Research article

Microalgal solutions in the cultivation of rotifers and artemia: scope for the modulation of the fatty acid profile

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ABSTRACT

The microalgae *Aurantiochytrium* sp. (AUR), *Isochrysis* sp. (ISO), and *Nannochloropsis* sp (NAN) were studied as possible alternative feeds to well established commercial compound feeds for both rotifers (*Brachionus plicatilis*) and *Artemia franciscana*. Fatty acid (FA) composition —relative (in % of total FAs) and absolute (in mg/g dw)— was determined in order to assess their potential for providing essential FAs. The FA profiles showed relevant differences between the four feeds (compound feed and the three microalgal species), but less stark than in the feeds themselves. Whereas *Isochrysis* sp. was relatively rich in DHA and poor in EPA, 18.6 ± 1.7% *vs* 0.6 ± 0.0%, respectively, *Nannochloropsis* sp. had the opposite pattern, 0.2 ± 0.3% *vs* 28.3 ± 0.7%. *Aurantiochytrium* sp. was rich in DHA (19.1 ± 0.2% corresponding to 89.8 ± 0.2 mg/g dw), but posed difficulties as a feed for both rotifers and artemia, given its low lipid incorporation and, in particular, poor DHA deposition. Rotifers fed the compound feed had the best combination of n3 PUFA levels (22.1 ± 0.1 mg/g dw), DHA contents (13.6 ± 0.4 mg/g dw), and DHA/EPA ratios (~3), being rotifers fed AUR and ISO feeds second best. Hence, these microalgae may deserve to be further explored as potential sources of specific FAs in rotifers and artemia.

1. Introduction

Fish aquaculture has greatly expanded in the last decades, being rotifers and *Artemia* sp. widely used as first foods in the culture of almost all marine fish species. It has been reported that rotifers are able to produce polyunsaturated fatty acids (PUFA), but synthesized amounts are too low to assure high fish larval survival. On the other hand, *Artemia* sp. are typically poor in essential FAs (Bell et al., 2003; Copeman et al., 2002; Czesny et al., 1999). Accordingly, both types of organism are deemed suboptimal for larval nutrition, particularly if compared to wild copepods (Han et al., 2000; Hanaee et al., 2005). These difficulties have stimulated the common practice of rotifer and *Artemia* sp. enrichment with phytoplankton or commercial products with high levels of essential FAs (Haché and Plante, 2011).

In this context, microalgae are an important resource, which is still insufficiently valorized (Matos et al., 2017). Microalgae contain a large variety of organic components, for instance, polyphenols and n3 PUFA, which may generate relevant bioactivities. The existence of substantial

concentrations of these components with possible beneficial health impact justifies the conduction of research on microalgae in different application fields, namely, as an ingredient to be incorporated in feeds for farmed fish. Among the most promising microalgae, *Aurantiochytrium* sp., *Isochrysis* sp., and *Nannochloropsis* sp. are known for their richness in eicosapentaenoic acid (EPA, 20:5 n3) and/or docosahexaenoic acid (DHA, 22:6 n3) (Chakraborty et al., 2007; Matos et al., 2017). While *Isochrysis* sp. is frequently rich in DHA, *Nannochloropsis* sp. exhibits high EPA content (Chakraborty et al., 2007). Regarding *Aurantiochytrium* sp., it belongs to the group of the thraustochytrids, which have been cultured and applied in the feed industry as an alternative source of PUFA (Visudtiphole et al., 2018). For instance, *Aurantiochytrium limacinum* is known as a thraustochytrid producing high quantities of DHA (Visudtiphole et al., 2018).

EPA and DHA are considered to have a positive impact on fish growth (Magalhães et al., 2020) as well as on human health (Simopoulos, 2002). These n3 PUFA may also have a positive effect on the development of the nervous system of infants (Cardoso et al., 2018) as well as a

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counterbalancing force with respect to the decline of neural tissues in older people (Cardoso et al., 2016). In addition, among other effects, EPA may act upon high-density lipoprotein, rendering much stronger its anti-inflammatory action (Tanaka et al., 2014). On the other hand, DHA is considered to be the starting molecule for different metabolic routes leading to beneficial compounds that mitigate inflammatory processes (Kuda, 2017).

Therefore, this study aimed at testing *Aurantiochytrium* sp., *Isochrysis* sp., and *Nannochloropsis* sp. as alternative feeds and n3 PUFA sources for rotifers (*Brachionus* spp.) and *Artemia franciscana*, thereby paving the way for future applications.

2. Materials and methods

2.1. Organisms and feed materials

Rotifers (*Brachionus plicatilis*) and artemia (*Artemia franciscana*) were selected as study organisms. The conduction of experiments with these non-vertebrate organisms does not require approval by an ethical committee. Nonetheless, it must be mentioned that all researchers and technicians involved in the maintenance, handling, and sampling of live animals were certified in Laboratory Animal Sciences, by the Federation of European Laboratory Animal Science Associations (FELASA) and the Pilot Station of Pisciculture at Olhão (EPPO) —where the experiments were conducted— is licensed for aquatic animal experiments with a scientific purpose, as overseen by the Portuguese National Competent Authority (Direção-Geral de Alimentação e Veterinária, DGAV).

Regarding the rotifers, the species *Brachionus plicatilis* with a total lorica length of 188.8 ± 39.0 μm was used. With respect to artemia, *Artemia franciscana* EG cysts (from INVE Aquaculture, Inc., Sep-Art Technology) were used. Regarding the diets, one compound feed (INVE), two commercial microalgae (*Isochrysis* sp., *Nannochloropsis* sp.), and one cultured microalga (*Aurantiochytrium* sp.) were used. The biomass of *Aurantiochytrium* sp. was attained through cultivation under heterotrophic conditions in a UniVessel® Glass (5L) vessel attached to a BioStat® B- plus system (Sartorius, Goettingen, Germany). These microalgae were freeze-dried at $-40\,°C$ and a pressure of 4×10^{-4} mbar for 48 h in a Heto Power Dry LL3000 freeze-dryer (Thermo Fisher Scientific, Waltham, MA, USA). On the other hand, the biomass of *Isochrysis* sp. (Phytobloom PROF Isochysis®) and *Nannochloropsis* sp. (Phytobloom prof Nannocloropsis®) was kindly provided by Necton S.A. (Necton, Companhia Portuguesa de Culturas Marinhas, S.A., Olhão, Portugal).

INVE feeds for rotifers and *Artemia franciscana*, DHA protein® (INVE, Belgium) and easy DHA® (INVE, Belgium), respectively, were used as reference in the experimental design, given their ability to ensure an adequate FA profile to fish reared in aquaculture hatcheries.

All used feeds were sent to IPMA laboratory at Lisbon for analysis.

2.2. Feeding experiments

For all organisms tested in the FA enrichment experiment, freeze-dried microalgae (AUR - *Aurantiochytrium* sp., ISO - *Isochrysis* sp., and NAN - *Nannochloropsis* sp.) and compound feeds (DHA protein® and easy DHA®, both from INVE) were used.

