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An attempt to improve the proximate composition of local *Artemia* strain (Wadi El Natrun, Egypt)

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Abstract

Background: Comparative analysis of essential amino acids and fatty acid of enriched and unenriched *Artemia spp.* collected from Wadi El Natrun, Elbeheira, Egypt, was investigated.

Results: The obtained results indicated that Leucine recorded the highest concentration (17.22%, 11.4%) in unenrichment *A. tunisiana* and *A. franciscana*, respectively followed by arginine (10.31%, 9.6%); however, glutamic acid recorded the lowest concentration (0.05%) in *A. tunisiana*. Statistical analysis showed no significant differences in some amino acid concentrations ($P < 0.001$) in both *A. tunisiana* and *A. franciscana* as arginine, valine, proline, and aspartic acid. Other amino acids were highly significantly fluctuated as glycine, leucine, histidine, phenylalanine, glutamic, and tyrosine; they were high in *A. franciscana*. Aspartic acid was significantly higher in unenrichment nauplii. Regarding the comparison between *A. franciscana* and enrichment *A. tunisiana*, the data showed non-significant improvement for the majority of the recorded amino acids. The analysis of unsaturated fatty acids n-3 and n-6 families after 18 and 24 h of enrichment *Artemia* was achieved. The composition of unsaturated fatty acids was significantly increased after enrichment to 79.7 mg/g DW instead of 46.5 in newly hatched nauplii. Saturated fatty acids were also significantly increased from 20 to 25.28 mg/g DW. The enrichment process was significant ($P < 0.001$) in nauplii enriched with fatty acids for 18 h. The more pronounced effect of duration was the amount of energy as it was 33.17 kJ/g after 18 h, while it was 45.693 kJ/g after 24 h incubation. The ratio of ascorbic acid, due to *Artemia* enrichment by vitamin C for 24 h, was increased from 853 to 3227 $\mu\text{g/g}$ dry weight after enrichment.

Conclusion: To conclude, enrichment with cod liver oil and vitamin C obviously affected the chemical composition of local Egyptian *Artemia* (*A. tunisiana*).

Keywords: *Artemia*, Fatty acids, Amino acids, Enrichment, Vitamin C

Background

Artemia is a cosmopolitan crustacean that has established itself worldwide in a harsh saline aquatic environment and gained a unique position in aquaculture system since it has several characteristics such as easy to handle, adaptable to wide environmental condition, non-selective filter feeder, and capable of growing at very high densities (Sivaji, 2016). Moreover, its high nutritive value meets both the dimensional (in terms of prey size) and nutritional requirements for the fish or finfish larva (Lubzens, Tandler, & Minkoff, 1989). The nutritional value of

Artemia can be improved by the enrichment process, thereby incorporating fatty acids and other compounds (vitamins, minerals, and antibiotics) into the larval rearing protocols.

Artemia strains from different geographical origins vary considerably in their nutritive values (Heydari & Akbary, 2011). These variations may have great influence on survival and growth of cultured fish and crustaceans (Salma, Davoodi, Shamsaei, & Kamali, 2013). Japanese, American, and European researchers studied these problems, and they confirmed variations in the nutritional value when using different geographical sources of *Artemia* for fish and shrimp species (Leger, Naessens-Foucquaert, & Sorgeloos, 1987). The situation became more critical when very significant differences in

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production yields were obtained with distinct batches of the same geographical origin of *Artemia* (Robin, Le Mili-naire, & Stephan, 1987). The important factor affecting the nutritional value of *Artemia* as food source for marine larval organisms is their contents of essential fatty acids, eicosapentaenoic acid (EPA, 20:5 n-3), and even more importantly docosahexaenoic acid (DHA, 22:6 n-3). In contrast to freshwater species, most marine organisms do not have the capacity to biosynthesize EPA from lower chain unsaturated fatty acids (Robin et al., 1987; Van Stappen, 1996).

Leger et al. (1986) and Robin et al. (1987) summarized the advantages of *Artemia* enrichment as they have higher energy content and contain all essential fatty acids n-3, which is generally absent in nauplii from all strains. Through enrichment techniques, other nutrients, prophylactics, and therapeutics may pass to predators via *Artemia* nauplii. Larvae fed on enriched *Artemia* are indeed healthier and more resistant to stressful conditions such as infections and weaning of fish, when transferring post larvae from hatchery tanks to nursery ponds.

Exploitation of *Artemia* in Egypt was started when Abdel Rahman and Abdel Razek (1992), Abdel Rahman (1993, 1994) evaluate some of local *Artemia* resources. In the same interval, by the help of FAO agency surveyed natural Egyptian *Artemia* from different sites; most of these sites are manmade salinas located on the Mediterranean coast, while only two sites are located in inland areas. The coastal sites are Port-Fouad, Balteim, El Max, and Bourg El Arab Salinas, while the inland sites are Wadi El Natrun and Fayom salina beside Qaroun Lake. Cross-breeding tests revealed that *Artemia* from Wadi El Natrun is belonging to *Artemia tunisiana*. The investigation completed by FAO (1994) established that the production of *Artemia* in Egypt is technically and economically feasible when *A. franciscana* has been inoculated in Port Fouad salina.

The main target of this paper was to investigate the effect of enrichment with cod liver oil and vitamin C on the chemical composition of local Egyptian *Artemia* (*A. tunisiana*) which has some deficiencies in order to introduce superior species to battle the imported ones and finally grow to be a successful economic activity.

Methods

Enrichment of *Artemia tunisiana*

This experiment was done in two labs; the enrichment part was done in the Central laboratory, Zoology Department, Faculty of Science, Tanta University, Egypt, and the biochemical analysis was done in the National Institute of Oceanography and Fisheries Central Laboratories Unit, Alexandria, Egypt. The dried cysts of the brine shrimp *Artemia*

franciscana were obtained from King British Aquarium Accessories Co Ltd., Bradford, UK, while local *Artemia* cysts (*A. tunisiana*) were collected from El Hamra Lake, Wadi El Natrun, Elbereira, Egypt.

