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Nutritional enrichment of Moina mongolica using different dietary strategies: Effects on growth, biochemical composition and functional nutrients

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Abstract

Moina mongolica Daday is an important live feed organism for marine aquaculture due to its high nutritional value, particularly its enrichment with essential fatty acids such as eicosapentaenoic acid (EPA). However, the nutritional quality of *M. mongolica* is highly dependent on dietary composition. This study evaluated the effects of different diets on the biochemical composition, fatty acid profile, amino acid composition, and carotenoid accumulation of *M. mongolica*. Four dietary treatments were designed: *Nannochloropsis oculata* (T1), *Saccharomyces cerevisiae* (T2), *N. oculata* + *S. cerevisiae* (T3), and *N. oculata* + *S. cerevisiae* + *Rhodopseudomonas palustris* (T4).

The results showed that dietary composition significantly affected the nutritional characteristics of *M. mongolica*. The T4 group exhibited the highest crude protein content (64.58%), whereas the T1 group showed the highest crude lipid content (28.56%). Fatty acid analysis revealed that the T1 group had the highest levels of polyunsaturated fatty acids (PUFA), especially ω3-PUFA, ω6-PUFA, EPA (C20:5n-3), linoleic acid (LA, C18:2n-6), and arachidonic acid (ARA), while the T2 group showed the lowest PUFA nutritional quality. The combined dietary treatments (T3 and T4) resulted in intermediate fatty acid profiles, with T4 showing improved PUFA composition compared with T3. In addition, dietary treatments significantly influenced total amino acid contents, with the highest amino acid level observed in the T4 group; however, the proportions of essential amino acids and umami amino acids remained relatively stable among treatments. Carotenoid analysis demonstrated that *M. mongolica* accumulated both astaxanthin and canthaxanthin, and their contents were closely associated with dietary pigment sources. The T4 group exhibited the highest canthaxanthin content, while astaxanthin accumulation was comparable to that in the T1 group.

Overall, dietary manipulation significantly regulates the nutritional quality of *M. mongolica*, particularly its protein content, lipid composition, PUFA profile, and carotenoid accumulation. The combined supplementation of *N. oculata*, *S. cerevisiae*, and *R. palustris* represents a promising dietary strategy for producing nutritionally enhanced *M. mongolica* as a high-quality live feed for marine aquaculture.

1. Introduction

With the rapid expansion of marine aquaculture, the development of high-quality live feeds has become increasingly important for the successful seedling production of economically valuable marine species. The nutritional quality of feeds organisms directly influences the growth, survival, and physiological performance of cultured larvae and juveniles (Radhakrishnan et al. 2020; Samat et al. 2020). However, traditional live feeds, particularly *Artemia* resources derived from salt ponds, have become increasingly limited due to over exploitation and environmental changes, highlighting the urgent need to develop alternative live feed resources with high nutritional value and stable production capacity (Jin et al. 2023).

Since *Moina mongolica* Daday was first reported in China in 1988, extensive studies have been conducted on its biological characteristics and cultivation techniques. Due to its appropriate body size,

rapid reproduction, strong salinity tolerance, and high nutritional value, *M. mongolica* has gradually become an important alternative live feed organism for the artificial propagation of marine and brackish-water fish, shrimp, and crab species (He et al. 2001; Suhaimi et al. 2022). Compared with conventional live feeds, dietary manipulation can further improve the nutritional quality of cladocerans and enhance their suitability for larval rearing (Hena et al. 2025; Ashaari et al. 2024). Recent studies have also demonstrated that enrichment of live feeds can modify their fatty acid composition and subsequently influence the performance of cultured fish larvae (Choi et al. 2021).

The nutritional value of live feed organisms is largely determined by their biochemical composition, particularly their fatty acid profiles. Polyunsaturated fatty acids (PUFAs), especially highly unsaturated fatty acids (HUFAs) such as eicosapentaenoic acid (EPA, C20:5n-3) and arachidonic acid (ARA, C20:4n-6), are important nutrients for marine fish and crustacean larvae (Tocher 2010), because they play crucial roles in growth, development, immunity, and physiological regulation (He et al. 2001; Glencross 2009; Ramos-Llorens et al. 2023). Previous studies have demonstrated that the biochemical composition of cladocerans is strongly influenced by dietary sources and enrichment strategies (Turcihan et al. 2022; Li and Liu 2021; Lu et al. 2026). Therefore, nutritional manipulation of live-feed organisms through dietary enrichment is an effective strategy for improving their feeding value in aquaculture (Abdel-Tawwab et al. 2021).

