Reproduction and cultivation of fish in aquaculture is inevitably associated with trauma to the skin of fish, infection with saprophytic, opportunistic and pathogenic microorganisms during transportation, sorting, dense planting, and transition to new feed. All these factors reduce the immune status of the fish organism and lead to the occurrence of various diseases that affect all metabolic processes. Currently, antibiotics of various groups with a wide spectrum of action are used to combat infectious diseases, as well as for prevention purposes. Due to the specific lifestyle of aquatic organisms, antibiotics are introduced into the fish organism in aquaculture using medicinal baths, injections, or orally with feed [1].

Recently, with the development of nanotechnology, various nanoparticles have been used to deliver drugs to animals. One of these developments is complexes of β-cyclodextrin (CD) derivatives for the delivery of drugs and prophylactic drugs to fish. Due to their tiny size and porous nature, β- cyclodextrin derivatives can bind poorly soluble drugs within their matrix and improve their bioavailability [2; 3].

Cyclodextrins and their derivatives have found application in pharmaceuticals as complexing agents that mask unpleasant odors and tastes, increasing the solubility, stability, and bioavailability of included molecules [4; 5]. They can be used to deliver drugs to specific sites, which prevents the degradation of drugs and proteins and prolongs the elimination of drugs [6-11]. The combination of cyclodextrin carriers with various polymers with a large number of covalent intramolecular bonds can cause a change in the properties of the drug compared to a simple drug-CD complex [8; 12].

A possible component of such a complex is a heteropolysaccharide. chitosan, which is characterized by good water solubility, biocompatibility and biodegradability, mucoadhesion, antimicrobial activity [13-16]. All these properties of chitosan make it attractive for use in agriculture and veterinary medicine. In this regard, scientific research on the creation and improvement of the effectiveness of such cyclodextrin drug complexes with antibiotics in the therapy of commercial fish is relevant.

One of the groups of antibiotics that fit into the porous structure of β-cyclodextrin derivative complexes are fluoroquinolones II generation, aimed at suppressing and destroying infections of various etiologies [8; 17], including pathogens of infectious lesions of cuts, burns and other types of wounds and damage to the outer skin.

Currently, ciprofloxacin is widely used in Russian aquaculture, while levofloxacin, also a fluoroquinolone, is a new agent for treating fish that has not been sufficiently studied, but is promising, since resistance to antibiotics used in aquaculture in fish is a fairly common phenomenon [18-20].

Purpose. The aim of the work was to study the effect of chitosan-ß-cyclodextrin complexes containing 10, 15 or 20% levofloxacin on the microflora of cut wounds and the composition of the microflora of the large intestine of fish using the qPCR method of juvenile hybrid Russian and Siberian sturgeon (*Acipenser queldenstadtii*, Brandt et Ratzeburg, 1833, and *Acipenser baeri stenorrhea Nikolsky*). In accordance with the principles of rational antibiotic therapy and to find the lowest effective concentration, complexes with different antibiotic contents were used in the work.

Materials and methods

The experiment was conducted in the research laboratory Advanced biotechnologies in aquacultureof the department "Genetics, breeding, feeding of animals and aquaculture" of the Federal State Budgetary Educational Institution of Higher Education Vavilov University in Saratov in the conditions of an aquarium installation. During the study, the effect of the chitosan-ß-cyclodextrin complex was studied. The studied chitosan-ß-cyclodextrin complex was synthesized and provided by the department "Chemical Enzymology" of the Lomonosov Moscow State University and and was a light yellow powder consisting of amphiphilic polymer particles have been developed that dissolve extremely slowly in water, since they contain chitosan and cyclodextrin, and they can be added to fish feed. Encapsulated fluoroquinolone will be released in a prolonged manner in the intestines of fish.

Chitosan and cyclodextrin are biocompatible, safe, and have mucoadhesive effects in the gastrointestinal tract. Cyclodectrin forms inclusion complexes with antibiotics and is used as a molecular container. The particles release the drug into the water extremely slowly (namely, 85% of levofloxacin is stored on the carrier in an hour), and therefore the entire antibiotic can be eaten by fish in the form of particles with food. At the same time, the bioavailability and effectiveness of antibacterial and healing effects increased.

