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Compound feed with cardioprotective effect for sturgeon fish

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Abstract. The authors carried out an experimental work on developing a new compound feed for growing sturgeons with possible prevention of cardiovascular diseases arising under the influence of adverse abiotic factors during artificial cultivation. The importance of modern cultivation of valuable fish species is explained by the need to create effective methods for the practical implementation of feeding technology and minimizing the effects of side factors, including the cardioprotective components with antihypertensive effects. As a result of the research work, a reasonable idea was obtained that herbal agents can be used as cardioprotective components, namely, the common barberry plant (*Berberis vulgaris* L.) possessing a cardioprotective effect when added to feed mass fraction of 3.0% The physiological indicators of the body were studied on the basis of blood indicators and a cardiosomatic index, which complement the general characteristic of abnormalities or heart disease, and also determine the physiological status of the fish. In the course of studies, it was found that when feeding sterlet weighing 100 g, the feeding rate makes 4% of the body weight of the developed feed and with a barberry content of 0.3% it ensures stabilization of the cardiovascular system, supports the heart and can be used as a preventive compound feed.

Keywords: sturgeons, cardioprotective supplements, cardiosomatic index, physiological-biochemical indicators, sterlet

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Научная статья

Комбикорм с кардиопротекторным действием для осетровых рыб

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Аннотация. Авторами проведена экспериментальная работа по разработке нового комбикорма для выращивания осетровых рыб с возможностью обеспечения профилактики сердечно-сосудистых заболеваний, возникающих под воздействием неблагоприятных факторов среды при искусственном интенсивном выращивании. Актуальность данного направления современного выращивания ценных видов рыб обусловлена необходимостью создания эффективных способов практической реализации технологии кормления и минимизации воздействий побочных факторов, в том числе с включением компонентов, обладающих кардиопротекторным действием. В результате проведенной экспериментальной работы получено обоснованное представление, что в качестве кардиопротекторных компонентов могут быть использованы фитосредства, а именно растение барбарис обыкновенный (*Berberis vulgaris* L.), обладающее кардиопротекторным действием при добавлении в корм массовой доли 3,0 %. Изучение физиологических показателей состояния организма осетровых осуществлялось на основе измерений показателей крови и кардиосоматического индекса, которые дополняют общую характеристику отклонений в развитии или заболевания сердца, а также определяют физиологический статус рыбы. В ходе исследований установлено, что при кормлении стерляди массой 100 г норма кормления разработанным кормом составляет 4 % от массы тела и при содержании в комбикорме барбариса 3,0 % обеспечивает улучшение показателей состояния сердца, стабилизацию работы сердечной мышцы и может использоваться в качестве профилактического комбикорма, улучшающего ключевые хозяйственно важные признаки объектов аквакультуры.

Ключевые слова: осетровые, кардиопротекторные добавки, кардиосоматический индекс, физиолого-биохимические показатели, стерлядь

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Introduction

The fishing industry has accumulated extensive experience in using various compound feed formulations for economically important fish species, through which artificial rearing and breeding fish have achieved a significant progress (standards, guidance, etc. have been developed), including obtaining marketable fish products.

Though many feed formulations are considered quite effective, most of them also have significant drawbacks (side effects). These include, for example, wide addition of salt in high content to compound feeds in order to increase growth rates, which leads to a decrease in the satiety reflex, to constant food intake and maximization of the peak of feeding activity. As a result of rapid growth, disorders of the cardiovascular system occur widely in fish, along with a number of different physiological complications, which consequently leads to increasing losses in the fish cultivation. The cost of such losses is especially high for fish farms that grow economically valuable species (sturgeons) that need long time to develop.

One of the solutions to the problems that arise during fish farming and associated with deteriorations in the cardiovascular system (which results in a general decrease in the efficiency of the rearing process and an increase in mortality rate) can be the application of special components – cardioprotectors into the formulations of the produced feed, activity of which would be aimed at increasing the stability of the cardiovascular system in fish reared under the influence of adverse factors. Besides the above-mentioned high concentration of sodium chloride in fish feeds, there are other factors such as unavoidable handling during cultivation and risk of heat shock (especially in pond culture) [1-4], causing an increase in heart rate, blood pressure, systolic volume and cardiac output [5-9], resulting in higher cardiosomatic index.

