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THE RELATIVE PROPORTION OF T-AND B-LYMPHOCYTES AND THEIR FUNCTIONAL ACTIVITY OF FEMALE-NURSERY CARPS DEPENDING ON THE LEVEL OF VITAMIN A IN THE RATION

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One of the most actual scientific and practical issues of contemporary pond fish farming is to increase the resistance of fish to diseases and negative anthropogenic environmental factors. In this regard, the development of effective methods of carps feeding, considerable attention is given to the search of the immune status of fish. In the specialized fish farms fish breeding is closely connected with its feeding, which aim is to get maximal number of high quality products in the shortest terms for the least feed cost. It is important to ensure a complete fish ration with vitamins, especially vitamin A.

The purpose of the search ascertain the influence of different levels of vitamin A in the ration of female-nursery carps on the activity of T- and B-cell immune units. The search was conducted on three groups of female-nursery scaly carps, to which during before-spawning period, vitamin A was carried in, in the form of retynilacetate at doses of 2500 IU and 5000 IU. It was determined that additional input into the

content of the ration of female-nursery carps vitamin A is finding out the stimulating effect on the number of T-and B-lymphocytes, as well as on their functional activity through the redistribution of lymphocyte receptor system in the direction of increasing their avidity. In particular, the number of T-lymphocytes (total, active, theophylline-resistant and-susceptible) and B-lymphocytes in the blood of female-nursery carps, which in addition to the feed were fed with vitamin A was greater than that of female-nursery carps, who consumed only a standard feed. It was found out a greater number of T-and B-lymphocytes in the blood of female-nursery carps from experimental groups compared to the control.

Keywords: CELLULAR STAGES OF IMMUNITY, T-LYMPHOCYTES, B-LYMPHOCYTES, FEMALE-NURSERY, CARP, DOSE, VITAMIN A, RETYNILATSETAT

ВІДНОСНА КІЛЬКІСТЬ Т- І В-ЛІМФОЦИТІВ ТА ЇХ ФУНКЦІОНАЛЬНА АКТИВНІСТЬ У САМОК-ПЛІДНИКІВ КОРОПА ЗАЛЕЖНО ВІД РІВНЯ ВІТАМІНУ А У РАЦІОНІ

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Однією з найбільш актуальних науково-практичних проблем сучасного ставкового рибориства є підвищення резистентності риб до захворювань і негативних техногенних факторів зовнішнього середовища. У зв'язку з цим при розробці ефективних методів годівлі коропів значна увага приділяється дослідженню імунного статусу риб. В умовах спеціалізованих рибницьких господарств вирощування риби тісно пов'язане з її годівлею,

метою якої є отримання максимальної кількості продукції високої якості в найкоротші терміни за мінімальних витрат кормів. При цьому важливе значення має повноцінне забезпечення раціону риб вітамінами, зокрема вітаміном А.

Мета дослідження полягала у з'ясуванні впливу різного рівня вітаміну А у раціоні самок-плідників коропа на активність Т- і В-клітинної ланки імунітету. Дослідження

проведено на трьох групах самок-плідників лускатого коропа (*Cyprinus carpio* L.), яким у переднерестовий період до комбікорму вносили вітамін А у формі ретинілацетату у дозах 2500 ІО і 5000 ІО. Встановлено, що додаткове введення у склад раціону самок-плідників коропа вітаміну А виявляє стимулювальний вплив на кількість Т- і В-лімфоцитів, а також на їх функціональну активність за рахунок перерозподілу рецепторного апарату лімфоцитів у бік збільшення їхньої авідності. Зокрема, кількість Т-лімфоцитів (загальних, активних, теофілін-резистентних і теофілін-чутливих) і В-лімфоцитів у крові самок-плідників коропа, яким додатково до

комбікорму згодовували вітамін А була більша, ніж у самок-плідників, які споживали лише стандартний комбікорм. При цьому у крові самок-плідників коропів дослідних груп, порівняно до контрольної виявлено більшу кількість Т- і В-лімфоцитів з низькою і середньою авідністю, і меншу кількість недиференційованих лімфоцитів крові.

