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Effects of lipid source, ascorbyl palmitate concentration, and enrichment duration on the biochemical composition of *Artemia urmiana* nauplii

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Abstract

The biochemical composition of *Artemia urmiana* nauplii can be modified through enrichment with different lipid sources and ascorbyl palmitate (AP), although the magnitude of these changes may depend on AP concentration and enrichment duration. This study investigated the effects of lipid source, AP concentration, and enrichment duration on the vitamin C content, total lipid content, and fatty acid profile of *A. urmiana* nauplii. Newly hatched nauplii were enriched using either a commercial ICES30/4 emulsion or sturgeon ovary oil in combination with 10%, 20%, or 30% AP for 12 or 24 h. Unenriched nauplii served as the control. Vitamin C content was quantified by high-performance liquid chromatography (HPLC), whereas total lipid content and fatty acid composition were determined using standard lipid extraction, fatty acid methylation, and gas chromatography procedures. The overall vitamin C content was significantly higher in nauplii enriched with ICES30/4 than in those enriched with sturgeon ovary oil, reaching 767.46 ± 67.00 μg g^{-1} dry weight. Among the AP concentrations tested, 20% yielded the highest mean vitamin C content (839.33 ± 81.13 μg g^{-1} dry weight), while enrichment for 24 h resulted in the highest mean value (843.96 ± 56.31 μg g^{-1} dry weight). The highest vitamin C content was observed in nauplii enriched with ICES30/4 containing 20% AP for 24 h (1063.80 ± 48.00 μg g^{-1} dry weight), compared with 294.30 ± 35.21 μg g^{-1} dry weight in the control. Sturgeon ovary oil resulted in a significantly higher total lipid content than ICES30/4. Both lipid source and enrichment duration significantly affected the DHA/EPA ratio, whereas AP concentration had no significant main effect on this parameter. The n-3/n-6 fatty acid ratio was significantly influenced by lipid source, AP concentration, enrichment duration, and their interactions. Overall, the results demonstrate that lipid source and enrichment duration, together with AP concentration, influence the biochemical composition of *A. urmiana* nauplii. The findings provide a basis for optimizing enrichment protocols to modify vitamin C content and selected fatty acid characteristics of this live feed.

Keywords: *Artemia urmiana*; live feed enrichment; ascorbyl palmitate; vitamin C; fatty acid composition; DHA/EPA ratio; n-3/n-6 ratio.

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Introduction

Live feeds remain an essential component of larviculture because many fish and crustacean larvae have limited digestive capacity during the early stages of exogenous feeding. Among the live organisms used in hatcheries, *Artemia* is particularly important because its cysts can be stored for long periods, hatched on demand, and subsequently enriched with selected nutrients before being offered to larvae. The nutritional quality of live feeds, however, can vary considerably and may not always satisfy the requirements of developing fish and crustaceans. Consequently, enrichment of *Artemia* and other zooplankton has become an established practice in hatchery production (Radhakrishnan *et al*., 2020; Pan *et al*., 2022, Efatpanah *et al*., 2024, Kim *et al*., 2026, Yanes-Roca *et al.,* 2026**)**).

The nutritional limitation of unenriched *Artemia* is particularly evident in relation to 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). These fatty acids play important roles in membrane structure, development, growth, stress responses, and physiological regulation in aquatic animals. Accordingly, enrichment protocols have traditionally focused on transferring marine oils or concentrated HUFA emulsions to *Artemia* nauplii before their use as live feed.

Recent research continues to demonstrate that the nutritional composition of *Artemia* can be modified substantially through enrichment. Nieves-Soto *et al*. (2021), for example, demonstrated that enrichment duration affected the incorporation and subsequent decline of ARA, EPA, and DHA in *Artemia franciscana*, suggesting that both nutrient uptake and metabolic utilization or oxidation may influence the final fatty acid profile. More recently, Roo *et al*. (2023) showed that the fatty acid profile of enriched *Artemia* could be optimized by manipulating the EPA content of the enrichment emulsion. These studies emphasize that enrichment duration and the composition of the enrichment medium should be considered together rather than independently.

