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ORIGINAL ARTICLE

Comparative study of *Artemia* fatty acid composition collected from different Algerian saline sites

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Artemia is a small branchiopod crustacean Anostraca of aquaculture. Its interest remains among the most essential foods used in larviculture of fish and crustaceans. The determination of the fatty acid composition of brine shrimp cysts of certain Algerian populations from different origins allows us to assess the nutritional quality of these populations for their aquaculture exploitation. Sampling was carried out in two large areas, to the east: Chott Merouane (city of el Oued), Sebkhet Ez-Zemoule (city of Oum El Bouaghi), Lake El Bahira (city of Sétif), and the west: sebkhet Timimoune (city of Timimoun), Saline of Betioua (city of Oran) and Sidi Bouziane (city of Relizane). The overall percentage of total lipids contained in samples of brine shrimp cysts varied between 11.02% and 30.25% of dry weight. Maximum values were found in samples from Timimoun. Analysis of fatty acids by gas chromatography revealed the presence of twenty-one fatty acids. The contents of monounsaturated fatty acids were significantly ($p<0.05$) higher in the different strains studied compared to the contents of saturated fatty acids (82.80% *vs.* 21.963%), respectively. The high value of α-linolenic acid (ALA, 18:3n-3) was observed in the Chott Merouane strain with (55.04% ± 5.83%)

Keywords: *Artemia*, fatty acids, saltwork, cysts, nauplii.

Introduction

Nowadays, aquaculture's development requires an increased demand for live foods. As a result, the *Artemianauplius* remains the organism most used as live food for fish and crustacean larvae (Ben naceur et al., 2017; Sebesta et al., 2018).

Artemia is a crustacean that lives in halophilic ecosystems. It produces either nauplii or dormant eggs called cysts when environmental conditions are inadequate and can remain in this form for a long time (Gajardo and Beardmore 2012).

It's simple to raise brine shrimp. They are conserved throughout their life cycle. The latter allows them to be used in a variety of ways. Hence, after hatching, it also explains why the cyst generates the nauplius as an optimal prey in larval rearing.

In terms of nutritional value, it turns out that Artemia naturally has a high content of neutral lipids and a low content of long-chain polyunsaturated fatty acids (LC-PUFAs) such as 20:5n-3 (EPA), and especially 22:6n-3 (DHA). They are considered essential fatty acids for the normal development of marine fish (Sargent *et al.*, 1999). These fatty acids have a positive impact on fish growth (Magalhaes et al., 2020). In this regard, recourse to the enrichment of Artemia with fatty acids was used to adapt its lipid composition to the nutritional needs of marine larvae (Reis et al., 2017). This gave a positive effect on the growth of fish, their development and decreased the mortality rate (Kolman et al., 2018). Fatty acids play a crucial role in energy storage. Polyunsaturated (PUFA) and highly unsaturated (HUFA) fatty acids act more on the structure of the biological membrane (Gonçalves et al., 2017).

The biochemical composition of Artemia varies from strain to strain due to abiotic and genetic factors, as well as the food ingested (Léger et al., 1986; Navarro et al., 1992; Torrentera and Dodson 2004; Ruiz et al., 2008). The essential fatty acid (EFA) profile and the n-3 and n-6 series are of great importance to assess the dietary value of Artemia before its use (Sorgeloos et al., 2001). Indeed, docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6), α-linolenic acid (ALA, 18: 3n-3), and linoleic acid (LOA, 18:2n-6) are essential for most species in aquaculture. They are indispensable with varying proportions depending on the species' needs in culture (Glencross 2009). Generally, the aquatic animals' fatty acid composition (AG) reflects their diet. The study of fatty acids is not dealt with in extreme environments, specifically in hyper-saline biotopes. So, the knowledge of the biochemical composition of Artemia in different saltworks of Algeria is an aspect that allows studying the nutritional value of this branchiopod. The objective of this work is to study the biochemical characteristics. It identifies the nutritional quality of fatty acid intake in the branchiopod Artemia. These data may lead to a possible aquaculture operation.