Rotifers of the same lot of *B. plicatilis* were distributed to twelve 24 L tanks containing 10 L of salt water, rendering an organism concentration of 640/mL. For the first 12 h (overnight), the organisms received only oxygen and no feed (18 h fasting - previous feed was an yeast-based product, ω3Yeast60 from Bernaqua®). The salinity level was 20. Thereafter, salinity was increased to 37 and they were fed twice with the four alternative feeds (each feed for three distinct tanks). Three microalgal feeds were used during a 24 h enrichment (dissolved in seawater at a 12 mg/mL concentration) and the commercial feed was used accordingly to the manufacturer indications (dissolved in fresh water at a 12 mg/mL concentration, used during an 8 h enrichment period at a proportion of approximately 1 g of feed per million rotifers). During the enrichment

experiment, oxygen varied between 14.7 and 23.0 mg/L and temperature and salinity were regulated, remaining temperature constant at $26.0 \pm 0.0\,°C$ and salinity constant at 37 —it must be noted that this salinity was chosen for enrichment, and not for rotifer growth purposes. After the enrichment period, rotifer samples were taken from each set of three tanks (in triplicate).

Regarding *Artemia franciscana*, recently hatched organisms were distributed to twelve 24 L tanks containing 10 L water, rendering an organism concentration of 189/mL. No feeding occurred prior to the trial's onset. Then, artemia were fed twice with the four alternative feeds (each feed for three distinct tanks). Three microalgal feeds were used during a 24 h enrichment (previously dissolved in seawater at a 12 mg/mL concentration) and the compound feed was used accordingly to the manufacturer indications (dissolved in fresh water at a 12 mg/mL concentration, used during a 6 h enrichment period at a proportion of approximately 3 g of feed per million artemia). During the enrichment experiment, oxygen did not drop below 12.5 mg/L and temperature and salinity were regulated, remaining temperature constant at $25.3 \pm 0.0\,°C$ and salinity constant at 37. After 24 h, artemia samples were taken from each set of three tanks (in triplicate).

All samples were kept in liquid nitrogen until freeze-drying and after being freeze-dried kept at -80 °C before being sent in dry ice boxes to IPMA laboratory at Lisbon for analysis.

2.3. Fatty acid profile

Fatty acid methyl esters (FAME's) were prepared from the freeze-dried microalgae, feeds, and the experimental organism (rotifers and artemia) biomass by acid-catalysed transesterification using the methodology described by Bandarra et al. (1997). The FAMEs were identified by comparing their retention time with those of several Sigma-Aldrich standards (PUFA-3, Menhaden oil, and PUFA-1, marine source from Supelco Analytical). The LOD is 1 mg/100g. Results were calculated in % of total fatty acids on the basis of peak areas and results in mg/g were attained through the internal standard (10 mg/ml of heneicosanoic acid, 21:0) method. Analyses were always done in triplicate.

2.4. Statistical analysis

The Kolmogorov-Smirnov's test and Levene's F-test were applied to data in order to check whether normality and variance homogeneity, respectively, were acceptable for parametric tests. Once verified these assumptions, one-way ANOVA using the Tukey HSD post-hoc test to determine the difference in the fatty acid profiles between the alternative feeds either in the case of the rotifer or of the artemia experiment was applied. Alternatively, a factorial ANOVA using the Tukey HSD to determine the difference in the fatty acid profiles between organisms and between alternative feeding groups was used. The significance level (α) was always 0.05. Analysis was done with the specialized software STATISTICA 6 (Stat-sof, Inc. USA, 2003).

3. Results

3.1. Fatty acid profile of feeds

3.1.1. Rotifer feeds

The fatty acid composition (in % of total fatty acids and in mg/g dry weight) of the studied rotifer feeds, comprising the microalgae *Aurantiochytrium* sp., *Isochrysis* sp., and *Nannochloropsis* sp. as well as the commercial feed (INVE) are shown in Tables 1 and 2.

The importance of PUFA varied between only $28.0 \pm 0.3\%$ for *Aurantiochytrium* sp. and more than the double, $57.0 \pm 0.7\%$ for *Isochrysis* sp. This wide variation is mainly due to the high variability of the n3 PUFA, since the n6 PUFA contents were relatively low and differed not much (the highest n6 PUFA content was recorded for *Nannochloropsis* sp., $13.1 \pm 0.6\%$). As a consequence of these differences, while saturated FA (SFA)

Table 1. Fatty acid profile (in % of total fatty acids) of the compound feeds (Inve Rot and Inve Artem) and of the microalgae (*Aurantiochytrium* sp., *Isochrysis* sp., and *Nannochloropsis* sp.) used to feed the rotifers and artemia.

Fatty acid (% total fatty acids)	Inve Rot	Inve Artem	*Aurantiochytrium* sp.	*Isochrysis* sp.	*Nannochloropsis* sp.
16:0	27.0 ± 0.9[a]	15.0 ± 0.6[b]	28.0 ± 0.1[a]	9.5 ± 0.4[c]	16.8 ± 0.7[b]
18:0	4.6 ± 0.2[a]	3.5 ± 0.1[a]	0.8 ± 0.1[b]	0.1 ± 0.2[c]	0.1 ± 0.1[c]
Σ SFA	34.3 ± 0.7[b]	24.0 ± 0.7[c]	48.1 ± 0.4[a]	22.3 ± 0.9[d]	24.3 ± 0.8[c]
16:1 n7	6.1 ± 0.2[b]	4.9 ± 0.2[a]	11.9 ± 0.0[c]	4.3 ± 0.1[a]	22.0 ± 1.2[d]
18:1 n9	16.1 ± 0.7[a]	19.2 ± 0.5[a]	0.0 ± 0.0[c]	7.4 ± 0.2[b]	7.8 ± 0.1[b]
18:1 n7	1.7 ± 0.1[b]	2.4 ± 0.1[b]	10.7 ± 0.0[a]	1.2 ± 0.0[c]	0.2 ± 0.2[d]
Σ MUFA	25.1 ± 0.5[b]	29.1 ± 0.8[a]	22.9 ± 0.0[c]	18.8 ± 0.4[d]	30.8 ± 1.1[a]
18:2 n6	7.6 ± 0.1[a]	7.3 ± 0.2[a]	0.1 ± 0.0[d]	4.1 ± 0.1[b]	3.5 ± 0.1[c]
20:4 n6	1.0 ± 0.1[b]	1.2 ± 0.0[b]	0.1 ± 0.0[d]	0.4 ± 0.0[c]	9.2 ± 0.2[a]
22:5 n6	0.2 ± 0.4[b]	0.6 ± 0.5[b]	7.4 ± 0.2[d]	2.9 ± 0.2[c]	0.1 ± 0.1[a]
Σ n6 PUFA	8.9 ± 0.4[b]	9.9 ± 0.6[b]	7.9 ± 0.1[a]	7.7 ± 0.2[a]	13.1 ± 0.6[c]
18:3 n3	1.1 ± 0.1[b]	1.5 ± 0.0[b]	0.0 ± 0.0[a]	6.9 ± 0.2[c]	0.0 ± 0.1[a]
18:4 n3	0.8 ± 0.1[b]	1.0 ± 0.0[b]	0.0 ± 0.0[c]	19.6 ± 0.4[a]	0.0 ± 0.0[c]
20:5 n3	0.2 ± 0.4[a]	7.7 ± 0.1[b]	0.6 ± 0.1[a]	0.6 ± 0.0[a]	28.3 ± 0.7[c]
22:6 n3	20.8 ± 1.0[a]	20.7 ± 0.3[a]	19.1 ± 0.2[a]	18.6 ± 1.7[a]	0.2 ± 0.3[b]
Σ n3 PUFA	31.3 ± 0.6[b]	33.9 ± 0.1[b]	20.0 ± 0.3[d]	47.5 ± 0.7[a]	28.6 ± 0.9[c]
Σ PUFA	40.6 ± 1.1[b]	44.9 ± 0.6[b]	28.0 ± 0.4[a]	57.0 ± 0.7[c]	42.2 ± 1.4[b]
Σ n3/Σ n6	3.52 ± 0.14[b]	3.44 ± 0.23[b]	2.53 ± 0.01[c]	6.17 ± 0.11[a]	2.19 ± 0.03[d]

Values are presented as average ± standard deviation. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between the relative (%) FA profiles of used feeds.