Culture conditions

Both strains were hatched following standard procedures (Sorgeloos et al. 2001). Newly hatched *Artemia* nauplii (stage 0) (200,000 nauplii/l) were divided in batches in 5-l tanks. Enrichment protocol followed the method of Treece (2000). Briefly, 0.5 ml of the enrichment emulsion (assuming a density of 200 *Artemia* per ml) was added per liter of the incubation water at the onset of the enrichment period. Newly hatched *Artemia* nauplii served as the control. The enrichment medium maintained at 25 ± 2 °C. The enrichment emulsion was added at a dose of 0.5 g/l that was applied at two time intervals: after hatching and 18 h post hatching. Strong aeration was applied to maintain dissolved oxygen levels above 4 mg/l. Enrichment nauplii were harvested at 18 h and 24 h post hatching. The harvested samples were divided into three groups: group 1 for weighing, group 2 for measuring length, and group 3 for thoroughly rinsed and stored at temperatures below 10 °C in order to assure that highly unsaturated fatty acids (HUFAs) are not metabolized during storage. These samples were later used for further analysis.

Standard method for enrichment of *Artemia* nauplii with vitamin C

Enrichment with vitamin C is the second step to enrichment with HUFAs. Therefore, in order to enrich the live food with vitamins, fatty acid emulsion was used as carrier of this component. Fatty acid emulsion was prepared and 10% of Ascorbyl palmitate *w/w* was added to the fatty acid emulsion (Treece, 2000). Mixing was done using an electric mixer until the vitamin C is dissolved. The emulsion containing vitamin C was preserved in refrigerator until use. Nauplii were enriched with two doses of this mixture at 0 and 18 h during the process of enrichment.

Sample analysis

Proximate composition

Moisture, crude protein, crude lipid, and ash were measured using standard methods (Association of Official Analytical Chemists (AOAC), 1990).

Moisture

Moisture was determined by placing a thin layer of *Artemia* of known weight in an aluminum pan, and then placed in a drying oven at 90 °C. Samples were removed after 24 h, placed in a desiccator for 30 min to cool

down, and weighed. This procedure was repeated every 2 h until a constant weight was reached.

Protein content

Total organic nitrogen was determined by the Kjeldhal method.

Total lipids

Lipid extraction and analysis were performed using the Soxtec system, which is a modification of the Soxhlet principle. Crude freeze-dried samples were ground to a fine powder, weighed (approximately 3.0 g), placed in extraction thimbles, covered with cotton wool, and inserted in the Soxtec machine. Pre-weighed extraction cups with boiling chips and approximately 25–50 ml of hexane were also placed in the Soxtec system. Extraction was performed for 60 min in the “Boiling” position and, subsequently, for 30–45 min in the “Rinsing” position. After evaporation of the solvent, extraction cups were placed in the drying oven (at 100 °C) for 30 min. Cups were cooled in a desiccator and weighed. Crude fat could then be calculated as shown by Goddard (1992).

Ash

Crucibles were first washed, dried, incinerated (550 °C for 2 h), cooled in desiccator, and weighed. Samples were added to crucibles, weighed, and placed in a muffle furnace (250 °C for 2 h). Temperature was then raised to 550 °C and samples were left overnight. After cooling in a desiccator, crucibles with samples were again weighed and ash content calculated.

Carbohydrates

This group of nutrients—e.g., sugars and starches—was estimated as the difference between the sum of the other constituents and the original dry weight of the sample.

Amino acid analysis

Total amino acids Total amino acids were determined as described by Shahidi et al. (1990) and Blackburn (1978).

Free amino acids

For determination of free amino acids, freeze-dried samples were homogenized using a Polytron PT 3000 (Brinkmann Instruments, Rexdale) homogenizer in a small 50-ml centrifuge tube with 20 ml of ice-cold 6% perchloric acid for 2 min in an ice bath (Yamanaka, 1989).

Fatty acid analysis

Fatty acid composition of lipids was determined using gas chromatography (GC). Lipids were extracted with

chloroform and methanol, based on the method described by Bligh and Dyer (1959).

Statistical analysis

All results were represented as mean \pm SE ($n = 3$). Statistical comparisons between groups were made using a one-way analysis of variance (ANOVA) as part of an SPSS and a post hoc test followed by Dunnett’s multiple comparison tests. All p values were paired two-tailed, with p values < 0.05 considered significant.

Results

Proximate composition

The average proximate composition (in % dry basis) in milligrams per gram DW of the newly hatched *Artemia tunisiana*; Borg El Arab, El Mex, and Port Saied strains; and the imported American strain (*A. franciscana*) are shown in Table 1. The results showed that the Port Saied strain had the highest lipid composition followed by *A. franciscana* while the lowest lipid composition was scored by *A. tunisiana*. No differences were recorded in protein content in *Artemia* collected from the Wadi El Natrun, Borg El Arab, and San Francisco Bay strains. On the other side, the Port Saied strain had the highest protein content followed by El Mex. No differences in carbohydrate composition from all strains (~14%) except for Port Saied, it recorded 10.8%. The fiber content in both Port Saied and *A. franciscana* had the lowest values (4.9 and 3.7%, respectively) and decreased than the rest of the strains.

In the case of enrichment of *A. tunisiana* as shown in Fig. 1, the more noticeable elevated component was the lipid which increased to 21.53% after 24 h from enrichment process. The proximate composition of ash, carbohydrate, and fibers were significantly decreased in enrichment samples while the moisture ratio was significantly increased ($p < 0.05$).