Vitamin C is another important nutrient that can be incorporated into *Artemia* through enrichment. Ascorbic acid is involved in collagen synthesis, antioxidant defense, tissue development, stress resistance, and immune function in aquatic animals. However, conventional ascorbic acid is relatively unstable in aqueous environments. Lipid-soluble derivatives such as ascorbyl palmitate can therefore be useful for bioencapsulation through lipid-based enrichment media. Noshirvani *et al*. (2006) previously demonstrated that enrichment of *A. urmiana* with an emulsion containing ascorbyl palmitate significantly increased tissue ascorbic acid concentrations.

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The choice of enrichment lipid is also important because the fatty acid profile of the enrichment medium can be reflected in the enriched *Artemia*. Hafezieh *et al*. (2008) demonstrated that ICES/30/4 could substantially enhance the lipid and n-3/n-6 fatty acid composition of *A. urmiana*. More recent evidence indicates that *Artemia* possesses considerable metabolic flexibility in response to different lipid sources. Samaee and Estévez (2025) reported substantial changes in fatty acid profiles of *A. franciscana* following enrichment with different plant oils, indicating that both dietary incorporation and endogenous fatty acid metabolism may contribute to the final fatty acid composition.

A recent comprehensive review by Ramena *et al*. (2025) further emphasized that enrichment with oil emulsions, microalgae, probiotics, yeast, lecithin, and micronutrients can modify the nutritional value of *Artemia*. The authors highlighted enrichment duration, emulsion composition, lipid concentration, and oxidation as important variables affecting the efficiency of nutrient transfer.

Previous work examined the use of enriched *Artemia urmiana* in Persian sturgeon larviculture and demonstrated that enrichment could modify both the nutritional characteristics of the live feed and the subsequent performance of sturgeon larvae (Hafezieh *et al*., 2008, 2010). An earlier conference contribution also reported experiments involving ICES30/4 and sturgeon ovary oil, three ascorbyl palmitate levels, and 12- and 24-h enrichment periods (Hafezieh *et al*., 2010). These studies provide an important background for the present work. The present article focuses specifically on the biochemical characterization of the resulting *A. urmiana* nauplii, including vitamin C concentration, total lipid, major fatty acid groups, and DHA/EPA and n-3/n-6 ratios, rather than on subsequent larval growth, survival, or salinity tolerance.

Against this background, the present study evaluated the effects of two lipid sources, three concentrations of ascorbyl palmitate, and two enrichment durations on the biochemical composition of *A. urmiana* nauplii. Particular emphasis was placed on vitamin C accumulation and changes in lipid and fatty acid characteristics relevant to the use of *Artemia* as a live-feed vehicle in aquaculture. By characterizing these responses under different combinations of enrichment source, ascorbyl palmitate concentration, and exposure time, the study provides a consolidated assessment of the biochemical characteristics of enriched *A. urmiana* nauplii.

Materials and methods

Hatching of Artemia urmiana cysts

A. urmiana cysts were incubated in 300-L conical hatching tanks containing seawater adjusted to a salinity of 30 g L^{-1}. Water temperature was maintained at 28°C, pH at approximately 8.0, and strong continuous aeration was supplied throughout the hatching period. Newly hatched nauplii were collected approximately 24 h after incubation and

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immediately transferred to the enrichment experiments.

Experimental enrichment design

The experiment was conducted using two enrichment lipid sources:

1. Commercial ICES30/4 enrichment emulsion (INVE, Belgium)
2. Sturgeon ovary oil

Each lipid source was combined with three concentrations of ascorbyl palmitate (AP):

- 10%
- 20%
- 30%

Two enrichment durations were evaluated:

- 12 h
- 24 h

An unenriched group was maintained as the control.

