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Research Article

Survival and larval development of cinnamon freshwater prawn *Macrobrachium acanthurus* fed different concentrations of vitamin A

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ABSTRACT. A feeding trial was performed to determine the effects of three concentrations of vitamin A (VA, 5, 10, and 20 mg) on the survival and larval development of the cinnamon freshwater prawn *Macrobrachium acanthurus*. Microcapsules with the respective concentrations of the vitamins were prepared and used to enrich *Artemia* nauplii and fed twice daily to triplicate groups of 20 larvae at stage 3. Survival and stage of development were registered daily to produce survival curves and index of development. Results show that including 10 mg of VA improved the survival and development of larvae, while 20 mg caused high mortality and slower development after stage 6. The present results indicate that VA is necessary for *M. acanthurus* larvae, and its use may aid in overcoming the bottleneck associated with the larval rearing stage, thus promoting continued culture supplementation of this species.

Keywords: *Macrobrachium acanthurus*; *Artemia* nauplii; larval development; larval rearing; retinol; retinoic acid; survival

INTRODUCTION

The cinnamon freshwater prawn *Macrobrachium acanthurus* is a native species of America and distributes on the Atlantic coastline, from North Carolina, USA, and down to Rio Grande du Sul, Brazil. Natural populations are disappearing due to several factors, such as overfishing, pollution from industrial and municipal wastes, and water competition for human consumption (Hernández et al. 2024). For the conservation of this species, its culture has been suggested for several years (Kutty & Valenti 2010). However, larval rearing has not been successful due to high mortalities during the development process, and few advances have been made in this regard (Hernández et al. 2024). The nutrient requirements for this stage have not been determined, though few attempts have been made with vitamin C and methionine (Hernández et al. 2015).

On the other hand, vitamin A (VA) is the generic term for all the molecules with the qualitative biological activity of all-*trans*-retinol (Beare-Rogers et al. 2001). It includes the retinol itself, retinal and retinyl esters (NRC 2011). Another compound derived from retinol is retinoic acid, which is not considered VA as it cannot be converted into retinol metabolically (Combs 1998). This vitamin plays an important role in several metabolic processes of vertebrates, such as vision, growth, reproduction, embryonic development, bone development, and general health maintenance (Halver 2002, Hernández & Hardy 2020). In crustaceans, VA has been related to the vision process (Cronin & Jinks 2001). Still, recent findings indicate that it is also related to growth (Shiau & Chen 2000, Hernández et al. 2009, Fuertes et al. 2014, Huang et al. 2022) and sexual maturation (Liñan-Cabello & Paniagua-Michel 2004, Liñan-Cabello et al. 2004, Mengqing et al. 2004) of different species of decapods.

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Regarding the larval stage, the presence of several retinol-derived molecules has been reported in the zoea and mysis stages of *Penaeus ensis* (Gu et al. 2002) and during the whole larval development of the giant prawn *Macrobrachium rosenbergii* (Pakdeenarong & Damrongphol 2006). These reports show that VA, particularly retinoic acid, may play a similar role in the larval development of crustaceans (Alabat 2009), as has been reported for vertebrates (Gauthier et al. 2023). However, the molecular mechanisms are still to be described. Therefore, the objective of the present work was to determine the effect of VA inclusion in the supplementation of live food in the survival and larval development of the cinnamon freshwater prawn.

MATERIALS AND METHODS

Experimental organisms

Larvae of *M. acanthurus* were obtained from broodstock maintained in the Laboratorio de Producción Acuícola, FES Iztacala, UNAM, by following the process reported by Frías et al. (2023). After hatching, larvae were transferred to a 2-L flask with water at a salinity of 19, continuous aeration, and a temperature of 29°C until the feeding trial started.

