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RESEARCH

Effect of microalgae feed supplementation on growth performance and feeding efficiency of tilapia fry

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Abstract

Search for novel best-performing starter feed ingredients is one of the main challenges of modern aquaculture industry. The goal of this article is to evaluate the effect of microalgae supplementation enriched with omega-3 polyunsaturated eicosapentaenoic acid and the powerful antioxidant fucoxanthin on growth performance of Red tilapia fry (*Oreochromis mossambicus* × *Oreochromis niloticus*). We formulated three experimental diets, using two strains of microalgae from our culture collection – *Vischeria magna* SBV-108 (Eustigmatophyceae), rich in eicosapentaenoic acid and *Mallomonas furtiva* SBV-13 (Chrysophyceae), rich in fucoxanthin – and their combination. *Vischeria magna* SBV-108 is a new strain which biochemical properties and growth characteristics have not been previously studied. Coppens Supreme-15 was used as a control diet. The best growth performance was recorded in eicosapentainoic acid enriched Diet 1 (10% w/w *V. magna*), that significantly ($P<0.05$) overperformed control feed on absolute growth, average growth, specific growth rate, feed conversion rate, protein efficiency ratio, protein productive value and energy productive value. Diet 3 (5% w/w *V. magna* and 5% w/w *M. furtiva*) and Diet 2 (10% w/w *M. furtiva*) were not significantly different from control at most parameters. Generally, supplementation of feed with microalgae rich in omega-3 eicosapentaenoic acid significantly improved Red tilapia fry growth parameters and overall feed performance, while supplementation of feed with microalgae rich in antioxidant fucoxanthin was not beneficial compared to the control.

Keywords Microalgae · EPA · Omega-3 · Fucoxanthin · Fish feed supplement · Aquaculture

Introduction

Growing aquaculture demands novel and sustainable feed ingredients, especially for starter feeds (Tacon and Metian 2015). Quality of fish protein, healthy fat (enhanced level of polyunsaturated fatty acids, PUFA) and low level of contaminants are among the most important characteristics of quality feed. The growing demand for fishmeal and diminishing fish capture have resulted in the high price of fish meal. Thus, it is essential to evaluate different protein sources as cheaper substitutes to replace fish meal and/or expensive plant protein concentrates as a dietary protein source in aquafeeds (Takeuchi et al. 2002). Plant ingredients have increased in use lately to replace fishmeal for the purpose of cost reduction, improvement of sustainability and maintaining the requirement of high-quality protein. This has resulted into a steady decline of dietary fishmeal inclusion levels in aquafeeds (Carter and Hauler 2000; Shepherd and Jackson 2013). Replacing the traditional finite marine ingredients, fishmeal and fish oil, in diets with sustainable alternatives of terrestrial origin leads to lower content of polyunsaturated fatty acids omega-3 (n-3 PUFA), including alpha-linolenic acid (LNA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3) in farmed fish in comparison to wild-caught fish (Silva

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et al. 2014), posing a significant challenge for the aquaculture industry (Sprague et al. 2016). In this regard, single cell products are commonly considered as a possible substitute for fishmeal and plant protein concentrates in aquafeeds due to their ability to be cultured on a wide variety of substrates and wastes (Nandeesha et al. 1998) and as a source enriched in essential fatty acids (Sarker et al. 2016a, b). The high protein content and high production rate of algae in tropical areas, especially in developing countries hold promise as a possible alternative protein source for aquafeeds. It has been shown that many fish including carnivorous species can ingest algae as a part of diet formulation (Nandeesha et al. 1998). Therefore, using algae in fish diets might result in better utilization of artificial diets in cultured fish and enhance fish health (Mustafa and Nakagawa 1995). Usually only limited number of algae and cyanobacteria are used as feed in fish aquaculture. The most popular is *Arthrospira* (*Spirulina*) (Lu et al. 2002; Takeuchi et al. 2002; Lu and Takeuchi 2004; Abdel-Tawwab and Ahmad 2009; Sarker et al. 2016b). However, the problem of a low content of polyunsaturated fatty acid and especially EPA content in the feed remains unsolved. Omega-3 fatty acids – mainly EPA and DHA – are beneficial for fish health and growth performance, especially at early stages of development (Watters et al. 2012), while also enhancing the nutrition value of fillet (Sarker et al. 2016a). Nowadays, the nutritional quality of fish with regard to the content of EPA and DHA is becoming a determining factor of consumer acceptance, due to the awareness of the general public of the benefits of omega-3 fatty acids for human health. Lastly, the search for compounds with antioxidant activity (astaxanthin, fucoxanthin, phycocyanin, β-carotene etc.), protecting against certain diseases associated with the formation of free radicals and improving the quality of farmed fish (Bermejo et al. 2008) receives a lot of attention. Generally, these works are also associated with the study of cyanobacteria from the genus *Arthrospira* (Bhat and Madyastha 2000; Reddy et al. 2000; Wang et al. 2007) or microalgae *Haematococcus pluvialis* (Chlorophyceae) (Sheikhzadeh et al. 2012).

The purpose of this research is to study the effect of algae supplementation enriched with eicosapentaenoic acid and fucoxanthin on the growth and development of Red tilapia fry.

Materials and methods

Microalgae cultivation

We used the heterokont microalgae *Mallomonas furtiva* Gusev, Certnerová, Škaloudová & ŠkaloudSBV-13 (Chrysophyceae) and *Vischeria magna* Kryvenda, Rybalka, Wolf & Friedl SBV-108 (Eustigmatophyceae).

Mallomonas furtiva was grown on the modified WC medium (Guillard and Lorenzen 1972) with a 10-fold amount of nitrate and phosphate (WC*10). The WC*10 medium contained 0.85 g L^{-1} $NaNO_3$; 0.114 g L^{-1} $K_2HPO_4.3H_2O$; 0.0126 g L^{-1} $NaHCO_3$; 0.0368 g L^{-1} $CaCl_2.2H_2O$; 0.0212 g L^{-1} $Na_2SiO_3.9H_2O$; 0.5 g L^{-1} Tris; 0.037 g L^{-1} $MgSO_4.7H_2O$; trace elements (4.36 mg L^{-1} Na_2EDTA; 3.15 mg L^{-1} $FeCl_3.6H2O$; 0.01 mg L^{-1} $CuSO_4.5H_2O$; 0.022 mg L^{-1} $ZnSO_4.7H_2O$; 0.01 mg L^{-1} $CoCl_2.6H_2O$; 0.18 mg L^{-1} $MnCl_2.4H_2O$; 0.006 mg L^{-1} $Na_2MoO_4.2H_2O$; 1 mg L^{-1} H_3BO_3) and vitamins (0.1 mg L^{-1} thiamine, 0.0005 mg L^{-1} biotin).