Amino acid composition

Table 2 summarizes the free amino acids of unenrichment and enrichment (24 h) *A. tunisiana* comparable with *A. franciscana* as gram of amino acid per 100 g of protein in the sample. Leucine recorded the highest concentration (17.22 and 11.4%) in unenrichment *A. tunisiana* and *A. franciscana* respectively followed by arginine (10.31 and 9.6%). The lowest amino acid was glutamic acid (0.05%) and recorded with *A. tunisiana*. One-way ANOVA analysis shows non-significant differences in some amino acid concentration ($p < 0.05$) in both *A. tunisiana* and *A. franciscana* as arginine, valine, proline, and aspartic acid. While others were highly significantly fluctuated as glycine, leucine, histidine, phenylalanine, glutamic, and tyrosine, they were high in *A. franciscana*. As demonstrated in Table 2, some amino

Table 1 Average proximate composition (in % dry basis) mg/g DW of the newly hatched different *Artemia* strains

Strain	Moisture	Ash	Protein	lipid	Carbohydrate	Fiber	Sum (%)
<i>A. tunisiana</i>	64.3	7.54	54.46	12.59	13.8	8.71	97.1
Borg El Arab	69.52	5.27	53.81	13.96	14.18	8.21	95.43
El Mex	72.16	6.43	56.17	14.4	13.6	8.5	99.1
Port Saied	70.25	7.67	57.8	16.86	10.82	4.9	98.05
<i>A. franciscana</i>	63.8	7.11	53.5	15.7	13.7	3.7	98.76

acids were not affected by enrichment as arginine, glycine, valine, proline, and leucine. Others were affected; they were significantly higher as histidine, phenylalanine, glutamic, and tyrosine. In the case of aspartic acid, it was significantly higher in unenrichment nauplii. Regarding to the comparison between *A. franciscana* and enrichment *A. tunisiana*, the data showed non-significant improvement for the majority of the recorded amino acids.

Fatty acid composition and the effect of enrichment duration (18–24 h)

As demonstrated in Fig. 2, higher amounts of total essential fatty acids in the n-3 and n-6 families after 18 and 24 h of enrichment were achieved, for example, 18:2 ω -6 (linoleic acid), 18:3 ω -3 (linolenic acid), 18:4 ω -3 (octadecatetraenoic acid), 20:3 ω -3 (eicosatrienoic acid) beside 20:5 ω -3 (eicosapentaenoic acid) and 22:6 ω -3 (docosahexaenoic acid). The composition of unsaturated fatty acids was significantly increased after enrichment to 79.7 mg/g DW instead of 46.5 in newly hatched nauplii. Saturated fatty acids were also significantly increased from 20 to 25.28 mg/g DW.

As the fatty acids were taken up by *Artemia*, their profiles changed according to the duration of the enrichment

period from 0 to 18 h in Fig. 2. The enrichment process as depicted in Fig. 2 was significant ($P < 0.001$) in nauplii enrichment with fatty acids for 18 h, while the fatty acid composition was not significantly different due to the duration from 18 to 24 h, except for the two fatty acids 18:2 ω -6 and 18:3 ω -3. Newly hatched *Artemia* had 64 mg total fatty acids/g dry weight with no detectable levels of DHA (22:6 ω -3). After enrichment for 18 h, the total fatty acids increased significantly to 97 mg/g, then elevated to 112 mg/g dry weight after 24 h. The level of DHA in *Artemia* was significantly increased to reach 1.45 mg/g after 18 h, while another peak was noted (1.86 mg/g) after 24 h. The second important fatty acid (EPA) reached 8.13 mg/g DW after 24 h; it was 1.3 mg/g in unenrichment nauplii. The highest fatty acid was 18:1 ω -9 (oleic acid), it was 35.85 mg/g, followed by palmitic acid (16:0) which was 16.3 mg/g DW, then 18:3 ω -3 (13.46 mg/g DW) after 24 h of enrichment. The lowest fatty acid concentration was 14:0 (myristic acid) and did not change by 18 h enrichment, while slightly increased after 24 h of enrichment. When comparing the fatty acid composition between *Artemia franciscana* and *tunisiana* nauplii, the result as shown in Fig. 2 showed that there were significant differences between fatty acid composition between both species without enrichment; it was higher in *A. franciscana*

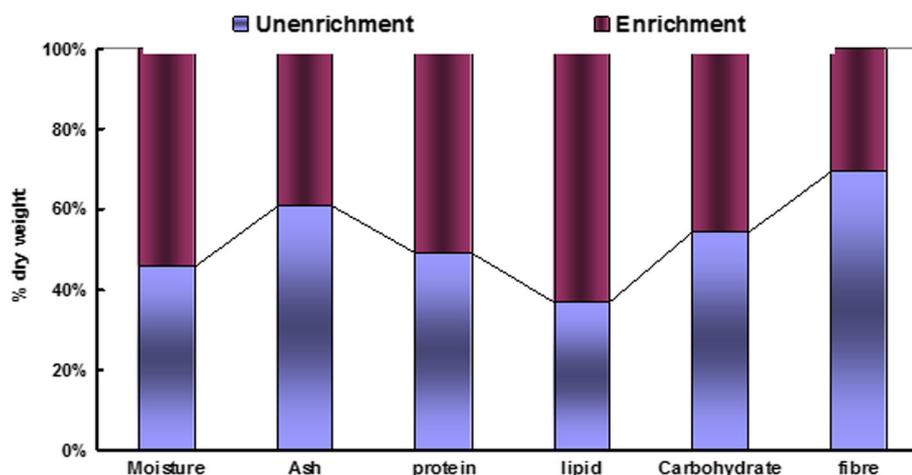
**Fig. 1** Biochemical composition (% dry weight) in newly hatched (unenrichment) and enrichment (24 h) *Artemia tunisiana* nauplii

Table 2 Amino acid composition (g amino acids/100 g) (% dry basis) of *A. franciscana* (A) and unenriched *Artemia tunisiana* (B), and enriched with HUFA + vitamin C (C) for 24 h

Amino acids	<i>A. franciscana</i> (A)	<i>A. tunisiana</i>	
		Unenriched (B)	Enriched (C)
Arginine	9.6	10.31	10.75
Glycine	4.3	1.7	2.04
Valine	6.31	6.41	5.7
Proline	5	5.10	7.21
Leucine	11.4	17.22	14.8
Histidine	2.7	1.04	3.11
Phenylalanine	8.6	2.49	4.76
Glutamic acid	9.76	0.05	1.5
Aspartic acid	9.3	9.8	4.31
Tyrosine	4.6	0.36	2.8

especially with 18:0, 18:1 ω-9, 18:3 ω-3, and 20:5 ω-3. Concerning enrichment, the results were opposite where *A. tunisiana* scored a highly significant increase in fatty acid composition except for 14:0, 16:0, and 18:1 ω-9, and 18:3 ω-3.

