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CELLULAR AND HUMORAL IMMUNITY OF CARP AT THE ACTION OF BIOLOGICALLY ACTIVE ADDITIVES

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Aim. To investigate the impact of L-arginine and ethanol extract of purple echinacea on cellular and humoral links of immunity of carps. **Methods.** Determination of the population composition of lymphocytes, immunological, clinical and statistical methods. **Results.** The data about the qualitative composition of T-lymphocytes and their subpopulations and B-lymphocytes in the blood of carps at the action of L-arginine and purple echinacea are presented. It was established that feeding carps with starch paste with the addition of L-arginine and ethanol extract of purple echinacea activated the cellular link of their immunity, in particular, it enhanced the number of T-lymphocytes (active and theophylline-resistant) in blood. Feeding starch paste with ethanol extract of purple echinacea per os promoted the decrease in the population of theophylline-sensitive T-lymphocytes in blood of carps and had a stimulating effect on the B-lymphocyte link of the immunity. Conclusions. Feeding carps with additives to starch paste – L-arginine and ethanol extract of purple echinacea – activates cellular and humoral links of immunity and affects the functional state of immunocompetent cells, which is confirmed by the increase in the relative number of T- and B-lymphocytes in blood, and the re-distribution of avidity towards enhancing the receptor apparatus of plasmatic membranes of T-lymphocytes.

Keywords: carp, L-arginine, purple echinacea, blood, T- and B-lymphocytes.
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INTRODUCTION

A relevant problem of modern development of fisheries is the elaboration of the ways of affecting the processes of immunity formation, targeted at enhancing the resistance and adaptation capacity of fish.

In recent 50 years, the global volumes of fisheries increased by 50 million tons [1, 2]. At the same time, priority relevance was attributed to technologically complicated industrial forms of intensifying fish farming, envisaging high concentration of fish per unit of the area and thus requiring provision of complete feed for them.

It is known that high productivity and preservation of animals, birds, and fish require providing the ratios with sufficient amount of fats, carbohydrates, mineral elements, and vitamins, which is first and foremost related to proteins and aminoacids [3–6]. A number of aminoacids, arginine in particular, are not capable of being synthesized in the organism, which requires introducing their synthetic analogs to the combined feeds.

Numerous studies demonstrate [7–9] that L-arginine has a broad spectrum of biological impact on the organism. At the same time, the biologically active substances, present in the raw vegetative materials, for instance, in purple echinacea, both have a positive impact on morphological indices of the blood of animals, and change the activity of enzymes of protein, lipid, and carbohydrate exchanges [10, 11], and, according to the data of [12], have immunostimulating properties. However, there are no scientific data regarding the action of purple echinacea on cellular and humoral links of the immunity of carps, there are insufficient scientific investigations on norming the level of arginine in the combined feeds for fish, the available information is contradictory and requires further confirmation.

The determination of the impact of biologically active substances on biochemical processes, forming the basis

of the formation and functioning of the immune system of fish, is extremely important for scientific studies, as it will allow elaborating theoretical foundations for enhancing the immune function and adaptation capability in the organism of different species. At the same time, it is noteworthy that the use of biologically active substances, added to the standard combined feeds for fish, is more efficient compared to other methods of immuno-modulating impact on their organism.

MATERIALS AND METHODS

The experiment was conducted in cooperation with the Lviv division of the Institute of Fisheries, NAAS in the village of Liubin Velyky, Horodotsky district, Lviv region. The experiment was conducted using three groups of two-year-old carps, five fish per group. Carps of the control group were fed starch paste per os, cal-culated as 1 % per 1 kg of bodyweight. In the course of 10 days, carps of the first group were given identical paste with the addition of L-arginine in the amount of 1 mg/kg of bodyweight daily, and carps of the second group – the paste with the addition of 50 % ethanol extract of purple echinacea in the amount of 1 ml/kg of fish bodyweight. Carps were kept in the aquarium conditions.

After the experiment, blood samples of carps were taken. The relative number of T- and B-lymphocytes and their subpopulations, antigen-binding lymphocytes were studied in blood lymphocytes. According to the density of receptors and respective number of bound erythrocytes, lymphocytes were differentiated into non-differentiated, low-avid, medium-avid, and highly avid ones [13].

