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The Effect of Vitamin C-Fortified Artemia on Growth and Survival of *Sepia pharaonis* Larvae

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Abstract

Vitamin C is a water-soluble compound that can involve in the repair of tissue and the enzymatic production of certain neurotransmitters. The aims of the present study were the evaluation of the effects of vitamin C on the growth performance and survival rate of Pharaoh Cuttlefish **(***Sepia pharaonis*) for 25 days. *Sepia pharaonis* were randomly allocated in 20 aquaria (21 fish per tank) and triplicate groups of *Sepia pharaonis* (initial weight 4.5±0.11g) were fed with supplemented Biomar feed. The larvae in the control treatment were fed without Biomar. The larvae in the experimental treatment were fed with Biomar in addition to vitamin C. The results clearly showed that in fish fed vitamin C, body weight had significantly increased in comparison to control treatment (P>0.05). Also, vitamin C had a positive effect on fish survival rate there was no significant difference between treatments. The best results on survival rate were obtained by feeding vitamin C which had a great effect on the survival of this species in captivity.

Keywords: *Sepia pharaonis*, Vitamin C, Survival, Bodyweight

1 Introduction

Mollusks have a significant share in global markets and their global catch and reproduction are of particular importance; For example, *Sepia pharaonis* is important as a commercial species for local fisheries in the southeast of Asia, north of the Indian ocean, Yemen sea, and Suez canal [1]. according to reports, the annual catch of *S. pharaonis* in the Chinese Sea is about 150,000 tonnes. Also, S. pharaonis is 16% of cephalopods caught by offshore trawl and 10% of cephalopods caught by offshore fixed net in Gulf of Thailand and Andaman Seas [2]. These organisms play a very important role in the food chain of the seas, and their larvae are a great food source for many invertebrates and vertebrates [3, 4]. Mollusks are also considered as an indicator of total production and an indicator of water quality [5].

Sepia pharaonis (figure 1) is a species of mollusk that is commercially caught and distributed in large parts of the world from the Red Sea to the coasts of Japan and Australia [6. 7]. This species has an elliptical and spongy skeleton in its back part, which forms its internal skeleton. It is one of the most important commercial species in the markets of these areas on the northern shores of the Indian Ocean and along the coasts of the Arabian Sea, the Sea of Oman, and the Persian Gulf [8]. *Sepia pharaonis* is a short-lived species sensitive to overfishing and environmental fluctuations but can regenerate and replenish its reserves in a short time [6]. Due to the simultaneous migration of this species in spawning seasons to coastal areas and shrimp hunting, many breeders of this species are implicitly caught during shrimp hunting. Therefore, it is necessary to take action to reproduce this species and rebuild its stocks. Today, the use of live food is widely practiced inbreeding shrimp larvae and farmed fish, especially saltwater fish [7].

Figure 1: *Sepia pharaonis*

The use of live food in larval nutrition of many marine aquatic animals to improve the nutritional status, growth rate and reduce larval mortality is one of the most significant advances in aquaculture [9]. Artemia is one of the most important and relatively widespread species of crustaceans, widely used in research and aquaculture. The reasons are high nutritional value and digestibility, size variation, easy production method, and tolerance to various salinities. The size of Artemia is very appropriate considering the size of the mouth of aquatic animals. This organism supports the larvae of many fish and shrimp as a starting food [10]. Many nutrients, vitamins, minerals, and medicines can be provided to aquaculture by enrichment or Artemia. The most important

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characteristic of Artemia is its non-selective filter-feeding which facilitates the easy entrance of desired materials into the Artemia culture environment and further consumption by feeding the aquatic culture by this enriched Artemia [11]. The most important factor for using Artemia as a live food is its nutritional value, which contains 66% protein and 14% fat and almost all the essential amino acids and most fatty acids in the desired level. Environmental stresses can lead to tissue damage, reduced growth and reproduction, health and ultimately reduced survival chances [12-20]. Various methods have been proposed to deal with these environmental stressors like the use of various disinfectants to improve environmental conditions in cases of biological contaminants such as fungi [21-23].