Table 2. Fatty acid profile (in mg/g dry weight) of the compound feeds (Inve Rot and Inve Artem) and of the microalgae (*Aurantiochytrium* sp., *Isochrysis* sp., and *Nannochloropsis* sp.) used to feed the rotifers and artemia.

Fatty acid (mg/g dry weight)	Inve Rot	Inve Artem	*Aurantiochytrium* sp.	*Isochrysis* sp.	*Nannochloropsis* sp.
16:0	52.8 ± 1.5[b]	67.2 ± 4.0[b]	131.5 ± 0.8[a]	9.1 ± 1.0[d]	23.4 ± 1.5[c]
18:0	8.9 ± 0.2[b]	15.7 ± 1.2[a]	4.1 ± 0.2[c]	0.1 ± 0.2[d]	0.2 ± 0.2[d]
Σ SFA	67.3 ± 1.0[c]	107.3 ± 7.0[b]	225.0 ± 1.5[a]	21.7 ± 3.1[e]	33.7 ± 2.3[d]
16:1 n7	11.8 ± 0.4[b]	22.0 ± 1.4[c]	55.7 ± 0.3[e]	4.1 ± 0.5[aa]	30.6 ± 2.7[d]
18:1 n9	31.0 ± 1.1[b]	86.1 ± 6.1[a]	0.0 ± 0.0[e]	7.2 ± 1.0[d]	10.9 ± 0.7[c]
18:1 n7	3.3 ± 0.0[c]	10.8 ± 1.5[b]	50.1 ± 0.2[a]	1.1 ± 0.2[d]	0.2 ± 0.2[e]
Σ MUFA	48.9 ± 0.5[c]	130.5 ± 9.5[a]	107.3 ± 0.6[b]	18.3 ± 2.6[d]	42.7 ± 3.0[c]
18:2 n6	14.9 ± 0.2[b]	32.6 ± 3.1[a]	0.3 ± 0.0[d]	4.0 ± 0.6[c]	4.9 ± 0.3[c]
20:4 n6	1.9 ± 0.1[c]	5.3 ± 0.4[b]	0.5 ± 0.0[c]	0.4 ± 0.1[c]	12.7 ± 0.4[a]
22:5 n6	0.9 ± 0.8[a]	2.7 ± 2.4[b]	35.2 ± 0.0[c]	2.8 ± 0.3[b]	0.1 ± 0.2[a]
Σ n6 PUFA	18.0 ± 0.7[b]	44.3 ± 6.0[a]	37.5 ± 0.0[a]	7.5 ± 0.8[c]	18.1 ± 0.3[b]
18:3 n3	2.2 ± 0.2[b]	6.6 ± 0.5[a]	0.0 ± 0.0[c]	6.7 ± 0.9[a]	0.0 ± 0.1[c]
18:4 n3	1.6 ± 0.2[c]	4.3 ± 0.4[b]	0.2 ± 0.0[d]	19.1 ± 2.7[a]	0.0 ± 0.0[d]
20:5 n3	15.2 ± 0.6[c]	34.6 ± 3.2[b]	2.5 ± 0.0[d]	0.6 ± 0.1[e]	39.3 ± 1.3[a]
22:6 n3	39.6 ± 0.7[b]	92.7 ± 7.6[a]	89.8 ± 0.2[a]	18.0 ± 2.2[c]	0.2 ± 0.4[d]
Σ n3 PUFA	61.6 ± 1.7[c]	152.0 ± 13.7[a]	94.8 ± 0.2[b]	46.0 ± 5.5[d]	39.6 ± 1.0[e]
Σ PUFA	80.9 ± 3.0[c]	201.3 ± 20.1[a]	132.4 ± 0.3[b]	55.3 ± 0.6[d]	58.4 ± 1.4[d]
Σ n3/Σ n6	3.52 ± 0.14[b]	3.44 ± 0.23[b]	2.53 ± 0.01[c]	6.17 ± 0.11[a]	2.19 ± 0.03[d]
Σ FA	197.1 ± 3.2[b]	439.1 ± 22.4[a]	464.7 ± 2.1[a]	95.3 ± 3.8[d]	134.8 ± 3.9[c]

Values are presented as average ± standard deviation. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between the absolute (mg/g dw) FA profiles of used feeds.

were dominant in the case of the thraustochytrid, PUFA represented the main FA share in the other three feeds. Another consequence and second main aspect was a relatively high n3/n6 ratio in the various feeds. However, this ratio was as low as 2.53 ± 0.01 in the case of *Aurantiochytrium* sp. and as high as 6.17 ± 0.11 in *Isochrysis* sp. A third main issue relates to the considerable variability observed within the main classes of the SFA, monounsaturated FA (MUFA), and PUFA.

Particularly, within SFA, the palmitic acid (16:0) content varied between 9.5 ± 0.4% in *Isochrysis* sp. to its threefold percentage (27–28 %) in the compound diet and *Aurantiochytrium* sp. This largely determined the variation of the total SFA. Regarding MUFA, each of the three main

FAs, 16:1 n7, oleic acid (18:1 n9), and 18:1 n7, is specifically abundant in particular feeds. Concerning n6 and n3 PUFA, it is worth comparing their main FAs in parallel. Namely, the C20 FAs —arachidonic acid (20:4 n6) and EPA— were more abundant in *Nannochloropsis* sp. than in the other three feeds used in the rotifers trial. For EPA, in particular, its concentration did not surpass 1 % for these three feeds, but reached 28.3 ± 0.7% in *Nannochloropsis* sp. The C22 FAs —22:5 n6 and DHA— were almost absent in this microalgal species (≤0.2 %), but were abundant in *Aurantiochytrium* sp., totalling more than 26 %. In the cases of the INVE diet and *Isochrysis* sp., however, DHA levels near 20 % were coupled with 22:5 n6 levels below 3 %. In the case of *Isochrysis* sp., it was also observed a

noteworthy high value of stearidonic acid (18:4 n3), 19.6 ± 0.4%, far exceeding all other feeds.

With respect to FA levels in mg/g feed dw, a distinct picture can be drawn as a result of different lipid contents in each feed, as inferred from the total FA methyl ester (FAME) levels in each rotifer feed. Indeed, total FAME varied from 95.3 mg/g dw in *Isochrysis* sp. and 134.8 mg/g dw in *Nannochloropsis* sp. to 197.1 mg/g dw in INVE (compound feed) and to 464.7 mg/g dw in *Aurantiochytrium* sp. Therefore, several FA contents became higher in the latter feed with respect to other feeds. Despite the relative low total FAME content in *Nannochloropsis* sp., this microalgal feed offers the highest absolute contents of two FAs, both C20 FAs, arachidonic acid and EPA, 12.7 ± 0.4 mg/g dw and 39.3 ± 1.3 mg/g dw, respectively. Even the leanest feed, *Isochrysis* sp., is the richest source of two FAs —among those feeds fed to rotifers—, the alpha-linolenic acid (18:3 n3), reaching 6.7 ± 0.9 mg/g dw, and stearidonic acid, equalling 19.1 ± 2.7 mg/g dw. The commercial feed (Inve Rot) was the richest feed —among those fed to rotifers— in stearic acid (18:0), oleic acid, and linoleic acid (18:2 n6), all of them C18s. It should also be stressed that DHA varied from less than 1 mg/g dw in *Nannochloropsis* sp. to 89.8 ± 0.2 mg/g dw in the thraustochytrid feed.