Though the sturgeon farming technique has reached a certain level of effectiveness (recirculating aquaculture systems, recirculating water supply units, etc.), the physiological factor of nutrition remains underestimated in terms of potential enhancement of cultivation productivity by reducing stress factors during the farming process. It helps to improve health of the farmed fish, therefore reduces mortality and production costs.

In modern aquaculture it is necessary to develop new compound feed formulations that could maintain normal physiological status of the cardiovascular system in sturgeons cultivated in the conditions in which the impact of adverse factors is likely to occur during commercial farming process.

Therefore, the purpose of this study is aimed at screening such plant-derived natural components that could be applied to compound feed formulas without undergoing deep processing and in relatively low concentrations, which would not reduce the nutritional properties of the feeds. Adding such components into the fish feeds would contribute to their production efficiency by improving the preservation of the normal physiological state of the body.

There is a number of natural substances that could be considered as components with a potentially cardioprotective effect.

According to the literature, barberry contains vitamins (A, C, E, B group), carotene, alkaloids, pectin, tannin, carbohydrates (fructose, glucose, sucrose, fiber), organic acids (tartaric, malic, citric), tannins and essential oils. Among 22 alkaloids reported so far in the berries, berberine and berbamine are considered the most valuable for their choleretic effect and supporting liver and gallbladder function. Barberry is beneficial to the circulatory system: it lowers the blood pressure; normalizes the heart rhythm; enhances blood clotting activity; strengthens capillary walls and stops bleeding; disposes cholesterol plaques [10]; prevents the development of cardiovascular diseases. Moreover, attention is particularly drawn to a substance called berberine [5], which is found in large quantities in barberry. Berberine lowers blood pressure and normalises heart rate. Along with anti-inflammatory and antiproliferative effects, berberine also possesses antihypertensive effects [6]. Research results show that berberine prevents the heart failure, lowers the cholesterol level, and increases the resistance to stress [7, 8].

The practice of using berberine in aquaculture also shows a positive effect on the health state of farmed fish in general, growth rate and resistance to bacterial infections. For example, adding berberine to the fish feed in a ratio of 50 mg/kg significantly increased the growth rate of bream, while immunological parameters were higher than that in fish consumed high-fat feed [6].

Also for the treatment or prevention of metabolic syndrome is used a composition [9] containing a polyphenolic extract of grapes, in an amount effective in reducing blood pressure, which is administered to the body.

Moreover, a combination of water-alcohol polycomponent extract from 21 phytocomponents was used for prevention and adjuvant therapy of arterial hypertension [11]. This botanical blend possessed cardioprotective, sedative and hypotensive effects. It also showed a positive therapeutic effect as decreasing tachycardia and the blood pressure.

The excessive salt intake in salt-sensitive individuals has been reported to induce a significant increase in blood pressure, while in salt-resistant individuals, blood pressure (BP) values remained normal or increases to a small extent. A possible solution to this problem could be the application of a plant-based salt substitute to diet. Another invention [12] suggested to use herbs Salicornia spp. as

an alternative for salt, containing the aerial part and roots of the plant in the form of a ready to serve green powder.

Today has been developed a grower feed formula for sturgeons, which includes glasswort *Salicornia* spp. The feed claimed to solve the problems of normalizing the state of the cardiovascular system during the period of intensive growth [13]. Furthermore, a compound feed for freshwater shrimp has been improved with glasswort, giving the feed salty flavour, which along with other features, met the requirement in saltiness of this species [14].

A method for prevention of hypertension was developed based on using Patrimin [15], a purified dry extract qualified as a triterpene glycoside derivative obtained from the roots of *Patrinia intermedia* (Horn.) Roemet Schul. of the Valerianaceae family. According to the authors, the preparation exhibited sedative, hypotensive, anticoagulant and lipid-lowering effects. The study on the antihypertensive effect of the preparation included the analysis of the decrease in blood pressure and heart rate. The main drawbacks of the method for prevention of hypertension based on the Patrimin preparation is the absence of study on changes in the biochemical composition of blood. Another disadvantage of the preparation is its alcohol extract origin, which may cause changes in the effect of the active compound and possible allergic reaction in patients.