Ключові слова: КЛІТИННА ЛАНКА, ІМУНІТЕТ, Т-ЛІМФОЦИТИ, В-ЛІМФОЦИТИ, САМКА-ПЛІДНИК, КОРОП, ДОЗА, ВІТАМІН А, РЕТИНІЛАЦЕТАТ

ОТНОСИТЕЛЬНОЕ КОЛИЧЕСТВО Т-И В-ЛИМФОЦИТОВ И ИХ ФУНКЦИОНАЛЬНАЯ АКТИВНОСТЬ У САМОК-ПРОИЗВОДИТЕЛЕЙ КАРПА В ЗАВИСИМОСТИ ОТ УРОВНЯ ВИТАМИНА А В РАЦИОНЕ

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Одной из наиболее актуальных научно-практических проблем современного прудового рыбоводства является повышение резистентности рыб к заболеваниям и негативным техногенным факторам внешней среды. В связи с этим при разработке эффективных методов кормления карпов значительное внимание уделяется исследованию иммунного статуса рыб. В условиях специализированных рыбоводческих хозяйств выращивания рыбы тесно связано с ее кормлением, целью которого является получение максимального количества продукции высокого качества в кратчайшие сроки при минимальных затратах кормов. При этом важное значение имеет полноценное обеспечение рациона рыб витаминами, в частности витамином А.

Цель исследования заключалась в выяснении влияния разного уровня витамина А в рационе самок-производителей карпа на активность Т-и В-клеточного звеньев иммунитета. Исследование проведено на трех группах самок-производителей чешуйчатого карпа (*Cyprinus carpio* L.), которым в преднерестовый период в комбикорма вносили витамин А в форме ретинилацетата в дозах 2500 ІО и 5000 ІО. Установлено, что дополнительное введение в рацион самок-

производителей карпа витамина А оказывает стимулирующее влияние на количество Т-и В-лимфоцитов, а также на их функциональную активность за счет перераспределения рецепторного аппарата лимфоцитов в сторону увеличения их авидности. В частности, количество Т-лимфоцитов (общих, активных, теофиллин-резистентных и теофиллин-чувствительных) и В-лимфоцитов в крови самок-производителей карпа, которым дополнительно к комбикорму скармливали витамин А была больше, чем у самок-производителей, которые употребляли только стандартный комбикорм. При этом в крови самок-производителей карпов опытных групп, по сравнению с контрольной выявлено большее количество Т- и В-лимфоцитов с низкой и средней авидностью и меньшее количество недифференцированных лимфоцитов крови.

Ключевые слова: КЛЕТОЧНОЕ ЗВЕНО, ИММУНИТЕТ, Т-ЛИМФОЦИТЫ, В-ЛИМФОЦИТЫ, САМКИ-ПРОИЗВОДИТЕЛИ, КАРП, ДОЗА, ВИТАМИН А, РЕТИНИЛАЦЕТАТ

Introduction. Now there is no clear answer to the question about the role of

vitamin A in immune reactions and also about the mechanism of its action. It can be assumed that the protective function of vitamin A in fish is realized through mucous membranes and through the interstitial fluid, which referred to the formation of vitamin has a direct relationship [1–3]. Discovery of the nuclear receptor of active metabolites of vitamin A-trans, cis-retinoic acid (retinoic acid receptor RAR and retinoic X receptor RXR), which regulate the transcription of genes had given a fundamental understanding of the mechanisms by which these substances affect the immune system [4–6].

