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Scientific Article

Can commercial aquafeeds improve the nutritional value of brine shrimp? Proximal composition and lipid profile of alternative enhancers

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ABSTRACT

Artemia, commonly known as brine shrimp, holds a significant role as live prey in ornamental and commercial fish aquaculture. This study addressed the nutritional deficiency in adult *Artemia* biomass, exploring alternative enrichers available in Brazil. Evaluating a commercial aquafeed (Polinutre Poli Camarão 400PL) and a lipid emulsion (BioViv HUFA Continum), adult *Artemia* were subjected to different enrichment protocols, including periods of 1, 12, and 24 hours with each enricher, as well as a test group raised 100% on the commercial feed. Protein content for *Artemia* exclusively fed the commercial aquafeed (CF100) reached values very similar to those provided by the main commercial products available worldwide and other commonly used live prey. Enrichment time effects were observed from 12 h onwards, showing positive impacts on protein accumulation. Lipid content peaked at 12 h, followed by a decrease at 24 h. Further studies are needed to assess whether the combination of feed-based protocols followed by 12-h lipid emulsion enrichment can surpass the current results, providing high levels of protein content while maintaining a complete lipid profile.

Keywords: *Artemia*; Aquaculture; Protein content; Fatty acid profile; Alternative feeds.

Os *aquafeeds* comerciais podem melhorar o valor nutricional do camarão-de-salmoura? Composição proximal e perfil lipídico de melhoradores alternativos

RESUMO

Este estudo aborda a deficiência nutricional na biomassa de *Artemia* adulta, explorando enriquecedores alternativos disponíveis no Brasil. Avaliando ração comercial (Polinutre Poli Camarão 400PL) e uma emulsão lipídica (BioViv HUFA Continum), *Artemia* adultas foram submetidas a sete protocolos de enriquecimento. A análise da composição revelou melhorias significativas na densidade energética e no teor de proteína para *Artemia* alimentada exclusivamente com ração comercial (CF100), com valores muito semelhantes aos dos principais produtos comerciais disponíveis, incluindo Algamac-3050, Super Selco, e DHA Protein Selco, além de diversas opções alternativas, como farinha de arroz e soja, esterco suíno e uma mistura de microalgas. Efeitos do tempo de enriquecimento foram observados a partir de 12 horas, mostrando impactos positivos no acúmulo de proteínas. O conteúdo lipídico atingiu o pico às 12 horas, seguido por uma diminuição às 24 horas. O estudo sugere que as alternativas mais eficazes entre as avaliadas são os tratamentos CF100 e LE12, mas que estudos adicionais são necessários para avaliar se a combinação de protocolos, fornecendo altos níveis de proteína enquanto mantêm um perfil lipídico completo.

Palavras-chave: *Artemia*; Aquicultura; Teor de proteínas; Perfil de ácidos graxos; Alimentos alternativos.

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INTRODUCTION

Commonly referred to as brine shrimp, *Artemia* sp. represents a genus of crustaceans that holds a fundamental role in both ornamental and commercial aquaculture due to its ease of propagation, and widespread acceptance across diverse aquatic species (Bengtson et al., 2018; Kandathil Radhakrishnan et al., 2020, Wang et al., 2022; Wee et al., 2021). Despite the popularity of *Artemia* as a live feed, literature addressing nauplii stages are more abundant than those available for adult brine shrimp. This gap becomes critical given the reliance of numerous adult ornamental species, including seahorses (Planas et al., 2020; Planas et al., 2021), mandarin fish (Pratoomyot et al., 2016; Wang et al., 2022), besides a range of freshwater species (Lipscomb et al., 2020), and related zoological institutions (Wee et al., 2021) on larger live prey.

Despite its extensive application as a food source, *Artemia*'s nutritional profile is acknowledged to be deficient, particularly in terms of essential fatty acids (Ramos-Llorens et al., 2023). Brine shrimp are non-selective filtering microcrustaceans whose metabolic rate depends on the environmental temperature; therefore, their nutritional value is not constant, with possible variations regarding geographic region, culture time, temperature, and feeding (Kara et al., 2004; Moraiti-Ioannidou et al., 2007). In this sense, there are several research scoping alternative food items or formulas that could enrich *Artemia* via bioencapsulation, ensuring a more balanced nutritional profile to achieve better results and productive viability (Conceição et al., 2010; Kandathil Radhakrishnan et al., 2020; Novelli et al., 2016; Samat et al., 2020). Among the options presented so far, good results were achieved from commercial formulas rich in essential fatty acids and with a mix of pure microalgae, such as *Isochrysis galbana*, *Pavlova lutheri*, and *Nannochloropsis oculata* (Planas et al., 2008; Planas et al., 2017). While established commercial enrichers are available, the limited accessibility to such technologies via importation, notably in Latin America, and its short storage period underscore the need to evaluate alternatives in the local market.