In previous studies on the mass cultivation of *M. mongolica*, microalgae and yeast have been commonly used as primary dietary sources. However, the specific nutritional effects of different microalgal species and microbial combinations on the biochemical composition of *M. mongolica* remain insufficiently understood. *Nannochloropsis oculata*, a marine microalga widely applied in aquaculture hatcheries, has attracted considerable attention due to its rapid growth, high lipid productivity, and abundant long-chain polyunsaturated fatty acids, particularly EPA (C20:5n-3), which can effectively enhance the nutritional quality of live feeds (Glencross 2009; Matsui et al. 2022). Photosynthetic bacteria (PSB), especially *Rhodopseudomonas palustris*, have also been widely investigated as functional microbial resources in aquaculture. Due to their high protein content, vitamin content, carotenoid accumulation, and bioactive compounds, PSB can serve as nutritional supplements or live feed enhancers, contributing to improved nutritional composition and physiological quality of aquatic organisms (Kuo et al. 2023; Li et al. 2023). However, information regarding the application of *R. palustris* as a dietary component for enriching *M. mongolica* remains limited.

Therefore, this study investigated the effects of different dietary combinations on the nutritional quality of *M. mongolica*. Four dietary treatments, including *N. oculata*, yeast (*Saccharomyces cerevisiae*), *N. oculata* combined with yeast, and *N. oculata* combined with yeast and *R. palustris*, were applied to cultivate *M. mongolica*. The proximate composition, amino acid profiles, fatty acid composition, and carotenoid contents (astaxanthin and canthaxanthin) of *M. mongolica* were analyzed and compared. The objective of this study was to evaluate the effects of dietary manipulation on the nutritional value of *M. mongolica* and provide theoretical support for improving the quality of live feeds used in marine aquaculture seedling production.

2. Materials and Methods

2.1. Experiment Material

The *Moina mongolica* used in this study was maintained at the East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (Shanghai, China). The stock population was maintained through continuous parthenogenetic reproduction in seawater with a salinity of 15.5–16.5‰.

Seawater was prepared by mixing bittern with tap water and filtered through degreasing cotton before use. *Nannochloropsis oculata* produced by heterotrophic fermentation was obtained from Guangdong Yingyu Biotechnology Co., Ltd. (Guangdong, China), with a density of (80–100) × 10^8 cells·mL^{-1}. Commercial high-activity dry yeast (*Saccharomyces cerevisiae*) was purchased from Angel Yeast Co., Ltd. (Hubei, China). The photosynthetic bacterium *Rhodopseudomonas palustris* was obtained from Yantai Muhairen Biotechnology Co., Ltd. (Shandong, China), with a cell density of (100–120) × 10^8 cells·mL^{-1}.

2.2. Culture conditions of Moina mongolica

The *M. mongolica* population was expanded in 150-L white plastic tanks at 27 ± 0.5°C, with temperature maintained using thermostatic heaters. The culture water was maintained at a salinity of 15.5–16.5‰ under continuous aeration and natural photoperiod conditions.

During the culture period, *M. mongolica* was fed daily with *Nannochloropsis oculata* (300 × 10^4 cells mL^{-1}). The feeding amount was adjusted according to the population density and feeding activity of *M. mongolica* to maintain satiation. The feeding level was considered appropriate when the culture water became clear approximately 3 h after feeding. One-third of the culture water was renewed daily.

When sufficient biomass was obtained, *M. mongolica* was collected a 150 um plankton net and rinsed with clean seawater to remove impurities before the experiment.

2.3.Experimental design and dietary treatments

The experiment consisted of four dietary treatments with three replicates per treatment, using a total of 12 experimental tanks. After cleaning, *M. mongolica* individuals were randomly distributed into 30-L seawater tanks at an initial density of 5–6 ind. mL^{-1}. The experimental conditions were maintained at 27 ± 0.5°C, salinity of 15.5–16.5‰, a light intensity of 2000–2500 lx, and a photoperiod of 12 h light:12 h dark. During the experiment, 10 L of fresh seawater was added to each tank daily, and continuous aeration was provided to maintain dissolved oxygen above 5 mg L^{-1}.

Based on preliminary feeding trials, four dietary treatments were established as follows: T1, *Nannochloropsis oculata* at 300 × 10^4 cells mL^{-1}; T2, yeast at 30 mg L^{-1}; T3, *N. oculata* at 150 × 10^4 cells mL^{-1} combined with yeast at 30 mg L^{-1}; and T4, *N. oculata* at 150 × 10^4 cells mL^{-1} combined with yeast at 30 mg L^{-1} and *Rhodopseudomonas palustris* at 150 × 10^4 cells mL^{-1}.

The diets were supplied four times daily at 06:00, 12:00, 18:00, and 24:00 h in equal proportions. The feeding trial lasted for 7 days. At the end of the experiment, *M. mongolica* was collected using a 150 um plankton net, rinsed with distilled water, gently dried with absorbent paper, and stored at − 20°C for subsequent analyses of proximate composition, amino acid profiles, fatty acid composition, astaxanthin, and canthaxanthin contents.

