Received: 16 March 2020 | Revised: 22 April 2020 | Accepted: 26 April 2020

DOI: 10.1111/are.14679

ORIGINAL ARTICLE

Aquaculture Research WILEY

Effects of different salinities on amino acid profile in *Artemia franciscana*

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Funding information
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Abstract

The effects of different salinity levels, including 10–15, 30–35, 70–75, 110–115 and 150–155 ppt, were evaluated on survival and the body amino acids (BAA) of *Artemia franciscana*. The results were expressed as total essential (TE), non-essential (NE) and total amino acids (TAA); also, the ratio of the TE to NE (ENAA) on days 3 and 13 of the culture is reported. The study of changes in *Artemia* BAA showed that with the increase in the water salinity, the TE, NE and TAA increased significantly on days 3 and 13 of the culture. However, the highest ratio of ENAA was observed in the 110 gL^{-1} salinity treatment on day 3 (1.067 ± 1.25). Regarding the effects of different salinity treatments on the survival rate of *Artemia*, it was observed that, generally, an increase in the water salinity would reduce the survival rate of this species. This reduction was observed on day 3 of culture in the salinity treatments above 120 (66.66 ± 1.68%) and below 30 gL^{-1} (89.66 ± 0.34%) and on day 13 of culture in the salinity treatments below 30 (11.86 ± 0.13%) and above 70 gL^{-1}. Basically, it can be concluded that *A. franciscana* culture at a salinity of 110 gL^{-1} can lead to the biological enrichment of *Artemia* in terms of amino acid profile.

KEYWORDS

amino acid quantity, *Artemia franciscana*, biochemical osmolyte, salinity stress, survival rate

1 | INTRODUCTION

Increasing the culture of high-quality and healthy larvae is the key to success in the aquaculture industry (Richard, Engrola, Palma, Simes, & Conceição et al., 2015). This success depends to a large extent on one's knowledge of fish food requirements at the larval stages and the use of high-quality and appropriate food items (Campoverde & Estevez, 2017). However, in some larvae, due to the lack of a basic and well-evolved digestive system, some specialized gastric and digestive enzymes which are effective on the hydrolysis of proteins, such as pepsin and hydrochloric acid, are inactive; thus, these larvae are not able to digest the complex protein components in the diet (Rathore, Yusufzai, Katira, & Jaiswal, 2016). These important factors are found in polypeptide-to-peptide conversion for weeks in some species and for months, even after metamorphism and anthropogenic evolution, in some other species. In other words, protein digestion in the intestine is done extracellularly through the pinocytosis of the enterocyte cells of the intestine. Therefore, the use of hydrolysed protein compounds, such as amino acids, can improve the uptake of larvae (Tonheim, Espe, Hamre, & Rønnestad, 2005). On the other hand, the percentage of leaching is so high in many formulated foods, including amino acids (Rathore et al., 2016; Richard, Engrola, Palma, Simes, & Conceição, 2015) that nutrient leaching is performed in many microdiets which are used to feed the larvae after one or two minutes of immersion in water (Hamre, 2006). Hence, the use of live food has been extensively considered in this area (Nhu, Dierckens, Nguyen, Tran, & Sorgeloos, 2009). *Artemia franciscana* has the highest osmotic regulation in the animal phylum, and because of its unique adaptive mechanisms in adverse environmental conditions, it can be used as an animal model in the ecological, biological, and physiological studies (Conceicao et al., 2010). *Artemia* as a live food is used widely in the larviculture, due to high

nutritional value, small size, no need to change of food types from nauplius to adult and cost-effective culture. *Artemia* is used in the various forms in aquaculture, which include decapsulated cysts, newly hatched nauplius, meta-nauplius, post-larval, and adult. The use of *Artemia* in the nauplius and meta-nauplius stages is the most common in larviculture compared with the other stages. Because of the visibility of colored nauplius by larvae and higher energy content that was reduced by *Artemia* growth. Due to the more protein and lower lipid content than the nauplius stage, the post-larvae and adult forms have a great advantage in aquaculture nutrition as *Artemia* biomass, especially in the cultivation of shrimp and ornamental fish (Sorgeloos, Dhert, & Candreva, 2001).

Any change in the environmental factors that disrupt the homeostasis or the steady state of the body of an aquatic organism can be considered as a stressor (Jisha & Babu, 2014). The biomolecules and biochemical reactions have been used as short-term indicators of the organisms' response to stress and an adaptive mechanism to measure the effects of environmental factors (Pandey, Patel1, & Mishra, 2015). Osmolytes, such as sugars, organic acids, and amino acids, are effective biochemical constituents in the process of compatibility to environmental stresses (Ashraf & Harris, 2004). As an efficient way to adapt to salinity stress, the aquatic organisms use organic intracellular materials to balance the osmotic pressure during the evolution process (Nostro, Ninham, Carrett, Dei, & Baglioni, 2015). The effective mechanisms to cope with hypo-osmotic stress include reducing the intracellular ions, the amino acid synthesis rate, and protein catabolism, on the one hand, and increasing the amino acid oxidation, protein synthesis and the release of amino acids to the cytosol, on the other hand (Lu et al., 2015; Mazzarelli, Santos, Amorim, & Augusto, 2015). In this regard, the cellular mechanisms involved in osmotic regulation when exposed to high salinity can include an increase in the intracellular concentrations through increased protein catabolism, increased release of amino acids into the cell, and the hydrolysis of blood proteins to increase amino acids (Kimmel & Bradley, 2001).

