E3S Web of Conferences **381**, 01073 (2023) https://doi.org/10.1051/e3sconf/202338101073
AQUACULTURE 2022

Use of compound feed with the probiotic feed supplement based on Bacillus bacteria for sterlet producers

Vadim Grigoriev[1], *Angelika* Kovaleva[1,*], *Peter* Geraskin[2], *Marina* Sorokina[1], *Alexander* Korchunov[1], *Dmitry* Rudoy[3,4], and *Anastasiya* Olshevskaya[3]

[1]Federal Research Centre the Southern Scientific Centre of The Russian Academy of Sciences, Chekhov Street, 41, Rostov-on-Don, 344006, Russia
[2]Astrakhan State Technical University, Tatishchev St., 16, Astrakhan, 414056, Russia
[3]Don State Technical University, Gagarin sq. 1, Rostov-on-Don, 344003, Russia
[4]FSBSI "ARC "Donskoy", Nauchny Gorodok Str.3, Zernograd, 347740, Russia

Abstract. The effect of feed with probiotics B-1895 and Subtilis-C on the growth and physiological condition of sterlet producers in the RCD was studied. It was found that the weight gain in the first experimental group was higher by 13.3 % and in the second group by 53.3 % in comparison with the control. A positive effect of the probiotics studied on the stabilization of the physiological state of the fish that experienced little stress at the beginning of the experiment has been revealed. As a result, the normalization of physiological and biochemical parameters with a decrease in blood levels of haemoglobin, cholesterol, triglycerides and beta-lipoproteids is noted. No significant effect of the probiotics studied on the generative function was found. Besides a positive effect of the probiotic «Subtilis-C» on the prevention of oocyte resorption was shown. **Key words:** sterlet, feed, probiotics, weight, growth, physiology, blood

1 Introduction

Fishmeal is the main component of feed in aquaculture and the main source of protein in the diet of farmed fish. But nowadays, its use in feeds is becoming increasingly difficult (shortages have arisen) and more expensive. Therefore, this component in feeds is often replaced by vegetable ingredients, which are characterized by lower nutritional value, incomplete amino acid composition, imbalance of minerals, which can adversely affect the health and immune protection of fish [1-4]. These disadvantages of adapted feed formulations can be corrected by the addition of probiotic organisms.

Additionally, with the intensification of cultivation systems, there is an increased risk of disease in hydrobionts and significant losses for farmers. The use of antibiotics in these cases leads to disturbances in the bacteriocenosis of fish, a general decrease in immunity as well as the occurrence of antibiotic-resistant strains of pathogenic microorganisms. To reduce the negative effects of antibiotics, the introduction of probiotic additives in feed has been

[*] Corresponding author: anhramova@yandex.ru

practiced for the past three decades, which, along with prebiotics and synbiotics, also have great potential for the prevention and treatment of various types of diseases in fish [5].

Their activity in the host is multidirectional. It can be the competition for nutrients with pathogenic microorganisms, synthesis of antibacterial compounds, immune stimulation, destruction of toxins and allergens, reduction of cholesterol levels in blood, excretion of heavy metals [6-10].

As positive results of the using of probiotic preparations in fish feed, a marked improvement in digestion, better digestion of feed, and, consequently, an increase in weight gain of animals, poultry, fish due to the increased activity of digestive enzymes has been observed [11-13]. More often than other probiotics in aquaculture, different species of Bacillus are used because they are FDA GRAS certified, which is one of the main criteria for products eaten by humans [14]. It is necessary to mention other, no less important, properties of probiotics in aquaculture, namely their stimulating role in increasing the survival rate of products at all stages of fish breeding. Thus, the use of the probiotic "Subtilis" as such a stimulant, has been shown to increase the survival rate of eggs, embryos and larvae, reduce mortality in the larval stage of development, increase viability in the early stages of ontogenesis, as well as improve natural immunity [15]. In addition, there are also publications on the effects of probiotics on the reproductive ability of fish such as Nile tilapia Oreochromis niloticus [16], oil catfish Ompok pabda [17], African catfish Clarias gariepinus [18] and European eel A. Anguilla [19].

Probiotics stabilise and diversify the microbial community and thereby optimise reproductive performance through the activation of hormones, enzymes and gene transcription [20]. This translates into increased individual fecundity and improved vitellogenic oocyte quality [21]. Indeed, feeding with B.subtilis at a dose of 1010 CFU g-1 for 90 days increased the individual fecundity and the number of mature females of O. Niloticus [16].

