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Effectiveness of biologically active substances added to sturgeon feeds

Elena N. Ponomareva[1], Anzhelika V. Kovaleva[1], Vadim A. Grigoriev[1] and Nuhkadi I. Rabazanov[2,3]
[1]Federal Research Centre the Southern Scientific Centre, Russian Academy of Sciences, Rostov-on-Don, Russia
[2]Caspian Institute of Biological Resources, Dagestan Federal Research Centre, Russian Academy of Sciences, Makhachkala, Russia
[3]Dagestan State University, Makhachkala, Russia

Principal contact
Anzhelika V. Kovaleva, Candidate of Biological Sciences, Senior Researcher, Federal Research Centre, Southern Scientific Centre, Russian Academy of Sciences; 41 Chekhov Ave., Rostov-on-Don, Russia 344006.
Tel. +79275679776
Email anhramova@yandex.ru
ORCID https://orcid.org/0000-0002-8503-6461

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Abstract
The purpose of this research was to study the effect of the vitamin-mineral complex E-selenium and the probiotic preparation Bacell when added to industrial feed on the fish-breeding-biological and physiological-biochemical parameters of juvenile sterlet-beluga hybrids when reared in cages.
The duration of the experiment was 45 days. In experimental option 1, the fish were fed with basic compound feed with the addition of E-selenium (300 µg selenium/kg of feed), in experimental option 2, the fish were fed with basic compound feed with the addition of E-selenium (300 µg selenium/kg of feed) and the Bacell preparation (0.2 % by weight of dry food). The control variant was fed with basic feed.
The absolute increase in the experimental variants was 13.0–25.0 % higher than in the control. Hematological parameters varied – in some cases with a high degree of significance. In particular, hemoglobin increased by 5.6–13 %, blood serum protein by 0.42–12.6 %, cholesterol by 9.8–16.3 % in the experimental variants and by 70.5 % in the control, due to the presence of a high degree of stress in control fish. At the same time, in fish experiments the effects of stress factors were suppressed by antioxidants.
Preventive doses of microelements, vitamins and probiotics reduce and stabilise peroxide processes and contribute to the maintenance of good physiological condition of fish kept in artificial conditions.

Key Words
Hybrid of sterlet and beluga, vitamins, microelements, probiotic, food, sturgeon.

Эффективность добавления биологически активных веществ в корма осетровых рыб

Елена Н. Пономарева[1], Анжелика В. Ковалева[1], Вадим А. Григорьев[1], Нухкади И. Рабазанов[2,3]

[1]Федеральный исследовательский центр Южный научный центр Российской академии наук, Ростов-на-Дону
[2]Прикаспийский институт биологических ресурсов Дагестанского Федерального Исследовательского центра Российской Академии Наук, Махачкала, Россия
[3]Дагестанский государственный университет, Махачкала, Россия

Контактное лицо
Анжелика В. Ковалева, кандидат биологических наук, старший научный сотрудник, ФГБУН «Федеральный исследовательский центр Южный научный центр Российской академии наук»; 344006 Россия, г. Ростов-на-Дону, пр. Чехова, 41.
Тел. +79275679776
Email anhramova@yandex.ru
ORCID https://orcid.org/0000-0002-8503-6461

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Резюме
Цель настоящих исследований заключается в изучении влияния витаминно-минерального комплекса Е-селен и пробиотического препарата Бацелл при кормлении промышленным комбикормом на рыбоводно-биологические и физиолого-биохимические показатели молоди гибрида стерляди и белуги при выращивании в садках.
Срок проведения эксперимента составил 45 суток. В опытном варианте 1 кормление рыб осуществлялось базовым комбикормом с добавлением Е-селена (300 мкг селена/кг корма), в опытном варианте 2 – базовым комбикормом с добавлением Е-селена (300 мкг селена/кг корма) и препарата Бацелл (0,2 % от массы сухого корма). Контрольный вариант кормили базовым комбикормом.
Абсолютный прирост в опытных вариантах был на 13,0–25,0 % выше, чем в контроле. Гематологические показатели различались и в некоторых случаях с высокой степенью достоверности. В частности, гемоглобин увеличился на 5,6–13 %, белок сыворотки крови – на 0,42–12,6 %, холестерин – на 9,8–16,3 % в опытных вариантах и на 70,5 % в контрольном, что обусловлено наличием высокой степени стресса у контрольных рыб. В то же время у рыб в опытах действия факторов стресса купируют антиоксиданты.
Профилактические дозы микроэлементов, витаминов и пробиотиков, в первую очередь, направлены на снижение и стабилизацию перекисных процессов и поддержание хорошего физиологического состояния рыб, содержащихся в искусственных условиях.

