Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2019, 9(3), 168-173

ORIGINAL ARTICLE

Influence of seasonal factors on carp fish immune reactivity

O.P. Rudenko, R.P. Paranjak, N.A. Kovalchuk, L.P. Kit, N.I. Hradovych, B.V. Gutyj, B.M. Kalyn, O.P. Sukhorska, A.A. Butsiak, S.I. Kropyvka, V.V. Petruniv, L.M. Kovalska

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies
Lviv, Pekarska Str., 50, Lviv, 79010, Ukraine
E-mail: olgarudenko86@ukr.net

Received: 26.09.2019. Accepted: 26.10.2019

The article presents data about influence of seasonal factors on immune reactivity in carp scaly, carp framey, and carp. We studied the blood that was taken from the heart of fish in different seasons: spring (May), summer (August) and autumn (October). The research was carried out at the Lviv department of the Institute of Fisheries of the National Academy of Agrarian Science, Velykyj Ljubin in three groups of two-year-old fish. The received data give reason to think that the number of T- and B-lymphocytes in the blood of carp fish and their functional activity to a large extent depends on the influence of seasonal factors (temperature of the medium, oxygen concentration, duration of light day). This is points to the probable increase in the number of T-lymphocytes (common, active, theophylline-sensitive and theophylline-resistant) and a decrease in B-lymphocytes in the blood of investigated fish in the summer and, especially in the autumn period of research. In this case, an increase in the functional activity of T-lymphocytes was revealed due to the redistribution of the receptor apparatus of the immunocompetent cells. In particular, a reduction in the number of blood of inactive T-lymphocytes in functionally and an increase in cells with low and medium receptor density. Regarding the degree of differentiation of B-lymphocytes, the decrease in their amount in the blood of the studied individuals occurred due to the increase of "zero" and low-conductivity EAS-RUL and reduction of the subpopulation with average receptor density. We suggested that the abiotic factors can modulate the immune response of carp fish.

Keywords: immune reactivity; fish; carp; abiotic factors

Introduction

Seasonal factors – Is one of the main abiotic influences on living organisms and the environment of their existence. By the main abiotic factors of the aqueous medium include temperature, oxygen content, light daytime, feed resources and pH of the aqueous medium (Aspen-Baxter, 2007; Grynevych et al., 2018). The temperature regime is the main abiotic factor. The temperature regime affects the body of fish directly - changing the intensity of the enzymatic processes occurring in the organism, the activity of food intake, metabolism (Fediaiev, 2003; Ahmad et al., 2014). Carp is the main type of industrial fish in Ukraine. Abiotic environmental factors greatly affect the morphological, immunological and physiological and biochemical features of the carp organism (Hrytsyniak et al., 2008).

The optimal temperature range for growth and development of the carp varies from 25 to 27 °C. Under conditions of increase or decrease in water temperature, the intensity of nutrition and assimilation of food in it is decreasing (Oleksiienko & Hrytsyniak, 2007). Relatively high temperatures are a favorable factor for the growth of cyanobacteria, high loading of organic matter, densification of plankton in an aquatic environment (Neofitou & Klaoudatos, 2008). Moreover, the excessive loading of organic matter into the aquatic environment increases the biological consumption of oxygen, reducing its content in the aquatic environment, which can lead to mass death of fish (Boet et al., 1999; Winemiller et al., 2008). Oxygen is important for metabolic processes. Like all aerobic organisms, fish react sensitively to the influence of reactive oxygen (Kondratiev et al., 2001).

Biological rhythms significantly affect the hematological status of the organism. Namely, clearly expressed daily and yearly (seasonal) influences on fluctuations of all blood parameters (Shah, 2009). Seasonal fluctuations in the concentration of hemoglobin are potentially related to changes in water temperature and changes in oxygen concentration in water (Cameron, 2010; Akinrotimi et al., 2011). In conditions of winter fasting in the organism of fish the temperature and resource-deficient stress is developing. At the beginning of stress in the organism there is a short-term state of hypoxia (Tymochko et al., 1998).