Another way is the addition of supplements to aqueous diets such as mineral supplements, digestive supplements (multi enzymes), and vitamin supplements such as vitamins C and E to strengthen the immune system [24-25]. Artemia can be used as a carrier of some nutrients such as long-chain unsaturated fatty acids, vitamins, especially vitamin C [26]. Food quality is an important factor in the growth performance, survival, and maintenance of fish health and its strength. Vitamins are organic compounds used for further growth and survival and increase the level of health in fish diets in conditions of intensive breeding. Ascorbic acid (a form of vitamin C) is an essential food additive, both in formulated and in live food [27]. Vitamin C is the most unstable water-soluble vitamin and is one of the most important nutrients in fish farming. This vitamin is effective in the formation of steroid hormones and increases the body's resistance to stress and infections. Vitamin C is added directly to formulate foods, although, in the case of live foods, enrichment methods should be used to deliver this vitamin to the animal [26]. Vitamin C also plays an important role in increasing and maintaining immune responses and adaptation, biological activities, such as preventing body deformation, growth and survival, and physiological factors such as resistance to stress, intoxication, and immune activity in larvae of various species. Aquaculture is improved by taking vitamin C supplements [28]. Considering the effect of vitamin C on other aquatic animals and the low survival of larvae of this species in breeding tanks, the present study evaluates the effect of Artemia enrichment with vitamin C on *Sepia pharaonis*.

2 Materials and Methods

In this study, 420 *Sepia pharaonis* with an average weight of 4.5 g were studied for 25 days in Chabahar city, Iran. Each fiberglass tank was equipped with an aeration system and the physicochemical conditions of the water were kept similar in all tanks. Following the methodology (Static-renewal test condition) to prevent the effect of metabolites and organic matter of *Sepia pharaonis* 50% of water in all tanks was renewed on daily basis. . During the experimental period, physicochemical factors of water were monitored and controlled constantly in all treatments. Artemia cysts were incubated under standard incubation conditions (water temperature 28°C, salinity 35 g/l, the light intensity of 2000 lux, and pH of 8 g/l) in 200liter conical tanks with intense aeration and after 24 hours the nauplii were collected using their positive phototropism Immediately after washing, they were transferred to 2-liter conical enrichment containers (300 Nauplii per ml of water), At this stage, A. franciscana nauplii were washed with saltwater and stocked into the enrichment tanks (2 l) at 150 nauplii per milliliter. Cod liver oil (EPA 6.84% and DHA 5.98%), ascorbyl 6-palmitate (Serva, USA), and α-tocopherol acetate (Sigma, USA) were used as lipid, vitamins C sources. The enrichment emulsion was prepared according to the method described by Kolkovski et al. [29]. Vitamins were added as a percentage of fish oil to the emulsion. The enrichment solution was given (2 ml per liter) in two portions at 12-h intervals. After 24 h incubation during the enrichment, the Artemia were washed with salt water (28 ppt) to discard non-absorbed lipids and were then kept aerated at 4°C until they were served to fish [30]. Immobile fish with no gill cap movement were considered dead and removed from the water. Mortality was recorded and registered daily for 25 days and determined by the end of the experiment. The normality of the data was assessed using the Kolmogorov-Smirnov test. An independent t-test was used to compare the two experimental and the control treatments. Mini Tab 18 and Excel 2019 were used to draw the charts.

3 Results and Discussion

Figure 2 shows that the use of vitamins can increase the survival of larvae. Also, the survival rate of control treatment and experimental treatment in this study was significantly different (P≤0.05). Comparing both treatments after 25 days showed that consumption of vitamin C-fortified artemia did not have much effect on increasing the growth of these larvae (Figure 3). The body size of the two groups did not show a significant difference (P≥0.05).

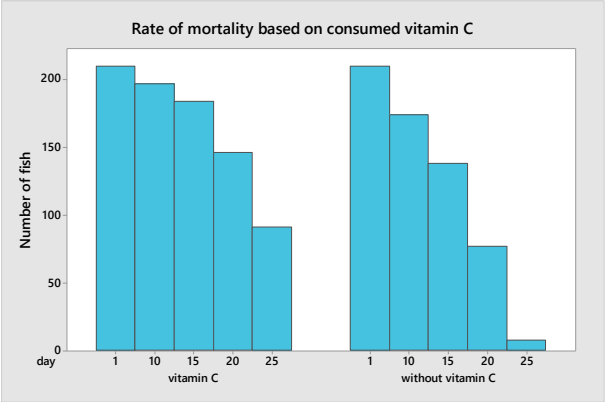

Figure 2: The effect of vitamin C-fortified artemia on survival of *S. pharaonis* larvae

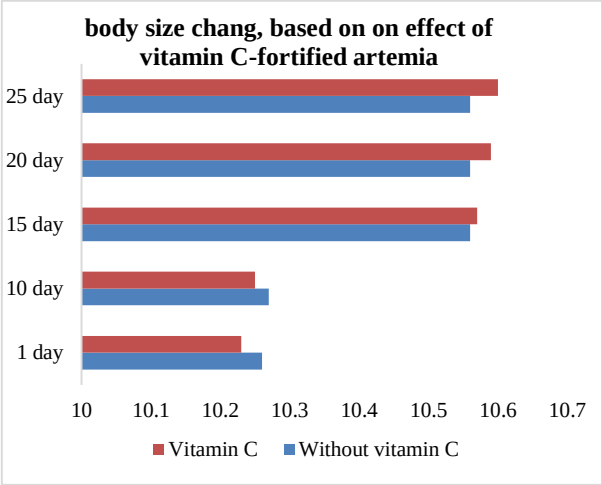

Figure 3: The effect of vitamin C-fortified artemia on the growth of *S. pharaonis* larvae

The results also showed (Figure 4) that the larvae that consumption of vitamin C-fortified artemia showed more weight gain than the control treatment and showed a significant difference between the two groups (P≤0.05).