The complex was created in the following way: A solution of carbonyldiimidazole (CDI) in DMSO of 50 mg/ml in a five-fold molar excess was added to a solution of hydroxypropyl cyclodextrin (HPC) in water of 5 mg/ml slowly dropwise with intensive stirring. The mixture is heated to 60 ° C for 2 hours. Then, activated HPCD was added to a 5 kDa chitosan solution in a sodium acetate buffer (0.02 M, pH 5) with intensive stirring. The solution was incubated for 12 hours at 50 °C. Purification from unreacted impurities was carried out using dialysis against water (3.5 kDa cut-off weight) for 24 hours. Then LF in 0.01 M HCl (30% by weight of polymer) was added to the solution. The system was incubated for 12 hours at 40°C. Purification from unreacted impurities was carried out using dialysis against water (3.5 kDa cut-off weight) for 24 hours. The samples were freeze-dried.

The degree of modification was calculated according to spectrophotometric titration of amino groups (before and after modification) with 2,4,6-trinitrobenzenesulfonic acid (TNBS) using 1 M TNBS solution in 1 M sodium borate buffer (pH 9.2). The content of amino groups in modified chitosan is assumed to be 100%.

Fourier infrared spectroscopy (FTIR). The IR spectra of the sample suspension were recorded using a Bruker Tensor 27 spectrometer equipped with a liquid nitrogencooled MCT (mercury-cadmium telluride) detector. The samples were placed in a BioATRII thermostatic cell with a ZnSe ATR element (Bruker, Bremen, Germany).

The FTIR spectra were obtained in the range from 850 to 4000 cm−1 with a spectral resolution of 1 cm−1. 50 scans were accumulated and averaged for each spectrum. Spectral data was processed using the Bruker Opus 8.2.28 software system (Bruker, Bremen, Germany).

The synthesis of nanospanges particles with LF includes: 1) obtaining chitosan particles grafted with CD 2) loading of LF. Chitosan-CD conjugates were obtained by the modification reaction of 5 kDa chitosan amino groups with activated OH CD groups to form the chitosan product:CD = 1:4 (according to the analysis of IR spectra).

The average particle size is 120±30 nm according to the analysis of the trajectory of nanoparticles.

In the process of conducting the research, before the experiment, 5 groups of sturgeons (n=10) were formed using the pair-analogue method and placed in aquariums. The experimental scheme is presented in Table 1.

Table 1.

Scheme experience

Group	Fish condition	Feeding type
1 control (K1)	Damaged	Basic diet of quality feed (BD_{QF})
2 control (K2)	Damaged	Basic diet of low-quality feed (BD_{LQF})
1 experienced	Damaged and receiving treatment	BD_{QF} + complex with 20% levofloxacin
2 experienced	Damaged and receiving treatment	BD_{QF} + complex with 15% levofloxacin
3 experienced	Damaged and receiving treatment	BD_{QF} + complex with 10% levofloxacin

To obtain a pronounced effect from the introduction of the chitosan-ß-cyclodextrin complex, the fish were fed with expired compound feed with a peroxide value of 24.68±2.22 (ORN) for 10 days before the experiment, which suggested a model digestive disorder and intestinal dysbiosis. The fish received feed with the preparation daily (3 times a day). Individuals of the 1st and 2nd control groups did not receive the studied complex; in addition, individuals of the 2nd control group continued to receive low-quality feed during the experiment. The experimental groups received high-quality feed with the chitosan -ß- cyclodextrin complex in different dosages of levofloxacin (the first - a complex with 20% levofloxacin, the second - with 15% levofloxacin and the third - with 10% levofloxacin) for 7 days. The composition of the combined feed for sturgeons (OR) is balanced in terms of nutrients.

The simulated wounds were dorsal skin incisions 2 cm long and 0.5 cm deep.

The total microbial count (TMC) was determined in fish wound swabs using the culture method (the method of serial dilutions followed by sowing on meat-peptone agar (MPA)) and the composition of the microflora of the large intestine of fish by the method qPCR (real-time PCR).