Like other hematological parameters, the count of white blood cells in fish depends on various abiotic and biotic factors, such as water temperature, ecological stress, age (Holland et al., 2002; Hollick & Chen, 2008). In particular, in the winter period, a differential count of leukocytes is characterized by a higher proportion of lymphocytes, and a smaller proportion of granulocytes.

The population of lymphocytes in fish performs the function of adaptive immunity, as well as lymphocytes of mammals. It distinguishes subpopulations, similar to superficial markers and functions to T- and B-lymphocytes of mammals (Khariv et al., 2017). The literature suggests that the fish have the same mechanisms of immunity as mammals, only their manifestation

depends on the temperature of the body of fish, which in turn fluctuates in accordance with the temperature of water, the dissolution of oxygen, the duration of the light day (Kondratiev et al., 2001; Kondratiev & Kitashova, 2002).
The purpose of this search was to evaluate the influence of seasonal variations of abiotic parameters on the characteristics of immune reactivity in carp scaly, carp framey and carp, in particular, to determine the parameters of the cellular resistance level during the spring, summer and autumn periods of their cultivation.

Materials and methods

The research was carried out at the Lviv department of the Institute of Fisheries of the NAAS, Velykyj Ljubin in three groups of two-year-old fish. Carp scaly and framey were grown adjacent in one pond, and carp - separately, in the nearby location. Two-year-old fish were grown under semi-intensive technology using grain mixtures. The hydrochemical regime in the ponds was maintained within the limits of fishery norms, by introducing fertilizers into water in relation to needs. Special control was carried out by limiting factors, in particular, on the content of Oxygen in water, permanganate oxidation, whose values were not allowed below 2.5 mg/L for Oxygen, and above 19.0 ml of oxidation. The development of feed hydrobionts was stimulated by the introduction of humus into the water with a rate of 2 t/ha and the creation of favorable hydrobiological indicators of the reservoir.
The material used for research was blood that was taken from the heart of fish in different seasons: spring (May), summer (August) and autumn (October).
In blood samples, the total number of T-lymphocytes (E-RUL) was determined - determined in the reaction of spontaneous rosacea with red blood cells of ram (Jondal M. et al., 1972), their subpopulations are T-helperies (Th-RUL; Surovas V.M. et al., 1980); the number of "active" T-lymphocytes (TA-RUL; Wansbrough-Jones M. et al., 1979); the number of T-cells with predominantly suppressor activity (Ts-RUL) – subtracting the number of theophylline-resistant T-cells from the total number of T-lymphocytes, the immunoregulatory index (IRI) was calculated from the ratio Th/Ts, B-lymphocytes (EAS-RUL) – In the reaction of complementary socket formation with erythrocytes of a ram (Chernushenko E.F. et al., 1979). When counting the number of T- and B-lymphocytes and their regulatory subpopulations on fixed and painted smears of blood, lymphocytes with low, medium and high density of receptors were determined (Gutyj et al., 2017; Kushnir et al., 2019).