Microcapsule elaboration and *Artemia* nauplii enrichment

According to Ando et al. (2004), microcapsules were elaborated by dissolving gelatin and gum Arabic (10% each) in 40 mL of distilled water at 50°C. Then, 20 mL of oleic acid was added to the solution containing gelatin and gum Arabic, and the mixture was blended for 5 min. Then, 60 mL of distilled water at 50°C was added. Distilled water at 0°C until it was added to the mixture to obtain a total of 150 mL. Three treatments were used: 5 (VA5), 10 (VA10), and 20 mg (VA20) of VA, which correspond to 9,000, 18,000, and 36,000 international units (IU), respectively. Retinyl palmitate (1,800,000 IU g^{-1}, Sigma-Aldrich, St. Louis, USA) was used as a VA source. The concentrations were dissolved in the 20 mL of oleic acid before the microcapsules were prepared. For the control group, larvae were fed with nauplii enriched with the oleic acid microcapsules, and there was no addition of VA. Microcapsules produced with this method were stored for only 3 days at 4°C in amber flasks to prevent degradation of the VA. Subsequently, a new batch was prepared.

Artemia cysts (Biogrow, ProAqua, Sinaloa, Mexico) were hatched in 500 mL of saline water at 30 for 24 h, and newly hatched nauplii were collected to perform the enrichment with the VA. For the enrichment, glass beakers were used with 50 mL of saline water and nauplii at a density of 100 ind mL^{-1}. Then, 5 mL of each microcapsule solution (5, 10, and 20 mg) was added to the beakers and covered with aluminum foil to avoid VA degradation. The solution was aerated for 30 min to ensure that the microcapsules were adhered to the body and appendages of nauplii. After the 30 min of enrichment, the nauplii were filtered and slightly washed with saline water and offered to *M. acanthurus* larvae.

Feeding trial

For the feeding trial, plastic flasks of 500 mL were used, and 20 three-day post-hatching larvae were randomly allocated in each flask. Each concentration of VA was fed to triplicate groups of larvae, and a control group without vitamin supplementation was established. The larvae were fed twice daily, at 10:00 and 18:00 h, and nauplii were maintained at a concentration of 4 ind mL^{-1} in each flask. Fifty percent of water exchange was performed daily in the morning to remove the excess nauplii and maintain the salinity and water quality before the feeding. During all the trials, conditions were salinity 19, temperature 29 ± 1°C, and dark:light cycles of 12:12 h. The feeding trial lasted 43 days until the last organism molted into postlarvae in the VA20 treatment.

Survival and larval development

Survival was monitored daily, and all alive larvae were counted each morning during the water exchange. Counting was performed manually by taking the larvae with a plastic pipette from one flask to a new one previously prepared. Just one individual from each replicate flask was observed in a stereoscopic microscope to determine the larval stage and avoid losses for handling (Nikon, model SMZ1500, Tokyo, Japan), and morphological features were compared with the reports of Choudhury (1970) and Quadros et al. (2004) to determine the developmental stage. Daily revisions were performed until all individuals reached the postlarvae stage.

Development index

Data from the larval stage were used to determine the index of development by using the formula reported by Villegas & Kanazawa (1979):

$$\text{Index of development (ID)} = \Sigma A / N$$

where A is the absolute value assigned to each larval stage, and N is the number of individuals observed.

Absolute values were assigned according to the larval stages of the individuals observed: value 1 was given to the first stage of development, value 2 to the second, and so on until stage 10. For the case of postlarvae, the value assigned was 11.

Statistical analysis

Survival curves were analyzed with the Kaplan-Meier test, while data of final survival (percentage) were transformed using arcsine, and then, a one-way ANOVA was performed. Significant differences were evaluated post hoc using Fisher´s LSD test by setting the error at 5% for each set of comparisons. All analyses were performed with the software Prism v.10.3.0 for Mac (GraphPad Software, San Diego, CA, USA).

RESULTS

Survival curves of the control and VA-supplemented groups are shown (Fig. 1), with the best performance observed in the group fed nauplii with VA10, followed by those larvae fed VA5 and the control group. Including 20 mg of VA resulted in a significantly sharp decrease in larval survival after 9 days from the start of the feeding trial, which was reflected in the final survival (Fig. 2), showing significantly lower values compared to VA5 and VA10. The highest final survival rate was observed in the VA10 group, reaching a mean of 40%, with no significant differences compared to the mean survival rate (30%) of the group fed VA5 supplementation.

