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Research article

Biotransformation of aquaculture wastewater into aquatic feed protein source: *Chlorella sorokiniana* nutritional value and safety risk assessment

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A R T I C L E I N F O

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A B S T R A C T

Aquaculture wastewater contains high concentrations of nitrogen and phosphorus compounds, which can be used as nutrients for microalgal growth. In this study, the ability of *Chlorella sorokiniana* (*C. sorokiniana*) to purify aquaculture wastewater from an intensive recirculating aquaculture system (RAS) carp sp. farm was evaluated. We then assessed the safety risk of *C. sorokiniana* cultured from aquaculture wastewater and conducted an 8-week fish feeding trial to evaluate its nutritional value as a feed protein source. The three diets were supplemented with 0 (FM, control), 5% (MM5) or 15% (MM15) *C. sorokiniana* to replace the fish meal. A total of 180 healthy gibel carp (*Carassius gibelio*) of similar size were randomly selected into 9 tanks (20 fish/tank, 3 tanks/group). At the end of *C. sorokiniana* purifying aquaculture wastewater, DIC and DTC gradually decreased by 80.6% and 16.5%, respectively, whereas DOC increased by 52.2%. The change curve of COD_{Mn} was similar to that of DOC, and the removal rates of NH_4-N, DTN, DIP and DTP were 93.5%, 86.8%, 36.0% and 26.6%, respectively. The heavy metals and antibiotics contents of *C. sorokiniana* were low or not detected and conformed to the requirements of the aquatic feed ingredient standards. The ARA, EPA and total polyunsaturated fatty acids contents of *C. sorokiniana* were 13.67, 33.82 and 76.81% of the total fatty acids content, respectively. At the end of the fish feeding trial, we found that the replacement of fishmeal with *C. sorokiniana* did not affect the growth of the fish or the amino acids contents of the muscle but promoted the body colour values of the fish and the relative content of n-3 polyunsaturated fatty acids in the muscle. In addition, 5% dietary *C. sorokiniana* can upregulate the relative expression of *cat* and increase the activity of CAT in the liver; upregulate the relative expression of the proinflammatory factor *inf-γ* and the anti-inflammatory factors *il-4* and *tgf-β*; and reduce the relative abundance of pathogenic bacteria, such as *Citrobacter*, *Staphylococcus* and *Pseudomonas,* in the gut of gibel carp. However, 15% dietary *C. sorokiniana* significantly increased the relative expression of *inf-γ* and *hsp70* in the liver and only reduced the relative abundance of *Citrobacter*. Overall, *C. sorokiniana* has the ability to remove nutrients from aquaculture wastewater and can be an alternative protein source for fish. On the basis of growth performance, antioxidant capacity, fatty acid contents of muscle, and the gut microbiota, 5% dietary *C. sorokiniana* is recommended.

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1. Introduction

Fish account for one-third of the animal protein consumed by humans (Han et al., 2018). To satisfy market demand, global aquaculture production reached a record 122.6 million tons in 2020 (FAO, 2022). Sustainable aquaculture development remains critical to meet the growing demand for aquatic foods; however, many challenges, such as water pollution, arise with this rapid development of aquaculture (Jiang et al., 2022).

During the production of aquaculture, waste, including uneaten food or undigested components that are discharged as faeces, is also produced, leading to an increase in nitrogenous and phosphorous compounds in the water (Ahmad et al., 2021). A typical aquaculture pond was reported to release approximately 80% of the nitrogen and phosphorus into a reservoir in semiarid northeastern Brazil (Moura et al., 2016). Approximately 7.3×10^6 kg of total nitrogen from aquaculture ponds is estimated to be discharged to Taihu Lake in China, accounting for 7.3% of agricultural pollutant emissions (Nie et al., 2020). Faeces and various excretions of aquatic animals and uneaten feed emitted into the environment have been reported to cause pollution, resulting in aquatic ecosystem degradation and biodiversity loss (Islam, 2005; Ottinger et al., 2016). Hence, it is important to reuse nutrient-rich aquaculture wastewater to promote the responsible and sustainable development of aquaculture.

The rapid development of aquaculture has also led to an increasing demand for high-quality protein as a source of ingredients for fish feed. Over 70% of global fishmeal is used by the aquaculture sector (Jannathulla et al., 2019). However, fishmeal is an unsustainable resource and is in short supply (Olsen and Hasan, 2012). The price of fishmeal is also increasing, and it is forecast to be 28% higher by 2030 than it was in 2005 (FAO, 2022), leading to high-cost pressure on the aquaculture industry. Hence, sustainable and available protein sources are urgently needed to replace fishmeal in aquatic feed (El-Ouny et al., 2023; Li et al., 2023).

Microalgae are primary producers and can rapidly absorb dissolved nitrogen and phosphorus in wastewater. Microalgal biomass is considered an effective method for recovering nutrients from wastewater (He et al., 2023). Chlorella has a high removal efficiency of ammonia, nitrite and orthophosphate of up to 80%–90% and a high growth rate, with a cell density that can increase more than tenfold in 14 days (Nasir et al., 2023). It can also metabolize and accumulate products with high nutritional value, such as protein, fatty acids, carotenoids, and vitamins (Rodriguez-Garcia and Guil-Guerrero, 2008). For example, *Chlorella vulgaris* is considered a high-quality, single-cell protein source that can improve muscle quality by increasing the muscle crude protein content and muscle and skin colour values of largemouth bass (*Micropterus salmoides*) (Yu et al., 2022). It has also been reported that dietary *Chlorella sorokiniana* (*C. sorokiniana*) not only substitutes for fishmeal to significantly increase the feed intake and growth of rainbow trout (*Oncorhynchus mykiss*) but also promotes the antioxidant capacity and survival rate of fish infected with *Aeromonas salmonicida* (Chen et al., 2022).

C. sorokiniana is one of the most widely used microalgae for CO_2 fixation, wastewater remediation, and the production of value-added products (Rani and Ojha, 2021). It was proven that *C. sorokiniana* can be a potential ingredient for aquatic feed and can be beneficial to the health of aquatic animals (Chen et al., 2021; Liu et al., 2022b). Moreover, Chlorella can absorb heavy metals easily; for example, cadmium (Cd) and copper (Cu) with an adsorption rate by this microalga of up to 99% (Zhao et al., 2023). Additionally, Chlorella has great potential for removing most antibiotics from wastewater (Chu et al., 2023; Li et al., 2022b), but some antibiotics have also been shown to be toxic to Chlorella and fish (Ma et al., 2024). Hence, the potential risks associated with the use of aquaculture wastewater cultured with Chlorella in aquatic feed must be considered, including heavy metals and antibiotics.

In the present study, the green microalga *C. sorokiniana* cultured with aquaculture wastewater was tested for its ability to replace fishmeal in gibel carp (*Carassis auratus gibelio*) diets. First, the ability of *C. sorokiniana* to remove nutrients was evaluated, and the microalgal biomass and main nutrient contents were measured. To make aquaculture more sustainable, microalgae were subsequently used as feed ingredients to replace fishmeal, the safety of *C. sorokiniana* diets was considered, and a fish feeding trial was conducted to assess the influence of *C. sorokiniana* on the growth performance, quality and health of the fish. This study contributes to increasing the knowledge of the application of microalgae in aquaculture feed, especially microalgae produced from nutrients lost during fish farming practices.

2. Materials and methods

2.1. Microalgal cultivation and diets preparation

The microalga *C. sorokiniana* was produced in batches in a runway tank supplemented with fish farm wastewater collected from a containerized, high-density carp sp. aquaculture system and biotransformed sludge. The length, width and depth of the runway tank were 42 m, 2.6 m and 0.3 m, respectively. A total of 33 m^3 of fish farm wastewater was filtered with a 112 μm plankton net and injected into the runway tank at the initial stage of culture. A total of 1.74 kg of NH_4Cl was added to provide nutritional supplements for *C. sorokiniana* cultivation, and the cultivation lasted for 7 days. The biomass of microalgae inoculated on Day 0 was 0.02 g/L. The culture light was natural light (from 6:00 to 19:00 every day), and the light reached a maximum value of approximately 3000 μmol/m^2/s at noon. The temperature of the water was measured every day and ranged from 27.4 to 35.6 °C. During microalgal cultivation, the water temperature, pH, dissolved oxygen (DO), conductivity, total dissolved solids (TDS), turbidity, dissolved total carbon (DTC), permanganate index (COD_{Mn}), dissolved total nitrogen (DTN) and dissolved total phosphorus (DTP) of aquaculture wastewater and the ash-free dry weight, chlorophyll *a*, chlorophyll *b* and carotenoids of *C. sorokiniana* are determined via the methods described by He et al. (2023).

The microalgae were harvested on Day 7 and freeze-dried to obtain the fish-feed ingredients. The fish-feed formulation and chemical

Table 1
Ingredients and proximate composition of the diets (% dry matter).