RESULTS AND DISCUSSION

T-lymphocytes play a key role in the formation of specific cellular immunity [14]. The study on T-cellular link of fish immunity demonstrated the increase in the relative number of T-active and T-theophylline-resistant lymphocytes in the blood of carps of the second experimental group (Table 1). Mainly it occurred due to low-avid and especially highly avid cells, and to somewhat lesser degree – due to the increase in the number of their medium avid forms on the background of a decrease in the number of non-differentiated cells. These changes may be explained by increased migration of T-lymphocytes from thymus into peripheral lymphoid organs and the stimulation of the processes of their differentiation under the impact of the extract of purple echinacea which, as a complex, promotes the increase in the immune status of carps. Here the blood of carps of the mentioned group demonstrates the decrease by 3 % (compared to the control) in the relative number of T-theophylline-sensitive lymphocytes. The decrease in their number of blood may testify to the stabilization of the immune aggression processes in the organism of carps at the action of purple echinacea.

Table 1. The indices of T-cellular immunity and their functional activity in the blood of carps ($M \pm m$; %; $n = 3–4$)

Index	Group of fish		
	Control	First experimental	Second experimental
T-general, 0	51.0 ± 3.21	52.33 ± 2.73	52.33 ± 1.45
3–5	32.67 ± 1.20	26.67 ± 4.84	30.0 ± 1.15
6–10	11.0 ± 1.15	13.33 ± 2.73	11.67 ± 1.45
M	5.33 ± 1.86	7.67 ± 2.33	6.0 ± 1.15
%	49.0 ± 3.21	47.67 ± 2.73	47.67 ± 1.45
T-active, 0	74.0 ± 1.15	72.67 ± 2.85	72.0 ± 2.52
3–5	17.67 ± 1.76	17.33 ± 1.45	18.67 ± 2.03
6–10	5.33 ± 1.20	6.67 ± 1.76	5.67 ± 2.40
M	4.5 ± 1.5	5.0 ± 3.0	5.5 ± 0.5
%	26.0 ± 1.15	27.33 ± 2.85	28.0 ± 2.52
T-theophylline-resistant, 0	72.67 ± 1.67	72.33 ± 1.45	71.0 ± 2.52
3–5	22.0 ± 1.00	19.67 ± 0.88	21.0 ± 2.08
6–10	4.67 ± 0.33	6.0 ± 1.15	5.33 ± 0.33
M	1.0 ± 0.01	3.0 ± 1.0	4.0 ± 0.01
%	27.33 ± 1.67	27.67 ± 1.45	29.0 ± 2.52

Note. In this and following tables: 0 – non-differentiated cells, 3–5 – cells with low density of receptors, 6–10 – cells with medium density of receptors and morulas (M) – cells with high density of receptors.

Table 2. The number of B-lymphocytes in the blood of carps ($M \pm m$; %; n = 3–4)

Index	Group of fish		
	Control	First experimental	Second experimental
0	57.67 ± 1.67	57.67 ± 0.88	57.0 ± 1.15
3–5	30.67 ± 0.33	30.33 ± 1.20	30.0 ± 0.58
6–10	8.33 ± 1.20	9.33 ± 0.33	9.0 ± 1.15
M	3.33 ± 0.67	2.67 ± 0.67	3.33 ± 0.88
%	42.33 ± 1.67	42.33 ± 0.88	43.0 ± 1.15

Similar changes were revealed in the blood of carps of the first experimental group. For instance, feeding carps with L-arginine, added to the starch paste, led to the increase in the relative number of T-active and T-theophylline-resistant lymphocytes in their blood. However, in this case a higher number of the mentioned subpopulations of T-lymphocytes in the blood of carps of the first experimental group, compared to the control, was conditioned by the increase in the number of cells with medium and high density of receptors. These data testify to the impact of the addition of L-arginine and the extracts of purple echinacea to starch paste on the increase in the functional activity of immunocompetent blood cells of carps at the expense of the redistribution of their avidity towards the increase in the receptor field. There are scientific data [15] proving that arginine strengthens the immune system due to the increase in the activity of the T-cellular immunity, in particular, via enhancing the proliferation of T-lymphocytes, the increase in their receptor activity and activation of mitogenesis processes.

The implementation of humoral immune response in the organism involves the participation of B-lymphocytes, which get differentiated into antibody producers under the impact of antigen stimulus [16]. Positive impact of feeding starch paste with the addition of the ethanol extract of purple echinacea on the indices of specific immune protection of fish is evident in the increase in the relative number of B-lymphocytes in the blood of carps of the second experimental group compared to the control (Table 2). At the same time, a tendency towards the increase in the number of their medium-avid forms has been revealed in the blood of carps of this group.