Regarding larval development, Table 1 shows when larvae molt from one stage to another. Development was faster in the VA10 group, where stage 6 was reached on day 15 of the feeding trial, and the first postlarvae appeared on day 24. The slowest development was observed in the VA20 treatment, which took 43 days to reach the postlarvae stage. The development index during the feeding trial is shown (Fig. 3). A similar trend was observed among treatments until day 17, after which larvae fed with VA10 molted between stages faster than those in the other groups. In contrast, the group fed with VA20 showed slower stage moltings after day 20, taking an additional 20 days to progress through three stages before reaching the postlarvae stage.

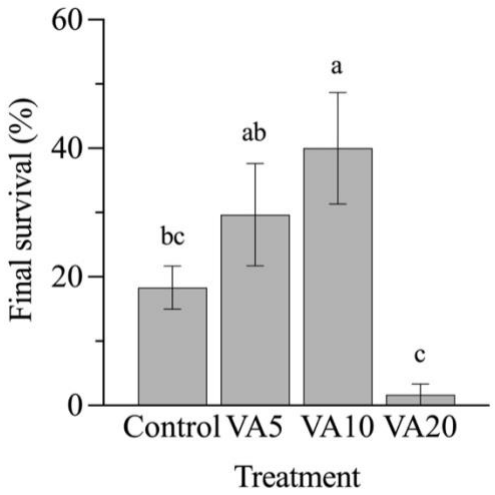

Figure 1. Survival of *Macrobrachium acanthurus* during larval development and fed different vitamin A (VA) concentrations. Each point represents the mean of three replicate groups ± standard error. Differences were found among the curves of the group VA20 compared to the other treatments ($P < 0.05$).

DISCUSSION

VA has been reported to play an important role in vision, growth, and reproduction in several species of crustaceans, particularly in decapods. Even though some VA-related molecules (such as retinoic acid) have been detected during the larval stage of certain decapods (Gu et al. 2002, Pakdeenarong & Damrongphol 2006), there are no reports on how VA influences the survival and larval development of crustaceans. It was not possible to assess the VA contents on the *Artemia* nauplii biomass. Still, the findings show that the different concentrations of retinyl palmitate have a differential effect on survival and development with an optimal inclusion level of 10 mg (18,000 IU) to enrich *Artemia* nauplii.

Reported survival rates and periods to reach the postlarval stage for *M. acanthurus* include 8.5% in 25 days (Choudhury 1971), 2% in 44 days (Dobkin 1971), and 20% in 33 days (Hernández et al. 2015). The inclusions of VA5 and VA10 improved the survival rates (30 and 40%, respectively) and reduced the time to reach the postlarval stage. Lack of VA caused low survival. Recently, Gauthier et al. (2023) reported that inhibition of retinoic acid synthesis induced abnormal development of limbs of *Ulca pugilator*, which may have happened in larvae fed the control treatment. On the other hand, the inclusion of 20 mg of VA resulted in a sharp increase in mortality after day 10 of the feeding trial, leading to a final survival of 1.6%. Excessive VA has been reported to negatively impact the survival rates of several species of fish larvae (Hernández & Hardy 2022). Lack or excess of VA influences the production of retinoic acid (Alabat 2009),

