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A study of the possibility of using animal feed additives and probiotic feed additives in the diet of fish

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Abstract. The shortage of fish meal and fish oil provokes the search for alternative sources of these feed components. Insects that are part of the diet of animals, birds and fish, rich in protein and fat, can serve as such an alternative. The article presents the results of testing of compound feed for fish containing the fat of the larvae of the black soldier fly *(Hermetia illucens)* and a feed additive with probiotic activity. The use of the fat of the larva of the black soldier fly *(Hermetia illucens)* and a probiotic feed additive allows increasing the survival rate of fish from 90 to 95%, increase the conversion of feed and the average daily increase. The calculation of the economic efficiency from the use of compound feed, which includes feed additives of animal origin and additives with probiotic activity, showed an increase in profit when replacing compound feed made according to a standard recipe with compound feed with new feed additives. The economic effect of using compound feed with new feed additives amounted to more than 650 thousand rubles per year on small and medium-sized trout farms up to 20 tons per year. **Keywords:** compound feed; aquaculture; feed additives; probiotic; probiotic supplement; alternative protein sources; insect fat; animal protein; insects; black soldier; Hermetia illucens.

1 Introduction

The modern feed industry in Russia is an important link in the development of animal husbandry and aquaculture, which requires constant systematic development of the feed and raw materials base. The growth in demand and prices for compound feed, the increase in the falsification of feed raw materials, in particular fish meal and fish oil [1,2], forces us to look for alternative sources of feed raw materials that are not inferior in quality and price to existing ones.

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Marine zooplankton, namely Antarctic krill and calanus, as well as squid, mollusks, and other crustaceans, are considered as alternative sources of protein and fat [3-5]. Such an alternative has a number of disadvantages: their active use causes a risk of changing food chains; the high cost of the final product; the need to reduce fluorides [3, 4].

Vegetable components are also considered as alternative sources of protein, such as soy flour, protein concentrate from lupine and peas, oil cake and meal of oilseeds, corn stillage, wheat gluten, etc.

Typical soy flour contains from 44 to 48% dry protein and, according to various data, can replace from 25 to 100% fishmeal [5-7]. However, feeds with a high content of soy flour are associated with the anti-nutritional effects of oligosaccharides and trypsin inhibitors [5]. Therefore, when using soy flour, additional processing of soy is necessary in order to neutralise the effects of oligosaccharides and trypsin inhibitors on the body of animals and fish.

Alternative sources of protein and fat of animal origin can also be animal waste and by-products (blood, skin, feather, bones). Some components are already widely used in compound feeds for various animal species [3, 4]. But nevertheless, due to the existing disadvantages, their use is limited: low availability of amino acids in feather flour, which leads to a violation of the amino acid balance in the feed; low content of long–chain polyunsaturated fatty acids of the Omega-3 series; high levels of iron in blood meal, which can lead to the oxidation of important components of the diet, such as astaxanthin; relatively high melting point of lipid fractions, which limits the use of cold-water fish species in feed. In addition, the nutritional value of such products is not stable and depends on the solubility of some fractions, processing conditions, etc. [4].

Insects are gaining more and more popularity in the world as alternative sources of protein. Insects, especially in the larval stage, are rich in protein and fat and are actively eaten by omnivores in nature. Most often, grasshoppers, locusts, flour crunch, fly larvae (housefly, black soldier fly (BSF) are used for feed production [8-10].

The literature analysis shows that there are technical possibilities for the production of insects on an industrial scale and to use them as a high-protein resource in fish feeding. The sources [10-15] present the results of studies on the successful replacement of fish and soy flour with silkworm flour, flour crunch (mealworm) and grasshoppers.

Larvae of the black soldier fly *(Hermetia illucens)* showed good results in feeding chickens, pigs, rainbow trout, channel catfish and blue tilapia [10, 16-19]. Larvae can replace 25% of fish meal and 38% of fish oil for feeding rainbow trout. All studies were focused on the use of the protein part of the larva, while the effect of the fat fraction on the body of animals, birds and fish has not been studied enough.

The fatty acid analysis of larval fat was investigated by the author and presented in a scientific article [20]. Most of the fatty acid composition is occupied by lauric acid (more than 40%), which has a bactericidal effect, suppressing the development of pathogenic microflora [20, 21]. The second fatty acid contained in the larva is oleic. In large quantities, it is found in fish oil and olive oil, which has a positive effect on the animal's body and participates in vital processes.