Amino acids are among the types of biological molecules that play an important role in maintaining the osmotic balance and compatibility to salinity stress in zooplankton (Nakamura, Iwaizumi, & Yamada, 2007). In the osmotic regulation process, the concentration of extracellular solutions is regulated during the process of synthesis and/or degradation of the amino acids (kimmel & Bradley, 2001). Therefore, any change in the amino acid content, as one of the most important osmolytes in zooplankton, makes the modification of the nutrient content possible without live food enrichment methods; this can be used for feeding larviculture management. Although few studies have been conducted on the role of amino acids in the osmotic regulation process of *A. franciscana* (Castro et al., 2009; Nakamura et al., 2007), it seems that water salinity stress may affect the quantity of amino acids (Helland et al., 2000). Thus, the present study was designed to study the effects of different levels of salinity on the survival rate and amino acid profiles of *A. franciscana*.

2 | MATERIALS AND METHODS

2.1 | The design of the *Artemia franciscana* culture system and treatment

The culture system consisted of 15 glass aquariums of 35 × 35×35 cm and a volume of 10 l. The aquariums were dewatered using aerated municipal water with artificial marine salt. The water salinity was measured by a digital multimeter (Extech EC 400). The environmental factors were measured every two days using related sensors, including a multimeter (Extech EC 400) to measure the dissolved oxygen, temperature, and pH; the salinity parameter was measured by a salinity meter (AZ8371). The aeration of water in each aquarium was also done by a central air pump using air rock. The dissolved oxygen content was kept at a normal level (above 5 mgL^{-1}). The water temperature was also kept constant at about 26 ± 1.1°C, using 100 W (HT2002) aquarium heaters during the experiment period. Also, during the experiment, pH was kept in the range of 7.4–8.4. Light intensity changes in the culture system were based on the environmental changes in the form of 12 hr of light and 12 hr of darkness. In this study, the effects of different salinity levels on the quality and quantity of *A. franciscana* amino acids were examined in five treatments (10–15, 30–35, 70–75, 110–115, and 150–155 gL^{-1} salinities). All treatments had three replications. Experiments were carried out in 13 days (before maturation and formation of reproductive organs).

2.2 | Hatching and introduction of nauplii to the culture system

Artemia franciscana cysts were obtained from the Salt Lake Aquafeed Company with a hatching percentage of 90%. 1.5-L plastic conical-cylindrical containers filled with de-chlorinated water were used to incubate the cysts. To hatch the cysts, 1 g of *A. franciscana* cyst was weighed and transferred to the incubators with a capacity of 1.5 liters, including 1 L of 32 gL^{-1} saltwater made by sea salt (Reef Crystal). The cysts were hatched for 24 hr at 28°C, pH = 8, under slow aeration and the light of a lamp (1000 lux). Finally, the *A. franciscana* nauplii were isolated after hatching, and, in the first instar, the cyst shells were separated from the non-hatched ones (Khalili, Emadi, & Negarestan, 2007). The number of nauplii in each incubator was counted and then introduced to the experimental treatments (10 L volume) with a concentration of 10 *A. franciscana* per ml. The *A. franciscana* were harvested at the end of the 13th day.

2.3 | Feeding management

The yeast without mannoprotein was prepared with methods described by Marques, Francois, Dhont, Bossier, and Sorgeloos (2004) and Talebi, Esmaili Fereydoni, Abdi, & Manafifar (2013). *A. franciscana* was fed by using yeast 6 times daily for 2 hr and based on water

turbidity. The first feeding was administered at 8:00 a.m. and the last feeding at 6:00 p.m. every day. The amount of yeast needed to feed the *A. franciscana* in each aquarium was calculated based on the number of live *A. franciscana*. Therefore, the number of *A. franciscana* in each aquarium was counted daily; then, the amount of yeast needed to feed was calculated and it was approximately 8 g of yeast for each 24,000 *A. franciscana* daily. After weighing, the above-mentioned amount was dissolved in water (2 g of yeast in 15 ml of water) and added to the aquarium water (Asgari, Najd Gerami, Zare, & Manaffar, 2016). The yeasts were prepared daily and stored at 4°C until subsequent use.

2.4 | Survival rate, sampling, and analysis of amino acid content

The survival of the *A. franciscana* was assessed on days 3 and 13 for all the treatments. In order to calculate the survival, the mean number of live *A. franciscana* per milliliter was calculated. The mean number was then generalized to the total volume of aquarium water. In order to calculate the survival rate, the number of live *A. franciscana* in each aquarium was divided by the number of stored *A. franciscana* at the beginning of the experiment, and the percentage was calculated (Khalili et al., 2007).

Artemia franciscana were sampled on day 3 and at the end of day 13 post-stock. For the sampling of each treatment, about 9,000 *A. franciscana* were taken by an 80-μm grid on day 3 and transferred to 2.5-ml microtubes. At the end of day 13, all the water in each aquarium was drained, and the *A. franciscana* were taken by an 80-μm grid. Then, they were stored in 5-ml microtubes in a freezer at −80°C until the analysis of amino acids.

All the *A. franciscana* were analysed using the Waters Pico-Tag method and high-performance liquid chromatography (HPLC). The samples were prepared prior to analysis by the method described by White, Hart, & Fry (1986). The HPLC system used for the analysis of amino acids (Knauer) was measured by column 18 and fluorescence detector (RF5300 Knauer) at a wavelength of 570 nm. Crude protein of *Artemia* was analysed following the official method of AOAC (2000). Pearson's bivariate correlation test was used to investigate the correlation between amino acids. One-way ANOVA was used to compare the data, and Tukey's post hoc test was used to compare the means. The tests were evaluated at $p<0.05$.