From Table 3, it can be seen that all of the enrichment procedures listed result in significant increases in total fatty acids compared to newly hatched *Artemia* nauplii except for 6:0. An increase in the total fatty acids equates to an increase in the caloric value of the feed (i. e., increase in energy content from 23.050 to 45.693 kJ/g). The essential highly unsaturated fatty acids (HUFAs) 20:5 n-3 or eicosapentaenoate (EPA) and 22:6 n-3 or docosahexaenoate (DHA) are significantly higher in *Artemia* nauplii that have been enriched.

Table 3 assumes the effect of enrichment duration on some parameters. There was no effect of duration on

Table 3 Average total protein, lipid (% DW), DHA, and EPA fatty acid composition (mg/g DW) and DHA:EPA ratios in control and enriched *Artemia tunisiana* at 18 and 24 h

Parameters	Unenriched		Enriched	
	0	18	18	24
Time (hours)	0	18	18	24
Protein	54.46	57.3	57.8	57.8
Lipid	12.59	21.53	22.29	22.29
Protein/lipids	4.326	3.03	2.68	2.68
C22:6n3 (DHA)	0	1.45	1.86	1.86
C20:5n3 (EPA)	1.3	7.62	8.13	8.13
DHA/EPA	0	0.19	0.23	0.23

total protein or lipid concentration Fig. 3. A slight effect was noticed in the concentration of DHA and EPA fatty acids as illustrated in Fig. 4. On the other area, the length and stages were affected, for instance, 100% of nauplii achieved stage (1) after 18 h from incubation, while after 24 h 37% reached stage (2). The length also was changed from 574 ± 85 μm after 18 h from enrichment to 646 ± 160 μm after 24 h Fig. 5. The enrichment duration had no significant effect ($P < 0.001$) on the weight of the specimens. After 18 h, it scored 2.79 ± 0.083 μg while after 24 h it gained only 0.04 μg/individual. The more pronounced effect of duration was the amount of energy (kJ/g); as mentioned in Fig. 6, it was 33.17 kJ/g after 18 h, while it was 45.693 kJ/g after 24 h from incubation.

Ascorbic acid

The ratio of ascorbic acid due to enrichment by vitamin C was increased from 15% (853 μg/g dry weight) with newly hatched nauplii to 85% (3227 μg/g dry weight) after enrichment for 24 h (Fig. 7). One-way ANOVA showed highly significant differences ($P < 0.05$) due to enrichment.

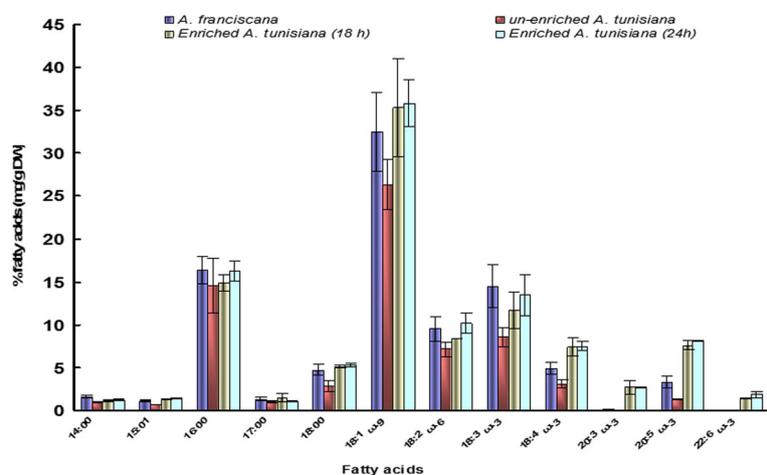


Fig. 2 Fatty acid composition of unenrichment *A. franciscana*, *A. tunisiana*, and enrichment *A. tunisiana* for 18 and 24 h