Materials and Methods

Sampling

Study zone

We collected the cyst samples used in this study from six hyper-saline lakes in Algeria:

- Saline of Sidi Bouziane (35° 51′ 07″ N-0° 39′ 02″ E), city of Relizane and saline of Bethioua (35° 41′ 21″ N-0° 18′ 15″), city of Oran, located in the northwest of Algeria.
- Lake El Bahira (35° 50′ 07″ N-5° 15′ 04″ E), and Sebkhet Ez-Zemoul (35° 52′ 57″ N-6° 32′ 54″ E) located in the high plateau of northeastern Algeria in a semi-arid region.
- Chott Marouane (34° 02′ 45″ N- 6° 02′ 06″ E), city of El Oued and sebkhet Timimoune (29° 10′ N, 0° 04′ E), city of Adrar located in the Sahara in an arid climate.

Treatment of cysts

Cysts were harvested from the shores of saline lakes and transported in brines. Immediately, they were cleaned with fresh water, separated from impurities between sieves of 125 and 400 µm meshes under a vacuum. Then they were dried in thin layers in an oven at 30°C for 48 h until constant weight. The samples were stored in sterile 50 ml vials under vacuum and preserved (Sorgeloos et al., 1986).

Dosage of lipids and determination of fatty acid profile

Before lipid extraction, the cysts were hydrated in distilled water under aeration until the cysts were spherical. Then they were decapsulated according to the protocol of Sorgeloos et al., (1986). Afterward, the cysts were dried in an oven at 38°C until they had a constant weight. Fatty acids were extracted with chloroform/methanol (2:1 v/v) according to Folch et al., (1957).

Fatty acid methyl esters (FAME) were prepared from six samples. Fatty acid assays were analyzed on an HP (Hewlett Packard) Agilent 6890N gas chromatograph (GC), equipped with a flame ionization detector (FID), and with an HP-5MS capillary column (30 m 0.25 mm id and 0.25 µm). The temperature of the injector and detector were 250°C. The oven was programmed at 70°C initial temperatures and 5 minutes of initial time. Subsequently, the temperature was increased to 130°C at 10°C/min, then increased to 200°C at 3°C/min for 2 min and held at 220°C for 4 min and increased to 280°C at 10°C/min for 7min. The total cycle time was 60 min. The carrier gas used was helium purity min). CGL analysis of FAME was performed in three replicates. Fatty acid identification was performed by comparing the relative retention time of the FAME peaks of our samples with those obtained by Alltech (Carolean Industrial Drive, State College, PA). Results were expressed as the relative percentage of DIZ response area, then as milligrams per gram of dry weight.

Statistical analysis

The data collected in this randomized design were subjected to an analysis of variance (SAS Institute, 2008). The treatment means were separated using Duncan's multiple range tests. Freedom contrasts' single degree was used to test the overall effects of the effect of the area on fatty acid composition. The level at which differences were considered significant was $p<0.05$.

Results

The percentage of total lipids and the fatty acid composition of the decapsulated cysts of the six strains are shown in Table 1.

Table 1. Fatty acid composition (% of total fatty acids) of decapsulated Artemia cysts collected from different areas in Algeria (means ± SD).