Results

The study of sturgeon wound microflora revealed a significant effect of the chitosan-ß-cyclodextrin complex with levofloxacin on the MFC of cut wounds. As can be seen from the results presented in Table 2, in the control groups (K1 and K2), the inflammatory process in the wounds continued until the end of the experiment (day 8).

In group K1, the MFC significantly decreased by day 8. At the same time, in group K2, the MFC value increased by day 8 and was 10 times higher compared to group K1, which indicates the effect of feed quality on the growth and development of fish wound microflora.

Table 2.

Cyclodextrin complexes with antibiotic and chitosan on the microflora of sturgeon cut wounds

Groups	Day	
	1	8
	OMCH, CFU/ml	
K1	$5.0 \cdot 10^{5} \pm 0.2$	$1.0 \cdot 10^{5} \pm 0.4^{*}$
K2	$5.0 \cdot 10^{5} \pm 0.2$	$1.0 \cdot 10^{6} \pm 0.4^{*\bullet}$
1 experimental - 20% levofloxacin	$5.0 \cdot 10^{5} \pm 0.2$	$1.0 \cdot 10^{2} \pm 0.2^{*\bullet\circ}$
2 experimental - 15% levofloxacin	$1.0 \cdot 10^{5} \pm 0.1$	$1.0 \cdot 10^{2} \pm 0.2^{*\bullet\circ}$
3 experimental - 10% levofloxacin	$5.0 \cdot 10^{5} \pm 0.4$	$2.0 \cdot 10^{3} \pm 0.8^{*\bullet\circ}$

Note: $p \leq 0.05$ relative to *the value of 1 day in its group; $^{\bullet}$ relative to the value in group K1; $^{\circ}$ relative to the value in group K2.

In the 1st group of fish, which received a 20% dose of antibiotic, a significant decrease in the TMC relative to the 1st day was established by the 8th day - by 1000 times. In the fish of the 2nd experimental group, which received 15% levofloxacin, the results of the TMC count on the 8th day were comparable with the results in the 1st experimental group and also lower than K1 by 1000 times, and K2 - by 10,000 times. In the 3rd experimental group, which received a therapeutic complex with a minimum dose of 10% levofloxacin with food, the wound contamination decreased by the end of the observations by 100 times compared to the 1st day, by 100 and 1000 times compared to K1 and K2, respectively.

As can be seen from Table 2, on the 8th day, the greatest suppressive effect on the microflora of fish wounds was exerted by complexes with 15 and 20% antibiotic content.

The results of the assessment of the diversity of representatives of the microflora of the large intestine of sturgeons under the influence of the chitosan -ß- cyclodextrin complex with 20% levofloxacin content, presented in Table 3, indicate a greater diversity of representatives of the bacterial community in the control group compared to the experimental ones.

Table 3.

Microflora of the large intestine of sturgeon fish under the influence of a complex of cyclodextrin with chitosan and levofloxacin

Identifiable groups of microorganisms	Microorganism content, genomes/g			
	Groups of fish participating in the experiment			
	K1	1 experimental - 20% levofloxacin		
	Day			
	1	1	8	14
Bacteroides (p. *Prevotella* And *Porphyromonas*)	$2.4\times10^{3}\pm0.7$	$2.1\times10^{3}\pm0.7$	$1.2\times10^{3}\pm0.4$	$2.0\times10^{3}\pm0.4$
Eubacteria (genus *Eubacterium*)	$5.0\times10^{5}\pm0.9$	$1.8\times10^{5}\pm0.7$	$4.9\times10^{4}\pm0.9$*	$2.7\times10^{3}\pm0.4$* •
Clostridia (p. *Lachnobacterium* And *Clostridium*)	$7.0\times10^{5}\pm0.9$	$1.8\times10^{5}\pm0.9$*	$8.0\times10^{4}\pm0.9$*	$4.8\times10^{3}\pm0.9$* •
Lactobacilli (genus *Lactobacillus*)	$8.4\times10^{2}\pm0.9$	< p.d.o.	< p.d.o.	$5.3\times10^{2}\pm0.7$
Lactate - utilizing bacteria (p. *Megasphaera* spp., *Veillonella* spp., Dialister *spp.*)	< p.d.o.	< p.d.o.	< p.d.o.	< p.d.o.
Peptostreptococci (genus *Peptostreptococcus*)	$7.5\times10^{5}\pm0.9$	$4.3\times10^{2}\pm0.5$	$4.2\times10^{2}\pm0.5$	$7.3\times10^{3}\pm0.4$* •
Enterobacteriaceae (family *Enterobacteriaceae*)	$3.4\times10^{4}\pm0.8$	$5.4\times10^{4}\pm0.9$	$4.2\times10^{3}\pm0.9$*	$1.4\times10^{6}\pm0.9$* •