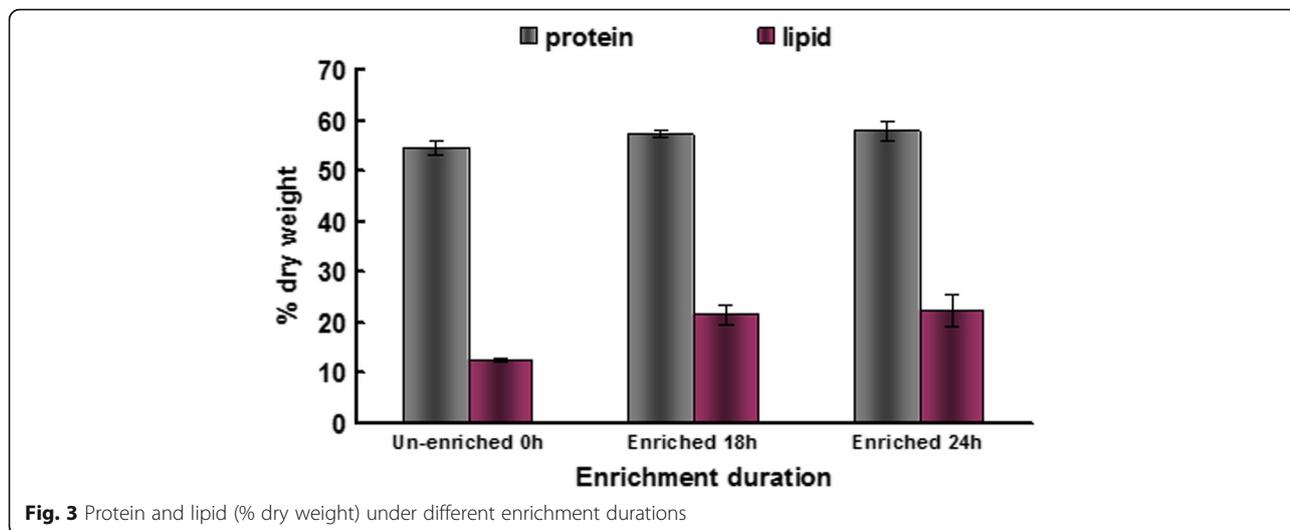


Fig. 3 Protein and lipid (% dry weight) under different enrichment durations

Discussion

Egypt has varieties of *Artemia* strains that can be utilized for feeding of larval stages of different fish and crustacean species in hatcheries (Zaki & Abdel Rahman, 1985; Abdel Rahman, 1995). Generally, the results of crude protein, ash, and carbohydrates were within the ranges compiled by Leger et al. (1986) from 15 references. Heydari and Akbary (2011) analyzed the proximate composition of *Artemia* from Urmia Lake, Iran, and they recorded values which resemble to those in *A. tunisiana* except for protein which was higher (61.7% protein) while other proximate composition were nearly the same (11.44% lipid and 6.78% ash on a dry weight basis). Ash content in all investigated strains (7.5, 5.2, 6.4, 7.6, and 7.11%) for *Artemia* Wadi El Natrun, Borg El

Arab, El Mex, Port Saied, and San Francisco Bay strain respectively was analogous to the values (7.6 and 7.1% Great Salt Lake *Artemia*) recorded by García-Ortega et al. (1998) and Estevez et al. (1998) and 8.17% with San Francisco Bay (Han, Geurden, & Sorgeloos, 2000). On the other hand, nauplii collected from PR China and France recorded high ash content of 21 and 15% (Lavens, Léger, & Sorgeloos, 1989). This is probably a consequence of the wild population’s feeding regime which is mainly based on organic particulate matter that can cause ash accumulation in the digestive tube, thus increasing the ash proportion and lowering that of other nutritional elements (Gozalbo & Amat, 1988).

Protein composition of all studied *Artemia* strains by Leger et al. (1986) ranged from 53.81 to 57.8%. The

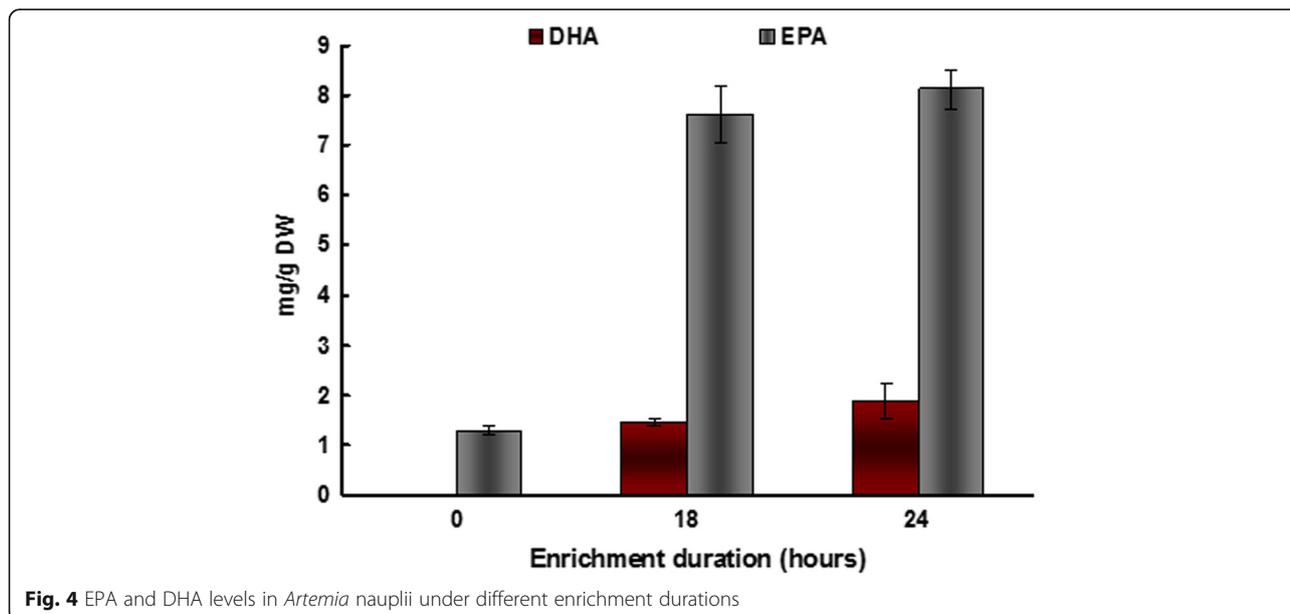


Fig. 4 EPA and DHA levels in *Artemia* nauplii under different enrichment durations