Ключевые слова
Гибрид стерляди и белуги, витамины, микроэлементы, пробиотик, корм, осетр.

INTRODUCTION

In order to improve fish breeding biotechniques, one of the directions of improvement is the use of adaptogenic agents, which include antioxidants that regulate the intensity of lipid reoxidation [1–7]. Vitamins and trace elements, in particular, vitamin E (α-tocopherol) and the trace element selenium, which forms enzymes with proteins, as well as selenium in organic form, selenomethionine (SeMet) possessing antioxidant properties, are used to regulate the reoxidation of polyunsaturated fatty acids and stabilize cell membranes in animals [8].

The most common form of vitamin E, alpha-tocopherol, and the chemical element selenium (Se), which enhances the effects of other antioxidants, are combined in E-Selenium. It protects the body's cells from reactive oxygen species O_2 and suppresses the activity of free radicals, thereby reducing the level of lipid peroxidation (LPO). This complex supports liver function, normalizes the reproductive processes of animals, and participates in the functioning of the thyroid gland. The use of an inorganic form of selenium – sodium selenite, which is hundreds of times more effective than vitamin E, helps reduce the need for vitamin E in animals and fish [9–13].

The administration of injections and feed supplements of selenium with various vitamins to farm animals (cows, sheep, chickens) has been studied extensively. However, very little research has been carried out on the effects of vitamin E and selenium on fish, and especially sturgeon, and the results that exist are scanty and do not give the whole picture of the action of the complex [14; 15].

There are studies of the effect of E-selenium on juveniles and fingerlings of trout, which led to enhanced growth and normalization of metabolism due to the activation of phospholipids and triacylglycerols [16]. Our earlier studies have shown that the E-selenium complex regulates gonadogenesis and improves the reproductive system of sturgeon fish species, which often exhibit lipid metabolism disorders when reared in a closed water system [17], and also accelerate the dynamics of oocyte maturation of female sturgeons [18; 19].

The progression of pathological condition in fish when reared in artificial conditions can be determined not only by keeping them in a small confined space, but also by the use of easily oxidized polyunsaturated fatty acids in feed. Selenium-containing preparations in complex with vitamins seem to be effective enough to prevent lipid overoxidation in fish feed [20].

Fish are able to accumulate selenium from water and food and concentrate it mainly in the liver and kidneys, where detoxification processes occur. The need for it ranges from 150 to 250 mcg/kg of feed. It is more advisable to introduce selenium into artificial food together with vitamin E, since there is a higher chance of protecting the body from the harmful effects of oxidized fats [12]. Research by A.V. Zhigin and co-authors [21] it was noted that when selenium-containing preparations were added to the feed, it was possible to reduce the concentration of lipids in the body while maintaining active linear growth and development of trout.

In 2012, preliminary studies were carried out to study the effect of various concentrations (300 μg/kg and 2000 μg/kg of feed) of E-selenium on the physiological state of young Russian-Lena sturgeon were carried out at the scientific and experimental base of the Southern Scientific Center of the Russian Academy of Sciences [22].

Hybrid rearing in a closed water supply unit (CWSU) under optimal water environment parameters revealed a positive effect of feed with vitamin and mineral supplement E-selenium on the physiological state of experimental fish.