The above literatures demonstrate quite a wide potential of probiotics in improving the quality parameters of farmed fish, including reproductive characteristics.

The objective of this work was to study the effect of «Subtilis-C» and B-1895 preparations based on Bacillus subtilis, as part of feed on the functional state and reproductive performance of the studied fish.

2 Material and research methods

The studies were conducted in the aquarium complex of coastal scientific expeditionary base of the SSC RAS «Kagalnik» (Rostov Oblast, Azov district). The object of the study was sterlet producers weighing 2.0-2.2 kg. The fish were kept in pools of 2 m3 of closed water supply in the amount of 20 specimens in each experimental group. The conditions of keeping the fish in all groups were the same: the temperature during the whole period of the experiment was within 20.9-23.4 0C, which is the optimum for sturgeons [22, 23], the oxygen content was within the norm - 6.9-7.4 mg/l, pH - 6.72-6.94.

The probiotic supplement «Subtilis-C», a microbial mass of spore-forming bacteria Bacillus subtilis (BKM B-2250), Bacillus subtilis (BKM B-2287) and Bacillus licheniformis (BKM B-2252) was used for experiments. The bacterial content was at least 1 × 109 CFU per 1 g, and a synbiotic preparation based on B. subtilis strain (VKPM) B-1895, the number of colony-forming units in the preparation based on B. subtilis was 3 × 1010 at the time of study. For feeding, a pelleted compound feed, prepared according to GOST10385-2014 for sturgeon fish was used [24]. Fat emulsion with probiotic additives was introduced into the unfattened pellet until the crude fat content in the pelleted feed reached 12%. Before introduction, the fat emulsion was heated to 40 Co, a probiotic was added and a fat antioxidant was administered.

https://doi.org/10.1051/e3sconf/202338101073

The experimental options are shown in Table 1.

Table. 1. Research scheme.

Experimental groups	Duration of experiment, days	Diet
1 variant	65	compound feed with probiotic "B-1895" (0,1 %)
2 variant	65	compound feed with probiotic "Subtilis-S" (0,02 %)
Control	65	compound feed without probiotics

The rates of probiotic supplementation were determined on the basis of literature data and manufacturers' recommendations [25]. Experimental groups of fish were fed with probiotic preparation at the dose of 1 g per 1 kg of feed (variant 1) and 0.2 g per 1 kg of feed (variant 2). The duration of the experiment was 65 days. The fish were fed twice a day, in the morning and in the evening, the daily feeding rate was 0.5% of the total weight of the planted fish.

In the period of monitoring the weight of fish, gonads maturity stage (GMS) were determined using SonoScape SSI-600 ultrasound scanner, blood was taken from the tail vein to determine physiological and biochemical parameters.

To assess the physiological condition of the fish, blood parameters were examined: erythrocyte sedimentation rate (ESR), haemoglobin content. In addition, the levels of total protein, cholesterol, beta-lipoproteins, triglycerides in blood serum were measured using the methods we published earlier [26]. All findings were processed using variation and statistical methods [27]. Elements of statistical analysis with determination of arithmetic mean values and their standard error ($M \pm m$) were used. The significance of differences was assessed using Student's t-test. Differences were considered statistically significant at $p < 0.05$.

3 Results and discussion

The results of the fish growth study showed a higher growth rate in both variants of the experiments compared to the control group. If for the studied period in the control group of fish the growth by weight was 6.9 %, in the experimental - 8.5 and 10.9 % in the first and second respectively (figure 1). In relation to the control, the weight gain in the first variant was higher by 13.3 % and in the second by 53.3 %, which is comparable with the results obtained for juvenile and two-year-old Russian sturgeon. Thus, in the conditions of the basin workshop of the Temryukskiy sturgeon fish-farming hatchery, the growth of the average weight of young Russian sturgeon of the experimental group receiving the same preparation as in our experiment (variant 1), the preparation based on Bacillus subtilis (B-1895) in an amount of 0.1 % was higher by 19.2 % in comparison with the control group of fish [25]. Approximately the same results were obtained for two-year-old Russian sturgeon grown in cages on production compound feed OT-7 with synbiotic preparation «Pro-Stor». The weight gain of the experimental fish was 19.8% higher than that of the control fish [28]. The survival rate for the whole period of the experiment was 100%.

