Using of dry biomass of methanotrophic bacteria for enrichment of starter artificial feeds when growing juveniles of broad whitefish *Coregonus nasus* (Pallas, 1776)

Polina Zenkovich[1*], *Aleksandr* Litvinenko[1], *Ruslan* Zenkovich[1], *Lyudmila* Litvinenko[1], and *Marina* Korentovich[1]

[1]Northern Trans-Ural State Agricultural University, Tyumen, Russia

Abstract. A research was conducted to study the effect of starter artificial feeds enriched with dry biomass of methanotrophic bacteria (*Methylococcus capsulatus*) and a complex of fatty acids on growth, survival, fish productivity, feed conversion efficiency and the biochemical composition of tissues juveniles of broad whitefish (*Coregonus nasus*). Using of an experimental diet (10 % microbial protein, linseed oil, premix drug "Arfit") made it possible to increase the growth rate of juveniles of broad whitefish. The final weight of fish in the experiment was 17.2% higher than the control. The condition factor was 3.1% higher compared to the control, fish productivity was 13.0% higher, and there was a 4.0 % decrease in feed costs for ichthyomass gain. At the end of the experiments the content of crude fat and crude protein was higher by 12.4% and 3.0%, respectively, compared to the control. The total content of amino acids in the tissues of juveniles of broad whitefish, when microbial protein and a complex of fatty acids were added to the diet, increased by 5.4% compared to the control.

1 Introduction

Fishmeal is the main component of artificial feed for fish. Currently, there is a shortage of fishmeal in the world, the prices of which are constantly rising. An alternative to replace missing fishmeal is dry biomass of methanotrophic bacteria (MB). Converting methane, a potent greenhouse gas, into a highly nutritious animal feed ingredient is an attractive opportunity offered by methanotrophic bacteria. MB produce single-cell protein with a comparable or even superior nutritional profile compared to the most traditional protein sources. This protein can be produced with less environmental impact [1]. MB are used for inclusion in aquaculture feeds [2-5].

In the early stages of development of family *Coregonidae*, it is important to use starting artificial feed with the required content of protein (40-50%), fat (8-12%) and fiber (no more than 5%). MB, containing more than 70% protein and a significant amount of essential

* Corresponding author: zenkovich.pa@edu.gausz.ru

amino acids, is an alternative to fishmeal. It is also rich in fats, but differs from fishmeal in the absence of highly unsaturated fatty acids (HUFAs) of the linolenic (omega-3) and linoleic (omega-6) families [6]. To compensate for the missing HUFAs in MB, a complex of fatty acids can be used. The normal development and vital activity of family *Coregonidae* requires a sufficient supply of essential amino acids from food. An insufficient amount of them in the diet leads to an increase in feed costs per unit of fish weight gain and a decrease in the efficiency of protein use.

The purpose of the research was to establish the effect of starter artificial feeds enriched with MB and a complex of fatty acids on growth, survival, fish productivity, feed conversion efficiency, and also to study the biochemical composition of juveniles of broad whitefish.

2 Materials and methods

The objects of the research were larvae and juveniles of broad whitefish *Coregonus nasus* (Pallas, 1776). Experimental work was carried out in a recirculation system at "Sobsky Fish Hatchery" (Kharp village, Yamalo-Nenets Autonomous Okrug, Russian Federation). Larvae of broad whitefish were obtained from females from the natural population. Feeding of juveniles of broad whitefish was carried out in 6 pools (3 experimental and 3 control). The area of one pool is 2.78 m^2. Useful volume is 0.7 m^3. The water level was 25 cm. The initial density of fish was 11000 specimens per m^3. Control fish were fed with starter artificial feed firm Coppens, and experimental fish were fed with feed firm Coppens, enriched with 10% MB (*Methylococcus capsulatus*, strain GBS-15) and a complex of fatty acids (linseed oil, premix "Arfit"). "Arfit" is a therapeutic and prophylactic premix drug. It is used as an enrichment additive for feeding larvae, juvenile fish and crustaceans. It contains a phytocomplex and a concentrate from *Artemia* cysts, containing essential HUFAs (omega-3 and omega-6). In combination with linseed oil, it will help replenish the HUFAs missing in MB. Experimental and control fish were fed 18 times a day. Feed application rates were calculated according to the feed company tables in accordance with the average weight of juveniles and water temperature.