In parallel, the range of commercial feed options in aquaculture has slowly increased over the years (Tacon & Metian, 2015). These feeds usually present balanced levels of nutrients and adequate lipid profiles, besides additives such as probiotics, essential oils, and vitamin premix. Whereas *Artemia* are non-selective filtering crustaceans, the use of commercial feeds in adequate granulometry can be promising in the culturing and improve its nutritional value as a food source (Marinho-Soriano et al., 2011). Thus, even if the target species does not accept inert food, *Artemia* might act as a nutrient conductor, outsourcing the offer in the different rearing stages.

Thus, this study aimed to tackle these intricate challenges by conducting a thorough analysis of the nutritional composition and lipid profile of potential maintenance diets designed for adult ornamental species relying on fresh or live food sources. By exploring the influence of alternative enrichers available on the local market, the study aimed to provide insights, laying the groundwork for customized feeding solutions to enhance the nutritional quality of *Artemia* and similar live feeds. Intending to evaluate and compare the effect of alternative enhancers available in Brazil on the proximate composition of cultured brine shrimp, a commercial aquafeed (Polinutre Poli Camarão 400PL) and a lipid emulsion designed for the aquarium hobby (BioViv HUFA Continum) were tested. The objective was to establish a bioencapsulation protocol that enhances nutrient retention, particularly focusing on protein and lipids, by testing whether these two locally available products could serve as viable options for improving the nutritional profile of *Artemia*, given the limited availability of specialized enrichers in Brazil.

METHODS

Treatments evaluated in this study are described in Table 1.

BioViv HUFA Continum is available from Continuum Aquatics and is presented in the form of lipid emulsion, providing essential lipid and vitamin complex, essential omega-3 fatty acids, DHA, and EPA. Limits are not specified on the label.

Commercial feed Polinutri Poli Camarão 400PL is available from the Brazilian company Polinutri Alimentos S.A., presented as 0.4-mm granules, containing 13% moisture, 40% protein, 8% lipids, 12.5% ashes, 4.5% of fiber content, 18–28% Ca, 12.5% P, 150 UI of vitamin E, 150 mg of vitamin C and 0.2 mg of Se.

Artemia used in this study were grown for 20 days according to the conditions proposed by Lavens and Sorgeloos (2018) and fed oat flour, except for sample CF100, in which feeding was based on the commercial feed throughout the culturing period. At harvest, live adult *Artemia* were filtered using a 200-µm mesh, weighed, and redistributed randomly and homogeneously among six 10-L plastic containers; each container received 150 g of adult *Artemia*. Recipients were provided aeration throughout the different enrichment times. For control samples and CF100, 150 g of live adult *Artemia* were washed in running water and conserved shortly after harvesting.

For treatments CF1, CF12, and CF24, 4 g of diluted commercial feed was used per container, previously homogenized in 500 mL of fresh water, configuring a dilution of 0.4 g per liter. For treatments LE1, LE12, and LE24, the use of 0.5 mL of

Table 1. Alternative enriching formulas and protocols tested for adult *Artemia* sp.*.

Treatment	Description
Control	Adult *Artemia* fed oat flour
CF1	Adult *Artemia* enriched for 1 h with the commercial feed Polinutre Poli Camarão 400PL
CF12	Adult *Artemia* enriched for 12 h with the commercial feed Polinutre Poli Camarão 400PL
CF24	Adult *Artemia* enriched for 24 h with the commercial feed Polinutre Poli Camarão 400PL
LE1	Adult *Artemia* enriched for 1 h with commercial emulsion BioViv HUFA Continum
LE12	Adult *Artemia* enriched for 12 h with commercial emulsion BioViv HUFA Continum
LE24	Adult *Artemia* enriched for 24 h with commercial emulsion BioViv HUFA Continum
CF100	Adult *Artemia* fed exclusively on commercial feed Polinutre 400PL for 20 days