Vitamin A plays an important role in the immune system and is necessary for the optimal functioning of innate and adaptive immunity [7]. Interest in vitamin A as immunoregulator is associated with a sensitivity of animals with deficiency of this vitamin to infections resulting from the depression of cellular and humoral immunity [8]. Regulation of immune function is carried out entirely-trans-and 9-cis-retinoic acid [9]. These molecules play a central role in the regulation of development, differentiation and apoptosis of immune system that are essential for the proper functioning of innate and adaptive immunity [8, 9]. It was found out that vitamin A deficiency leads to a decrease of cellular and humoral immunity. On the one hand, there is a dysfunction of lymphocytes, natural killer-cells and neutrophils, and the second — the inhibition of cell proliferation processes and antibody production. All this leads to an increased risk of various infections and diseases [9]. In the condition of vitamin A deficiency, humoral immune response to T-dependent antigens is suppressed, the activity of cytotoxic lymphocytes, IL-2 synthesis are reduced, synthesis of IL-10 and IL-12 is increased. Vitamin A deficiency attenuates innate immunity by preventing from the normal regeneration of damaged mucosa with infection and reduces the function of neutrophils, macrophages and natural killer-cells. Vitamin A is also required for adaptive immunity and plays an important role in the development of both T-helper cells and B-cells. In particular, vitamin A deficiency

diminishes antibody-mediated responses Th-2 cells, although some aspects of the Th-1-are mediated to immunity are also reduced [10, 13].

Taking into consideration the above mentioned, the aim of our study was to find out the effect of different levels of vitamin A in the ration in the form of retynil acetate on the activity of T-and B-cell links of immune system of female-nursery carps.

Materials and methods

Experiment was done on three groups of female-carps nursery at the age of six yers weighing 5.5–6 kg, which were grown in the experimental ponds of Lviv Search Station of the Institute of Fisheries NAAS. The temperature in the ponds ranged from normal. Hydrochemical indices of water and oxygen regime in a pond were within normal limits. Fish was caught by trawl from ponds. Fish of the first group who consumed a standard feed comprised control. 2500 IU of vitamin A in the form of 3.44 % of oil solution of retynilacetate was added to the standard feed of carps from the second group, and 5000 IU of vitamin A — to the third group of fish (JSC «Technologist», town Umanj). The experiment lasted for two months (May-June), after which five carps from each group were subjected to decapitation. For biochemical search blood samples were taken from them that were used in subsequent studies. In the stabilized by heparin blood it was determined the total number of T cells (TE-RUL), the number of active (TA-RUL) — in rosette reaction with ram erythrocytes and their subsets — T-helper and T-lymphocytes with predominantly suppressor activity by subtracting the number of teofil-resistance of T cells from the total T-lymphocytes, B-lymphocytes (EAS-RUL) in the reaction of complementary rosette with ram erythrocytes. When counting the number of T- and B-lymphocytes and their regulative subpopulations of fixed and painted blood smears lymphocytes was determined with low, medium and high density (more than 10 cells) of receptors and functionally undifferentiated lymphocytes.

The obtained numerical data are statistically worked on using Microsoft EXCEL. Digital data were processed statistically using Student's t criteria.

Results and discussion

As the results of research the total number of TE-RUL and their functional activity in the blood of female-nursery carps depends on the level of vitamin A in the ration. From the data in Table 1 we can see that the blood of female-nursery carps of the second and third groups when additionally received

accordingly 2500 and 5000 IU of vitamin A, the total number of TE-RUL is greater than carp of the second who consumed a standard feed ($p<0.05$, $p<0.001$). The increase in the total number of TE-RUL in the blood of females-nursery carps of research groups occurred due to redistribution of receptor avidity of immune system cells. In particular, the increase quantity in TE-RUL with low and average level of avidity ($p<0.05$, $p<0.01$) and decrease number of undifferentiated in functionally cells attitude ($p<0.05$; $p<0.001$).