Another important component in the fat of the larva of the black soldier fly *(Hermetia Illucens)* is vitamin E up to 25 mg/100 g. [22]. The addition of vitamin E to fish feed leads to an increase in the growth rate, improves their physiological condition, and reduces feed costs.

In addition to feed additives with a high protein content, probiotics are increasingly used in the feed industry. They have a positive effect on the general condition of the animal and fish, have an immunomodulatory effect, promote germination in weight, increase survival, feed conversion, and improve the organoleptic properties of meat [23-28].

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Thus, the purpose of the study is to conduct research on the effect of compound feed for fish, which contains the fat of the larva of the black soldier fly *(Hermetia illucens)* and a probiotic feed additive on fish breeding biological indicators of fish cultivation, their microbiota and calculate the economic efficiency of the use of the above feed additives in the fish diet.

2 Materials and Methods

Experimental studies on the effect of the fat of the larva of the black soldier fly *(Hermetia illucens)* and probiotic grown on the cake of the larva, as part of the production feed, on the growth, survival, and general condition of trout were carried out in standard planting pools. Cultivation and feeding were carried out according to the existing technology [3].

The standard compound feed for production fish RGM – 8B was taken as a basis [3]. The rate of introduction of larval fat into feed was 0.1-0.5%, of probiotic additives 0.10-0.15%. For obtaining a feed additive with probiotic activity for feeding fish, bacteria of the Bacillus amyloliquefaciens B–1895arm strain (see Bioresource Center All-Russian Collection of Industrial Microorganisms (BRC ARCIM) SRC "Kurchatov Institute" GosNIIgenetics accepted the culture of Bacillus amyloliquefaciens B–1895arm for national patent deposit. The date of deposit is December 28, 2021. The depositor is FSBEI HE DSTU. VKPM registration number: B–14100) are grown directly on a moist substrate with pronounced prebiotic properties: the black soldier fly larvae cake (*Hermetia illucens*), obtained after pressing fat with a screw press.

The new formula of compound feed for trout is presented in Table 1. A patent for the invention RU 2777768 C1 was obtained for this formulation.

Table 1. Formulation of compound feed for trout.

Ingredient	Contain, %
Fish meal	30-40
Meat and bone meal	7-9
Blood meal	4-6
Sunflower meal	19-21
Soy meal	19-21
Yeasts	5.4-5.5
Vegetable oil	3.6-4.0
Premix PF-2B	0.9-1.1
Fish oil	1.0-1.9
Fat of the larva of the black soldier fly *(Hermetia illucens)*	0.1-0.5
Probiotic supplement grown on the cake of the larva of the black soldier fly *(Hermetia illucens)*	0.10-0.15

The production compound feed was made by the well-known method of wet pressing. All feed components were mixed in a mixer, then sent to a laboratory granulator press for the subsequent formation of granules of various diameters and lengths at a temperature not exceeding 60 °C.

The effectiveness of the use of production compound feed with the addition of fat of the larva of the black soldier fly (*Hermetia illucens*) and probiotic was determined by survival rates, growth rate, mass accumulation coefficient, feed costs.

The study of the state of the microbiota of trout fed with standard compound feed and compound feed with the fat of the larva of the black soldier fly (*Hermetia illucens*) and probiotic was carried out as follows: 10 fish were selected from each group by the blind method and delivered to the laboratory chilled on ice for 1 day. Then the gastrointestinal tract was extracted from each fish and the contents of the intestine were extracted into a

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sterile container. Then the obtained samples were thoroughly mixed and a series of consecutive decimal dilutions were prepared. The determination of the number of microorganisms contained in the samples of intestinal contents was carried out by surface seeding, in the amount of 3 repetitions for each nutrient medium for each studied dilution.

3 Results and Discussion

3.1 Application of Black Soldier Fly Larva Fat (Hermetia Illucens) and Probiotic Feed Additive in Mixed Feeds for Trout

Table 2 presents the fish-breeding and biological indicators of trout cultivation on production compound feed with the addition of fat from the larva of the black soldier fly (*Hermetia illucens*) and probiotics.

