Experience of using dry biomass of methanotrophic bacteria in the enrichment of artificial feeds for Siberian sturgeon juveniles of the Ob population

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Abstract. The article discusses the results of a study on the enrichment of artificial feeds with dry biomass of methanotrophic bacteria of 10 and 20% concentration and fatty acids (treatment "Arfit", linseed oil). The data on the increase in body length and weight, the survival rate of Siberian sturgeon juveniles, depending on the applied preparations introduced, are presented. The best results were obtained when 10% of dry biomass of methanotrophic bacteria and HUFA were added to the feed (acceleration of weight growth by 44%, linear growth by 38%). The presented results are aimed at developing some elements of the technology for enriching artificial feeds with microbiosynthesis products.

1. Introduction
Fishmeal is the most valuable protein component of animal origin, but its production has decreased by almost 60% over the past 30 years. In addition to the reduction in production volumes, the price of fishmeal is also growing (on average, by 15-20% per year). Based on the available data, we can talk about a shortage of fishmeal on the market of protein components for compound feeds used both in aquaculture and in animal husbandry. The result of such deficit is expressed in the falsification of fishmeal, which directly affects the growth rate and health of fish [1-2].

In this regard, technologies aimed at finding an alternative to fishmeal are gaining relevance. An example of such alternative can be the use of dry biomass of methanotrophic bacteria – Methylococcus capsulatus (haprin) in artificial mixed feed. These bacteria are grown on natural gas hydrocarbons, which is promising for regions with rich methane deposits (such as the Tyumen region, including the Khanty-Mansi and Yamalo-Nenets Autonomous regions).

Haprin is a dry inactivated microbial mass with a high content of protein (about 70%), amino acids and nucleic acids. The biochemical composition of haprin is similar to fishmeal, but the fatty acid composition of methanotrophic bacteria differs from fishmeal in the absence of HUFAs of the linolenic (omega-3) and linoleic (omega-6) families [3].

In this regard, for the most balanced diet for all biochemical components, it is necessary to enrich the starting artificial feeds, in addition to haprin, with fatty acids, for example, linseed oil, with the "Arfit" premix treatment. This preparation, consisting of Artemia cyst oil and a phytocomplex of medicinal plants, is used as a feed enrichment additive for larvae, juvenile fish and crustaceans. It has a large amount of HUFA, as well as vitamins A, E, F, trace elements and carotenoids.
The expediency of using methanotrophic bacteria in the composition of starting and production feeds for various fish species (carp, perch, salmon, whitefish) has been proven in a number of fish-breeding studies carried out in the 80-years last century, and at the present time. The results obtained showed a positive effect on the growth rate and survival of fish [2-6].

The aim of the study is to reveal the possibility of using dry biomass of methanotrophic bacteria when feeding Siberian sturgeon juveniles from the Ob population.

2. Materials and methods.
Experimental work was carried out in August-September 2021 at the enterprise "New Aquaculture" (Tyumen) while performing routine maintenance on artificial reproduction of Siberian sturgeon of the Ob population (Acipenser baerii Brandt, 1869). The experiments used eggs obtained from one female Siberian sturgeon of the Ob population of natural origin at «Yugorsky Fish Hatchery» (Khanty-Mansiysk) in the third decade of June 2021. On both farms, artesian water was used as a source of water supply with help of recirculation system (RAS).

For research work start artificial feed of firm Coppens (Netherlands), fraction No. 0.5-0.8; 0.8-1.2. were used.

The period of keeping the Siberian sturgeon prelarvae lasted 8-10 days, then the larvae were fed by Artemia nauplii. Then the control individuals were transferred to the start artificial food. The experimental individuals were transferred to the following diets:
1. Starter artificial feed enriched with methanotrophic bacteria (100 g / kg feed), Line-seed oil, phyto-complex and "Arfit" (experiment AH100).
2. Starter artificial feed enriched with methanotrophic bacteria (200 g / kg feed), line-seed oil, phyto-collection and "Arfit" (experiment AH200).

Each variant of the experiment and control was performed in three replicates.

The rearing of larvae and juveniles was carried out in 9 rectangular pools with a working area of 3.2 m² at a water temperature of 18.8-19.3 °C and a content of oxygen dissolved in water of 6.8-8.1 mgO₂ / l. The water temperature and the content of oxygen dissolved in the water were within optimal limits for rearing Siberian sturgeon juveniles.

In the process of analyzing the results of the experiment, the following methods of statistical processing were used: comparison of absolute and arithmetic mean values (by weight, absolute (zoological) length, survival rate); calculation of absolute and average daily gains, specific rate of weight growth (according to the formula of Schmalhausen and Brody) [7], coefficients of body weight variability (Cv), fish productivity, standard deviations and errors, reliability of differences according to Student's t-test at $\alpha = 0.05$ [8].

