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Long-term effects of vermicompost manure leachate (powder) inclusions on growth and survival, biochemical composition, total carotenoids, and broodstock reproductive performance of *Artemia franciscana* (Kellogg, 1906)

Saeid Vahdat[1] · Abolghasem Esmaeili Fereidouni[2] · Mohammad Kazem Khalesi[2]

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Abstract Long-term effects of different levels of dietary vermicompost manure leachate powder (VCL) were investigated on the nauplii growth and survival, biochemical composition, total carotenoids, and reproductive performance of broodstock *Artemia franciscana* in laboratory cultures. The instar I nauplii were fed in five treatments including 100% of the microalga *Dunaliella salina* (Alg; control group, with a density of 18×10^6 cells mL^{-1}) and mixtures of 75% Alg-25% VCL, 50% Alg-50% VCL, 25% Alg-75% VCL, and 100% VCL for 3 weeks. At maturity, 35 pairs (males and females) were individually isolated from each treatment and transferred to 50-mL falcon tubes in which the reproduction and longevity of females were monitored until mortality. Results showed that the total length of *Artemia* in the control, 75 Alg-25 VCL, and 50 Alg-50 VCL treatments was significantly different from the other groups at the end of the 2nd and 3rd weeks. The pre-puberty survival rate was severely affected by the increased dietary levels of VCL declining from 52% (control) to 6.7% (100 VCL). The adults' body protein levels in the control and 75 Alg-25 VCL (54–57%) were similar, but both groups were significantly different from the other treatments. However, the lipid content (13.7–19.8%) and total carotenoids (36.5–47.7 μg mg^{-1}) were significantly different between treatments. Many broodstocks' reproductive characteristics were affected by the diet so that the total number of offspring in the control was markedly higher than the other groups. However, the number of offspring per brood revealed no differences between the control and 75 Alg-25 VCL treatment,

✉ Abolghasem Esmaeili Fereidouni
a.esmaeili@sanru.ac.ir

[1] Faculty of Animal Sciences and Fisheries, Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran

[2] Fisheries Department, Faculty of Animal Sciences and Fisheries, Sari Agricultural Sciences and Natural Resources University (SANRU), Km 9, KhazarAbad, P.O.Box 578, Sari, Iran

but both were significantly dissimilar with the other groups. The interval between two successive brood productions detected in 100 VCL was almost double those in the other groups. The lifetimes of *Artemia* were not significantly different in the treatments received varied algal diets with the lowest lifespan in 100 VCL treatment. By increasing VCL to over 25%, the growth, survival, and particularly females' reproductive performance decreased significantly so that *Artemia* had to be fed only up to 25% VCL (with 75% Alg). These findings indicate an inefficiency of VCL powder supply in long-term feeding to *A. franciscana*. Moreover, the use of only 25% VCL is apparently appropriate in small-scale laboratory cultures.

Keywords Vermicompost manure leachate powder (VCL) · *Artemia franciscana* · Reproductive performance · Life span

Abbreviations

VCL Vermicompost manure leachate powder

Alg Algae

Introduction

Artemia accounts for an important live feed in the aquaculture industry for the feeding stages of aquatic species (Lavens and Sorgeloos 2001; Anh et al. 2009). Reductions occurring in the natural resources and also increasing demand for *Artemia* cysts have resulted in the application of various techniques to increase both extensive, intensive, and super-intensive mass production in large earthen salt ponds, outdoor environments, as well as in indoor, recirculation, and biofloc systems (Baert et al. 1996; Naegel 1999; Zmora et al. 2002; Zmora and Shpigel 2006; Van Hoa et al. 2011; Ronald et al. 2014; Bossier et al. 2016). *Artemia* species are mostly fed with agricultural by-products, organic manures, brans (rice, wheat, corn), and/or industrially processed feed consisting of a variety of pelleted soy protein, whey, algal dried powder, yeasts, and bacteria (single-cell proteins) as the sole foodstuffs and/or supplemental feeds along with other sources such as microalgae (Basil et al. 1995; Zmora and Shpigel 2006; Anh et al. 2009; Ownagh et al. 2015). Different live and dried unicellular algae such as *Dunaliella* are commonly used as food for *Artemia*; however, the high costs and laborious task of algal production are considered as the major restrictions in the mass culture of *Artemia* (Coutteau et al. 1992; Lavens and Sorgeloos 1996; Naegel 1999; Maldonado-Montiel et al. 2003). On the other hand, low growth and survival rates were reported when agricultural by-products (e.g., wheat bran, rice bran, and soybean meal) were substituted as the sole diets for *Artemia* (Sorgeloos 1980; Anh et al. 2009; Ownagh et al. 2015).

Organic manures (e.g., cow, cattle, sheep, pigs, horses, and poultry) are considered cheap inputs that may contribute to improved crop yields; however, responses to these fertilizers can vary not only due to their own characteristics, but also to the regional environmental conditions, salinity, and treatments applied to these wastes. When these organic matters are decomposed, they turn into manures which can be used to produce natural foods such as bacterial and fungal communities, yeast, and different kinds of microorganisms to support the growth and reproduction of crustaceans (Ronsivalli and Simpson 1987; Baert et al. 1997; Zmora et al. 2002; Chakrabarty 2009). The extracts derived from organic manure may directly be consumed and/or enrich the culture water through the production of autotrophic phytoplankton and/or heterotrophic microbial communities (Muendo et al. 2006).

In recent years, the use of such bio-fertilizers as vermicompost has revealed a growing trend in the aquaculture industry (Chakrabarty et al. 2010; Kaur and Ansal 2010; Kumar et al. 2012; Bansal et al. 2014). This is mainly because of high diversity of both major and minor nutrients, enzymes, vitamins, antibiotics, and growth promoters in vermicompost (Edwards et al. 2011). The manure (containing 80% moisture) is derived from the biological activity of the omnivorous red earthworm *Eisenia foetida* in a semi-aerobic process to feed on a wide range of waste organic materials including cow dung, poultry waste, and piggery agricultural wastes (Gunadi et al. 2002). The worm excrements produce nutritive manure which is considered as one of the richest bio-organic manures (Edwards et al. 2011). Additionally, the degradable ability of vermicompost manure makes it possible to greatly reduce degraded water quality due to extreme biological oxygen demand (BOD) as well as production of some undesirable gases (H_2S, NH_3). Vermicompost is comparatively found to be more nutritious concerning greater amounts of carbon and phosphorus, lesser potassium and comparable nitrogen compared to cow dung and other farmyard manures (Edwards et al. 2011). The ratio of carbon to nitrogen (C/N) in vermicompost ranges between 15 and 20, humus amounts to about 20% of the dry weight, and the size of grains varies between 1 and 5 mm. The leachate derived from vermicomposting, often called “worm tea,” can be used as a liquid fertilizer because it contains large amounts of nutrients that might contribute to the plant development (Arancon et al. 2008). Since this manure is completely biodegradable and digestible, the best alternative to minimize the harmful effects of organic manures is to utilize fully decomposed/digested organic manures compared to undigested and/or semi-digested ones.

