

# Content in nine minerals and seven vitamins of rotifers (*Brachionus plicatilis*) fed commercial diets and two forms of *Nannochloropsis oculata*

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## Abstract

The objective of the present study was to determine the effects of spray-dried and fresh forms of the microalgae *Nannochloropsis oculata* and other commonly used commercial diets on vitamin and mineral compositions of rotifer produced under commercial conditions. Experimental rotifer groups were fed Protein-Plus<sup>®</sup> (PP treatment), Inactive Baker's Yeast<sup>®</sup> (INBY treatment), Algome<sup>®</sup> (Algome treatment), freshly cultured *N. oculata* (FA treatment), and spray-dried *N. oculata* (SDN treatment). At the end of the experiment, seven essential vitamins, nine macro- and micro-minerals, and two pigments were analyzed in diets and rotifers. All the treatments tested provided adequate levels of Ca, P, Mg, and Co to the rotifers, but insufficient levels of I, Zn, and vitamins B2 and E. Moreover, PP diet produced rotifers with high levels of vitamins B1, B3 and B9, and P; the SDN diet produced rotifers with high levels of P and Cu; the Algome diet produced rotifers high in vitamins B3 and B9, and Ca; the INBY diet produced rotifers with high levels of vitamin B1 and Ca; and the FA diet produced rotifers with more vitamin B6, Se, and Cu. In conclusion, specific minerals or vitamins corrections are required in these feeds for

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rotifers to adequately meet the nutritional needs for marine larvae.

#### KEYWORDS

microalgae, minerals, rotifer, rotifer diets, vitamins

## 1 | INTRODUCTION

Marine fish larvae require certain amounts of essential minerals and vitamins at different life stages. Each mineral and vitamin has important roles during both larval and on-growing stages of farmed fish. Those essential nutrients must be delivered to larvae by copepods, rotifers, and *Artemia* (live preys) or micro-diets to ensure that their needs are covered. Studies on nutrients contents of live preys have been considered in the last decades (Hamre, 2016; Mæhre et al., 2013). Non-enriched live preys may lack nutrients that are essential for fish larvae, such as highly unsaturated fatty acids (HUFAs), essential amino acids (EAA), vitamins, or minerals (Eryalçin, 2018; Eryalçin, 2019; Hamre, Srivastava, et al., 2008). The optimum dietary contents of essential FAs (Eryalçin et al., 2017; Eryalçin et al., 2013; Izquierdo et al., 1989, 2001) and amino acids (Aragão et al., 2004; Srivastava et al., 2006) for marine fish larvae have been well studied in several species. Accordingly, during live preys feeding and enrichment procedures, mainly essential FAs and amino acids contents are considered (Turcihan et al., 2021, 2022). However, more recent studies revealed that other nutrients such as vitamins and minerals play important roles in marine larval physiology (Eryalçin et al., 2020; Hamre et al., 2013; Izquierdo et al., 2017). For instance, deficiencies or excess in some minerals and vitamins such as retinol (vitamin A) (Fernández et al., 2008),  $\alpha$ -tocopherol (vitamin E) (Atalah et al., 2012), vitamin D3 (Sivagurunathan et al., 2022), vitamin K (Sivagurunathan et al., 2023), ascorbic acid (vitamin C) (Betancor et al., 2012; El Kertaoui et al., 2017; Izquierdo et al., 2019; Izquierdo & Betancor, 2015; Ruiz, Betancor, et al., 2019; Ruiz, Hernández-Cruz, et al., 2019), iodine (I) (Hamre et al., 2013; Hamre, Mollan, et al., 2008), selenium (Se) (Hamre, Mollan, et al., 2008; Hamre, Srivastava, et al., 2008; Izquierdo et al., 2017; Saleh et al., 2014), manganese (Mn) (Izquierdo et al., 2017; Terova et al., 2018), copper (Cu) (Penglase et al., 2015; Tseng et al., 2023), zinc (Zn) (Izquierdo et al., 2017; Terova et al., 2018), or the calcium/phosphorous (Ca/P) ratio (El Kertaoui et al., 2019) may affect growth, survival, stress resistance, or bone development (Moren et al., 2011). Moreover, the micro-nutrients contents in enriched zooplankton may be very inconstant due to the large variety of diets and methods commercially available (Hamre et al., 2013). However, less attention has been paid to the determination of minerals and vitamins contents of live preys, apart from some pioneering research (Hamre, 2016; Penglase et al., 2011, 2015; Srivastava et al., 2011).

Rotifers (*Brachionus plicatilis*) are the first live prey utilized in marine fish larval production, and they have critical role in larval survival by delivering essential nutrients for larval requirements. However, the nutritional composition of rotifer feeds and enrichments is not sufficiently characterized and seems to differ among commercial products (Hamre, Mollan, et al., 2008; Hamre, Srivastava, et al., 2008; Mæhre et al., 2013). Besides, the gut/whole body ratio of rotifer is so small that short-term enrichments could not be sufficient to correct rotifers nutritional profiles to fulfil the nutritional needs of fish larvae. Therefore, it is necessary to obtain more information on the micronutrients such as minerals and vitamins of rotifers diets in order to enhance successful larval feeding by rotifer (Hamre et al., 2013; Izquierdo & Koven, 2010).

The nutritional quality of rotifers is affected by two main processes: feeding and enrichment, which depend on different procedures and commercial formulations for feeds and enrichment products (Hamre, 2016). Among the wide variety of feeding products for rotifers, baker's yeast and the fresh microalgae *Nannochloropsis oculata* are the most commonly used in commercial hatcheries. Microalgae are particularly used to attain high rotifer densities and, added to larval rearing tanks, to maintain water quality and rotifers nutritional value. The microalgae *N. oculata* is

more frequently employed due to its suitable size (2–4  $\mu\text{m}$ ), easy production and the good content in essential FAs for larvae, such as eicosapentaenoic acid, protein, and energy (Carvalho et al., 2020; Eryalçin et al., 2015; Fang et al., 2004; Sarker et al., 2018). Moreover, this alga is also high in antioxidants such as  $\alpha$ -tocopherol,  $\beta$ -carotene, and astaxanthin (Brown et al., 1999; Durmaz, 2007; Neto et al., 2018) and contains various vitamins and minerals (Nicoletti, 2016). Furthermore, these algae also show antibacterial properties when distributed in the water that contribute to control the pathogenic bacterial community (Cremen et al., 2007). Other commercial products with high quality, such as PP (Aquafaune Bio-Marine Inc., Hawthorne, USA) or Algome<sup>®</sup> (MarinBio, Aydin, Turkey), have been developed, but their high prices promoted the search for novel products, such as inactive baker's yeast or spray-dried microalgae (Eryalçin, 2019; Khudiyi et al., 2018; Ran et al., 2015). Despite the efficiency of these products in rotifer culture has been previously demonstrated, there is not sufficient information on the vitamin and mineral contents of the rotifers fed with them.