Nauplii were stocked at a density of approximately 300 individuals mL^{-1} in 2-L conical enrichment vessels. Each treatment was conducted in triplicate, resulting in 39 experimental vessels including the control groups. At the end of each enrichment period, nauplii were collected, washed with clean water, and immediately frozen at −80°C until biochemical analysis.

Enrichment media

The fatty acid composition of the two lipid sources used in the enrichment experiment is presented in Table 1. The substantially different fatty acid profiles of the two enrichment sources provided an opportunity to evaluate whether the source of lipid affected the nutritional characteristics of the enriched nauplii.

Table 1: Lipid and fatty acid composition of the enrichment sources used in the experiment.

Component	ICES30/4	Sturgeon ovary oil
Total lipid (%)	60.00	54.00
ARA (%)	0.78	5.00
EPA (%)	6.29	7.55
DHA (%)	20.90	2.76
DHA/EPA	3.32	0.36
Saturated fatty acids (%)	32.17	28.80
Monounsaturated fatty acids (%)	21.87	41.28
n-6 (%)	0.78	5.00
n-3 (%)	27.19	10.31
n-3/n-6	34.85	2.06

Determination of vitamin C

Vitamin C concentration in *A. urmiana* nauplii was determined by high-performance liquid chromatography (HPLC). Samples were prepared according to the analytical procedure used for the determination of ascorbic acid in biological tissues. A HPLC system manufactured by Kenauer, Germany, equipped with appropriate detection at 254 nm, was used. Vitamin C concentrations were expressed as µg g^{-1} dry weight.

Determination of total lipid and fatty acids

Total lipid was extracted according to the method of Folch *et al*. (1957), using

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a chloroform: methanol solvent system. The extracted lipid was quantified gravimetrically and expressed as a percentage of dry weight. Fatty acid methyl esters (FAMEs) were prepared using boron trifluoride in methanol according to Metcalfe and Schmitz (1961). The FAMEs were analyzed using a DANI-1000 gas chromatograph equipped with a flame ionization detector and a BPX70 capillary column. Helium was used as the carrier gas. Individual fatty acids were identified by comparison with appropriate standards. The principal fatty acid groups evaluated included saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-3 and n-6 fatty acids, as well as EPA and DHA. The DHA/EPA and n-3/n-6 ratios were subsequently calculated.

Statistical analysis

The effects of enrichment source, AP concentration, and enrichment duration were analyzed using three-way analysis of variance (ANOVA). Where significant differences were detected, Tukey's multiple-range test was used for pairwise comparisons. Statistical analyses were performed using SPSS version 14.0. Differences were considered statistically significant at $p<0.05$.

Results

The effects of enrichment source, ascorbyl palmitate concentration, and enrichment duration on the biochemical composition of *Artemia urmiana* nauplii are summarized in Table 2. The results are presented for vitamin C, total lipid, saturated fatty acids, monounsaturated fatty acids, the DHA/EPA ratio, and the n-3/n-6 ratio.

Table 2: Mean ± SD of total lipid, vitamin C, and selected fatty acid concentrations in *Artemia urmiana* nauplii under different enrichment conditions.