Increasingly, veterinarians are choosing probiotics to maintain the normal physiological state of farmed animals. They perfectly complement the vitamins and microelements contained in the feed. Probiotics stimulate the growth of beneficial microorganisms and inhibit the development of pathogenic bacteria, which helps maintain the natural immunity of the animal, including in aquaculture [23]. Maxim E.A. and co-authors note that when feeding the drug “Bacell” to juvenile sterlet, survival rate increases by 5.7 % and weight gain by 9.1 % [24].

However, it is possible that when selenium is used in combination with probiotics, part of the selenium, when interacting with intestinal microflora, will turn into an insoluble form and will be removed from the body without bringing any benefit. This effect has been noted in cattle. In this case, a lack of selenium in the body, complicated by its low level in the environment, negative living conditions, and low-quality feed, can provoke oxidative stress [25].

It is worth considering that aerobic spore-forming bacteria (*Bacillus subtilis*), which are a component of the probiotic, use free oxygen to maintain vital functions and growth. This can enhance the antioxidant properties of selenium, which in turn will have a positive effect on the physiological status and aquaculture quality of fish. A similar interaction was found in *Bacillus ferreus*, which have the ability to reduce selenium compounds [12].

Previously, studies were carried out on young individuals of a hybrid of sterlet and beluga in conditions of a recirculation installation. Positive results were obtained and recommendations were given for the use of the E-selenium drug in combination with the bacterial drug Bacell [26]. Currently, research is continuing; however, the experimental hybrid was kept in cages. The effect of E-selenium and Bacellus in the composition of commercial feed on fish farming on biological and physiological parameters of juveniles was studied.

MATERIAL AND METHODS

Experiments on the use of complex E-selenium and the probiotic Bacell in feeding young sterlet and beluga hybrids were conducted in 2023 in the conditions of the cage complex of the Southern Scientific Centre, Russian Academy of Sciences (Kagalnik village, Azov region). The period of experimentation amounted to 45 days. Two experimental groups were allocated: in experiment 1, the fish were fed with basic compound feed with the addition of E-selenium (300 μg of selenium per 1 kg of compound feed), in experiment 2 – with basic compound feed with the addition of E-selenium (300 μg of selenium per 1 kg of compound feed) and probiotic drug Bacell (0.2 % of dry feed weight). Drug input rates were determined earlier on other sturgeon species. In the control variant, the hybrids were fed only basic mixed fodder without the addition of experimental preparations. Feeding was carried out 2 times a day. The calculation of the feed dose was carried out according to accepted norms in all variants at the same time.

The hydrological analysis of cage water was carried out 3 times a day. The water temperature during the experiment varied within the range of 18.7–21.2 °C, the

content of dissolved oxygen O_2 in the water was 73–83 % and the pH values of the environment were 5.1–7.2 units. These parameters are optimal for sturgeon farming.

Each of the three groups was kept in a separate square-shaped cage with a capacity of 1 m^3 of water. The sample from each experimental cage for studying growth and physiological characteristics was 30 fish per cage. The average weight of fish in the experiment variants at the beginning of the experiment was close in value and amounted to 77.3–81.2 g, differing by 1.5–3.5 % compared to the control.

Growth studies provide insight into the efficiency of the rearing process, taking into account animal housing conditions and the level of digestion of feed. Feeding adequacy also plays an important role. During the experiment, changes in growth rate and fatness of fish were observed.

Weighing, measurement of the studied fish and calculations of mass characteristics (absolute gain (g) and average daily gain (g), etc.) were made in accordance with existing methods.

Average daily gain (specific growth rate, C_w) – change in weight of fish for each day of the period, expressed in percent – was determined according to the formula:

$$C_w = \frac{2(M_t - M_0)}{(M_t + M_0)t} 100\%.$$

where
M_0 — initial mass, g;
M_t— final weight, g;
t — duration of cultivation, days.

The mass accumulation coefficient K_m was calculated according to the formula:

$$K_m = \frac{(M_t^{1/3} - M_0^{1/3}) \cdot 3}{t}$$

where
M_0 — initial mass, g;
M_t— final weight, g;
t — duration of cultivation, days.

