Comparative Evaluation of Algae, Yeast, and Mixed Diets on Growth Performance and Proximate Composition of *Artemia* franciscana in a 30-Day Culture System

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Abstract

This study investigated the effects of dietary supplementation with microalgae (Chlorella vulgaris), yeast (Saccharomyces cerevisiae), and a 1:1 combination of both on the growth performance and proximate composition of Artemia franciscana over a 30-day culture period. Four feeding treatments were evaluated: a control (natural seawater microbial load), yeast-only (2 g/L), algae-only (2 g/L), and a mixed diet (1 g/L yeast + 1 g/L algae). The results revealed that dietary treatment significantly influenced all measured parameters (p ≤ 0.05). The mixed diet group achieved the highest growth performance, with a final length of 7.4 ± 0.4 mm, biomass of 7.5 ± 0.3 mg, weight gain of $300.5 \pm 9.6\%$, and survival rate of $88.4 \pm 2.6\%$. Algae-fed Artemia also showed notable improvement (final length: 6.7 ± 0.5 mm; biomass: 6.8 ± 0.4 mg; weight gain: 270.3 \pm 11.2%; survival: 81.1 \pm 2.3%), followed by the yeast group (final length: 6.3 \pm 0.3 mm; biomass: 6.3 ± 0.3 mg; weight gain: $254.5 \pm 10.6\%$; survival: $78.7 \pm 2.7\%$). The control group recorded the lowest values across all metrics, with a final biomass of 1.7 ± 0.1 mg and survival of 52.4 ± 3.5%. Proximate composition analysis further confirmed the nutritional benefits of the mixed diet, which produced the highest crude protein (53.7 ± 1.2%) and lipid content (15.4 ± 0.3%) with reduced ash (11.3 \pm 0.6%) and moisture levels (75.3 \pm 0.5%). These findings demonstrate that a combined yeast-algae diet provides complementary nutrients that enhance Artemia growth and nutritional quality. These results highlight the potential of mixed yeast algae diets as a sustainable and cost-effective strategy for producing nutritionally enriched Artemia in aquaculture systems.

Keywords: Artemia nauplii, Chlorella vulgaris, Saccharomyces cerevisiae, Growth performance, Proximate composition.



Introduction

Artemia franciscana (A. franciscana), commonly known as brine shrimp, has become an essential component in aquaculture, particularly as a live feed for the early life stages of fish and crustaceans (Jacob et al., 2025). Its widespread application is attributed to a combination of biological and practical advantages. These include its remarkable tolerance to varying salinity levels, the easy of harvesting from dormant cysts, and the ability to culture large populations under relatively simple conditions (Hassan et al 2025; Sivamurugan et al., 2025). More importantly, A. franciscana serves as a reliable first feed due to its ideal size, digestibility, and enrichment potential. It can effectively deliver a variety of nutritional and therapeutic compounds such as essential fatty acids, vitamins, minerals, and immune-boosting agents, making it a versatile compound for enhancing larval development and health in aquaculture operations (Andrade-Bustamante et al., 2025; Al Sulivany el al., 2025).

While *A. franciscana* is widely regarded for its utility, its intrinsic nutritional value is not always aligned with the specific requirements of marine and freshwater larvae (Al Bassam *et al.*, 2023; Dinesh *et al.*, 2025). In particular, its baseline levels of polyunsaturated fatty acids (PUFAs), protein, and other bioactives may be suboptimal without dietary enhancement (Glencross *et al.*, 2025). The nutritional quality of *A. franciscana* is largely determined by the composition of its feed during culture, which has led to continuous efforts in technical feeding strategies (Santos *et al.*, 2025). Conventionally, enrichment practices are limited to short-term exposures, often ranging from 18 to 48 hours (Marumure *et al.*, 2025). Although effective to a certain extent, such methods usually modify only the outer lipid layer of *A. franciscana* without producing deeper physiological improvements that a long-term nutritional approach (Mohamad Hassan *et al.*, 2024; Dutta *et al.*, 2025).

Microalgae, especially species like *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis* spp, have long been the preferred feed choice in hatcheries (Bakshi *et al.*, 2025). These algae are known for their high concentrations of essential fatty acids like eicosapentaenoic acid (EPA), natural pigments, and a suite of micronutrients that support both the nutritional profile and physiological resilience of *A. franciscana* (Khanzadeh *et al.*, 2025). However, the scalability of microalgal production remains a challenge. High cultivation costs, sensitivity to contamination, and the need for specialized infrastructure can limit their consistent use, especially in low-resource or small-scale hatcheries (Manoharan *et al.*, 2025).