Results

Significant interest is the study of immunobiological reactivity of the cellular link of a specific immune response in carp fish in connection with seasonal factors.
The conducted searches showed that the total number of T- and B-lymphocytes, as well as their regulatory populations in the blood of scaly carp, carp framey and carp in the three growing seasons to a large extent depend on seasonal factors.
As we can see from the data presented in Table 1, the total number of T-lymphocytes in the carp framey and carp in summer and especially autumn growing periods is greater ($P < 0.001$) than in spring period. At the same time, in the blood of scaly carp, a tendency towards a decrease in TE-RUL in the summer and an increase in the autumn period of the study was established. In particular, the number of TE-RUL with low and average density of receptors in the blood of carp framey and carp species in the given period of research was greater ($P < 0.05–0.001$), and the “zero”, undifferentiated in the functional aspect of the cells, is lower ($P < 0.001$) than in control. At the same time, these changes were expressed to a greater extent in the blood of carps.
Thus, the number of TE-RUL with average receptor of density in the summer and autumn periods was increased respectively by 2.6 ($P < 0.001$) and 4.6 times ($P < 0.001$), and by “zero” – by 12.3 ($P < 0.001$) was decreased and 25.4% ($P < 0.001$) compared to the spring research period.
From the data presented in Table 1, we see that the total amount of TA-RUL in the blood of carp scaly in the autumn period is greater ($P < 0.001$), and in the summer – less ($P < 0.05$) than in spring. At the same time, in the autumn period, in comparison with spring, the number of T-active lymphocytes in the blood of carp was also higher ($P < 0.001$).
It is known that T-lymphocytes are divided into functionally distinct populations, the main of which are T-helper (Th) and cytotoxic (Tc or CTL) cells.
There is probably a greater number of theophylline-resistant T-lymphocytes in the blood of carp scaly and carp in the autumn and in the carp in the summer period of the research. Regarding the degree of their differentiation, changes in the number of Th-lymphocytes in the blood of carp scaly and carp in these periods of research were due to an increase ($P < 0.05–0.001$) of the population of low-avid Th-cells and a decrease ($P < 0.05–0.001$) – “zero”, undifferentiated in the functional sense of theophylline-resistant T-lymphocytes.
Changes in the amount of TA-RUL in the blood of the carp frames during the experiment were not probable. An increase in the number of TA-RUL in the blood of carp scaly and carp in the autumn period occurred on the background of an increase in the number of low avid ($P < 0,01–0,001$) and a decrease ($P < 0,001$) of the number of undifferentiated in functional cells.
At the same time, it should be noted that the decrease in the number of T-active lymphocytes in the blood of carp scaly in the summer was due to a decrease ($P < 0.01$) of TA-RUL with low density of receptors and an increase ($P < 0.05$) of “zero” cells.
As the research has shown (Table 1), the number of theophylline-sensitive T-lymphocytes in the blood of carp in the summer and autumn periods was increased respectively by 2.4 ($P < 0.001$) and 3.2 times ($P < 0.001$), and in the frame carps – in 2.0 ($P < 0.001$) and 2,3 times ($P < 0.001$) in comparison with their number in the spring research period. At the same time, in the blood

of framey carp and carp in the summer and autumn period, the amount of Th was higher (P < 0.05–0.001), and in the spring period it was lower (P < 0.05) than in scaly carps.

Table 1. Relative number of T-common lymphocytes and their individual subpopulations in the blood of carp fish (M ± m, n = 5)

Indexes	Group of fish	Spring (contr.)	Summer	Autumn
TE-RUL 0	SC(contr.)	69.8±1.43	73.6±1.20	67.0±1.18
	RC	67.6±0.5	61.6±0.8^{ooo}	56.4±0.81^{ooo}
	C	76.0±1.30	63.7±1.45^{ooo}	50.6±1.4^{ooo}
3-5	SC(contr.)	23.2±1.39	16.3±0.66^{oo}	26.2±1.31
	RC	21.4±0.44	27.6±0.66^{ooo}	30.4±0.5^{ooo}
	C	21.0±0.89	27.0±1.15^{oo}	34.4±0.67^{ooo}
6-10	SC(contr.)	7.0±0.83	8.0±1.52	6.4±0.81
	RC	9.40±0.40	8.66±0.88	12.4±1.1^{o}
	C	3.00±0.63	8.0±0.57^{ooo}	14.0±1.51^{ooo}
M	SC(contr.)	-	2.1±0.57	-
	RC	1.6±0.24	-	-
	C	-	-	-
%	SC(contr.)	30.2±1.40	26.4±1.20	33.0±1.18
	RC	32.0±0.50	38.3±0.8^{ooo}	43.6±0.81^{ooo}
	C	24.0±1.30	36.3±1.45^{ooo}	49.4±1.4^{ooo}
TA-RUL 0	SC	71.6±1.43	79.3±2.3^{o}	62.2±0.37^{ooo}
	RC	63.2±1.52	66.4±1.76	64.0±1.48
	C	73.8±0.96	73.6±2.33	62.2±2.15^{oo}
3-5	SC	23.4±0.97	17.3±1.45^{oo}	30.4±1.2^{oo}
	RC	31.8±1.31	27.3±3.38	27.8±0.86^{o}
	C	18.4±0.67	21.0±1.73	30.6±2.06^{ooo}
6-10	SC	4.6±0.87	3.0±0.57	7.0±1.14
	RC	5.0±0.44	5.33±1.45	7.8±0.86^{o}
	C	7.4±0.50	4.3±0.3^{ooo}	6.8±0.91
%	SC	28.4±1.43	20.7±2.33^{o}	37.8±0.37^{ooo}
	RC	36.8±1.52	33.6±1.76	36.0±1.48
	C	26.2±0.96	26.3±2.3	37.8±2.15^{ooo}
Th-RUL 0	SC	81.0±0.94	81.3±0.88	77.4±0.92^{o}
	RC	75.4±0.81	77.0±0.57	74.0±0.70
	C	82.2±0.58	78.7±0.88^{o}	70.4±0.41^{ooo}
3-5	SC	14.0±0.44	12.7±0.88	19.4±0.5^{ooo}
	RC	17.2±0.37	17.6±0.32	18.6±0.92
	C	12.4±0.50	15.7±0.87^{o}	21.2±0.58^{ooo}
6-10	SC	5,0±0,54	6.0±1.52	3.4±0.5
	RC	7.4±0.37	5.3±0.88	7.4±0.67
	C	5.20±0.48	5.6±0.80	7.6±0.74^{o}
M	SC	-	-	-
	RC	-	-	-
	C	-	-	-
%	SC	19.0±0.94	18.7±0.66	22.6±0.92^{o}
	RC	24.6±0.81	23.0±0.57	26.0±0.70
	C	17.8±0.58	21.3±0.80^{o}	29.6±0.40^{ooo}
Indexes Ts %	SC	11.4±1.31	7.7±0.6^{o}	10.8±1.7
	RC	7.8±0.58	15.3±0.6^{ooo}	17.6±1.4^{ooo}
	C	6.2±0.96	15.0±1.5^{oo}	19.8±1.46^{ooo}
IRI	SC	1.78±0.21	2.45±0.21	2.43±0.40
	RC	3.23±0.37	1.50±0.07^{ooo}	1.52±0.17^{oo}
	C	3.06±0.35	1.44±0.17^{oo}	1.52±0.10^{oo}