In accordance with Cong Lan et al., 2018, studying the effect of curcumin on stroke prevention in an experimental rat model with spontaneous hypertension [16], an oral administration of curcumin (100 mg/kg/day) delayed the onset of stroke compared with the control group that received saline.

The range of biological activity of curcumin so far is considerably wide and relevant [17], it could be noted solely as a powerful antioxidant, improving lipid metabolism, and therefore, contributes to the normalization of the state of blood vessels and presents a positive effect on the functions of the cardiovascular system.

Nevertheless, the abovementioned treatment and preventative approaches have some disadvantages: complexity of using combined biologically active additives, risk of allergic reactions to one of the components, lack of detailed information about duration of application, not considering the problem of salt sensitivity, and the use of separated components, such as curcumin, is complicated by low bioavailability and absorption in the gastrointestinal tract.

Based on the foregoing, it is necessary to assess the effect of berberine contained in the Common barberry as a component of compound feed, which is the core purpose of this study – to evaluate the possibility of optimizing the growing process based on feed formulations with the studied component – cardioprotector Common barberry (*Berberis vulgaris* L.) and to estimate changes the state of the cardiovascular system of sterlet (*Acipenser ruthenus*) fed with the studied compound feed.

Materials and methods

Experimental work in growing and feeding with the proposed compound feed was carried out in the summer 2022. Experiments were conducted on sterlet species (*Acipenser ruthenus*) weighing 100 g obtained from cage fish farm in the Astrakhan region. The fish were sparsely stocked into 400 l tanks. The water temperature and oxygen content in the water were maintained at 8.0 mg/l, 24-25 °C at a water flow rate of 2 l/min.

In the course of rearing the water quality parameters were measured using generally approved hydrochemical methods.

Prior to the start of the experiment, a group of species were randomly selected from the general sample for physiological and biological studies, as well the evaluation of cardiosomatic index, then the general sample was divided by random selection into a control group and two experimental groups.

The physiological state of reared sterlet was assessed using hematological and biochemical parameters [18], sampling was carried out *in vivo* according to the recommended methods, in compliance with the basic principles of asepsis and antisepsis.

The assessment of the adaptive reaction of the organism was carried out according to the indicators of the physiological and biochemical parameters of blood, changes in the relative mass of the heart.

Using length and weight parameters of fish, a number of derived features and coefficients were calculated.

The absolute weight gain was calculated by the formula:

$$WG_{abs} = m_f - m_0,$$

where WG_{abs} is the absolute weight gain; m_f is the final weight, g; m_0 is the initial weight, g.

The average daily weight gain was determined as:

$$DWG = W_{abs} / t,$$

where t is the duration of the experiment, days.

The specific growth rate was calculated by using the following formula:

$$SGR = [(m_f / m_0)^{1/t} - 1] \cdot 100\%.$$

The mass accumulation coefficient was calculated for more accurate results of the growth rate determination:

$$C_m = ((m_f^{1/3} - m_0^{1/3}) \cdot 3) / t.$$

Additionally, Fulton's condition factor was determined according to the following formula:

$$Q_F = W \cdot 100 / l^3,$$

where Q_F – Fulton's condition factor; W is the weight of fish, g; l is the total length, cm.

Feed input is the total amount of feed added to the rearing tanks. Feed conversion ratio (FCR) is the amount of feed it takes to increase fish weight, taking into account the survival rate during the rearing period. Its value for various feeds is established empirically. The calculation of the feed coefficient is carried out according to the formula:

$$FCR = C^f / (M_1 - M_0),$$

where C^f is the total feed intake, g (kg); M_1 is the body weight at the beginning of feeding trial, g (kg); M_0 is the body weight at the end of feeding trial, g (kg).

The cardio-somatic index of fish (CSI, ‰) was calculated using the equation:

$$CSI = x / y \cdot 1\,000,$$

where x is heart weight, g; y is total body weight, g.

Fish were fed according to three schemes: control and experimental groups 1, 2. The experiment lasted 30 days from the moment of feeding.

The recipes included the following components: fish meal, pumpkin and hemp oil press-cakes, soybean meal, feed yeast, oatmeal, corn, wheat, premix, fish oil. Besides, the developed compound feed contained barberry, with the following content of the component, wt. %: experimental 1 - 1%, experimental 2 - 3%.