An alternative feed option that has gained considerable attention is *Saccharomyces cerevisiae* (*S. cerevisiae*). Yeast offers practical advantages, including affordability, stability, and ease of storage and use (Al-Sahlany *et al.*, 2025). Rich in protein ranging from 45% to 55% along with B-vitamins and other growth-enhancing compounds, yeast can significantly improve *A. franciscana* survival and growth. Nonetheless, its deficiency in long-chain PUFAs like DHA and EPA restricts its use as a standalone enrichment source (Dinesh *et al.*, 2025).





In response to these limitations, researchers have begun exploring the potential benefits of using mixed diets that combine different feed sources (Sohel *et al.*, 2025). The integration of algae and yeast, in particular, has shown promise for achieving complementary nutritional effects. Algae contribute fatty acids and pigments, while yeast enhances protein and vitamin content (Wichaphian *et al.*, 2025). This combination may provide a more holistic diet that supports both growth and physiological development. However, most of these studies have been confined to short enrichment durations, leaving a gap in understanding how mixed diets influence *A. franciscana* physiology and composition over an extended culture period.

To fill the existing knowledge gap, this study evaluated the long-term impact of three dietary treatments, *C. vulgaris*, *S. cerevisiae*, and their 1:1 combination, on the growth, survival, and proximate composition of *A. franciscana*. Over a 30-day culture period, the trial aimed to assess how these feeding regimes influence both performance and nutritional quality. The results are expected to support the development of efficient and cost-effective live feed strategies for sustainable aquaculture practices.

Materials and Methods

Experimental Design and Animal Source

The experiment was conducted to evaluate the effects of three dietary treatments: microalgae (C. vulgaris), yeast (S. cerevisiae), and a combination of both, on the growth performance and proximate composition of A. franciscana. Artemia cysts were procured from a certified commercial supplier (INVE Aquaculture, Thailand) and stored at 4°C until hatching. Before the trial, cysts were hydrated and decapsulated using standard protocols as described by Van Stappen (1996), then hatched under optimal conditions in filtered seawater (salinity 35 ppt, temperature 28 ± 1 °C, continuous aeration, and light) (Al Sulivany et al., 2024).

Culture Conditions

Upon hatching, nauplii were transferred to 3-L transparent culture tanks at a stocking density of 100 nauplii/mL. Each treatment group was conducted in triplicate. All tanks were maintained at $28 \pm 1^{\circ}$ C with constant aeration, salinity at 35 ppt, and pH between 7.8–8.2. Photoperiod was maintained at 16:8 h (light: dark) using overhead fluorescent lighting. Daily monitoring of water temperature, pH, and salinity ensured stable rearing conditions throughout the 30-day trial. Water exchange (40%) was performed daily using pre-aerated seawater to maintain water quality and remove accumulated waste. No antibiotics or antimicrobials were used during the experiment.

Feed Preparation and Feeding Protocol

To investigate the nutritional impact of different diets on *A. franciscana*, four distinct feeding treatments were designed: a control, a yeast-based diet, an algal diet, and a combination of yeast and algae. The control group relied solely on the microbial content and dissolved organic matter naturally present in the seawater, including ambient



plankton and bacterial populations, which offered minimal but measurable nutritional value. For the formulated diets, *S. cerevisiae* (baker's yeast) was obtained from a local commercial source, gently oven dried at 40°C to preserve essential nutrients, and ground into a fine powder for uniform dispersion. The algal feed was composed of powdered *C. vulgaris*, valued for its high protein content and digestibility, and acquired from a certified algae production facility. In the mixed-diet treatment, both yeast and algal powders were blended in equal parts by weight (1:1) and freshly prepared each day to maintain feed quality and nutritional integrity. All feed types were administered at a concentration of 2 grams per liter of culture medium, with the mixed group receiving 1 gram of each component. Feeding was carried out three times daily—at 08:00, 14:00, and 20:00—using a consistent schedule adapted from Asem *et al.* (2025), designed to support steady nutrient availability and promote uniform growth. Feed rations were adjusted at five-day intervals in response to visible biomass increases, and tanks were cleaned of residual feed and metabolic waste before each water exchange to maintain hygienic and stable culture conditions.