Note. In this and the following table ^{o} – P < 0.05; ^{oo} – P < 0.01; ^{oooo} – P < 0.001 – probable differences in the parameters of summer and autumn period carps in comparison with the spring period of the research.

From the data presented in the table, the reduction of the magnitude of the immunoregulatory index in the blood of the framey carp and carp attracts attention in more than twice (P < 0.01–0.001) in the summer and autumn periods of the research compared with spring. Thus, in scaly carps, higher lymphocytic activity was recorded in the summer and autumn periods compared to spring.

Thus, the obtained results of the research indicate a significant influence of seasonal factors on lymphocytic activity in the blood of carp fish.

In the research of immune T-systems combine both quantitative and functional characteristics of its individual components. One of the important parameters of functional activity of peripheral blood lymphocytes is the Blast Transformation Index (RBT). As we can see from the obtained data (Table 1), the number of blast cells in the blood of scaly carp and carp in the autumn period is greater ($P < 0.001$) than in spring. At the same time, in the blood of scaly carp in the summer, the inhibition of the blast transfection of T-lymphocytes occurs in the T-lymphocytes of the FSH mitogen, compared with the spring search period ($P < 0.05$). The probable increase in the number of blast cells in the blood of the framey carps indicates that activation of cell proliferation processes, enhancement of the myogenesis of lymphocytes and increase of their functional activity passes.

In the analysis of the data presented in Table 2. attention is paid to the probably smaller number of B-lymphocytes in the blood of the framey and scaly carps and carps in the autumn period, compared with spring.

Table 2. The relative number of B-lymphocytes (EAS-RUL) in carp fish (M ± m; n = 5)