3.1.2. Artemia feeds

The relative FA profile of the compound feed (INVE) supplied to artemia was poorer in SFA than the compound feed used in the rotifers' trial, thus not distinguishing itself from the SFA content in *Nannochloropsis* sp. profile (Table 1). The SFA content of Inve Artem, *Isochrysis* sp., and *Nannochloropsis* sp. was in the 22.3–24.3 % interval. This fact may be ascribed to a palmitic acid content in the INVE diet for artemia between this FA levels in *Isochrysis* sp. and *Nannochloropsis* sp., 15.0 ± 0.6%. Regarding MUFA, both INVE feeds were rich in oleic acid and, as a consequence, in MUFA. In the case of the commercial feed for artemia, the MUFA level does not differ from this parameter in *Nannochloropsis* sp. The similarity of the two INVE feeds is also observed in the case of n6 PUFA and DHA. For EPA, there is a contrast, leading to a content in INVE feed supplied to artemia that was higher than that of *Aurantiochytrium* sp. and *Isochrysys* sp., a difference inexistent in the case of INVE given to rotifers. Remaining aspects of the FA composition did not differ between both compound feeds.

With respect to the absolute FA profiles (expressed in mg/g feed dw), a stronger contrast emerges between the two INVE feeds, since while the rotifer feed had a total FAME content of 197.1 mg/g dw, the artemia feed contained 439.1 mg of total FAME per g dw (Table 2). This high level almost matches the total FAME concentration in *Aurantiochytrium* sp., 464.7 mg/g dw.

As in the case of the rotifer trial (see 3.1.1.), there is wide variability in the FA profiles of the four feeds, leading to a possible variable nutritional impact on artemia.

3.2. Fatty acid profile of rotifers and artemia

The fatty acid composition (in % of total fatty acids and in mg/g dry weight) of the rotifers and *Artemia franciscana* before the experiment (control) and fed the alternative diets (INVE Rotifers, INVE Artemia, AUR, ISO, and NAN) is displayed in Table 3 and Table 4.

It should be noted that, particularly in the case of rotifers, enrichment is affected by organism condition. After the enrichment period, rotifers were active and, besides a lower number of rotifers in the AUR and ISO treatments, no major differences in the populations associated to the different feeds were observed.

The obtained relative fatty acid profiles in the case of the studied rotifers showed relevant differences with respect to the control rotifers (prior to trial) and between the four groups (compound, AUR, ISO, and NAN), but less stark than in the feeds themselves. The control rotifers were poorer in palmitic acid, total SFA, and palmitoleic acid (16:1 n7) and richer in total n6 PUFA as well as total PUFA. Indeed, the diet rotifers (INVE, AUR, ISO, and NAN) exhibited increases in the palmitic acid relative content between approximately 50 % in ISO rotifers to almost 90 % in AUR rotifers (from 9.1 ± 0.4% to 16.8 ± 0.9% of total FA). The enrichment in SFA was more modest, being the highest increase from 19.0 ± 0.7% to 21.5 ± 2.3% (INVE) with respect to control rotifers. On the other hand, the total n6 PUFA content fell from 19.9 ± 0.3% in control rotifers to 12.5–14.7 % of total FA in the rotifers fed the various feeds. This decline was more pronounced in the case of the total PUFA content, which was above 50 % of total FA (58.4 ± 1.7%) in the control rotifers, but below this threshold in the trial rotifer groups (37.1–49.7 %).

Table 3. Fatty acid profile (in % total fatty acids) of the different rotifer and artemia groups before the trial (Control) and as a function of their feed (INVE: Inve/compound; AUR: *Aurantiochytrium* sp.; ISO: *Isochrysis* sp.; and NAN: *Nannochloropsis* sp.).

Fatty acid (% total fatty acids)	Rotifers					Artemia				
	Control	Inve	AUR	ISO	NAN	Control	Inve	AUR	ISO	NAN
16:0	9.1 ± 0.4[a]	14.8 ± 1.6[cd]	16.8 ± 0.9[d]	12.8 ± 0.7[c]	15.9 ± 1.4[d]	8.3 ± 0.9[a]	9.5 ± 0.3[a]	12.6 ± 0.8[bc]	9.8 ± 0.1[a]	10.3 ± 0.2[ab]
18:0	4.6 ± 0.1[ab]	4.1 ± 0.6[ab]	3.6 ± 0.4[a]	3.9 ± 0.1[a]	3.7 ± 0.2[a]	5.8 ± 0.2[bc]	3.7 ± 0.0[a]	5.0 ± 0.1[b]	6.6 ± 0.1[c]	6.8 ± 0.7[c]
Σ SFA	19.0 ± 0.7[a]	21.5 ± 2.3[b]	28.6 ± 1.6[d]	24.0 ± 0.5[bc]	25.8 ± 2.2[cd]	18.6 ± 1.0[a]	17.3 ± 0.3[a]	23.1 ± 0.8[bc]	21.8 ± 0.3[bc]	20.6 ± 1.5[ab]
16:1 n7	2.2 ± 0.1[a]	6.8 ± 0.4[c]	7.7 ± 0.4[cd]	4.6 ± 0.3[b]	11.5 ± 0.8[e]	7.5 ± 1.1[c]	7.4 ± 0.5[c]	9.0 ± 0.6[d]	7.6 ± 0.2[cd]	9.0 ± 0.6[d]
18:1 n9	7.3 ± 0.3[b]	15.4 ± 1.5[d]	2.8 ± 0.2[a]	6.2 ± 0.2[b]	7.4 ± 0.3[b]	19.5 ± 0.2[e]	18.1 ± 0.5[e]	12.9 ± .4[c]	17.9 ± 0.4[e]	19.0 ± 1.7[e]
18:1 n7	3.5 ± 0.3[ab]	2.3 ± 0.2[a]	9.6 ± 0.4[c]	4.8 ± 0.1[b]	5.7 ± 0.1[b]	12.7 ± 0.1[cd]	9.5 ± 0.2[c]	14.5 ± 0.4[d]	13.5 ± 0.1[d]	13.9 ± 1.3[d]
Σ MUFA	20.7 ± 0.5[a]	27.8 ± 1.5[bc]	24.7 ± 1.1[ab]	21.8 ± 0.4[a]	29.8 ± 1.1[c]	41.7 ± 0.7[e]	36.5 ± 1.2[d]	37.4 ± 0.7[d]	40.8 ± 0.8[e]	42.9 ± 1.7[e]
18:2 n6	4.8 ± 0.2[bc]	8.3 ± 0.5[d]	1.7 ± 0.1[a]	3.9 ± 0.3[b]	4.1 ± 0.3[b]	9.0 ± 0.1[e]	7.4 ± 0.4[d]	5.4 ± 0.2[c]	7.4 ± 0.1[d]	7.7 ± 0.5[d]
20:4 n6	5.0 ± 0.1[d]	2.5 ± 0.1[ab]	2.3 ± 0.1[a]	2.5 ± 0.1[ab]	5.5 ± 0.4[d]	2.8 ± 0.3[b]	2.2 ± 0.1[a]	3.0 ± 0.1[b]	2.6 ± 0.1[ab]	3.8 ± 0.4[c]
22:5 n6	8.2 ± 0.4[a]	3.3 ± 0.1[c]	8.3 ± 0.4[a]	4.8 ± 0.2[b]	3.0 ± 0.3[c]	0.3 ± 0.0[e]	1.9 ± 0.0[d]	3.2 ± 0.1[c]	0.3 ± 0.2[e]	0.1 ± 0.2[e]
Σ n6 PUFA	19.9 ± 0.3[a]	14.7 ± 0.1[b]	13.4 ± 0.5[bc]	12.5 ± 0.6[cd]	14.3 ± 1.0[b]	12.8 ± 0.5[c]	12.1 ± 0.1[cde]	12.1 ± 0.2[cde]	11.0 ± 0.2[e]	12.0 ± 0.4[de]
18:3 n3	0.7 ± 0.1[b]	1.0 ± 0.0[b]	0.1 ± 0.1[a]	2.4 ± 0.1[c]	0.2 ± 0.0[a]	9.4 ± 0.1[g]	5.0 ± 0.2[d]	6.3 ± 0.2[e]	7.9 ± 0.1[f]	7.8 ± 0.6[f]
18:4 n3	0.2 ± 0.0[a]	0.3 ± 0.3[a]	0.1 ± 0.1[a]	7.3 ± 0.2[d]	0.0 ± 0.0[a]	1.5 ± 0.0[b]	0.9 ± 0.0[b]	0.9 ± 0.0[b]	2.7 ± 0.1[c]	1.2 ± 0.1[b]
20:5 n3	5.5 ± 0.0[ab]	6.9 ± 0.9[b]	1.2 ± 1.6[a]	3.0 ± 0.1[a]	9.2 ± 1.2[bc]	10.7 ± 0.9[cd]	10.7 ± 0.5[cd]	9.3 ± 0.4[bc]	8.9 ± 0.3[bc]	12.4 ± 1.4[d]
22:6 n3	23.7 ± 1.2[a]	20.3 ± 2.5[a]	21.3 ± 2.3[a]	13.8 ± 0.8[b]	7.4 ± 1.0[c]	0.3 ± 0.2[d]	11.8 ± 0.3[b]	7.3 ± 0.4[c]	0.6 ± 0.1[d]	0.0 ± 0.0[d]
Σ n3 PUFA	38.1 ± 1.4[a]	33.4 ± 3.7[ab]	27.1 ± 3.7[cd]	36.5 ± 0.7[a]	22.4 ± 2.0[de]	22.9 ± 1.1[de]	30.1 ± 0.2[bc]	24.3 ± 0.7[cde]	20.8 ± 0.4[e]	21.6 ± 1.7[de]
Σ PUFA	58.4 ± 1.7[a]	48.6 ± 3.9[b]	40.8 ± 4.2[cd]	49.7 ± 0.6[b]	37.1 ± 3.1[cde]	38.1 ± 1.4[cde]	43.7 ± 0.1[bc]	38.0 ± 0.8[cde]	33.9 ± 0.6[e]	35.3 ± 1.7[de]
Σ n3/Σ n6	1.92 ± 0.04[abc]	2.27 ± 0.23[cd]	2.03 ± 0.21[bc]	2.92 ± 0.15[e]	1.57 ± 0.04[a]	1.78 ± 0.02[ab]	2.48 ± 0.00[d]	2.00 ± 0.05[bc]	1.89 ± 0.01[abc]	1.81 ± 0.14[ab]