Growth and Survival Assessment

Sampling was conducted at the end of the 30-day culture period. Ten individuals were randomly sampled from each replicate for length measurements using a calibrated stereomicroscope with an ocular micrometre. Final biomass per replicate was determined by collecting, filtering, and oven-drying the entire *A. franciscana* biomass at 60°C for 24 hours, then weighing on an analytical balance (±0.001 g).

Weight gain (%) was calculated using the following formula:

Weight Gain (%) = ((Final Weight – Initial Weight) / Initial Weight) \times 100 (Owais *et al.*, 2024).

Initial average weight was determined from a subsample (n = 30) on day 0 (mean: 1.8 \pm 0.1 mg). Survival rate was estimated based on the following formula:

Survival rate = Nt /No x 100%

Where (Nt) is the number of Artemia at the end of the experiment, and (No) is the number of nauplii at the start of the experiment.

Proximate Composition Analysis

On the final day of the experiment (day 30), *A. franciscana* specimens from each replicate tank were harvested for biochemical analysis. The collected biomass was first gently rinsed with distilled water to remove residual salts, feed particles, and debris. The samples were then oven-dried at a stable temperature of 60°C until a constant weight was achieved, ensuring all moisture was removed without compromising the integrity of proteins or lipids. Once dried, the samples were finely ground using a clean mortar and pestle to produce a uniform powder. The homogenized material was stored in airtight polyethylene containers and refrigerated at 4°C to prevent oxidation or degradation before analysis. The proximate composition was assessed by determining four key nutritional parameters: moisture content, crude protein, crude lipid, and ash. All analyses were





conducted in triplicate for each replicate to ensure the reliability and reproducibility of results. Moisture content was quantified by weighing a known amount of each sample and drying it in a hot air oven at 105°C for 24 hours. The percentage of moisture was calculated by the weight difference before and after drying, as per the procedures outlined in AOAC (1990). Crude protein levels were measured using the Kjeldahl method, which involves acid digestion to convert organic nitrogen into ammonium sulfate, followed by distillation and titration. The total nitrogen content was then multiplied by a conversion factor of 6.25 to estimate crude protein concentration. Crude lipid was extracted using the Soxhlet apparatus, with petroleum ether serving as the organic solvent. Each dried sample was placed in a cellulose thimble and subjected to continuous solvent extraction for a minimum of 6 hours to ensure complete lipid recovery. Ash content was determined by incinerating a known weight of dried sample in a pre-weighed ceramic crucible at 550°C for 6 hours using a muffle furnace. The inorganic residue remaining after combustion represented the total mineral content of the sample. All proximate values were calculated on a dry-weight basis and expressed as percentages of the total sample weight. The analytical methods used were consistent with the standard protocols provided by the Association of Official Analytical Chemists (AOAC, 1990), widely recognized for accuracy and reliability in nutritional studies.

Statistical Analysis

Data were tested for normality and homogeneity of variance using Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) was conducted to identify significant differences among treatments, followed by Tukey's HSD post hoc test at a 95% confidence level (p < 0.05). All statistical analyses were performed using SPSS software (version 26.0).

Results

The growth performance and proximate composition of *A. franciscana* were significantly influenced by the different feeding treatments over the 30-day experimental period (Tables 1 and 2). The mixed diet (1 g/L yeast + 1 g/L algae) yielded the highest growth metrics, with a final length of 7.4 ± 0.4 mm, final biomass of 7.5 ± 0.3 mg, weight gain of $300.5 \pm 9.6\%$, and survival rate of $88.4 \pm 2.6\%$, all of which were significantly greater than the other treatments (p < 0.05). The algae-only diet (2 g/L) resulted in intermediate growth, with a final length of 6.7 ± 0.5 mm, biomass of 6.8 ± 0.4 mg, weight gain of $270.3 \pm 11.2\%$, and survival rate of $81.1 \pm 2.3\%$, significantly outperforming the yeast-only treatment (p < 0.05). The yeast-only group (2 g/L) exhibited lower but still improved growth (final length: 6.3 ± 0.3 mm; biomass: 6.3 ± 0.3 mg; weight gain: $254.5 \pm 10.6\%$; survival: $78.7 \pm 2.7\%$) compared to the unfed control (p < 0.05). The control group, which received no feed, displayed the poorest performance in all measured parameters (final length: 4.2 ± 0.3 mm; biomass: 1.7 ± 0.1 mg; weight gain: $210 \pm 9\%$; survival: $52.4 \pm 3.5\%$) (Fig. 1).