	El Bahira	Timimoune	Chott Merouane	Sidi Bouziane	Betioua	Ez-Zemoule
% total lipids	11.136 ± 0.193^{c}	23.433 ± 0.200^{b}	30.063 ± 0.650^{a}	11.493 ± 0.314^{c}	13.42 ± 0.370^{c}	11.516 ± 0.319^{c}
Fatty acids			**% of total fattyacids**			
C10:0	1.79 ± 0.03^{b}	0.11 ± 0.02^{b}	0.123 ± 0.05^{b}	0.15 ± 0.01^{b}	0.100 ± 0.02^{b}	0.090 ± 0.01^{b}
C11:0	ND	ND	ND	1.78 ± 0.02^{a}	ND	ND
C13:0	0.443 ± 0.088^{a}	0.593 ± 0.389^{a}	ND	0.533 ± 0.112^{a}	0.450 ± 0.041^{a}	0.517 ± 0.066^{a}
C14:0	ND	ND	2.14 ± 0.04^{b}	ND	ND	ND
C15:0	ND	1.463 ± 0.087^{a}	ND	ND	ND	ND
C16:0	12.423 ± 0.159^{c}	20.844 ± 0.487^{b}	20.241 ± 1.06^{b}	21.21 ± 2.091^{b}	29.379 ± 0.508^{a}	22.588 ± 2.383^{b}
C17:0	ND	ND	3.901 ± 0.349^{a}	1.267 ± 0.409^{b}	ND	0.484 ± 0.136^{c}
C18:0	3.460 ± 0.229^{b}	3.589 ± 0.158^{b}	3.901 ± 0.430^{b}	0 ± 0.159^{c}	4.464 ± 0.044^{b}	6.732 ± 0.852^{a}
C20:0	3.847 ± 1.537^{a}	0.333 ± 0.366^{b}	ND	4.599 ± 0.73^{a}	4.433 ± 1.372^{a}	ND
C16:1 n3	7.854 ± 0.147^{c}	2,38 ± 0,266^{b}	2,185 ± 0,302^{b}	19,954 ± 1,391^{a}	19,364 ± 0,467^{a}	10,6 ± 0,761^{b}
C16:1 n9	7.84 ± 0.03^{a}	ND	ND	ND	ND	ND
C16:1 n7	3.24 ± 0.02^{c}	38.02 ± 0.72 ^{a}	ND	ND	16.9 ± 0.64^{b}	ND
C16:1 n5	4.34 ± 0.04^{a}	ND	ND	ND	ND	ND
C18:1 n9	47.364 ± 0.434^{a}	38.032 ± 2.106^{b}	9.784 ± 1.927^{b}	22.716 ± 7.334^{c}	21.538 ± 1.396^{c}	48.204 ± 2.756^{a}
C18:1 n11	ND	0.800 ± 0.089^{a}	ND	ND	ND	0.827 ± 0.121^{a}
C18:1 n10	ND	1.967 ± 0.335^{b}	ND	1.333 ± 0.692^{b}	13.567 ± 3.491^{a}	13.333 ± 1.605^{a}
C18:1 n8	ND	0.287 ± 0.181^{b}	ND	ND	1.867 ± 0.527^{a}	0.48 ± 0.225^{b}
C18:1 n6	0.2 ± 0.376^{a}	1.323 ± 0.729^{a}	2.143 ± 1.875^{a}	0.4 ± 0.498^{a}	1.1 ± 0.882^{a}	0.8 ± 0.671^{a}
C18:3 n3	34.43 ± 1.931^{b}	22.73 ± 1.207^{b}	55.04 ± 5.835^{a}	16.670 ± 2.429^{b}	32.26 ± 1.273^{b}	25.18 ± 1.234^{b}
C20:4 n6	3.67 ± 0.815^{c}	2.033 ± 0.677^{bc}	4.867 ± 0.89^{ab}	11.33 ± 3.811^{a}	5.633 ± 0.829^{ab}	7.067 ± 1.531^{a}
C22.6 n3	ND	ND	ND	5,64 ± 0,01^{a}	ND	ND
C20:5	ND	ND	ND	0,05 ± 0,02^{b}	0,09 ± 0,01^{a}	ND
Total SFA	21.963 ± 2.146^{d}	26.822 ± 1.59 ^{c}	30.182 ± 2.348 ^{b}	27.609 ± 3.521^{c}	38.726 ± 2.068^{a}	30.321 ± 3.802^{b}
Total MUFA	70.838 ± 1.047^{a}	82.8 ± 3.706 ^{c}	14.112 ± 1.875^{d}	44.403 ± 9.915^{b}	74.33 ± 6.763^{b}	74.244 ± 6.139^{a}
Total PUFA	38.1 ± 2.012 ^{d}	28.433 ± 1.275 ^{b}	59.907 ± 6.725^{a}	36.66 ± 6.241 ^{b}	24.246 ± 1.356^{c}	25.587 ± 1.387^{c}
UFA/SFA	4.96 ± 0.05^{a}	4.14 ± 0.652^{c}	2.433 ± 0.15^{c}	2.93 ± 0.221 ^{c}	2.54 ± 0.181^{c}	3.292 ± 0.251^{b}
Total PUFA n-3	42.32 ± 1.245^{d}	32.45 ± 1.354 ^{d}	57.12 ± 3.215 ^{a}	42.264 ± 2.567^{b}	55.91 ± 1.956^{c}	35,78 ± 1,321^{d}
Total PUFA n-6	3.870 ± 0.488^{d}	3.356 ± 0.658 ^{b}	7.010 ± 1.279 ^{a}	11.73 ± 0.752 ^{c}	6.733 ± 1.023^{b}	7.867 ± 1.541^{c}
Total PUFA n-9	55.204 ± 1.522^{a}	38.032 ± 1.112 ^{c}	9.784 ± 0.956^{e}	22.716 ± 3.254^{c}	21.538 ± 1.562^{d}	48.204 ± 2.031^{b}
n3/n6	10.93 ± 1.254^{b}	9.68 ± 0.250^{a}	8.04 ± 0.678^{a}	3.6 ± 1.041^{c}	8.3 ± 1.321^{c}	4.54 ± 1.020^{c}
ratio16:0/16:1	1.58	8.75	9.28	1.06	1.51	2.13