In view of the recent studies denoting the importance of vitamins and minerals for larval nutrition and the lack of information on the contents of these nutrients in rotifers, the aim of the present study was to determine the composition of different feeds and their effect on rotifers nutritional value for marine fish larvae. For that purpose, a trial was conducted by feeding rotifers with several dietary protocols and analyzing their contents in vitamins (A, E, B1, B6, B2, B3, and B9), minerals (Ca, P, I, Se, Mn, Cu, Zn, magnesium [Mg], and cobalt [Co]), and antioxidants (lutein and  $\beta$ -carotene).

## 2 | MATERIALS AND METHODS

### 2.1 | Rotifer cultures and feeding procedures

Rotifers (*Brachionus plicatilis*, L-type CCAP strain number: 5010/1, lorica size 200–250  $\mu\text{m}$ ) were produced in 1000-L circular tanks with baker's yeast (*Saccharomyces cerevisiae*, Pakmaya, Turkey). Stock cultures were maintained in 1-L Erlenmeyer flasks at a density of 30 rotifers/mL in semi-continuously conditions. Salinity (26‰ ppt [parts per thousand]), dissolved oxygen ( $6.5 \pm 0.7$  parts per million [ppm]), and temperature ( $26 \pm 0.5^\circ\text{C}$ ) were daily determined by multiparameter OxyGuard H04PP Handy pH meter (Oxyguard International, Denmark).

The rotifer feeding trial was conducted at the facilities of Akvatek Aquaculture Company, İzmir, Turkey. Initial rotifer stock density was 600–700 rotifers/mL in all experimental culture tanks. Non-enriched rotifers were cultured by baker's yeast (*Saccharomyces cerevisiae*) following the commercial protocol described in the previous paragraph. Enriched rotifers were fed for 16 days semi-continuously with different diets following the proportions recommended by the products companies. Thus, 650 million rotifers previously fed with baker's yeast were placed in a 1000-L tank and fed one of the following diets at the proportions indicated in brackets: Protein-Plus<sup>®</sup> (PP treatment; 0.3–1.0 g/10<sup>6</sup> rotifers, Aquafaune Bio-Marine Inc., Hawthorne, USA), Inactive Baker's Yeast<sup>®</sup> (INBY treatment 0.3–0.4 g/10<sup>6</sup> rotifers, Simbiyotek Inc., İstanbul, Turkey), Algome<sup>®</sup> (Algome treatment, 1 g/10<sup>6</sup> rotifers containing dried *Schizochytrium* sp., MarinBio, Aydin, Turkey), freshly cultured algae *N. oculata* (FA treatment; density of 10<sup>7</sup> cell/mL/10<sup>6</sup> rotifers), and spray-dried *N. oculata* (SDN treatment; 0.5–0.6 g/10<sup>6</sup> rotifers). Temperature ( $24 \pm 0.5^\circ\text{C}$ ), water salinity (26‰ ppt [parts per thousand]), light (100  $\mu\text{E m}^{-2} \text{s}^{-1}$  light intensity), and 12 L:12D photoperiod were maintained during experiment. Each diet was tested in triplicates.

### 2.2 | Vitamins analysis

#### 2.2.1 | Water-soluble vitamin analysis

One gram of sample was extracted with 10 mL of hot Milli-Q water (80°C) for 10 min. The supernatant was collected into a flask after centrifugation at 7500 g for 5 min. The residue was further extracted twice with 5 mL of hot

Milli-Q water (80°C). The combined extract was diluted with water at a ratio of 1:5 (v/v), and 1 mL of extract was passed through a 0.45- $\mu$ m nylon filter and transferred into a high-performance liquid chromatography (HPLC) vial. The water-soluble vitamins in the samples were analyzed with ultra-performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS) (Waters Corp., Milford, MA, USA) as described by a Waters Corp. Application Note.14 Sample extracts were separated by using a reversed phase Acquity UPLC (flow rate of 0.3 mL/min) on a BEH C18 column (Waters Corp., Dublin, Ireland), having a 1.7- $\mu$ m particle size (i.d. = 2.1 mm, length = 100 mm) at 40°C. The injection volume was set to 20  $\mu$ L. The binary mobile phase consisted of solvent A, composed of formic acid 0.1% in HPLC grade water, and solvent B, composed of formic acid 0.1% in methanol. The solvent gradient was programmed as follows: isocratic elution of 1% B and 99% A, 0–3 min; linear gradient elution to 75% B and 25% A, 3–5 min; linear gradient elution to 1% B and 99% A, 5–5.1 min; and isocratic elution of 1% B and 99% A, 5.1–8 min (Ball, 2005; Eitenmiller et al., 2016).

## 2.2.2 | Fat-soluble vitamins and pigment analysis

The contents in vitamin E and A were analyzed by HPLC (AOAC, 2011; Manz & Phillip, 1981). DL- $\alpha$ -tocopherol (Calbiochem, No:90669, Sigma-Aldrich, USA) and vitamin A retinol acetate (Calbiochem, 613,420, Sigma-Aldrich, USA) were used as standards for analysis of vitamins E and A, respectively.

Around 10 mg of freeze-dried material from each sample were extracted at room temperature by homogenizing with  $\text{CHCl}_3$ :MeOH (1:1) using a bead beater (Bead Mill<sub>24</sub>, Fisher Scientific, USA) in a 2-mL Lysing matrix Y tubes (3  $\times$  1 min cycles) in duplicate. That extract was dried with  $\text{N}_2$  gas, and the residue dissolved in 1.0 mL MeOH for HPLC analysis. Extraction was performed under weak light to minimize carotenoid decomposition. Carotenoid analysis was performed using an Agilent 1200 series HPLC with a YMC Carotenoid column (5  $\mu$ m, 2  $\times$  250 mm, 181 YMC Co. Ltd., Japan) eluting with 50-mM  $\text{NH}_4\text{OAc}$  in MeOH/tertiary butyl methyl ether (TBME) linear gradient 5% to 65% B in 30 min at 0.2 mL/min flow rate for 60 min. Standard curves for astaxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, canthaxanthin, fucoxanthin, lutein, lycopene, and zeaxanthin at 450 nm were used for carotenoid quantification. The concentration of unknown carotenoids was calculated using lutein as a standard (Banskota et al., 2019).

## 2.3 | Mineral analysis

Minerals were analyzed according to AOAC method 999.10 (AOAC, 2011). Freeze-dried samples (1 g) were weighed in a Teflon microwave digestion vessel, and 5 mL of concentrated  $\text{HNO}_3$  and 1 mL of  $\text{H}_2\text{O}_2$  were added. The vessel was then closed and placed into a microwave oven (Kenwood, Japan). The digested samples were allowed to cool and transferred to a 50-mL volumetric flask and made up to a final volume of 50 mL with the deionized water. Minerals were determined using an inductively coupled plasma-mass spectrometer (ICP-MS) (Elan DRCE, Perkin-Elmer, Norwalk, CT, USA). The following standard isotopes were used to quantify the minerals: Ca, 44; Cu, 63; Co, 59; Mg, 26; Mn, 55; P, 31; Se, 78; and Zn, 66.