Table1. Final length, biomass, weight gain, and survival rate of *A. franciscana* fed different diets for 30 days.

Treatment	Final Length (mm)	Final Biomass (mg)	Weight Gain (%)	Survival Rate (%)
Control	4.2 ± 0.3^{d}	1.7 ± 0.1^{c}	210±0.09 ^c	52.4 ± 3.5^{c}
Yeast (2 g/L)	6.3 ± 0.3^{c}	6.3 ± 0.3^{b}	254.5 ± 10.6^{b}	$78.7 \pm 2.7^{\rm b}$
Algae (2 g/L)	6.7± 0.5 ^b	6.8± 0.4 ^b	270.3 ± 11.2^{b}	81.1 ± 2.3^{b}
Mixed (1+1 g/L)	7.4 ± 0.4^{a}	7.5± 0.3 ^a	300.5 ± 9.6^{a}	88.4 ± 2.6a

Note: Values are presented as mean \pm standard deviation (n = 3). Means within the same row that do not share a common superscript letter (a, b, c, d) are significantly different (p \leq 0.05) according to Tukey's HSD test. All treatments began with a uniform initial length of 3.1 \pm 0.2 mm and an initial biomass of 1.8 \pm 0.1 mg.

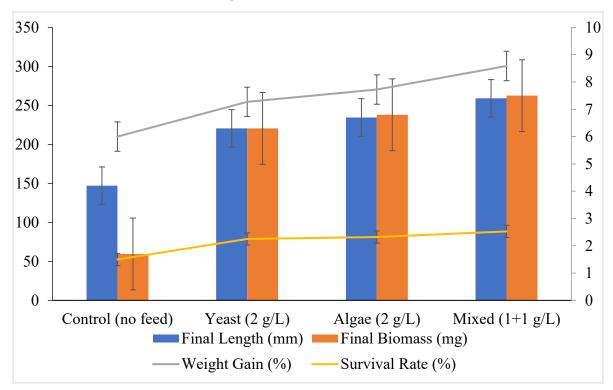


Figure1. Final length, biomass, weight gain, and survival rate of *A. franciscana* fed different diets for 30 days.

The mixed diet also produced the highest crude protein content (53.7 \pm 1.2%) and crude lipid content (15.4 \pm 0.3%), both of which were significantly greater than the other treatments (p \leq 0.05). The yeast-only diet resulted in comparable protein levels (53.3 \pm 1.4%) but lower lipids (10.1 \pm 0.6%), while the algae-only treatment yielded higher lipids (14.2 \pm 0.7%) but slightly lower protein (48.7 \pm 1.6%) compared to the mixed diet (p \leq 0.05). The control group had the lowest protein (37.21 \pm 1.1%) and lipid (7.4 \pm 0.3%) content, along with the highest ash (15.3 \pm 0.6%) and moisture (79.4 \pm 0.6%) levels, all of





which differed significantly from the fed groups ($p \le 0.05$). These results demonstrate that dietary supplementation, particularly with a mixed yeast-algae regimen, significantly enhances both the growth and nutritional quality of *A.franciscana* (Fig. 2).

Table2. Proximate composition (crude protein, crude lipid, ash, and moisture) of *A. franciscana* fed different diets for 30 days.

Treatment	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Moisture (%)
Control	37.21± 1.1 ^d	7.4 ± 0.3^{c}	15.3 ± 0.6^{a}	79.4 ± 0.6^{a}
Yeast (2 g/L)	53.3 ± 1.4^{b}	$10.1 \pm 0.6^{\rm b}$	12.6 ± 0.3^{b}	77.7 ± 0.7 ^b
Algae (2 g/L)	$48.7 \pm 1.6^{\circ}$	14.2 ± 0.7^{a}	13.3± 0.4 ^b	76.6 ± 0.8^{c}
Mixed (1+1 g/L)	53.7 ± 1.2^{a}	15.4 ± 0.3^{a}	11.3 ± 0.6^{c}	75.3 ± 0.5^{d}

Note: Values are presented as mean \pm standard deviation (n = 3). Means within the same row that do not share a common superscript letter (a, b, c, d) are significantly different (p \leq 0.05) according to Tukey's HSD test