The fatness coefficient (Ky) characterises the fatness or "fleshiness" of fish. The fatness factor is defined as the ratio of weight to body length according to the formula of T. Fulton:

$$K_y(\phi) = P*100 / L^3$$

where
P — fish mass, g;
L — length, cm (in sturgeons in this experiment the entire length of the individual was measured).

To study the physiological and biochemical status of the hybrids, biological material (whole blood) was obtained intravitally from the tail vein. Each sample was formed from the blood of 3 individuals. To understand the physiological state of the juveniles, the following indicators were determined: erythrocyte sedimentation rate, hemoglobin concentration in whole blood, as well as the concentration of total protein and cholesterol in the blood serum. The erythrocyte sedimentation rate was determined by the Panchenkov method, hemoglobin – by the unified cyanmethemoglobin photometric method, its level was determined using the apparatus “Mini-GEM-540”. Serum protein content was determined by the biuret method using a set of reagents, Olvex diangnostikum. Blood cholesterol was determined by the enzymatic method using a set of Olvex diangnostikum reagents [27].

The values of the physiological characteristics of sturgeon species living in the natural environment were considered as the norm. Thus, the normal level of hemoglobin concentration is 50–80 g/l, serum protein – 28–40 g/l, cholesterol 1–2.8 mmol/l and erythrocyte sedimentation rate (ESR) 2–4 mm/hour [28].

The results of the analyses were processed using the Microsoft Excel software package for descriptive statistics with the calculation of a two-sample t-test with different variances, as a result of which the level of significance (p) was determined for the compared unequal small samples [29].

RESULTS OBTAINED AND THEIR DISCUSSION

Examples of the use of preventive doses of E-selenium as an additive in feeding farm animals, including fish [3; 11] indicated a slight increase in size and weight indicators, as well as an improvement in the physiological state of experimental animals.

According to early studies conducted at the aquatic complex of the Southern Scientific Centre, Russian Academy of Sciences in 2012 on a hybrid of Russian and Lena sturgeon, the addition of E-selenium at a dose of 300 μg/kg in feed contributed to an increase in the growth rates of juveniles by 3–5 % compared to the control option [17].

When feeding hybrid of sterlet and beluga juveniles with food containing the E-selenium complex (option 1) and the E-selenium + Bacell complex (option 2), positive dynamics were revealed. The absolute increase in the experimental variants was 13.0–25.0 % higher than in the control (table 1, fig. 1). Other growth indicators of hybrid juveniles were also higher.

The hematological parameters of fish at the beginning and end of the study differed and some analytical results revealed significant differences (Table 2). The number of fish in the sample was 15 specimens. (no=15) and each collective blood sample contained blood from 3 individuals).

The erythrocyte sedimentation rate (ESR) at the beginning of the experiment was low in all variants of the experiment (the range of reference values for ESR in fish from natural habitats is 2–4 mm/h). By the end of the study, ESR – in all variants of the experiment – still increased (reliability $p < 0.05$) in fish in experiment 1. The addition of vitamins, microelements and probiotics probably had an effect on some blood factors that determine the erythrocyte sedimentation rate (viscosity blood, degree of erythrocyte aggregation).

A high level of hemoglobin was found in control fish at the beginning of the experiment in comparison with individuals in experiment 2 and experiment 1 (differences are unreliable). Taking into account the optimal hydrological and hydrochemical parameters of the environment, it is possible to note the increased level of oxidative metabolism in fish in the control variant. By the end of the experiment, the concentration of hemoglobin in blood increased from the initial level in fish of experimental group 2 (by 13.0 %), to which a complex preparation (E-selen + probiotic) was added to the feed. In experiment 1, where E-selen was used, hemoglobin concentration increased insignificantly by 5.6 %. The increase in hemoglobin values indicates the effect of the probiotic and

vitamin and mineral supplements in the feed intended for the experimental groups of fish on the change in hemoglobin level (although the differences registered are not reliable). The values were within the reference values for fish from natural environment (50–80 g/l).