Indicators of EAS-RUL	Group of fishes	Periods of research		
		spring	summer	autumn
0	SC	71.8±1.11	73.7±1.20	77.2±0.58^{oo}
	RC	69.6±1.16	66.3±1.45	76.8±0.37^{ooo}
	C	72.4±0.24	69.7±1.45	74.6±0.5^{oo}
3-5	SC	19.0±0.44	18.3±0.88	19.0±0.44
	RC	22.4±1.24	22.0±1.15	28.2±0.37^{oo}
	C	16.2±0.37	20.33±0.88^{oo}	20.8±0.37^{ooo}
6-10	SC	9.20±0.86	8.0±0.57	3.8±0.37^{ooo}
	RC	8.0±0.70	9.1±0.57	5.0±0.31^{oo}
	C	9.8±0.48	9.0±0.57	4.6±0.67^{ooo}
M	SC	-	-	-
	RC	-	2.6±0.6	-
	C	1.6±0.24	-	-
%	SC	28.2±1.11	26.3±1.20	22.8±0.58^{oo}
	RC	30.4±1.16	33.7±1.45	23.2±0.37^{ooo}
	C	27.6±0.24	30.3±1.45	25.4±0.55^{oo}

At the same time, in the blood of fish during this period, a greater ($P < 0.01–0.001$) number of "zero" and low – avid EAS-RUL and smaller ($P < 0.01–0.001$) – antigen-binding B-lymphocytes with average receptor density were detected .

Discussion

Some authors argue that seasonal cycles are important and modulate the behavior of fish. The results of these searches indicate that not all immune factors are suppressed by low temperatures (Valero et al., 2014). Moreover, some of them depend to a large extent on the season more than others. The number of lymphocytes in the peripheral blood of fish is an order of magnitude higher than in the blood of mammals, it depends on the season, the physiological state. At low water temperatures, the rate of antibody formation in fish lymphocytes is decreased. It also reduces the ability of lymphocytes to destroy of antigen (Hansen & Zapata, 1998; Tavares-Dias & Moraes, 2007; Zou et al., 2010).

It is known that the population of T-lymphocytes consists of several subpopulations, the cells of which differ in their functional state. Therefore, the use of "active" rosette formation in the tests allows the determination of the subpopulation of T cells with high affinity receptors to indicator cells (erythrocytes) and actively interacts with them without additional sensitization.

Our research have shown that the total number of T- and B-lymphocytes, as well as their regulatory populations in the blood of scaly carp, framey carp and carp in the three seasons of their cultivation are largely dependent on the influence of abiotic factors. According to the literature it is known that the number of antigen-destroying lymphocytes in carp at a water temperature below 4 °C is likely to decrease, and at a temperature of 22–26 °C – Increases (Fagbenro et al., 2013).

We found an increase in the total number of T-lymphocytes in the blood of carp fish during the summer and autumn growing periods. These changes occurred due to the redistribution of the receptor apparatus of the immunocompetent cells. These data give reason to assert about the significant immune-stimulating effect of seasonal factors in the summer and autumn periods on the total number of T-lymphocytes and their functional activity in the blood of framey carp and carp, and, to a lesser extent, in scaly carp.

The total amount of TA-RUL in the blood of scaly carp in the autumn period is greater, and in the summer - less than in spring. At the same time, in the autumn period, in comparison with spring, a greater number of T-active lymphocytes was also recorded in the blood of carps. These data indicate the influence of seasonal factors on the amount and functional activity of TA-RUL in the blood of investigated fish, which coincides with the data of other authors (Zou et al., 2007). There were significantly more theophylline-resistant T-lymphocytes in the blood of scaly carp and carp in the autumn and in the carp in the summer period of the research. With regard to the degree of their differentiation, changes in the number of Th-lymphocytes in the blood of

scaly carp and carp in these periods of research were due to an increase in the population of low-avid Th-cells and reduction – "zero", undifferentiated in the functional relation of theophylline-resistant T-lymphocytes.

T-suppressors, besides performing of helper and cytotoxic functions, can also inhibit the immune response. To date, there are different views on the nature and physiological role of cells, which are called T-suppressors (Haugarvoll et al., 2008; Koppang et al., 2010). The conducted searches showed that the number of theophylline-sensitive T-lymphocytes in the blood of carps and in framey carp in the summer and autumn periods was increased compared with their number in the spring search period.

A decrease in the size of the immunoregulatory index in the blood of the framey carp and carp in the summer and autumn periods of the search compared with spring was noted.

Blast lymphocyte transformation is the process of activation of small lymphocytes, which are in a state of rest relatively low active or inactive T- and B -lymphocytes of peripheral blood (Apatenko, 1994).