## 2.4 | Statistical analysis

All data were tested for normality and homogeneity of variances with Levene's test and treated using one-way ANOVA. Means were compared by Duncan's test ( $p < 0.05$ ) using SPSS software (SPSS for Windows 11.5; SPSS Inc., Chicago, IL, USA). All values presented as percentage were arc cosine transformed before performing any statistical test. Logarithmic and linear regression models were used to determine the effect of dietary minerals and vitamins on rotifer contents.

### 3 | RESULTS

#### 3.1 | Vitamin contents in rotifer diets

The highest ( $p < 0.01$ ;  $p$  value =  $1.0e^{-20}$ ) vitamin B1 content was found in INBY, followed by Algome, then PP and, with a minimum content, in SDN and FA (Table 1). For vitamin B2, the highest ( $p < 0.01$ ;  $p$  value =  $4.95e^{-08}$ ) content was found in Algome, followed by PP, then INBY and SDN, and with a very low content FA (Table 1). For vitamin B3, the highest ( $p < 0.01$ ;  $p$  value =  $4.93e^{-29}$ ) content was found in INBY, followed by PP, then SDN and Algome, and, finally, FA. Both PP and Algome showed the highest ( $p < 0.01$ ;  $p$  value =  $2.66e^{-18}$ ) vitamin B6 content, followed by SDN and INBY, and closely FA.

Vitamin A was highest ( $p < 0.01$ ;  $p$  value =  $1.31e^{-06}$ ) in PP followed by SDN and FA, whereas this vitamin was not identified in Algome or INBY. Vitamin E was highest in SDN, followed by PP and in much lower contents in FA, INBY, and Algome ( $p < 0.01$ ;  $p$  value =  $1.51e^{-28}$ ). Overall, PP was characterized by the highest contents in vitamins B6, B9 and A; SDN was particularly high in vitamins C and E; Algome was high in B2 and B6 but lack vitamin A and C; INBY was high in B1 and B3 but lacked vitamin A, and FA showed very low contents in all vitamins except vitamin A (Table 1). Lutein and  $\beta$ -carotene were only found in PP, SDN, and FA (Table 1). Lutein was higher ( $p < 0.01$ ;  $p$  value =  $1.65e^{-18}$ ) in SDN, whereas  $\beta$ -carotene was highest in SDN followed by PP and then FA ( $p < 0.01$ ;  $p$  value =  $1.72e^{-13}$ ).

#### 3.2 | Mineral contents in rotifer diets

The highest ( $p < 0.01$ ;  $p$  value =  $2.94e^{-15}$ ) Ca content was found in PP diet, followed by SDN, INBY, Algome, and then FA (Table 2). The highest ( $p < 0.01$ ;  $p$  value =  $3.04e^{-20}$ ) Mg content was found in SDN, followed by PP, Algome, and then, INBY and FA (Table 2). Phosphorus was highest ( $p < 0.01$ ;  $p$  value =  $1.67e^{-33}$ ) in PP and Algome, followed by SDN, INBY, and FA.

**TABLE 1** Water-soluble vitamins, fat-soluble vitamins, and pigments contents of the rotifer diets.

	PP	SDN	Algome	INBY	FA
Water-soluble vitamins (mg/kg) DM					
Vitamin B1	15.4 ± 3.6 <sup>c</sup>	0.27 ± 0.1 <sup>d</sup>	28.4 ± 3.0 <sup>b</sup>	36.7 ± 1.0 <sup>a</sup>	0.36 ± 1.0 <sup>d</sup>
Vitamin B2	25.1 ± 0.1 <sup>b</sup>	12.1 ± 1.0 <sup>c</sup>	40.3 ± 1.0 <sup>a</sup>	13.6 ± 4.0 <sup>c</sup>	3.4 ± 1.0 <sup>d</sup>
Vitamin B3	367.1 ± 1.0 <sup>b</sup>	256.6 ± 2.0 <sup>c</sup>	266.6 ± 1.0 <sup>c</sup>	458.9 ± 2.0 <sup>a</sup>	16.8 ± 2.0 <sup>d</sup>
Vitamin B6	27.2 ± 1.0 <sup>a</sup>	4.2 ± 6.0 <sup>b</sup>	25.0 ± 2.0 <sup>a</sup>	3.5 ± 2.0 <sup>b</sup>	2.4 ± 3.0 <sup>c</sup>
Vitamin B9	2.24 ± 0.02 <sup>a</sup>	1.41 ± 0.01 <sup>b</sup>	1.19 ± 0.01 <sup>c</sup>	1.35 ± 0.01 <sup>b</sup>	0.35 ± 0.01 <sup>d</sup>
Fat-soluble vitamins and pigments (mg/kg) DM					
Vitamin A	0.32 ± 0.00 <sup>a</sup>	0.23 ± 0.05 <sup>b</sup>	-	-	0.13 ± 0.01 <sup>c</sup>
Vitamin E	156.6 ± 1.0 <sup>b</sup>	202.4 ± 4.0 <sup>a</sup>	0.7 ± 1.0 <sup>d</sup>	0.6 ± 0.2 <sup>d</sup>	7.8 ± 3.0 <sup>c</sup>
Lutein	190.1 ± 0.004 <sup>b</sup>	1842.0 ± 0.001 <sup>a</sup>	-	-	296.3 ± 0.001 <sup>b</sup>
$\beta$ -carotene	152.6 ± 0.001 <sup>b</sup>	330.7 ± 0.002 <sup>a</sup>	-	-	52.3 ± 0.001 <sup>c</sup>

Note: Different superscripts for values of a given nutrient indicate significant differences in order;  $a > b > c > d$  ( $p < 0.05$ ,  $n = 3$ ).

Abbreviations: Algome<sup>®</sup>, Algome treatment; FA, freshly cultured algae; INBY, Inactive Baker's Yeast; PP, Protein-Plus<sup>®</sup>; SDN, spray-dried *Nannochloropsis oculata*.

Iodine was highest ( $p < 0.01$ ;  $p$  value =  $8.56e^{-10}$ ) in PP, followed by SDN and FA, but it was not detected in Algome and INBY. The highest Co content was found in SDN and Algome, followed by PP and INBY and with the lowest values in FA ( $p < 0.01$ ;  $p$  value =  $1.49e^{-13}$ ). Selenium was highest ( $p < 0.01$ ;  $p$  value =  $3.64e^{-09}$ ) in INBY, followed by Algome and FA, whereas very little contents were found in PP and SDN. The highest Mn values were found in INBY, followed by PP, SDN, Algome, and FA ( $p < 0.01$ ;  $p$  value =  $8.12e^{-14}$ ). The highest Cu level was found in SDN, which was three times higher than in PP and Algome diets, and was followed by Cu contents in FA. The lowest Cu content was found in INBY. High Cu level only observed in SDN diet ( $p < 0.01$ ;  $p$  value =  $1.24e^{-10}$ ). Algome diet showed the greatest content of Zn ( $p < 0.01$ ;  $p$  value =  $4.73e^{-18}$ ) followed by INBY, then PP, SDN, and FA. Overall, PP diet was high in Ca, P, and I; SDN was high in Mg, Cu, and Co; Algome was high in P, Zn, and Co; and INBY was high in Se and Mn, whereas FA was low in all the minerals analyzed.