Table 1. Growth indicators of a hybrid of sterlet and beluga in an experiment with the addition of E-selenium and the probiotic Bacell
Таблица 1. Показатели роста гибрида стерляди и белуги в эксперименте с добавлением Е-селена и пробиотика Бацелл

Indicators Показатели	**Experience 1** Опыт 1	**Experience 2** Опыт 2	**Control** Контроль
Initial mass, g Масса начальная, г	77,3 ± 8,2	81,2 ± 9,0	78,5 ± 10,2
Final mass, g Масса конечная, г	108,8 ± 6,9*	120,4 ± 10,3*	96,3 ± 9,7
Absolute increase, g Абсолютный прирост, г	31,50	39,2	17,80
Average daily increase, g Среднесуточный прирост, г	0,70	0,81	0,45
Average daily increase, % Среднесуточный прирост, %	0,75	0,86	0,25
Mass accumulation coefficient, units. Коэффициент массонакопления, ед.	0,033	0,039	0,019
Number of fish, n Количество рыб, экз.	30	30	30
Duration of cultivation, days. Продолжительность выращивания, сут.		45	

*Note: * – differences in comparison with the initial indicators are significant at p < 0.01*
*Примечание: * – различия по сравнению с исходными показателями достоверны при p < 0,01*

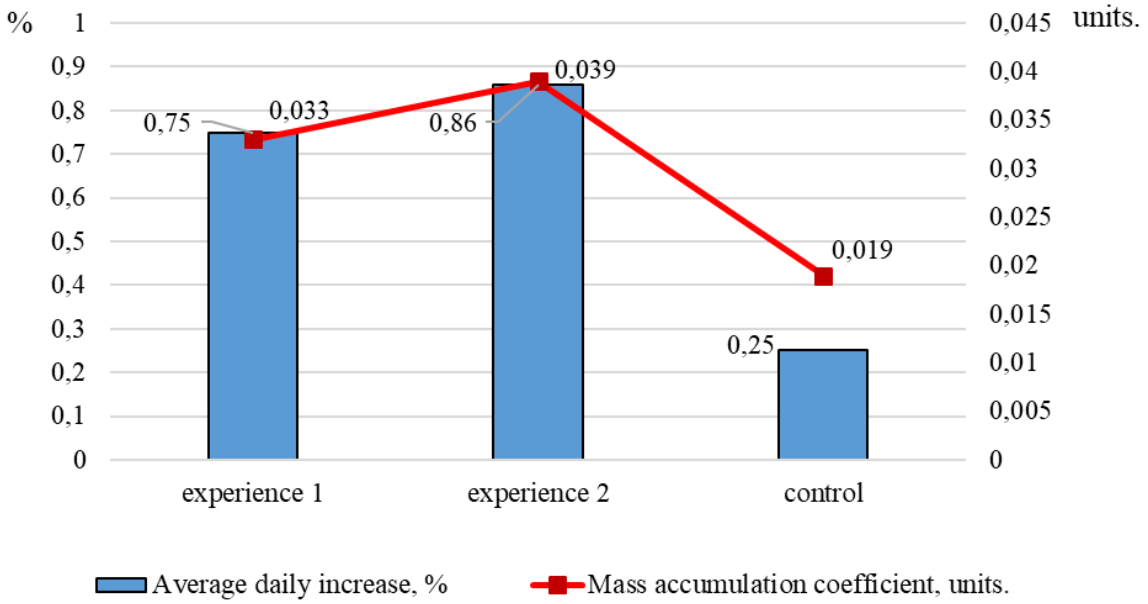

Figure 1. Average daily growth (%) and mass accumulation coefficient of the sterlet-beluga hybrid during the experiment
Рисунок 1. Среднесуточный прирост (%) и коэффициент массонакопления гибрида стерляди и белуги в период проведения эксперимента

Protein concentration in the blood of all groups of fish at the beginning of experimental works was low and did not differ significantly ($p > 0.05$). By the end of the study, its level increased in fish from experiment 2, compared to experiment 1 and control ($p > 0.05$). The same fish at the end of the experiment had higher weight due to higher level of protein metabolism, expressed in increased concentration of total protein in blood (Table 2).