Ingredients	FM	MM5	MM15
Fishmeal	20.00	16.40	9.20
Chlorella sorokiniana	0.00	5.00	15.00
Soybean meal	18.00	18.00	18.00
Rapeseed meal	18.00	18.00	18.00
L-Met	0.27	0.27	0.27
Wheat flour	20.00	20.00	20.00
Fish oil	2.90	2.90	2.90
Soybean oil	2.90	2.90	2.90
Cellulose	9.43	8.03	5.23
Vitamin premix	0.39	0.39	0.39
Mineral premix	5.00	5.00	5.00
Carboxymethyl cellulose	3.00	3.00	3.00
Choiline chloride	0.11	0.11	0.11
Total	100.00	100.00	100.00
Chemical composition			
Crude protein	32.54	31.89	31.22
Crude lipid	7.48	7.69	7.35

Fishmeal: Seafood white fishmeal imported from United States; Soybean meal and Rapeseed meal: Purchased from DBN Feed Co.Ltd, Wuhan, Hubei, China; Vitamin premix (mg kg^{-1} diet): Vitamin B_1, 20; Vitamin B_2, 20; Vitamin B_6, 20; Vitamin B_{12}, 0.02; folic acid, 5; calcium patothenate, 50; inositol, 100; niacin, 100; biotin, 0.1; cellulose, 3522; Vitamin A, 11; Vitamin D, 2; Vitamin E, 100; Vitamin K, 10; Mineral premixes (mg kg^{-1} diet): NaCl, 500.0; $MgSO_4{\cdot}7H_2O$, 8155.6; $NaH_2PO_4{\cdot}2H_2O$, 12,500.0; KH_2PO_4, 16,000.0; $Ca(H_2PO_4)\cdot 2H_2O$, 7650.6; $FeSO_4{\cdot}7H_2O$, 2286.2; $C_6H_{10}CaO_6{\cdot}5H_2O$, 1750.0; $ZnSO_4{\cdot}7H_2O$, 178.0; $MnSO_4{\cdot}H_2O$, 61.4; $CuSO_4{\cdot}5H_2O$, 15.5; $CoSO_4{\cdot}7H_2O$, 0.91; KI, 1.5; Na_2SeO_3, 0.60; Wheat flour, 899.7.

composition are shown in Table 1. In the feed, *C. sorokiniana* was added at different concentrations, 0 (FM), 5% (MM5) and 15% (MM15), to progressively replace the fishmeal. All the ingredients were mixed well with water and shaped into pellets via a laboratory granulator (Fishery Mechanical Facility Research Institute, Shanghai, China). The pellets were dried in an oven and stored at 4 °C. The amino acid profiles of the diets are shown in Table 2, and the main fatty acids compositions are shown in Table 3.

2.2. Fish and aquaculture management

All the fish experiments were approved under principles for the use and care of experimental animals to avoid suffering (IHB, CAS, Protocol No. 2016-018). The gibel carp were purchased from Guanqiao hatchery (Wuhan, China). All the fish were reared in a 1500 L tank for two weeks. Prior to the feeding trial, the fish fasted for 24 h. A total of 180 healthy fish (initial weight 8.06 ± 0.04 g) were randomly selected from the 1500 L tank and placed into nine 400 L tanks (20 fish/tank, 3 tanks/group). The fish were fed twice a day to apparent satiation (8:30, 16:30), and the fish feeding trial lasted for eight weeks under the following standard conditions: photoperiod of 8:16 (L:D), water temperature of 27 ± 2 °C, ammonia nitrogen content under 0.5 mg/L, dissolved oxygen over 7.5 mg/L, and pH between 6.5 and 7.0.

2.3. Sample Collection

After eight weeks, all the fish from each tank were anaesthetized with 80 mg/L MS-222 (Sigma–Aldrich, St. Louis, MO, USA) and weighed. Three fish from each tank were weighed and stored at −20 °C for chemical composition analysis, measurement of body length and estimation of growth. To assess the condition factor (CF), the livers of these three fish were collected and weighed to determine the hepatosomatic index (HSI). Three different fishes were prepared to measure their body colour via a KonicaMinolta CR-400 tristimulus colorimeter (Minolta, Osaka, Japan). Blood samples were subsequently drawn from the caudal vein with heparinized syringes and placed in a 1.5 ml tube. The blood cells were removed by centrifugation at 3000×*g* for 10 min at 4 °C. The supernatant, designated plasma, was carefully removed from the cell pellet via a Pasteur pipette. Afterwards, the liver and dorsal muscle were removed for further analysis, and the contents of the intestine were collected for microbiotic analysis.

2.4. Biochemical analysis

The moisture, crude lipid and crude protein contents of the *C. sorokiniana*, experimental diets and whole fish were determined via the methods described by the AOAC (2003). In brief, the moisture content was measured to a constant weight in a 105 °C oven, the crude lipid content was analysed via a Soxtec system (Soxtec, 2055; FOSSTecator, Haganas, Sweden), and the crude protein content was analysed via a KD-310-Autoanalyzer (OPSIS, Furulund, Sweden). The amino acids of *C. sorokiniana*, the experimental diets and the dorsal muscle were determined with an A300 amino acid analyser (MembraPure, Hennigsdorf, Germany) following the methods described by Liu et al. (2019). The fatty acids contents of *C. sorokiniana*, the experimental diets and the dorsal muscle were determined via gas chromatography–mass spectrometry (GC–MS, 7890A, AgilentTechnologies, USA) according to the methods described by Fei et al. (2020). The heavy metals of *C. sorokiniana*, the experimental diets and the dorsal muscle were detected by an OPTIMA 8000DV Inductively Coupled Plasma Mass Spectrometry machine (PerkinElmer, USA) at Akeruide Testrust Company (Wuhan, China). The antibiotic contents of *C. sorokiniana* and the experimental diets were also tested at Akeruide Testrust Company (Wuhan, China).

The activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the plasma were measured via commercial assays (A007–1, A001–3 and A005; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The reduced glutathione (GSH) and malondialdehyde (MDA) concentrations were also determined via commercial assays (A006–2 and A003-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Table 2
Amino acid profile of diets (% dry matter).

Amino acid	FM	MM5	MM15
EAA			
Thr	1.07	1.04	1.01
Val	1.44	1.44	1.43
Met	0.70	0.57	0.66
Ile	1.19	1.10	1.08
Leu	2.07	1.93	1.92
Phe	1.25	1.11	1.18
His	0.76	0.72	0.62
Lys	1.74	1.64	1.53
Arg	1.62	1.53	1.55
NEAA			
Asp	2.81	3.48	3.97
Ser	1.05	1.40	1.42
Glu	5.22	3.91	3.79
Gly	1.40	2.02	1.88
Ala	1.44	2.19	1.94
Tyr	0.73	1.14	1.06
Pro	1.79	1.47	1.44

EAA: Essential amino acid; NEAA: Non-essential amino acid.

Table 3
Main fatty acid composition of diets (% of total fatty acids).

Fatty acids	FM	MM5	MM15
C14:0	2.88	2.51	2.24
C15:0	0.58	0.51	0.47
C16:0	9.37	8.39	7.84
C17:0	0.59	0.48	0.41
C18:0	3.35	2.81	2.39
∑SFA	16.78	14.71	13.36
C16:1n-7	4.04	4.40	5.21
C18:1n-9	15.14	14.52	13.27
C20:1n-9	1.34	1.53	1.09
∑MUFA	20.52	20.45	19.56
C18:3n-3	4.44	4.46	5.28
C20:5n-3 (EPA)	4.13	9.34	11.91
C22:6n-3 (DHA)	10.80	9.41	7.41
∑n-3PUFA	19.38	23.21	24.60
C18:2n-6 (LA)	37.33	34.63	32.43
C18:3n-6 (LNA)	0.11	0.12	0.11
C20:2n-6	0.31	0.29	0.26
C20:4n-6 (ARA)	1.00	1.67	3.30
C22:2n-6	4.57	4.92	6.38
∑n-6PUFA	43.32	41.64	42.47
ΣPUFAs	62.70	64.85	67.08
Σn-3/Σn-6	0.45	0.56	0.58

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LNA: linolenic acid; ARA: arachidonic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ∑n-3 PUFA: n-3 polyunsaturated fatty acids; ∑n-6 PUFA: n-6 polyunsaturated fatty acids.

2.5. Quantitative real-time PCR analysis

Total RNA extraction, reverse transcription and quantitative real-time PCR were conducted according to the methods of Liu et al. (2022a). The reference gene β-actin was used to standardize the calculation of the relative expression of genes. The primers used for quantitative RT–PCR are shown in Table S1.

2.6. Intestinal microbiotic analysis

The methods of extracting DNA and amplifying the intestinal microbiota via PCR followed the methods of Fu et al. (2023). Purified

amplicons were pooled in equimolar amounts and paired-end sequenced via an Illumina MiSeq platform (Illumina, San Diego, USA) according to standard protocols by Shanghai Majorbio Biopharm Technology Co., Ltd., and the data were analysed via the Majorbio Cloud Platform (www.majorbio.com).