Table 2. Fish-breeding and biological indicators of trout cultivation on production compound feed with the addition of fat of the larva of the black soldier fly (*Hermetia illucens*) and probiotic.

Indicators	Prototype	The proposed compound feed
Initial weight, g	50.93±9.80	50.84±10.10
Final weight, g	91.67±6.90	96.60±3.80
Total gain, g	40.74	45.76
Average daily gain, g	1.16	1.31
Cultivation, day	35	35
Survival rate, %	90	95
Feed ratio, units	1.1	1.1

The results of the experiments showed that the introduction of the fat of the larva of the black soldier fly (*Hermetia illucens*) and a probiotic grown on the cake of the larva into the production feed for trout had a positive effect on the growth, survival and general condition of the fish. In the experiment, the mass of fish increased by 1.9 times, in the control by 1.8 times with a survival rate of 95% and 90%, respectively.

The results of the organoleptic evaluation in accordance with GOST 7631 showed that the feed additive in the form of fat of the larva of the black soldier fly (*Hermetia illucens*) and probiotics as part of the production feed for trout does not have a negative effect on the general condition of the fish: the surface is clean, the natural color inherent in this type of fish, with a thin layer of mucus; there were no signs of diseases, the scales are shiny, tightly fitting to the body.

Table 3 shows the content of various groups of microorganisms in the contents of the intestines of fish.

Table 3. The content of various groups of microorganisms in the contents of the intestines of fish, KOE/g.

Group of microorganisms	Prototype	The proposed compound feed
Lactobacillus	$8.4 \pm 0.4 \cdot 10^5$	$8.2 \pm 0.4 \cdot 10^5$
Enterococcus	$1.6 \pm 0.2 \cdot 10^3$	$2.7 \pm 0.3 \cdot 10^3$
E.coli	$3.3 \pm 0.2 \cdot 10^2$	$4.0 \pm 0.2 \cdot 10^2$
Citrobacter, Enterobacter, and others	$4.7 \pm 0.3 \cdot 10^3$	$4.4 \pm 0.6 \cdot 10^3$
Vibrio	$2.5 \pm 0.2 \cdot 10^4$	$3.2 \pm 0.1 \cdot 10^4$
Bacillus	$5.0 \pm 0.4 \cdot 10^3$	$7.5 \pm 0.8 \cdot 10^3$

Table 3 shows that the fat of the larva of the black soldier fly (*Hermetia illucens*) and the probiotic grown on the cake of the larva does not have a negative effect on the microbiota of fish.

According to the results of the experiments, in order to increase the growth rate of trout and its survival, it is recommended to partially replace fish oil with the fat of the larva of the black soldier fly (*Hermetia illucens*) and add a probiotic grown on the cake of the larva as part of the production feed for trout. The composition of the production compound feed increases the survival rate of trout, and also contributes to an increase in the mass of fish without negatively affecting its general condition. The patent "Production compound feed for trout" RU 2777768 C1 [29] was obtained for this recipe of compound feed.

3.2 Calculation of the Economic Effect of Replacing Fish Meal and Fish Oil with Protein and Fat of the Larva of the Black Soldier Fly (Hermetia Illucens)

In order to determine the cost of the manufactured new compound feed and compare it with the prototype, the unit cost was calculated, as well as the wholesale and retail price was calculated.

The total cost of compound feed consists of material costs M_o, basic wages Z_o and additional wages D_o, insurance contributions to state extra-budgetary funds for social purposes in the State budget B_{roc}, general production costs P_{gp}, general economic expenses P_{ge}, commercial expenses P_{com}. Calculations were carried out in accordance with the methodological recommendations [30].

Material costs are the costs of purchasing raw materials for the manufacture of compound feed (Table 4).

Table 4. The need for raw materials and materials per unit of production.

Ingredient	Price, rub/tons	Content in standard compound feed, %	Content in the new compound feed, %	Raw material costs for standard compound feed, rub/t	Raw material costs for new compound feed, rub/t
Fish meal	90 000	35	35	31 500	29.75
Meat and bone meal	40 000	8	8	3 200	31 500
Blood meal	45 000	5	5	2 250	3 200
Sunflower meal	30 000	20	20	6 000	2 250
Soy meal	45 000	20	20	9 000	6 000
Yeasts	35 000	5,2	5,2	1 820	9 000
Vegetable oil	40 000	3,8	3,8	1 520	1 820
Premix PF-2B	450 000	1,0	1,0	4 500	1 520
Fish oil	300 000	2,0	1,7	6 000	4 500
Fat of the larva	1 000 000	-	0,3	0	5 100
Sum	-	-	-	65 790	67 890

Table 5 shows the need for staff and remuneration.