2.4 Proximate composition, fatty acid and amino acid analyses.

The proximate composition and biochemical profiles were analyzed following previously described methods (AOAC International 2005; Liu et al., 2021). Moisture content was measured by oven drying at 105°C to constant weight, ash content was determined by combustion at 550°C, crude protein content was analyzed using the Kjeldahl method, and crude lipid content was determined using Soxhlet extraction.

Determination of fatty acid content: according to GB/T 5009.168–2016 method (Determination of fatty acids in food) using gas chromatography to determine the relative content of each fatty acid calculated by normalising the peak area of the chromatographic peak (Christie, 1982; General Administration of Quality Supervision 2016).

Amino acid determination: 16 kinds of amino acids except tryptophan and cystine were determined according to the method of GB/T 5009.124–2016 (Determination of amino acids in food) using Biochrom 20 amino acid auto analyser (AOAC, 2005).

2.5 Astaxanthin content

Astaxanthin and canthaxanthin contents in freeze-dried *M. mongolica* biomass were determined using high-performance liquid chromatography (HPLC) (Shanghai Zhuangrun International Trade Co., Ltd., Shanghai, China), following methods described by Li et al. (2021).

2.6 Statistical analysis

The data obtained were expressed as mean ± standard error (Mean ± SE) and analyzed using SPSS 18.0 statistical software (SPSS, Chicago, IL, USA). All data were checked for normality and homogeneity using the Kolmogorov-Smirnov test and Levene's test, respectively. Means were compared by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. $P < 0.05$ was considered to be statistically significant, while $P > 0.05$ was considered not significant difference.

3. Results

3.1. Effects of different diets on proximate composition of Moina mongolica

The proximate composition of *M. mongolica* fed with experimental diets is presented in Table 1. The results indicated that *M. mongolica* contained relatively high levels of crude protein and crude lipid, suggesting its potential nutritional value as a live feed organism. Dietary composition significantly affected the crude protein and crude lipid contents of *M. mongolica*, whereas no significant differences were observed in moisture and ash contents among dietary treatments ($P > 0.05$).

The crude protein content of *M. mongolica* varied significantly among treatments ($P < 0.05$). The highest crude protein content was observed in the T4 diets, followed by the T2 and T3 diets, while the T1 diets exhibited the lowest value. The crude protein content in the T4 diets was significantly higher than that in the T1 diets ($P < 0.05$), whereas no significant differences were detected between the T4 diets and the T2 or T3 diets ($P > 0.05$). Dietary treatments also significantly influenced crude lipid content ($P < 0.05$). The highest crude lipid content was detected in the T1 diets, which was significantly higher than those in the T2 and T3 diets ($P < 0.05$), but showed no significant difference compared with the T4 diets ($P > 0.05$). The T2 diets exhibited the lowest crude lipid content among all treatments ($P < 0.05$), while the T3 diets showed an intermediate value.

Table 1
Effect of different diets on the comparison of basic nutritionin of *Moina mongolica* Daday. (n = 3).

Groups	Moisture /%	Crude protein/%	Crude lipid/(%·)	ash content/%
T1	91.08 ± 0.12	61.25 ± 0.56^{a}	28.56 ± 0.85^{c}	7.84 ± 0.62
T2	91.07 ± 0.20	64.35 ± 1.02^{b}	17.39 ± 0.39^{a}	7.99 ± 1.00
T3	91.05 ± 0.11	62.91 ± 0.49^{ab}	27.04 ± 0.62^{b}	7.86 ± 1.07
T4	91.04 ± 0.19	64.58 ± 1.30^{b}	27.64 ± 0.93^{c}	7.93 ± 0.56

Note: Values presented as means (n = 3) and pooled SEM. Different superscript lowercase letters within each row represent significant differences (Duncan's test;$P < 0.05$)

3.2. Effects of different diets on the fatty acid composition of Moina mongolica

A total of 23 fatty acids were detected in *M. mongolica*, including 9 saturated fatty acids (SFAs), 6 monounsaturated fatty acids (MUFAs), and 8 polyunsaturated fatty acids (PUFAs) (Table 2). DHA (C22:6n-3) was not detected in any treatment. Dietary treatments markedly altered the fatty acid profiles of *M. mongolica*. Notably, C20:0 and C20:2 were detected at trace levels in the T1, T3 and T4 diets but were absent in T2.

The proportions of total SFAs (ΣSFA) and total MUFAs (ΣMUFA) differed significantly among treatments ($P < 0.05$). ΣSFA was highest in T1 (52.02%) and lowest in T2 (33.90%), whereas ΣMUFA showed the opposite pattern, increasing from 34.16% in T1 to 60.32% in T2. These variations were mainly associated with changes in the predominant fatty acids C16:0, C14:0, and C16:1.