2.7. Statistical analyses

All values are presented as the means ± standard errors (SEMs), and the data were analysed by SPSS 22. Normality and homoscedasticity assumptions were confirmed before one-way analysis of variance (ANOVA). Duncan's multiple range test was used to detect differences among the three treatments. *P* values less than 0.05 indicated significant differences.

3. Results and discussion

3.1. Physicochemical parameters of aquaculture wastewater

The parameters of water temperature, pH, dissolved oxygen (DO), conductivity, total dissolved solids (TDS) and turbidity are shown in Fig. S1. As shown in Fig. 1, at the beginning of microalgal cultivation, dissolved inorganic carbon (DIC) was the main component of dissolved total carbon (DTC). At the end of microalgal cultivation, DIC and DTC gradually decreased by 80.6% and 16.5%, respectively, while dissolved organic carbon (DOC) increased by 52.2% and became the main component of DTC. The results revealed that dissolved organic matter was released when the microalga *C. sorokiniana* was cultured in aquaculture wastewater. The change curve of COD_{Mn} was similar to that of DOC, indicating that the chemical oxygen demand required for *C. sorokiniana* culture gradually increased. In this study, ammonia nitrogen (NH_4-N) was always the main component of total dissolved nitrogen (DTN). After three days of *C. sorokiniana* cultivation, the concentrations of NH_4-N and DTN in the effluent were 0.64 mg/L and 2.05 mg/L, respectively, and the removal rates were 93.5% and 86.8%, respectively. When NH_4Cl was added, both NH_4-N and DTN increased rapidly and then decreased rapidly. The change in the dissolved total phosphorus (DTP) content was highly similar to that of the DTC. At the beginning of the experiment, the proportion of dissolved inorganic phosphorus (DIP) in the total dissolved phosphorus was greater. The removal rates of DIP and dissolved total phosphorus (DTP) were 36.0% and 26.6%, respectively. The concentration of dissolved organophosphorus (DOP) increased significantly after the addition of NH_4Cl and then decreased slowly, with a removal rate of 19.5%. At the end of cultivation, DOP accounted for a greater proportion of DTP.

3.2. Microalgal growth, cellular pigments, nutritional composition, heavy metals and antibiotics in C. sorokiniana

As shown in Fig. 2, at the end of culture, the maximum dry weight without ash, chlorophyll *a*, chlorophyll *b* and carotenoid contents of *C. sorokiniana* were 0.24 g/L, 2051.9 μg/L, 693.5 μg/L and 537.8 μg/L, respectively. The present study revealed that microalgae can be used as bioremediation agents for aquaculture wastewater with high production of biomass and bioactive substances, such as chlorophyll-a, chlorophyll-b and total carotenoids, which represent important benefits to human and animal health (Stevens et al., 2021). Similar results were reported for the scaled-up production of Spirulina with aquaculture wastewater, in which 2480 μg/L chlorophyll-a, 2460 μg/L chlorophyll-b and 807 μg/L carotenoids were produced (Cardoso et al., 2021b).

The crude lipid and crude protein contents of the dry matter of *C. sorokiniana* were 6.2% and 50.4%, respectively. Microalgae, such as Chlorella and Spirulina, have high nutrient removal efficiency and metabolically produce proteins and lipids (Daneshvar et al., 2018;

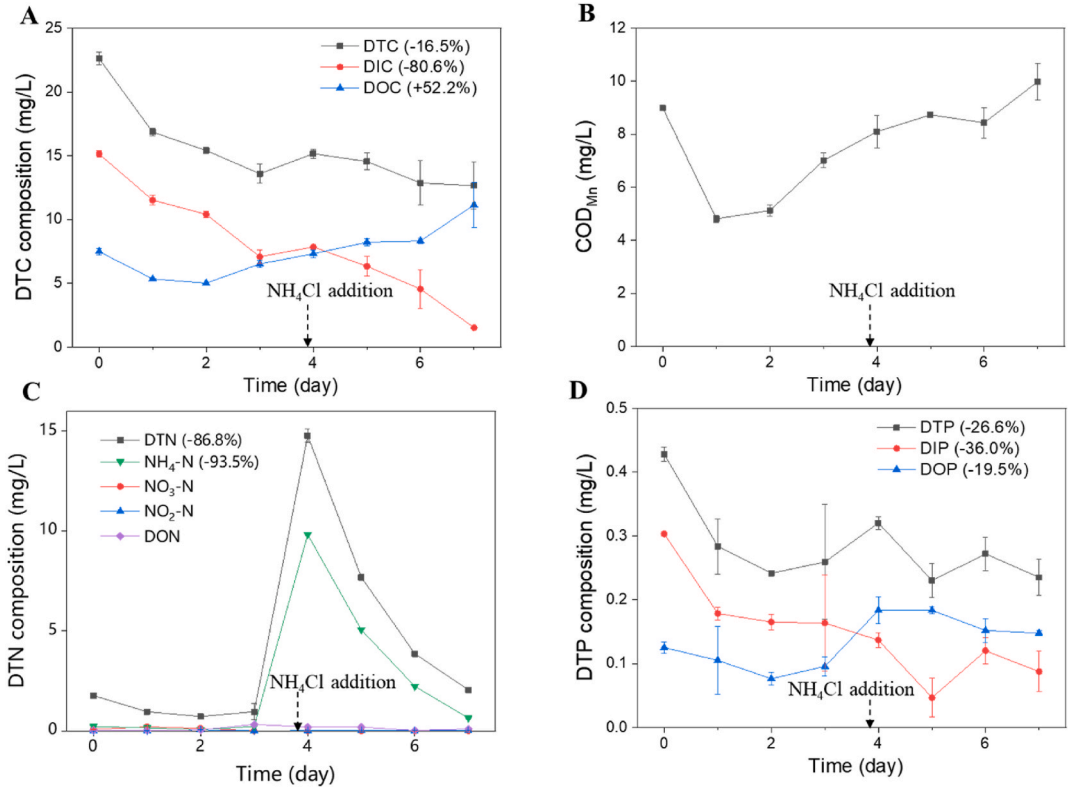

Fig. 1. Changes in DTC composition (A), COD_{Mn} (B), DTN composition and DTP composition in aquaculture wastewater during the cultivation of *Chlorella sorokiniana*. DTC, dissolved total carbon; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon. COD_{Mn}, chemical oxygen demand using potassium permanganate (KMnO4) as oxidant; DTN, total dissolved nitrogen; NH_4-N, ammonia nitrogen; NO_3-N, nitrate nitrogen; NO_2-N, nitrite nitrogen; DON, dissolved organic nitrogen. DTP, Dissolved total phosphorus; DIP, dissolved inorganic phosphorus; DOP, dissolved organophosphorus.

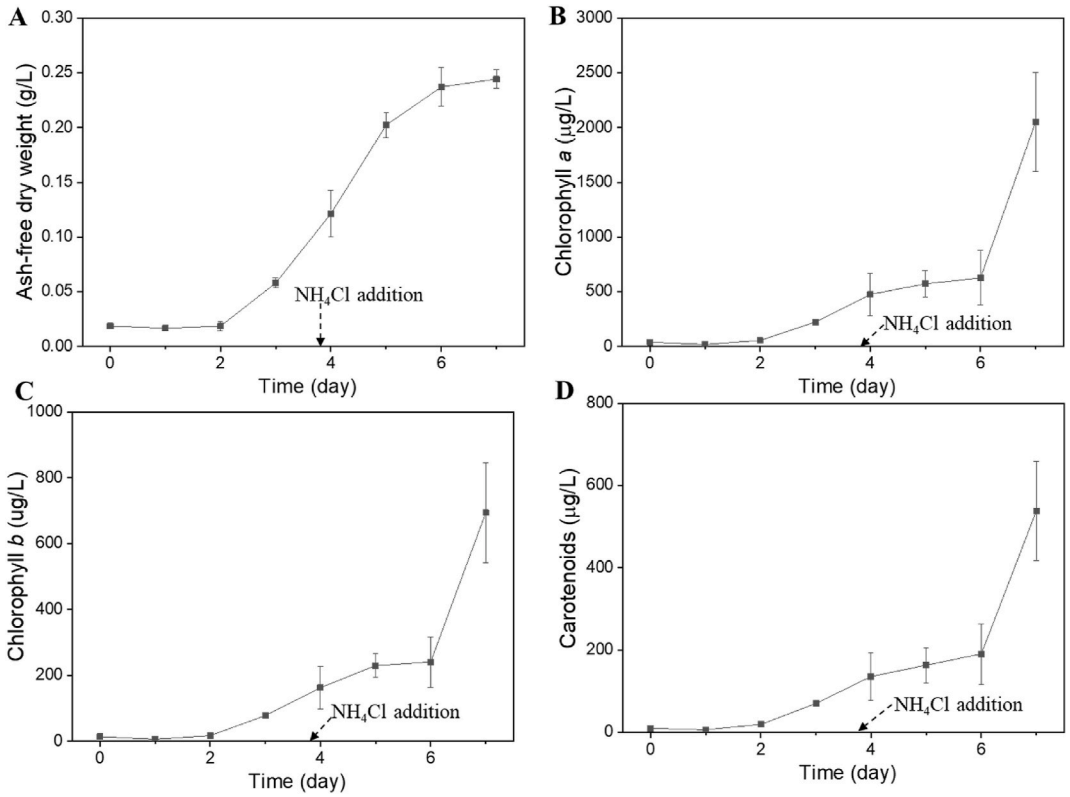

Fig. 2. Ash-free dry weight (A), chlorophyll *a* (B), chlorophyll *b* (C) and carotenoids (D) of *Chlorella sorokiniana* during the treatment of aquaculture wastewater.