3 | RESULTS

3.1 | *Artemia franciscana* survival rate

The survival rates of the *A. franciscana* measured under salinity increase on day 3 and day 13 are shown in Table 1. According to the results of one-way ANOVA statistical analysis, it can be stated that the survival rate of the *A. franciscana* was significantly affected by the salinity treatments (p <0.05). Accordingly, the lowest survival rate was observed on day 3 of culture in the 150 gL^{-1} salinity treatment (66.66 ± 0.86%) while the highest survival rate was observed in the 30 gL^{-1} salinity treatment (98.00 ± 0.33%). On day 13, the highest and the lowest survival rates were recorded in the 70 (34.66 ± 0.33%) and 10 (11.86 ± 0.13%) gL^{-1} salinity treatments respectively. No significant difference was observed between the 30, 110, and 70 gL^{-1} salinity treatments (p >0.05).

TABLE 1 The survival rate of *Artemia fransciscana* under different levels of culture salinity on day 3 and day 13

Salinity treatments (ppt)	Survival rate (%)	
	3rd day	13th day
10–15	89.66 ± 0.34^{ab}	11.86 ± 0.13^{a}
30–35	98.00 ± 0.33^{b}	31.83 ± 0.60^{b}
70–75	97.66 ± 0.33^{b}	34.66 ± 0.33^{b}
110–115	98.55 ± 0.32^{b}	33.89 ± 0.76^{b}
150–155	66.66 ± 0.86^{a}	12.76 ± 1.31^{a}

Note: Mean ± SE values (n = 3) with different superscript in each column are significantly different (p <0.05)

3.2 | *Artemia franciscana* amino acid compounds

The results of the analysis of body amino acids on days 3 and 13 of the *A. franciscana* culture are presented in Tables 2 and 3 respectively. These results are shown in the form of the quantity of amino acids in different saline treatments, the total essential (EAA), and non-essential amino acids (NEAA) as well as the ratio of total essential to non-essential amino acids.

3.3 | The third day of culture

Based on the results of one-way ANOVA statistical analysis, the quantity of each of the amino acids, except the aspartic acid, of the *A. franciscana* culture on day 3 was significantly affected by the salinity treatments (p <0.05). According to the results presented in Table 2, in all the essential amino acids, except for isoleucine and valine, generally, an increase in the culture water salinity led to an increase in the amino acid content (mg/g dry weight). Among the EAAs, arginine had the highest amount of EAA in the 30, 70, 110, and 150 gL^{-1} salinity treatments. The results of the one-way ANOVA showed that the quantity of essential amino acids on the third day of culture was significantly different in salinity treatments (p <0.05). The highest and the lowest total amount of whole-body EAA was found in the *A. franciscana* which were exposed to 150 g/L and 10 gL^{-1} salinity treatments respectively. In other words, as the salinity of the culture water increased, the amount of total essential amino acids in the body of the *A. franciscana* increased on the third day. The results of the correlation between the EAA on the third day of the culture showed that there was a significant correlation between the *A. franciscana* whole-body amino acids of histidine and leucine (p <0.01, y = 9,231.2x–0.94, R^2 = 0.904), phenylalanine (p <0.05,

TABLE 2 The analysis of body amino acid profile (mgg^{-1} dry weight) and crude protein (gkg^{-1} dry weight) of *Artemia fransciscana* under different levels of culture salinity after 3 days post-hatching