Despite previous studies on the growth and reproductive performances of *Artemia* with a variety of enriched diets and organic fertilizers, no study has ever examined the potential application of vermicompost leachate (VCL) on the growth and reproductive characteristics of cultured *Artemia* during total life span in long-term trials. Therefore, this study examined the effects of different levels of vermicompost leachate (powder) on the growth and survival of nauplii, body biochemical composition, total carotenoids, reproductive performance, and longevity of *A. franciscana* (Kellogg 1906) broodstock in small-scale laboratory cultures.

Materials and methods

Preparation of vermicompost manure leachate powder (VCL)

The experiments were conducted at the Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran in 2015–2016. The raw vermicompost used in this study had been originated from the cow dung manure composted in a process of 6 months at a local farm. The biochemical composition of raw vermicompost was determined according to AOAC (2002).

To prepare the VCL powder, first 55 g of raw vermicompost manure was mixed in 1.0 L of freshwater and incubated with strong aeration at room temperature (25 °C) for 24 h allowing the contents of manure to be completely dissolved in water (Edwards et al. 2011). Then, all the mixture (containing dissolved proteins) was completely discharged and the remaining leachate was sieved through a 20–50 μm filter followed by oven-drying at 40 °C for 24 h. Finally, 4.0 g of dried extract (powdered) was dissolved in 600 mL of water and fed to *A. franciscana* at different treatments during the entire life cycle (long-term period) according to Coutteau et al. (1992) protocol. An initial assessment showed that *A. franciscana* was capable of feeding the powdered leachate both in nauplii and mature stages so that the gut content was quite full of manure particles. The colored intestine of *Artemia* depending on their respective diet provided evidence of ingested food particles offered. The concentrations of ammonia, nitrate, and phosphate in vermicompost leachate were measured based on AOAC (2002).

Cyst hatching and *Artemia* culture procedures

The dried cysts of *A. franciscana* were obtained from the Institute of Artemia and Aquatic Animals Research (Urmia University, Iran). The cysts were hydrated in tap water for 1 h, and then incubated under standardized hatching conditions (28 °C, 33 g L^{-1}; pH 8–8.1) (Sorgeloos et al. 1986).

Experimental design

After hatching, *Artemia* Instar-I nauplii ($n = 1500$) were transferred directly to 25 cylindroconical glass tubes containing 1.0 L diluted Urmia Lake water containing 750 mL of 33 g L^{-1} salinity at a density of 2 nauplii mL^{-1} (five replications per treatment) (Abatzopoulos et al. 2003). During the first 2 days, the nauplii were fed with the halotolerant green algae *Dunaliella salina* (18×10^6 cells mL^{-1}; Coutteau et al. 1992). From the third day, the experimental *Artemia* were fed in five different treatments (each with five replications) based on the following percentages of dietary combinations:

$$T1\ (control) : 100\ D.salina\ (\mathrm{Alg}); T2 : 75\ \mathrm{Alg}{-}25\ \mathrm{VCL}; T3 : 50\ \mathrm{Alg}{-}50\ \mathrm{VCL}; T4 : 25\ \mathrm{Alg}{-}75\ \mathrm{VCL}; and\ T5 : 100\ \mathrm{VCL}.$$

In all treatments, the daily feeding rate containing *D. salina* was based on a concentration of 18×10^6 cells mL^{-1}. The different levels of VCL powder replacement rates were adjusted according to Coutteau et al. (1992) (Table 1). Water salinity during the pre-maturity period was increased gradually to 100 g L^{-1} at a rate of 10–15 g L^{-1} every alternate day in such a way that the animals were not stressed (i.e., a salinity of 100 g L^{-1} was reached after 4–5 days for all treatments). The density of the animals was reduced to one individual (meta-nauplius) per 4 mL in the culture media (8 L) after day 5 (Abatzopoulos et al. 2003). A gentle aeration was

Table 1 Daily feeding rate of *Artemia franciscana* with the microalgae *Dunaliella salina* (Alg) and vermicompost leachate powder (VCL) during the culture period (Coutteau et al. 1992; the numbers show the amounts of algae and VCL used for 100 *Artemia*)

Culture days	*D. salina* (mL)	VCL powder (mL)
3, 4	0.413	0.413
5, 6	0.625	0.625
7	0.826	0.826
8	1.060	1.060
9	1.7	1.7
10, 11	2	2
12, 13	2.5	2.5
14, 15	3	3
16, 17	3.5	3.5
18, 19	4.25	4.25
20 and more	5	5

applied from the bottom of culture containers. Approximately 50% of the culture medium was replaced by fresh medium every 3 days. A constant photoperiod of 12 h light/12 h dark was applied for all treatments. This procedure was continued until the animals reached sexual maturity in which morphological differentiation began in all treatments.

Some physicochemical parameters of the culture medium during the rearing period of *Artemia* prior to maturity were as follows (American Public Health Association 1995): water temperature 26 °C, dissolved oxygen 5.9–6.1 mg L^{-1}, pH 7.5–7.8, salinity 100 g L^{-1}, total alkalinity 174 mg L^{-1} $CaCO_3$, total hardness 268 mg L^{-1} $CaCO_3$, Ca^{2+} 36 mg L^{-1}, and So_4^{2-} 212 mg L^{-1}.

Growth, survival, and sex ratio of *Artemia* prior to maturity

The growth rate of *Artemia* was measured at different treatments through total length, furca and antennae lengths (mm) on days 8, 15, and 21 of the rearing period with random sampling of 10 animal using stereomicroscope (Model; Nikon SMZ, 1500, Tokyo, Japan). The survival rate in each replicate before maturity was calculated according to the following equation:

$$Survival\ rate\ (\%) = [final\ number\ of\ Artemia/initial\ number\ of\ stocked\ nauplii] \times 100$$

The sex ratios of *Artemia* in each replicate were determined on the basis of gender (the number of males and females) at different treatments.

Artemia reproductive characteristics during the reproductive period

The period required for the sexual maturity of *Artemia* during pre-adult stage (pre-reproductive period) was determined through daily separation of broodstock from the culture when 50% of total population reached the adult stage showing the onset of morphological differentiation. To study the females' reproductive performance upon the onset of sexual maturity, 35 broodstocks (male and female) were isolated from rearing cultures, in which each pair (a pair of male and female) was individually placed in cylindroconical falcon tubes (50 mL). Each falcon was taken as a replication and a total of 175 pairs ($n = 35 \times 5 = 175$) of *Artemia* broodstock were isolated from the five treatments (the dietary treatments were randomly assigned). The individual falcons were not aerated in this stage. The broodstock at individual cultures were daily fed with *D. salina* (18×10^6 cells mL^{-1}) and VCL powder according to Table 1. The amount of VCL powder for broodstock feeding of T5 (100 VCL) was calculated as 10 mL, the amounts of which in the other treatments were daily added to the individual cultures based on the percentage of replacement rates (Coutteau et al. 1992).