Table 1

Number of TE RUL and their functional activity in the blood of female-nursery carps, depending on the level of vitamin A in the diet, % ($M\pm m$, $n=5$)

Index	Group of fishes		
	I	II	III
Total (TE-RUL), 0	60.0 \pm 0.32	57.2 \pm 0.86*	56.0 \pm 0.62***
3–5	28.6 \pm 0.51	30.2 \pm 0.37*	31.0 \pm 0.32**
6–10	7.8 \pm 0.24	8.2 \pm 0.38	9.6 \pm 0.51*
M	3.6 \pm 0.51	4.4 \pm 0.51	3.8 \pm 0.49
%	40.0 \pm 0.32	42.8 \pm 0.86*	44.4 \pm 0.68***

Note: 1.* — $p<0,05$; ** — $p<0,01$; *** — $p<0,001$ compared with a group of fish that were consumed only combined feed; 2.° — $p<0,05$; °° — $p<0,01$ compared between the 2nd and 3rd groups

The research of the total number of «active» T-lymphocytes (TA-RUL) showed (tabl. 2) that in blood of female-nursery carps of the second and third groups whose ration was enriched with, respectively 2500 and 5000 IU of vitamin A, is much higher than in blood of female-nursery carps of the first group who had consumed the standard feed ($p<0.001$). Thus in the blood of female-nursery carps of experimental groups compared with

the carps of the first group it was found out a greater number of TA-RUL with low avidity ($p<0.01$; $p<0.001$) and less quantity of undifferentiated cells ($p<0.01$; $p<0.001$). It was also found out an increase in the total number of TA-RUL-blood of female-nursery carps of the second groups and compared to the third group ($p<0.01$), and also reduction of undifferentiated T-lymphocytes ($p<0.05$).

Table 2

Number of TA-RUL and their functional activity in the blood of female-nursery carps, depending on the level of vitamin A in the diet, % ($M\pm m$, $n=5$)

Index	Group of fishes		
	I	II	III
Total (TA-RUL), 0	79.8 \pm 0.40	76.8 \pm 0.43**	75.0 \pm 0.32*** °
3–5	18.0 \pm 0.32	20.2 \pm 0.37**	21.4 \pm 0.51***
6–10	2.4 \pm 0.42	3.0 \pm 0.45	3.4 \pm 0.43
M	0	0	0
%	20.2 \pm 0.37	23.2 \pm 0.37***	25.0 \pm 0.32*** °°

Thus, the addition to the standard feed of carps vitamin A shows a stimulating effect on the number and functional activity of TA-RUL and TE-RUL in the blood of fish from research groups.

In determining the amount of theophylline-resistant populations of T-lymphocytes in the blood of carp, depending on the level of vitamin A in their ration, it was found out similar changes in determining TE-

and TA-RUL. Thus, from the above mentioned data in table 3, we see that in the blood of carps of the third group, which ration was added with vitamin A, the total number of Th-lymphocytes and theophylline-resistant T lymphocytes with low receptor density was greater and undifferentiated — less ($p<0.05$) than their number in the blood of carps of the first group who consumed the standard feed. These results show immunostimulating influence on supplement of vitamin A on the quantity and functional activity of the indicated populations of T lymphocytes. It should be noted that the number and functional activity of T-lymphocytes-helpers in the blood

of carps of the second group was greater than that of carps of the first group. However, these differences were unreliable.

He results shown that the number of theophylline-sensitive T-lymphocytes in the blood of carps of the second and third groups which additionally consumed, respectively, 2500 and 5000 IU of vitamin A was greater than that of carps of the first group who have got only the standard combined feed. It should be noted that the difference in the number of theophylline-sensitive T lymphocytes in the blood of carps of the third group were trustworthy ($p<0.05$). However, this does not significantly affect on the immunoregulatory index (correlation).