The dietary treatments also significantly affected the PUFA profiles ($P < 0.05$). The contents of ARA, EPA, ΣPUFA, and Σn-3 PUFA followed the same order: T1 > T4 > T3 > T2. The highest and lowest Σn-6 PUFA contents were observed in T1 and T2, respectively, whereas no significant difference was detected between T3 and T4 ($P > 0.05$). The EPA/ARA ratio was significantly higher in T1 than in the other treatments, followed by T4, while no significant difference was observed between T2 and T3.

Table 2
Effects of different diets on fatty acid compositions of *Moina mongolica*.

Fatty acids	***T1***	***T2***	***T3***	***T4***
Capric acid (C10:0)	1.58 ± 0.03	0.99 ± 0.01	1.26 ± 0.04	1.20 ± 0.02
Lauric acid (C12:0)	0.59 ± 0.01	0.78 ± 0.01	0.74 ± 0.01	0.62 ± 0.02
Tridecanoic acid (C13:0)	0.33 ± 0.02	0.59 ± 0.02	0.43 ± 0.01	0.41 ± 0.01
Myristic acid (C14:0)	10.52 ± 0.02	6.93 ± 0.01	8.11 ± 0.09	7.97 ± 0.01
Pentadecanoic acid (C15:0)	1.63 ± 0.03	2.25 ± 0.01	2.14 ± 0.06	2.26 ± 0.01
Palmitic acid (C16:0)	35.25 ± 0.05	20.06 ± 0.05	29.07 ± 0.08	27.22 ± 0.02
Heptadecanoic acid (C17:0)	0.05 ± 0.00	0.10 ± 0.00	1.15 ± 0.08	0.06 ± 0.00
Stearic acid (C18:0)	2.03 ± 0.01	2.20 ± 0.03	2.55 ± 0.04	2.43 ± 0.03
Arachidic acid (C20:0)	0.03 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	0.06 ± 0.00
Monounsaturated fatty acids (MUFA)	52.02 ± 0.08^{d}	33.90 ± 0.01^{a}	45.50 ± 0.26^{c}	42.22 ± 0.07^{b}
Myristoleic acid (C14:1)	0.13 ± 0.02	0.25 ± 0.01	0.19 ± 0.01	0.26 ± 0.01
cis-10-Pentadecenoic acid (C15:1)	0.06 ± 0.00	0.57 ± 0.01	0.33 ± 0.01	0.36 ± 0.01
Palmitoleic acid (C16:1)	26.27 ± 0.03	41.48 ± 0.02	36.18 ± 0.21	34.23 ± 0.03
cis-10-Heptadecenoic acid (C17:1)	0.08 ± 0.00	0.16 ± 0.01	0.15 ± 0.01	0.08 ± 0.00
Elaidic acid (C18:1ω9t)	0.03 ± 0.00	0.14 ± 0.01	0.11 ± 0.01	0.06 ± 0.00
Oleic acid (C18:1ω9c)	7.59 ± 0.05	17.72 ± 0.03	10.45 ± 0.09	14.83 ± 0.04
Polyunsaturated fatty acids (PUFA)	34.16 ± 0.04^{a}	60.32 ± 0.03^{d}	47.39 ± 0.27^{b}	49.81 ± 0.06^{c}
trans-Linoleic acid (C18:2ω6t)	0.11 ± 0.00	0.05 ± 0.01	0.11 ± 0.01	0.10 ± 0.01
Linoleic acid (C18:2ω6c, LA)	3.05 ± 0.02	1.45 ± 0.01	1.49 ± 0.02	1.54 ± 0.01
γ-Linolenic acid (C18:3ω6)	0.68 ± 0.01	0.31 ± 0.01	0.47 ± 0.01	0.42 ± 0.01
α-Linolenic acid (C18:3ω3, ALA)	0.15 ± 0.01	0.18 ± 0.01	0.19 ± 0.00	0.36 ± 0.01
Eicosadienoic acid (C20:2)	0.08 ± 0.01	0.00 ± 0.00	0.10 ± 0.02	0.09 ± 0.01
Eicosatrienoic acid (C20:3ω6)	0.15 ± 0.01	0.15 ± 0.01	0.12 ± 0.04	0.08 ± 0.00
Arachidonic acid (C20:4ω6, ARA)	2.06 ± 0.01^{d}	1.00 ± 0.01^{a}	1.26 ± 0.01^{b}	1.39 ± 0.04^{c}

Note: Values presented as means (n = 3) and pooled SEM. Different superscript lowercase letters within each row represent significant differences (Duncan's test;$P < 0.05$)