Cardoso et al., 2021a). As shown in Fig. 3, *C. sorokiniana* cultured from aquaculture wastewater contains all the essential amino acids and nonessential amino acids. Except for aspartic acid (Asp), the contents of other amino acids in *C. sorokiniana* cultured from aquaculture wastewater were lower than those in Chlorella meal (Demeter Bio-Tech Co., Ltd., Wuhan, China) and fishmeal (seafood white fishmeal imported from the United States), and all the amino acids contents were lower than those in *A. platensis* (Lvfuyuan Company, Ordos, China). However, we found that *C. sorokiniana* cultured from aquaculture wastewater was rich in fatty acids, especially polyunsaturated fatty acids. The contents of arachidonic acid (ARA), eicosapentaenoic acid (EPA), n-3 polyunsaturated fatty acids and total polyunsaturated fatty acids in *C. sorokiniana* cultured from aquaculture wastewater were greater than those in Chlorella meal and fishmeal. Compared with the fatty acid composition (% of total fatty acids) of fish oil reported by Hossain et al. (2021), the relative contents of linoleic acid (LA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), n-3 polyunsaturated fatty acids and polyunsaturated fatty acids in *C. sorokiniana* increased by 3.17, 12.16, 19.11, 13.82 and 47.23, respectively.

As microalgae are known to absorb heavy metals through their cell walls (Aslam et al., 2019), the risk of microalgal contamination with heavy metals was assessed. As shown in Table 4, the copper (Cu), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) contents of *C. sorokiniana* were 2.34, 0.56, 0.77, 0.26, and 0.15 mg/kg, respectively. Compared with those in fishmeal diets, the Cu contents in diets supplemented with *C. sorokiniana* decreased, whereas the As, Cd, Pb and Hg contents increased as the level of *C. sorokiniana* added increased; however, the dietary heavy metals were safe for fish (AQSIQ, 2001; NRC, 2005; EU, 2019). Cardoso et al. (2020) reported that Spirulina harvested from aquaculture could be used in food and feed, since the biomass did not contain heavy metals such as Pb, Cd, nickel (Ni), chrome (Cr), Cu, zinc (Zn), or As.

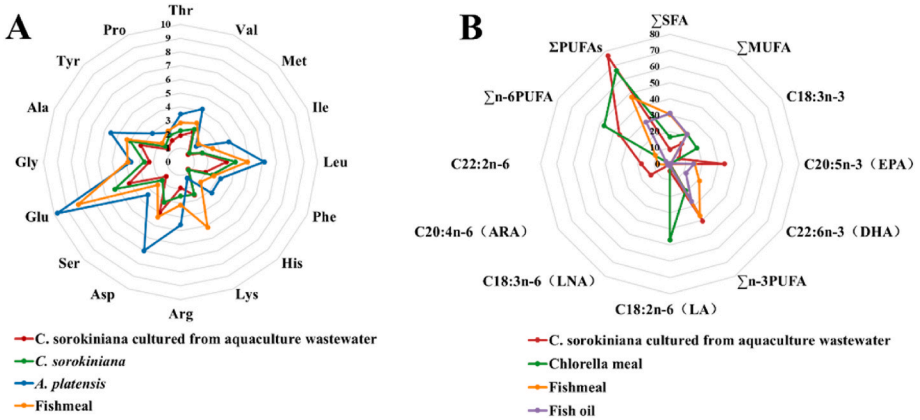

Fig. 3. The radar chart of amino acids (A) and fatty acids (B) of *C. sorokiniana* cultured from aquaculture wastewater, Chlorella meal, *A. platensis* and fishmeal.

Table 4
Heavy metals and antibiotics in *Chlorella sorokiniana* and feed; Statutory maximum levels of AQSIQ and EU as well as maximum tolerable level recommended by the NRC are also shown.

	C. sorokiniana	FM	MM5	MM15	AQSIQ	EU	NRC
Cu (mg/kg)	2.34	4.52	3.79	3.15			
As (mg/kg)	0.56	0.33	0.33	0.36	2	4	5
Cd (mg/kg)	0.77	0.18	0.19	0.21	0.5	0.5	10
Pb (mg/kg)	0.26	0.11	0.54	0.66	5	10	10
Hg (mg/kg)	0.15	0.003	0.004	0.008	0.1		
Florfenicol (μg/kg)	ND	ND	ND	ND			
Sulfonamide(μg/kg)	ND	ND	ND	ND			

The removal of antibiotics by microalgae has been extensively reported (Bai et al., 2022). The residues of antibiotics commonly used in aquaculture were detected in *C. sorokiniana* and all the experimental diets. Our analyses revealed that florfenicol and sulfonamide antibiotics were, however, under the detection limits of the method (0.1 and 20.1 μg/kg, respectively) and were detected in both *C. sorokiniana* and its feed. Antibiotic residues not only negatively affect the metabolism of cultured objects but also increase drug resistance (Bai et al., 2022). The present study revealed a low risk of contamination by toxic agents; therefore, wastewater from fish farms can be a source of nutrients for the culture of *C. sorokiniana*.

3.3. Effects of C. sorokiniana on the growth performance and body colour of fish

The feed rate (FR) was significantly greater in the MM15 group than in the FM group ($P < 0.05$, Fig. 4A). The substitution of fishmeal with *C. sorokiniana* promoted the feed intake of gibel carp, which was consistent with previous studies showing that Chlorella meal can replace between 75% and 100% of fishmeal with an increase in the feed intake of gibel carp (Shi et al., 2017), since Chlorella meal is rich in large amounts of bioactive substances, such as fatty acids, polysaccharides, vitamins and pigments, which can promote fish feeding (Abdel-Tawwab et al., 2022; Chen et al., 2022). However, the specific growth rate (SGR) did not significantly differ among the three groups (Fig. 4B); obviously, the feed efficiency (FE) significantly decreased as the quantity of *C. sorokiniana* added increased (Fig. 4C). The results indicated that *C. sorokiniana* inclusion at 5% and 15% can replace 18% and 54% of the inclusion of the fishmeal in the feed, respectively, without affecting the growth of gibel carp. Similarly, a previous study reported that Chlorella meal can replace 10%–40% of fishmeal in feed, with no effect on the growth of largemouth bass (Xi et al., 2022). However, some studies have shown that Chlorella meal can improve the growth of several carnivorous fish, such as rainbow trout, Korean rockfish (*Sebastes schlegeli*) and olive flounder (*Paralichthys olivaceus*) (Bai et al., 2001; Rahimnejad et al., 2017; Arteaga et al., 2021). The hard cell wall of Chlorella causes a significant decrease in the digestibility of dry matter, crude protein and energy in gibel carp, which results in increased feed intake to meet the growth requirements of the fish (Tibbetts et al., 2017). Chlorella meal can be used as a protein source in aquatic feed instead of fishmeal without affecting the growth of the fish; however, to improve growth and feeding efficiency, technological advancements, such as breaking the cell wall via physical or chemical methods, are needed to improve the utilization of Chlorella in fish feed.