For all populations, the results showed the existence of 21 fatty acids. The most dominant were: linolenic acid (C18:3n3), palmitic acid (C16:0), palmitoleic acid (C16:1n3), oleic acid (C18:1n9) and arachidic acid (C20:4n6).

The total lipid contents varied significantly ($p<0.05$) between the cysts taken from Lake El Bahira and those from Chott Merouane (11.13% *vs.* 30.06%), respectively.

The values of palmitic acid (C16:0) were higher in the population of the saline of Betioua (29.37%) compared to those of the populations of Lake El Bahira of the order of (12.423%).

The proportions of monounsaturated fatty acid (MUFA) varied between 14.11% and 74.24%, respectively, for the populations of Chott Merouane and Sebkhet Ez-Zemoul.

Polyunsaturated fatty acids (PUFAs) had high concentrations in the populations of Chott Merouane (59.90%).

Saturated fatty acid (SFA) levels showed almost identical levels for all populations.

Arachidonic fatty acid (C20:4n6) was high in the populations of Sidi Bouziane (11.33%). The lowest content was recorded in the population of Sebkhat Timimoune (2.03%). The highest rate of linolenic acid (C18:3n3) was found in the populations of Chott Merouane (55.04%). For palmitoleic acid (16:1n3), the values of Betioua and Sidi Bouziane were very close (19.36% and 19.95%) and remained high compared to the other populations.

In the n3 and n6 series, palmitoleic acid (C16:1n3) was higher in the populations of Sidi Bouziane (19.95%), oleic acid (C18:1n6) was dominant in the population of Chott Merouane (2.14%). Linolenic acid (C18:3 n3) was present in all populations with significantly different values ($p<0.05$) ranging from 55.04% in Chott Merouane against 16.67% in Sidi Bouziane. On the other hand, oleic acid C18:1(n-6) was available with low values of 0.2% for the populations of Lake El Bahira and 2.14% of the population of Chott Merouane, docosahexaenoic acid (C22:6 n3) was recorded only in the population of Sidi Bouziane (5.64%). Eicosapentaenoic acid (C20:5) was present in low proportions (0.05% and 0.09%) in the two populations of Sidi Bouziane and Betioua, respectively.

The n3/n6 ratio was between 10.93% and 3.60% in the population of El Bahira's Lake and Sidi Bouziane, respectively.

The difference ratio in monounsaturated fatty acids between Sebkhet Ez-Zemoul and Chott Merouane was estimated at 80.99%, concerning polyunsaturated fatty acids, the difference ratio between El Bahira's lake and Chott Merouane was estimated at 65.278%. The statistical study ($p<0.05$) revealed that the brine shrimp cysts collected in the Chott Merouane area presented significantly high levels of N-3 compared to those recorded in the Sebkhet Timimoune area (57.12% *vs.* 32.45%), respectively.

The ratio of C16:0/C16:1 was greater than 1 in all populations.