### 3.3 | Vitamin and pigment contents in rotifers biomass

Vitamin B1 was one of the water-soluble vitamins with a higher variation in its contents in rotifers. Thus, the highest ( $p < 0.01$ ;  $p$  value =  $3.65e^{-12}$ ) vitamin B1 levels were found in rotifers fed INBY, almost doubling the contents in non-enriched rotifers (Table 3). However, vitamin B1 was reduced to the lowest values in SDN, Algome, and FA rotifers (Table 3). Vitamin B2 did not markedly changed, being slightly increased in PP and SDN rotifers and lowest in INBY rotifers. Similarly, vitamin B3 was not markedly affected, the highest contents were found in PP and Algome rotifers, keeping the levels of non-enriched rotifers, whereas FA and INBY rotifers showed reduced contents of this vitamin ( $p < 0.01$ ;  $p$  value =  $3.19e^{-07}$ ). On the contrary, FA rotifers showed the highest levels of vitamin B6, doubling the contents of non-enriched rotifers ( $p < 0.01$ ;  $p$  value =  $1.69e^{-08}$ ) (Table 3). Regarding vitamin B9, only PP and Algome rotifers maintained the levels found in non-enriched rotifers, being significantly higher ( $p < 0.01$ ;  $p$  value =  $1.86e^{-10}$ ) than the contents of this vitamin in SDN, INBY, and FA rotifers were reduced over a 50% (Table 3). A significant logarithmic regression was found between the dietary vitamin B6 levels and the content in the rotifers of vitamin B3 ( $p$  value = 0.06,  $R^2 = 0.97$ ; Figure 1) or vitamin B9 ( $p$  value = 0.02,  $R^2 = 0.96$ ; Figure 1).

**TABLE 2** Macro- and micro-minerals contents of the rotifer diets.

Macro-minerals (g/kg) DM	PP	SDN	Algome	INBY	FA
Ca	1.41 ± 0.13 <sup>a</sup>	1.32 ± 0.08 <sup>b</sup>	0.93 ± 0.07 <sup>d</sup>	1.20 ± 0.11 <sup>c</sup>	0.27 ± 0.06 <sup>e</sup>
P	11.29 ± 0.32 <sup>a</sup>	10.50 ± 0.29 <sup>b</sup>	11.48 ± 0.27 <sup>a</sup>	7.53 ± 0.08 <sup>c</sup>	1.65 ± 0.12 <sup>d</sup>
Ca:P	0.12 ± 0.02	0.12 ± 0.01	0.08 ± 0.01	0.16 ± 0.01	0.16 ± 0.02
Mg	2.54 ± 0.07 <sup>b</sup>	6.54 ± 0.21 <sup>a</sup>	2.15 ± 0.16 <sup>b</sup>	1.31 ± 0.9 <sup>c</sup>	1.25 ± 0.12 <sup>c</sup>
Micro-minerals (mg/kg) DM	PP	SDN	Algome	INBY	FA
I	1.4 ± 0.7 <sup>a</sup>	1.3 ± 0.2 <sup>b</sup>	<0.01	<0.01	0.9 ± 0.7 <sup>b</sup>
Se	0.3 ± 0.1 <sup>d</sup>	0.2 ± 0.6 <sup>e</sup>	0.8 ± 0.6 <sup>b</sup>	1.4 ± 0.2 <sup>a</sup>	0.46 ± 0.2 <sup>c</sup>
Cu	10.28 ± 0.6 <sup>c</sup>	30.82 ± 0.6 <sup>a</sup>	10.8 ± 0.6 <sup>c</sup>	4.97 ± 0.3 <sup>d</sup>	19.78 ± 0.2 <sup>b</sup>
Zn	54.5 ± 0.6 <sup>c</sup>	25.3 ± 0.6 <sup>d</sup>	140.53 ± 0.6 <sup>a</sup>	120.03 ± 3.6 <sup>b</sup>	5.16 ± 0.3 <sup>e</sup>
Mn	36.13 ± 0.1 <sup>b</sup>	15.28 ± 0.6 <sup>c</sup>	5.54 ± 0.6 <sup>d</sup>	43.53 ± 0.2 <sup>a</sup>	3.05 ± 0.6 <sup>e</sup>
Co	5.4 ± 0.5 <sup>b</sup>	6.9 ± 0.9 <sup>a</sup>	6.7 ± 0.6 <sup>a</sup>	4.9 ± 0.1 <sup>b</sup>	1.7 ± 0.2 <sup>c</sup>

Note: Different superscripts for values of a given nutrient indicate significant differences in order;  $a > b > c > d$  ( $p < 0.05$ ,  $n = 3$ ).

Abbreviations: Algome<sup>®</sup>, Algome treatment; FA, freshly cultured algae; INBY, Inactive Baker's Yeast; PP, Protein-Plus<sup>®</sup>; SDN, spray-dried *Nannochloropsis oculata*.

**TABLE 3** Water-soluble vitamins, fat-soluble vitamins, and carotenoids contents of rotifers biomass before and after feeding the commercial diets, with copepods values given in the literature for comparison.

	Non-enriched rotifers	PP rotifers	SDN rotifers	Algome rotifers	INBY rotifers	FA rotifers	Copepods <sup>A</sup>
Water-soluble vitamins (mg/kg DW)							
Vitamin B1	11.0 ± 0.1	12.8 ± 0.4 <sup>b</sup>	0.4 ± 0.0 <sup>d</sup>	0.4 ± 0.1 <sup>d</sup>	19.0 ± 0.1 <sup>a</sup>	0.7 ± 0.0 <sup>c</sup>	13-23
Vitamin B2	3.7 ± 0.2	4.7 ± 0.3 <sup>a</sup>	4.2 ± 0.1 <sup>a</sup>	3.2 ± 0.1 <sup>b</sup>	2.7 ± 0.3 <sup>c</sup>	3.3 ± 0.1 <sup>b</sup>	14-27
Vitamin B3	32.1 ± 2.3	31.0 ± 2.0 <sup>a</sup>	23.9 ± 1.0 <sup>b</sup>	29.3 ± 0.1 <sup>a</sup>	21.6 ± 0.2 <sup>c</sup>	20.1 ± 1.0 <sup>c</sup>	100-150
Vitamin B6	1.1 ± 0.1	0.9 ± 0.0 <sup>c</sup>	1.3 ± 0.0 <sup>b</sup>	1.0 ± 0.0 <sup>b</sup>	0.9 ± 0.0 <sup>c</sup>	1.8 ± 0.01 <sup>a</sup>	2-6
Vitamin B9	390 ± 2.0	300.0 ± 3.0 <sup>a</sup>	210 ± 5.0 <sup>b</sup>	300.0 ± 9.0 <sup>a</sup>	180.0 ± 4.0 <sup>c</sup>	170 ± 8.0 <sup>c</sup>	-
Fat-soluble vitamins & pigments (mg/kg DW)							
Vitamin A	-	-	0.05 ± 0.00	-	-	-	-
Vitamin E	1.2 ± 0.1	1.8 ± 0.3 <sup>c</sup>	2.6 ± 0.01 <sup>a</sup>	0.3 ± 0.01 <sup>d</sup>	2.3 ± 0.01 <sup>b</sup>	1.9 ± 0.10 <sup>c</sup>	110
Lutein	0.12 ± 0.01	1.78 ± 0.00 <sup>b</sup>	4.03 ± 0.01 <sup>a</sup>	0.13 ± 0.00 <sup>c</sup>	0.10 ± 0.00 <sup>c</sup>	0.16 ± 0.2 <sup>c</sup>	-
β-carotene	-	3.70 ± 0.00 <sup>a</sup>	2.74 ± 0.00 <sup>b</sup>	-	-	-	-