Table 5. The need for staff and remuneration.

Staff category	**Number of people**	**Remuneration for 1 hour, rub**	**Labor intensity of the manufactured unit of production, hour**	**Total salary, rub**
Technician	2	180	0.3	132
Engineer	2	300	0.3	75
Food safety technologist	1	350	0.3	90
Chief technologist	1	400	0.3	120
Sales manager	2	200	0.3	60
Accountant	1	200	0.3	90
Director	1	300	0.3	108
Total *З₀*				675

The calculation of the cost of production is presented in Table 6.

Table 6. Unit cost calculation.

Calculation article	**The formula from the calculation**	**The amount for standard compound feed, rub**	**The amount for a new compound feed, rub**
Material costs	M_0	65 790.00	67 890.00
Basic salary	Z_0	675.00	675.00
Additional salary, including bonuses	$D_0=Z_0 \cdot 0.4$	270.00	270.00
Insurance contributions to state extra-budgetary social purpose funds*	$B_{year}=(Z_0 + D_0) \cdot 0.3$	283.50	283.50
General production expenses	$P_{gp}=Z_0 \cdot 6.5$	4 387.50	4 387.50
General economic expenses	$P_{ge}=Z_0 \cdot 2.8$	1 890.00	1 890.00
Production cost	$C_{pr}=(M_0+Z_0+D_0+B_{year}+P_{gp}+P_{ge})$	73 396.00	75 396.00
Commercial expenses	$P_{com}=C_{pr} \cdot 0.05$	3 664.80	3769.80
Full cost price	$C_n=C_{pr}+P_{com}$	76 961.00	79 166.00

To determine the price, it is necessary to calculate the planned profit. To do this, it is necessary to determine the level of profitability of the new compound feed $P_к$, which is usually in the range of 15 to 30%. Let us take the average profitability from this range: 22.5%. The profit was determined by the following formula (1):

$$P = C_p \cdot \frac{P_c}{100} \tag{1}$$

The wholesale price of compound feeds is calculated according to the formula (2):

$$C_o = C_p + P \tag{2}$$

The sale price is determined by the formula (3):

$$C_{pr} = C_o + VAT \quad (3)$$

where VAT is value added tax, which is determined in accordance with the current legislation of the Russian Federation (in 2023, VAT = 20%).

The results of calculations according to formulas (1-3) are summarized in Table 7.

Table 7. Calculation of the sales price of compound feeds according to standard and new recipes for 1 ton.

Article of calculation	The formula from the calculation	The amount for standard compound feed, rub. $C_{pr.sf}$	The amount for a new compound feed, rub. $C_{pr.nf}$
Profit, rub.	$P=C_n \cdot \frac{P_c}{100}$	17 316.18	17 812.31
Product price, rub/t	$C_0=C_n+P$	94 277.00	96 978.00
Sale price, rub/t	$C_{pr}=C_0+VAT$	113 132.38	116 373.73

The calculation of the sale price of new and standard compound feed showed that the new compound feed is more expensive by 3241.35 rub/t. Despite the higher price of the new compound feed, when using the new compound feed, the survival rate of trout and its growth increases.

The costs of growing rainbow trout and obtaining economic benefits were calculated in accordance with the methodology presented in [31].

The annual productivity of small and medium-sized fish farms is up to 20 tons/year of fish products. Based on the experimental data presented in Table 7, we calculate the amount of compound feed required for the production of 20 tons of rainbow trout. For the calculation, we will give tons in kg.

For growing 20000 kg of trout, a standard compound feed q_{sf} will be necessary:

$$q_{sf} = \frac{0{,}04481 \cdot 20000}{0{,}04074} = 22\ 000 \text{ kg.}$$

where 0.04481 kg is the amount of feed needed to increase the weight of trout by 0.04074 kg with a feed ratio of 1.1 units.