Cists were acquired from the Brazilian company Artemia Salina, from Rio Grande do Norte, Brazil, sold as *Artemia salina*.

emulsion per container was adopted, configuring 1 drop per liter, following the instructions provided on the label. The containers were identified according to the enrichment time, 1 h, 12 h, and 24 h, which were inoculated and enriched at the same initial time. All containers were identical, comprising conical plastic structures with 23 cm in height and 28 cm in diameter. Recipients were all exposed to the same natural environmental conditions so the only difference between the innocuous was the harvest time.

Natural seawater was sourced from the local bay near the Núcleo de Pesquisa e Desenvolvimento do Litoral Norte, Instituto de Pesca in Ubatuba (SP), Brazil, with recorded conditions of 27ºC, 35 ppm salinity, and pH of 8.3. All recipients were kept on an outdoor patio, provided a natural photoperiod (13:11), but protected from direct sunlight by shady areas. In the stipulated time for each treatment, *Artemia* was once again filtered with the 200-µm mesh and washed in running fresh water to remove any excess product.

All samples were analysed on lipid profile and chemical composition according to the methodology described by AOAC (1965). Due to the high moisture content of the samples, crude protein (CP) and lipid content (EE) were determined after lyophilization. The analyses were carried out in triplicate from subsamples of the biomass of each treatment. Protein content was determined by the Kjedhal's method, by determining the total nitrogen with the conversion of the result into CP by factor 6.25, using the defatted dry extract of the sample. Total lipids were cold extracted by the Folch's method. Carbohydrates (Nifext fraction) were determined by difference, and the total caloric value was calculated from the corresponding caloric coefficients for proteins and lipids, which are 4 and 9 kcal/g, respectively. Moisture and ash were determined from the weight difference after dehydration and burning of non-lyophilized subsamples in the oven and muffle, respectively.

For the identification of fatty acids (FA), 1-g samples of each treatment, frozen at -20ºC in pre-identified *falcons*, were used in duplicate. Lipids were extracted with chloroform:methanol:water (2:1:0.5) according to Folch et al. (1957), as described in Saini et al. (2021). The lipid extract was methylated with acetyl chloride (5% HCl in methanol) (Christie, 2003). The FA composition was analysed as methyl esters using a Scion model 436 gas chromatograph equipped with a flame ionizer (FID) and auto-injector, using hydrogen as carrier gas with a flow rate of 22 mL/min. The capillary column for analysing the FA was a CP Wax 52 CB, 0.25-µm thick, 0.25-mm internal diameter, and 30-m long. The following temperature program was used: 170°C for 1 minute, followed by a ramp of 2.5°C/minute until reaching 240°C, and a final waiting time of 5 minutes, summing up 31 minutes. The temperature was 250°C in the injector and 260°C in the FID. FA standards in the form of methyl esters (FAME) (Supelco, 37 components and Larodan) were used to identify FA based on retention time. Quantification was carried out by normalizing the area, expressing the result as a percentage of the area of each acid over the total area of FA (%).

Statistical analysis

The experimental design followed a completely randomized design. Each treatment generated three biological replicates, with analyses performed in triplicate. All statistical analyses were conducted using the R software version 3.5.1. Means and standard deviations were calculated assuming a significance value of 5%. Data was submitted to an analysis of variance (ANOVA) to identify global mean variations, with the subsequent application of Bartlett's test assessing the homogeneity of variances. Further evaluations leading to means comparison proceeded via either Tukey's or Games-Howell's test, contingent on variance homogeneity. Interpretation hinged

on *p*-values and confidence intervals, deeming significant differences if *p*-values fell below a predefined threshold (e.g., α = 0.05). Model validation included scrutinizing the normality of residuals and the independence of data.

RESULTS

The means values of the proximate composition of each treatment are expressed in Table 2. Overall, adult *Artemia* fed exclusively on commercial feed Polinutre 400PL for 20 days (CF100) showed significant improvement in energy density (caloric coefficient) and protein content and lower levels of carbohydrates and lipids (Fig. 1).