The daily offspring production (cysts + nauplii) at different treatments was monitored in each falcon tube. After females spawned in each individual falcon, pairs of male and female broodstock were instantly isolated from relevant falcons and transferred to new ones (with the same conditions) to determine their subsequent spawning events. This procedure continued in each falcon until the death of female broodstock, while following the death of a male broodstock, a new one was introduced to facilitate observation of the subsequent reproduction. In this stage, the water salinity was 100 g L^{-1} and other water quality factors such as temperature, pH, alkalinity, and hardness were maintained constant as the previous step.

To evaluate the reproductive performance of female broodstock at different feeding treatments, the following characteristics were investigated: total number of offspring produced (cysts + nauplii), the number of nauplii produced, the percentage of encysted embryos, the number of broods, the interval between two consecutive spawning, number of offspring per brood, number of offspring per female per day, pre-reproductive and reproductive periods, and the extent of period after the last reproduction to death (post-reproductive period). The total longevity was calculated as the sum of three periods including pre-reproductive, reproductive, and post-reproductive durations.

Body biochemical composition and total carotenoids

To determine the moisture, protein, lipid, total carbohydrate, and ash contents of whole body of adult *Artemia*, proper amounts from each treatment were randomly sampled and minced for analysis according to AOAC (2002). Moisture was determined by oven drying at 105 °C for 24 h. Crude protein (N × 6.25) was determined by the Kjeldahl method after an acid digestion using an auto-Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Crude lipid was estimated by the ether-extraction method using Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Ash content was measured using a muffle furnace at 550 °C for 24 h. Total carbohydrate was calculated according to 100 − [(lipid (%) + protein (%) + ash (%)].

To calculate the total carotenoids, the adult *Artemia* was harvested from each treatment and place into the tubes containing 5.1 mL of pure ethanol, which were foiled separately and kept in the dark (5 °C, 24 h). Total carotenoids (μg mg^{-1}) for each treatment were calculated using a spectrophotometer at an absorption maximum of 450 nm based on the following formula (Britton 1995):

$$\textit{Total carotenoid}\ \left(\mu g\ mg^{-1}\right) = 1 \times 10^4\ (OD_{450}/2.62\%) \times (V/W)$$

where OD is the optical density (at 450 nm of 1.0 cm cuvette path), *V* is the volume of fluid in the cuvette (1 mL), *W* is the weight (mg), and 2.62% is the absorption coefficient of 1.0% beta-carotene at 450 nm.

Bacterial load of the culture media

The bacterial load at the different feeding treatments was examined on day 21 by sampling 1.0 mL of the culture water diluted in six sequential intervals. The leachate contained bacteria ranging from 0.2 to 3 μm in size. Then, the resulting cultures of the last dilution were pour-plated in plate count agar and incubated at 37 °C for 48 h. Finally, the numbers of aerobic bacterial colonies were counted and calculated using the following equation expressed as $\log_{10}$ CFU mL^{-1}.

$$\mathrm{CFU} = \textit{colony count} \times \textit{inverted dilution factor}$$

Statistical analyses

Prior to analysis, Kolmogorov-Smirnov and Bartlett's tests were applied to verify the normality and homogeneity of variances (Sokal and Rohlf 1981). Then, the results of growth, body composition, and reproductive performance were analyzed using a standard one-way analysis of variance (ANOVA) by SPSS software (Version 22). Tukey test was applied in order to determine the significant differences between the means at a significance level of $P < 0.05$.

Results

The biochemical composition of raw vermicompost and nutrient concentrations in the leachate

The raw vermicompost used in this study contained 10.68, 13.73, 74, and 23%, respectively, of protein, lipid, ash, and moisture contents. Moreover, the concentrations of ammonia, nitrate, and phosphate in the VCL were 0.07, 388, and 0.7 mg L^{-1}, respectively.

Bacterial load in the culture media

On day 21, the bacterial load (73.50×10^6 CFU mL^{-1}) in the *Artemia* culture of 100 VCL was significantly different ($P < 0.05$) from those ($56–60 \times 10^6$ CFU mL^{-1}) in the other treatments while there were no significant differences ($P > 0.05$) between the other groups (Fig. 1).

Total body length, the furca and antennae lengths

Total first-week body length of the reared *Artemia* showed significant differences ($P < 0.05$) among different treatments. At the end of the second week, however, changes in the body length in 100 Alg, 25 Alg-75 VCL, and 50 Alg-50 VCL treatments showed no significant

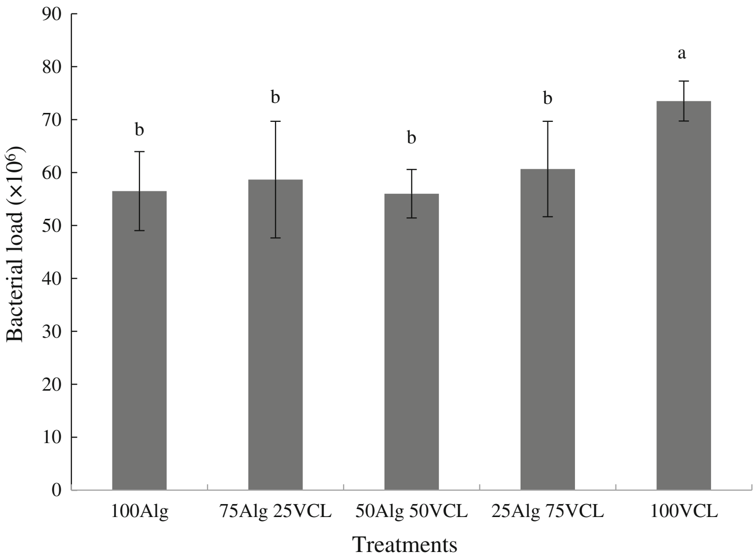


Fig. 1 Total bacterial load ($n \times 10^6$ CFU mL^{-1}; mean ± SD) in the culture medium of *Artemia franciscana* at different treatments on day 21

differences ($P > 0.05$), and all these three treatments were statistically dissimilar ($P < 0.05$) with the other feeding groups. The 100 Alg reached a body size of 5 to 5.5 mm in the 3rd week showing significant differences ($P < 0.05$) with 25 Alg-75 VCL and 50 Alg-50 VCL treatments (4.4 and 4.7 mm, respectively) (Fig. 2). Furca length revealed marked differences ($P < 0.05$) among the different feeding levels during the first week of growing with the least value recorded in 100 VCL treatment. At the end of the second week, the furca length of the *Artemia* fed 100 Alg, 75 Alg-25 VCL, and 50 Alg-50 VCL showed no significant differences ($P > 0.05$); however, all the above groups (100 Alg, 75 Alg-25 VCL, and 50 Alg-50 VCL) differed considerably ($P < 0.05$) with the other treatments. Similar trends were observed in the furca lengths in the third week. The length of antennae displayed a relatively similar trend in all treatments during the different weeks of culture (Fig. 3).