3. The results of the study
Summary data on the weight gain of experimental and control individuals are presented in Table 1.

|     | 13.08.21 | 20.08.21 | 26.08.21 | 02.09.21 |
|-----|----------|----------|----------|----------|
| Pool №1 ($\bar{m}$, g) | 1,08 | 3,39 | 5,67 | 7,51 |
| Pool №2 ($\bar{m}$, g) | 1,33 | 3,36 | 7,54 | 12,48 |
| Pool №3 ($\bar{m}$, g) | 1,40 | 4,76 | 8,09 | 12,88 |
| Average | 1,27 | 3,84 | 7,10 | 10,96 |
| Standard deviations | 0,74 | 2,51 | 4,80 | 7,50 |
| Standard errors | 0,08 | 0,26 | 0,48 | 0,61 |
| Student's t-test at $\alpha = 0.05$ | - 0,03 | 0,0002 | 0,00003 |
| Initial body length, cm | 6,03 |
| Final body length, cm | 16,48 |
The initial average weight of larvae before switching to food enriched with dry biomass of methanotrophic bacteria of 10% concentration and HUFA was 1.27 ± 0.74 g; before adding dry biomass of methanotrophic bacteria of 20% concentration and HUFA to feed - 1.70 ± 0.90 g, in control - 1.70 ± 0.94 g.

The results of growing sturgeon juveniles that consumed artificial feed enriched with dry biomass of methanotrophic bacteria of 10% concentration and HUFA to an average weight of 10.96 ± 7.5 g showed an acceleration in weight growth (44% higher than the control). The data obtained in relation to the control turned out to be statistically significant at p = 0.00003. An increased rate of linear growth was also noted - 38% higher than in the control (at p = 0.000001).

Graphically, data on the rate of weight growth of juvenile Siberian sturgeon are presented in Figures 1-3.

![Figure 1](image1.png)  
**Figure 1.** Weight growth rate of Siberian juveniles in the AH100 experiment.

![Figure 2](image2.png)  
**Figure 2.** Weight growth rate of Siberian sturgeon juveniles in the AH200 experiment.
Figure 3. Weight growth rate of Siberian sturgeon juveniles in control.

At the same time, there was an increase in the mass of Siberian sturgeon fed with artificial feeds enriched with methanotrophic bacteria in a 20% concentration (9.13 ±5.69 g) by 20% compared to the control group (7.6±6.29 g). The data are reliable at a significance level of p = 0.01. In addition, there was an increase in the linear growth of experimental individuals (by 16.6% more than the control) at p = 0.001.

The absolute average daily gain with the addition of 10% haprin and HUFA to feed was 0.48 g; 20% concentration - 0.37 g, in control - 0.29 g. The relative average daily gain in the experiment, where artificial feed was enriched with 10% dry biomass of methanotrophic bacteria, was 7.9%; 20% - 6.9%; in control - 6.3%.

The specific rate of weight growth of Siberian sturgeon juveniles was the highest after feeding with artificial feeds enriched by dry biomass of methanotrophic bacteria of 10% concentration and HUFA, and amounted to 0.11. In the variant of the experiment, where the feed was enriched by haprin of 20% concentration and HUFA, the specific rate of weight growth was 0.08, in the control it was 0.07.

The mean for the period coefficient of variability (Cv) differed in the experimental (10% concentration of haprin + HUFA; 20% concentration of haprin + HUFA) and control groups and was 63.52; 55.55 and 74.87% respectively. Consequently, the smallest coefficient of variability was observed when methanotrophic bacteria of 20% concentration and HUFA were added to artificial feed.

Fish productivity during rearing of Siberian sturgeon juveniles fed on feeds enriched by dry biomass of methanotrophic bacteria of 10% concentration and HUFA was the highest and amounted to 16.8 kg / m² (44% more than in the control). With the addition of 20% dry biomass of methanotrophic bacteria and HUFA to the feed, the fish productivity was 14.0 kg / m² (20% more than in the control).

During the experimental work in all variants of the experiment and in the control, an equally high survival rate of individuals was noted - about 97-98%. Maximum elimination was noted during the transition to enriched feed.

4. Conclusions
1. According to the results of the study, it is possible to determine the optimal concentration of methanotrophic bacteria (haprin) in the amount of 10% in the composition of artificial compound feeds for juvenile sturgeon fish species. Juveniles that consumed feed enriched with 10% haprin and HUFA showed a statistically significant (p = 0.00003) accelerated rate of weight and linear growth - almost 1.44 and 1.38 times higher than in the control, respectively.
2. There was an increase in the mass of Siberian sturgeon juveniles that consumed artificial feed enriched by methanotrophic bacteria (haprin) of 20% concentration and HUFA, by 1.2 times compared with the control group (at \( p = 0.01 \)).

3. The survival rate of Siberian sturgeon juveniles was equally high (97-98%) in both experimental and control groups.

4. An increase in fish productivity was revealed when juveniles of Siberian sturgeon were fed with artificial feeds enriched by 10% dry biomass of methanotrophic bacteria and HUFA (by 44%), 20% of microbial protein and HUFA - by 20%.

**Recommendations**

1. Partial replacement of fishmeal with methanotrophic bacteria will increase the competitiveness of the Russian feed market.

2. Inclusion of methanotrophic bacteria in the artificial feed in the amount of 10% and HUFA for juvenile sturgeon fish species will provide accelerated rates of linear-weight growth, as well as a high survival rate of juveniles.

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