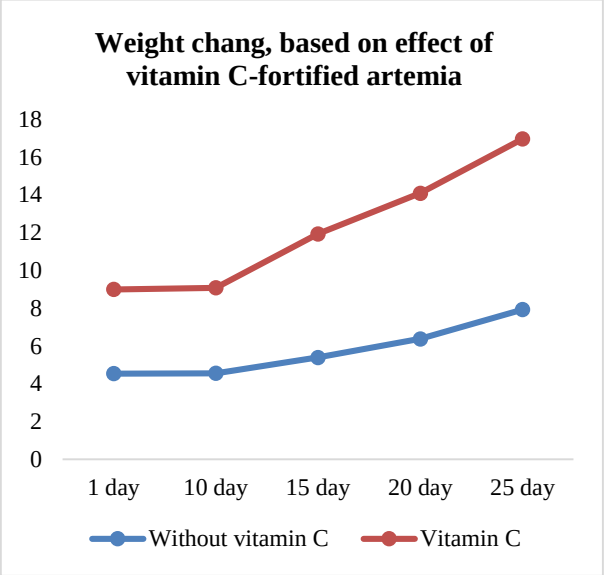

Figure 4: The effect of vitamin C-fortified artemia on the weight of *S. pharaonis* larvae

One of the primary goals of aquaculture is to produce different aquatic species for food production as well as stocks rehabilitation [31]. In the present study, *Sepia pharaonis* larvae fed vitamin C in the diet had a significant difference in terms of body weight gain, survival percentage compared to the control treatment. The results showed that the addition of vitamin C to the diet of *Sepia pharaonis* had greatly improved the growth indices of the species, although the effect was not significant. Not many nutritional studies have been performed on *Sepia pharaonis* larvae. *Sepia pharaonis* is one of the most important cephalopods with economic value in southern Iran. indifferent seasons of the year, Significant amounts of *Sepia pharaonis* eggs are observed in different regions of the southern coast of the country in different seasons of the year, of which, small amounts hatch and survive in nature, and many of them are abolished [32]. Vitamin C plays an important role in various physiological and biochemical processes of the fish body and various studies have mentioned, the positive role of this vitamin on the growth performance of fish and the beneficial role of vitamin C on growth indices has been widely reported by various researchers. The effect of vitamin C on the growth and survival of sturgeon larvae Reddy and Ramesh [33], Papp et al [34], and Sadowski et al [35] showed that Vitamin C in food does not have a significant effect on the growth of *Acipenser baerii*, which is completely consistent with the results of the present study and shows that this vitamin, although has a positive effect on fish weight but does not have a significant effect on its growth. A review of previous studies also shows that the diet containing vitamin C did not have a significant effect on the survival rate of rainbow trout fry [36, 37] In contrast, the effect of vitamin C on survival in *Labeo rohita* has been proven [38]. By adding vitamin C to *Sepia pharaonis* diet in the present study, there was a statistically significant difference in survival ratio between treatments. In general, vitamin C is an essential component of the diet of many species of fish and participates in metabolic processes, including mineral metabolism. This vitamin can allow nutrients by preventing hormonal changes and maintaining the strength of the immune system due to its role as a powerful biological antioxidant in protecting cells against oxidative damage as well as reducing stress due to congestion and manipulation. To be spent on protein production and increasing fish growth and survival rate.

Authors' contributions

Mohammad Forouhar Vajargah: Investigation, Methodology, Validation; Ghasem Hashemi Supervision – Conceptualization; Mehdi Bibak: Visualization – Software; Ahmad Mohammadi Yalsuyi: Writing- Data analysis

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Data availability

All data in this article will be available upon request.

Competing interests

The authors declare that they have no conflict of interest.

Ethical issue

The study protocol and ethics of this work have been approved by the Ethical Committee of the Gorgan University. Authors are aware of and comply with, best practices in publication ethics specifically about authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language. Also, all procedures performed in studies involving human participants were by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures performed in this study involving animals were following the ethical standards of the institution or practice at which the studies were conducted.

Consent to participate

No human or animal specimens were used in this work.

Consent to publish

All the authors agreed to publish the data in this journal.

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