Values are presented as average ± standard deviation. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between the relative (%) FA profiles of studied organisms.

Table 4. Fatty acid profile (in mg/g dry weight) of the different rotifer and artemia groups before the trial (Control) and as a function of their feed (INVE: Inve/compound; AUR: *Aurantiochytrium* sp.; ISO: *Isochrysis* sp.; and NAN: *Nannochloropsis* sp.).

Fatty acid (mg/g dry weight)	Rotifers					Artemia				
	Control	Inve	AUR	ISO	NAN	Control	Inve	AUR	ISO	NAN
16:0	3.0 ± 0.1[a]	10.9 ± 0.6[c]	9.9 ± 0.4[c]	6.3 ± 0.6[b]	9.6 ± 1.5[bc]	8.4 ± 2.7[bc]	23.1 ± 0.2[e]	18.5 ± 2.6[d]	9.9 ± 0.5[c]	10.0 ± 1.9[c]
18:0	1.5 ± 0.0[a]	3.1 ± 0.2[c]	2.2 ± 0.0[bc]	1.9 ± 0.1[b]	2.2 ± 0.4[bc]	5.8 ± 1.0[d]	9.0 ± 0.1[f]	7.3 ± 0.6[e]	6.7 ± 0.5[e]	6.6 ± 0.8[e]
Σ SFA	6.3 ± 0.2[a]	15.6 ± 1.6[b]	16.9 ± 0.7[bc]	11.9 ± 0.9[b]	15.6 ± 2.5[b]	18.7 ± 4.9[bc]	42.3 ± 0.4[e]	34.0 ± 3.9[d]	21.9 ± 1.0[c]	20.1 ± 4.8[c]
16:1 n7	0.7 ± 0.0[a]	4.9 ± 0.1[bcd]	4.3 ± 0.3[bc]	2.3 ± 0.2[b]	7.0 ± 1.5[cde]	7.5 ± 2.6[de]	18.0 ± 0.7[g]	13.2 ± 1.9[f]	7.7 ± 0.6[de]	8.8 ± 2.1[e]
18:1 n9	2.4 ± 0.1[a]	11.4 ± 0.1[c]	1.6 ± 0.1[a]	3.1 ± 0.1[ab]	4.5 ± 0.9[b]	19.4 ± 3.8[d]	44.3 ± 0.2[e]	19.0 ± 1.8[d]	18.0 ± 0.7[d]	18.2 ± 1.8[d]
18:1 n7	1.2 ± 0.1[a]	1.7 ± 0.1[b]	5.6 ± 0.3[c]	2.4 ± 0.1[b]	3.4 ± 0.7[bc]	12.7 ± 2.5[d]	23.3 ± 0.0[e]	21.2 ± 2.1[e]	13.5 ± 0.8[d]	13.4 ± 1.3[d]
Σ MUFA	6.9 ± 0.1[a]	20.0 ± 0.8[c]	14.5 ± 0.6[bc]	10.8 ± 0.6[b]	18.1 ± 3.4[bc]	41.5 ± 9.2[d]	89.1 ± 0.8[f]	55.0 ± 5.7[e]	41.1 ± 1.6[d]	41.4 ± 6.0[d]
18:2 n6	1.6 ± 0.0[a]	6.0 ± 0.0[b]	1.0 ± 0.1[a]	1.9 ± 0.1[a]	2.5 ± 0.7[a]	8.9 ± 1.8[c]	18.0 ± 0.4[d]	8.0 ± 0.8[c]	7.4 ± 0.5[bc]	7.5 ± 1.1[c]
20:4 n6	1.7 ± 0.0[a]	1.8 ± 0.0[ab]	1.3 ± 0.0[a]	1.3 ± 0.1[a]	3.4 ± 1.0[cd]	2.8 ± 0.3[bc]	5.4 ± 0.1[e]	4.5 ± 0.4[de]	2.7 ± 0.1[bc]	3.6 ± 0.5[cd]
22:5 n6	2.7 ± 0.1[b]	2.3 ± 0.2[b]	4.7 ± 0.0[a]	2.4 ± 0.2[b]	1.8 ± 0.5[b]	0.3 ± 0.0[c]	4.6 ± 0.2[a]	4.7 ± 0.2[a]	0.3 ± 0.2[c]	0.1 ± 0.2[c]
Σ n6 PUFA	6.6 ± 0.1[a]	10.2 ± 0.4[bcd]	7.6 ± 0.1[ab]	6.2 ± 0.1[a]	8.8 ± 2.4[abc]	12.7 ± 2.2[d]	29.7 ± 0.5[f]	17.8 ± 1.2[e]	11.0 ± 0.4[cd]	11.5 ± 1.7[d]
18:3 n3	0.2 ± 0.0[a]	0.7 ± 0.1[a]	0.1 ± 0.0[a]	1.2 ± 0.1[a]	0.1 ± 0.0[a]	9.4 ± 2.0[b]	12.3 ± 0.3[c]	9.3 ± 0.9[b]	7.9 ± 0.4[b]	7.6 ± 1.7[b]
18:4 n3	0.1 ± 0.0[a]	0.2 ± 0.2[a]	0.1 ± 0.1[a]	3.6 ± 0.2[d]	0.0 ± 0.0[a]	1.5 ± 0.4[b]	2.3 ± 0.0[c]	1.3 ± 0.1[b]	2.7 ± 0.2[c]	1.0 ± 0.1[b]
20:5 n3	1.8 ± 0.0[a]	4.7 ± 0.2[b]	0.1 ± 0.0[a]	1.5 ± 0.1[a]	5.7 ± 2.0[b]	10.6 ± 1.3[c]	26.2 ± 0.6[e]	13.6 ± 1.1[d]	9.0 ± 0.2[c]	12.0 ± 2.4[d]
22:6 n3	7.9 ± 0.3[c]	13.6 ± 0.4[e]	11.7 ± 0.2[d]	6.8 ± 0.7[c]	4.5 ± 1.3[b]	0.3 ± 0.2[a]	28.8 ± 0.1[f]	10.7 ± 0.7[d]	0.6 ± 0.0[a]	0.0 ± 0.0[a]
Σ n3 PUFA	12.7 ± 0.3[a]	22.1 ± 0.1[c]	14.6 ± 0.2[b]	18.1 ± 1.0[bc]	13.7 ± 3.9[b]	22.7 ± 3.6[c]	73.6 ± 1.2[e]	35.7 ± 2.3[d]	20.9 ± 0.8[c]	20.9 ± 4.2[c]
Σ PUFA	19.5 ± 0.3[a]	32.7 ± 0.4[cd]	22.4 ± 0.3[b]	24.6 ± 1.1[bc]	22.7 ± 6.3[b]	37.7 ± 6.5[d]	106.9 ± 2.2[f]	55.7 ± 3.6[e]	34.1 ± 1.3[d]	34.2 ± 6.2[d]
Σ n3/Σ n6	1.92 ± 0.04[abc]	2.27 ± 0.23[cd]	2.03 ± 0.21[bc]	2.92 ± 0.15[e]	1.57 ± 0.04[a]	1.78 ± 0.02[ab]	2.48 ± 0.00[d]	2.00 ± 0.05[bc]	1.89 ± 0.01[abc]	1.81 ± 0.14[ab]