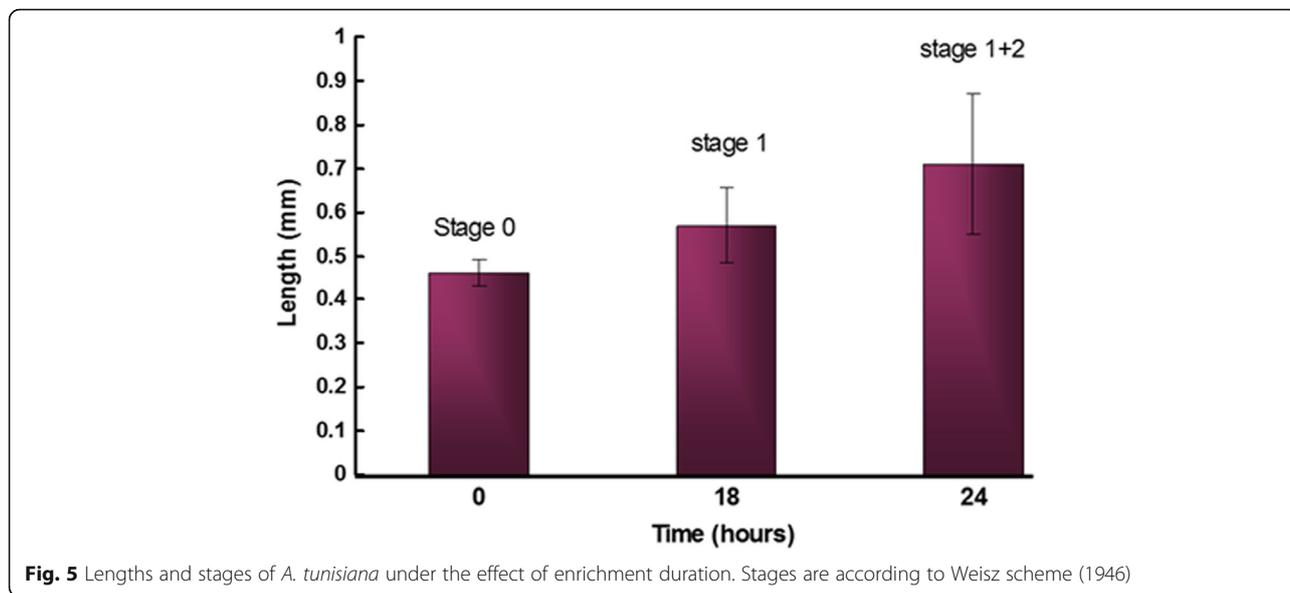


Fig. 5 Lengths and stages of *A. tunisiana* under the effect of enrichment duration. Stages are according to Weisz scheme (1946)

protein content in the same studied Egyptian strains (El Mex, Borg El Arab, and Port Saied) was measured before by Abdel Rahman (1995); he recorded nearly the same values (56.23–57.63%) of the investigated strains. And also they were relatively similar to the other wild *Artemia* fed on rich protein sources such as *Spirulina* Maldonado-Montiel & Rodriguez-Canche, 2005). The previous authors recorded 53, 50, and 58% protein for different *Artemia* strains fed on different protein sources. Comparable to *A. franciscana*, the Egyptian strain under the present study showed no significant differences between them (53.5%); its protein content has been previously studied by many authors and was found to meet the essential requirement of many fish species (Claus, Benijts, Vandeputte, & Gardner, 1979). Other studies with other strains recorded protein content of

61, 56.2, and 54.03% in nauplii from Great Salt Lake strain (García-Ortega et al., 1998; Moraiti-Ioannidou, Castritsi-Catharios, Miliou, & Sorgeloos, 2009).

Some confusion appears when reviewing amino acid profile of *Artemia* because of different methods of analysis or reporting data. Leger et al. (1986) has suggested that other factors such as the developmental stages of the animals, population-based differences, and the type, quality, and quantity of food available can affect the amino acid composition in *Artemia*. Related to the present data, both *A. tunisiana* and *A. franciscana* nauplii contained sufficient levels of the 10 amino acids. Protein contents of *A. tunisiana* was not significantly different from that of *A. franciscana* strain; on the other side, the content of some amino acids as phenylalanine,

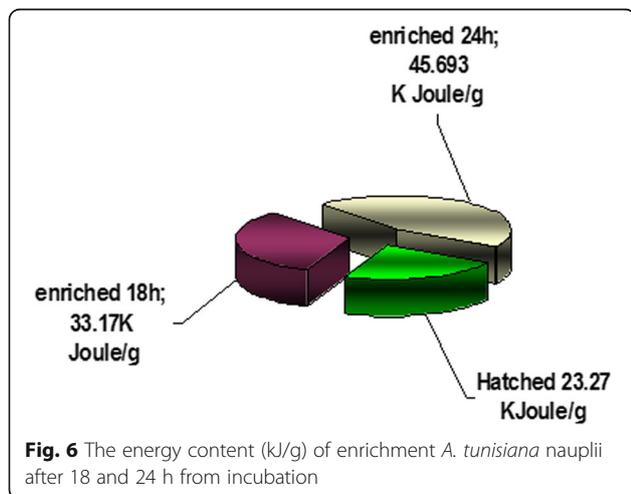


Fig. 6 The energy content (kJ/g) of enrichment *A. tunisiana* nauplii after 18 and 24 h from incubation

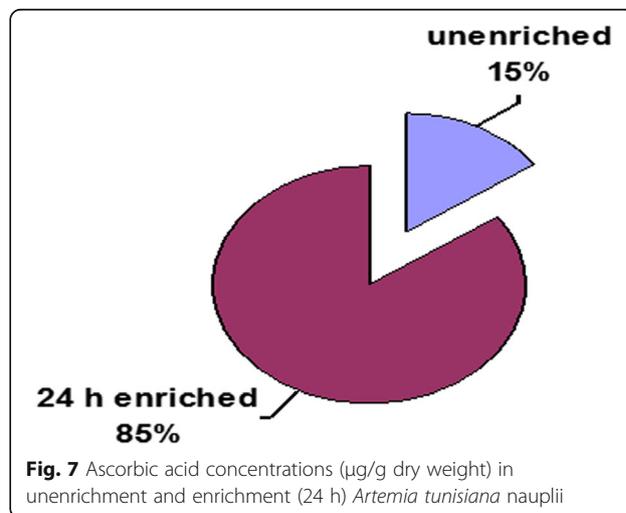


Fig. 7 Ascorbic acid concentrations (µg/g dry weight) in unenrichment and enrichment (24 h) *Artemia tunisiana* nauplii

glutamic acid, and tyrosine were significantly higher in *A. tunisiana*. Abdel Rahman (1995) who accomplished the same comparison with other Egyptian strains gave the same conclusion (same protein content but with different concentrations of amino acids). Leucine in both unenrichment (17.22%) and enrichment (14.8%) *A. tunisiana* was above the minimum requirement of fish (Seidel et al. 1980) and also above its value in *A. franciscana*. Glycine (1.7%) was lower than that recorded in other Egyptian *Artemia* strains (Abdel Rahman, 1995) or the herein *A. franciscana* (4.3%), but this type of amino acid is non-essential, so in terms of protein quality *A. tunisiana* could be utilized as an adequate dietary protein source for cultured fish larvae.