The presence of surface membrane receptors to complements in B-cells allows detecting lymphocytes, forming "rosettes" with sheep erythrocytes, which have an erythrocyte-antigen complex on the membranes [17]. In general, the increase in the relative number of B-lymphocytes in the blood of carps of the second experimental group as the main antibody-forming cells reflects the activation of a humoral link of immunity in the organism. Evidently, this occurs due to the regulatory impact of the addition of the extract of purple echinacea on the differentiation of T- and B-lymphocytes in the organism of carps, and the increase in the number of functionally specialized cellular populations, in particular, T-theophylline-resistant lymphocytes, initiating the processes of proliferation of B-cells, and participating in the production of non-specific factors of protection – immunoglobulins.

CONCLUSIONS

Feeding carps with additives to starch paste – L-arginine and ethanol extract of purple echinacea – activates cellular and humoral links of immunity and affects the functional state of immunocompetent cells, which is confirmed by the increase in the relative number of T- and B-lymphocytes in blood, and the re-distribution of avidity towards enhancing the receptor apparatus of plasmatic membranes of T-lymphocytes.

Стан клітинного та гуморального імунітету коропа за дії біологічно активних добавок

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Мета. Дослідити вплив L-аргініну та спиртового екстракту ехінацеї пурпурової на клітинну та гуморальну ланки імунітету коропів. **Методи.** Визначення популяційного складу лімфоцитів, імунологічні, клінічні і статистичні методи. **Результати.** Представлено дані щодо кількісного складу Т-лімфоцитів і їхніх субпопуляцій та В-лімфоцитів у крові коропів за дії L-аргініну і ехінацеї пурпурової. Встановлено, що згодовування коропам крохмального клейстеру з добавкою L-аргініну та спиртового екстракту ехінацеї пурпурової активує клітинну ланку їхнього імунітету, зокрема, підвищує в крові кількість Т-лімфоцитів (активних і теофілінрезистентних). Згодовування per os крохмального клейстеру

зі спиртовим екстрактом ехінацеї пурпурової сприяє зменшенню в крові коропів кількості популяції теофілінчутливих Т-лімфоцитів і проявляє стимулюючий вплив на В-лімфоцитарну ланку імунітету. **Висновки.** Згодовування коропам добавок до крохмального клейстеру L-аргініну та спиртового екстракту ехінацеї пурпурової активує клітинну і гуморальну ланки імунітету й впливає на функціональний стан імунокомпетентних клітин, про що свідчить збільшення у крові відносної кількості Т- і В-лімфоцитів й перерозподіл авідності у бік зміцнення рецепторного апарату плазматичних мембран Т-лімфоцитів.

Ключові слова: короп, L-аргінін, ехінацея пурпурова, кров, Т- та В-лімфоцити.

Состояние клеточного и гуморального иммунитета карпа под воздействием биологически активных добавок

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Цель. Исследовать влияние L-аргинина и спиртового экстракта эхинацеи пурпурной на клеточное и гуморальное звенья иммунитета карпов. **Методы.** Определение популяционного состава лимфоцитов, иммунологические, клинические и статистические методы. **Результаты.** Представлены относительно количественного состава Т-лимфоцитов, их субпопуляций и В-лимфоцитов в крови карпа под воздействием L-аргинина и эхинацеи пурпурной. Установлено, что скармливание карпу крахмального клейстера с добавкой L-аргинина и спиртового экстракта эхинацеи пурпурной активирует клеточное звено их иммунитета, в частности, увеличивает в крови количества Т-лимфоцитов (активных и теофиллинрезистентных). В то же время скармливание per os крахмального клейстера со спиртовым экстрактом эхинацеи пурпурной способствует уменьшению в крови карпа количества популяции теофиллинчувствительных Т-лимфоцитов и проявляет стимулирующее влияние на В-лимфоцитарное звено иммунитета. **Выводы.** Скармливание карпу добавок к крахмальному клейстеру L-аргинина и спиртового экстракта эхинацеи пурпурной активирует клеточное и гуморальное звенья иммунитета и влияет на функциональное состояние иммунокомпетентных клеток, о чем свидетельствует увеличение в крови относительного количества Т- и В-лимфоцитов и перераспределение авидности в сторону укрепления рецепторного аппарата плазматических мембран Т-лимфоцитов.

Ключевые слова: карп, L-аргинин, эхинацея пурпурная, кровь, Т- и В-лимфоциты.

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