The condition factor (CF) was not affected by the addition of *C. sorokiniana* (Fig. 4D). The hepatosomatic index (HSI) was significantly lower in the MM15 group ($P < 0.05$) than in the FM group (Fig. 4E). The HSI is usually measured to assess the condition of the liver

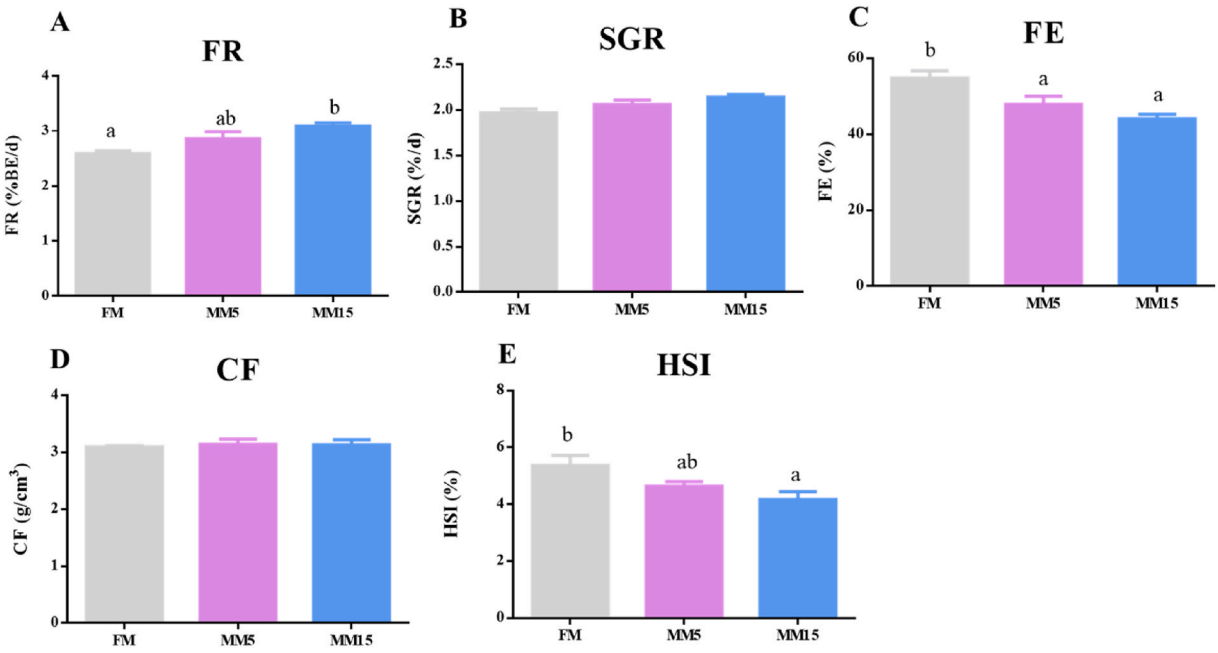

Fig. 4. The growth performance of gibel carp. (A) feeding rate (FR, %/body weight day) = 100 × (feed intake in dry matter)/[days × (initial body weight + final body weight)/2]; (B) specific growth rate (SGR, %/d) = 100 × [ln(final body weight) − ln(initial body weight)]/days; (C) feed efficiency (FE,%) = (final body weight − initial body weight)/feed intake in dry matter; (D) condition factor (CF, g/cm^3) = 100 × (body weight)/(body length)^3; (F) hepatosomatic index (HSI,%) = 100 × (liver weight)/(whole body weight). Data are indicated as mean ± SE (n = 3). Bars with different lower-case letters represent the differences among groups ($P < 0.05$).

and body and the impact of stressors on fish. High levels of *C. sorokiniana* lead to a decrease in the HSI, indicating that fish may not grow well or face stressful environments (Gonino et al., 2019). This hypothesis agreed with the results that fish had no significant growth advantage despite increasing feed intake.

The body colour of the skin of gibel carp is shown in Fig. 5. There was no difference in the lightness (L) or redness (a) of the skin among the three groups, but supplementation with *C. sorokiniana* significantly increased the yellowness (b) of the skin of gibel carp, and yellowness is an important index for analysing colouration in fish (Liu et al., 2019). Microalgae contain natural carotenoid additives, such as in Chlorella, which contains 0.4% (on a dry basis) carotenoids that can effectively improve the colour of fish skin and muscles (Gouveia et al., 2002). In the present study, the carotenoids content of *C. sorokiniana* was 537.8 μg/L, and the addition of *C. sorokiniana* to the diet was expected to increase the skin yellowness of gibel carp. Consistent with the findings of this study, the yellowness of both the skin and the fillets increased in largemouth bass fed Chlorella diets, and the carotenoids contents of the plasma, liver and fillets also increased (Xi et al., 2022).

3.4. Effects of C. sorokiniana on the whole-body composition of fish and amino acids, fatty acids and heavy metals contents of muscle

The moisture, crude protein and crude lipid contents of whole fish did not differ among the three groups, and the amino acids contents in the muscle were not affected by the addition of *C. sorokiniana* (Table 5). The protein and lipid contents depend on the level of amino acids in the feed (Khoklang et al., 2024), whereas the essential amino acids content decreases with supplementation with *C. sorokiniana*, resulting in a trend towards lower crude protein and crude lipid contents in the fish with the addition of *C. sorokiniana*. These results are consistent with studies on rainbow trout (Liu et al., 2022b) and pacific white shrimp (*Litopenaeus vannamei*) (Li et al., 2022a).

Table 5
The chemical composition of whole fish, the protein and amino acid profile in the muscle of gibel carp fed the experimental feeds.

Diets	FM	MM5	MM15
Chemical composition of fish (% fresh fish)			
Moisture	72.32 ± 0.82	73.26 ± 0.27	73.47 ± 0.60
Crude protein	14.77 ± 0.47	13.33 ± 0.26	13.43 ± 0.32
Crude lipid	8.70 ± 0.30	8.73 ± 0.14	7.67 ± 0.43
Amino acid profile in muscle (% dry matter)			
Essential amino acid			
Thr	2.38 ± 0.06	2.48 ± 0.01	2.36 ± 0.13
Val	3.01 ± 0.06	3.18 ± 0.04	3.03 ± 0.20
Met	1.40 ± 0.07	1.44 ± 0.12	1.50 ± 0.04
Ile	2.61 ± 0.04	2.79 ± 0.03	2.67 ± 0.17
Leu	4.51 ± 0.09	4.79 ± 0.06	4.57 ± 0.27
Phe	2.39 ± 0.07	2.57 ± 0.05	2.44 ± 0.14
His	2.44 ± 0.06	2.54 ± 0.05	2.36 ± 0.14
Lys	5.39 ± 0.11	5.62 ± 0.07	5.33 ± 0.31
Arg	3.26 ± 0.04	3.49 ± 0.02	3.28 ± 0.19
EAA	27.40 ± 0.51	28.91 ± 0.33	27.52 ± 1.55
Non-essential amino acid			
Asp	6.51 ± 0.15	6.77 ± 0.09	6.51 ± 0.34
Ser	2.10 ± 0.05	2.20 ± 0.01	2.10 ± 0.13
Glu	7.75 ± 1.67	9.88 ± 0.15	9.50 ± 0.57
Gly	2.45 ± 0.08	2.66 ± 0.11	2.58 ± 0.14
Ala	3.59 ± 0.11	3.82 ± 0.02	3.71 ± 0.22
Tyr	1.73 ± 0.08	1.86 ± 0.03	1.79 ± 0.09
Pro	1.78 ± 0.04	2.03 ± 0.03	1.94 ± 0.14
TAA	53.30 ± 1.81	58.11 ± 0.46	55.66 ± 3.15

Date presented are Means ± SE (n = 3).

The fatty acids profiles (% of total fatty acids) of the muscles of gibel carp are shown in Table 6. The relative contents of saturated fatty acids (SFAs) were lower in the MM5 and MM15 groups ($P < 0.05$); however, arachidonic acid (ARA) and n-3 polyunsaturated fatty acids (PUFAs)