Although the mean composition values of *Artemia*, except for CF100, provided similar data regardless of the type or time of enrichment, when comparing only the treatments that aim to enrich *Artemia* at different times (1, 12, and 24 h), it is possible to observe significant effects of enrichers from 12 h onwards ($p < 0.05$). As for protein levels, time showed a positive effect

Table 2. Proximal analysis of *Artemia* samples submitted to each protocol proposed. Moisture, lipids, ash, crude protein, and carbohydrate contents are expressed in percentage (g•100g^{-1} dry weight); caloric coefficient is expressed in Kcal/kg*.

Treatment	Moisture Mean ± SD	Lipids Mean ± SD	Ashes Mean ± SD	Crude protein Mean ± SD	Carbohydrates Mean ± SD	Caloric coefficient Mean ± SD
Control	90.15 ± 0.06 ab	13.36 ± 0.33 a	12.91 ± 0.31 a	36.27 ± 0.36 a	37.46 ± 0.32 a	265.27 ± 4.36 a
CF1	89.56 ± 0.02 a	13.41 ± 0.09 a	17.71 ± 0.08 b	34.87 ± 0.61 a	34.01 ± 0.55 ab	260.19 ± 3.27 a
CF12	89.55 ± 0.01 a	14.61 ± 0.01 a	16.30 ± 0.10 b	36.56 ± 0.21 a	32.54 ± 0.25 b	277.69 ± 0.89 bc
CF24	88.58 ± 0.09 a	14.26 ± 0.29 a	16.17 ± 0.11 b	36.78 ± 0.46 a	32.80 ± 0.53 b	275.44 ± 4.43 b
LE1	89.35 ± 0.06 a	13.44 ± 0.22 a	16.24 ± 0.04 b	34.20 ± 0.49 a	36.11 ± 0.56 a	257.76 ± 3.94 a
LE12	90.00 ± 0.08 ab	15.13 ± 0.34 a	16.23 ± 0.11 b	35.76 ± 0.13 a	32.88 ± 0.52 b	279.20 ± 3.57 c
LE24	90.66 ± 0.04 ab	14.94 ± 0.30 a	19.05 ± 0.16 c	35.97 ± 0.15 a	30.04 ± 0.53 c	278.30 ± 3.26 bc
CF100	92.44 ± 0.08 b	09.64 ± 0.36 b	14.38 ± 0.69 ab	56.58 ± 0.94 b	19.40 ± 1.28 d	313.12 ± 7.04 d

SD: standard deviation; *values followed by different letters in the same column differ by Tukey's test ($p < 0.05$).