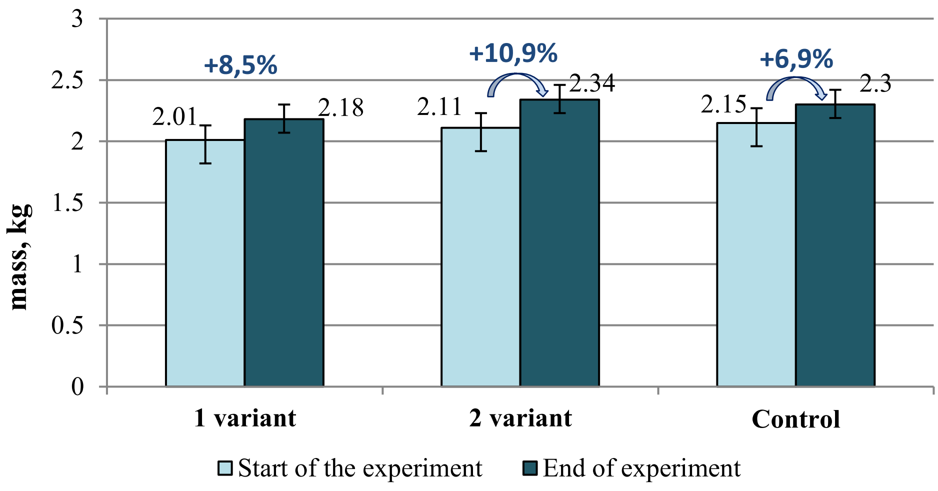

Fig. 1. Changes in sterlet producer weight using feed with probiotic.

A comparison of the producers' gonad maturation at the beginning and at the end of the experiment showed an increase in the number of fish with stages II and IV in variant 1 and in the control. The increase in the number of fish at stage II of gonad maturity is due to the fact that individuals at the final stages were overripe and further resorption of the gonads. The number of fish with stage III GSS decreased (variant 1 and control) or remained at the same level (variant 2).

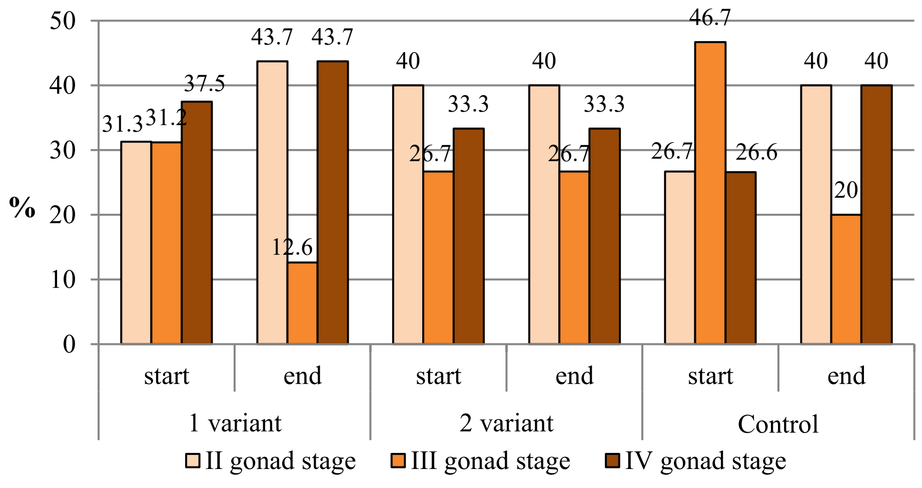

Fig. 2. Gonad maturity stages of sterlet producers at the beginning and end of the experiment.

In general, there were no major changes in the state of maturity of the gonads in all variants of the experiment. This proves that the probiotics used have no significant effect on the growth of the reproductive system of fish.

To assess the effect of the studied preparations on the physiological state of fish, we also analyzed haematological and biochemical blood parameters.

It is known that in the process of evolutionary development blood has acquired a number of important properties that reflect changes in the body, providing regulation and optimization of its relationship with the environment. Thus, haematological indicators can

https://doi.org/10.1051/e3sconf/202338101073

serve as indicators of the organism's reactions to stress, as also allow detecting the impact of unfavorable environmental factors on fish [29]. In addition, blood parameters can be used to judge about changes in the physiological state of fish, including their use to assess the impact of various feed additives on the organism [30-32]. As one of the haematological indices, we used a red blood cell count, which in all studied groups was within the reference values for sturgeons and was: at the beginning of the experiment, on average, 3.7; 4.5 and 4.8 mm/h in the 1st, 2nd variants and the control, respectively; at the end of the experiment the mean values were 3.95; 4.4 and 4.0, accordingly. The differences in these parameters at the beginning and end of the experiment were not significant and, respectively, were not statistically confirmed.