Values are presented as average ± standard deviation. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between the absolute (mg/g dw) FA profiles of studied organisms.

AUR rotifers' FA profile was distinguished from the FA profile determined in the INVE rotifers by very low oleic and linoleic acid contents —also lower than in the ISO and NAN groups— and high 18:1 n7 and 22:5 n6 contents, thereby generating a n3/n6 ratio of 2.03 ± 0.21, lower than in ISO rotifers, 2.92 ± 0.15, but higher than in NAN rotifers, 1.57 ± 0.04. Both ISO and NAN rotifers differed from INVE in lower oleic, linoleic acid, and DHA contents, 6.2–7.4 % *vs* 15.4 ± 1.5%, 3.9–4.1 % *vs* 8.3 ± 0.5%, and 7.4–13.8 % *vs* 20.3 ± 2.5%, respectively. The low n3/n6 ratio of NAN rotifers is mainly due to their very low DHA content, since their EPA content was quite high, 9.2 ± 1.2%. Moreover, the arachidonic acid and 16:1 n7 contents were higher in NAN rotifers than in all other groups, 11.5 ± 0.8% *vs* 4.6–7.7 % and 5.5 ± 0.4% *vs* 2.3–2.5 %, respectively. It is also worth noting that ISO rotifers were relatively poor in palmitic acid and 16:1 n7, but slightly richer in alpha-linolenic acid than all other rotifers.

As to *Artemia franciscana*, the organisms before trial (control artemia) were specifically characterized by being rich in linoleic and alpha-linolenic acids. Regarding the former FA, its content was reduced from 9.0 ± 0.1% to 5.4–7.7 % of total FA in the trial artemia organisms. As to the latter, reductions were all greater than 15 % and, in the case of INVE artemia, alpha-linolenic content prior to trial (control) was halved to only 5.0 ± 0.2%. The remaining FA did not differ much between control and trial artemia.

In addition, it should be remarked that all artemia organisms were poorer in palmitic acid, 22:5 n6, and DHA than the trial rotifers, 8.3–12.6 % *vs* 12.8–16.8 %, 0.1–3.2 % *vs* 3.0–8.3 %, and <12 % *vs* 7.4–21.3 %, respectively. On the other hand, trial artemia were richer in oleic acid, alpha-linolenic acid, and EPA than the trial rotifers, 12.9–19.0 % *vs* 2.8–15.4 %, 5.0–7.9 % *vs* 0.1–2.4 %, and 8.9–12.4 % *vs* 1.2–9.2 %, respectively. As observed in the rotifers, AUR artemia had lower oleic and linoleic acid contents and higher 22:5 n6 content than the other artemia groups. Furthermore, ISO and NAN artemia had less DHA than the INVE artemia, <1 % *vs* 11.8 ± 0.3%. Again, as in rotifers, arachidonic acid and EPA were relatively high in the NAN group, 3.8 ± 0.4% and 12.4 ± 1.4%, respectively. The n3/n6 ratios in artemia were similar to those in the rotifers, being its range somewhat narrower, 1.78–2.48.

Finally, it should be remarked that the absolute values of the control rotifers and artemia (prior to the feeding experiments) were mostly very low, a consequence of low fat reserves. Their values were usually lower than their counterparts (either INVE, AUR, ISO, and NAN rotifers or INVE, AUR, ISO, and NAN artemia) with the exception of oleic acid and some PUFA. However, even in these cases, the control absolute values were always near the low end of the variation range in the various groups.

More specifically, for rotifers, there were FA whose absolute concentrations increased greatly in all diet groups, such as palmitic acid, total SFA, and palmitoleic acid. In the case of palmitic acid, its content was twofold the control group in ISO rotifers or even higher in the other three groups. Other FA also increased its level after feeding, namely, stearic acid, total MUFA, total n3 PUFA, and total PUFA, but with more modest increases. In other cases, only some groups registered enrichment with respect to control rotifers. In fact, this was the case of oleic acid and INVE rotifers, with 11.4 ± 0.1 mg/g dw *vs* 2.4 ± 0.1 mg/g dw in control rotifers. This was largely replicated by the total MUFA parameter. INVE rotifers also showed enrichment in linoleic acid, EPA, DHA, and total PUFA levels with respect to control rotifers. NAN rotifers were clearly enriched in arachidonic acid —doubled its content in control— and EPA, 5.7 ± 2.0 mg/g dw *vs* 1.8 ± 0.0 mg/g dw in control rotifers. This enrichment was not significantly different from that experienced by the INVE rotifers, reaching 4.7 ± 0.2 mg/g dw. In the case of DHA, its level in control, 7.9 ± 0.3 mg/g dw, augmented 50 % to 11.7 ± 0.2 mg/g dw in AUR rotifers and 70 % to 13.6 ± 0.4 mg/g dw in INVE rotifers.