Leger et al. (1985) and Leger et al. (1987) concluded that the main factor affecting the nutritional value of *Artemia* for marine shrimp larvae was the content of the highly unsaturated fatty acid (HUFA) eicosapentaenoic acid 20:5 n-3 (EPA). It was found that ω 3 polyunsaturated fatty acids (PUFAs) are required for lobsters (Castell & Covey, 1976), prawns (Guary, Kayama, Murakami, & Ceccaldi, 1976; Kanazawa, Teshima, & Ono, 1979), and several marine fish, including plaice (Owen, Adron, Sargent, & Cowey, 1972), red sea bream (Yone & Fujii, 1975a), and turbot (Cowey, Owen, Adron, & Middelton, 1976).

The present analysis showed a slight difference in lipid composition (%) between examined strains except for Port Saied and San Francisco Bay strains. The total lipid of *A. tunisiana*, Borg El Arab, and El Mex strain (\approx 12.59–14%) resembled another *Artemia* sp. (9–13%) studied by Anh et al. (2009). Triantaphyllidis et al. (1995) and Han et al. (2000) established that the lipid composition of hatched nauplii is not affected by diet or environmental conditions. The differences in lipid profile that are observed between strains can reflect either genetic characteristic or the lipid profile of the food of the parental population. Several authors have demonstrated that the fatty acid profile of *Artemia* adults and their offspring clearly reflects the composition of the parental diet, despite the strain (Navarro & Amat, 1992). It was stated that the essential fatty acids contained in *Artemia* nauplii are the principle value of *Artemia* to fish (Watanabe, Oowa, Kitajima, & Fujita, 1978). Many publications include values for 15 or more different fatty acids in *Artemia* nauplii, but according to Leger et al. (1986) only six fatty acids (16:0, 16:1 ω 7, 18:1 ω 9, 18:2 ω 6, 18:3 n-3, and 20:5 n-3) actually make up about 80% of the total fatty acid pool in an *Artemia* sample. When calculating the ratio of these six fatty acids during this work, the result showed the same ratio with *A. franciscana* (77.5%) and enriched *Artemia tunisiana* for 18 or 24 h (79.4 and 84.9% respectively) while this ratio differed in the case of unenrichment

of *A. tunisiana*, it was 58.9%. The same author compiled data in almost 150 fatty acid analyses of nauplii from about 20 different *Artemia* sources and came to the following conclusions. Oleic acid (18:1 ω 9) is often the most abundant fatty acid. The present species (*A. tunisiana*) and the imported one (*A. franciscana*) recorded the high concentration of oleic acid (18:1 n9) comparable to other fatty acids, it scored 32.5, 26.35, 35.3, and 35.85 mg/g DW for *A. franciscana* and *A. tunisiana* unenriched and enriched (18 and 24 h respectively). It also has the most stable occurrence. Together with palmitic (16:0), it accounts for 40–50% of the total fatty acids in *Artemia*. The level of 16:0 is fairly constant over different strains (\approx 15%) as showed in the herein species; other studies recorded 13.2–19.4 mg/g DW in Great Salt Lake strain (Lavens et al., 1989), 12.6 mg/g DW in PR China (Han et al., 2000), 15.7% in *Artemia parthenogenitica* (Triantaphyllidis et al., 1995), and 14.4 mg/g DW in *A. persimilis* (Han et al., 2000). The levels of EPA (20:5 ω 3) seem to be inversely related to linolenic acid.

The concentration of EPA in freshly hatched nauplii of *A. tunisiana* was low (1.3 mg/g DW) and lower than the tested *A. franciscana* (3.3 mg/g DW). The low concentration of EPA was observed also in Great Salt Lake (3.5 mg/g DW), PR China (1.4 mg/g DW), and Urmia, Iran strain (2.7 mg/g DW), *A. persimilis* (0.0) (García-Ortega et al. 1998; Dhert et al. 1993; Triantaphyllidis et al. 1995) and Han et al. (2000) respectively. Fujita et al. (1980) reported that marine fish require EPA and DHA in its diet while freshwater fish require linolenic acid (18:3 n-3). Therefore, the local strain (*A. tunisiana*) in its original state is not an ideal food for larvae of marine fish and crustaceans so it is urgent to enrich it to improve the levels of these fatty acids to suit the feeding of marine creatures. Studies in Japan and the multidisciplinary International Study on *Artemia* revealed that the concentration of the essential fatty acid eicosapentaenoic (EPA) in *Artemia* nauplii was determining its nutritional value for larvae of various marine fishes and crustaceans (Leger et al., 1986). Beneficially, EPA (20:5 ω 3) was increased by enrichment from 1.3 mg/g DW to six times its original concentration (7.6 mg/g DW) after 18 h, while no significant differences between 18 and 24 h. Comparable with *A. franciscana*, EPA (3.3%) is significantly lower than the enriched *tunisiana*. EPA was measured in other strains of *A. franciscana* by many authors and scored 4.4, 5.5, and 4.1% (Dhert et al., 1993 and Estevez et al., 1998) while others scored high EPA contents 29.6–53% (Han et al., 2000). Before enrichment, the local Egyptian *Artemia (tunisiana)* was classified into freshwater type according to Watanabe et al. (1978), which contain low levels of 20:5 n3, but after enrichment

and the improvement of EPA ratio, it became suitable for the marine fish.