Vischera magna was grown on the modified 3NBBM medium, which contained: 0.75 g L^{-1} $NaNO_3$; 0.075 g L^{-1} K_2HPO_4; 0,175 g L^{-1} KH_2PO_4; 0.025 g L^{-1} NaCl; 0.025 g L^{-1} $CaCl_2.2H_2O$; 0.075 g L^{-1} $MgSO_4.7H_2O$; trace elements (4.5 mg L^{-1} Na_2EDTA; 0.582 mg L^{-1} $FeCl_3.6H2O$; 0.03 mg L^{-1} $ZnCl_2.7H_2O$; 0.012 mg L^{-1} $CoCl_2.6H_2O$; 0.246 mg L^{-1} $MnCl_2.4H_2O$; 0.006 mg L^{-1} $Na_2MoO_4.2H_2O$).

Microalgae were cultivated in laboratory incubator shaker Multitron (Infors HT) at 24°C, constant shaking at 150 rpm and 5% CO_2 in air supply. Light intensity was 160 µmol photons m^{-2} s^{-1} with 16:8 h light/dark photoperiod. 20% inoculum was used and the culture was grown for 14 days. Microscopic examination and measurements of optical density of culture at 680 nm were performed daily.

After laboratory cultivation, cultures were inoculated into semi-commercial flat panel photobioreactor Lumian AGS 260 (Fig. 1) with total working volume 260 L. The reactor was operated in batch mode. Cultures were grown simultaneously in different panel groups. Each panel was filled with 12 L of fresh medium and inoculated with 3 L culture, which then had been growing for 14 days. Lighting was provided at 16:8 h light:dark photoperiod with light intensity of 120 µmol photons m^{-2} s^{-1} for days 1-7 of cultivation and 235 µmol photons m^{-2} s^{-1} for days 8-14 of cultivation; CO_2 was sparged for 30 min a day; pH 7 and temperature 24°C were automatically controlled by integrated photobioreactor sensors. Each second day a 20 mL sample was taken from the culture for microscopic observation, dry mass evaluation and nutrient consumption analysis. After 14 days of cultivation, 12 L of culture was harvested using a flow centrifuge (CEPA LE, Biorus) and the remaining 3 L was filled up with fresh medium and used as a new inoculum for the next batch. The concentrated pellet was lyophilized for 48-72 h. The obtained powder was weighed and stored in a low-temperature at minus 80°C.

Molecular studies of *V. magna*

The total DNA of the unilgal culture SBV-108 was extracted using InstaGeneTM Matrix according to the manufacturer's protocol. Fragments of ITS2 rDNA (256 bp) was amplified using primers ITS1-ITS4 from White et al. (1990).

Fig. 1 Lumian AGS260 photobioreactor used for microalgae cultivation

Amplification of all studied fragments was carried out using the premade mix ScreenMix (Evrogen, Russia) for the polymerase chain reaction (PCR). The conditions of amplification for ITS2 rDNA fragments were: an initial denaturation of 5 min at 95°C, followed by 35 cycles at 94°C for denaturation (30 s), 52°C for annealing (30 s) and 72°C for extension (50-80 s), and a final extension of 10 min at 72°C. The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1.5%), colored with SYBR Safe (Life Technologies, USA). Purification of DNA fragments was performed with the ExoSAP-IT kit (Affymetrix, USA) according to the manufacturer's protocol. ITS2 rDNA fragments were decoded from two sides using forward and reverse PCR primers and the Big Dye system (Applied Biosystems, USA), followed by electrophoresis using a Genetic Analyzer 3500 sequencer (Applied Biosystems). Sequences were edited manually and assembled using BioEdit v7.1.3, and MegaX (Kumar et al. 2018). Newly determined sequence and GenBank sequences of 13 other taxa from the genus *Vischeria* of different morphological groups were included in the alignment. Maximum likelihood of phylogeny (ML) was constructed using MegaX (Kumar et al. 2018) with the Subtree-Pruning-Regrafting tree rearrangements algorithm (SPR). The bootstrap analysis with 1,000 replicas was used. Trees were viewed and edited in the programs FigTree (ver 1.4.2) and Adobe Photoshop CC (19.0). For the annotation ITS2 sequence, was used tool ITS2-Annotation (http://its2-old.bioapps.biozentrum.uni-wuerzburg.de/cgi-bin/index.pl?annotator). It returns by definition of the ITS2 the sequence between the conserved 5.8S and 28S (or equivalent) rRNA funding region.

Dry mass and nutrient consumption analysis of microalgae

To obtain dry mass and nutrient consumption data, 20 mL sample of culture was taken every two days. For dry mass analysis, the biomass was separated by centrifugation (15279 × *g*, 3 min), lyophilized and homogenized, then weighted on high-precision balances. Remaining supernatant was used for measurement of nitrate and phosphate concentrations.

Nitrate and phosphate concentrations were measured using a DR 6000 (HACH-Lange) UV-Vis spectrophotometer. Nitrate standard Permachem NitraVer 5 Powder Pillows and phosphate standard Permachem PhosVer 3 Powder Pillows, purchased from HACH, were used for calibration. 12 mL of supernatant was used for each analysis. Calculation of concentration was performed using the standard programs of spectrophotometer: № 357 «N Nitrate UV» and № 490 «P React. PV» respectively.

Chemical analysis of the microalgae biomass

Fatty acid composition analysis and EPA content determination

For EPA content and fatty acid composition determination, 600 µL of nonadecanoic acid in hexane (375 ppm) used as the internal standard, three glass beads and 2 mL of HCl methanol solution (1.25 M) was added to 10 mg of freeze-dried biomass. The sample was vortexed for 1 min and incubated for 2 h at 96.0 ± 0.1 °C in a water bath with vortexing every 30 min for 15 s. After 2 h incubation the sample was cooled to room temperature and 1 mL saturated NaCl solution was added into a mixture. After the phase separation, a 100 µL aliquot of the organic phase was sampled, diluted ten times with hexane, and analyzed by GC/MS (Thermo Scientific Trace GC Ultra DSQ II) with temperature programming (140 °C for 5 min, heated to 280 °C at 10 °C min^{-1} , isotherm 280 °C for 15 min; sample injection volume of 1 µL with 1:20 split; flow rate of helium > 99.9999% was 1.2 mL min^{-1} ; column HP-5MS 30 m × 0.25 mm × 0.25 µm).