Body biochemical composition and total carotenoid content of *Artemia*

The protein contents (56.9 and 54.6%, respectively) were almost similar ($P > 0.05$) for 100 Alg and 75 Alg-25 VCL treatments and both showed significant differences ($P < 0.05$) with the other groups (41.9–50.15%). The total amount of lipid showed significant differences ($P < 0.05$) among all treatments so that the highest and lowest contents, respectively, were recorded in 100 VCL (19.8%) and 75 Alg-25 VCL (13.7%) treatments. No differences ($P > 0.05$) were found in the amounts of total carbohydrates, fiber, and ash among treatments. The total amount of body carotenoids was almost the same ($P > 0.05$) in the diets of 100 Alg and 75 Alg-25 VCL, but both were considerably different ($P < 0.05$) from the other levels (Table 2).

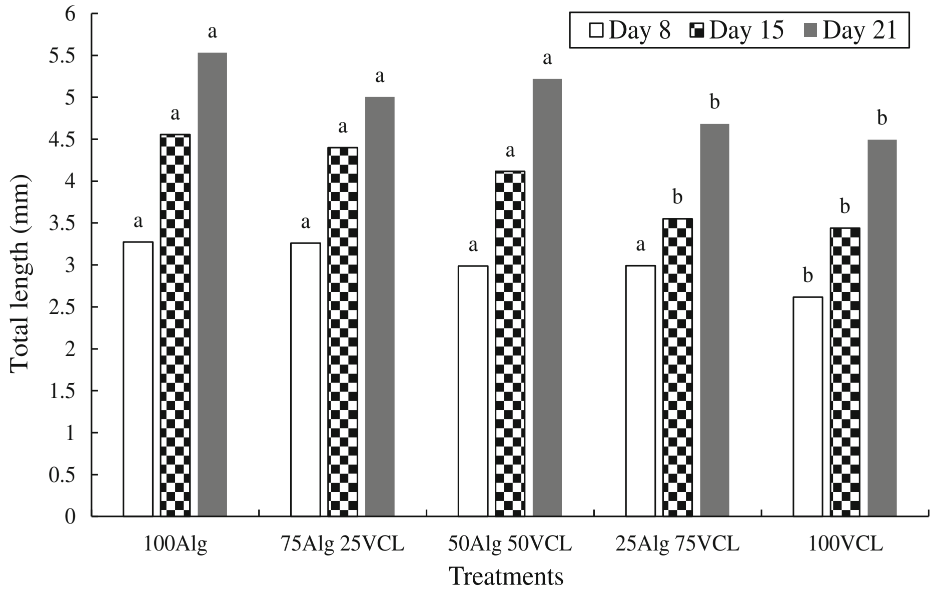


Fig. 2 Total length (mm) of *Artemia franciscana* nauplii cultured at different treatments until pre-reproduction period after 8, 15, and 21 days post-culture

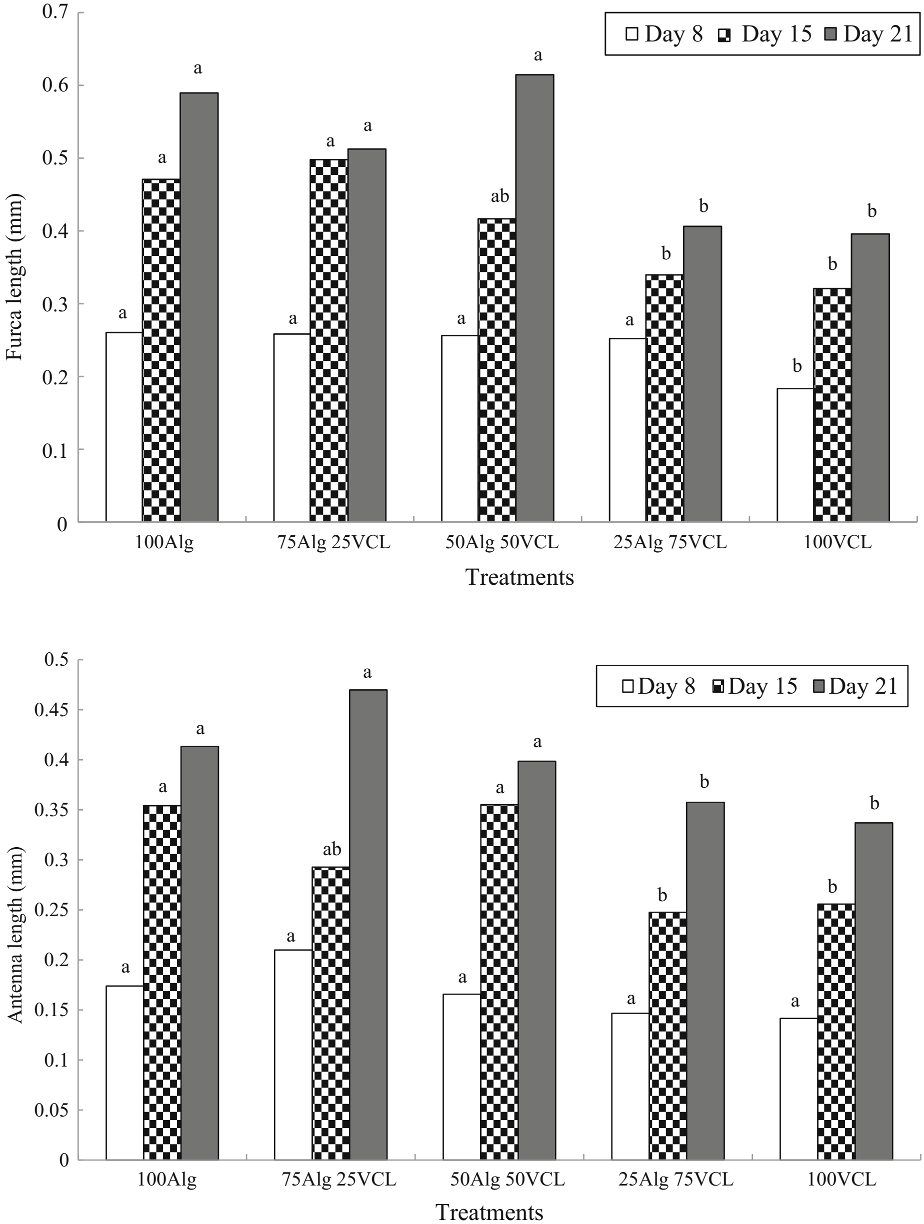


Fig. 3 Furca and antenna lengths (mm) of *Artemia franciscana* cultured at different treatments until pre-reproduction period after 8, 15, and 21 days post-culture

Fecundity, reproductive model, and total longevity of females

The process of reproduction at different treatments showed that a considerable number of reproductive parameters such as the number of offspring, the rate of cyst formation, number of broods, number of offspring per brood, the production of offspring per female per day,

Table 2 Final proximate composition of whole body (expressed in percent dry weight) and total carotenoids (μg mg^{-1}) of *Artemia franciscana* fed diets containing different levels of the alga *Dunaliella salina* and vermicompost leachate (VCL) powder