Initial body weight (W_0), final body weight (W_t), total length (TL) and numbers of larvae were recorded during the trial period (t) to compute the following indices [7]:

- Weight gain (WG, mg) = $W_t - W_0$.
- Average daily gain (ADG, mg) = WG/t.
- Specific growth rate (SGR, %/day) = $[\mathrm{Ln}\ W_t - \mathrm{Ln}\ W_0/t] \times 100$.
- Feed conversion ratio (FCR) = FI/WG.
- Condition factor (K) = $[Wt/TL^3] \times 100$.
- Survival rate (S%) = [Final larvae number/ initial larvae number] × 100.
- Fish productivity (kg/m^2) = [Final mass of fish – initial mass of fish] / area of pools.

Biochemical analyzes were carried out in a specialized laboratory. The mass fraction of protein was determined by the Kjeldahl´s mineralization method. The fat content was determined by distilling it off using a Soxhlet apparatus, then the gravimetric method was used. Crude fiber was determined by extraction method [8]. The determination of free amino acids was carried out according to the method [9] using capillary electrophoresis "Kapel" - 105M."

The statistical analyses were used software program Statistica 13.3 and Microsoft Excel 2019 for Windows. Data are presented as mean values (M); standard error of mean (m) coefficient of variation (Cv, %). The level of significance and differences between variants were estimated by Student-index (tst) under level of determination $p \leq 0.05$.

3 Results

3.1 Abiotic environmental factors

During experimental work abiotic environmental factors were close to optimal. At the beginning of growing the water temperature was 11.8 °C and then it gradually increased to 16.1 °C. The content of oxygen dissolved in water during experimental work varied in the range of 7.5-9.8 mg/l.

The pH value was in the range of optimal values: in the incoming water – 6.8-7.2, in the outgoing water it was 6.9-7.3. The ammonium concentration was within the acceptable range: in the incoming water – 0.01-0.06 mg/l, in the outgoing water it was in the range from 0.10 to 0.27 mg/l. The concentration of nitrites in the water entering the pools was in the range of 0.001-0.007 mg/l. The nitrite concentration in the outgoing water was 0.01-0.09 mg/l. The concentration of nitrates in the incoming water was at a level of 0.2 to 0.3 mg/l, in the outgoing water – 0.6-1.7 mg/l. The phosphate concentration in the incoming water was 0.08-0.24 mg/l. The phosphate concentration in the outgoing water ranged from 1.32 to 4.08 mg/l.

3.2 Growth rates of larvae and juveniles of broad whitefish

Data on the rates of linear-weight growth of broad whitefish juveniles are given in Table 1.

Table 1. Dynamics of indicators of length and body weight of broad whitefish juveniles.

	10 % MB + HUFA	**Control**
Initial body weight (g)	0.369±0.020	0.374±0.022
Final body weight (g)*	1.50±0.06	1.28±0.06
Cv (%)	32.0±1.7	38.1±1.9
Initial total length (cm)	2.93±0.05	2.97±0.06
Final total length (cm)*	4.85±0.07	4.66±0.08
Weight gain (g)	1.13±0.03	0.90±0.11
Specific growth rate (%/day)	8.3±0.8	7.2±1.0
Condition factor	1.31±0.09	1.27±0.09
Fish survival (%)	97.7±0.33	96.8±0.38
Feed conversion ratio	0.76±0.02	0.79±0.03
Fish productivity (kg/m^2)	1.30±0.04	1.15±0.06
Duration of feeding (days)	17	17

* the table shows statistically significant differences at $p < 0.001$

The initial average mass of juvenile broad whitefish in the experimental group was 0.369±0.020 g, and in the control group – 0.374±0.022 g. The initial average length of broad whitefish juveniles in the experimental group was 2.93±0.05 cm, while in the control group it was 2.97±0.06 cm.