EPA was identified by selected ion monitoring (SIM) of ions with mass-to-charge ratios 67, 74, 79, 87, and 91. Quantitative analysis was performed by external standard calibration method, using Sigma-Aldrich Eicosapentaenoic acid ethyl ester standard PHR1790.

The column was calibrated with fatty acid methyl ester standard samples Supelco 47885 and 47040 to determine the fatty acid composition. The fatty acid methyl esters were quantified by total ion current (EI, 70 eV) in the mass range 50–550 Da.

Fucoxanthin content determination in *M. furtiva* biomass

10.0 mg of lyophilized biomass and 1.0 mL of HPLC-grade acetonitrile was added and the suspension was shaken at room temperature for 20 min at 1800 rpm. Then the mixture was centrifuged for 3 min at 15000 ×*g*. The supernatant (0.8 mL) was collected for further analysis. Analysis was performed on HPLC system (Nexera X2, Shimadzu) with SPD

M20A diode array detector and HPLC Discovery C18 150 × 4.6 mm, 5 µm column (Supelco). Isocratic elution with water-acetonitrile mixture, 1:3, for 12 min was carried out at 1 mL min^{-1}, monitoring absorbance at 450 nm. Solutions of Sigma-Aldrich fucoxanthin standard PN F6932 (3.2 mg in 25.0 mL of acetonitrile) at various dilutions (up to 50 × in case of minimal concentration) were used for calibration.

Protein content determination

Protein content was determined using the method of Lowry et al. (1951). 5 mg of oven-dried microalgae sample was mixed with 25 mL of deionized water. Mixed reagent was prepared by adding 1 mL of 1% potassium sodium tartarate to 50 mL of 0.2 g L^{-1} solution of sodium carbonate in 0.1 N NaOH. Then, 0.5 mL of sample was added with 0.5 mL of 1 N sodium hydroxide and it was kept at 100°C in a water bath for 5 min. Then 2.5 mL cooled mixed reagent and 0.5 mL Folin reagent were added to the sample, and kept in a dark place for 30 min. Protein content was then determined by measuring the absorbance of solution at 750 nm.

Lipid content determination

Total lipid content was determined using the method of Bligh and Dyer (1959) with modifications. 200 mg of dry biomass collected at the 14 day of cultivation was mixed with 3.2 mL methanol and homogenized. After homogenization, 4 mL chloroform and 4 mL methanol were added and mixed for 15 min. After mixing, 4 mL chloroform and 4 mL NaCl solution (0.3% w/v) were added. The upper fraction containing methanol and NaCl solution was discarded. Lower fraction containing chloroform and lipids was evaporated and lipid content was determined gravimetrically.

Carbohydrate content determination

Carbohydrate content was determined using the method of Dubois et al. (1956). 5 mg of oven-dried microalgae sample was mixed with 25 mL deionized water. 1 mL aliquot of sample, 1 mL 5% phenolic solution and 5 mL concentrated sulphuric acid were added to the test tube and kept in an ice bath for cooling. After cooling, the optical density was measured at 488 nm.

Diet preparation

Experimental diets were formulated by wet mixing of homogenized commercial feed (Coppens Supreme-15) with lyophilized microalgae biomass. The resulting mass was dried at temperature 50°C, homogenized and sifted to obtain required grain size. Three experimental diets were prepared using this method: Diet 1 contained 10% w/w of *V. magna* biomass; Diet 2 contained 10% w/w *M. furtiva* biomass; Diet 3 contained 5% w/w *V. magna* biomass and 5% w/w *M. furtiva* biomass. Homogenized Coppens Supreme-15 was used as control diet, its composition is listed in Table 1.

Tilapia growing

Experiments were performed in Aquaculture Research Facility of Astrakhan State Technical University (ARF ASTU), Russia. The experimental design and fish handling protocols were approved by the Animal Care Ethical Committee of Astrakhan State Technical University and were carried in accordance with EU Directive 2010/63/EU for animal experiments. Red tilapia fry *Oreochromis mossambicus* × *Oreochromis niloticus* from the first breed produced by ARF ASTU were obtained. At the beginning of the experiment, tilapia fry with initial weight 0.56±0.04 g were randomly stocked into twelve 10 L rectangular tanks at density 10 fry per tank. Each tank was equipped with individual water biofilter and temperature control system. 5% of water was changed daily. Water quality parameters were checked regularly to ensure that the water quality remained within required ranges: temperature 29-30°C, pH 7-8, dissolved oxygen 8-10 mg L^{-1}, total ammonia nitrogen <0.8 mg L^{-1}, nitrite concentration <0.15 mg L^{-1}, nitrate concentration 100-250 mg L^{-1}, conductivity 800-3000 µS cm^{-1}. 16 h light:8 h dark photoperiod was used to simulate natural conditions.

The experiment was conducted for 15 days. Experimental diets were randomly assigned between tanks, three tanks per diet. Fish were hand fed four times per day at 7:00-7:30, 11:00-11:30, 15:00-15:30 and 19:00-19:30, daily amount of feed was 7.5% of fish body weight (El-Dahhar 2007).

Table 1 Composition of Coppens Supreme-15 (from manufacturer's specification)

Component	Content
Protein (%DW)	46
Fat (% DW)	15
Crude Fiber (% DW)	1.8
Ash (% DW)	8.1
Total Phosphorus (% DW)	1.06
Vitamin A (IE)	10000
Vitamin D (IE)	2790
Vitamin E (mg)	200
Vitamin C (mg)	490
Gross Energy (KJ g^{-1})	21
Digestible Energy (KJ g^{-1})	19.2

Water quality parameters control

Temperature, pH, conductivity and dissolved oxygen in fish tank water were measured twice a day at 07:00 and 19:00 using a pre-calibrated multiparameter water-quality meter (HI2030-02 edge, Hanna Instruments).

Nitrate, nitrite and total ammonium nitrogen concentrations in fish tank water were measured daily using UV-Vis spectrophotometer (DR 6000, HACH-Lange). For nitrate concentration determination, reagent NitraVer 5 Nitrate Reagent Powder Pillow and protocol Method 8039 from HACH-Lange were used. For nitrite concentration determination, reagent NitriVer 2 Nitrite Reagent Powder Pillows and protocol Method 8153 from HACH-Lange were used. For total ammonium nitrogen determination reagent set Nitrogen, Total, LR, Test 'N Tube Reagent Set and protocol Method 10071 from HACH-Lange were used.