Components	Treatments				
	100 Alg	75 Alg-25 VCL	50 Alg-50 VCL	25 Alg-75 VCL	100 VCL
Crude protein	56.9 ± 1.4 a	54.6 ± 1.2 a	50.15 ± 1.5 b	46.9 ± 1.3 c	41.9 ± 1.1 d
Crude lipid	14.5 ± 0.1 d	13.7 ± 0.3 e	17.4 ± 0.2 c	19 ± 0.04 b	19.8 ± 0.15 a
Ash	17.3 ± 1.4	16.8 ± 2.6	16.5 ± 1.8	16.7 ± 2.1	17.6 ± 1.9
Fiber	0.5 ± 0.1	0.47 ± 0.1	0.46 ± 0.1	0.48 ± 0.04	0.4 ± 0.1
Total carbohydrates	10.8 ± 2.6	14.63 ± 1.5	15.5 ± 3.1	16.9 ± 2	20.3 ± 0.8
Total carotenoid	45.9 ± 0.13 a	47.73 ± 0.02 a	40.65 ± 0.13 b	40 ± 0.04 b	36.47 ± 0.1 c

Values within the same row not sharing common letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD ($n = 3$)

and duration of females' reproduction were affected by the dietary regimens containing *D. salina* and VCL levels leading to significant differences ($P < 0.05$) between the treatments. The duration of pre-reproduction period varied between 21.4–24.5 days (3–3.5 weeks) in all groups; however, feeding *Artemia* with diets of 100 Alg and 75 Alg-25 VCL rendered no significant differences ($P > 0.05$), but both led to marked dissimilarities ($P < 0.05$) with the other groups. Except the group fed with 100 VCL, a similar trend ($P > 0.05$) was also observed in the females' reproductive duration. This period lasted 51 days for 100 Alg, 46.8 days for 75 Alg-25 VCL, and the shortest period (only 26 days) was noticed in the group fed with 100 VCL. Apart from the group fed with 100 VCL, the period between the last reproductions until the death of females showed no significant differences ($P > 0.05$) between the treatments, though females in all treatments died within 1.3–4.8 days after last spawning (Table 3).

The highest total number ($n = 1051$) of offspring was produced by the females fed with 100 Alg showing significant differences ($P < 0.05$) with the other groups, while the lowest value ($n = 65$) was obtained in 100 VCL. Except the group fed with 100 VCL, a 25% drop in the algal diet and its replacement with dietary VCL resulted in a decreased offspring production of approximately 25% in *Artemia* broodstock. Premature survival rate of nauplii showed significant differences ($P < 0.05$) between groups during the nearly 3-week feeding. Almost a survival rate of 6.7% in VCL-fed *Artemia* was obtained within 3 weeks, and the highest survival rate (52.5%) was recorded in 100 Alg with significant differences ($P < 0.05$) with the other groups (Table 3).

The percentage of encysted embryos (86%) was much higher ($P < 0.05$) in broodstock fed 100 VCL diet, but much lower total production of offspring ($n = 65$) was observed in females fed this diet compared to those (496–1051 offspring) fed with other diets. Similarly, other reproductive factors such as the number of broods, interval between two successive brood production, and number of offspring per brood displayed significant differences ($P < 0.05$) together with a decreasing trend in the groups whose broodstock diets contained elevated amounts of vermicompost (especially in diets containing 75 and 100% of VCL). However, the broodstock received diets containing 100 Alg and 75 Alg-25 VCL were not markedly different ($P > 0.05$) in such main indices as percentage of encysting, interval between two successive brood

Table 3 Mean values (± SD) of reproductive performances, pre-maturity survival rate, and total lifespan in *Artemia franciscana* under feeding regimes containing different levels of the alga *Dunaliella salina* and vermicompost leachate (VCL) powder

Characteristics	Treatments				
	100 Alg	75 Alg-25 VCL	50 Alg-50 VCL	25 Alg-75 VCL	100 VCL
Total number of offspring	1051.4 ± 356.7 a	795.1 ± 418.3 b	566.2 ± 256.1 c	496.4 ± 190.8 c	65.2 ± 37.7 d
Min-max mean offspring production	14–184.8	23–205	46–163	48–159	8–59.5
Number of nauplii	842.5 ± 336.7 a	617.4 ± 393.6 b	372.3 ± 207.6 c	297.6 ± 161.6 c	10.2 ± 1.2 d
Percentage of encysted embryos	21 ± 10 d	25 ± 13 d	36 ± 11.1 c	43 ± 17 b	86 ± 12.5 a
Number of broods	8.5 ± 2.6 a	6.78 ± 2.8 b	5.5 ± 2.1 b	5.68 ± 1.65 b	1.87 ± 0.9 c
Days between broods	6 ± 2.3 b	7 ± 2.1 b	8.03 ± 2 b	7 ± 2.7 b	16.1 ± 8.2 a
Offspring per brood	123.69 ± 25.9 a	117.27 ± 26.4 ab	102.94 ± 22.6 bc	87.4 ± 23.1 c	34.89 ± 7.4 d
Offspring per female per day	19.42 ± 3.8 a	15.95 ± 4.2 b	11.31 ± 2.5 c	10.31 ± 3.6 c	1.5 ± 0.04 d
Pre-reproductive period (days)	21.46 ± 4.1 b	21.44 ± 3.8 b	23.35 ± 3.3 a	24.44 ± 3.9 a	24.28 ± 3.9 a
Reproductive period (days)	50.96 ± 8.1 a	46.78 ± 7.8 a	44.55 ± 11.5 a	44.32 ± 7.4 a	26.60 ± 9.6 b
Post-reproductive period (days)	3.96 ± 3.1 ab	4.63 ± 2.6 a	4.86 ± 4.18 a	4.12 ± 3.7 ab	1.25 ± 0.6 b
Pre-mature survival (%)	52.5 ± 3.55 a	45.9 ± 3.3 b	36 ± 3.9 c	29.5 ± 2.3 d	6.7 ± 3.1 e
Female: Male ratio	3.5 (± 0.8): 1 bc	3.3 (± 0.7): 1 b	4.1 (± 0.9): 1 a	2.8 (± 0.2): 1 c	1.9 (± 0.6): 1 d
Survival rate (%) of females in individual falcons for 45 days	67.2 ± 5.6 a	65.3 ± 5.3 a	46.4 ± 4.9 b	36.9 ± 2.3 c	8.85 ± 6.8 d
Total life span (days)	74.43 ± 8.3 a	70.8 ± 11.3 a	69.1 ± 10.5 a	68.7 ± 7.6 a	50.86 ± 8.7 b

Different letters within the same rows denote significant differences ($P < 0.05$)

production, number of offspring per brood, pre-reproduction duration, and the duration of reproduction period. Total longevity in different treatments varied in the range of 50–74 days and, except the broodstock fed with 100 VCL (50 days), no significant differences ($P > 0.05$) were observed among the broodstock fed with other diets (Table 3).

The average daily offspring production per female per day by *A. franciscana* was highest in the first and fourth weeks at all treatments (especially between the 2nd to 4th weeks) and showed a downward trend from the fifth week onwards (Fig. 4).