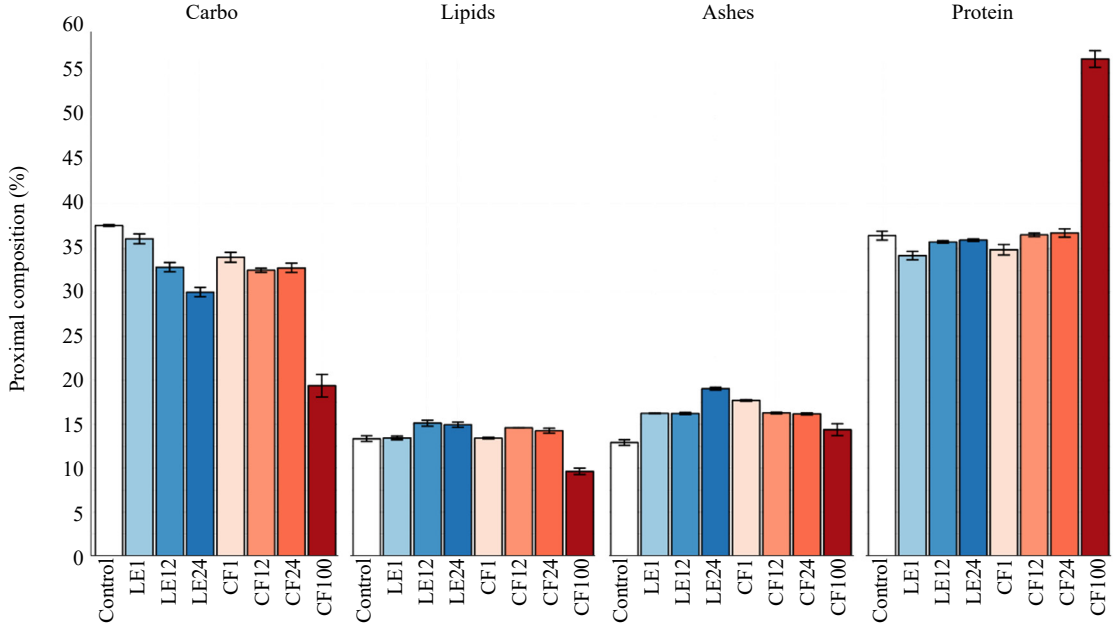


Figure 1. Effect of enrichment time on proximal composition. Proximal analysis of *Artemia* samples submitted to each protocol proposed. Crude protein, lipids, ash, and carbohydrate contents are expressed in percentage (g·100g^{-1} dry weight). Shades of blue indicate treatments based on lipid emulsion and red shades, commercial aquafeed.

on the accumulation of nutrients (Fig. 2a). For lipid content, incorporation differed from those observed for the other nutrients, with the accumulation peak being observed at 12 h of enrichment, followed by a decrease in the contents at 24 h (Fig. 2b).

Regarding lipid profile, 30 FA were detected among the treatments, in addition to an unidentified chain. Considering the difference in the lipid content of the samples and aiming to standardize the data, the values obtained as a percentage between the chains by the chromatogram were adjusted according to the lipid content of each sample and are expressed in percentage to the natural matter or $g \cdot 100g^{-1}$.

As expected, the higher lipid content of samples based on *Artemia* reflected the accumulation of smaller chains with fewer unsaturation, with emphasis on peaks of C18:1n9 (oleic) and C18:2n6 (linoleic), common to all treatments. The most common concentrations found were palmitic acid (12–14%), oleic (32–39%), and linoleic (26–29%) to the total lipid content. In treatment CF100, accumulations were mainly based on palmitic (12–13%), 7-octadecenoic (26–27%), linoleic (9–10%), and γ linolenic acids (22%).

Among the lipid profiles, there was a significant difference ($p < 0.05$) between the accumulation of FA when categorized by saturation and chain size (Table 3, Fig. 3). All treatments showed a higher proportion of long-chain polyunsaturated fatty acids (PUFA and HUFA).

When considering only the most prominent FA in the nutrition of aquatic organisms–oleic, linoleic, α-linolenic, arachidonic, eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids–, it is possible to notice two trends of accumulation among treatments (Figs. 4a and 4b). In general, except for CF100, treatments based on *Artemia* sp. tend to a marked accumulation of linoleic and α-linolenic acids. For CF100, no measurable concentrations of α-linolenic, arachidonic, or DHA were observed, but higher rates of EPA accumulation were found. For treatments CF12, CF24, LE1, LE24, and CF100, the presence of DHA was not identified (Fig. 4b). Regarding enrichment time (1 h, 12 h, and 24 h), there was no statistical difference between the different times or between the enrichers evaluated for oleic, linoleic, α-linolenic, arachidonic, EPA, and DHA acids when compared to the control group.

DISCUSSION

The proximal composition of enriched *Artemia* differed from that reported by Balachandar and Rajaram (2019), Segade et al. (2016), Woods (2003), and Woods and Valentino (2003), showing 7.30% lower protein content and 5.64% higher values for lipids and carbohydrates (19.71%). For CF100, however, although still below the values found by Segade et al. (2016), protein content was very similar to those observed by Anh et al. (2009), Balachandar and Rajaram (2019), Woods (2003), and Woods and Valentino (2003).