Note: Different superscripts for values of a given nutrient indicate significant differences in order; a > b > c > d ( $p < 0.05$ ,  $n = 3$ ).

Abbreviations: Algome<sup>®</sup>, Algome treatment; FA, freshly cultured algae; INBY, Inactive Baker's Yeast; PP, Protein-Plus<sup>®</sup>; SDN, spray-dried *Nannochloropsis oculata*.

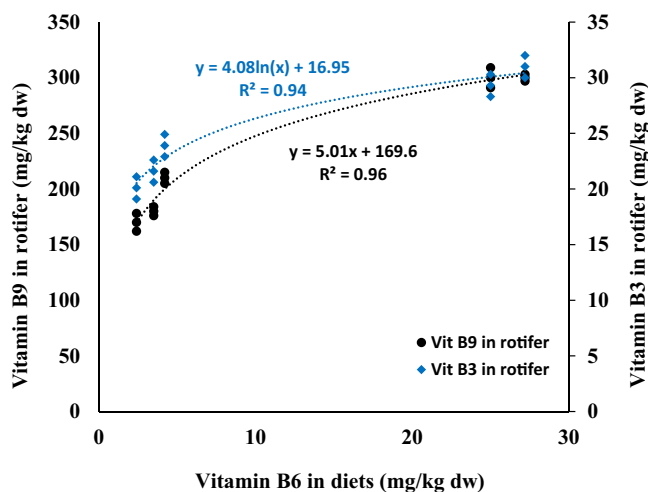
<sup>A</sup>Copepods data are from, Hamre, Mollan, et al., 2008 and Rønnestad et al., 1995.

Consequently, a direct significant linear regression was found between the contents of vitamins B3 and B9 in the rotifers ( $p$  value = 0.01,  $R^2 = 0.97$ ; Figure 2).

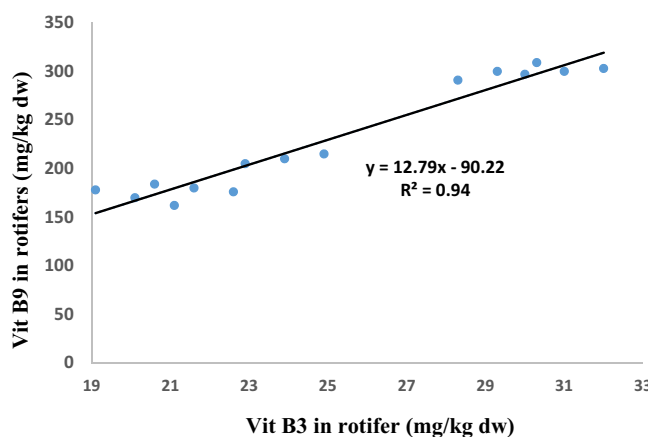
Vitamin A was only detected in rotifers fed SDN diets, whereas vitamin E was particularly increased in SDN rotifers ( $p < 0.01$ ;  $p$  value =  $7.01e^{-15}$ ). Lutein content was found to be high in SDN group rotifers, whereas β-carotene levels were higher in rotifers fed PP ( $p < 0.01$ ;  $p$  value =  $4.72e^{-19}$ ) (Table 3).

### 3.4 | Mineral contents in rotifers biomass

Ca ( $p < 0.01$ ;  $p$  value =  $9.89e^{-21}$ ), I and Zn levels were the highest ( $p < 0.01$ ;  $p$  value =  $8.04e^{-10}$ ) in rotifers fed INBY and increased in relation to the non-enriched rotifers. Mn ( $p < 0.01$ ;  $p$  value =  $4.42e^{-21}$ ) and P levels were significantly ( $p < 0.01$ ;  $p$  value =  $2.33e^{-26}$ ) highest in rotifers fed SDN diet, and also increased in relation to non-enriched rotifers. Cu levels of rotifers were similar in SDN and FA groups and significantly higher than other groups ( $p < 0.01$ ;  $p$  value =  $2.77e^{-11}$ ). Algome<sup>®</sup> diet only enhanced Mg ( $p < 0.01$ ;  $p$  value =  $1.98e^{-06}$ ) and Co ( $p < 0.01$ ;  $p$  value =  $3.41e^{-05}$ ) levels in rotifers compared with other rotifer groups ( $p < 0.05$ ). Finally, Se level was found to be significantly higher in FA groups ( $p < 0.05$ ;  $p$  value =  $2.73e^{-06}$ ) (Table 4). Calcium contents in rotifers followed a significant exponential regression with the Se levels in the diet ( $p < 0.01$ ;  $R^2 = 0.91$ ; Figure 3).



**FIGURE 1** Logarithmic regression between the dietary vitamin B6 levels and the content in the rotifers of vitamin B3 and B9 (blue dot represents vitamin B3 in Rotifer, black dot represents vitamin B9 in Rotifer).