Table 3

The relative amount of Th and Ts-lymphocytes in the blood of female-nursery carps, depending on the level of vitamin A in the diet, % ($M\pm m$, $n=5$)

Index	Group of fishes		
	I	II	III
Th theophylline-resistant, 0	76.6 \pm 0.75	75.6 \pm 0.93	74.2 \pm 0.49*
3–5	20.2 \pm 0.49	20.6 \pm 0.40	22 \pm 0.45* °
6–10	3.2 \pm 0.49	3.8 \pm 0.66	3.4 \pm 0.62
M	0	0	0
%	23.4 \pm 0.75	24.4 \pm 0.93	25.8 \pm 0.49*
Ts theophylline-sensitive, %	16.6 \pm 0.81	18.4 \pm 0.40	18.6 \pm 0.24*
IRI	1.43 \pm 0.12	1.33 \pm 0.07	1.40 \pm 0.02

The research results testify the stimulating influence of the additional quantity of vitamin A in the content of feed on the quality and functional activity of T-lymphocytes (total, active and theophylline-resistant).

The research of B-lymphocytes in the blood reflects the level of humoral links of immunity. As a result it was found out that their number in the blood of female-nursery carps, as well as the number of T-lymphocytes, significantly depends on the level of vitamin A in their ration. As you can see from the results, presented in table 4, the

number of EAC-RUL in blood of female-nursery carps of the second and third groups was greater and the number of undifferentiated in functionally cells relation is less than carps of the first group ($p<0.05$). These data indicate the immunostimulating influence of additional introducing of vitamin A in the ration of carps on the activity of humoral link of immune response of fish. By the degree of differentiation of B lymphocytes in the blood of female-nursery carps of experimental groups compared to the control, a greater number of low-input EAS-RUL was found out ($p<0.001$).

Table 4

The relative amount of B-lymphocytes (EAS-RUL) in the blood of female-nursery carps, depending on the level of vitamin A in the diet, % ($M\pm m$, $n=5$)

Index	Group of fishes		
	I	II	III
B-lymphocytes (EAS-RUL), 0	59.6 \pm 0.93	56.8 \pm 0.74*	56.8 \pm 0.37*
3–5	28.4 \pm 0.25	30.4 \pm 0.25***	31.2 \pm 0.37***
6–10	8.0 \pm 0.32	8.6 \pm 0.81	8.6 \pm 0.51
M	4.0 \pm 0.55	4.2 \pm 0.37	3.6 \pm 0.51
%	40.4 \pm 0.93	43.2 \pm 0.74*	43.2 \pm 0.37*

Increasing of the number of EAC-RUL in the blood of female-nursery carps of research groups can be explained by the influence of additional introducing of vitamin A in their ration on the amount of theophylline-resistant populations of T-lymphocytes, that activate lymphopoiesis and differentiation of B-lymphocytes.

Thus, the research have shown that the number of T and B lymphocytes and also their functional activity in the blood of carps varies depending on the level of vitamin A in the ration. Increasing the number and functional activity of T-and B-lymphocytes in the blood of carps can be explained as a direct and indirect influence of vitamin A on the expression of T-and B-lymphocytes on the plasma membrane. This is evidenced by the results of research by other authors testify it. Particular, we found out that vitamin A works in synergy with protective activity of immune cells. All these elements are necessary for production of antibodies. It was found that deficiency of vitamin A decreases natural function of killer [7]. There is evidence that deficiency of vitamin A leads to a decrease in the number of T-lymphocytes, disrupted the mitogen and antigen-induced DNA synthesis, increases the number of 0-cells, reduced the correlation of T-lymphocytes /B-lymphocytes (number of B lymphocytes does not change) occur in other disorders of the activity of immune system [2, 3].

Conclusion

Retynilacetate introduction into the ration of carps leads to increase number of T-and B-lymphocytes in their blood and increases the functional activity of immune competentive cells through the redistribution of lymphocyte receptor system in the direction of increasing their avidity.

Prospects for further research. It is advisable to continue the study of the influence of vitamin A on the immune system, in particular its influence on humoral protective factors in the blood of female-nursery carps.

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