The ratio of DHA in freshly hatched *Artemia tunisiana* was 0.0; in addition, its value in the studied *A. franciscana* was also low (0.04 mg/g DW). The low ratio of DHA is explained by McEvoy et al. (1995) who publicized that *Artemia* converts docosahexaenoic acid (DHA) into EPA. The DHA, which was rarely found in the present strain, is known to promote survival and growth especially in crab cultures (Levin & Sulkin, 1984; Leger et al., 1987). Takeuchi et al. (1994) suggested that the appropriate level of DHA should be around 1% DW. In their study, any amount higher than 1% DHA resulted in a high percentage of abnormal fish, together with high mortality. Taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to manipulate the nutritional value of HUFA-deficient *Artemia*, because brine shrimp nauplii that have molted into the second instar stage (i.e., about 8 h following hatching) are non-selective particle feeders. A simple method had been developed to incorporate different kinds of products into the *Artemia* prior to feeding to predator larvae. So enrichment of the local strain (*A. tunisiana*) improved and corrected the value of DHA to be 1.45 mg/g DW after 18 h and 1.86 mg/g DW after 24 h from enrichment. Sargent et al. (1999) suggested that species-specific requirements for DHA exist among marine finfish larvae but several other studies suggested that much higher levels of DHA (or n-3 HUFAs) could reduce larval survival (Planas & Cunha, 1999).

Copeman et al. (2002) found that yellow tail flounder fed high DHA/EPA (8:1) had a higher growth and survival than those fed a DHA/EPA ratio of 1.9:1. So many contradictions are postulated about the function, concentration, and mechanism of DHA. Estevez et al. (1999) and Furuita et al. (1999) recorded no significant difference in the growth of Japanese flounder and turbot larvae when they were fed with different dietary ratios of DHA and EPA.

Several studies have focused their attention on the importance of other nutrients such as vitamins (Merchie et al., 1997 and Halver, 2002). For example, vitamin C (ascorbic acid) is considered as an essential nutrient during larviculture (Lavens and Sorgeloos, 1996). Vitamin C is found as ascorbic acid 2-sulfate (AAS) in *Artemia* cyst, during the hatching process, the AAS is hydrolyzed into free ascorbic acid and become available to predators.

Fish treated with HUFA + vitamin C gained better growth and lower mortality value in fish indicated that ascorbic acid supplementation enhanced resistance to stress, temperature, and salinity (Merchie et al., 1995). *Clarias gariepinus* larvae fed ascorbate-supplemented

diet exhibited significantly low mortality than those larvae fed an ascorbate-free diet (Merchie et al., 1995). Better growth was observed among tilapia fingerlings (Anadu, Anozie, & Anthony, 1990) and plaice (Rosenlund, Joergenses, Waagboe, & Sadnes, 1990) fed diets supplemented with ascorbic acid. Although it is possible that HUFA alone may have improved growth performance in trout (as reported in *chonos chonos* by Gapasin, Bombeo, Lavens, Sorgeloos, & Nelis, 1998), the synergistic effect of vitamin C cannot be neglected. The present *Artemia* enriched with HUFA + vitamin C to investigate its ability to increase its contents of ascorbic acid and to what extent was the rising of its level. The result showed good evidence to increase vitamin C ratio by enrichment from 853 to 3227 µg/g dry weight.

The total energy values of the unenriched *A. tunisiana* (23,270 J/g) was in close agreement with those of Paffenhofer (1967) who found a level of 24,900 J/g organic substance for an unidentified brine shrimp strain and with Vanhaecke et al. (1983) who recorded 23,250 J/g with freshly hatched nauplii collected from various geographical origins. Enrichment elevated the energy content from 23,270 J/g to 33,170 and 45,693 J/g after 18 and 24 h of enrichment respectively. The nutritional effectiveness of a food organism is in the first place determined by its ingestibility and as a consequence by its size and form. So feasibility and duration of enrichment process should also be under consideration when preparing *Artemia* nauplii as a food. Enrichment for 18 and 24 h was tested to score the lowest duration for suitable enrichment with keeping the size as petite as possible. Generally, enrichment processes for *Artemia* nauplii significantly elevated the majority of the fatty acids found in the nauplii. Most notable is the elevations of C20:5 n-3 (EPA) as well as infusion of C22:6 n-3 (DHA). Both of these have been implicated to be essential for larval growth and development in a number of fish species (Watanabe, Kitajima, & Fujita, 1983). The result indicated that 18 h enrichment achieved good and enough nutritional value compared to 24 h. For 18 h, the length of enriched nauplii was ≈ 0.5 ml while after 24 h their lengths achieved ≈ 0.65 ml. The size is not critical for crustacean larvae, which can capture and tear apart food particles with their feeding appendages. For marine fish larvae that have a very small mouth and swallow their prey in one bite, the size of the nauplii is particularly critical.

Conclusions

Enrichment with cod liver oil and vitamin C obviously had a positive effect on the chemical composition of local Egyptian *Artemia* (*A. tunisiana*).

Abbreviations

DHA: Docosahexaenoate; EPA: Eicosapentaenoate; FAO: Food and Agriculture Organization; HUFAs: Highly unsaturated fatty acids

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

E-SR wrote the manuscript, contributed to the design of the study, and carried out the data analysis. FS coordinated the design of the study and was responsible for the execution of the experiment. ME-G helped to write the manuscript. FA-R contributed to the design of the study, contributed to the literature review, and critically revised the manuscript. Finally, MMM provided the idea for the study, coordinated the study, and critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Authors were asked to participate in a satisfaction survey on a voluntary basis. Participants were informed that this survey was part of a research project. This study was approved by the Social Science Ethical Committee of Faculty of Science, Tanta University & Research and complied with the Egyptian Code of Conduct for Scientific Practice, National Institute of Research, Egypt.

Competing interests

The authors declare that they have no competing interests.

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