Fatty acids	*T1*	*T2*	*T3*	*T4*
Eicosapentaenoic acid (C20:5ω3, EPA)	7.53 ± 0.05^{d}	2.63 ± 0.01^{a}	3.37 ± 0.09^{b}	3.99 ± 0.05^{c}
Total PUFA (∑PUFA)	13.81 ± 0.11^{d}	5.78 ± 0.02^{a}	7.11 ± 0.01^{b}	7.97 ± 0.02^{c}
Total ω3-PUFA (∑ω3-PUFA)	7.69 ± 0.06^{d}	2.82 ± 0.00^{a}	3.56 ± 0.08^{b}	4.35 ± 0.06^{c}
Total ω6-PUFA (∑ω6-PUFA)	6.04 ± 0.04^{c}	2.97 ± 0.02	3.44 ± 0.08	3.52 ± 0.04^{d}
EPA/ARA	3.67 ± 0.04^{c}	2.64 ± 0.03	2.67 ± 0.09	2.88 ± 0.12

Note: Values presented as means (n = 3) and pooled SEM. Different superscript lowercase letters within each row represent significant differences (Duncan's test;$P < 0.05$)

3.3. Effects of different diets on the amino acid composition of Moina mongolica

Sixteen amino acids were detected in *M. mongolica* across all dietary treatments, whereas tryptophan and cysteine were not determined (Table 3). The amino acid profiles were characterized by relatively high proportions of flavor-related amino acids and fish essential amino acids (EAAs), which accounted for 51.60–52.34% and 45.59–46.20% of total amino acids (TAA), respectively. Among the EAAs, phenylalanine was the most abundant, followed by leucine, lysine, and threonine, whereas methionine showed the lowest content. Among the non-essential amino acids, glutamic acid and aspartic acid were predominant.

Dietary composition significantly affected the absolute amounts of TAA, flavor-related amino acids, and EAAs ($P < 0.05$). All three parameters showed the same ranking among treatments: T4 > T2 > T3 > T1. However, the proportions of flavor-related amino acids and EAAs relative to TAA did not differ significantly among treatments ($P > 0.05$), indicating that dietary treatments primarily affected the total amino acid content rather than the overall amino acid profile.

Table 3
Effects of different diets on amino acid compositions of *Moina mongolica*.

Amino acid	***T1***	***T2***	***T3***	***T4***
(Met)*	0.017 ± 0.001	0.031 ± 0.001	0.022 ± 0.001	0.027 ± 0.001
(Phe)*	0.355 ± 0.001	0.381 ± 0.001	0.360 ± 0.007	0.388 ± 0.001
(Thr)*	0.201 ± 0.001	0.219 ± 0.002	0.211 ± 0.006	0.238 ± 0.001
(Val)*	0.163 ± 0.002	0.177 ± 0.001	0.169 ± 0.002	0.185 ± 0.001
(Ile)*	0.120 ± 0.001	0.131 ± 0.001	0.121 ± 0.002	0.141 ± 0.001
(Leu)*	0.293 ± 0.002	0.320 ± 0.001	0.316 ± 0.005	0.348 ± 0.004
(Lys)*	0.262 ± 0.001	0.287 ± 0.001	0.276 ± 0.010	0.320 ± 0.001
(Arg)*	0.258 ± 0.001	0.281 ± 0.001	0.271 ± 0.003	0.316 ± 0.001
(His)*	0.098 ± 0.001	0.109 ± 0.001	0.103 ± 0.006	0.122 ± 0.001
(Pro)	0.223 ± 0.002	0.225 ± 0.001	0.219 ± 0.002	0.253 ± 0.003
(Tyr)	0.198 ± 0.001	0.207 ± 0.002	0.202 ± 0.001	0.225 ± 0.003
(Asp)	0.416 ± 0.002	0.448 ± 0.001	0.424 ± 0.007	0.494 ± 0.001
(Ser)	0.212 ± 0.002	0.231 ± 0.001	0.217 ± 0.006	0.251 ± 0.001
Glu)	0.585 ± 0.003	0.632 ± 0.001	0.614 ± 0.012	0.682 ± 0.002
(Gly)	0.204 ± 0.004	0.221 ± 0.001	0.218 ± 0.006	0.244 ± 0.003
(Ala)	0.270 ± 0.001	0.292 ± 0.001	0.279 ± 0.011	0.314 ± 0.002
Total amino acids	3.878 ± 0.007[a]	4.192 ± 0.003[c]	4.022 ± 0.008[b]	4.544 ± 0.017[d]
Total Fresh Amino Acids	2.030 ± 0.003[a]	2.181 ± 0.001[c]	2.097 ± 0.014[b]	2.346 ± 0.009[d]

Amino acid	*T1*	*T2*	*T3*	*T4*
Total essential amino acids in fish	1.768 ± 0.002[a]	1.936 ± 0.002[c]	1.849 ± 0.010[b]	2.085 ± 0.003[d]
Flavour amino acids/total amino acids	52.34 ± 0.07	52.03 ± 0.06	52.14 ± 0.25	51.60 ± 0.01
Total essential amino acids in fish/total amino acids	45.59 ± 0.05	46.20 ± 0.02	45.97 ± 0.27	45.86 ± 0.10