The final mass of broad whitefish juveniles in the experimental pools reached 1.50±0.06 g and exceeded the final mass of the control group (1.28±0.06 mg). An increase in the linear growth of experimental individuals was also noted.

Specific growth rate in the experimental groups of whitefish was 8.3%, in the control group – 7.2%.

The maximum value of condition factor was noted on the first day of the experiment: in the experimental groups – 1.51; in control – 1.43. By the end of the study, the condition factor in the experimental groups was 1.32, in the control groups – 1.26.

The survival rate of juvenile broad whitefish the entire period of the experiment remained high both in the experimental groups – 97.7%, and in the control – 96.8%.

A decrease in the feed conversion ratio was noted in individuals fed artificial feed enriched with MB (10%) and HUFA. The feed conversion ratio in the control was 0.79±0.03, while when experimental fishes were fed by artificial feed enriched with MB (10%) and HUFA, the feed conversion ratio was 0.76±0.02.

Fish productivity when growing broad whitefish juveniles fed on feed enriched with MB (10%) and HUFA was the highest and amounted to 1.30±0.04 kg/m^2, in the control – 1.15±0.06 kg/m^2.

3.3 Biochemical composition of larvae and juveniles of broad whitefish

According to studies of the biochemical composition of the tissues of larvae and juveniles broad whitefish, protein and fat were identified as the main components, which accounted for 11.8-13.1% and 3.3-5.6% of wet weight, respectively (table 2). Crude fiber accounted for less than 2% of the wet weight of the sample.

Table 2. Fat and protein content in the tissues of broad whitefish larvae and juveniles during the period of research in the experiment and control, % of wet weight.

Period	Experiment option	Crude fat, %*	Crude protein, %
Start of experimental work	Experiment	3.30±0.13	12.55±0.41
	Control	3.30±0.10	11.77±0.23
End of experimental work	Experiment	5.63±0.67	13.09±0.36
	Control	5.01±0.53	12.71±0.08

* the table shows statistically significant differences at $p \leq 0.05$

Both in the experiment and in the control there was an increase in crude fat and protein towards the end of the experiment.

The content of amino acids in the tissues of larvae and juveniles of broad whitefish is presented in Table 3. Indicators for histidine (all samples were <0.5%) and tryptophan (all samples <0.1%) were excluded from the analysis.

In the experiment, the highest concentration was observed for glutamic acid and glutamine (1.81±0.205%), lysine (0.87±0.022%); in third place in terms of content in the tissues of juveniles of broad whitefish was glycine (0.84±0.009%). The content of other amino acids was located in the following sequence: alanine (0.76±0.031%), leucine and isoleucine (0.76±0.009%) > asparagine and aspartic acid (0.70±0.046%) > arginine (0.69±0.100 %) > threonine (0.63±0.030%) > proline (0.62±0.017%) > valine (0.58±0.009%) > serine (0.57±0.062%) > phenylalanine (0.48±0.029 %) > methionine (0.29±0.010%) > cysteine (0.14±0.006%).

In the control, the highest concentrations were observed for glutamic acid and glutamine (1.36±0.095%), lysine (0.91±0.062%), asparagine and aspartic acid (0.88±0.135%). The content of other amino acids is located in the following sequence: glycine (0.78±0.039%) > leucine and isoleucine (0.74±0.047%) > alanine (0.73±0.042%) > arginine (0.65±0.045%) > threonine (0.62±0.030%) > proline (0.56±0.026%) and valine (0.56±0.025%) > serine (0.55±0.030%) > phenylalanine (0.50±0.070%) > methionine (0.26±0.012%) > cysteine (0.14±0.008%).