Chemical analysis of fish and feed

Chemical analysis of fish and feed was performed using the same methods. At the start of experiment 10 fry from the same batch as tested fish were used for initial body composition determination. At the end of experiment, all living fish were used for final body composition determination. Dry mass and water content were determined by drying samples for at least 4 h at 103 °C until constant weight (ISO 6496). Ash content was determined by incineration using a muffle furnace for 4 h at 550 °C (ISO 5984). Crude protein (N x 6.25) was analyzed by the Kjeldahl method (ISO 5983). Crude fat was measured by Soxlet extraction (ISO 5986). Carbohydrate content was calculated as difference between dry weight and the sum of crude protein, crude fat and ash contents.

Growth performance and feed efficiency data analysis and calculation

Growth performance and feed efficiency were evaluated using formulae from Sarker et al. (2016a, b, 2018).

1) The Absolute Growth was calculated as the difference between the average individual initial (W_0) and final (W_1) body weight per fish in grams.
2) Absolute Growth Rate (AGR) was determined as follows:

$$\text{Absolute Growth Rate} = \frac{W_1 - W_0}{T}$$

where: W_0 = initial body weight of fish (g); W_1 = final body weight of fish (g); T = length of experiment (days).

3) Specific Growth Rate (SGR) was determined as follows:

$$\text{Specific Growth Rate} = \frac{[\ln W_1 - \ln W_0]}{T} \times 100$$

where: W_0 = initial body weight of fish (g); W_1 = final body weight of fish (g); T = length of experiment (days).

4) Feed Conversion Rate (FCR) was determined as follows:

$$\text{Feed Conversion Rate} = \frac{\text{Feed intake (g)}}{\text{Weight gain(g)}}$$

5) Survival was determined by accounting of dead fish during daily tank cleaning procedure:

$$\text{Survival} = \frac{\text{Initial number of fish in tank}}{\text{Final number of fish in tank}} \times 100$$

6) Protein Efficiency Ratio (PER) was calculated as follows:

$$\text{Protein Efficiency Ratio} = \frac{W_1 - W_0}{P_i}$$

where: W_0 = initial body weight of fish (g); W_1 = final body weight of fish (g); T = length of experiment (days); P_i = feed protein intake (g).

7) Protein Productive Value (PPV) were calculated as follows:

$$\text{Protein Productive Value} = \frac{P_1 - P_0}{P_i} \times 100$$

where: P_0 = initial content of protein in fish (g); P_1 = final content of protein in fish (g); P_i = feed protein intake (g)

8) Energy Productive Value (EPV) was calculated as follows:

$$\text{Energy Productive Value} = \frac{E_1 - E_0}{E_i} \times 100$$

where: E_0 = initial content of protein energy in fish (kJ); E_1 = final content of energy in fish (kJ); E_i = feed energy intake (kJ).

9) Energy content in fish was calculated as follows:

$$\text{Energy Content} = 23.66 \times P + 39.57 \times F + 17.17 \times C$$

where P, F and C are protein, fat and carbohydrate content, respectively, in wet weight.

Statistical analyses were performed using STATISTICA statistical software (StatSoft). Data on growth performance, feed efficiency and fish body composition were subjected to one-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons of several groups. Data was expressed as the mean ±SEM of three replicate groups (n=3).

Values without letters or with the same letters, which are on the same line, indicate that there was no significant difference ($p > 0.05$), whereas those with different letters indicate a significant difference ($p < 0.05$).

Results

Strain description of *V. magna*

During our studies we used two strains of algae from two classes. *Mallomonas furtiva* SBV-13 (previously mentioned as *Mallomonas* sp. SBV-13) belongs in the class Chrysophyceae. Its description was given previously (Petrushkina et al. 2017; Gusev et al. 2018). The second strain, *Vischeria magna* SBV-108, was not described previously. Here we provide its morphological description and confirmation of identification by molecular methods.

Strain description of *Vischeria magna* SBV-108

Phylum: Heterokontophyta
Class: Eustigmatophyceae
Order: Eustigmatales
Family: Chlorobotryaceae
Genus: *Vischeria*

Vischeria magna (J.B. Petersen) Kryvenda, Rybalka, Wolf & Friedl (Syn: *Eustigmatos magnus* (J.B. Petersen) D.J. Hibberd; *Pleurochloris magna* J.B. Petersen). Fig. 2.

Strain: *Vischeria magna* (SBV-108) is deposited also in the culture collection of the Institute of Biology, Komi Scientific Center, Ural Branch of the Russian Academy of Sciences under the name SYKOA E-07-09 (SYKOA, Syktyvkar, Russia, https://ib.komisc.ru/sykoa/eng/collection/280/).

Representative ITS2 rDNA sequence: SUB14164378 Vischeria PP178161.

Locality: Russia, Subpolar Urals, Mount Barkova, 65°12′34″ N, 60°15′45″E, 630 m above sea level in soil of the herb-grass-willow community 20 VII 2009. Detailed characteristics of the habitat is given in the Patova et al. (2023), including soil chemistry.

Morphological description: *Vischeria magna* – single-celled coccoid alga. The cells are solitary, round in shape, with a thin and elastic membrane, sometimes slimy. The chloroplast is single, strongly lobed, with the pyrenoid of polyhedral shape. In old cells, one large (or several) vacuole is formed. Cells are 6-34 μm in diameter. Reproduction by zoospores (2-8) and spherical or angular autospores (2-4).

The strain SYKOA E-07-09 (=SBV-108) did not show significant morphological changes during cultivation compared to natural populations. The size and shape of the cells of the studied strain correspond to those given in the keys (Ettl 1978). The average cell size is about 10-11 μm.

Comparison with other species of the genus based on ITS2 rDNA showed that the strain belongs to the *Vischeria magna* clade. Despite some differences in the nucleotide composition, CBC between type strain SAG 2554 (Kryvenda et al. 2018) and SBV-108 was not detected and the identification of this strain as *V. magna* was confirmed (Fig. 3).

Cultivation and chemical analysis of microalgae

During cultivation in the AGS-260 photobioreactor *V. magna* achieved final dry mass concentration of 4 g L^{-1} which corresponds to the productivity of 0.29 g L^{-1} day^{-1}, while *M. furtiva* showed 2 g L^{-1} final culture density and 0.14 g L^{-1} day^{-1} productivity. Nutrients were fully utilized during 14-day growth period by both strains. The growth curves and the curves of nutrient consumption of tested strains are shown in Fig. 4.

Chemical composition of *V. magna* biomass is listed in Table 2. Under experimental cultivation conditions, strain reached high lipid content (38.6% DW) and its fatty acid profile was dominated by omega-7 palmitoleic acid (50 mg g^{-1} DW) and omega-3 eicosapentaenoic acid (46.1 mg g^{-1} DW).