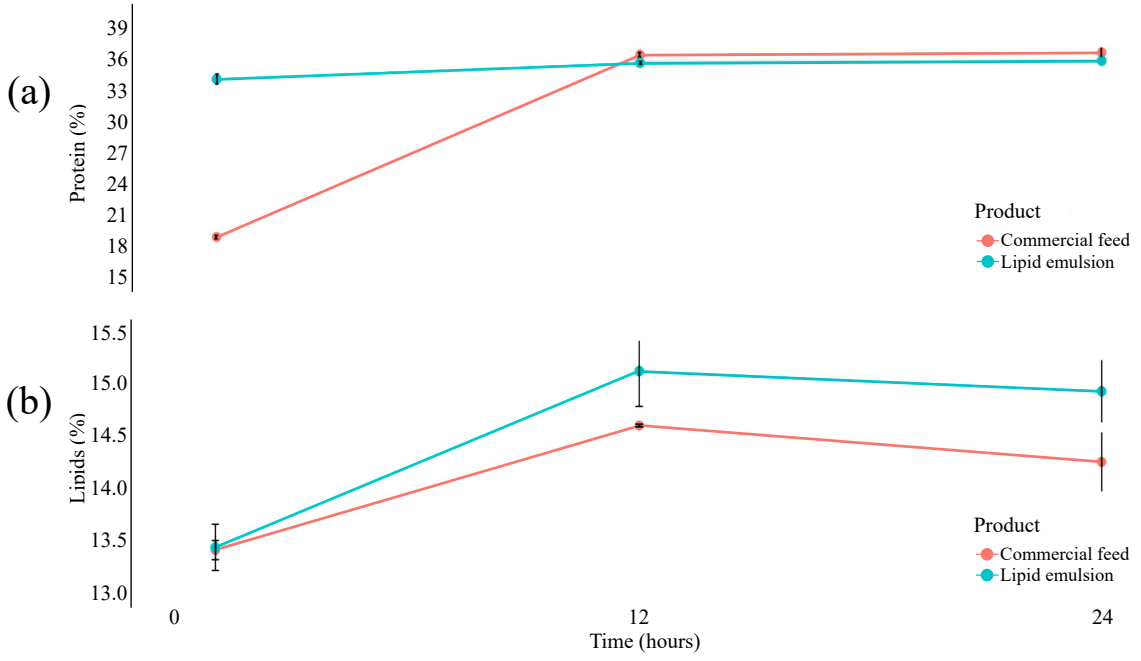
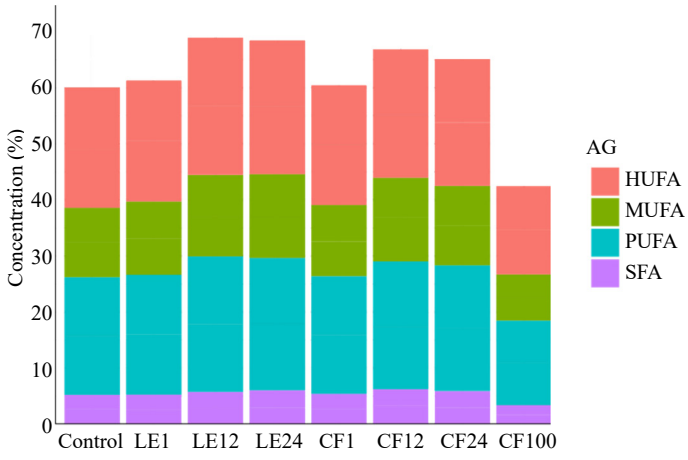


Figure 2. Effect of time on the accumulation (a) of protein and (b) lipids in the proximal composition of cultured brine shrimp by the products evaluated. Black bars indicate the standard deviation of the data. Each treatment was evaluated in triplicates.

Table 3. Comparison of the accumulation of fatty acids categorized by saturation and chain length for the different treatments. The values are expressed in percentage to the wet weight*.

	SFA		MUFA		PUFA		HUFA	
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Control	2.62	± 0.22 a	6.18	± 0.26 a	10.48	± 0.46 a	10.74	± 0.60 a
CF1	2.71	± 0.18 a	6.36	± 0.26 a	10.48	± 0.54 a	10.7	± 0.64 a
CF12	3.12	± 0.34 a	7.47	± 0.62 b	11.37	± 1.62 b	11.47	± 1.70 bc
CF24	2.96	± 0.08 a	7.08	± 0.11 bc	11.21	± 0.24 b	11.3	± 0.26 ab
LE1	2.64	± 0.06 a	6.54	± 0.11 ac	10.67	± 0.27 a	10.8	± 0.30 a
LE12	2.88	± 0.07 a	7.29	± 0.20 bc	12.06	± 0.32 b	12.25	± 0.38 bc
LE24	3.03	± 0.12 a	7.48	± 0.29 bc	11.79	± 0.55 b	11.91	± 0.57 bc
CF100	1.69	± 0.70 b	4.12	± 1.76 d	7.55	± 2.18 c	7.87	± 2.26 d

*Means followed by different letters in the same column differ by the Games-Howell's test; SFA: the sum of the percentages of the saturated fatty acid chains; MUFA: the sum of the percentages of the mono-unsaturated fatty acids chains; PUFA: the sum of the percentages of the polyunsaturated fatty acid chains, with chains shorter than 20 carbons; HUFA: the sum of the percentages of polyunsaturated fatty acid chains with chains equal to or greater than 20 carbons.