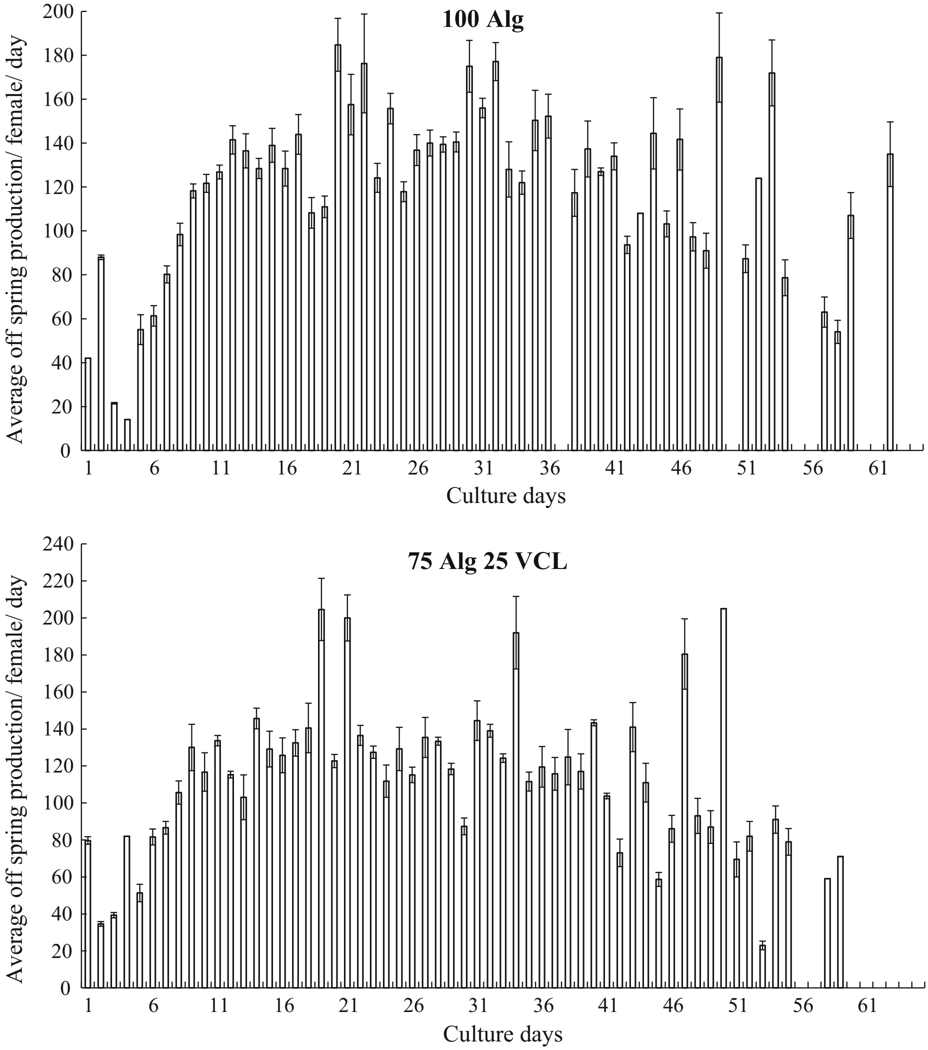
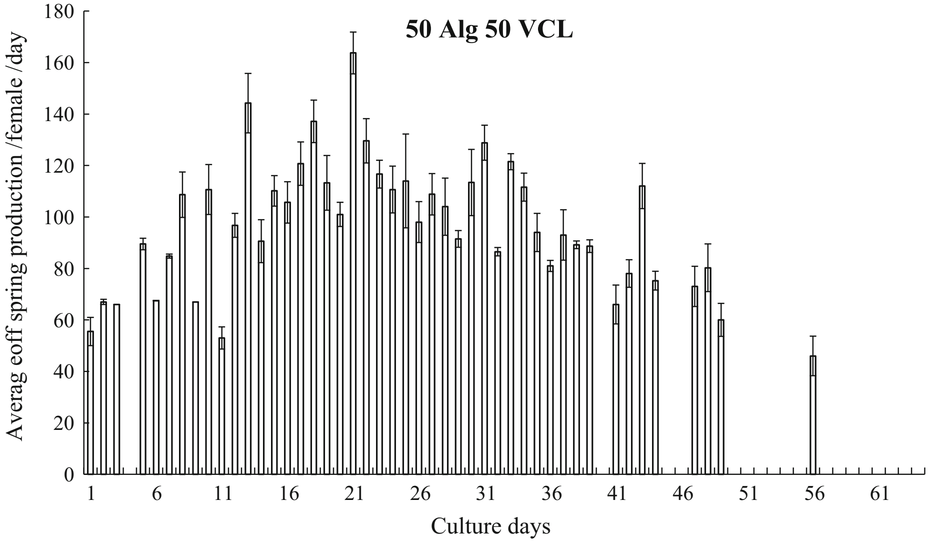


Fig. 4 Average (mean ± SE) daily offspring production per female over the experimental period in *Artemia franciscana* at feeding regimes containing different levels of the alga *Dunaliella salina* and vermicompost leachate (VCL) powder [SE = SD/√*n*; *n* = 35 females]



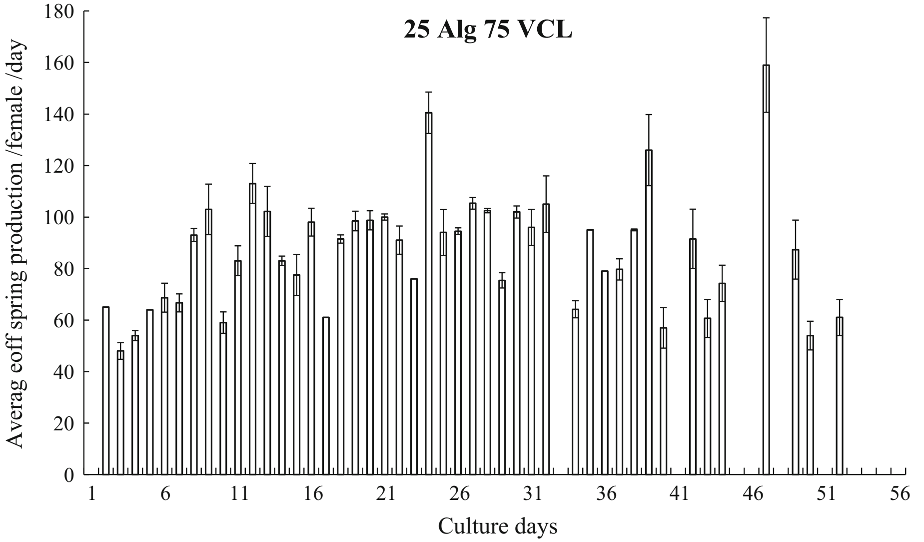


Fig. 4 (continued)

Discussion

The chemical characterization of VCL

The chemical composition of VCL in this study displayed relatively low amount of ammonia (less than 0.04%) as opposed to that used by Ranalli et al. (2001). The high nitrate levels in vermicompost show that the fertilizer had properly been undergone both ammonification and nitrification processes. The pH of VCL was close to the neutral value (around 6.7), which did not correspond to the results reported by Atiyeh et al. (2002) and Tejada et al. (2008), who obtained a pH value of 6 for VCL from cow manure. However, the pH value is lower than in