Parameter	ICES30/4	Sturgeon ovary oil	10% AP	20% AP	30% AP	12 h	24 h
Total lipid (% DW)	16.47 ± 1.05	19.63 ± 1.36	17.85 ± 1.41	18.54 ± 2.42	17.68 ± 2.18	17.72 ± 2.10	18.32 ± 1.96
Vitamin C (μg g^{-1} DW)	767.46 ± 67.00	675.60 ± 55.11	537.85 ± 54.23	839.33 ± 81.13	787.60 ± 70.20	600.10 ± 37.39	843.96 ± 56.31
ARA, C20:4n-6 (mg g^{-1} DW)	2.35 ± 0.21	1.85 ± 0.22	2.05 ± 0.15	2.24 ± 0.17	2.02 ± 0.21	2.09 ± 0.18	2.11 ± 0.20
EPA, C20:5n-3 (mg g^{-1} DW)	6.94 ± 0.35	5.16 ± 0.37	5.17 ± 0.30	5.56 ± 0.21	6.42 ± 0.20	5.42 ± 0.18	6.68 ± 0.29
DHA, C22:6n-3 (mg g^{-1} DW)	3.47 ± 0.35	1.58 ± 0.28	2.18 ± 0.16	2.74 ± 0.23	2.66 ± 0.24	2.14 ± 0.23	2.91 ± 0.18
DHA/EPA	0.49 ± 0.03	0.30 ± 0.02	0.40 ± 0.02	0.40 ± 0.03	0.39 ± 0.03	0.37 ± 0.02	0.42 ± 0.03
Total saturated fatty acids	31.38 ± 2.80	32.98 ± 2.03	31.38 ± 1.21	32.97 ± 2.22	32.19 ± 1.41	31.28 ± 1.71	33.08 ± 1.51
Total monounsaturated fatty acids	44.81 ± 3.90	46.76 ± 3.24	46.85 ± 1.77	45.24 ± 3.12	44.27 ± 2.24	47.40 ± 2.56	44.17 ± 2.20
Total n-3 fatty acids	10.46 ± 0.70	6.75 ± 0.65	7.36 ± 0.46	9.36 ± 0.44	9.09 ± 0.44	7.56 ± 0.42	9.65 ± 0.48
n-3/n-6	5.70 ± 0.24	2.91 ± 0.21	3.91 ± 0.15	4.37 ± 0.13	4.62 ± 0.15	3.88 ± 0.21	4.73 ± 0.20

Note: Values represent marginal means of the experimental factors. Different superscript letters in the original statistical table indicated significant differences at $p<0.05$.

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Vitamin C concentration

The concentration of vitamin C in *A. urmiana* nauplii was significantly affected by enrichment source, AP concentration, and enrichment duration. Nauplii enriched with ICES30/4 had a significantly higher overall vitamin C concentration (767.46±67.00 μg g^{-1} dry weight) than those enriched with sturgeon ovary oil. Among AP concentrations, the 20% treatment produced the highest overall vitamin C concentration (839.33±81.13 μg g^{-1} dry weight). The 24-h enrichment period also produced the highest overall vitamin C concentration (843.96±56.31 μg g^{-1} dry weight). The highest vitamin C concentration among the individual treatment combinations was recorded in nauplii enriched with ICES30/4 containing 20% AP for 24 h, reaching 1063.80±48.00 μg g^{-1} dry weight. In contrast, the unenriched control contained only 294.30±35.21 μg g^{-1} dry weight. These results demonstrate the strong capacity of the enrichment process to increase vitamin C accumulation in *A. urmiana* nauplii.

Total lipid

Lipid source significantly affected the total lipid content of the enriched nauplii. Nauplii enriched with sturgeon ovary oil contained 19.63±1.36% lipid on a dry-weight basis, whereas nauplii enriched with ICES30/4 contained 16.47±1.05%. Thus, the sturgeon ovary oil treatment resulted in a significantly higher total lipid content.

Saturated fatty acids

No significant differences were observed in total saturated fatty acid content as a result of enrichment source, AP concentration, or enrichment duration. This indicates that the experimental treatments primarily modified selected unsaturated fatty acid characteristics rather than producing a consistent change in the overall saturated fatty acid fraction.

Monounsaturated fatty acids

The concentration of monounsaturated fatty acids was significantly affected by AP concentration and enrichment duration. The highest overall MUFA concentration was observed with 10% AP (46.85±1.77 mg g^{-1} dry weight), whereas the 12-h enrichment period resulted in the highest overall concentration (47.40±2.56 mg g^{-1} dry weight). The highest value among the combined treatments was recorded in nauplii enriched with sturgeon ovary oil, 10% AP, and 12 h enrichment, reaching 51.48±2.80 mg g^{-1} dry weight.