HUFA: the sum of the percentages of polyunsaturated fatty acid chains with chains equal to or greater than 20 carbons; MUFA: the sum of the percentages of the mono-unsaturated fatty acids chains; PUFA: the sum of the percentages of the polyunsaturated fatty acid chains, with chains shorter than 20 carbons; SFA: the sum of the percentages of the saturated fatty acid chains.

Figure 3. Effect of enrichment time on the accumulation of fatty acids categorized by saturation and chain length for the different treatments. The values are expressed in percentage to the wet weight.

Protein concentrations were similar to those found in live and frozen mysis and adult *Artemia* enriched for 24 hours with the main commercial products available, including Algamac-3050, Super Selco, and DHA Protein Selco, besides a variety of alternative feedings, such as rice and soy flour and pig manure. Yet, both protein and lipid content of CF100 were higher than those presented by Pratoomyot et al. (2016) for adult farmed *Artemia* fed a mix of microalgae. These variations can be related to the quantitative and qualitative availability of enrichers under the effect of time, as well as the accumulation of nutrients in the composition of *Artemia* regarding its need to mobilize body reserves. As a result, a gradual increase in nutrient accumulation can be observed at the initial times, followed by a drop in values as the time of harvesting increases, as observed for protein and lipid content in this and other studies (Anh et al., 2009; Segade et al., 2016).

There are only a few studies on the proximal composition of *Artemia* biomass, especially for the FA profile. Although there is no precise information regarding lipid requirements for most fish species, it is agreed that it is essential for HUFA chains to be supplied in the diet of marine fish, especially EPA and DHA, since PUFA and HUFA cannot be synthesized by fish *de novo*, although some species can convert 18-carbon PUFA to longer HUFA chains (Planas et al., 2021). Among the alternatives evaluated in this study, all treatments showed detectable EPA profiles in their composition. For DHA, however, only the treatments based on unenriched *Artemia* (control), *Artemia* enriched with commercial feed for 1 hour (CF1), and *Artemia* enriched with commercial emulsion for 12 hours (LE12) showed significant concentrations. Similarly, when evaluating farmed *Artemia* fed a mix of microalgae, Pratoomyot et al. (2016) did not detect measurable amounts of DHA.

Brine shrimp usually has naturally high concentrations of α-linolenic acid (ALA) and linoleic acid (LNA) and relatively low levels of arachidonic acid (ARA), EPA, and DHA (Joshua et al., 2022). Contents of FA chains found in this study differed