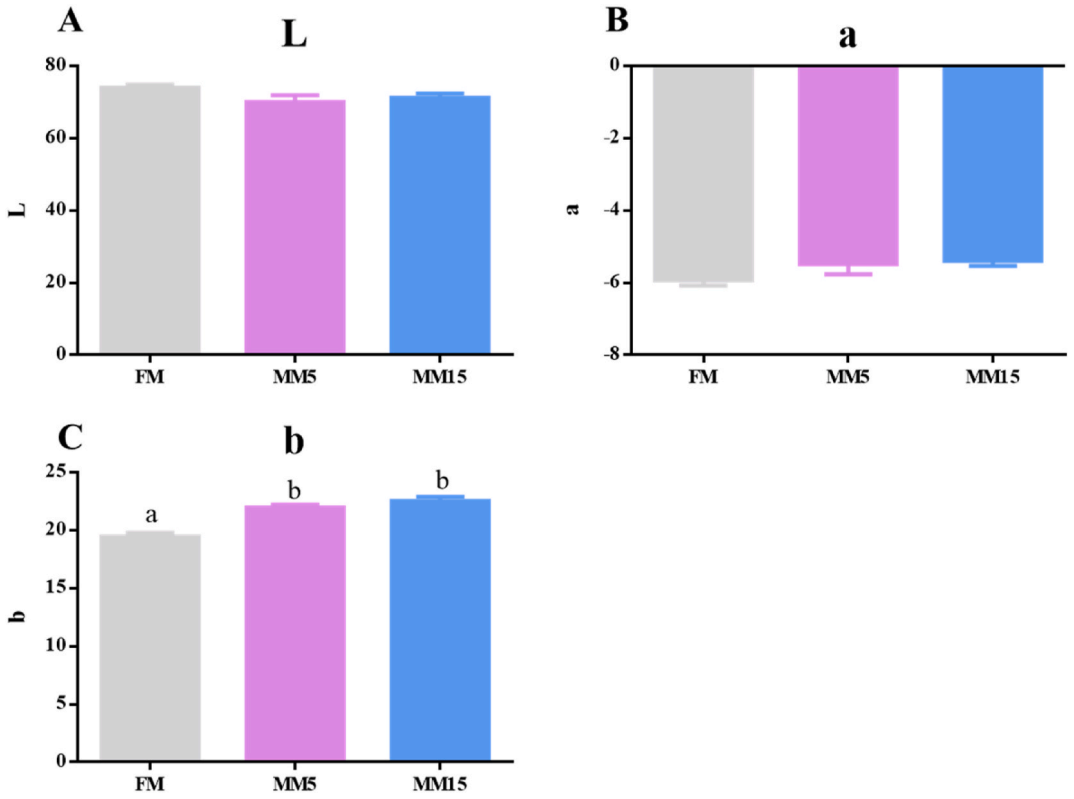

Fig. 5. The effects of *Chlorella sorokiniana* on body color of gibel carp. L, lightness; a, redness; b, yellowness. Data are indicated as mean ± SE (n = 9). Bars with different lower-case letters represent the differences among groups ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Fatty acid profile (% of total fatty acids) in the muscle of gibel carp fed the experimental feeds.

Fatty acids	FM	MM5	MM15
C14:0	1.47 ± 0.02^{b}	1.32 ± 0.04^{a}	1.30 ± 0.04^{a}
C15:0	0.47 ± 0.01^{b}	0.44 ± 0.01^{ab}	0.42 ± 0.01^{a}
C16:0	8.98 ± 0.09^{b}	8.79 ± 0.06 ^{ab}	8.58 ± 0.11^{a}
C17:0	0.42 ± 0.01^{b}	0.37 ± 0.01^{a}	0.37 ± 0.01^{a}
C18:0	3.12 ± 0.09	3.02 ± 0.03	2.92 ± 0.07
∑SFA	14.47 ± 0.19^{b}	13.94 ± 0.11^{a}	13.59 ± 0.20^{a}
C16:1n-7	7.30 ± 0.16^{a}	7.81 ± 0.04^{b}	7.74 ± 0.08^{b}
C18:1n-9	19.95 ± 0.41^{b}	18.97 ± 0.30^{ab}	18.39 ± 0.32^{a}
C20:1n-9	3.12 ± 0.06	2.98 ± 0.06	3.00 ± 0.07
∑MUFA	30.36 ± 0.56	29.76 ± 0.29	29.13 ± 0.42
C18:3n-3	3.17 ± 0.01	3.38 ± 0.16	3.46 ± 0.14
C20:5n-3 (EPA)	3.64 ± 0.03	4.01 ± 0.17	4.02 ± 0.17
C22:6n-3 (DHA)	18.19 ± 0.30	18.75 ± 0.35	18.83 ± 0.19
∑n-3PUFA	24.99 ± 0.30^{a}	26.14 ± 0.14^{b}	26.32 ± 0.26^{b}
C18:2n-6 (LA)	25.7 ± 0.16	25.13 ± 0.17	25.74 ± 0.25
C18:3n-6 (LNA)	0.34 ± 0.01	0.36 ± 0.01	0.34 ± 0.01
C20:2n-6	0.63 ± 0.02	0.57 ± 0.02	0.60 ± 0.03
C20:3n-6	1.28 ± 0.05	1.33 ± 0.02	1.33 ± 0.01
C20:4n-6 (ARA)	2.22 ± 0.15^{a}	2.79 ± 0.15^{b}	2.96 ± 0.21^{b}
∑n-6PUFA	30.18 ± 0.11	30.17 ± 0.28	30.97 ± 0.36
ΣPUFAs	55.17 ± 0.40^{a}	56.30 ± 0.31^{ab}	57.29 ± 0.53^{b}
Σn-3/Σn-6	0.83 ± 0.01^{a}	0.87 ± 0.01^{b}	0.85 ± 0.01^{ab}

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LNA: linolenic acid; ARA: arachidonic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ∑n-3 PUFA: n-3 polyunsaturated fatty acids; ∑n-6 PUFA: n-6 polyunsaturated fatty acids.
Date presented are Means ± SE (n = 3), Values in the same row with different superscript letters are significantly different ($P < 0.05$).

were higher in the groups fed *C. sorokiniana* feed ($P < 0.05$). The total PUFA content was significantly greater in the MM15 group than in the FM group ($P < 0.05$), and the Σn-3/Σn-6 ratio was significantly greater in the MM5 group ($P < 0.05$). The present study revealed that the relative content of eicosapentaenoic acid (EPA) in *C. sorokiniana* cultured from aquaculture wastewater reached 33.82%, and the relative contents of n-3 and n-6 polyunsaturated fatty acids were as high as 40.67% and 36.14%, respectively. The dietary PUFA content increased in proportion to the quantity of *C. sorokiniana* in the feed, resulting in higher contents of ARA, n-3 PUFAs and total PUFAs in the muscle. These results demonstrated that using *C. sorokiniana* cultured from aquaculture wastewater in fish feed can improve the nutritional value of the muscle of fish. Dietary Chlorella significantly improved the fatty acid composition of gilthead seabream (*Sparus aurata*) (Karapanagiotidis et al., 2022).

The Pb and Cd contents in the fish muscle were below the detection limit of our methods in all three groups, and the As, Cu and Hg contents in the muscle did not differ among the three groups (Table 7). The metal levels in the fish muscles were all below the maximum permissible levels of the local standard (maximum permitted concentrations: As = 6 mg/kg; Cd = 2 mg/kg; Pb = 6 mg/kg; and Hg = 0.5 mg/kg) (CFS Centre for Food Safety, 2008), and the muscles were safe as human food. These results are consistent with the low risk associated with the use of *C. sorokiniana* as a feed ingredient because it contains few toxic substances.

Table 7
Heavy metals in muscles of gibel carp (mg/kg).

	FM	MM5	MM15	Limits in aquatic products
As	0.21 ± 0.03	0.18 ± 0.02	0.17 ± 0.01	≤6
Cu	1.95 ± 0.08	1.60 ± 0.24	1.75 ± 0.24	NA
Hg	0.13 ± 0.01	0.12 ± 0.01	0.10 ± 0	≤0.5
Pb	ND	ND	ND	≤6
Cd	ND	ND	ND	≤2

3.5. Effects of *C. sorokiniana* on the antioxidant and inflammatory signalling of fish

The activity of CAT in the plasma was significantly greater in the MM5 and MM15 groups than in the FM group ($P < 0.05$, Fig. 6A), and the activity of GPX in the plasma was also greater in the MM15 group ($P < 0.05$, Fig. 6C). The replacement of fishmeal with *C. sorokiniana* had no effect on SOD activity or the contents of GSH and MDA in the plasma (Fig. 6B–D, Fig. 6E). Moreover, compared with that in the FM group, the relative expression of *cat* in the liver was significantly upregulated in the MM5 group ($P < 0.05$, Fig. 7A), and the relative expression of *sod*, *gpx*, *keap1* and *bach1* in the liver was not affected by the different treatments (Fig. 7A). The relative expression of *hsp70* significantly increased in the MM15 group ($P < 0.05$, Fig. 7A).

The innate immune system, which includes a variety of antioxidant enzymes, is an important barrier for maintaining immune homeostasis (Zhang et al., 2023). SOD can scavenge superoxide anions, GPX can reduce the content of hydrogen peroxide and organic peroxide, and CAT mainly promotes the decomposition of hydrogen peroxide and prevents peroxide damage. These are the three important antioxidant enzymes that constitute the first line of defence in protecting organisms (Muñoz et al., 2000; Wang et al., 2022). The inclusion of 5% and 15% *C. sorokiniana* in the feed improved the plasma CAT activity of gibel carp, and the relative expression of the *cat* gene in the liver also significantly increased. GPX activity in the plasma of gibel carp also increased when they were fed a 15% *C. sorokiniana* diet. These results indicate that *C. sorokiniana* can significantly increase the antioxidant capacity of fish. GSH is considered to balance redox reactions by eliminating excess reactive oxygen species (ROS), which can protect cells from oxidative stress (Yu et al., 2023). MDA is an important indicator of lipid peroxidation, and the accumulation of MDA causes cell damage and biotoxicity (Parvez and Raisuddin, 2005). The *C. sorokiniana* diets had no effects on GSH or MDA, indicating that the replacement of fishmeal with *C. sorokiniana* did not cause oxidative stress or the accumulation of lipid peroxides in the fish. Chlorella is considered an antioxidant because it contains bioactive substances such as carotenoids, flavonoids and polyphenols, which scavenge free radicals (Reis et al., 2022). HSP70 is an indicator of stress and health status in fish, and the relative expression of the hsp70 gene in the liver was significantly upregulated in gibel carp fed 15% *C. sorokiniana* diets, indicating that 15% *C. sorokiniana* may cause heat shock reactions in the liver.