3.4 Effects of different dietary compositions on carotenoid (Astaxanthin and Canthaxanthin) contents in Moina mongolica

The contents of astaxanthin and canthaxanthin in *Moina mongolica* from the four experimental groups are presented in Fig. 1. As shown in Fig. 1, M. *mongolica* contained detectable levels of both astaxanthin and canthaxanthin. In all four dietary treatments, the canthaxanthin content was significantly higher than the astaxanthin content ($P < 0.05$). The highest astaxanthin content was observed in *M. mongolica* fed *Nannochloropsis sp*. in the T1 group, which was significantly higher than those in the other three groups ($P < 0.05$). In contrast, the T2 group, which was fed yeast, exhibited the lowest astaxanthin content, significantly lower than those of the other three groups ($P < 0.05$). The astaxanthin contents in the T3 and T4 groups were also significantly lower than that in the T1 group ($P < 0.05$).

For canthaxanthin, the highest content was detected in *M. mongolica* from the T4 group, which was significantly higher than those in the other three groups ($P < 0.05$), whereas the T2 group exhibited the lowest canthaxanthin content, significantly lower than those in the other three groups ($P < 0.05$).

4. Discussion

Dietary composition significantly affected the proximate composition of *M. mongolica*, particularly crude protein and lipid contents, whereas moisture and ash contents remained relatively stable (Radhakrishnan et al. 2020; Samat et al. 2020). The highest lipid content but lowest protein content was observed in the *N. oculata*-fed T1 group, whereas the yeast-fed T2 group showed the opposite pattern. This difference likely reflects the distinct nutritional characteristics of the dietary ingredients. *N. oculata* is generally rich in lipids, particularly polyunsaturated fatty acids, whereas *S. cerevisiae* provides a relatively protein-rich and lipid-poor microbial biomass. Similar dietary-dependent changes in protein and lipid composition have been reported in cladocerans, indicating that the biochemical composition of live feed can be effectively modulated through dietary manipulation (Li and Liu 2021; Suhaimi et al. 2022; Turcihan et al. 2022). The highest crude protein content was detected in the T4 group, which received the combined diet of *N. oculata*, *S. cerevisiae*, and *R. palustris*. The additional inclusion of *R. palustris* may have contributed to the increased protein accumulation because photosynthetic bacterial biomass can provide substantial microbial protein. More importantly, the response observed in T4 suggests that combining nutritionally complementary microorganisms may improve the overall nutrient composition of

M. mongolica. Similar effects of dietary manipulation on the nutritional quality of *M. mongolica* have been reported in recent studies (Ashaari et al. 2024; Hena et al. 2025). Overall, these findings indicate that the proximate composition of *M. mongolica* is strongly influenced by the nutritional composition of its diet. The combined *N. oculata*–yeast–*R. palustris* diet produced a relatively favorable protein–lipid profile, suggesting that dietary combinations may be more effective than single-source diets for producing nutritionally enriched *M. mongolica*.

Fatty acid composition is considered one of the most important nutritional characteristics determining the quality of live feeds in aquaculture (Tocher et al. 2003). Particularly, long-chain polyunsaturated fatty acids (LC-PUFAs), including eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6), are important for larval development, growth, immune regulation, and physiological homeostasis in marine fish because their endogenous biosynthesis can be limited (Glencross 2009; Ramos-Llorens et al. 2023). Therefore, the nutritional value of live prey organisms largely depends on their ability to accumulate these essential fatty acids.

In the present study, DHA was not detected in *M. mongolica* under any dietary treatment. This observation is consistent with the limited capacity for DHA accumulation reported for some live-feed organisms (Ramos-Llorens et al. 2023). Similar characteristics have also been reported in other cladoceran species, suggesting that the absence or extremely low level of DHA may be an inherent metabolic feature of *M. mongolica* rather than solely determined by dietary supply (Wade et al. 2020). Although DHA was deficient, *M. mongolica* showed considerable EPA accumulation, and dietary composition significantly affected EPA content. In this study, the EPA content was highest in the T1 group fed *N. oculata* and lowest in the yeast-fed T2 group. This difference may be attributed to the distinct fatty acid profiles of the dietary organisms. *N. oculata* is well known as an EPA-rich microalga, whereas *S. cerevisiae* contains relatively limited amounts of PUFA. Therefore, dietary microalgae supplementation could effectively enhance the PUFA nutritional quality of *M. mongolica*. The present results further demonstrated that dietary composition significantly altered the proportions of SFA, MUFA, and PUFA in *M. mongolica*. The yeast-fed group exhibited the highest MUFA content but the lowest PUFA level, whereas *N. oculata*-fed *M. mongolica* showed increased PUFA accumulation, especially higher levels of EPA, LA, and ARA. These results indicate that fatty acid profiles of *M. mongolica* are strongly influenced by dietary fatty acid sources, supporting previous findings that biochemical composition of zooplankton can be modified through nutritional manipulation (Samat et al. 2020; Turcihan et al. 2022).