The analysis of the artemia absolute contents also points to INVE (compound) feed as particularly efficient at enriching the FA profile of these organisms. Indeed, besides those FA whose absolute contents augmented in all diet groups, INVE artemia displayed enrichment of palmitoleic and oleic acids, total MUFA, linoleic and arachidonic acids, total n6 PUFA, alpha-linoleic, EPA, DHA, total n3 PUFA, and total PUFA in comparison to the control. Noteworthy cases are oleic acid, from 19.4 ± 3.8 mg/g dw in control to 44.3 ± 0.2 mg/g dw, linoleic acid, from 8.9 ± 1.8 mg/g dw in control to 18.0 ± 0.4 mg/g dw, EPA, and DHA. Concerning these latter two, other artemia groups showed signs of substantial enrichment. However, the compound feed was more efficient in this regard. While AUR and NAN artemia had increased their EPA level from 10.6 ± 1.3 mg/g dw in control to 13.6 ± 1.1 and 12.0 ± 2.4 mg/g dw, respectively, INVE artemia reached more than the double, 26.2 ± 0.6

mg/g dw. Moreover, prior to trial beginning, artemia organisms were very poor in DHA, 0.3 ± 0.2 mg/g dw (control), but were much enriched in this key FA by the INVE and AUR feeds, to 28.8 ± 0.1 and 10.7 ± 0.7 mg/g dw, respectively.

4. Discussion

4.1. Fatty acid profile of feeds

A more detailed analysis of the FA composition of the feeds may offer some glimpses into the metabolic paths in each microalga species. In fact, results suggest some specificities of the FA elongation and desaturation machinery of these microalgae. While *Nannochloropsis* sp. microalgae might desaturate and elongate 18:2 n6 and 18:3 n3 to arachidonic acid and EPA, *Aurantiochytrium* sp. apparently accumulates, after desaturation and elongation, 22:5 n6 and DHA. In addition, the FA profile of microalgae depends on cultivation parameters and growth phase at the time of harvest, as observed with *I. galbana* (Durmaz et al., 2008). For instance, it has been claimed an inverse relation between EPA and DHA concentrations and cultivation temperature (Tasselli and Doimi, 1990).

Therefore, the microalgae studied as alternatives to the compound feed are all different, offering variable lipid molecules and, as a consequence, being liable to have a distinctive nutritional impact on rotifers and artemia.

4.2. Fatty acid profile of rotifers and artemia

The FA profile of the animals offers invaluable information that needs analysis. The abundance of 18:1 n7 and C22 FAs and scarcity of oleic and linoleic acids in AUR rotifers largely reflects their feed. Likewise, the abundance of 16:1 n7 and C20 FAs in NAN rotifers corresponds to the FA profile of *Nannochloropsis* sp. The same correspondence is clear when the FA profiles of *Isochrysis* sp. and ISO rotifers are compared, namely, regarding palmitic, 16:1 n7, and alpha-linolenic acids, or when the FA composition of INVE feed is compared to that of INVE rotifers. Despite these similarities, there were relevant deviations between the feeds' profiles and the rotifers' profiles. In fact, very high palmitic acid contents in INVE and AUR feeds or EPA content in NAN feed as well as high alpha-linolenic acid content and very high stearidonic acid content in ISO feed were much reduced in the respective rotifer groups. In the opposite direction, the very low oleic acid content in AUR feed or DHA content in NAN feed contrasted with somewhat higher contents in the corresponding rotifers. It is worth mentioning that while, for *Nannochloropsis*-fed *A. salina*, there was no ability to synthesize DHA from its EPA-rich feed (Chakraborty et al., 2007), *Brachionus plicatilis* fed with *Nannochloropsis oculata* were able to synthesize DHA in non-negligible quantities (Birkou et al., 2012). The sets of observations in the current study suggest that either there was a previous influence of the rotifer FA composition prior to trials (control) —despite the 12 h period without feeding— or relevant biosynthetic pathways had an effect on the final rotifer FA profiles. The previous hypothesis supports all deviations with exception of oleic acid, since the control rotifers had a starting FA profile that deviated from the diets in the mentioned FA examples. Hence, it is possible that such deviations would disappear by extending the feed experiments.

The absolute FA profiles of rotifers and artemia reflected both the relative composition as well as the total FAME content. The absolute FA profiles before the beginning of the feeding trial were characterized by very low contents and, as a consequence, low total FAME content, thus enabling a FA enrichment in multiple directions. Moreover, taking into account total FAME content, trial artemia were fatter than trial rotifers, 95.7–238.3 mg/g dw *vs* 47.3–68.3 mg/g dw, and compound feeds led to fatter organisms than AUR feeds, 68.3–238.3 mg/g dw *vs* 53.8–144.7 mg/g dw. Moreover, AUR feeds were, in turn, fatter than ISO and NAN feeds, whose total FAME content were in the 47.3–97.1 mg/g dw interval. While the fatter artemia cannot be ascribed to feeds, since they were

identical in both types of organism with exception of the compound feed and control artemia animals were already fatter than control rotifers, the fatter compound and AUR organisms largely mirror the total FAME content of the feeds themselves. However, the fat present in the compound feeds was much more extensively accumulated in both organisms than that of AUR feeds. For instance, in the case of the rotifers, AUR feed had a very high total FAME level of 464.7 mg/g dw and compound feed had 197.1 mg/g dw, but AUR rotifers contained only 53.8 mg/g dw, which compares to 68.3 mg/g dw in the INVE rotifers.

A more painstaking analysis of all absolute FA compositions reveals that the deposition of particular FAs in the animal biomass deviates from the average across FAs. Some FAs seem to be either poorly absorbed or converted into other FAs. This is the case for palmitic and stearidonic acids in rotifers and artemia and DHA in artemia. The low levels of palmitic and stearidonic acids in the control organisms and DHA in control artemia may not suffice as explanation. For instance, very rich sources of DHA (compound and AUR feeds), containing approximately 90 mg/g dw feed, led to artemia whose DHA content was in the 10.7–28.8 mg/g dw range. As a reference, it may be mentioned that total FAME levels of 439.1–464.7 mg/g dw in these feeds led to total FAME contents of 144.7–238.3 mg/g dw in artemia. In addition, to a lesser extent, in the specific case of *Nannochloropsis* sp. feeds, its EPA was poorly accumulated in rotifers and artemia (from 39.3 ± 1.3 mg/g dw feed to 5.7–12.0 mg/g dw). This was observed in *Nannochloropsis*-fed *A. salina* previously (Chakraborty et al., 2007). In the rotifer *B. plicatilis*, a significant reduction of EPA by 60 % was observed (Birkou et al., 2012). Arachidonic acid concentration was also lower than expected —on the basis of other FAs' accumulation— in NAN artemia (from 12.7 ± 0.4 mg/g dw feed to 3.6 ± 0.5 mg/g dw artemia) and 22:5 n6 level was lower than expected in AUR artemia (from 35.2 ± 0.0 mg/g dw feed to 4.7 ± 0.2 mg/g dw artemia). For stearidonic acid, its content in ISO feed, 19.1 ± 2.7 mg/g dw, declined to 2.7–3.6 mg/g dw in both types of animals. On the other hand, there were FAs which had higher absolute contents in the animals than in the feeds, thus opposing the observed general trend. In artemia, alpha-linolenic content clearly exceeded this FA level in the feeds, 7.6–12.3 mg/g dw artemia *vs* < 6.7 mg/g dw feed. The control artemia was only moderately rich in this FA, 9.4 ± 2.0 mg/g dw. Without *de novo* synthesis of alpha-linolenic acid, feed levels should lead to larger declines of this FA content in the trial groups. Such was also the case of oleic and linoleic acids in artemia with exception of compound feed, <10.9 mg/g dw feed *vs* 18.0–19.0 mg/g dw artemia and <4.9 mg/g dw feed *vs* 7.4–8.0 mg/g dw artemia, respectively, or 18:1 n7 in artemia with exception of AUR feed, <10.8 mg/g dw feed *vs* 13.4–23.3 mg/g dw artemia. All these were C18 FAs. However, the opposite variation in the case of stearidonic acid, also a C18 FA, should prevent generalizations. Other similar situations albeit more circumscribed were observed for EPA in AUR and ISO artemia and DHA in NAN rotifers.