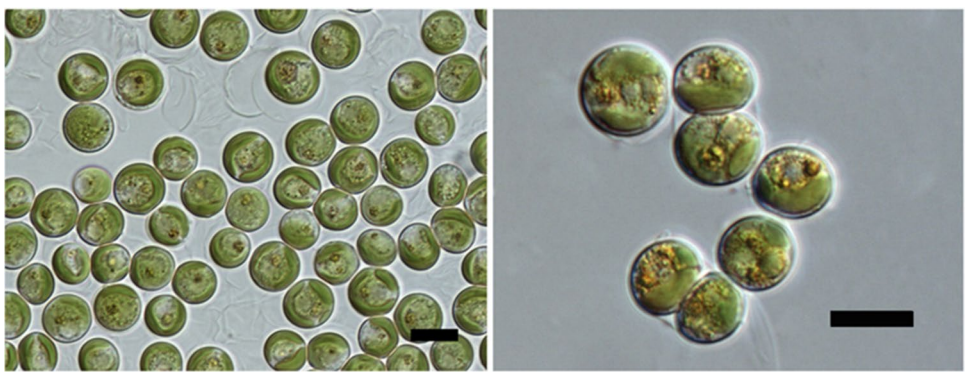

Fig. 2 Vegetative cells of *Vischeria magna* (SBV-108). Scale bars: 10 μm

The chemical composition of *M. furtiva* biomass is listed in Table 3. Under experimental cultivation conditions, *M. furtiva* biomass contained 41.3 %DW protein, 15.4 %DW total lipids, 15.7 %DW total carbohydrates and 2.6 %DW fucoxanthin respectively.

Chemical composition of tested diets

Composition of tested diets is listed in Table 4. Addition of *V. magna* biomass to Diets 1 and 3 resulted in slightly higher lipid content and lower protein content, while Diet 2, containing only *M. furtiva* biomass, was almost identical to the control feed by these parameters. Addition of microalgae led to slight increase in carbohydrate content of all formulated diets compared to control feed, while gross energy content remained almost the same.

Growth performance

Final weight of all fish exceeded 1 g at the end of experiment, which means that all tested diets were effective. No fish died during experiment. The best performing were fish fed on Diet 1 (10% w/w *V. magna*) with average final weight 1.27 g, which is almost 25% higher than performance of the control group (1.03 g) ($p<0.05$). Final weight of fish fed on Diet 3 (5% w/w *V. magna* + 5% w/w *M. furtiva*) and Diet 2 (10% w/w *M. furtiva*) was not significantly different

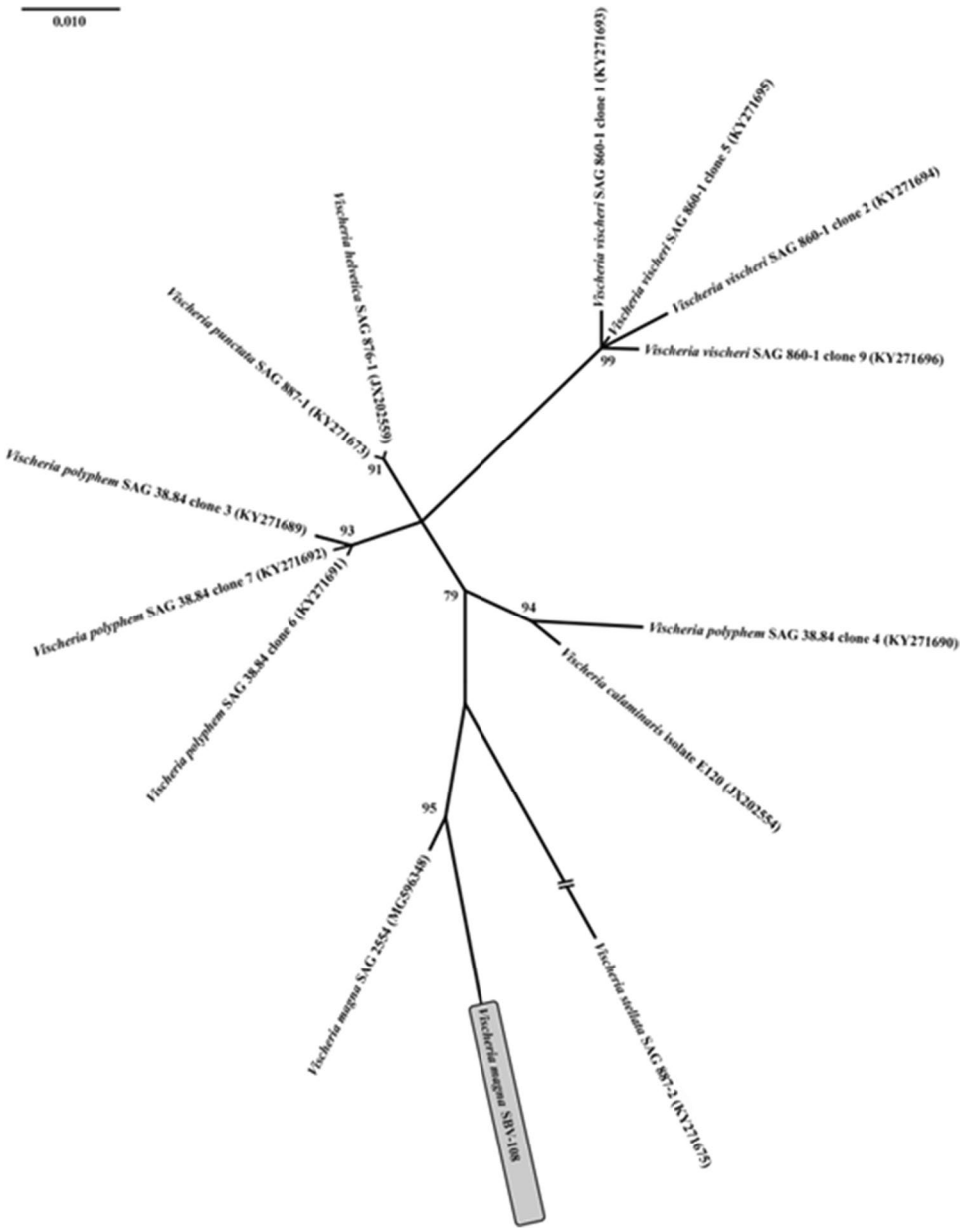

Fig. 3 Unrooted maximum likelihood tree of the ITS2 rDNA of species from the genus *Vischeria*. The maximum likelihood bootstrap values are shown. Scale bar represents: substitution per site