ARA is another important PUFA involved in immune regulation through its conversion into eicosanoids, including prostaglandins and leukotrienes (Wei et al. 2015). In the present study, ARA content was significantly higher in the T1 group than in the yeast-based diet group, which may be associated with the relatively high ARA content of *N. oculata*. Similarly, LA, an essential precursor of LC-PUFA biosynthesis, was also enhanced by microalgae supplementation. These results suggest that *N. oculata* represents an effective dietary source for improving the HUFA profile of *M. mongolica*. Interestingly, the mixed dietary treatment containing *N. oculata*, yeast, and *R. palustris* resulted in a more balanced fatty acid profile, with moderate levels of SFA, MUFA, and PUFA. This may be attributed to the complementary nutritional

characteristics of different dietary components. Although yeast provides abundant protein and contributes to biomass production, its low PUFA content limits its ability to enhance the fatty acid nutritional value of live feeds. Conversely, microalgae provide essential fatty acids but may not fully satisfy all nutritional requirements alone. The incorporation of *R. palustris* may further improve the nutritional balance by supplying microbial biomass and carotenoid-related functional nutrients (Kuo et al. 2023; Li et al. 2023).

Overall, the present study indicates that dietary manipulation is an effective strategy to regulate the fatty acid composition and nutritional quality of *M. mongolica* (Lu et al. 2026; Yu et al. 2025). Among the tested diets, *N. oculata* alone produced the highest PUFA nutritional value, whereas the combined diet containing *N. oculata*, yeast, and *R. palustris* provided a more balanced nutritional profile. These findings suggest that mixed dietary strategies may be suitable for production of nutritionally enhanced *M. mongolica* as a live feed for marine fish and crustacean larvae.

Dietary composition can substantially influence the protein content and amino acid profile of live feeds, thereby affecting their nutritional quality for aquaculture larvae. In the present study, all dietary treatments resulted in the detection of 16 amino acids in *M. mongolica*, with essential amino acids (EAAs) and flavor-related amino acids accounting for relatively stable proportions of total amino acids. These findings indicate that although dietary composition altered the absolute amino acid content, the overall amino acid profile of *M. mongolica* remained relatively conserved. Methionine was the least abundant EAA in *M. mongolica* in the present study, particularly in the T1 group fed *N. oculata*. This may be associated with the relatively low protein content and limited methionine contribution of the microalgal diet. In contrast, the inclusion of yeast and *R. palustris* increased the total amino acid content of *M. mongolica*. Yeast is a relatively protein-rich feed ingredient, whereas photosynthetic bacteria can provide substantial microbial biomass. Thus, the combination of microalgae, yeast, and photosynthetic bacteria may provide complementary nutrients and promote the accumulation of protein-derived amino acids in *M. mongolica*. Similar nutritional complementation among different live-feed dietary sources has been reported in aquaculture (Li and Liu 2021; Suhaimi et al. 2022). The T4 group exhibited the highest total amino acid content, as well as the highest absolute contents of flavor-related amino acids and EAAs, suggesting that the combined diet provided a favorable protein nutritional profile for *M. mongolica*. However, the proportions of flavor-related amino acids and EAAs relative to total amino acids did not differ significantly among dietary treatments. This suggests that dietary manipulation primarily affected the quantity rather than the relative composition of amino acids in *M. mongolica*. The relatively stable EAA/total amino acid ratio further indicates that the nutritional quality of its protein was maintained across dietary treatments. From an aquaculture perspective, the high proportion of EAAs in *M. mongolica* supports its potential as a live feed for marine fish and crustacean larvae. Nevertheless, the relatively low methionine content observed in the present study, particularly under the microalgae-only diet, suggests that targeted amino acid enrichment may further improve its nutritional value.

Carotenoids are important functional nutrients in aquaculture because many cultured animals, particularly fish, have limited or no capacity for de novo carotenoid synthesis and therefore depend

largely on dietary sources for pigmentation and related physiological functions. Astaxanthin and canthaxanthin are among the major carotenoids associated with pigmentation in aquatic animals and may also contribute to antioxidant defense and other physiological processes (Roo et al. 2023; Ashaari et al. 2024; Li and Liu 2021). However, compared with studies focusing on carotenoid deposition and pigmentation in cultured fish and crustaceans, relatively little attention has been paid to carotenoid accumulation in live-feed organisms.