Table 3. Average amino acid values in the studied samples of larvae and juveniles of broad whitefish (% of wet weight)

Amino acid	Start of experimental work		End of experimental work*		CV, %
	experiment	control	experiment	control	
Alanine	0.64±0.012	0.64±0.092	0.76±0.031	0.73±0.042	8.6
Arginine	0.68±0.031	0.54±0.037	0.69±0.100	0.65±0.045	11.0
Valine	0.51±0.017	0.55±0.032	0.58±0.009	0.56±0.025	5.4
Glycine	0.69±0.040	0.80±0.038	0.84±0.009	0.78±0.039	8.3
Leucine and Isoleucine	0.65±0.012	0.61±0.115	0.76±0.009	0.74±0.047	10.5
Lysine	0.76±0.030	0.61±0.097	0.87±0.022	0.91±0.062	17.1
Cysteine	0.12±0.020	0.18±0.023	0.14±0.006	0.14±0.008	15.5
Methionine	0.24±0.001	0.24±0.003	0.29±0.010	0.26±0.012	8.9
Proline	0.54±0.003	0.55±0.055	0.62±0.017	0.56±0.026	6.2
Serine	0.67±0.021	0.46±0.067	0.57±0.062	0.55±0.030	15.5
Threonine	0.54±0.003	0.57±0.046	0.63±0.030	0.62±0.030	7.0
Phenylalanine	0.48±0.022	0.42±0.070	0.48±0.029	0.50±0.070	7.5
Asparagine and Aspratic Acid	0.70±0.043	0.89±0.135	0.70±0.046	0.88±0.135	13.4
Glutamine and Glutamic Acid	0.96±0.070	1.33±0.125	1.81±0.205	1.36±0.095	25.3

* the table shows statistically significant differences at $p \leq 0.05$

A comparison of the amino acid content in the tissues of broad whitefish larvae and juveniles in the experiment and the control as a whole for the entire period of the study, presented in Figure 1, indicates that by the end of the experimental work lysine, phenylalanine, aspargine and aspartic acid in the experimental fish samples had values lower than in control. The content of other amino acids in the tissues of larvae and juveniles of broad whitefish was higher than in the control. The maximum differences were noted for glutamine and glutamic acid.

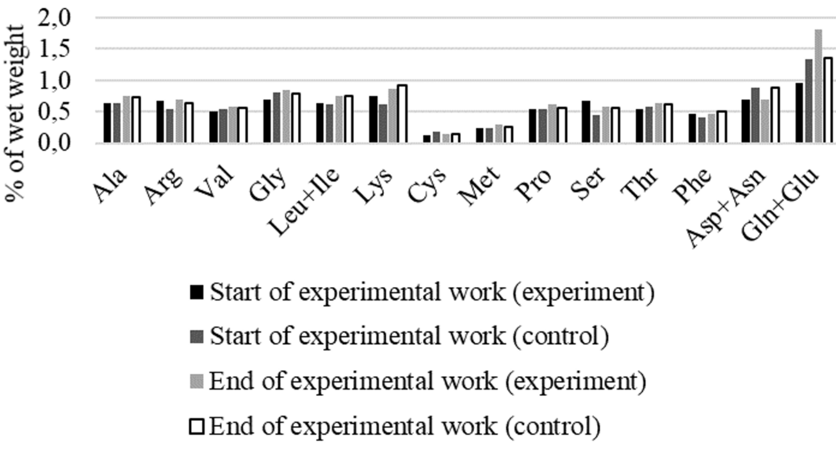

Fig 1. Average content of amino acids in the tissues of broad whitefish larvae and juveniles in experimental and control pools.

The coefficient of variation (Cv, %) of the obtained data on the content of amino acids in the tissues of larvae and juveniles of broad whitefish, which is in the range of 5.4-25.3%, indicates a low level of variability of such amino acids as valine, proline, threonine and phenylalanine, as well as about the high level of variability in glutamic acid with glutamine and lysine. The remaining amino acids have an average level of variability.