**FIGURE 2** Linear regression between the contents of vitamins B3 and B9 in the rotifers.

## 4 | DISCUSSION

### 4.1 | Vitamins and pigments contents

Thiamine (vitamin B1) is essential in fish for reproductive performance, immune system or lipid metabolism (Ghiasi et al., 2017; Honeyfield et al., 2016). In marine fish larvae, vitamin B1 must be important for growth, neural development, and correct pigmentation. Riboflavin (vitamin B2) plays important roles in carbohydrates, lipids, and proteins metabolism and would be important in marine fish larvae for eye and scales development (Souto et al., 2008; Waagbø, 2010). However, requirements for these two vitamins have not been yet determined in marine fish larvae. In the present study, in comparison with non-enriched rotifers, only the Protein-Plus and the INBY diets were able to maintain or even increase vitamin B1 contents in the rotifers (12.8 and 19 mg/kg, respectively) until levels like those found in copepods (13–23 mg/kg, Hamre, Srivastava, et al., 2008; van der Meeren et al., 2008). However,



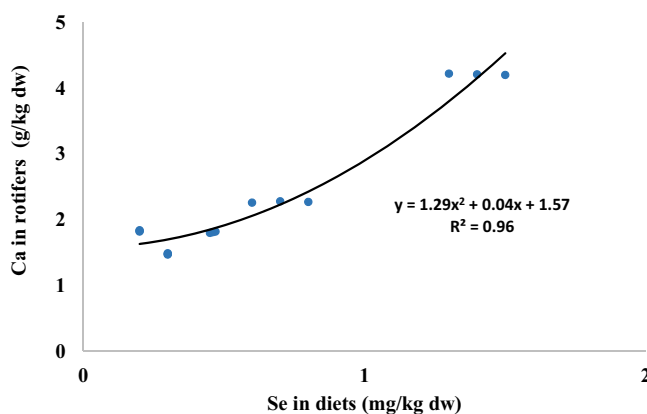
**TABLE 4** Mineral contents of rotifers biomass before and after feeding the commercial diets, with copepods values given in the literature for comparison.

Macro-minerals (g/kg DM)	Non-enriched rotifers	PP rotifers	SDN rotifers	Algome rotifers	INBY rotifers	FA rotifers	Copepods <sup>A</sup>
Ca	1.62 ± 0.01	1.48 ± 0.01 <sup>d</sup>	1.83 ± 0.01 <sup>c</sup>	2.27 ± 0.01 <sup>b</sup>	4.21 ± 0.01 <sup>a</sup>	1.81 ± 0.01 <sup>c</sup>	1.1–2.4
P	13.43 ± 0.01	14.67 ± 0.01 <sup>b</sup>	18.40 ± 0.01 <sup>a</sup>	11.46 ± 0.00 <sup>c</sup>	11.21 ± 0.00 <sup>c</sup>	11.62 ± 0.01 <sup>c</sup>	12.4–15.0
Ca:P	1:8	1:10	1:10	1:5	1:3	1:6	1:8
Mg	3.45 ± 0.01	3.83 ± 0.01 <sup>c</sup>	4.16 ± 0.01 <sup>c</sup>	6.38 ± 0.01 <sup>a</sup>	5.77 ± 0.01 <sup>b</sup>	3.89 ± 0.01 <sup>c</sup>	2.4–3.1
Micro-minerals (mg/kg DM)	Non-enriched rotifers	PP rotifers	SDN rotifers	Algome rotifers	INBY rotifers	FA rotifers	Copepods <sup>A</sup>
I	<0.01	<0.01	<0.01	<0.01	0.42 ± 0.03	<0.01	50–350
Se	0.3 ± 0.00	0.5 ± 0.01 <sup>c</sup>	0.7 ± 0.04 <sup>b</sup>	-	-	0.9 ± 0.00 <sup>a</sup>	3–5
Cu	4.6 ± 0.7	3.7 ± 0.3 <sup>c</sup>	14.2 ± 0.6 <sup>a</sup>	10.5 ± 0.2 <sup>b</sup>	4.4 ± 0.3 <sup>c</sup>	15.2 ± 0.6 <sup>a</sup>	12–38
Zn	16.9 ± 0.1	15.9 ± 0.7 <sup>c</sup>	12.0 ± 0.5 <sup>d</sup>	14.8 ± 0.2 <sup>c</sup>	24.6 ± 0.4 <sup>a</sup>	17.2 ± 0.7 <sup>b</sup>	340–570
Mn	29.0 ± 0.3	1.0 ± 0.4 <sup>c</sup>	2.2 ± 0.6 <sup>a</sup>	1.1 ± 0.3 <sup>c</sup>	1.4 ± 0.5 <sup>b</sup>	1.3 ± 0.5 <sup>c</sup>	8–25
Co	1.9 ± 0.01	1.7 ± 0.02 <sup>c</sup>	1.7 ± 0.03 <sup>c</sup>	2.2 ± 0.02 <sup>a</sup>	1.9 ± 0.03 <sup>b</sup>	1.5 ± 0.01 <sup>c</sup>	0.10

Note: Different superscripts for values of a given nutrient indicate significant differences in order; a > b > c > d ( $p < 0.05$ ,  $n = 3$ ).

Abbreviations: Algome<sup>®</sup>, Algome treatment; FA, freshly cultured algae; INBY, Inactive Baker's Yeast; PP, Protein-Plus<sup>®</sup>; SDN, spray-dried *Nannochloropsis oculata*.

<sup>A</sup>Copepods data are from Hamre, Mollan, et al., 2008 and Rønnestad et al., 1995.

**FIGURE 3** Exponential regression of calcium contents in rotifers with the selenium levels in the diet.

regardless the diet fed, vitamin B2 contents in the rotifers were lower than in copepods (28 mg/kg, van der Meeren et al., 2008) and lesser than the requirements of juvenile fish species for this vitamin, in agreement with previous studies (Hamre, Mollan, et al., 2008).

On the contrary, regarding vitamin B6, only feeding with freshly cultured algae increased vitamin B6 contents in the rotifers until levels (1.8 mg/kg) close to those found in copepods (2–6 mg/kg) (Hamre et al., 2013), in agreement with the synthesis and accumulation of this vitamin in fresh microalgae (Hemantkumar & Rahimbhai, 2019). Interestingly, the elevation of dietary vitamin B6 levels increased the content of niacin (vitamin B3) in the rotifers, following a logarithmic regression ( $y = 4.08\ln(x) + 16.95$ ,  $R^2 = 0.94$ ), in agreement with the role of vitamin B6 on the conversion of tryptophan to niacin (Shibata et al., 1995). Besides, folic acid (vitamin B9) content of rotifer was also increased proportionally to the vitamin B6 levels in the diet, following a significant logarithmic regression ( $y = 5.01x + 169.60$ ,  $R^2 = 0.96$ ). Indeed, folic acid levels in tissues are regulated by vitamin B6, which acts as a cofactor for several enzymes related to vitamin B9 metabolism and regulates B9 vitamers conversions (Franco et al., 2022). These increases in vitamin B3 and B9 levels in rotifers when vitamin B6 increased in the diet suggested that dietary B3 and B9 levels in the diets were insufficient for the rotifers. In agreement to the enhancing effect of dietary vitamin B6 on vitamins B3 and B9 contents on rotifers, there was also a significant lineal regression ( $y = 12.79x - 90.22$ ,  $R^2 = 0.94$ ) between the contents of these two vitamins in the rotifers. These three vitamins from the B group play crucial roles for the regulation of one carbon metabolites and must be extremely important during larval development, particularly for growth, neurological function, or red blood cell formation (Hansen et al., 2013; Liu et al., 2022). In juvenile fish vitamin B3 is necessary for growth, health, or antioxidant processes (Liu et al., 2022; Xun et al., 2019). However, their importance for larval nutrition has been scarcely studied, and therefore, their levels and proportions in rotifers and larval diets should be well defined in future studies. Both PP and Algome diets seemed to have an adequate level of vitamin B6 that allowed the rotifers fed with these diets to keep similar levels of vitamin B9 and B3 than the initial rotifers, whereas all the other diets tested led to rotifers with low contents in these two vitamins. However, vitamin B3 contents in rotifers of the present study were lower than those found in copepods (100–150 mg/kg).