DHA/EPA ratio

The DHA/EPA ratio was significantly affected by enrichment source and enrichment duration, whereas AP concentration did not exert a significant main effect. The overall DHA/EPA ratio was higher in nauplii enriched with ICES30/4 (0.49±0.03) than in those enriched with sturgeon ovary oil (0.30±0.02). The 24-h enrichment treatment produced a higher DHA/EPA ratio (0.42±0.03) than the 12-h treatment. The highest DHA/EPA ratio

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was observed in the ICES30/4 + 30% AP+24-h treatment, with a value of 0.60±0.03.

n-3/n-6 ratio

The n-3/n-6 ratio was significantly affected by enrichment source, AP concentration, enrichment duration, and their interactions. The overall n-3/n-6 ratio was significantly higher in the ICES30/4 treatment (5.70±0.24) than in the sturgeon ovary oil treatment. Among AP concentrations, 30% AP produced the highest overall n-3/n-6 ratio (4.63±0.13), while the 24-h enrichment treatment resulted in the highest value (4.73±0.20). The highest combined treatment value was observed in nauplii enriched with ICES30/4, 30% AP, and 24 h, reaching 6.29±0.24.

Discussion

The present study demonstrates that the biochemical composition of *Artemia urmiana* nauplii can be substantially influenced by the source of enrichment lipid, ascorbyl palmitate (AP) concentration, and enrichment duration. The responses observed for vitamin C, total lipid, monounsaturated fatty acids, DHA/EPA ratio, and n-3/n-6 ratio indicate that the nutritional characteristics of enriched nauplii depend not only on the composition of the enrichment medium but also on the duration of exposure.

Vitamin C enrichment

The concentration of vitamin C in the enriched nauplii was strongly affected by the enrichment lipid source, AP concentration, and enrichment duration. ICES30/4 produced a higher overall vitamin C concentration than sturgeon ovary oil, while 20% AP resulted in the highest mean vitamin C concentration among the three AP levels. The highest concentration was obtained after 24 h of enrichment. Most notably, the combination of ICES30/4, 20% AP, and 24 h resulted in 1063.80±48.00 μg g^{-1} dry weight, compared with 294.30±35.21 μg g^{-1} dry weight in the unenriched control. The higher vitamin C accumulation obtained with ICES30/4 may be related to the physical and chemical characteristics of the commercial enrichment emulsion. An effective enrichment medium must allow the nutrient to remain sufficiently dispersed in the water column and be readily ingested by *Artemia*. The lipid-soluble nature of AP may also facilitate its incorporation into *Artemia* through lipid droplets associated with the enrichment emulsion. Differences in lipid composition, particle characteristics, stability, and nutrient availability between ICES30/4 and sturgeon ovary oil could therefore explain the different vitamin C accumulation observed in the present study. The increase in vitamin C with enrichment duration is consistent with the principle that longer exposure provides additional opportunity for ingestion and accumulation of the supplemented nutrient. However, the absence of a simple proportional increase at all AP concentrations suggests that enrichment efficiency is not determined solely by the amount of

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AP supplied. Uptake, digestion, incorporation, metabolism, and possible degradation of the vitamin may all contribute to the final concentration measured in the nauplii. The high vitamin C concentration obtained in the ICES30/4+20% AP+24-h treatment is consistent with observations from the same research program and with the findings of Noshirvani *et al*. (2006), who demonstrated that enrichment of *A. urmiana* with an emulsion containing ascorbyl palmitate significantly increased tissue ascorbic acid concentrations. The present results further demonstrate that the response depends on the interaction between enrichment medium, AP concentration, and exposure time rather than on AP concentration alone. The lower mean value obtained with 30% AP compared with 20% AP is also noteworthy. Increasing the concentration of an enrichment component does not necessarily result in a proportional increase in its incorporation into *Artemia*. At higher concentrations, changes in emulsion properties, nutrient availability, ingestion efficiency, or metabolic regulation may reduce the efficiency of transfer. Therefore, optimization of the enrichment concentration is preferable to simply maximizing the concentration of the supplemented nutrient.