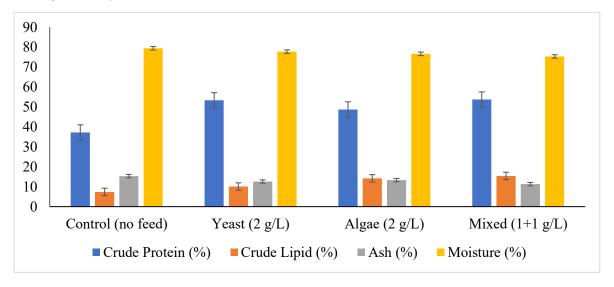


Figure 2. Proximate composition (crude protein, crude lipid, ash, and moisture) of *A. franciscana* fed different diets for 30 days.

Discussion

The results of the present study suggest that dietary supplementation can enhance the growth performance and biochemical composition of *A. franciscana*. Among the tested diets, the combination of *C.vulgaris* and *S. cerevisiae* (1:1) produced the most favorable outcomes, significantly outperforming both single-source diets and the control in all measured parameters.

The better performance observed in the mixed diet group is evident through the highest final length, biomass, weight gain, survival rate, and nutritional content, supporting the hypothesis that such an approach may compensate. The microalga *C. vulgaris* is known to be rich in essential fatty acids, pigments, and antioxidants, all of which are important





for maintaining membrane integrity, energy metabolism, and stress tolerance in aquatic organisms (Mendes et al., 2024; Panahi et al., 2025). However, microalgae alone may not provide sufficient protein density to support rapid biomass accumulation over extended culture periods (Sánchez-Bayo et al., 2020). On the other hand, S. cerevisiae, although protein-rich and abundant in B-vitamins, lacks long-chain polyunsaturated fatty acids (PUFAs), such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which are essential for larval development and immune modulation (Kour et al., 2019; Ilieva et al., 2024). The combination of these two feed types appears to bridge their nutritional gaps. Our findings align with previous research suggesting that co-feeding strategies incorporating microalgae and yeast can enhance growth and nutrient assimilation in zooplankton and larval stages of aquaculture species (Haris et al., 2024; Abreu et al., 2025). The observed increase in crude protein and lipid levels in A. franciscana under the mixed diet suggests improved nutrient assimilation, likely facilitated by the complementary availability of fatty acids and amino acids. The algae-only group also supported notable improvements in growth and lipid content relative to the yeast-fed and control groups. This is likely attributable to the presence of essential fatty acids and antioxidant compounds in Chlorella, which are vital for energy storage and larval development (Lopes et al., 2024; Yuslan et al., 2025). However, the slightly lower protein content in this group compared to the yeast and mixed treatments suggests that protein availability may have been a limiting factor for maximum growth potential. The yeast-only group achieved moderate improvements in growth and protein content but was less effective in promoting lipid accumulation (Kim et al., 2023). These results are consistent with earlier reports that identified yeast as a valuable but incomplete diet for A. franciscana due to its poor fatty acid profile (Sharma et al., 2025; Liang et al., 2025). Nonetheless, its positive contribution to survival and biomass compared to the control underscores its viability as a low-cost dietary supplement in hatchery systems. The control group, which relied solely on the background microbial load in the culture water, showed the lowest survival, weight gain, and nutrient content. This outcome indicates that while A. franciscana can survive temporarily on residual organic matter and autotrophic microbial communities, such a diet is insufficient for sustainable growth and optimal nutrient deposition (Anand et al., 2024; Chen et al., 2024). From a practical standpoint, the findings suggest that formulating diets that combine algae and yeast may represent a balanced and potentially cost-effective option solution for enhancing the productivity and quality of live feed in aquaculture hatcheries. Moreover, this feeding strategy may reduce dependence on expensive or logistically demanding single-feed systems, especially in settings with limited resources.

Conclusion

This study provides strong evidence that diet plays a crucial role in determining the growth performance and proximate composition of *A. franciscana* during extended culture. The mixed diet of *C. vulgaris* and *S. cerevisiae* resulted in the highest biomass





yield, percent of weight gain, survival, protein, and lipid content, demonstrating the advantages of a synergistic feeding approach. Both algae and yeast individually improved *A. franciscana* quality over the control, but neither alone matched the performance of the combined diet. These results highlight the potential of mixed microalgal-yeast diets as a cost-effective, nutritionally superior strategy for producing enriched *A. franciscana* suitable for aquaculture applications. Future studies could explore the effects of varying algal-yeast ratios or supplementing with additional functional ingredients to further enhance the nutritional profile and bioactive properties of *A. franciscana*.