The proposed compound feed in this study was prepared by wet pressing. The components in the specified percentage maintain their nutritional value without affecting the protein balance. All components according to the proposed formula were thoroughly mixed to obtain a homogeneous mass. The received mass was then dried, crushed and sieved to the required granule size of 2.5-3.0 mm.

Statistical analyses of the obtained results were performed by the method of variation statistics using Microsoft Excel 2016 program. The Student's *t*-distribution was used in order to calculate the confidence interval while the Student's *t*-test was used to assess the significance of differences between the mean sample values, the differences in studied parameters were considered significant at $p < 0.05$.

Results

The experimental data obtained show that the proposed compound feed displayed a positive effect on the growth performance of experimental sterlet. This reflected in the registered increase in absolute weight gain by 1.4 times in comparison with the control group with a reduction in feed input up to 11% and improvement in other biological indicators (mass accumulation coefficient, survival rate).

During the experiments, individuals of the experimental group actively consumed the given feed; weight gain was 12.17% of the initial weight. The highest absolute weight gains of experimental groups 1 and 2 of sterlets were by 1.2 and 1.9 times respectively higher than that of the control group. The average daily weight gain was higher in experimental 1 and 2 groups by 1.1 and 1.9 times in comparison with the control group. As for the specific growth rate the values of this comparison were 1.1 and 2.0 respectively.

The main indicator for assessing the state of the heart was the cardiosomatic index value. The value under unstable environmental conditions can vary up to 40% [19].

Heart weight varied within studied groups of fish. At the beginning of the experiment, the indicator was 0.129 ± 0.05 mg, and at the end of the experiment, the highest value was registered in the control group, which by 0.033 g and by 0.055 g higher than in experimental 1 and 2 groups, respectively. Based on the obtained data on the weight of the heart and the total weight of the fish, the cardiosomatic index of sterlet was calculated for the sampling at the beginning of the experiment and for each of the groups of fish at the end of the trial.

The initial mean of the cardio-somatic indexes in the sterlets were 1.54‰, at the end of the experiment, this means were 1.47 and 1.31, respectively, which, according to the results of the experiment, was by 21.8 and 30.3% lower, respectively, in comparison with the control. Such a decrease in the studied indicator could be suggested as an improvement in the state of the heart muscle (Table 1).

Table 1

Biological parameters of reared sterlet

Parameters	Control group	Experimental 1	Experimental 2
Initial body weight, g	85.51 ± 6.54	87.58 ± 1.94	78.00 ± 4.59
Final body weight, g	90.5 ± 7.9	93.3 ± 4.7	87.5 ± 5.3
Initial body length, cm	29,93 ± 0,73	30.39 ± 0.15	29.21 ± 0.55
Final body length, cm	31.10 ± 0.73	31.00 ± 0.19	29.53 ± 0.61*
Absolute weight gain, g	4.99	5.72	9.5
Absolute length gain, cm	1.17	0.61	1.59
Daily weight gain, g	0.17	0.19	0.32
Specific growth rate, %	0.19	0.21	0.38
Fulton's condition factor	0.3	0.31	0.3
Mass accumulation coefficient	0.0084	0.0095	0.0167
Heart weight, mg	0.170 ± 0.26	0.137 ± 0.024	0.115 ± 0.024*
CSI, ‰	1.88	1.47	1.31
FCR	2.62	2.56	2.34
Survival rate, %	100		
Duration of the experiment, days	30		

* Significant differences at $p < 0.05$.

Feed input were registered lower in experimental groups than in the control one. Sterlet survival in all three groups was 100%.

The results of monitoring blood parameters obtained from experimental sterlet given experimental compound feed did not show any syndromes of diseases or physiological deviations from the norm (Table 2).