Total lipid and fatty acid composition

Sturgeon ovary oil produced a significantly higher total lipid content in the nauplii than ICES30/4. This result is consistent with the relatively high lipid contribution of the sturgeon ovary oil and its fatty acid composition. The enrichment medium itself contained different proportions of saturated, monounsaturated, and polyunsaturated fatty acids, and these differences were reflected to some extent in the biochemical composition of the enriched nauplii. The fatty acid composition of the two enrichment sources differed markedly. ICES30/4 contained substantially higher DHA and total n-3 fatty acids, whereas sturgeon ovary oil contained higher proportions of monounsaturated fatty acids and ARA. These differences provide a plausible explanation for the contrasting fatty acid profiles observed in the enriched nauplii. Hafezieh *et al*. (2008) similarly demonstrated that enrichment with ICES/30/4 could increase the lipid and n-3/n-6 fatty acid characteristics of *A. urmiana*. The absence of a significant effect of the experimental factors on total saturated fatty acids suggests that this fraction was comparatively stable under the enrichment conditions tested. In contrast, monounsaturated fatty acids responded to AP concentration and enrichment duration. The highest mean MUFA content was observed with 10% AP and after 12 h of enrichment, while the combination of sturgeon ovary oil, 10% AP, and 12 h produced the highest individual value (51.48±2.80 mg g^{-1} dry weight). This pattern is consistent with the relatively high MUFA content of the sturgeon ovary oil. The response of fatty acids to enrichment should not, however, be interpreted solely as direct dietary transfer. *Artemia* can modify

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ingested fatty acids through endogenous metabolic processes, and the extent of modification may depend on developmental stage, nutritional status, enrichment duration, and the composition of the supplied lipid. Samaee and Estévez (2025) similarly demonstrated substantial changes in the fatty acid profile of *A. franciscana* following enrichment with different lipid sources, supporting the view that both dietary incorporation and endogenous metabolism contribute to the final fatty acid profile.

DHA/EPA ratio

The DHA/EPA ratio was significantly affected by enrichment source and enrichment duration, whereas AP concentration did not produce a significant main effect. Nauplii enriched with ICES30/4 had a higher overall DHA/EPA ratio than those enriched with sturgeon ovary oil, which is consistent with the substantially higher DHA concentration of ICES30/4 relative to its EPA concentration. The highest DHA/EPA ratio observed in the combined treatment was 0.60±0.03 for ICES30/4 containing 30% AP and enriched for 24 h. This result illustrates that the fatty acid balance of the enrichment medium can remain an important determinant of the nutritional profile of the resulting live feed even when the primary experimental objective is vitamin C enrichment. The significant effect of enrichment duration indicates that the fatty acid profile may continue to change during the enrichment period. Nieves-Soto *et al*. (2021) reported that the incorporation and subsequent decline of ARA, EPA, and DHA in *A. franciscana* varied with enrichment duration. Their findings support the interpretation that the final fatty acid composition reflects a dynamic balance between uptake and subsequent metabolic utilization or loss of fatty acids.

n-3/n-6 fatty acid ratio

The n-3/n-6 ratio was significantly affected by enrichment source, AP concentration, enrichment duration, and their interactions. The overall ratio was higher in nauplii enriched with ICES30/4 than in those receiving sturgeon ovary oil, reflecting the markedly higher n-3/n-6 ratio of the former enrichment source. The highest combined-treatment value was obtained with ICES30/4, 30% AP, and 24 h of enrichment (6.29±0.24). This finding indicates that the enrichment protocol can modify not only individual fatty acids but also the relative balance between n-3 and n-6 fatty acid families. Such balance is nutritionally relevant because both fatty acid families participate in membrane structure and physiological regulation, while their relative availability can influence the biochemical pathways through which long-chain fatty acids are utilized. The present findings are also in agreement with the earlier work of Hafezieh *et al*. (2008), who demonstrated that ICES/30/4 could improve the n-3/n-6 characteristics of *A. urmiana*. More recent studies have likewise shown that the fatty acid composition of enriched

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Artemia depends strongly on the composition of the enrichment medium and the duration of enrichment (Roo *et al*., 2023; Samaee and Estévez, 2025).