Abbreviations

SE Standard error M Mean

Declarations

Consent for Publication

We, the authors of this research article, confirm that the work is original, has not been published elsewhere, and is not under consideration by another journal. All authors have reviewed and approved the final manuscript for submission.

Competing Interests

The authors declare that they have no competing financial or otherwise interests that could influence the work reported in this manuscript.

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Author Contributions

B.S.A.A.S., M.O., and H.H.E. contributed to the work's concept and design. M. S. A and H. A. M. were responsible for writing. M.O., B.S.A.A.S., A. H., and U. U., drafted the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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التقييم المقارن للأنظمة الغذائية القائمة على الطحالب والخميرة والمزيج بينهما على أداء النمو والتركيب التقريبي لـ (Artemia franciscana) في نظام زراعي مدته 30 يوماً

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المستخلص

هدفت هذه الدراسة إلى تقييم تأثير الإضافة الغذائية للطحالب الدقيقة (كلوريلا فولجاريس) والخميرة (ساكارومايسس سيريفيسيا) ومزيج منهما بنسبة ١:١ على أداء النمو والتركيب التقريبي لـ (أرتميا فرانسيسكانا) على مدى فترة زراعة مدتها ٣٠ يومًا. تم تقييم أربع معاملات غذائية: مجموعة تحكم (محمل ميكروبي طبيعي لمياه البحر)، ومجموعة تتغذى على الخميرة فقط (٢ جم/لتر)، ومجموعة تتغذى على الطحالب فقط (٢ جم/لتر)، ومجموعة تتغذى على مزيج منهما (١ جم/لتر خميرة + ١ جم/لتر طحالب). أظهرت النتائج أن المعاملة الغذائية أثرت بشكل معنوى على جميع المقابيس المدروسة ≥ p) • • • • (حققت مجموعة النظام المختلط أعلى أداء للنمو ، حيث بلغ متوسط الطول النهائي 7.4 ± 7.5 ملم، والكتلة الحيوية 7.4 ± 7.5 ملجم، وزيادة الوزن 7.5 ± 7.5 ٩.٦٪، ومعدل بقاء ٨٨.٤ ± ٢.٦٪. كما أظهرت مجموعة الطحالب تحسنًا ملحوظًا (متوسط الطول النهائي: ٦.٧ \pm ٠.٠ ملم؛ الكتلة الحبوية: 1.7 ± 3.0 ملجم؛ زيادة الوزن: 1.7 ± 7.0 ؛ البقاء: 1.1 ± 0.0 ، تليها مجموعة الخميرة (متوسط الطول النهائي: 7.7 ± 7.7 ملم؛ الكتلة الحيوية: 7.7 ± 7.7 ملجم؛ زيادة الوزن: 70.50 ± 1..1 ٪؛ البقاء: ٧٨.٧ ± ٧٨.٧ ٪). سجلت مجموعة التحكم أدني القيم في جميع المقاييس، حيث بلغت الكتلة الحيوية النهائية ١٠٧ ± ١٠٠ ملجم و معدل بقاء ٢٠٤ ± ٥٣٪. كما أكد تحليل التركيب التقريبي الفوائد الغذائية للنظام المختلط، الذي أنتج أعلى محتوى من البروتين الخام (٣٠٧ ± ١٠٢٪) والدهون (١٥٠٤ ± ٣٠٠٪) مع انخفاض في مستويات الرماد (١١.٣ $\pm ٠٠٠٪) والرطوبة (٧٥.٣ <math>\pm ٥.٠٪)$. تُظهر هذه النتائج أن النظام الغذائي المختلط من الخميرة والطحالب يوفر مغذيات متكاملة تعزز نمو وقيمة (الأرتميا) الغذائية. كما تُبرز هذه النتائج إمكانية استخدام الأنظمة الغذائية المختلطة من الخميرة والطحالب كاستر اتيجية مستدامة وفعالة التكلفة لإنتاج (أرتيميا) مُغَذِّي في أنظمة الاستزراع المائي.

الكلمات المفتاحية: يرقات الأرتيميا، طحالب الكلوريلا، خميرة، أداء النمو، التركيب التقريبي.