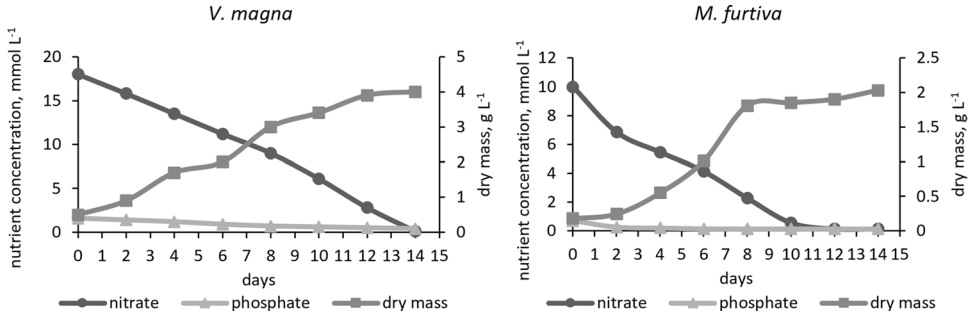

Fig. 4 Growth curve and nutrient consumption curve of tested strains

Table 2 Chemical composition of the *V. magna* biomass

Component	Content, % DW
Protein	23.2
Total lipids	38.6
Total carbohydrates	28.2
Ash	10.0
Fatty acid profile	Content, mg g^{-1} DW
C14:0	2.5
C16:1 (n-7)	50.1
C16:0	12.2
C18:2 (n-6)	13.8
C18:1 (n-9)	9.5
C20:4 (n-6)	6.3
C20:5 (n-3) EPA	46.1

Table 3 Chemical composition of the *M. furtiva* biomass

Component	Content, % DW
Protein	41.3
Total lipids	15.4
Total carbohydrates	15.7
Ash	25.0
Fucoxanthin	2.6
Fatty acid profile	Content, mg g^{-1} DW
C14:0	19.3
C16:0	7.9
18:1 (n-9)	29.5
C18:2 (n-6)	12.7
C18:4 (n-3)	10.4
C22:2 (n-6)	2.1

from control feed. Absolute growth and growth rate values exceeded control in all experimental groups, but only Diet 1 was significantly different. Specific growth rate was the highest for fish fed on Diet 1 (25% greater than control) followed by Diet 3, Diet 2 and control group (not significantly different). Fish were very active at feeding, especially on microalgae-containing Diets 1-3, which suggests presence of some attractants in microalgae. Summary of fish performance represented in Table 5 and Figs. 5 and 6.

Feed efficiency

Feed conversion rate as well as protein and energy productive values are among the most important properties of feeds. The best feed conversion ratios were achieved on Diet 1 (1.07), while Diet 2 (1.43) and Diet 3 (1.13) were not significantly different from control (1.56). Protein efficiency ratio was significantly higher for Diet 1 (2.84) compared to Diet 2 (1.94) and control (1.77), while Diet 3 value (2.23) was not significantly different from any other diet. Protein productive values were significantly improved with addition of microalgae in Diet 1 (26%) and Diet 3 (20%), while Diet 2 (18%) was not significantly different from control (14%). Energy productive value was 16%, 12%, 13% (Diets 1-3) and 11% (control), where only Diet 1 was significantly different from control feed. Experimental results presented in Table 6 and Figs. 7 and 8.

Table 4 Chemical composition of tested diets

Component	Control	Diet 1	Diet 2	Diet 3
M. furtiva biomass (% w/w)	0	0	10	5
V. magna biomass (% w/w)	0	10	0	5
Protein (%DW)	46.0	43.7	45.8	45.1
Lipids (%DW)	15.0	17.3	15.1	16.2
Carbohydrates (%DW)	8.0	9.9	8.9	9.3
Gross Energy (KJ g^{-1})	21	22	21	22

Body composition of tested fish

Effect of experimental diets on body composition of tilapia fry presented in Table 7. Protein, lipid, ash and energy content significantly increased in all fish compared to initial values, which suggest that all experimental diets were digested well. However, differences between tested Diets 1-3 and the Control diet were insignificant.

Table 5 Effect of experimental diets on performance of tilapia fry

Parameter	Control	Diet 1	Diet 2	Diet 3	F-value	P-value
Initial body weight (g)	0.57± 0.02^{a}	0.58± 0.01^{a}	0.58± 0.02^{a}	0.53± 0.01^{a}	1.72	0.167
Final body weight (g)	1.03± 0.03^{a}	1.27± 0.07^{b}	1.08± 0.05^{a,c}	1.10± 0.04^{a,c}	4.56	0.005
Absolute growth (g)	0.46± 0.04^{a}	0.70± 0.07^{b}	0.50± 0.05^{a,c}	0.57± 0.04^{a,c}	4.56	0.005
AGR (g day^{-1})	0.03± 0.002^{a}	0.05± 0.005^{b}	0.03± 0.003^{a,c}	0.04± 0.003^{a,c}	4.07	0.009
SGR (% day^{-1})	3.97± 0.31^{a}	5.16± 0.37^{b}	4.06± 0.32^{a,b}	4.81± 0.26^{a,b}	3.38	0.021
Survival rate (%)	100	100	100	100	-	-

Values are means ±SEM of three replicate groups (n = 3). Means within the same row not sharing a common letter are significantly different (P<0.05)

Discussion

In present work, we studied an enrichment of standard starter fish feed with microalgae biomass and describe a new strain of eustigmatophycean algae that is promising for EPA production. In the natural habitats microalgae are the essential feed for various juvenile hydrobionts including fish, mollusks and crustaceans. Moreover, some microalgae species are rich in biologically active compounds that improve growth rate and ensure proper development of juvenile fish, such as polyunsaturated fatty acids. Therefore, interest in the study and application of new algae in biotechnology is justified.

The main challenge of using microalgae biomass as aquaculture feed is the high cost of microalgae production. This is why microalgae are often used as a larval feed or as an enrichment of living diet (such as rotifers) in aquaculture. Only small number of microalgae species are studied as aquafeed additives, mostly cyanobacteria of genus *Arthrospira* (Lu et al. 2002; Takeuchi et al. 2002; Lu and Takeuchi 2004; Abdel-Tawwab and Ahmad 2009; Sarker et al. 2016b) and eukaryotic algae of genera *Chlorella* and *Schizochitrium* (Tadesse et al. 2003; Sarker et al. 2016a). We used microalgae of two classes, previously not studied as feed additives: *M. furtiva*, which has one of the highest known content of carotenoid fucoxanthin in biomass (Petrushkina et al. 2017; Khaw et al. 2022) and *V. magna*, which is known for one of the highest EPA content in microalgae, especially among freshwater and soil species (Table 8).