One of the important parameters of functional activity of peripheral blood lymphocytes is the Blast Transformation Index (RBTL) (Zaki, 2010). The results of our searches have shown that the number of blast cells in the blood of scaly carp and carp in the autumn period is greater than in spring. At the same time, in the blood of scaly carp in the summer time, the inhibition of the reaction of blast transfection of T-lymphocytes against the T-lymphocytes of the FSH mitogen was observed, compared with the spring study period. Together with this, in the blood of framey carp, compared with scaly in the spring and summer period of searches, an increase in the immune response of blood T lymphocytes to blast transformation into phytohaemagglutinin was observed.

The probable increase in the number of blast cells in the blood of the framey carps indicates that activation of cell proliferation processes, enhancement of the myogenesis of lymphocytes and increase of their functional activity passes.

In the study of the B-immune system, we found a significantly lower number of B-lymphocytes in the blood of the framey and scaly carp and carp in the autumn period, compared with spring. These changes in the amount of EAS-RUL in the blood of the carpal carp and carp were carried out on the background of a decrease in the number of "zero" and an increase in median intracellular B-lymphocytes. The results of the received research are in agreement with the data of other authors (Saha et al., 2002).

Conclusions

Thus, the results of experimental searches testify that the number of T- and B-lymphocytes in the blood of carp fish and their functional activity depends to a large extent on the influence of seasonal factors (temperature, oxygen concentration, light day duration). What it points to the probable increase in the number of T-lymphocytes (common, active, theophylline-sensitive and theophylline-resistant) and the decrease of B-lymphocytes in the blood of investigated fish in the summer and, especially, the autumn period of research. In this case, an increase in the functional activity of T-lymphocytes was revealed due to the redistribution of the receptor apparatus of the immunocompetent cells. In particular, a decrease in blood quantities of inactive T-lymphocytes in functional terms and an increase in cells with low and medium receptor of density.

Regarding the degree of differentiation of B-lymphocytes, the decrease in their amount in the blood of the experimental individuals occurred due to the increase of "zero" and low-avid EAS-RUL and reduction of the subpopulation with average receptor of density.

From the results of searches it can be argued that the influence of abiotic factors on the organism of carp fish can modulate their immune response.

References

Ahmad, T., Singh, S.P., Khangembam, B.K., Sharma, J.G., & Chakrabarti, R. (2014). Food consumption and digestive enzyme activity of Clarias batrachus exposed to various temperatures. Aquaculture Nutrition, 20(3), 265–272. doi: https://doi.org/10.1111/anu.12072/

Akinrotimi, O.A., Bekibele, D.O., & Orokotan, O.O. (2011). Selected haematological values of African catfish (Clarias gariepinus) raised in water recirculating system. International J Recirculating Aquaculture, 12, 1–12. doi: https://doi.org/10.21061/ijra.v12i1.1351.

Apatenko, V.M. (1994). Veterynarna imunolohiia ta imunopatolohiia. Kyiv.: Urozhai (in Ukrainian).

Aspen-Baxter, L. (2007). Biomes: Fresh Waters. Weigl Pub Inc

Boet, P., Belliard, J.et al. (1999). Multiple human impacts by the City of Paris on fish communities in the Seine river basin, France. Hydrobiologia, 410, 59–68. doi: https://doi.org/10.1023/A:1003747528595.

Cameron, J.N. (2010). The influence of environmental variables on the haematology of Pinfish, Lagodon rhomboids and mullet Mugil cephalus. Comparative Biochemistry and Physiology, 132, 175–192.

Cushing, C.E. & Allan, J.D. (2001). Streams: Their Ecology and Life. Journal of the North American Benthological Society, 21(1), 188. doi: https://doi.org/10.2307/1468308.

Fagbenro, O.A., Adeparusi, E.O., & Jimoh, W.A. (2013). Haematological profile of blood of African catfish (Clarias gariepinus, Burchell, 1822) fed sunflower and sesame meal based diets. Journal of Fisheries and Aquatic Science, 8, 80–86. doi: https://doi.org/10.3923/jfas.2013.80.86

Fediaiev, V.Ye. (2003). Stavkove rybnytstvo krainy: Mynule, sohodennia, maibutnie. Rybne hospodarstvo, 1, 38–39 (in Ukrainian).