	Salinity treatments (ppt)				
Amino acids	**10–15**	**30–35**	**70–75**	**110–115**	**150–155**
Essential amino acid (EAA)					
Arginine (Arg)	0.080 ± 0.00^{a}	0.832 ± 0.00^{bc}	0.764 ± 0.02^{b}	0.873 ± 0.02^{c}	0.872 ± 0.02^{c}
Histidine (His)	0.084 ± 0.00^{a}	0.085 ± 0.00 ^{ab}	0.085 ± 0.00^{ab}	0.086 ± 0.00^{b}	0.087 ± 0.00^{b}
Leucine (Leu)	0.150 ± 0.00 ^{a}	0.155 ± 0.00^{b}	0.155 ± 0.00^{b}	0.158 ± 0.00^{bc}	0.159 ± 0.00^{c}
Lysine (Lys)	0.131 ± 0.00 ^{a}	0.142 ± 0.00^{ab}	0.153 ± 0.00^{bc}	0.158 ± 0.00^{c}	0.162 ± 0.00^{d}
Methionine (Met)	0.090 ± 0.00^{ab}	0.091 ± 0.00^{ab}	0.088 ± 0.00^{a}	0.091 ± 0.00^{ab}	0.095 ± 0.00^{b}
Phenylalanine (Phe)	0.043 ± 0.00 ^{a}	0.047 ± 0.00^{b}	0.049 ± 0.00^{b}	0.055 ± 0.00^{c}	0.055 ± 0.00^{c}
Threonine (Thr)	0.058 ± 0.00 ^{a}	0.060 ± 0.00^{a}	0.064 ± 0.00^{b}	0.065 ± 0.00^{b}	0.065 ± 0.00^{b}
Isoleucine (Ile)	0.076 ± 0.00 ^{ab}	0.077 ± 0.00^{ab}	0.075 ± 0.00^{ab}	0.074 ± 0.00^{a}	0.075 ± 0.00^{ab}
Valine (Val)	0.140 ± 0.00 ^{b}	0.136 ± 0.00^{a}	0.139 ± 0.00^{ab}	0.138 ± 0.00^{ab}	0.139 ± 0.00^{ab}
Total EAA	0.852 ± 0.03 ^{a}	1.625 ± 0.05^{b}	1.572 ± 0.05^{b}	1.698 ± 0.05^{b}	1.709 ± 0.05^{b}
Non-essential amino acid (NEAA)					
Aspartic acid (Asp)	0.033 ± 0.00	0.034 ± 0.00	0.034 ± 0.00	0.036 ± 0.00	0.036 ± 0.00
Glutamic acid (Glu)	0.640 ± 0.00^{a}	0.640 ± 0.00^{a}	0.643 ± 0.00^{a}	0.656 ± 0.00^{b}	0.659 ± 0.00^{b}
Alanine (Ala)	0.354 ± 0.00^{b}	0.355 ± 0.00^{b}	0.350 ± 0.00^{b}	0.335 ± 0.00^{a}	0.348 ± 0.00^{b}
Glycine (Gly)	0.142 ± 0.00^{a}	0.180 ± 0.00^{b}	0.171 ± 0.00^{b}	0.186 ± 0.00^{bc}	0.205 ± 0.00^{c}
Proline (Pro)	0.112 ± 0.00^{b}	0.113 ± 0.00^{b}	0.110 ± 0.00^{a}	0.107 ± 0.00^{a}	0.108 ± 0.00^{a}
Tyrosine (Tyr)	0.161 ± 0.00^{bc}	0.176 ± 0.00^{c}	0.156 ± 0.00^{bc}	0.138 ± 0.00^{a}	0.147 ± 0.00^{ab}
Serine (Ser)	0.126 ± 0.00^{a}	0.127 ± 0.00^{ab}	0.132 ± 0.00^{c}	0.130 ± 0.00^{bc}	0.131 ± 0.00^{bc}
Total NEAA	1.568 ± 0.01^{a}	1.625 ± 0.03^{b}	1.596 ± 0.04^{b}	1.588 ± 0.04^{b}	1.634 ± 0.04^{b}
Total amino acid	2.240 ± 0.09^{a}	3.246 ± 0.00^{b}	3.168 ± 0.00^{b}	3.286 ± 0.15^{c}	3.340 ± 0.08^{c}
TEAA/TNEAA	0.544 ± 0.12^{a}	1.000 ± 0.00^{b}	1.002 ± 0.00^{b}	1.067 ± 1.25^{b}	1.045 ± 0.13^{a}
Crude protein	329.4 ± 28.5	344.4 ± 33.2	352.1 ± 30.1	354.9 ± 34.0	332.9 ± 29.6

Note: Mean ± SE values (n = 3) with different superscript in each row are significantly different (p <0.05).

y = 213.73x–18.01, R^2 = 0.781) and lysine (p <0.05, y = 9.19x–0.63, R^2 = 0.772), on the one hand, and between threonine and leucine (p <0.05, y = 0.975x + 0.0945, R^2 = 0.797), on the other hand.

Also, an increase was observed in the content of some non-essential amino acids, including glutamic acid, glycine, and serine, on the third day of the culture due to the increased water salinity. However, this trend was reversed for alanine and proline. Among the NEAA, aspartic acid and glutamic acid had the lowest and the highest values in all salinity treatments respectively. The lowest (p <0.05) and the highest amount of total NEAA were found in the *A. franciscana* in the 10 and 150 gL^{-1} water salinity respectively. The comparison of the total essential amino acid content, total non-essential amino acid content, and survival rate of *Artemia fransciscana* under different levels of culture salinity after 3 days is given in Figure 1a. The results of the correlation between the *A. franciscana* non-essential amino acids on the third day of the culture showed that there was a significant correlation between the aspartic acid and glycine (p <0.05, y = 15.139x–0.347, R^2 = 0.771), on the one hand, and between glutamic acid and proline (p <0.05, y = −0.258x + 0.277, R^2 = 0.794), on the other hand.

There was a significant difference between the total amino acids in different salinity treatments on the third day of the culture (p <0.05). The lowest value was observed in the 10 gL^{-1} of salinity treatment while the highest value was found in the 150 gL^{-1} salinity treatment. Also, the results showed that the ratio of total EAA to NEAA of *A. franciscana* whole-body on the third day of the culture was significantly affected by different salinity treatments. The highest and the lowest ratios were observed in the 110 and 10 gL^{-1} salinity treatments respectively. The findings of the correlation between the *A. franciscana* essential and non-essential amino acids showed that there was a significant correlation between histidine and aspartic acid (p <0.01, y = 91,154.1x–0.06, R^2 = 0.896), glutamic acid (p <0.01, y = 1.12x–0.06, R^2 = 0.899) and glycine (p <0.01, y = 19.23x–1.46, R^2 = 0.899); between leucine and aspartic acid (p <0.01, y = 0.362x–0.0216, R^2 = 0.894) and glycine (p <0.01, y = 6.382x–0.815, R^2 = 0.936); between phenylalanine and aspartic acid (p <0.01, y = 0.0046x + 0.034, R^2 = 0.912); and finally between isoleucine (p <0.01, y = 2.115x–0.0495, R^2 = 0.895) and proline.