The results of the haemoglobin analysis after feeding fish food with probiotics were very positive (Tabl. 2).

Table 2. Physiological and biochemical parameters of sterlet blood.

Parameter	1 variant		2 variant		Control	
	start	end	start	end	start	end
ESR. mm/h	3.7±0.75	4.0±0.67	4.5±1.23	4.4±0.55	4.8±0.82	4.0±0.77
Hemoglobin. g/l	113.6±7.0	82.0±3.3*	94.5±9.1	70.9±3.8*	85.4±12.9	83.1±4.0
Total protein. g/l	22.8±2.2	38.2±1.8*	30.4±2.4	37.0±2.6*	19.9±1.1	36.9±1.9*
Beta-lipoproteins. g/l	5.21±0.56	4.12±0.47	4.98±0.41	4.24±0.47	4.89±0.40	4.96±0.61
Cholesterol. mmol/l	2.85±0.30	2.57±0.25	2.69±0.17	2.44±0.23	2.55±0.30	3.06±0.30
Triglycerides. mmol/l	6.00±0.53	3.43±0.27*	6.68±0.33	3.85±0.58*	6.56±0.65	5.90±0.87

Note: Differences are significant at $p < 0.05$.

It is necessary to note that at the beginning of the experiment the individual values of haemoglobin, fluctuated greatly. From very high values (160 g/l, 149 g/l) to very low values (30 g/l), which exceeded the range of reference values for sturgeon in the natural environment (50-80 g/l) [33]. By the end of the experiment, the haemoglobin content in blood of the studied fish in all variants of the experiment and in the control decreased, with variants 1 and 2 with statistical confirmation of reliability of differences ($P < 0,05$). At the same time, there is a decrease in the error of mean values (m), indicating a decrease in the variability of individual values of haemoglobin concentration of the studied fish. The increased variability, in this case, is associated with the effect on the fish of unusual environmental conditions or modification factor [34].

In the period before the experiment, we recorded a decrease of oxygen content in the water to 5.4-5.6 mg/l, which is close to the critical value for sturgeon fish, which has a depressing effect on fish [35]. As a consequence, it can be considered as a factor negatively affecting fish. In addition, in the conditions of modern fish farming, there is usually a stress that worsens the physiological state of the farmed fish [36]. This is evidenced by increased concentrations of beta-lipoproteins, cholesterol and triglycerides in blood at the beginning of the experiment in all studied groups of fish. Cholesterol - as a precursor of corticosteroid hormones, including glucocorticoids, whose effect on adipose tissue is to increase triglycerides [37], with beta-lipoproteins having a transport function, supplying cholesterol for glucocorticoid synthesis. This metabolic rearrangement is associated with an activation of energy metabolism due to an increase in energy expenditure under stress.

At the end of the experiment, a statistically insignificant decrease in the content of cholesterol and beta-lipoproteins in the blood of experimental fish was observed, unlike control, where the values of these indicators either did not change as for beta-lipoproteins or increased by 20% as for cholesterol. At the same time, triglyceride concentration in both variants of experiments significantly ($p < 0,05$) decreased by around 75%. Such dynamics of the studied indicators indicates a corrective effect of probiotics on the physiological state of sterlets, so that the growth rate of these fish was higher than that of the control. In the control group, the physiological condition of the fish remained at the same level - with signs of stress effects.

The dynamics of total protein content in blood should be analysed separately. At the beginning of the experiment, the initial blood protein content of fish in the first experimental group and control was low, in the second - higher by 33 and 53%, respectively, than in the previous groups. By the end of the experiment, significant changes occurred - there was a statistically significant ($P < 0.05$) increase in protein concentration in all variants (Tabl. 2). They were most significant in the first variant of the experiment and in the control. While in the second variant of the experiment, due to stabilization of the physiological state of fish, protein metabolism was to a greater extent redirected towards somatic growth (higher increase among the studied fish), the causes were somewhat different in the fish of the 1st experimental group and the control. This is primarily the resorption of oocytes (an increase in the proportion of females with II SSH) and the release of protein components in the blood. If in control fish this is the primary factor, then in the experimental group 1 it is added to the factor of physiological condition stabilization.

Because of the probiotics presence in the digestive tract of fish, metabolic processes, synthesis of enzymes, amino acids and vitamins during digestion are increased, which is confirmed by earlier studies [8, 30].