The relative expression of inflammatory genes in the liver is shown in Fig. 4B. Compared with that in the FM group, the relative expression of *il-4* and *tgf-β* was significantly upregulated in the MM5 group ($P < 0.05$). The relative expression of *ifn-γ* significantly increased with increasing dietary *C. sorokiniana* ($P < 0.05$). There were no differences in the relative expression of *il-6*, *il-10* or *tnf-α* among the three groups. The immunity of fish is closely related to inflammation induced and regulated by proinflammatory and anti-inflammatory cytokines (Sun et al., 2018; Amoah et al., 2022). In the present study, the relative gene expression of the proinflammatory factor inf-γ and the genes encoding the anti-inflammatory factors *il-4* and *tgf-β* were significantly upregulated in the livers of the fish fed the 5% *C. sorokiniana* diet. However, only the relative expression of the proinflammatory factor inf-γ gene was upregulated in the livers of fish fed 15% *C. sorokiniana* diets, which is consistent with the finding that the relative expression of the *hsp70* gene was upregulated in the livers of fish fed the same diet. These results indicate that high levels of dietary *C. sorokiniana* may lead to stress responses and inflammation in fish.

The relative expression of *C3*, *IgM* and *lyz* was not affected by the addition of *C. sorokiniana* (Fig. 7C). IgM is the main immunoglobulin in fish and plays an important role in specific immunity, easily destroying invading bacteria and viruses via phagocytes and activating the complement system (Wang et al., 2019). C3 is produced mainly by the liver to activate the immune response (Amoah et al., 2022). Lyz is an important defence molecule in the innate immune system of fish and

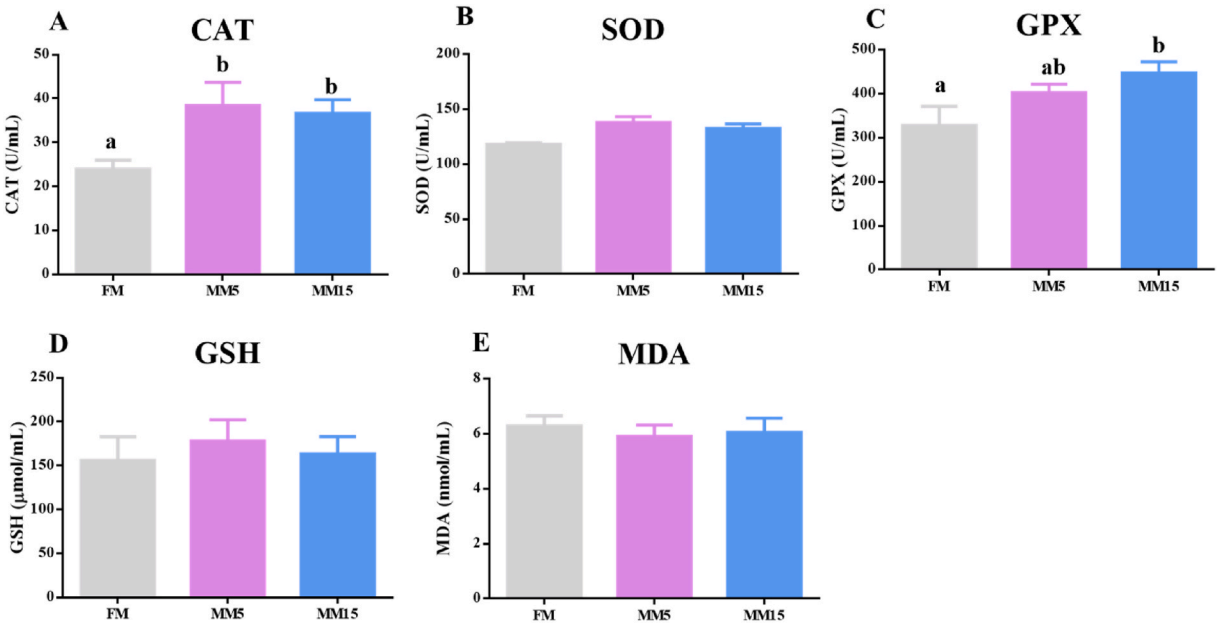

Fig. 6. The effects of *Chlorella sorokiniana* on plasma antioxidant indices of gibel carp. (A) catalase; (B) superoxide dismutase; (C) glutathione peroxidase; (D) reduced glutathione; (E) malonaldehyde; Data are indicated as mean ± SE (n = 6). Bars with different lower-case letters represent the differences among groups ($P < 0.05$).

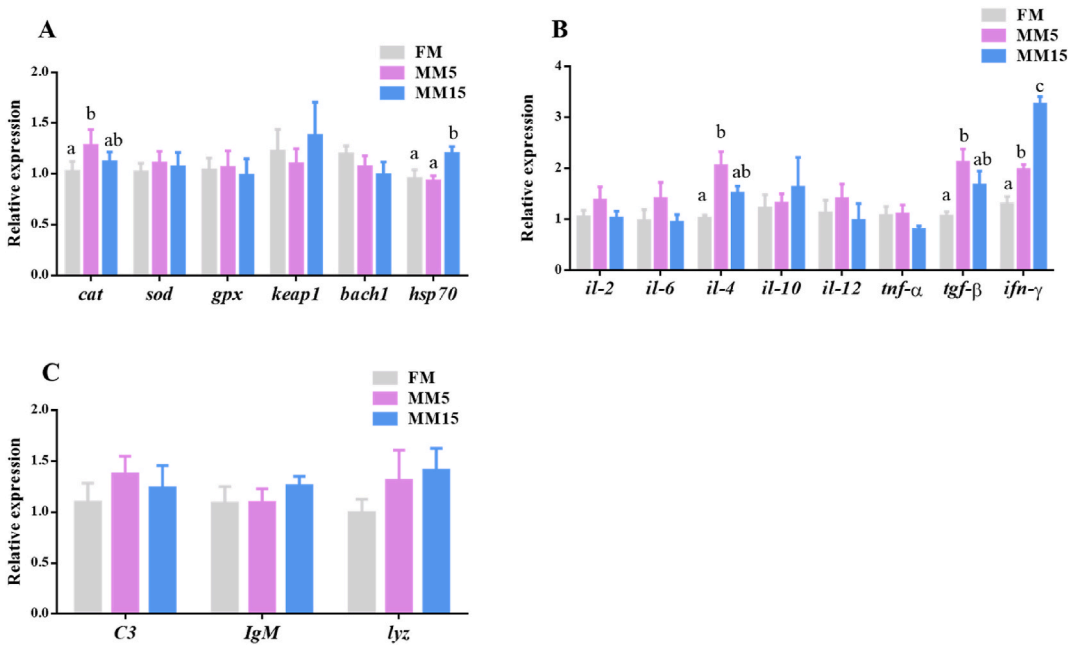

Fig. 7. The relative expression of antioxidant (A), inflammation (B) and immunity-related (C) genes in liver of gibel carp. Data are indicated as mean ± SE (n = 6). Bars with different lower-case letters represent the differences among groups ($P < 0.05$).

plays a major role in the hydrolysis and destruction of peptidoglycan glycosidic bonds (Magnadóttir, 2006). Chen et al. (2022) reported that Chlorella can increase the activity of lysozyme and C3 and IgM contents in the plasma of rainbow trout after infection with lipopolysaccharide (LPS); moreover, the survival rate of fish infected with *Aeromonas salmonicida* increased.

3.6. Effects of C. sorokiniana on the intestinal microbiotic composition of fish

The results of the microbiotic composition in the intestine are shown in Fig. S2. The microbial diversity of the fish fed the *C. sorokiniana* diets was not significantly different from that of the fish fed the FM diet on the basis of the Sobs and Shannon indices. The dominant phyla were Cyanobacteria, Actinobacteria, Fusobacteriota and Proteobacteria in the MM5 and MM15 groups, and the relative abundance of Cyanobacteria increased with increasing dietary *C. sorokiniana*; in contrast, the relative abundances of Actinobacteria, Fusobacteriota and Proteobacteria decreased (Fig. S2C). The dominant phyla in the FM group were Actinobacteria, Fusobacteriota, Proteobacteria and Bacteroidota (Fig. S2C). The dominant genera were *Cetobacterium*, *Mycobacterium* and *Bacteroides*, and the relative abundances at the genus level were 25.39%,

11.92% and 12.93%, respectively (Fig. S2D). *Norank_f_norank_o_-Chloroplast* was dominant at the genus level, followed by *Cetobacterium* and *Mycobacterium* in the MM5 and MM15 groups (Fig. S2D). The relative abundances of *Cetobacterium*, *Mycobacterium* and *Bacteroides* in the MM5 and MM15 groups were lower than those in the FM group.