3.4 | The thirteenth day of the culture

According to the results of one-way ANOVA statistical analysis, the amount of each amino acid of the *A. franciscana* on day 13

TABLE 3 The analysis of body amino acid profile (mgg^{-1} dry weight) and crude protein (gkg^{-1} dry weight) of *Artemia fransciscana* under different levels of culture salinity after 13 days post-hatching

	Salinity treatments (ppt)				
Amino acids	**10–15**	**30–35**	**70–75**	**110–115**	**150–155**
Essential amino acid (EAA)					
Arginine (Arg)	69.40 ± 0.25^{a}	82.50 ± 0.24^{b}	84.30 ± 0.23^{b}	88.40 ± 0.25^{c}	90.60 ± 0.26^{c}
Histidine (His)	26.10 ± 0.17^{a}	25.40 ± 0.17^{a}	26.30 ± 0.57^{a}	28.20 ± 1.15^{ab}	31.10 ± 0.57^{b}
Leucine (Leu)	77.70 ± 1.05 ^{a}	79.50 ± 0.52 ^{a}	92.30 ± 0.26 ^{a}	98.10 ± 0.57 ^{ab}	130.70 ± 0.56 ^{b}
Lysine (Lys)	45.90 ± 1.78 ^{a}	50.20 ± 4.61^{a}	60.30 ± 4.61^{a}	70.30 ± 4.62 ^{a}	131.20 ± 4.05 ^{b}
Methionine (Met)	16.20 ± 1.15^{a}	19.80 ± 1.73^{a}	23.10 ± 1.15^{ab}	21.30 ± 0.57^{a}	30.40 ± 1.46^{b}
Phenylalanine (Phe)	43.50 ± 0.57^{a}	45.60 ± 1.73^{a}	50.30 ± 3.05^{a}	58.20 ± 2.88^{ab}	77.30 ± 4.23^{b}
Threonine (Thr)	39.66 ± 1.76^{a}	47.13 ± 0.96^{a}	53.00 ± 1.15^{ab}	48.86 ± 1.45^{a}	71.20 ± 4.66^{b}
Isoleucine (Ile)	43.90 ± 0.69^{a}	45.60 ± 4.79^{a}	59.10 ± 0.46^{b}	58.30 ± 1.73^{b}	80.60 ± 2.02^{c}
Valine (Val)	80.00 ± 1.15^{b}	76.50 ± 0.57^{a}	75.00 ± 0.17^{a}	75.50 ± 0.57^{a}	76.84 ± 0.53^{ab}
Total EAA	442.36 ± 2.29^{a}	472.23 ± 2.26^{a}	523.72 ± 3.40^{a}	547.16 ± 3.27^{ab}	720.04 ± 4.90^{b}
Non-essential amino acid (NEAA)					
Aspartic acid (Asp)	1.36 ± 0.78^{a}	3.73 ± 0.08^{a}	3.80 ± 0.86^{a}	5.46 ± 1.74^{ab}	10.23 ± 1.79^{b}
Glutamic acid (Glu)	5.13 ± 2.46^{a}	25.40 ± 0.57^{b}	25.50 ± 4.04^{b}	40.20 ± 3.34^{c}	60.00 ± 3.46^{d}
Alanine (Ala)	115.00 ± 3.92^{c}	84.90 ± 5.39^{b}	63.40 ± 5.19^{ab}	46.60 ± 4.36^{ab}	40.03 ± 2.30^{a}
Glycine (Gly)	49.30 ± 3.32^{a}	42.16 ± 1.33^{a}	43.40 ± 0.57^{a}	45.46 ± 2.19^{a}	64.40 ± 2.30^{b}
Proline (Pro)	6.70 ± 1.73^{a}	18.30 ± 4.04^{ab}	40.20 ± 2.36^{bc}	50.20 ± 5.74^{c}	80.00 ± 7.62^{d}
Tyrosine (Tyr)	50.00 ± 4.69^{a}	52.01 ± 5.57^{a}	50.10 ± 5.61^{a}	55.30 ± 3.46^{ab}	50.60 ± 3.05^{b}
Serine (Ser)	17.90 ± 0.57^{a}	19.00 ± 0.11^{a}	20.20 ± 0.86^{a}	17.18 ± 1.94^{a}	24.50 ± 1.52^{b}
Cysteine (Cys)	0.80 ± 0.28^{a}	2.30 ± 0.23^{a}	3.00 ± 0.57^{ab}	4.00 ± 1.15^{ab}	6.30 ± 1.15^{b}
Total NEAA	246.59 ± 2.90^{a}	248.39 ± 3.30^{a}	249.00 ± 1.90^{a}	265.02 ± 4.70^{a}	336.96 ± 4.40^{b}
Total amino acid	688.66 ± 4.42^{a}	720.30 ± 3.73^{a}	773.26 ± 3.57^{a}	811.00 ± 3.75^{b}	1,056.83 ± 5.29^{c}
TEAA/TNEAA	1.79 ± 0.05^{a}	1.90 ± 0.12^{a}	2.10 ± 0.12^{ab}	2.07 ± 0.08^{ab}	2.14 ± 0.13^{b}
Crude protein	510.1 ± 41.4	524.4 ± 57.5	538.7 ± 49.1	542.1 ± 38.9	541.5 ± 50.7

Note: Mean ± SE values (n = 3) with different superscript in each row are significantly different (p <0.05).