Actinomycetes (*Mobiluncus*, *Corynebacterium*, *Atopobium*)	< p.d.o.	< p.d.o.	< p.d.o.	$4.3\times10^{2}\pm0.5$
Fusobacteria (genus *Fusobacterium*, *Sneathia*, *Leptotrichia*)	$2.6\times10^{4}\pm0.9$	< p.d.o.	< p.d.o.	$4.3\times10^{5}\pm0.8$*
Mycoplasmas (genus *Mycoplasma*, *Ureaplasma*)	< p.d.o.	< p.d.o.	< p.d.o.	< p.d.o.
Streptococci (p. *Streptococcus*)	< p.d.o.	< p.d.o.	< p.d.o.	< p.d.o.
Staphylococci (genus *Staphylococcus*)	$8.4\times10^{2}\pm0.9$	< p.d.o.	$5.4\times10^{2}\pm0.7$	$1.7\times10^{3}\pm0.9$* •
Fungi of the genus *Candida*	< p.d.o.	< p.d.o.	$8.7\times10^{2}\pm0.5$	< p.d.o.

Note: $p \leq 0.05$ relative to *value in group K1; • relative to the value on day 8 in the experimental group.

The data obtained in the control group describe the normal microflora of sturgeons grown in aquarium installations. Thus, in the control group of fish fed with quality food, the following genera were found: bacteroides, eubacteria, clostridia, peptostreptocci, enterobacteria , lactobacilli and staphylococci. At the same time, in the experimental group, where cyclodextrin preparations with an antibiotic were used after modeling a cut wound, on the 1st day differences in the diversity of microflora were observed in relation to lactobacilli, enterobacteria and fusobacteria , the number of genomes of which was below the limit of reliable detection.

Throughout the entire observation period, these representatives of the microflora were present in low quantities, which may indicate a natural imbalance (dysbacteriosis) caused by taking the antibiotic. Lactate -utilizing bacteria were not detected.

During the experiment, a decrease in the number of eubacteria and clostridia was observed, which is explained by the antimicrobial effect of levofloxacin and chitosan. At the same time, an increase in peptostreptococci , enterobacteria , fusobacteria and staphylococci was detected compared to normal microflora. This can be explained by the restoration of microflora after the use of the complex, including the detection of representatives of those genera, the number of genomes of which did not allow us to confirm their presence, at the same time, this may indicate a weakening of the immune system of fish after a long inflammatory process, which is injury, which requires additional attention during further sturgeon cultivation. Gradual recovery of the fish organism is confirmed by the presence of lactobacilli on the 14th day in the same amount as before injury and the use of the complex, as well as the absence of mycoplasmas, streptococci and Candida fungi.

Conclusions

A study of the wound microflora using the culture method showed that complexes with levofloxacin, used in the treatment of sturgeons, have a significant antimicrobial effect on the number of microorganisms compared to the control groups, with the best effect by the end of the experiment being provided by complexes with 15 and 20% levofloxacin content.

Molecular genetic research using the PCR method in real time determined the presence of bacteroids, eubacteria, clostridia, peptostreptocci, enterobacteria, lactobacilli and staphylococci in the composition of the normal flora. In 14 days the microflora of the large intestine of fish is characterized by a confident restoration of the number of lactic acid bacteria that regulate immune processes.

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