In the present study, both astaxanthin and canthaxanthin were detected in *M. mongolica*, with canthaxanthin generally occurring at higher levels than astaxanthin. Their concentrations varied among dietary treatments, indicating that carotenoid accumulation in *M. mongolica* is responsive to dietary composition. This observation is consistent with the established principle that carotenoid deposition in aquatic organisms is strongly influenced by dietary carotenoid sources and bioavailability (Liu et al. 2023; Li and Liu 2021). The highest astaxanthin content was observed in the T1 group, whereas canthaxanthin accumulation was highest in the T4 group. *N. oculata* contains carotenoid pigments, while *R. palustris* is also capable of producing carotenoid compounds (Li et al. 2023). Thus, the higher canthaxanthin accumulation observed in T4 may be associated with the complementary contribution of carotenoid-rich dietary components. In contrast, the relatively low carotenoid levels in the T2 group may reflect the limited carotenoid supply provided by the yeast-based diet. These results suggest that the carotenoid profile of *M. mongolica* can be modified through dietary manipulation.

The present findings are also consistent with our previous enrichment experiment, in which *M. mongolica* showed an appreciable capacity to accumulate exogenously supplied astaxanthin and canthaxanthin (Lu et al. 2026). Although the underlying mechanisms remain to be elucidated, these observations collectively indicate that *M. mongolica* may serve not only as a source of protein, amino acids, and fatty acids but also as a potential carrier of functional carotenoids in live-feed production. From an aquaculture perspective, the accumulation of carotenoids in live feeds may be relevant to marine larval production because carotenoids supplied through live prey can subsequently be transferred to cultured animals. Therefore, the combined use of *N. oculata*, yeast, and *R. palustris* may provide an effective strategy for producing nutritionally enriched *M. mongolica*. Nevertheless, the present study only demonstrates an association between dietary composition and carotenoid accumulation; the specific carotenoid sources, metabolic conversion pathways, and transfer efficiency from *M. mongolica* to target cultured animals require further investigation (Zuo et al. 2022).

5. Conclusion

Dietary composition significantly affected the proximate composition, fatty acid and amino acid profiles, and carotenoid accumulation of *Moina mongolica*. Feeding *Nannochloropsis oculata* resulted in the highest EPA and PUFA levels but the lowest crude protein content, whereas yeast alone produced the lowest PUFA and carotenoid levels. The combined diet of *N. oculata*, *Saccharomyces cerevisiae*, and *Rhodopseudomonas palustris* provided a more balanced nutritional profile, characterized by relatively high protein and lipid contents, amino acid abundance, and carotenoid accumulation. These results

indicate that mixed dietary supplementation may be a suitable strategy for improving the overall nutritional quality of *M. mongolica* for marine hatchery applications. Further enrichment with essential fatty acids, particularly EPA and DHA, may provide an additional approach to enhance its nutritional value as a live feed for marine fish larvae.

Declarations

Conflicts of Interest:

The authors declare no conflicts of interest.

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Author Contribution

B.Y. (Bingcong Ye): Data curation, Formal analysis, Investigation, Software, Visualization, Writing—original draft; J.L. (Jianxue Lu): Conceptualization, Methodology, Investigation, Software, Visualization, Writing—original draft. X.L. (Xin Liu): Formal analysis, Validation; X.W. (Yuxing Wei): Investigation, Methodology. Y.W. (Yuxin Wang): Formal analysis and data curation. Y.H. (Yanqing Huang): Conceptualization, Methodology, Resources, Visualization, Supervision, Writing—review & editing, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability:

The data that support the findings of this study are available on request from the corresponding author.

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Figures

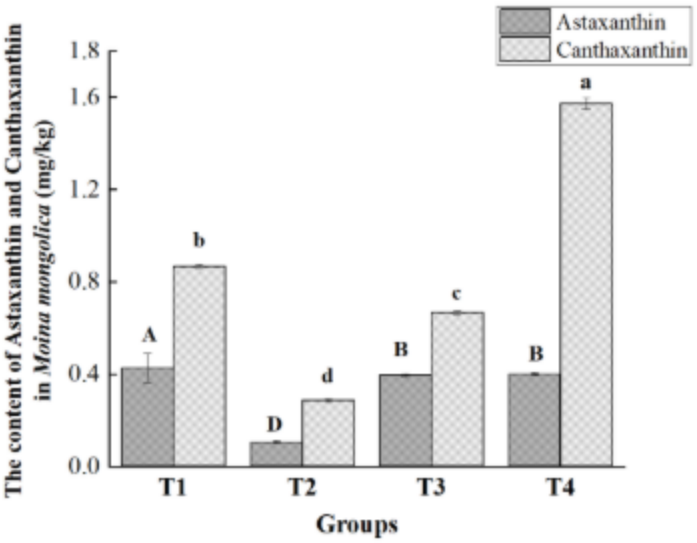


Figure 1

Effects of different diets on astaxanthin and canthaxanthin content of *Moina mongolica* Daday.
Note: Values presented as means (n=3) and pooled SEM. Different superscript lowercase letters within each row represent significant differences (Duncan's test;$P<0.05$)