At the same time, the cost of purchasing 22000 kg of standard compound feed will be (4):

$$c_{sf} = q_{sf} \cdot C_{pr.sf} \quad (4)$$

$$c_{sf} = 22\ 000 \cdot 113132 = 2\ 488\ 904 \text{ rub.}$$

Taking into account the survival rate of trout when fed with standard compound feed (90%), the mass of trout grown for sale can be determined:

$$m_{sf} = \frac{20000 \cdot 100}{90} = 18\ 000 \text{ kg.}$$

The market price of trout $Ц_ф$ for 2023 is 750 rub/kg.

Thus, the profit from the sale of trout grown on standard compound feed will be equal to (5):

$$P_{sf} = m_{sf} \cdot C_f - c_{sf} \tag{5}$$

$$P_{sf} = 18000 \cdot 750 - 2488904 = 11\,011\,096 \text{ rub/year.}$$

In the same way, the profit from the sale of trout grown on new compound feed will be calculated. For growing 20 tons of trout, the following mass of a new compound feed q_{nf} will be necessary:

$$q_{nf} = \frac{0{,}05034 \cdot 20000}{0{,}04576} = 22\,000 \text{ kg.}$$

where, 0.05034 kg is the amount of feed needed to increase the weight of trout by 0.04576 kg with a feed ratio of 1.1 units.

At the same time, the cost of purchasing 22000 kg of new compound feed will be equal to (6):

$$c_{nf} = q_{nf} \cdot C_{pr.nf} \tag{6}$$

$$c_{nf} = 22000 \cdot 116{,}374 = 2\,560\,228 \text{ rub.}$$

Taking into account the survival rate of trout when feeding with a new compound feed (95%), the mass of trout grown for sale can be determined:

$$m_{nf} = \frac{20000 \cdot 100}{95} = 19\,000 \text{ kg.}$$

Thus, the profit from the sale of trout grown on the new compound feed will be equal to (7):

$$P_{nf} = m_{nf} \cdot C_f - c_{nf} \tag{7}$$

$$P_{nf} = 19000 \cdot 750 - 2560228 = 11\,689\,772 \text{ rub/year.}$$

The difference P_o from the profit received when growing trout on standard and new compound feed is substracted (8):

$$P_o = P_{sf} - P_{nf} \tag{8}$$

$$P_o = 11689772 - 11011096 = 678\,676 \text{ rub/year.}$$

Thus, the economic effect of using the new compound feed in trout feeding on small and medium-sized farm fish farms amounted to 678676 rub/year.

4 Conclusion

Compound feed, which has in its formulation the fat of the larva of the black soldier fly (*Hermetia illucens*) and a probiotic feed additive, including a strain of bacteria *Bacillus* *amyloliquefaciens B–1895arm* and the cake of the larva of the black soldier fly (*Hermetia illucens*), obtained by squeezing fat from the biomass of the face on a screw press, showed good results in feeding rainbow trout: fish survival increased from 90 (standard formula of mixed feed for trout - experiment) to 95% (new formulation - control), in the experiment the mass of fish increased by 1.9 times, in the control by 1.8 times. The analysis of the fish microbiota also showed that the larva fat added to the feed and the probiotic feed additive do not have a negative effect.

The calculation of the economic effect of using the new compound feed is 678676 rubles per year on small and medium-sized trout farms up to 20 tons per year (with a need for compound feed up to 22 tons per year) due to improving the quality of feeding and productivity of fish cultivation.