Thus, we tested the performance of starter feed, enriched by natural microalgal EPA and fucoxanthin. Experimental data showed, that feeding all tested diets led to increase in fish body weight with 100% survival rate, which suggests that feed composition was suitable for tilapia. All diets, enriched with microalgae, showed the same or better performance compared to control commercial feed. However, the best performing was Diet 1 (10% w/w *V. magna* with high EPA content), where final body weight and specific growth rate exceeded control by 25% (Table 5), while Diets 2 and 3 containing *M. furtiva* biomass were not significantly different from the Control diet in the effect on fish performance. Supplementation by 10% w/w *V. magna* (Diet 1) also improved feed digestibility and showed the best feed conversion ratio, protein efficiency ratio as well as protein and energy productive values among all tested diets (Table 6).

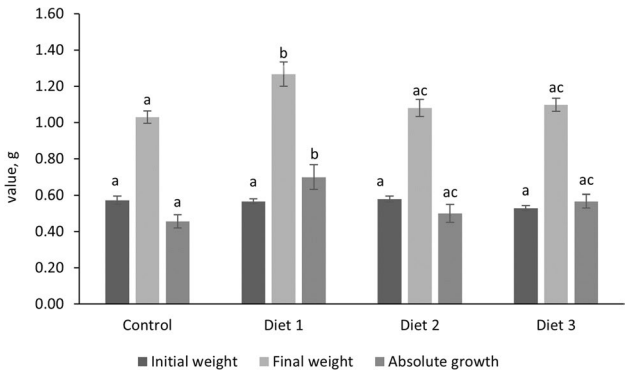

Fig. 5 Growth performance results: initial weight, final weight and absolute growth. Values are means of three replicate groups (n=3). Values across the bars not sharing a common letter are significantly different (P<0.05). The error bars represent the standard error of the mean

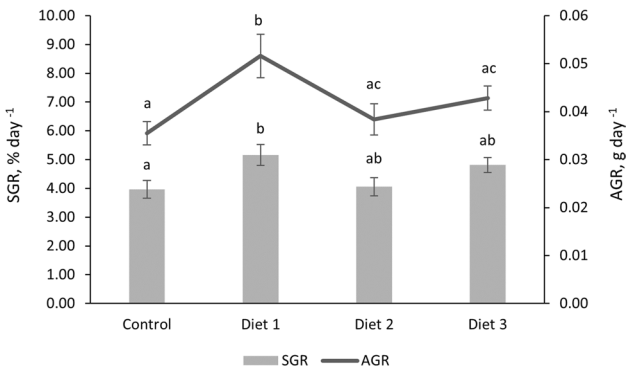

Fig. 6 Growth performance results: average growth rate (AGR) and specific growth rate (SGR). Values are means of three replicate groups (n=3). Values across the bars not sharing a common letter are significantly different (P<0.05). The error bars represent the standard error of the mean

Table 6 Efficiency of the experimental diets

Parameter	Control	Diet 1	Diet 2	Diet 3	F-value	P-value
FCR	1.56± 0.17^{a}	1.07± 0.11^{b}	1.43± 0.13^{a,b}	1.13±0.09^{a,b}	3.27	0.024
PER	1.77± 0.14^{a}	2.84±0.27^{b}	1.94±0.18^{a,c}	2.23±0.14^{a,c}	5.77	0.001
PPV (%)	14±1^{a}	26±2^{b}	18±1^{a,c}	20±1^{c}	13.36	<0.001
EPV (%)	11±1^{a}	16±1^{b}	12±1^{a,c}	13±1^{a,c}	4.62	0.004

Values are means ±SEM of three replicate groups (n = 3). Means within the same row not sharing a common letter are significantly different (P<0.05)

The effect of algae on growth performance has been previously studied in aquaculture important species like Mossambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*), Rainbow trout (*Oncorhynchus mykiss*) Atlantic salmon (*Salmo salar*) (Sommer et al. 1992; Lu et al. 2002; Tadesse et al. 2003; Abdel-Tawwab and Ahmad 2009; Sarker et al. 2016a; Sprague et al. 2016), African catfish (*Clarias gariepinus*) (Raji et al. 2020). In most cases positive effect after microalgae additions was revealed. Generally, microalgae were reported to enhance growth (Ju et al. 2008, 2009, 2012; An and Anh 2020; Annamalai et al. 2021; Ansari et al. 2021), can increase the survivability of farmed species (Nagappan et al. 2021) and have many health benefits for aquatic animals, such as having immune enhancing (Hayashi et al. 1994; Bahi et al. 2023) and anti-viral properties (Hayashi et al. 1996; Ma et al. 2020). The inclusion of microalgae in the diets was also considered to be essential for the efficacious utilization of nutrients by fish, and the increased growth rate was associated with improved physiological conditions such as protein assimilation, lipid metabolism, liver function and stress response (Mustafa and Nakagawa 1995; Ansari et al. 2021). It is well known that fish are not able to synthesize omega-3 fatty acids, and thereby, the omega-3 fatty acid content of fish majorly depends on feed and environment (Ju et al. 2017; Serrano et al. 2021). Previous researchers have attempted to enhance omega-3 fatty acid content in tilapia meat. Shapira et al. (2009) found that farmed tilapia had only slightly increased levels of docosahexaenoic acid (DHA) after being fed a diet with 7% linseed oil supplement. Tocher et al. (2001) and Karapanagiotidis et al. (2007) found that supplementing fish oil or vegetable oils to tilapia feed limitedly increased long chain omega-3 fatty acid concentrations in tilapia meat.

Various studies show that antioxidants are important for fish health. They protect lipids from oxidation by reactive oxygen species (Suárez-Jiménez et al. 2016), decrease muscle oxidative stress and fatigue (Powers et al. 2004) and modulate immunity of fish (Blount et al. 2003). Fucoxanthin is a natural antioxidant, commonly found in microalgae (class Bacillariophyceae and others), which are the primary natural feed for many fish species. However, effect of fucoxanthin enriched feed on fish growth and performance has not been extensively studied yet. Resent researches (Gammone and D'Orazio 2015; Miyashita and Hosokawa 2017) suggest that fucoxanthin affects energy expenditure and possess anti-obesity activity.