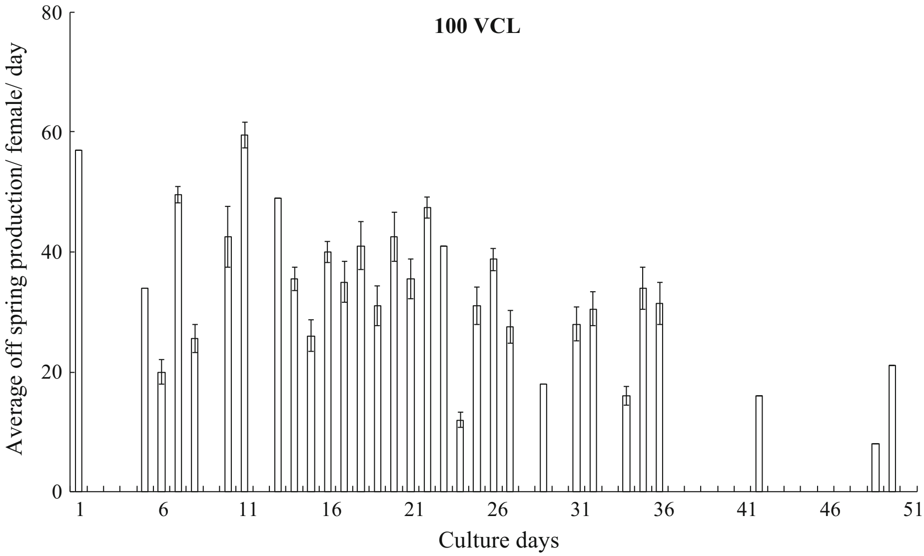


Fig. 4 (continued)

that (7.27) reported by Avila-Juarez et al. (2015). Therefore, the slightly decreased pH values of vermicompost compared to the traditional compost might be attributed to the mineralization of N and P, microbial decomposition of organic materials into intermediate organic, fulvic, and humic acids (Lazcano et al. 2008) and also concomitant production of CO_2 (Garg et al. 2006).

Growth, survival, biochemical composition, and total carotenoids of *Artemia*

The results showed that the type of diet clearly affects total body length of *A. franciscana* in the pre-puberty period; however, significant differences were not observed in *Artemia* fed 100 Alg and those (between 5 and 5.5 mm) that received up to 50 Alg-50 VCL. Additionally, differences in such characteristics as total body, furca, and antennae lengths were observed between treatments from the 1st week of rearing, a trend that continued until the 3rd week. On the other hand, *Artemia* body size decreased as the amount of VCL in the diet increased. Although of the highest estimates of growth and survival were obtained in the control, the same finding in the other treatments were comparable in some cases to that in the control group. The total body length of *A. franciscana* on day 21 was far lower than those recorded in the same species reported by Lavens and Sorgeloos (1996), and Bahmanpour et al. (2009). However, Bahmanpour et al. (2009) observed no significant differences in body morphology (total, furca, and antennae lengths) in two strains of *A. franciscana* (one from the Great Salt Lake in the USA and the other from Lake Maharloo in Iran) when fed a formulated yeast diet with *D. salina*. Although the highest growth rate (5.5 mm) was recorded for our *A. franciscana* in the control and 4.5–5 mm in the group fed a mix of Alg/VCL powder after 21 days, growth values of *A. urmiana* recorded in all treatments were reported to be lower than 7.82 and 7.76 mm (in groups fed the same alga and those received a mix of wheat bran/soybean, respectively) (Ownagh et al. 2015). Moreover, our observed growth rates (as above) were higher than those (4.93, 5.02, and 4.64 mm) of *A. franciscana* cultured using a commercially inert diet and *Chaetoceros* sp. for 11 days (Naegel 1999).

Several environmental (e.g., temperature and salinity) and nutritional factors (food quantity and quality) can affect the growth and survival rates of *Artemia* (Lavens and Sorgeloos 1996; Browne and Wanigasekera 2000; Baxevanis et al. 2004). In the present study, vermicompost manure contained high levels of ash (74%), which was not possibly much accessible to *Artemia* in the culture media. On the other hand, vermicompost manure contained a low protein level (10.68% of dry matter), which can be considered as one of the main reasons for the decreased growth rate of *Artemia*. Such a process was also observed in the *Artemia* body protein especially at high levels of VCL powder used. Such a trend was already reported in *Artemia* fed with different diets containing high indigestible fiber, which accumulates in the bottom and ultimately leads to limited access capabilities for *Artemia* (Lavens and Sorgeloos 1996).

In the present study, *Artemia* survival rate was strongly influenced by the diet type, in which a diet of 100 VCL caused an obvious reduction (less than 10%) up to pre-mature stage showing the nutritional inefficiency of VCL powder. Dwivedi et al. (1980) and Basil et al. (1989) commented that separate uses of organic manures or agricultural waste in the diet of *Artemia* reduced nauplii survival rates to less than 30–50% up to the maturity. This survival rate (30–45%) of *Artemia* was also observed in some mixed diets of the present study, including three mixed diets (with different percentages of algae and vermicompost), which is partly in line with the findings of Dwivedi et al. (1980) and Basil et al. (1989) concerning relatively low survival rate of *Artemia*. However, values of survival rates detected here are relatively lower than those reported by other studies possibly due to different culture environments, initial stocking density, and feeding conditions. For example, the survival rates after 21 days of culture obtained in the current study were not comparable to the data reported by Naegel (1999), who reported survival values of 72, 79, and 73.5% for *A. franciscana* reared using a commercial inert diet of Nestum (a baby food), enriched Nestum, and the microalgae *Chaetoceros* sp., respectively, for 11 days. Also, Ownagh et al. (2015) documented high survival rates of 70.3% (for *A. urmiana*) and 68.5% (for the parthenogenetic *Artemia*) using wheat bran as feed. Additionally, survival rates of 52–54% were noticed for biomass production of *Artemia* feeding on swine manure and food supplements (soybean powder and rice bran) in salt ponds by Anh et al. (2009) as well as a similar survival of 50% using poultry manure in ponds at San Crisanto, Yucatán, Mexico, by Maldonado-Montiel et al. (2003).

In this study, the high ash content (74%), relatively small amounts of protein (10.68%), and rather high fat content (13.73%) in vermicompost revealed obvious effects on the body composition of adult *Artemia*. The amount of protein was 57 and 54.6% (dry weight) as a result of feeding with 100 Alg and 75 Alg-25 VCL, respectively, but the amounts dropped significantly when VCL level gradually increased up to 100%. The lipid content in the adult *Artemia* fed diets containing different levels of VCL lied between 13.7–19.8% showing a significant increasing trend with elevations in the dietary VCL levels, which reflects the influence of dietary fat content. These data are slightly in accordance with the previous results reporting the effects of diet types on the biochemical compositions of *Artemia*. For example, Ronsivalli and Simpson (1987) by rearing *Artemia* for 15 days with rice bran and whey powder reported protein contents of 50 and 10%, lipid values of 5.9 and 3.1%, ash records of 9.9 and 6.7%, and carbohydrates amounts of 24 and 76%, respectively. Additionally, Naegel (1999) detected protein levels of 56.5, 43, and 41%, and lipid contents of 2.95, 16.5, and 20.3% in *A. franciscana* grown with the alga *Chaetoceros*, Nestum (powdered baby food), and enriched Nestum, respectively.