Relevance to the use of A. urmiana as live feed

The findings of the present study should be considered in the context of earlier work from the same research program. Hafezieh *et al*. (2010) investigated enriched *A. urmiana* primarily as a nutritional vehicle for Persian sturgeon larvae and reported effects on larval growth, survival, fatty acid composition, and salinity tolerance. An earlier conference contribution also examined combinations of ICES30/4, sturgeon ovary oil, AP concentration, and enrichment duration. Those studies addressed, in particular, the subsequent biological response of fish larvae to enriched live feed. In contrast, the present manuscript focuses on the biochemical characteristics of the *A. urmiana* nauplii themselves. This distinction is important because characterization of the live-feed vector provides information that can be used to interpret subsequent feeding responses. Nouri *et al*. (2011), for example, demonstrated that *A. urmiana* nauplii enriched with HUFA and vitamin C could affect growth, survival, and fatty acid composition of *Acipenser persicus* and *Huso huso* larvae. The nutritional characteristics measured in the present study therefore provide a biochemical basis for selecting enrichment protocols for subsequent feeding experiments. Overall, the results indicate that no single enrichment condition maximized all nutritional characteristics simultaneously. ICES30/4 was particularly effective for vitamin C accumulation and for maintaining a relatively high DHA/EPA and n-3/n-6 profile, whereas sturgeon ovary oil promoted higher total lipid and MUFA contents. Therefore, the selection of an enrichment protocol should depend on the specific nutritional objective and target species rather than on a single measure of enrichment efficiency. It should also be recognized that the present study evaluated the biochemical composition of the nauplii immediately following enrichment and did not directly assess nutrient retention after feeding, digestion, assimilation, or subsequent deposition in fish tissues. Further feeding trials are therefore required to determine the biological significance of the biochemical differences observed among enrichment treatments.

Conclusions

The present study demonstrates that lipid source, ascorbyl palmitate concentration, and enrichment duration can substantially influence the biochemical composition of *Artemia urmiana* nauplii. The effects were particularly evident for vitamin C concentration, total lipid, monounsaturated fatty acids, DHA/EPA ratio, and n-3/n-6 fatty acid ratio. ICES30/4 was particularly effective for vitamin C enrichment and produced a higher DHA/EPA and n-3/n-6 profile, whereas sturgeon ovary oil resulted in

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higher total lipid and monounsaturated fatty acid contents. The combination of ICES30/4, 20% ascorbyl palmitate, and 24 h of enrichment produced the highest vitamin C concentration (1063.80±48.00 μg g^{-1} dry weight), compared with 294.30±35.21 μg g^{-1} dry weight in the unenriched control. The highest DHA/EPA and n-3/n-6 ratios were obtained under enrichment conditions involving ICES30/4 and longer enrichment duration. These findings indicate that optimization of the enrichment medium and exposure period can substantially modify the nutritional characteristics of *A. urmiana* nauplii. The results provide a biochemical basis for selecting enrichment protocols according to the nutritional requirements of the intended aquaculture application. Because the present study focused on the biochemical composition of the nauplii rather than subsequent larval performance, the observed differences should not be interpreted as direct evidence of improved growth or survival of cultured fish. Further feeding experiments are warranted to determine how the nutritional differences identified among enrichment treatments affect nutrient assimilation, tissue fatty acid deposition, growth, survival, stress tolerance, and other physiological responses in target aquaculture species.

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