Discussion

The results obtained on the total lipid levels contained in the decapsulated cysts of Algerian *Artemia* populations were close to those reported in work on known populations, such as; the case of the species from the Grand Lac Salé and that of the Bay of San Francisco. The values obtained were 14.7% and 15.7%, respectively (Dendrinos and Thorpe 1987; Garcia-Ortega 1998).

The most abundant fatty acids in decapsulated Artemia cysts were palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1n-7), cis-vaccenic (C18:1n-7), oleic (C18:1n-9), linoleic (C18:2n-6), linolenic (C18:3n-3), and eicosa-pentaenoic (C20:5n-3) (Abatzopoulos et al., 2006; Naceur et al., 2013; Navarro et al., 1992; Ruiz et al., 2007). The results of our work confirm the presence of these acids in the Algerian populations.

The dosage of fatty acids in *Artemia* reveals annual variability between species (Leger et al., 1986, Navarro et al., 1992). This difference is probably due to the nature of the primary fauna and flora of the medium ingested by cyst-producing females.

It turned out that the percentage of unsaturated fatty acids, particularly, palmitic acid (C16:0) was present in the six Algerian populations in remarkable quantities. This may be due to a diet based on Chlorophyceae (Farhadian et al., 2013) since myristic acid (C14:0) and palmitic acid (C16:0) are the major and specific fatty acids of green algae (Brett et al., 2006).

Polyunsaturated fatty acids C22:6, C20:4, and C20:5 are considered essential fatty acids for the diet of crustacean and fish larvae (Sargent et al., 1997; Izquierdo et al., 2000). Their absence or deficiency in the diet would lead to their mortality (Glencross 2009). It would seem that all the Algerian populations analyzed have these elements.

Arachidonic acid is present in different concentration levels. This fatty acid plays an important role in improving larval growth and pigmentation in several species of marine fish. It provides precursors for the production of eicosanoids (Castell et al., 1994; Estevez et al., 1997; Sargent et al., 1995).

EPA and DHA are considered to have a positive impact on fish growth (Magalhaes *et al.*, 2020). According to (Gwangseok R. Yoon et al., 2022), EPA and DHA are important in the early life of fish. They act on growth and survival, which could be used as a factor to improve the survival rate in aquaculture. At the same time, amounts of EPA are greater than 4-5% in Artemia, which promotes

the survival and growth of marine species (Amarouayache et al., 2017). The quantities of EPA observed are found in the populations of the Betioua salt works (0.09%) and those of the Sidi Bouziane salt works (0.05%).

In general, *Artemia* contains low levels of EPA and DHA, so when used as food, it is enriched with lipids rich in essential fatty acids to meet the dietary requirements of fish larvae (Hawkyard et al., 2016). This enrichment is therefore essential to produce live foods with an effective nutritional profile (Feh'er et al., 2013).

According to the classification of Watanabe et al., (1978a), there are two categories of Artemia cysts: the freshwater type and the seawater type related to the fatty acid composition. The first category is characterized by a high concentration of linolenic acid (LNA) and a low concentration of eicosa-pentaenoic acid (EPA). On the other hand, the second category presents a higher concentration of EPA.

The data obtained for the Algerian populations show a high rate of LNA (C18:3), which is likely of the freshwater type. Navarro et al., (1993) explain that the C16:0/C16:1 ratio is a good index to characterize the samples in terms of marine or freshwater type. Given the high values of the C16:0/C16:1 ratio, all populations seem to belong to freshwater.

Conclusion

Biochemical analysis showed a dominance of saturated fatty acids. It turned out that brine shrimp accumulate certain fatty acids such as palmitic acid (C16:0) and linolenic acid (C18:3n3), which constitute a reserve of energy capable of ensuring their survival in the time of nutrient deficiency in the environment. Mono unsaturated and polyunsaturated fatty acids are present in large quantities. This could be an indicator of use as aquaculture feed. In addition, the determination of the fatty acid profile of *Artemia* cysts from Algeria reveals variability between the different populations. Ultimately, taking into account the fatty acid composition of the decapsulated cysts, the Algerian *artemia* is qualitatively good and would be an adequate source of food for fish larvae.

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