Grynevych, N., Sliusarenko, A., Dyman, T., Sliusarenko, S., Gutyj, B., Kukhtyn, M., Hunchak, V. Kushnir, V. (2018). Etiology and histopathological alterations in some body organs of juvenile rainbow trout Oncorhynchus mykiss (Walbaum, 1792) at nitrite poisoning. Ukrainian Journal of Ecology, 8(1), 402–408. doi: https://doi.org/10.15421/2018_228

Gutyj, B., Grymak, Y., Drach, M., Bilyk, O., Matsjuk, O., Magrelo, N., Zmiya, M., & Katsaraba, O. (2017). The impact of endogenous intoxication on biochemical indicators of blood of pregnant cows. Regulatory Mechanisms in Biosystems, 8(3), 438–443. doi: https://doi.org/10.15421/021768.
Gutyj, B., Khariv, I., Binkevych, V., Binkevych, O., Levkivska, N., Levkivskyj, D., & Vavrysevich, Y. (2017). Research on acute and chronic toxity of the experimental drug Amprolinsyl. Regul. Mech. Biosyst., 8(1), 41–45.
Gutyj, B., Leskiv, K., Shcherbatyy, A., Pritsak, V., Fedorovych, V., Fedorovych, O., Rusyn, V., & Kolomiiets, I. (2017). The influence of Metisevit on biochemical and morphological indicators of blood of piglets under nitrate loading. Regulatory Mechanisms in Biosystems, 8(3), 427–432. doi: 10.15421/021766
Gutyj, B., Martyshchuk, T., Bushueva, I., Semeniv, B., Parchenko, V., Kaplaushenko, A., Magrelo, N., Hirkovyy, A., Musiy, L., & Murska, S. (2017). Morphological and biochemical indicators of blood of rats poisoned by carbon tetrachloride and subject to action of liposomal preparation. Regulatory Mechanisms in Biosystems, 8(2), 304–309. doi:10.15421/021748
Hansen, J.D., & Zapata, A.G. (1998). Lymphocyte development in fish and amphibians. Immunol Rev, 166, 199–220.
Haugarvoll, E., Bjerkas, I., Nowak, B.F., Hordvik, I., & Koppang, E.O. (2008). Identification and characterization of a novel intraepithelial lymphoid tissue in the gills of Atlantic salmon. J Anat, 213(2), 202–210. doi: 10.1111/j.1469-7580.2008.00943.x
Holland, J.W., Pottinger, T.G., & Secombes, C.J. (2002). Recombinant interleukin-1 beta activates the hypothalamic-pituitary-interrenal axis in rainbow trout, Oncorhynchus mykiss. J Endocrinol, 175, 261–267.
Hollick, M.F., & Chen, T.C. (2008). Vitamin D deficiency: a worldwide problem with health consequences. American Journal of Clinical Nutrition, 87(4), 1080S-1086S. doi: 10.1093/ajcn/87.4.1080S.
Hrytsyniak, I.I., Nahorniuk, T.A., & Tarasiuk, S.I. (2008). Henetychna struktura porid i porodnykh hrup koropiv za okremymy henetyko-biokhimichnymy systemamy. Rybohospodarska nauka Ukrainy, 1, 29–35 (in Ukrainian).
Ivanov, A.A. (2003). The physiology of fish. Moscow: Mir (in Russian).
Khariv, M., Gutyj, B., Ohorodnyk, N., Vishchur, O., Khariv, I., Solovodzinska, I., Mudrak, D., Grymak, C., Bodnar, P. (2017). Activity of the T- and B-system of the cell immunity of animals under conditions of oxidation stress and effects of the liposomal drug. Ukrainian Journal of Ecology, 7(4), 536–541. doi: 10.15421/2017_157.
Kondratiev, I.A., & Kitashova, I.A. (2002). Modern views on the fish's immune system. Operation. Vestn. Mosk. University, Department of. Physiology of Microorganisms, biol. Faculty of Moscow State University. MV Lomonosov. Immunology, 2, 9–21 (in Russian)