The analysis of the microbial differences is shown in Fig. 8. At the phylum level, the relative abundance of Cyanobacteria in the MM5 and MM15 groups was significantly greater than that in the FM group (Fig. 8A, $P < 0.01$). The relative abundances of Proteobacteria and Firmicutes were obviously lower in the MM5 and MM15 groups than in the FM group (Fig. 8A, $P < 0.05$). The intestinal flora has important effects on health and the ability of fish to digest and absorb nutrients (Fan et al., 2023). The addition of *C. sorokiniana* significantly decreased the relative abundances of Proteobacteria and Firmicutes in the intestine.

The relative abundance of pathogenic bacteria at the genus level was analysed, and the results indicated that the relative abundance of *Citrobacter* was lower in the fish fed the *C. sorokiniana* diet than in the fish fed the FM diet (Fig. 8B, $P < 0.05$), the relative abundance of *Staphylococcus* was significantly lower in the MM15 group than in the FM group (Fig. 8C, $P < 0.05$), and the relative abundance of *Pseudomonas* was significantly lower in the MM5 group than in the FM group (Fig. 8D, $P < 0.05$). *Citrobacter* is a pathogen of fish that affects the physiological state of the blood and is characterized by typical haemorrhagic septicemia of the skin and viscera, which causes high mortality in fish (Xiong et al., 2020). *Staphylococcus* is a potential pathogen of fish that can also cause sepsis in fish, and biofilm formation, adhesion protein, and *Staphylococcus* enterotoxin are virulence-related factors affecting the pathogenicity of *Staphylococcus* (Pinheiro et al., 2015). White seabream (*Diplodus sargus*) has high mortality after infection with *Staphylococcus* (Saleh et al., 2021). *Pseudomonas*, which is also a harmful bacterium in the intestine of fish, plays a major role in outbreaks of fish diseases, leading to the occurrence of visceral white spot disease (Yan et al., 2022). A previous study revealed that *Pseudomonas* was significantly positively correlated with the contents of IL-8, tnf-α, IL-10 and MDA (Li et al., 2022). In the present study, the addition of *C. sorokiniana* reduced the relative abundances of *Citrobacter*, *Staphylococcus* and *Pseudomonas* in the intestine of gibel carp, thus reducing the risk of disease.

To meet the consumption demands of humans, the aquaculture industry has developed rapidly. China is the major producer of freshwater aquaculture production, and sufficient attention should be given to the ecological balance of aquaculture water and the protection of aquatic environments (Zhou et al., 2023). In recent years, microalgal cultivation has been found to be an efficient way to purify aquaculture wastewater and maintain the balance of aquatic environments, which can also successfully achieve a circular economy. In the present study, *C. sorokiniana* removed 86.8% of the nitrogen and 26.6% of the phosphorus in the aquaculture wastewater after 7 days of cultivation, and the biomass of *C. sorokiniana* increased from 0.02 g/L to 0.24 g/L. The conversion amounts of nitrogen and phosphorus in wastewater by Chlorella per unit were 61.50 mg/g and 0.86 mg/g, respectively. Chlorella can decrease nitrogen and phosphorus concentrations up to 97.3% and 53.8%, respectively, after 12 days of cultivation in an algal biofilm membrane photobioreactor, and the algal biomass concentration reached a maximum value of 92.32 mg/L (Peng et al., 2020). New culture models are needed to improve the purification efficiency of wastewater and increase the amount of algal biomass. Microalgae can convert nutrients and other substances into proteins, carbohydrates, lipids and other organic components, which can be used in feed ingredients, health products and pharmaceutical production(Ruiz et al., 2016). In this study, *C. sorokiniana* converted nutrients and produced 50.4% crude protein and 6.2% crude lipids, confirming the low risk associated with heavy metals and antibiotics, suggesting that *C. sorokiniana* is an alternative protein source to replace fishmeal. It can also increase the fatty acid content of muscle and the antioxidant ability of fish. Moreover, because the harvested *C. sorokiniana* biomass contains 537.8 μg/L carotenoids, *C. sorokiniana* can be used as an additive to colour fish and improve their economic value. Yu et al. (2022) reported similar results in largemouth bass when Chlorella was substituted for fishmeal. Microalgae can positively impact gut health by potentially

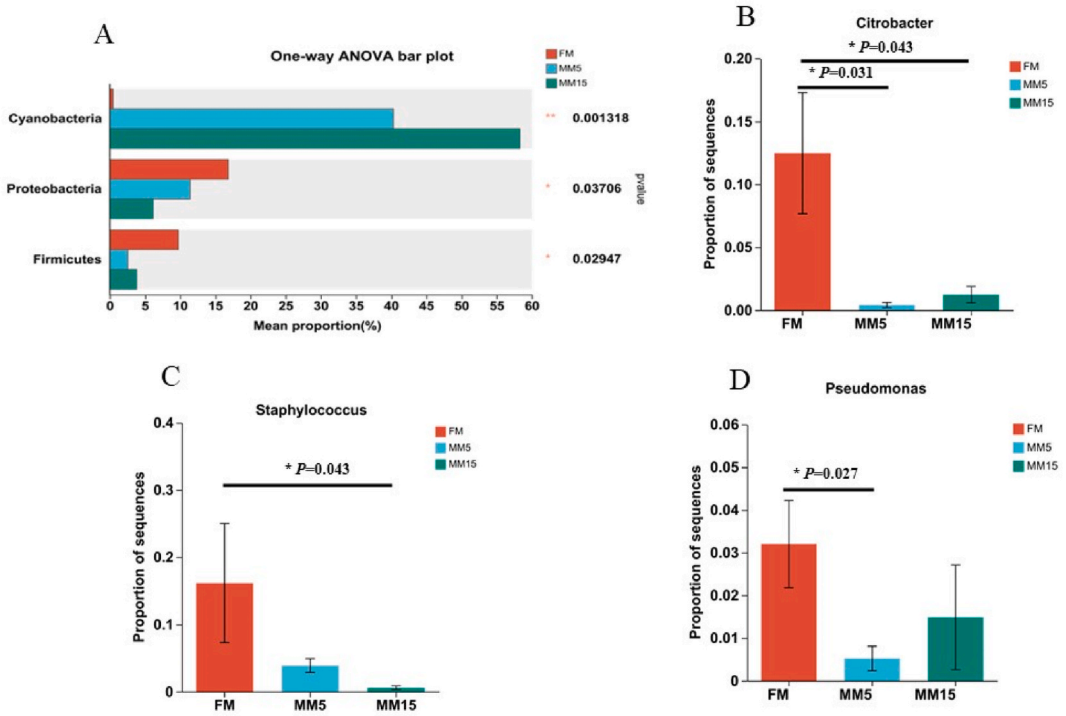

Fig. 8. Bacteria with significant differences in relative abundance at Phylum and Genus levels. (A) Bacteria with significant differences in relative abundance at Phylum level; (B–D) Pathogenic bacteria with significant differences in relative abundance at Genus level. Asterisk means there are significant difference among groups $^{*}P < 0.05$, $^{**}P < 0.01$.

modulating fish immune responses in intestinal tissues and improving resistance against infection (Albaqami, 2025). The present results showed that *C. sorokiniana* had positive influences on the gut microbiome in fish through reducing the relative abundance of pathogenic bacteria.

4. Conclusions

C. sorokiniana has excellent advantages in removing inorganic nutrients from aquaculture wastewater. Moreover, *C. sorokiniana* biomass were harvested, dried, and used as gibel carp feed ingredients to replace fishmeal. The nutritional value and safety risk of *C. sorokiniana* were subsequently evaluated. The present results indicated that *C. sorokiniana* cultured from aquaculture wastewater was safe as an aquatic feed ingredient. The replacement of fishmeal with *C. sorokiniana* did not affect the growth of gibel carp or the amino acids contents of their muscle. The use of microalgae in the fish diet increased the body colour of the fish and the relative content of polyunsaturated fatty acids in muscle. In addition, the addition of 5% *C. sorokiniana* to the diet can increase the antioxidant capacity, upregulate the expression of pro- and anti-inflammatory genes, and reduce the relative abundance of pathogenic bacteria in the gut of gibel carp. *C. sorokiniana* cultured with aquaculture wastewater can be used as a protein source to replace fishmeal, and the appropriate addition level was 5%, which can reduce the level of fishmeal by 3.6%.

CRediT authorship contribution statement

Cui Liu: Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Data curation. **Luohai Hua:** Formal analysis, Data curation. **Haokun Liu:** Writing – review & editing, Supervision, Conceptualization. **Lan Wang:** Investigation. **Xiaoming Zhu:** Supervision, Funding acquisition. **Céline Rebours:** Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Kevin Graham Harding:** Investigation. **Lu Tan:** Investigation. **Qiang Hu:** Supervision, Investigation. **Shouqi Xie:** Supervision, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.122510.

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