In the test samples, all analyzed amino acids at the end of the experimental work accounted for an average of 9.74±0.64% of the wet weight (Cv = 3.9%), in the control samples – 9.24±0.46% (Cv = 3.2%).

4 Discussion

Many researchers have been conducting experimental work on the complete or partial replacement of fishmeal with MB when feeding aquatic organisms in recent years. Supplementation of bacterial protein meal has been shown to be a good substitute for fishmeal in Japanese yellowtail [10], Black Sea bream [11], rainbow trout [12], barramundi [1], American eel [13] and white shrimp [14].

In our studies, the addition of MB with a complex of fatty acids to starter artificial feeds increased the growth rate of broad whitefish, survival rate, fish productivity and feed conversion efficiency. During 17 days of experimental work, juveniles of broad whitefish increased their body weight by 17.2%. The body length of the experimental fish was on average 4.1% higher than in the control. This determined higher values of the condition factor – by 3.1%, growth rate – by 15.2%, and fish productivity – by 13.0%. At the same time, the cost of experimental feed for fish weight gain decreased by 4.0%.

A study of the biochemical composition of the tissues of broad whitefish juveniles showed that in experimental fish at the end of the experiment there was an increase in the content of crude fat by 12.4% and crude protein by 3.0% compared to the control.

Amino acids are the main substrate for the synthesis of proteins and biologically active substances in fish ontogenesis, and in the early stages of fish development, they are the main source of energy. A lack of amino acids in the diet can disrupt metabolism, homeostasis, reproductive function, behavior in fish, and reduce immunity and resistance to disease [15].

Analysis of the amino acid content in the tissues of broad whitefish juveniles made it possible to establish that as they grew, both in the experiment and in the control, there was an increase in lysine, leucine and isoleucine, alanine and threonine. In addition, using of a diet with the addition of MB caused an increase in the content of glycine, proline and valine in the tissues of broad whitefish juveniles, as well as a significant increase in the content of glutamine and glutamic acid compared to the control.

In general, using of an experimental diet with the addition of MB led to an increase at the end of the experiments in the total content of amino acids in the tissues of juvenile broad whitefish to 9.74±0.64% or 5.4% higher compared to the control.

5 Conclusion

The results of our research showed the feasibility of adding MB with a complex of fatty acids to starter artificial feeds for broad whitefish juveniles. This is evidenced by an increase in growth rate, survival rate, higher condition factor, fish productivity, feed conversion efficiency, an increased content of crude protein and crude fat in the tissues of experimental juveniles, as well as an improvement in their amino acid composition.