Vitamin A is very important for vision, synthesis of protein, reproduction, larval development, and immunological responses (Hernandez & Hardy, 2020; Jiang et al., 2020; Wu et al., 2016). In the present study, despite the different dietary levels, vitamin A could only be detected in rotifers fed the SDN (0.05 mg/kg), in agreement with the detection of vitamin A in rotifers enriched with another microalgae, *Isochrysis galbana* (0.2 mg/kg, van der Meeren et al., 2008). These results suggested a better incorporation of vitamin A into the rotifers when they are fed with algae. Vitamin A content is also very low in copepods and zooplankton from natural environments mainly copepodites of *Temora longicornis* (0.2 mg/kg DW; Hamre, 2016), but both copepods and *Artemia* have high levels of carotenoids, which are precursors of vitamin A (Moren et al., 2004). In fact, excessive vitamin A dietary level unnecessarily accelerates the intramembranous ossification of vertebral centrums leading to a high incidence of fused and compressed vertebrae on gilthead sea bream larvae fed rotifers with more than 109.2 mg/kg of dry weight (Fernández et al., 2008). Vitamin A level in copepods is also limited in wild (Ajiboye et al., 2011; Hamre, 2016). Increased deformities are also observed in Senegalese sole larvae (*Solea senegalensis*) fed *Artemia* nauplii enriched with more than 2.1 mg/kg of dry weight (Fernández et al., 2008, 2009). Thus, rotifers must contain an optimum level of vitamin A to sustain an adequate larval development.

Vitamin E is a fundamental component of cell membranes with a strong antioxidant action, that plays essential roles in many biological processes (El-Sayed & Izquierdo, 2021). Dietary vitamin E levels have a profound effect on immune function (Montero et al., 1999), stress resistance (Montero et al., 2001), fish growth, and fillet quality (Lozano et al., 2017) or the occurrence of systemic granulomatosis in meager (Lozano et al., 2017; Ruiz, Betancor, et al., 2019; Ruiz, Hernández-Cruz, et al., 2019). In fish larvae, vitamin E requirements seem to be very high (800–1700 mg/kg in dry micro-diets), in relation to the great PUFA needs for larval development (Atalah et al., 2012; Betancor et al., 2012; Izquierdo et al., 2019; Ruiz, Betancor, et al., 2019; Ruiz, Hernández-Cruz, et al., 2019). Besides, the oxidative risk is particularly high in marine fish larvae due to the fast growth and elevated metabolic rate, the large water content in larval tissues, or specific rearing conditions such as light or water aeration (Izquierdo et al., 2019). The most active form of vitamin E is  $\alpha$ -tocopherol (Izquierdo & Betancor, 2015), which is also the most abundant tocopherol in copepods, where it reaches around 110 mg/kg (van der Meeren et al., 2008). In the present study, the highest vitamin E rotifers contents were obtained by feeding them with freshly cultured *Nannochloropsis*

*oculata* (18.9 mg/kg). These results agree well with the high  $\alpha$ -tocopherol content found in this alga (Durmaz, 2007) and denotes a better incorporation than from the SDN. Nevertheless, this vitamin E level is still far to lesser than that found in copepods or required by marine fish larvae.

In the present study,  $\beta$ -carotene was not detected in non-enriched rotifers, neither in rotifers fed with Algome, inactive Baker's Yeast or freshly cultured *Nannochloropsis oculata*, in agreement with the lack of  $\beta$ -carotene in rotifers from previous studies (van der Meeren et al., 2008). Feeding with PP or spray-dried *Nannochloropsis oculata* only slightly raised  $\beta$ -carotene contents in rotifers, despite the high levels found in these diets. Moreover, rotifers fed these diets did not show increased levels of vitamin A, suggesting that  $\beta$ -carotene is not a precursor of vitamin A in rotifers and would not be an essential ingredient in their diets. Moreover,  $\beta$ -carotene is not present in copepods or Artemia, which contain astaxanthin or canthaxanthin, respectively (Ajiboye et al., 2011; van der Meeren et al., 2008).

## 4.2 | Mineral contents

The analyzed macro-minerals contents in diets and rotifers were close to those found previously in rotifers and in copepods (Hamre, 2016). Nevertheless, since a large part of the copepods macro-minerals is included in the exoskeleton (Truong et al., 2020), which is not digested by early fish larvae, the macro-mineral contents in copepods may not necessarily be a good reference of the adequate dietary levels of these nutrients for marine fish larvae.

Ca contents in the rotifers showed larger variations than P, despite their contents in the diets were similar. Indeed, rotifers contents in Ca followed a significant exponential regression with dietary Se levels, in agreement with the effect of Se in Ca metabolism regulation. Se prevents the formation of reactive oxygen species (ROS), affecting the expression of Ca-regulated genes (Huang et al., 2019). Thus, ROS may damage mitochondria membranes and alter the transient storage organelle of  $\text{Ca}^{2+}$ , and hence Ca homeostasis (Adebayo et al., 2015). The effect of dietary Se on Ca tissue contents has been demonstrated in a wide range of animals, including birds (Huang et al., 2019), crustaceans (Satgurunathan et al., 2023), and mammals (Li et al., 2023), but this is the first time reported for rotifers. In gilthead seabream larvae, increase in dietary Se upregulates genes related with the Ca deposition in bone such as *osteocalcin* (Saleh et al., 2014). Apart from its several biological functions, Ca can be especially important for rotifers, since it induces the production of specific biopolymers, such as rotimer, which are essential for development and survival of monogonont rotifers (Balazs et al., 2021). In our study, Ca contents were highest in rotifers fed inactive Baker's yeast (4.21 g/kg), being slightly higher than those found previously for enriched rotifers (1.1–3.3 g/kg) or copepods (1.1–2.4 g/kg) (Hamre, 2016).

Phosphorous plays many important physiological roles, being part of cell wall structure, nucleic acid, and high energy molecules, such as ATP, ADP, and AMP (Lall & Kaushik, 2021). Diet is the main source of P for both marine vertebrates and zooplankton (Satoh et al., 2003). P contents in rotifers of the present study were 11.2–18.4 g/kg, being in the range of those described for rotifers in previous studies 8.3–19.9 g/kg (Hamre, 2016). The highest level of P (18.4 g/kg) was obtained in rotifer fed SDN diet, being slightly higher than those found in copepods (Hamre, 2016). No significant relations were found between dietary levels of Ca or P and the contents of these minerals in the rotifers, what could be related to the different molecular forms of these minerals among rotifers diets.