was significantly affected by the salinity treatments. According to the results presented in Table 3, in most of the essential amino acids, generally, an increase in the water salinity led to an increase in the amount of the EAA. For valine, however, the trend was almost descending. Among the EAA of the *A. franciscana* on day 13 of the culture, valine showed the highest value in the 10 gL^{-1} salinity treatment, arginine showed the highest value in the 30 gL^{-1} salinity treatment, leucine showed the highest value in the 70 and 110 gL^{-1} salinity treatments, and lysine showed the highest value in the 150 gL^{-1} salinity treatment. However, methionine had the lowest value in all treatments. The results of one-way ANOVA showed that there was a significant difference between the amount of EAA in different salinity treatments on day 13 of the culture (p <0.05). The lowest and the highest amount of *A. franciscana* essential amino acids was found in the 10 gL^{-1} and 150 gL^{-1} salinity treatments respectively. However, no significant difference was observed between 150 and 110 salinity treatments. With an increase in the water salinity of the treatments, the total EAA of the *A. franciscana* body also increased. The correlation between the EAA of the *A. franciscana* whole-body on day 13 showed that there was a significant correlation between histidine and phenylalanine (p <0.01, y = 5.6509x–100.19, R^2 = 0.968), on the one hand, and between leucine and isoleucine (p <0.01, y = 0.6795x–7.498, R^2 = 0.978), methionine (p <0.05, y = 0.2368x–0.1321, R^2 = 0.931), phenylalanine (p <0.01, y = 0.636x–5.878, R^2 = 0.986) and threonine (p <0.05, y = 0.5261x + 1.6451, R^2 = 0.911), on the other hand.

For non-essential amino acids, we observed a general increase in the content of amino acids with increased salinity. However, the increase in the salinity reduced the content of alanine. For NEAA, cysteine had the lowest value in all treatments. Alanine had the highest value in the 10, 30, and 70 gL^{-1} salinity treatments, tyrosine had the highest value in the 110 gL^{-1} salinity treatment, and proline had the highest value in the 150 gL^{-1} salinity treatment. Moreover, the results showed that there was a significant difference between the amount of NEAA in different salinity treatments on day 13 of the culture (p <0.05). The lowest and the highest amount of the *A. franciscana* total non-essential amino acids was observed in the 10 gL^{-1} and 150 gL^{-1} salinity treatments respectively. The comparison of the total essential amino acid content, total non-essential amino acid content, and survival rate of *Artemia franciscana*

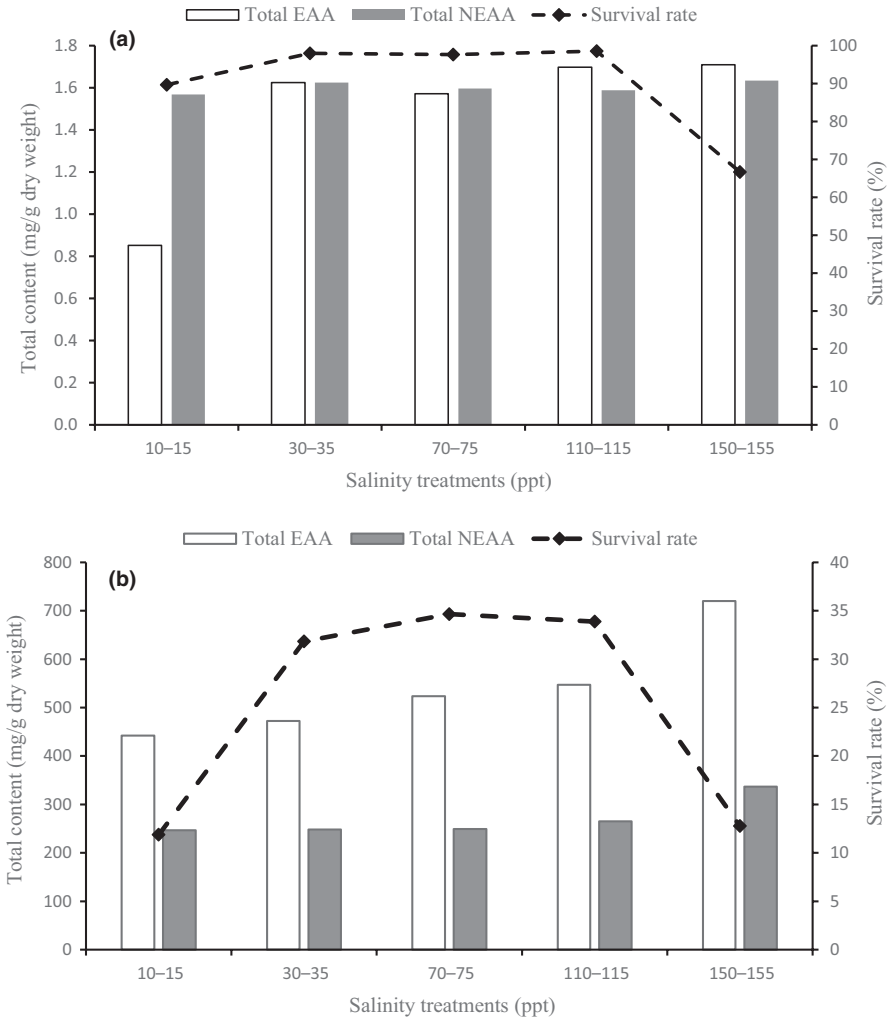

FIGURE 1 Comparison of the total essential amino acid (EAA, white bar) content, total non-essential amino acid (NEAA, grey bar) content and survival rate (%, dash line) of Artemia franciscana under different levels of culture salinity after 3 days (a) and 13 days (b) post-hatching

under different levels of culture salinity after 13 days is reported in Figure 1b. In general, the salinity increased the amount of NEAA. In addition, an examination of the results of the correlation between the NEAA of the *A. franciscana* whole-body on day 13 showed that there was a significant correlation between the aspartic acid, glutamic acid (p <0.01, $y = 6.0204x + 1.649$, $R^2 = 0.959$) and cysteine (p <0.01, $y = 0.6099x + 0.2817$, $R^2 = 0.969$); between glutamic acid, proline (p <0.01, $y = 1.3506x - 3.121$, $R^2 = 0.931$) and cysteine (p < .01, $y = 0.99x + 0.1598$, $R^2 = 0.982$); and finally between the proline and cysteine (p <0.01, $y = 0.0707x + 0.516$, $R^2 = 0.975$).