Total carotenoids in the body of *Artemia* showed a significant decreasing trend by increasing the amount of VCL to higher levels of 25%. It seems that reducing the amount of algal food (*D. salina*) at levels beyond 25% could potentially be a limiting factor in the amounts of body carotenoids. This is explained by the fact that *D. salina* is an important rich source of beta-carotene (12–14% of the dry weight) (Ben-Amotz et al. 2009). It is noteworthy here that *Artemia*, like other crustaceans, receives carotenoid resources through dietary intake, most of which is mobilized to the gonads and egg production.

Reproductive performance and longevity

The first-reproduction age of *Artemia* is considered as a determining factor in *Artemia* population dynamics under culture conditions. When the level of VCL powder reached an amount of 25% and higher, *A. franciscana* significantly required a longer time (by 20%) to reach maturity. The diets containing organic manures (with different percentages of incorporation) including cabbage leaves, cow, swine, and chicken manures used for three strains of *Artemia* (Tuticorin, USA, Belgian) were reported to show positive effects on the reproduction (shorter time required to maturity) and also on fecundity of *Artemia* (Basil et al. 1995). Also, a complete replacement of the algal diet with VCL powder resulted in a 50% reduction of females' reproductive period herein (from 50 to 26 days); however, no significant differences were observed between other treatments. Replacements of 25 and 50% of the alga with VCL powder reduced 24 and 54%, respectively, of the total number of offspring compared to those received 100% algal diet. Yet, some indices namely the interval between two consecutive spawning, number of offspring per brood, duration of reproductive period, and total life span of females did not differ significantly between the two groups of 75 Alg-25 VCL and control; nevertheless, all these indices diminished markedly at VCL powder at levels of above 25%. The effects of diets on reproductive performances of three strains of *Artemia* (Tuticorin, USA, Belgian) showed that the best performance among all the three strains was recorded in mixed diets (cabbage leaf + cow, swine, and chicken manures each with 25%) followed by 20% of each of cabbage leaves, and cow swine manures +40% of chicken manure (Basil et al. 1995). Accordingly, it appears that VCL powder is usable up to 25% only for *A. fransicana* reared in the present experiment and it cannot be fed to *A. fransiscana* as a suitable food source for long-term periods.

In the present investigation, the average daily offspring production by *A. franciscana* was highest in the first and fourth weeks at all treatments (especially between the 2nd to 4th weeks) and showed a downward trend from the fifth week onwards. Such a trend was also observed by Anh et al. (2009) wherein all groups displayed ascending patterns in fecundity from the 2nd to 4th weeks, which turned into descending modes from the 5th to 11th weeks. The highest fecundities of *Artemia* in their study were recorded, respectively, for soybean meal and rice bran treatments.

Based on the treatments examined in this study, the lipid content of *Artemia* fed these diets did not change significantly between treatments (in the range of 12–13.73%). However, protein content of *Artemia* fed the diets showed significant decreases with rising vermicompost levels (in order of the lowest to highest in the diet) from 55% in control, 45.25%, 38.5%, 21.75%, and to 10.1% in 100 VCL treatments. Reduced protein content by increasing the level of vermicompost can probably be one of the main reasons for reductions in several indices, especially reproductive performances, in *Artemia* breeders.

The C/N ratio in here for the treatment of 100 Alg was about 7.69, which is much lower than the normal range (C/N ratio of > 10; Avnimelech 1999) for the development of heterotrophic bacterial communities as well as a complementary food source for *Artemia*. The available N in the vermicompost manure of this study was between 1.7–1.8%, which is slightly lesser (1.5–2.6%) than those previously reported on this manure (Atiyeh et al. 2002). Accordingly, nitrogen sources constraints in the VCL powder can cause the lack of formation or progression of a biofloc based on the growth of heterotrophic bacterial communities. VCL, like other organic manures, is a rich source of bacteria, but the bacterial strain (*Firmicutes* sp. grown in VCL herein) might have not had adequate nutritional capacity as an alternative food source for *A. franciscana* during the whole life cycle, which remains to be verified more accurately in the future. The highest bacterial growth was detected in 100 VCL with significant difference with the other treatments. Intriago and Jones (1993) and Toi et al. (2013) commented that the bacteria (e.g., *Flexibacter* strain *Inp3*) was proven to be a proper food source for the growth and survival of *Artemia* and, in addition, could greatly help in better digestion of dietary algae in the gut of *Artemia*. Nevertheless, Seixas et al. (2009) reported that bacteria are not considered as high-energy food sources for *Artemia* compared to microalgae.

Although the vermicompost manure used herein had been dissolved in water for 24 h, it might, therefore, be possible that there were humic and fulvic acid compounds and/or other naturally occurring organic compounds in the VCL powder residues, which in turn can significantly reduce or inhibit bioavailability of metal uptake by aquatic organisms (Hollis et al. 1996; Tao et al. 2000). The high ability of vermicompost in binding to and uptake of metals is largely assigned to the presence of humus and negatively charged functional groups (e.g., aromatic rings, carbonyl groups, phenolic compounds, and hydroxy alcoholics) (Masini et al. 1998). On the other hand, VCL naturally contains immobilized compounds such as those disabling the enzymes e.g., lipase, protease, amylase, cellulase, and chitinase. Negative effects of high concentrations of long-term VCL powder usage has been demonstrated in many previous studies on plants showing such signs as reduced growth and biomass production, decreased germination, and increased death (Atiyeh et al. 2002; Lazcano and Domínguez 2010; Edwards et al. 2011). These materials are probably the major factors in reduced rate of growth and fecundity in *Artemia* fed diets containing VCL powder during the long period of this study, which remains to be verified more accurately in the future.

Conclusion

The results of current research revealed that the levels of VCL powder examined cannot be accounted for a suitable food source for rearing of *A. franciscana* in long-term periods (the whole life cycle). However, it is possible to use VCL powder as much as 25% in the diet of *Artemia* as the greater alternative levels rendered sharp declines in growth and reproductive performances of *Artemia*. According to the findings of this study, a diet based on 100 Alg followed by a diet of 75 Alg-25 VCL are recommended in small-scale laboratory cultures. Based on the findings of this study, further researches are suggested on the effects of vermicompost powder (mixed with other sources of food such as algae, other organic manures and agricultural by-products) in *Artemia* rearing earthen ponds (in long- and/or short-term applications). Such studies can also provide a more comprehensive judgment on the efficiency of vermicompost manure application in semi-natural environments of *Artemia* culture ponds, controlled systems, and even biofloc aquaculture systems.

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