Kondratiev, I.A., Kitashova, A., & Lange, M.A. (2001). Modern representation tion of the immune system of fish. Organization of the immune system of fish. Vestn. Mosk. University, Department of. Physiology of Microorganisms, biol. Faculty of Moscow State University. MV Lomonosov. Biology, 4, 11–23 (in Russian)
Koppang, E.O., Fischer, U., Moore, L., Tranulis, M.A., Dijkstra, J.M., Kollner, B. et al. (2010). Salmonid T cells assemble in the thymus, spleen and in novel interbranchial lymphoid tissue. J Anat, 217, 728–739. doi: 10.1111/j.1469-7580.2010.01305.x.
Kushnir, I.M., Kushnir, V.I., Gufriy, D.F., Gutyj, B.V., Vishchur, V.Ya., Bushueva, I.V., Kulish, S.M., Shcherbyna, R.O., Samura, T.A., & Stoyanovskyy, V.G. (2019). Subacute toxicity of the preparation “Biovir-P”. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 10(2), 674−680.
Neofitou, N., & Klaoudatos, S. (2008). Effect of fish farming on the water column nutrient concentration in a semi-enclosed gulf of the Eastern Mediterranean. Aquaculture research, 39(5), 482–490. doi: 10.1111/j.1365-2109.2008.01900.x.
Oleksiienko, O.O. & Hrytsyniak, I.I. (2007). Vnutrishnoporidna struktura ukrainskykh koropiv. Rybohospodarska nauka Ukrainy, 1, 21–27. http://nbuv.gov.ua/UJRN/rnu_2007_1_5/ (in Ukrainian).
Saha, N.R., Usami, T., & Suzuki, Y. (2002).Seasonal changes in the immune activities of common carp (*Cyprinus carpio*). Fish Physiology and Biochemistry, 26(4), 379–387. doi: https://doi.org/10.1023/B:FISH.0000009275.25834.67.
Shah, A.W. (2009). Impact of fish haematology of Anchar lake, Kashmir. Pakistan. Journal of Nutrition, 8(01), 42–45. doi: https://doi.org/10.3923/pjn.2009.42.45.
Tavares-Dias, M., & Moraes, F.R. (2007). Leukocyte and thrombocyte reference values for channel catfish (Ictalurus punctatus Raf.), with an assessment of morphological, cytochemical, and ultrastructural features. Veterinary Clinical Pathology, 36, 49–54.
Tymochko, M.F., Yeliseieva, O.P., Kobylinska, L.I., & Tymochko, I.F. (1998). Metabolichni aspekty formuvannia kysnevoho homeostazu v ekstremalnykh stanakh. Lviv (in Ukrainian).
Valero, Y., García-Alcázar, A., Esteban, M.Á., Cuesta, A., & Chaves-Pozo, E. (2014). Seasonal variations of the humoral immune parameters of European sea bass (Dicentrarchus labrax L.) Fish and Shellfish Immunology, 39, 185–187. doi: https://doi.org/10.1016/j.fsi.2014.05.011.
Winemiller, K.O., Agostinho, A.A. & Caramaschi, E.P. (2008). Fish ecology in tropical streams. Tropical streams ecology, 107–146.
Zaki, M.S. (2010). Assesment of the hazardous effect of lead pollution on Tilipia zilli, including hematological, biochemical and immunological and immunological parameters. Rep. Opin, 2, 82–87.
Zou, J., Bird, S., & Secombes, C.J. (2010). Antiviral sensing in teleost fish. Current Pharmaceutical Design, 16, 4185–4193.
Zou, J., Mercier, C., & Secombes, C. (2007). Discovery of multiple beta-defensin like homologues in teleost fish. Mol Immunol, 44(4), 638–647. doi: https://doi.org/10.1016/j.molimm.2006.01.012.

Citation:
Rudenko, O.P., Paranjak, R.P., Kovalchuk, N.A., Kit, L.P., Hradovych, N.I., Gutyj, B.V., Kalyn, B.M., Sukhorska, O.P., Butsiak, A.A., Kropyvka, S.I., Petruniv, V.V., Kovalska, L.M. (2019). Influence of seasonal factors on carp fish immune reactivity. *Ukrainian Journal of Ecology, 9*(3), 168-173.