The stabilization of the physiological state is also confirmed by the reduction of the dispersion in the variants with probiotics compared with the initial values, from 6.6 to 5.2 in variant 1 and from 7.1 to 5.3 in variant 2, indicating greater homogeneity of the sample, in contrast to the data in the control, where by the end of the experiment its value increased almost twofold (from 3.4 to 6.4).

Conducting experiments with «B-1895» and «Subtilis-C» based on Bacillus subtilis on sterlet producers reared in a recirculating aquaculture system revealed their positive effect on the studied fish. Compared to control, the weight gain of the first experimental group («B-1895») was higher by 13.3 % and that of the second group («Subtilis-C») by 53.3 %. At the same time, improvement of physiological condition of experimental fish was fixed, which was expressed in stabilization and moderate decrease of physiological and biochemical blood indices (except for serum protein), unlike the control group of fish, in which they either remained unchanged or increased slightly. In the protein system, the most positive changes were noted in the second group of experimental fish with the highest weight gain. In the remaining studied groups (the first and control) noted resorption of oocytes, which influenced the increase in the blood of these fish total protein. From the obtained results of the conducted studies, it follows that the most positive effect of the studied probiotics in fish feeding was shown by «Subtilis-C» preparation.

Acknowledgements

This publication was prepared within the framework of the State Order of SSC RAS, Grant No. 122020100328-1, using the Unique Scientific Installation "Modular Unit-Complex" and «Bioresource Collection of Rare and Endangered Fish Species No. 73602» of the SSC RAS. This study was supported by the grant from the Russian Science Foundation RSF No.№ 23-76-30006.

References

1. M.A.F. Nasr, R.M. Reda, T.A. Ismail, A. Moustafa, Animals (Basel) **11(3)**, 889 (2021) doi: 10.3390/ani11030889
2. G. Estruch, M.C. Collado, R. Monge-Ortiz et al, BMC Veter Res. **14(1)**, 302 (2018) doi: 10.1186/s12917-018-1626-6
3. Y. Gu, X. Xu, Y. Wu, T. Niu, Y. Liu, J. Li, G. Du, L. Liu, Metab Eng. **50**, 109-121 (2018) doi: 10.1016/j.ymben.2018.05.006.
4. M. Gu, Q. Jia, Z. Zhang, N. Bai, X. Xu, B. Xu, Fish Shellfish Immunol **77**, 264– 272 (2018) doi:10.1016/j.fsi.2018.04.004
5. S.H. Hoseinifar, Y-Z. Sun, A. Wang et al, Front. Microbiol. **9**, 2429 (2018) doi: 10.3389/fmicb.2018.02429.
6. E. Ringø, H. Van Doan, S.H. Lee, M. Soltani, S.H Hoseinifar, R. Harikrishnan, S.K. Song, J. Appl. Microbiol. **129(1)**, 116–136 (2020) doi: 10.1111/jam.14628.
7. M. Soltani, K. Ghosh, S.H. Hoseinifar, V. Kumar, A.J. Lymbery, S. Roy, E. Ringø, Rev. Fish. Sci. Aquac. **27(3)**, 331–379 (2019) doi:10.1080/23308249.2019.1597010
8. M.K. Koilybaeva, G.O. Ustenova, D.J. Batyrbaeva, J.S. Alibaeva, K.K. Mustafina, Bulletin of KazNMU **4**, 181-184 (2018)
9. I.V. Tkacheva, Current Issues in Veterinary Biology **1(41)**, 52-55 (2019) doi: 10.24411/2074-5036-2019-10015
10. S.Wuertz, A. Schroeder, K.M. Wanka, Water **13(10)**, 1348 (2021). doi: 10.3390/w13101348
11. S. H. Hoseinifar, M. Dadar, E. Ringø, Aquac. Res, **48(8)**, 3987–4000 (2017) doi: 10.1111/are.13368
12. F.K.A. Kuebutornye, Z.W. Wang, Y.S. Lu et al, Fish Shellfish. Immunol. **97**, 83–95 (2020) doi:10.1016/j.fsi.2019.12.046
13. R. Tarkhani, A. Imani, S.H. Hoseinifar et al, Fish Shellfish. Immunol. **98**, 661–669 (2020) doi:10.1016/j.fsi.2019.10.063
14. J. Olmos, M. Acosta, G.M. Almanza, V. Pitones, Archives of Microbiology, **202(3)** (2020) doi: 10.1007/s00203-019-01757-2.
15. G.V. Golovko, V.A. Chistyakov, M.A. Sazykina et al, The use of probiotic additive based on Bacillus subtilis "B-1895" in aquaculture, URL: http://subtilis.ru/use/fish
16. D.d.C. Dias, F.d.P.B. Furlaneto, F.R. Sussel et al, Acta Scientiarum. Anim. Sci. **42**, e47960 (2020) doi:10.4025/actascianimsci.v42i1.47960
17. M.L. Rahman, S. Akhter, M.K.M. Mallik, I. Rashid, Int. J. Curr. Res. Life Sci. **7**, 866–873 (2018)
18. P.S.W. Kusuma, D. Hariani, A.T Mukti, Turk. J. Fish. Aquat. Sci. **22**, TRJFAS19303 (2021) doi:10.4194/TRJFAS19303
19. M.C. Vílchez, S. Santangeli, F. Maradonna et al, Theriogenology **84(8)**, 1321–1331 (2015) doi: 10.1016/j.theriogenology.2015.07.011.
20. G. Gioacchini, E. Giorgini, L. Vaccari, O. Carnevali, *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics* (Hoboken, NJ, USA, John Wiley & Sons, Ltd, 2014) doi:10.1002/9781118897263.ch12
21. Md.A.A. Sumon, M.H.R.Molla, I.J.Hakeem et al, Fishes **7(4)**, 189 (2022) doi:10.3390/fishes7040189