The results of the statistical analysis showed that there was a significant difference between the total amino acids with different salinity treatments on day 13 of the culture (p <0.05). The lowest and the highest values were observed in the 10 gL^{-1} and 150 gL^{-1} salinity treatments. Also, a significant difference was observed between the ratio of the total EAA to NEAA of the *A. franciscana* on day 13 of the culture (p <0.05). The highest ratio was observed in the 150 gL-1 salinity treatment while the lowest ratio was observed in the 30 gL^{-1} salinity treatment. The results of the correlation between the *A. franciscana* essential and non-essential amino acids on day 13 showed that there was a significant correlation between arginine and alanine (p <0.05, $y = -2.8047x + 300.14$, $R^2 = 0.907$); between isoleucine and proline (p <0.01, $y = 1.9037x - 70.38$, $R^2 = 0.954$); between leucine and proline (p <0.01, $y = 1.298x - 85.13$, $R^2 = 0.941$); between lysine and aspartic acid (p <0.01, $y = 0.0928x - 1.726$, $R^2 = 0.943$); between methionine and proline (p <0.01, $y = 5.1594x - 75.253$, $R^2 = 0.957$) and cysteine (p <0.05, $y = 0.3715x - 4.953$, $R^2 = 0.907$); and finally between threonine and the aspartic acid (p <0.05, $y = 0.268x - 9.011$, $R^2 = 0.911$).

4 | DISCUSSION

Amino acids of live foods are important components of the nutrient profiles that meet the nutritional, metabolic, and evolutionary needs of larvae (Lindley, Phelps, & Davis, 2011); they are also considered as the dominant intracellular solutions in marine invertebrates and crustaceans. These organic osmolytes control intracellular osmotic pressure and maintain a balance between the tissue and haemolymph when the body is exposed to environmental stresses, such as salinity changes during a process called intracellular isosmotic regulation (Augusto, Greene, Laure, & McNamara, 2007; Pequeux, 1995; Shinji, Okutsu, Jayasankar, Jasmani, & Wilder, 2009). This mechanism

balances the osmotic pressure of the body fluids with the external environment, which will finally lead to compatibility and increased survival.

The content of biochemical compounds of zooplankton, including amino acids, is strongly influenced by enrichment methods (Aragao, Conceicao, Dinisa, & Fyhn, 2004). A variety of enrichment methods, including the addition of soluble amino acids to the culture medium water, the use of liposomal and phospholipid membranes, and the use of commercial enrichment solutions, exist for the *A. franciscana* body's amino acids, but these methods are not recommended due to their reducing the quality of the culture, low enrichment efficiency and high cost (Hawkyard, Laurel, & Langdon, 2014). In general, in fish enrichment methods, the cost and duration of the enrichment process are very important (Tamaru, Ako, Paguirigan, & Pang, 2011). For this reason, and due to their compatibility with the environmental stress, methods based on zooplankton enrichment can have a significant effect on reducing the costs associated with the larval feeding. In fact, the changes that take place in the amino acid content of zooplankton under the influence of water salinity are a type of biological enrichment, which is based on the natural mechanisms of the zooplankton's body response to environmental changes; therefore, methods based on zooplankton enrichment can be very useful in aquaculture (Gajardo & Beardmore, 2012).

The changes in the EAA and NEAA between the highest and lowest salinity treatments on day 13 (297.68 mgg^{-1} dry weight) were higher than those recorded on day 3 (1.23 mgg^{-1} dry weight). A similar trend was observed for the total NEAA on day 13 compared to day 3. Therefore, it can be stated that the amount of EAA and NEAA of the *A. franciscana* body was more affected on day 13 than on day 3 by the salinity changes. Also, on the third day of the culture, the most increases in the amount of total EAA observed when the salinity increased from 10 to 30 gL^{-1} (1.152) and, then, from 70 to 110 gL^{-1} (0.126). On day 13, the highest changes were observed as water salinity increased from 110 to 150 (190/32), followed by 30 to 70 gL^{-1} (51.47). For the NEAA, this change was also observed when the salinity treatment increased from 110 to 150 gL^{-1}. For the total amino acids, the highest changes were observed when the salinity was increased from 10 to 30 gL^{-1} (1.175) on the third day and from 110 to 150 gL^{-1} on the thirteenth day (250/83). In general, in this study, the *A. franciscana* body total amino acids increased as the salinity was increased from 10 to 150 gL^{-1}; moreover, the difference between the total body amino acids increased with the increase in the culture duration in different salinities. This indicates that the content of amino acids may be changed by the degree of water salinity and the culture duration. Similar results have been reported regarding the effects of water salinity on the content of amino acids in *A. franciscana* (Nakamura et al., 2007), *Calanus finmarchicus* (Lindley et al., 2011), *Litopenaeus vannamei* (Shinji & Wilder, 2012) and *Scylla paramamosain* (Lu et al., 2015).