The high lipid content of *Artemia* sp. has been frequently observed (Haché and Plante, 2011). Moreover, it has often been reported that these organisms are poor in long chain PUFA (Haché and Plante, 2011). This was also observed in the control artemia (Table 4). However, in unenriched nauplii, a relatively high EPA content has been determined and a rapid retroconversion of DHA to EPA by *Artemia* sp. has been shown (Bell et al., 2003; Han et al., 2001; Navarro et al., 1999). It is also apparent from previous experimental work (Chakraborty et al., 2007) that *Artemia* nauplii supplemented with PUFA from the feeds preferentially catabolize DHA relative to EPA. The aforementioned retroconversion could happen within 24 h and it may have occurred with the artemia analysed in the current study, given EPA and DHA contents in AUR and ISO artemia. However, it should be noted that apparently this retroconversion was more intense in ISO artemia than in AUR artemia. A similar contrast is also observable in the rotifers' case. Stearidonic acid may have also been converted to EPA in animals to which ISO diet was given. This higher propensity to convert other n3 PUFA to EPA in animals fed with ISO diet is, however, an artefact derived from the effect of the very low absolute n3 PUFA levels in the ISO diet (if compared with AUR

diet) on the metabolic machinery of artemia (and, to a lesser extent, rotifers).

This study does not support previous work that pointed to a better retention rate of EPA than DHA by rotifers regardless of the ratio available in their enrichment (Rodríguez et al., 1997). Likewise, the comparison to the control rotifers' results is not sufficient to support such conclusion. The experimental work on rotifers (Brachionus plicatilis) and Artemia salina by Haché and Plante (2011) also displayed a significant accumulation of C18 FAs by artemia. For instance, whereas A. salina enriched with a commercial compound product containing <1 % of Σ(18:1 n9, 18:1 n7, 18:2 n6, 18:3 n3) had 28.3 % of these same C18 FAs, the rotifers accumulated a total corresponding to only 8.6 % of the total FAs (Haché and Plante, 2011). Contrastingly, DHA from the same sources was much more concentrated in rotifers than in artemia, 9.8–34.4 % vs 8.3–23.2 %.

In general, there are previous studies which show that the FA composition of rotifers and artemia, including EPA and DHA levels, may be altered by their feed, even if exposition is only 24 h (Haché and Plante, 2011; Lewis et al., 1998). These same studies have found out that even when PUFA levels varied, the n3/n6 ratio remained relatively stable between distinct treatments for both rotifers and artemia. This was not the case in the current study. Particularly, in rotifers, NAN feeds produced a low n3/n6 ratio. The initial (control) composition of the organisms may also have an effect on the final outcome, being worth noting that artemia are frequently rich in C18 FAs, for instance, in Artemia salina this FA group was reported to recurrently represent >30 % of total FAs (Ben Naceur et al., 2010). Furthermore, there is always a complex interplay between feed composition and the metabolism of rotifers and artemia. It is possible that some FAs compete with others in accumulating in these animals.

The attained results do not support an association between low DHA deposition rates and oleic acid concentration in the feeds, since compound and AUR feeds had very different oleic acid contents. Hence, contrary to other studies on rotifers (Li and Olsen, 2015), there seems to be no strong competition between oleic acid and DHA incorporation. Likewise, no such competition in artemia seems to be deduced from current study's results. It is possible that because artemia contains substantial amounts of alpha-linolenic acid, it probably competitively inhibits EPA to DHA conversion (Chakraborty et al., 2007; Kanazawa et al., 1979). This requires further study. In addition, a competition between stearidonic acid and DHA in artemia and, to a lesser extent, in rotifers seems to be unsupported by the evidence, since no particular deposition of stearidonic acid was observed and AUR artemia (with low levels of stearidonic acid) also exhibited a poor accumulation of DHA. Finally, no competitive interaction of EPA or of arachidonic acid on DHA incorporation has been reported in the literature (Han et al., 2001).

Since, traditionally, high n3 PUFA levels and DHA/EPA ratios are the main factors in selecting the best feed, it may be concluded that rotifers fed the compound feed are the best food for fish larvae, being rotifers fed AUR and ISO feeds second best. Furthermore, compound feed was responsible for a more extensive FA enrichment with respect to control rotifers. The appraisal of NAN feeds is less favourable, thus differing from the assessment made by Birkou et al. (2012), which referred that the enrichment of Brachionus lipid with DHA using Nannochloropsis as feed could be of practical interest for aquaculture.

The main difficulty with Aurantiochytrium sp. —if compared with rotifers fed other thraustochytrids, such as Schizochytrium mangrovei (Estudillo-del Castillo et al., 2009) — may result from a large portion of this feed not being eaten by the animals, which represents a challenge for future experimental work. The particular abiotic conditions, such as salinity, and the optimal timing of the feeding periods may also be influential (Teixeira, 2004), thus warranting further research. Concerning artemia, further research aiming at finding the optimal time for their harvest may enable to circumvent its FA profile shortcomings (Nieves-Soto et al., 2020).

5. Conclusions

The fatty acid profiles showed relevant differences between the four feeds (compound, Aurantiochytrium sp., Isochrysis sp., and Nannochloropsis sp.), but less stark than in the feeds themselves. This was due to a complex interplay between feed composition and the metabolism of rotifers and artemia. Whereas Isochrysis sp. was relatively rich in DHA and poor in EPA, Nannochloropsis sp. had the opposite pattern. Aurantiochytrium sp. seems to pose difficulties as a feed for both rotifers and artemia, given the low fat incorporation and, in particular, poor DHA deposition, especially taking into account the high richness of this thraustochytrid in DHA. Taking into account that high n3 PUFA levels and DHA/EPA ratios are influential in selecting the best feed, it may be concluded that rotifers fed the compound feed are the best food for fish larvae, being rotifers fed AUR and ISO feeds best algal-fed solutions. Therefore, these microalgae deserve to be further explored as potential sources of specific FAs in rotifers and artemia. Future work should also focus on preparing more nutritionally balanced feeds using these microalgae as ingredients.

Declarations

Author contribution statement

Paulo M. C., Bandarra N. M.: Conceived and designed the experiments; Wrote the paper.

Cardoso C.: Analyzed and interpreted the data; Wrote the paper.

Coutinho J.: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Castanho S.: Performed the experiments; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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