The most promising feature of the biomass of *V. magna* SBV-108 is expressed in a very high concentration of EPA, one of the highest in freshwater algae. Maintaining high EPA

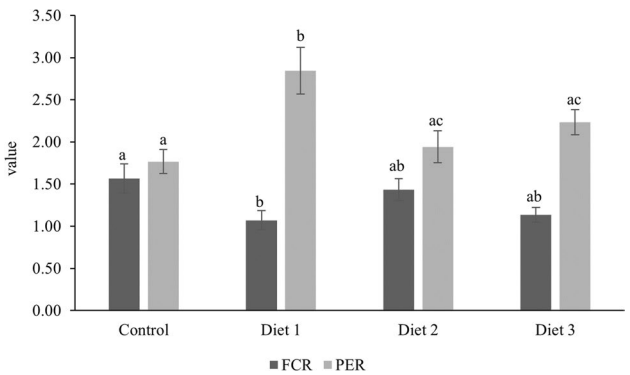

Fig. 7 Feed efficiency results: feed conversion rate (FCR) and protein efficiency ratio (PER). Values are means of three replicate groups (n=3). Values across the bars not sharing a common letter are significantly different (P<0.05). The error bars represent the standard error of the mean

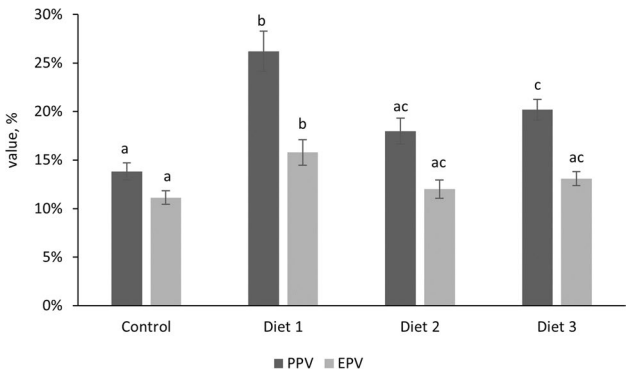

Fig. 8 Feed efficiency results: protein productive value (PPV) and energy productive value (EPV). Values are means of three replicate groups (n=3). Values across the bars not sharing a common letter are significantly different (P<0.05). The error bars represent the standard error of the mean

Table 7 Effect of experimental diets on body composition of tilapia fry

Parameter	Initial	Control	Diet 1	Diet 2	Diet 3	F-value	P-value
Crude protein (% DW)	63.1± 0.21	66.5± 0.25	68.6± 0.27	66.3± 0.21	67.2± 0.35	2.925	0.132
Crude fat (% DW)	7.9± 0.15	11.6± 0.60	11.8± 0.46	11.0± 0.72	10.9± 0.55	1.875	0.253
Carbohydrates (% DW)	16.6± 0.02	7.2± 0.05	4.6± 0.12	7.9± 0.08	7.5± 0.07	3.834	0.08
Ash (% DW)	12.4± 0.32	14.7± 0.25	15.0± 0.15	14.8± 0.19	14.4± 0.38	1.283	0.396
Energy (kJ g^{-1} wet weight)	1.89± 0.10	2.11± 0.22	2.38± 0.03	2.33± 0.14	2.34± 0.18	2.012	0.231

Values are means ±SEM of three replicate groups (n = 3). Tukey test was not performed as there was not significant difference between diets

and DHA content is especially important for starter feed, as fish omega-3 requirements may be twice as high in larvae as in adult fish, even within the same species, perhaps due to increased needs for neurological development (Izquierdo and Hernandez-Palacios 1997). In addition, feed supplementation with omega-3-rich microalgae is the primary way to increase omega-3 fatty acids content in the meat of aquaculture farmed fish (Stoneham et al. 2018). Ju et al. (2017) showed that addition of microalgae to the tilapia diet can lead to high omega-3 fatty acid content, especially DHA, in tilapia meat. Recently, some important studies of addition of the DHA-rich *Schizochytrium* sp. to the tilapia diet found that *Schizochytrium sp.* is a high-quality DHA-enriched candidate for complete substitution of fish oil in juvenile Nile tilapia feeds (Sarker et al. 2016a). However, up to now there are no commercial EPA-rich microalgae, which can be widely used as a full or supplemental feed for freshwater fish aquaculture. *V. magna* SBV-108 can be good candidate for this based on high and stable growth in the culture, high yield and high EPA content.

Conclusions

The present work showed that *Vischeria* biomass could be successfully used to improve performance of starter fish feeds. Microalgae serve as natural starter feed for many aquaculture species and contain essential components for proper growth and development of fry. However, while the supplementation of feed by fucoxanthin-rich microalgae, such as *M. furtiva,* could possibly improve health and immunity of fish, it had not led to any significant improvements of fish growth performance and feed efficiency in the present study. Contrary, supplementation of feed by 10% w/w *V. magna* biomass rich of omega-3 eicosapentaenoic acid, led to significant improvements of almost all performance

Table 8 EPA content in various microalgae strains

Strain	EPA content, mg g^{-1} DW	Reference
Nannochloropsis oceanica CY2	55.7	Chen et al. 2013
Vischeria magna SBV-108	46.1	This study
Phaeodactylum tricornutum	38.9	Jakhwal et al. 2022
Phaeodactylum tricornutum II242	38.6	Alonso et al. 1996
Pavlova sp. CS-50	35.2	Martínez-Fernández et al. 2006
Nannochloropsis oceanica IMET1	31.2	Jakhwal et al. 2022
Nannochloropsis oculata	30.8	Jakhwal et al. 2022
Phaeodactylum tricornutum NIVA BAC 2	28.4	Patil et al. 2007
Nannochloropsis oceanica NIVA 2/03	23.4	Patil et al. 2007
Porphyridium cruentum SRP-6	23.2	Cohen et al. 1992
Pavlova lutheri	23.1	Jakhwal et al. 2022
Aurantiochytrium sp. SD116:PfaA-D	18.7	Wang et al. 2020
Pavlova sp. NIVA 4/92	18.0	Patil et al. 2007
Chaetoceros sp. CS-256	15.4	Martínez-Fernández et al. 2006
Chaetoceros muelleri CS-176	10.4	Martínez-Fernández et al. 2006
Rhodomonas baltica	8.0	Wang et al. 2019
Porphyridium cruentum NIVA 1/92	6.1	Patil et al. 2007
Tetraselmis suecica NIVA 3/92	4.8	Patil et al. 2007
Rhodomonas baltica NIVA 5/91	4.4	Patil et al. 2007

and efficiency parameters by over 20%, which makes *V. magna* SBV-108 a great candidate as a supplement to starter aquafeed.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article;

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Competing interests The authors declare no competing interests.

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