Among the total amino acids in the body, some are most affected by changes in water salinity. In fact, it can be said that these amino acids play the most important role in osmotic regulation and balance in aquatic body fluids (Mazzarelli et al., 2015). In the present study, the highest changes in the amount of EAA affected by the salinity increase on day 3 were related to arginine and lysine respectively. On day 13, these changes were observed in lysine, leucine, isoleucine, phenylalanine, threonine, and arginine respectively. Regarding NEAA, most changes were observed in glycine and glutamic acid on day 3 and in proline, glutamic acid, glycine, tyrosine, and aspartic acid on day 13 respectively. According to the results, with the increasing compatibility of the *A. franciscana* with different salinities, more amino acids were changed. Among the EAA, arginine (22.7%), leucine (5.11%), lysine (4.9%) and methionine (2.9%) on the third day and leucine (12%), arginine (10%), lysine (9%), isoleucine (7.25%), phenylalanine (6.9%), threonine (6.5%) and histidine (3.4%) on the thirteenth day compared to other amino acids had the highest portion of the total EAA. For NEAA, tyrosine, proline, and glutamic acid accounted for 6.8%, 4.9%, and 3.9% of the total amino acids on the third day respectively; these acids had the highest amount among the NEAA. However, on day 13, glutamic acid (21.46%), glycine (5.84%), tyrosine (5.11%), serine (4.25%), and proline (3.65%) had the highest share compared to other NEAA. According to the results, it can be suggested that the above-mentioned amino acids probably play a more important role in adapting and balancing the salinity stress than the other amino acids of the *A. franciscana*. Shinji and Wilder (2012) also considered glycine, arginine and glutamic acid as the most important amino acids in the compatibility of *Litopenaeus vannamei* to environmental stresses. Burton and Feldman (1982) considered arginine as an important regulator in the osmotic regulation and compatibility of Copepod (*Tigropus californicus*) to salinity stress. Lu et al. (2015) also stated that proline, glutamic acid, glycine, and arginine showed more changes in response to hypo-osmotic stress in mud crabs than other amino acids.

Given that fish and, in particular, larvae growth are associated with high protein intake (Rojas-Garcia, Applebaum, Morais, & Rønnestad, 2016), maintaining a balance between the amino acid diet is an important factor in proper fish growth. Fish larvae require a significant amount of protein in their diets, and their intake of essential amino acids is high, so the amount of EAA in their diets can be considered as a growth-limiting factor as it will make protein synthesis difficult (Conceicao et al., 2010). Therefore, providing a proper ratio of EAA and NEAA in fish diets can not only maximize the yield and efficiency of protein utilization but also reduce nitrogen excretion (Peres & Oliva-Teles, 2006). Few studies have been conducted on the proper ratio of essential to non-essential amino acids in the diet of marine larvae (Green, Hardy, & Brannon, 2002). According to a study by Peres and Oliva-Teles (2006), the highest increase in the post-feeding fish growth was observed with a 50:50 (1:1) ratio of EAA to NEAA. In the present study, the ratio of EAA to NEAA in 30 (1.008) and 70 (1.026) gL^{-1} salinity treatments on day 3 of the culture were close to that of Peres and Oliva-Teles's (2006). The highest ratio of EAA to NEAA was observed on days 3 and 13 in 70 and 150 gL^{-1} salinity treatments respectively. However, no significant difference was found between them at the 110 gL^{-1} salinity treatment.

In the present study, the effects of the changes in the water salinity of the culture media on *A. franciscana* body's amino acid profile were examined and the results showed that the amount of *A. franciscana* body's amino acids will increase with the increase in the salinity. In addition, the difference in the changes in the amino acids increased as the *A. franciscana's* compatibility increased from day 3 to day 13. The difference in the total amino acids in the highest and lowest salinity treatments on day 13 was 421.17 units, which was much higher than that of day 3 (1.29). Based on the study results, it can be concluded that increasing water salinity and the duration of the culture can change the amount of most amino acids in *A. franciscana*. The highest amount of the total amino acids was recorded in the highest salinity treatment (150 gL^{-1} salinity). However, this trend was not true of the *A. franciscana's* survival rate since the highest survival rates were observed on days 3 and 13 in treatments other than 150 gL^{-1}. The correlation between the survival rate and the profile of EAA showed that on the third day of the *A. franciscana* culture, the highest survival rate was observed in the 110 gL^{-1} salinity treatment while the highest total amino acids were recorded in the 150 gL^{-1} salinity treatment. Given that the difference between the total essential amino acids in 110 and 150 gL^{-1} salinity treatments was only 0.011 mgg^{-1} of the dry weight, it can be concluded that the culturing the *A. franciscana* in 110 gL^{-1} salinity would be appropriate in terms of survival rate and EAA profile. On day 13, the highest amount of the total EAA was observed in the 150 gL^{-1} salinity treatment while the highest survival rate was recorded in the 70 gL^{-1} salinity treatment.

Finally, it can be concluded that *A. franciscana* cultured in 110 gL-1 salinity treatment are the most desired *A. franciscana* in terms of EAA profile, the ratio of total EAA to NEAA and survival rate. Therefore, similar to what was found in the present study, any culture of *A. franciscana* under110 gL^{-1} salinity can enrich the body of *A. franciscana* in terms of the content of the amino acids.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Khorramshahr University of Marine Science and Technology for their financial support to this research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors.

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How to cite this article: Zadehmohseni B, Zakeri M, Yavari V, Haghi M. Effects of different salinities on amino acid profile in *Artemia franciscana*. *Aquac Res*. 2020;00:1–9. https://doi.org/10.1111/are.14679