Table 2

Physiological and biochemical parameters of reared sterlet

Parameters	Control group	Experimental 1	Experimental 2
Hemoglobin, g/l,	59.75 ± 11.88	58.40 ± 11.72	59.75 ± 9.76
Total protein, g/l,	18.35 ± 3.8	19.3 ± 1.58	26.80 ± 1.98*
Erythrocyte sedimentation rate (ESR), mm/h,	2.25 ± 0.48	1.88 ± 0.43	1.63 ± 0.31*
Cholesterol, mmol/l	1.68 ± 0.18	1.55 ± 0.13	1.33 ± 0.13*
β-lipoproteins, g/l,	2.13 ± 0.43	2.13 ± 0.24	2.50 ± 0.46*
ALT, μmol/ ($s \cdot l$)	0.11 ± 0.08	0.08 ± 0.06	0.14 ± 0.2
AST, μmol/ ($s \cdot l$)	0.10 ± 0.01	0.06 ± 0.01	0.11 ± 0.1

* Significant differences at $p < 0.05$.

The studied indicators at the beginning the experiment were recorded: hemoglobin 53.7 ± 4.39 g/l, total protein 22.9 ± 2.16 g/l, ESR 1.18 ± 0.12 mm/h, cholesterol 3.20 ± 0.12 mmol/l, β-lipoproteins 1.17 ± 0.01 g/l.

An increase in hemoglobin concentration up to 59.75 g/l was observed in experimental individuals, which was corresponding to the norm. Hemoglobin performs the important respiratory function and plays the role of a transport element in respiration and the life activities of aquatic organisms, allowing gas exchange process between the respiratory apparatus and the tissues in organs. A normal hemoglobin concentration in blood contributes to the elimination of hypoxia risk caused by oxygen deficit, the last one may eventually lead to heart failure.

Among the biochemical parameters of blood, serum aminotransferase levels are the most specific indicators of necrosis in internal organs, especially in the liver, heart, and kidneys [20]. Aspartate aminotransferase (AST) is normally present in the blood at low concentrations, but when an organ tissue, for example, heart or liver, is infected or damaged, AST is released additionally into the bloodstream. Therefore, AST level in the blood is directly related to the degree of tissue damage [21, 22]. In this experiment, AST level remained low in all groups, the lowest AST value was noted in the fish of the second experimental group.

It is clear from the table that the ESR is higher in the control group, which may indicate inflammatory and pathological processes, induced more intensively in these individuals.

Plasma cholesterol concentration is one of the most important indicators in the current experiment and could serve as a risk indicator of alterations in cardiovascular system. The lowest mean of this indicator was found in the second experimental group – 1.33 mol/l with a significant difference when compared with the control group, which, among other things, reduces the risk of vascular dysfunction. Cholesterol levels at the end of the experiment significantly lowered in all groups in comparison with the initial means. However, the reductions of cholesterol level in the experimental groups this decrease were more noticeable.

The reference value of total serum protein was significantly higher in the second experimental group of sterlet ($p < 0.05$), which confirms the high quality of the proposed feed and indicates active nutrition.

Concentrations of β-lipoproteins in fish fed on the proposed compound feed were also statistically higher ($p < 0.05$).

Physiological and biochemical parameters of sturgeons grown on the proposed compound feeds were within the optimal reference values during the experimental period. The biochemical parameters of blood of sterlets in the experimental 2 group, fed on newly proposed feed showed an improvement in general, which confirms its high quality.

Conclusion

The key objective of aquaculture is the intensification of cultivated species' growth. However, this process must correlate with farming conditions, including a balanced diet, that contributes to maintaining the health state of cultivated fish. It is especially important to pay attention to the technologies and preventative approaches to deterioration of the cardiovascular system. Any failure to comply with this principle could lead to increased losses in fish farms even with formally high achieved growth rates.

This problem is especially significant for fish farms that grow economically important species (such as sturgeons), cultivation of which is already costly. Therefore, besides maintaining the production process with high technological effectiveness and proper environmental control, physiological factors of nutrition becomes one of the most important in terms of reducing losses and production costs.

The present experiment shows that the use of 0.3%

barberry preparation in compound feed significantly improved the health status of young sterlet and thus, improve the efficiency in their cultivation. The determination of cardio-somatic index in reared sterlet fish demonstrated significant decreases in this cardiac indicator in fish fed compound feed with barberry preparation, which serves as evidence of a significant level of cardioprotective effect of this additive.

Most importantly, the optimization of fish cultivation technology has been achieved and the studied compound feed could be recommended for use as it improved the health status while maintaining high values of key farming indicators of sturgeons during a 30-day feeding course with 3% cardioprotective component common barberry (*Berberis vulgaris* L.) in fish feed.

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