References

1. L. Wooley, Md R. Chaklader, L. Pilmer, F. Stephens, C. Wingate, M. Salini, G. Partridge, Gas to protein: Microbial single cell protein is an alternative to fishmeal in aquaculture. Science of The Total Environment **859(1)**, 1-43 (2023) https://doi.org/10.1016/j.scitotenv.2022.160141
2. L. Wooley, Md R. Chaklader, L. Pilmer, F. Stephens, C. Wingate, M. Salini, G. Partridge, Gas to protein: Microbial single cell protein is an alternative to fishmeal in aquaculture. Science of The Total Environment **859(1)**, 1-43 (2023) https://doi.org/10.1016/j.scitotenv.2022.160141
3. J. Sillman, L. Nygren, H. Kahiluoto, V. Ruuskanen, A. Tamminen, C. Bajamundi, Bacterial protein for food and feed generated via renewable energy and direct air capture of CO_2: Can it reduce land and water use? Global Food Security 22, 25-32 (2019) https://doi.org/10.1016/j.gfs.2019.09.007
4. B.D. Glencross, D. Huyben, J.W. Schrama, The application of single-cell ingredients in aquaculture feeds – a review. Fishes 5(2), 1-39 (2020) https://doi.org/10.3390/fishes5030022
5. P.A. Zenkovich, M.A. Korentovich, T.O. Shabalina, A.I. Litvinenko, L.I. Litvinenko, Experience of using dry biomass of methanotrophic bacteria in the enrichment of artificial feeds for Siberian sturgeon juveniles of the Ob population. IOP Conference Series: Earth and Environmental Science 949, 1-5 (2022) https://doi.org/10.1088/1755-1315/949/1/012136
6. I.N. Ostroumova, Biological basis of fish feeding (GosNIORH, Saint Petersburg, Russia, 2012)
7. R.S. Shadrack, I. Manabu, S. Yokoyama, S. Koshio, V.A. Miguel, Z. Yukun, K. Mzengereza, S. Seo, S. Dossou, M.F. El Basuini, Specific importance of low level dietary supplementation of Lypomyces starkeyi CB1807 yeast strain in red sea bream (Pagrus major). Annals of Animal Science, 1-29 (2022) https://doi.org/10.2478/aoas2022-0012
8. Fish, seafood and products made from them. Method for determining the mass fraction of protein, fat, water, phosphorus, calcium and ash by near-infrared spectroscopy. HOST RU 31795-2012 from July 1, 2013, Moscow, Standartinform (2013)
9. Determination of proteinogenic amino acids in compound feeds and raw materials. M-04-38-2009, Saint Petersburg, Lumex (2014)
10. A. Biswas, F. Takakuwa, S. Yamada, A. Furukawa, M. Shiratori, T. Koshiishi, H. Tomokane, H. Tanaka. Partial replacement of fishmeal by a protein-rich product from methanotrophic bacteria in juvenile red sea bream (Pagrus major). Aquaculture Nutrition **27**(**6**), 2726-2738 (2021) https://doi.org/10.1111/anu.13398
11. B. Xu, Y. Liu, K. Chen, L. Wang, G. Sagada, A.F. Tegomo, Y. Yang, Y. Sun., L. Zheng, S. Ullah, Q. Shao, Evaluation of Methanotroph (Methylococcus capsulatus, Bath) Bacteria Meal (FeedKind®) as an Alternative Protein Source for Juvenile Black Sea Bream, Acanthopagrus schlegelii. Frontiers in Marine Science **8**, 1-13 (2021) https://doi.org/10.3389/fmars.2021.778301
12. M. Rajesh, B.S. Kamalam, P. Sharma, V.C. Verma, A. Pandey, M.K. Dubey, A. Ciji, Md. Shahbaz Akhtar, N. Pandey, D. Sarma, S.J. Kaushik, Evaluation of a novel methanotroph bacteria meal grown on natural gas as fish meal substitute in rainbow trout, Oncorhynchus mykiss. Aquaculture Research **53**(**6**), 2159-2174 (2022) https://doi.org/10.1111/are.15735

13. W. Lu, H. Yu, Y. Liang, Sh. Zhai, Evaluation of methanotroph (Methylococcus capsulatus, Bath) bacteria protein as an alternative to fish meal in the diet of Juvenile American Eel (Anguilla rostrata). Animals **13**(**4**), 1-17 (2023) https://doi.org/10.3390/ani13040681
14. M.A.J. Nederlof, S.J. Kaushik, J.W. Schrama. Effect of different types of bacterial single cell protein on feed intake, digestibility, growth and body composition of Pacific white shrimp (Penaeus vannamei). Aquaculture Reports **33**, 1-10 (2023) https://doi.org/10.1016/j.aqrep.2023.101830
15. N. Kasozi, G. Iwe, K. Sadik, D. Asizua, V.T. Namulawa, Dietary amino acid requirements of pebbly fish, Alestes baremoze (Joannis, 1835) based on whole body amino acid composition. Aquaculture Reports **14**, 1-6 (2019) https://doi.org/10.1016/j.aqrep.2019.100197