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References

1. Global research of the feed market Alltech 2019. Animal husbandry of Russia **3**, 55-56 (2019)
2. E. Golovnya, Compound feed **3**, 70-72 (2014)
3. S.V. Ponomarev et al, *Industrial fish farming: textbook* (Lan, St. Petersburg, 2021)
4. A. Krogdal, Quality and efficiency **5**, 61-64 (2017)
5. J.P. Nunes Alberto, L.L. Dalen, G. Leonardi et al, Aquaculture Reports **27**, 101422 (2022) doi: https://doi.org/10.1016/j.aqrep.2022.101422
6. A.M. Watson, M.P. Napolitano, T.B. Schock et al, Aquaculture **529**, 735627 (2020) doi: https://doi.org/10.1016/j.aquaculture.2020.735627
7. V. Draganovic, Atze Jan van der Goot, R. Boom, J. Jonkers, Animal Feed Science and Technology **165(3 – 4)**, 238-250 (2011) doi: https://doi.org/10.1016/j.anifeedsci.2011.03.004
8. D. Rudoy, V. Pakhomov, A. Babajanyan et al, Lecture Notes in Networks and Systems book series (LNNS) **246**, 41–51 (2022) doi: 10.1007/978–3–030–81619–3_5
9. R.H. Janssen, Jean-Paul Vincken, L.A.M. van den Broek et al, J. Agricultural and Food Chemistry, 2275 (2017)
10. G.L. Newton, C.V. Booram, R.W. Barker, et al, J. Anim Sci. **44**, 395–400 (1977)
11. M.H. Moghadasian, Food Science and Nutrition **48(5)**, 402 – 410 (2008)
12. E. Ramos, *Insects: a hopeful food source. Ecological implications of minilivestock; role of rodents, frogs, snails, and insects for sustainable development* (Science Publishers, 2005)
13. M.J. Sánchez, Journal of Cleaner Production **6**, 112 – 126 (2016)

14. A. Téguia, M. Mpoame, Tropicultura **20(4)**, 187 – 192 (2002)
15. A.D. Zotte, Y. Singh, Zs. Gerencsér et al, Meat Science **193**, 108944 (2022) doi: https://doi.org/10.1016/j.meatsci.2022.108944.
16. O.M. Hale, Journal of the Georgia Entomological Society **8**, 16 – 20 (1973)
17. D. Pimentel, B. Berger, D. Filiberto et al, BioScience **54(10)**, 909–918 (2004)
18. D.C. Sheppard, G.L. Newton, G. Burtle, *Black soldier fly prepupae: a compelling alternative to fish meal and fish oil. A public comment prepared in response to a request by the National Marine Fisheries Service to gather information for the NOAA–USDA Alternative Feeds Initiative. Public comment on alternative feeds for aquaculture received by NOAA* (2008)
19. S. St–Hilaire, K. Cranfill, M.A. Mcguire et al, Journal of the World Aquaculture Society **38(2)**, 309 – 313 (2007)
20. A. Caligiani, A. Marseglia, G. Leni, S. Baldassarre et al, Food Research International, 812 (2018)
21. T.Y. Wibowo, H. Yohanes, F. Illaningtyas, et al, IOP Conference Series: Earth and Environmental Science **65**, 012046 (2017) doi:10.1088/1755–1315/65/1/012046
22. N.S. Liland, I. Biancarosa, P. Araujo et al, Plos One, 23 (2017)
23. M. Mazanko, E. Prazdnova, D. Rudoy, et al, E3S Web of Conferences **175**, 01010 (2020) https://doi.org/10.1051/e3sconf/202017501010
24. M.S. Mazanko, E.V. Prazdnova, M.P. Kulikov et al, Enzyme and Microbial Technology 155, 109980 (2022) https://doi.org/10.1016/j.enzmictec.2021.109980
25. A. Kuznetsova, S. Emelyantcev, D. Rudoy et al, Lecture Notes in Networks and Systems book series (LNNS) **246**, 102–110 (2022) doi: 10.1007/978–3–030–81619–3_11
26. M. AL–Dulaimi, A. Algburi, A. Abdelhameed et al, Pathogens **10**, 1574 (2021) https://doi.org/10.3390/pathogens10121574
27. N.A. Ushakova, V.G. Pravdin, L.Z. Kravtsova et al, Probiotics and Antimicrobial Proteins **13(4)** (2021) doi: 10.1007/s12602–021–09835–y
28. I.V. Popov, A. Algburi, E.V. Prazdnova et al, Animals **11(7)**, 1941 (2021) doi: 10.1007/s12602–021–09776–6
29. D.V. Rudoy, M.L. Chikindas, V.I. Pakhomov et al, *Patent for invention RU 2777768 C1 Russia, MPK A23K 50/80.: No. 2021137850 Bul. № 22* (2022)
30. *Order of the Ministry of Agriculture and Science of the Russian Federation from 06.06.2003 No. 792*, URL: https://docs.cntd.ru/document/902066049
31. V.I. Kryukov, A.V. Zarubin, *Fish farming. Cage trout farming in Central Russia. Textbook for agricultural universities* (Publishing house “Autograph”, Eagle, 2011)