The dynamics of cholesterol in the blood of all groups of fish at the beginning of the experiment was within the norm for sturgeons from the natural environment (1.0–2.8 mmol/l). During the period of the experiment, the dynamics of cholesterol in all variants was directed towards an increase in the indices but the reliability of differences between the initial level and the final result was revealed only in the control at $p < 0.05$. Since cholesterol is a precursor of corticosteroids that increase under stress, its increase in the blood of control fish appears to be due to this. At the same time in experimental 1 and 2 groups of fish the actions of stress factors are reduced by antioxidants. Thus, the importance of studying metabolic processes occurring in the body of

fish during cultivation in artificial conditions is undeniable, because it helps to timely monitor changes in the physiological state of the object and correct the conditions of maintenance and feeding. An important part of the feeding process is the introduction of additional components. In this case, probiotic supplements and vitamin-mineral complex were used.

Table 2. Dynamics of hematological parameters in a hybrid of sterlet and beluga during the experiment
Таблица 2. Динамика гематологических показателей у гибрида стерляди и белуги в период проведения эксперимента

Indicators Показатели	**Experiment 1** Опыт 1		**Experiment 2** Опыт 2		**Control** Контроль	
	beginning начало	**end** конец	**beginning** начало	**end** конец	**beginning** начало	**end** конец
ESR, mm/h СОЭ, мм/ч	1,2 ± 0,13	1,93 ± 0,25*	1,5 ± 0,26	1,88 ± 0,20	1,0 ± 0,12	1,3 ± 0,23
Hemoglobin, g/l Гемоглобин, г/л	70,65 ± 8,48	74,59 ± 2,26	62,15 ± 4,22	70,22 ± 5,39	87,61 ± 8,15	79,36 ± 4,55
Total protein, g/l Общий белок, г/л	24,05 ± 1,64	24,15 ± 1,57	24,81 ± 1,17	27,93 ± 1,51	23,55 ± 1,44	24,72 ± 1,61
Cholesterol, mmol/L Холестерин, ммоль/л	2,24 ± 0,16	2,46 ± 0,15	2,70 ± 0,19	3,14 ± 0,24	2,41 ± 0,66	4,11 ±0,51**

*Note: * – differences are reliable in comparison with initial indicators at p < 0.05, ** – differences are reliable in comparison with initial indicators at p < 0.10, and in comparison with final indicators of experiment 1 at p < 0.01*
*Примечание: * – различия достоверны по сравнению с исходными показателями при p < 0,05, ** – различия достоверны по сравнению с исходными показателями при p < 0,10, а также по сравнению с конечными показателями эксперимента 1 при p < 0,01*

CONCLUSIONS

It was revealed that the use of E-selenium and probiotic Bacell in fish feeding in the experiment led to an increase in fish weight. Absolute growth in experimental variants was 13.0–25.0 % higher in variants of experiment with E-selenium and complex E-selenium + Bacell than in the control. It was also shown that preventive doses of microelements, vitamins and probiotics primarily aimed at reducing and stabilizing peroxide processes and maintaining good physiological condition of fish kept in artificial conditions are effective. Unlike warm-blooded farmed animals, fish are cold-blooded and their response to supplements that stimulate growth and regulate metabolism may differ.

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AUTHOR CONTRIBUTIONS

Elena N. Ponomareva formulated the experimental plan and corrected the manuscript before submitting it to the Editor. Anzhelika V. Kovaleva and Vadim A. Grigoriev collected biological material, analysed the data and wrote the manuscript. All authors are equally responsible for plagiarism, self-plagiarism or other ethical transgressions.

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ORCID

Elena N. Ponomareva / Елена Н. Пономарева http://orcid.org/0000-0002-7129-6676
Anzhelika V. Kovaleva / Анжелика В. Ковалева http://orcid.org/0000-0002-8503-6461
Vadim A. Grigoriev / Вадим А. Григорьев http://orcid.org/0000-0002-3262-4198
Nuhkadi I. Rabazanov / Нухкади И. Рабазанов https://orcid.org/0000-0001-7664-6308