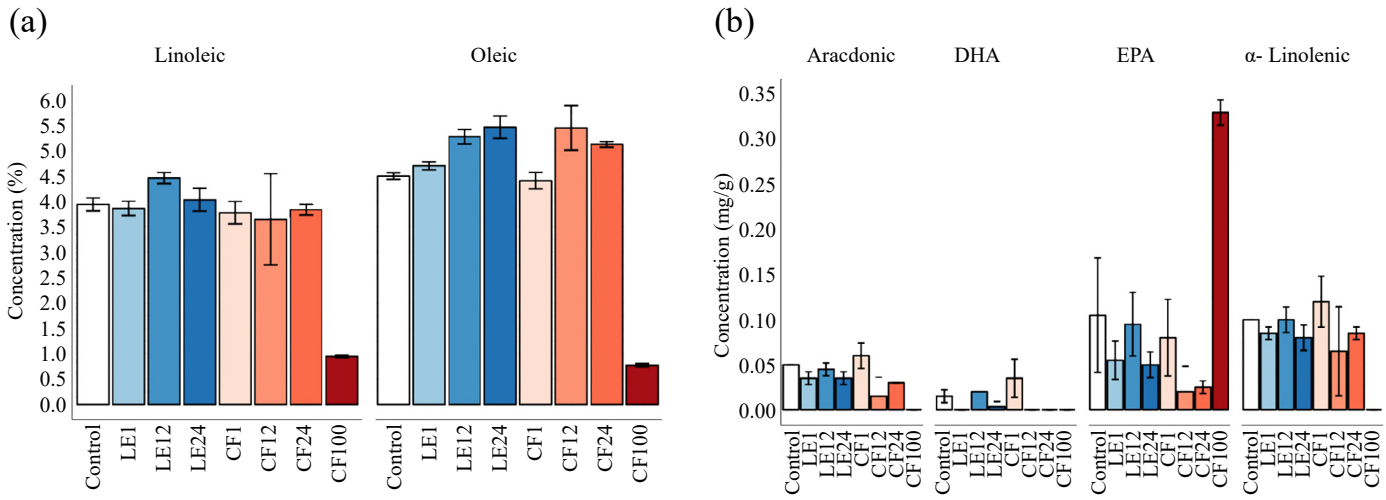


Figure 4. Effect of enrichment time on the accumulation of oleic, linoleic, α-linolenic, arachidonic, eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids. The values are expressed in the percentage of total lipid content in wet weight. (a) Accumulation of Linoleic and Oleic acids by treatment; (b) Fatty acids chain with 3 or more unsaturations.

from those indicated by Dendrinos and Thorpe (1987) and Ruiz et al. (2008), as higher values of C16:00, C18:1n9, C18:2n6 (LNA) and lower values of C18:3n3 (ALA), C20:5n3 (EPA) and C22:6 n3 (DHA) were found. For mono-unsaturated chains (MUFA), the FA profiles here described were in accordance with those pointed out by Balachandar and Rajaram (2019), Fábregas et al. (2001), Maldonado-Montiel and Rodríguez-Canché (2005), and Planas et al. (2021). As for C18:1n9 and LNA chains, the values found in this study were four to 10 times higher than the ones presented by those authors.

Regarding n-3 and n-6 FA requirements, on average, Mediterranean fish species require between 0.64 and 2.2% n-3 highly unsaturated fatty acid (n-3HUFA) in their broodstock diets (Izquierdo et al., 2001). For highly demanding species, such as seahorses, Nur et al. (2016) have observed that the minimum n-3 and n-6 FA requirements for *Hippocampus barbouri* reproduction are 5.13 and 14.83%, respectively. For mandarin fish (*Synchiropus splendidus*), Pratoomyot et al. (2016) had success in breeding with n-3 HUFA levels from 1.3 to 3.3%. In this study, CF100 showed an adequate concentration of n-3 for both commercial and ornamental fish species. For n-6, all treatments have reached the minimum limits.

Although there are numerous studies regarding the use of *Artemia* in aquaculture, there are not many that address the proximal composition of the *Artemia* biomass and the influence of enrichers beyond the nauplii stage (Anh et al., 2009; Balachandar & Rajaram, 2019; Fábregas et al., 2001; Woods, 2003). Considering that the enrichers evaluated in this work were not previously found in the literature, this study has contributed to the database of possible alternatives to be used in the enhancement of the nutritional value of *Artemia*. For future directions, the evaluation of the enrichers on the nauplii stage might be also an alternative for larviculture studies. As per the results, the use of commercial aquaculture feed in the culture of *Artemia* proved to be a promising option, with protein values close to those reached with imported formulas. The use of commercial feeds promotes higher rates of protein accumulation and might result in better yields on biomass production when compared to cultures based on natural productivity. Yet, despite the absence of DHA, for freshwater species, CF100 might dismiss the need for further enrichment, as the treatment showed an adequate concentration of n-3 and n-6.

Considering the overall results, the study suggested that the most effective alternatives among those evaluated are the CF100 and LE12 treatments. Further studies are needed to assess whether the combination of feed-based protocols followed by 12-hour lipid emulsion enrichment can surpass the current results, providing high levels of protein content while maintaining a complete lipid profile.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

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

FUNDING

Not applicable.

AUTHOR'S CONTRIBUTION

Conceptualization: Maganhe B; Formal **Analysis:** Maganhe B; **Investigation:** Maganhe B; **Resources:** Sanches EG; **Supervision:** Sanches EG; **Validation:** Sanches EG; **Data curation:** Sanches EG; **Writing – original draft:** Maganhe B; **Writing – review & editing:** Sanches EG, Maganhe B; **Final approval:** Maganhe B; Sanches E G.

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