Magnesium must be essential for fish larvae growth and development, for its roles in ATP structure and intracellular signaling, protein synthesis, cell replication, ion, oxidative phosphorylation, and energy metabolism (Hamre et al., 2018; Lall & Kaushik, 2021). The levels of this mineral in rotifers varied between 3.45 and 6.38 g/kg, being well in the range of those previously described (3.2–6.3 g/kg) and slightly higher than those found in copepods (Hamre, 2016). The Mg requirements for fish larvae are poorly studied but have been defined for post-larvae of some crustaceans (0.5 g/kg; Srinivasan et al., 2017).

In comparison with copepods, rotifers may be deficient on several micro-minerals such as Zn, Se, Cu, Mn, and I (Hamre, 2016; Hamre, Mollan, et al., 2008). Accordingly, fish larvae fed with rotifers reach lower mineral contents than those fed with copepods (Busch et al., 2010).

Iodine is a main component of thyroid hormone, which regulates development, growth, and metabolism. Iodine is one of the trace elements more critical in rotifers, since their contents in non-enriched (0.36–0.72 mg/kg) and even enriched rotifers (0.66–8.83 mg/kg) are much lower than those of copepods (50–350 mg/kg) (Hamre, 2016). In agreement, in the present study, even the highest I content (0.42 mg/kg) in the rotifers fed inactive baker's yeast was very low and insufficient to cover the requirements of larvae from marine fish such as cod (50 mg/kg, Penglase et al., 2013).

Selenium (Se) is an essential trace mineral that plays an important role in the antioxidant system in fish (Antony Jesu Prabhu et al., 2016; Domínguez et al., 2017, 2020). Se contents in rotifers ranged between 0.3 and 0.9 mg Se/kg in agreement with previous studies (Hamre, 2016) but were lower than in copepods (3–5 mg Se/kg) and, probably, were not able to match fish larvae requirements. Se requirements in marine fish larvae range between 1.4 and 3.0 mg Se/kg in rotifer and 6 mg Se/kg in micro-diet for growth promotion and 12 mg Se/kg in micro-diet for maximum antioxidant protection and lowest incidence of skeletal anomalies (Kim et al., 2014; Penglase et al., 2010; Saleh et al., 2014; Sivagurunathan et al., 2022; Tseng, Dominguez, et al., 2024).

Copper is an essential trace mineral important for development, growth, and survival that plays a role in Fe metabolism, cytochrome c oxidase production, antioxidation, collagen synthesis, and melanin production. Cu was one of the minerals that showed a larger variation among rotifers fed different diets (3.7–15.2 mg/kg), in agreement with other studies (4.8–128 mg/kg, Hamre, 2016). Only SDN and freshly cultured *Nannochloropsis oculata* were able to increase Cu content in the rotifers (14.2–15.2 mg/kg), until levels similar to those found in copepods (12–38 mg/kg, Hamre, 2016) and closely to those required by marine fish larvae. Cu requirements for fish larvae have been scarcely studied. Depending on the dietary levels of other antioxidant nutrients, larval gilthead seabream requires 17–20 mg/kg for normal growth and 21.5–22.6 mg/kg to reduce oxidative risk, promote docosahexaenoic, and eicosapentaenoic acid incorporation into larval lipids and prevent skeletal anomalies (Eryalçin et al., 2020; Tseng et al., 2023). Rotifers enriched with commercial products such as Multigreen and Ori-green may also result in low levels of Cu in rotifers (5 and 4 mg/kg DW) compared with natural zooplankton (Mæhre et al., 2013).

Dietary Zn promotes fish larvae growth, bone metabolism, and development (Ma & Yamaguchi, 2001). The Zn contents in rotifers ranged between 12 and 24.6 mg Zn/kg, being slightly lower than those described for enriched rotifers in previous studies (33–91 mg Zn/kg, Hamre, 2016) or those present in copepods (340–570 mg Zn/kg). Dietary requirements for Zn have been studied in gilthead seabream larvae fed micro-diets with six different Zn contents that varied between 79 and 248 mg/kg (Tseng et al., unpublished data). Larvae fed diets with 79 mg/kg showed the lowest growth and the highest incidence of skeletal anomalies, whereas the best growth, vertebrae mineralization, and skeleton health was obtained with 110 and 130 mg/kg dietary Zn (Tseng et al., unpublished data). Even the highest Zn levels (24 mg/kg), obtained in rotifers fed INBY, or those of rotifers in previous studies (Hamre, 2016), would be insufficient to cover seabream larvae requirements, and probably of most marine fish larvae given the high Zn contents of copepods.

Manganese plays vital roles in protein and energy metabolism, cellular defense for free radicals, bone mineralization, and metabolic regulation (Lall, 2022). In the present study, feeding rotifers with different quantities of Mn did not allow to maintain the initial Mn contents of non-enriched rotifers and led to lower Mn levels than in enriched rotifers from previous studies (5.9–20 mg/kg, Hamre, 2016). Besides, Mn levels in rotifers were also lower than those reported for copepods (8–25 mg/kg, Hamre, Mollan, et al., 2008, Hamre, Srivastava, et al., 2008; Hamre et al., 2013) or even lower than those necessary in *Artemia* to promote red sea bream larvae growth (42.8 mg/kg, Nguyen et al., 2008). Moreover, elevation of Mn levels in micro-diets from 6.3 to 200 mg/kg significantly improved gilthead seabream growth (Tseng, Izquierdo, et al., 2024). Therefore, Mn levels in rotifers of the present study were very low, regardless the diet fed. These results agree with previous studies where additions of different levels of Mn to rotifers diets results in relatively small increases in the rotifers, in comparison with other minerals (Nordgreen et al., 2013).

Among other functions, Co is needed for vitamin B<sub>12</sub> formation. Co supplementation increases the B<sub>12</sub> contents in rotifer (Yoshimatsu et al., 2006), but studies in Co requirements for larvae are very scarce. In juvenile mahseer (*Tor*

*putitora*) has been related to HSP70 activity (Younus et al., 2020). In our study, rotifer fed Algome diet showed highest Co level (2.2 mg/kg), which is higher than the contents reported for copepods (0.55 µg/kg, Fisher et al., 2000).

In summary, regardless the diet fed, all the rotifers contained adequate levels of Ca, P, Mg, and Co, close to those of copepods. The Protein-Plus diet produced rotifers with good levels of vitamins B1, B3 and B9, and P. Rotifers fed SDN had good levels of vitamin A, P, and Cu. The Algome diet produced rotifers high in vitamins B3 and B9, and Ca, whereas the INBY produced rotifers with good levels of vitamin B1 and Ca; and the freshly cultured algae *Nannochloropsis oculata* rotifers with more vitamin B6, Se, and Cu. Vitamin B2 was too low in all the rotifers and together with vitamins B6 and B3 should be increased in the diets tested, to achieve similar levels to those of copepods. All rotifers had very low contents of vitamin E, I, and Zn, lower than those found in copepods and unable to fulfill marine fish larvae requirements. In conclusion, specific minerals or vitamins corrections are required in these feeds for rotifers to adequate their nutritional value for marine larvae.

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## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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