22. V.K. Golovanov, Y.V. Gerasimov, D.S. Kapshay, O.L. Vasyura, Vestnik ASTU, Ser.: Fishery **1**, 62-68 (2014)
23. V.K. Golovanov, I.L. Golovanova, Vestnik ASTU, Ser.: Fishery **3**, 110-118 (2015)
24. *GOST 10385–2014 Interstate standard. Combined feeds for fish. General technical conditions* (2014) https://docs.cntd.ru/document/1200113022
25. A. V. Sevryukov, M. A. Morozova, Y. I. Levchenko, T. S. Kolmakova, V. A. Chistyakov, Current Issues of Veterinary Biology **4(20)**, 49-56 (2013)
26. G.F. Metalov, E.N. Ponomareva, P.P. Geraskin, A.V. Kovaleva, V.A. Grigoryev, Vestnik ASTU. Ser.: Fishery **4**, 128-135 (2017) doi: 10.24143/2073-5529-2017-4-128-135
27. S.P. Kozhevnikov, *Algorithms of biological statistics* (Udmurtian University Publishing Centre, Izhevsk, 2018)
28. A.D. Zhandalgarova, A.V. Polyakov, A.A. Bakhareva, Y.N. Grozesku, Izvestiya Samara Scientific Centre of the Russian Academy of Sciences **20(2)**, 107-111 (2018)
29. N.Y. Simon, Fisheries Science of Ukraine **1(39)**, 92-117 (2017) doi:10.15407/fsu2017.01.092
30. A. Gutsulyuk, Samara Scientific Bulletin **4(13)**, 113-116 (2015)
31. G.F. Metalov, O.A. Levina, V.A. Grigoryev, A.V. Kovalyova, Vestnik ASTU. Ser.: Fishery **3**, 146-152 (2013)
32. E.N. Ponomaryova, A.V. Kovalyova, A.N. Stepanova, N.A. Kondratova, Fish Farming and Fisheries **3**, 57-68 (2014)
33. G. F. Metalov, E. N. Ponomareva, V. A. Grigoryev, A. V. Dubovskaya, P. P. Geraskin, O. A. Levina, M. N. Sorokina, Vestnik ASTU. Ser.: Fisheries **4**, 117-131 (2018) doi: 10.24143/2073-5529-2018-4-117-131
34. L.M. Kostyleva, P.I. Kostylev, *Biometric methods in plant breeding* (Azov-Chernomorsk Engineering Institute, Donskoy State Agrarian University, 2017)
35. G.G. Matishov, E.N. Ponomareva, N.G. Zhuravleva et al, *Practical aquaculture* (Publishing house of the SSC RAS, Rostov n/a, 2011)
36. A.V. Basankin, M.P. Semenenko, V.M. Basankina, Collection of scientific works of KNTsZV **11(1)**, 226-229 (2022) doi: 10.48612/sbornik-2022-1-56
37. E.V. Artemova, Obesity and metabolism **14(2)**, 48-52 (2017) doi: 10.14341/omet2017248-52