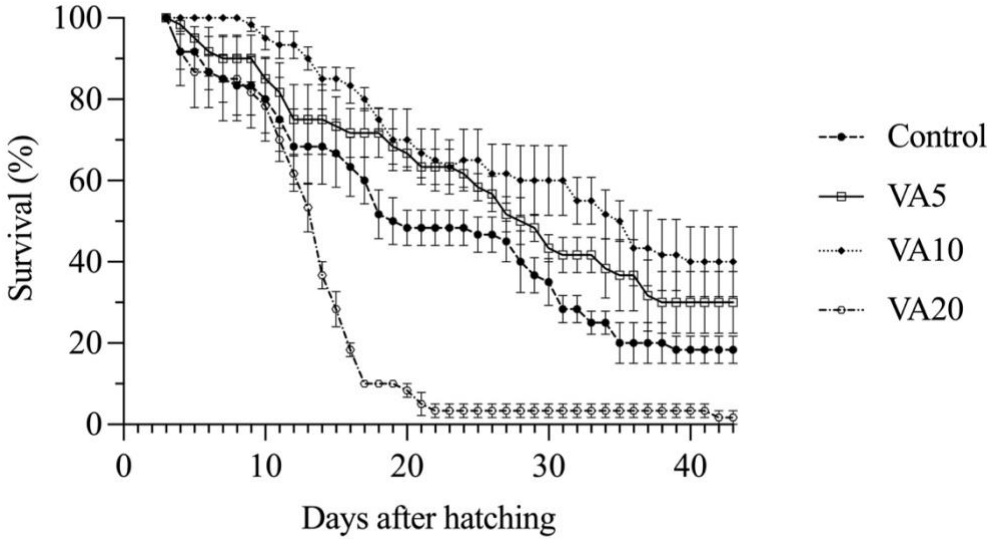

Figure 2. Final survival rates of *Macrobrachium acanthurus* after the larval development and fed different vitamin A (VA) concentrations. Each bar represents the mean of three replicate groups ± standard error. Different letters indicate significant differences ($P < 0.05$).

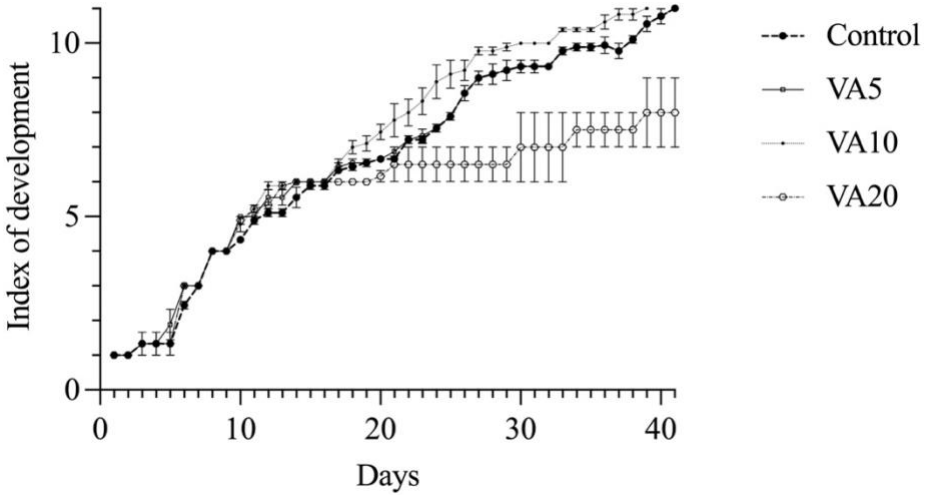

Figure 3. Index of development of *Macrobrachium acanthurus* during larval development and fed different concentrations of vitamin A (VA). Each point represents the mean of three daily observations ± standard error.

affecting the normal development of crustacean larvae, but the molecular mechanisms are still to be understood.

M. acanthurus undergoes an extended larval development (Mejía-Ortiz et al. 2016), which includes 10 stages characterized by morphological changes (Choudhury 1970). Including 10 mg of VA facilitated rapid metamorphosis of the larvae compared to the other treatments, as reflected in the development index. Stage 6 appears to be a critical period for larval development, with the highest mortality occurring during this stage. According to Quadros et al. (2004), the molting process from stage 6 does not result in a new stage as it does from stages 1 to 5. Morphological changes from stages 6 to 7 include pleopod development, but no reports detailing the internal changes occurring during this period. VA, through retinoic acid, seems to be part of the signaling system in invertebrates during the larval stage (Albalat 2009), with both deficiency and excess affecting normal development, as observed in the control and VA20 treat-

Table 1. Days to reach each larval stage of *Macrobrachium acanthurus*, fed with different vitamin A (VA) concentrations.

Larval stage	Time (days)			
	Control	VA5	VA10	VA20
1	1	1	1	1
2	3	3	3	3
3	6	5	6	6
4	9	8	8	8
5	11	10	9	9
6	15	12	11	11
7	17	16	15	20
8	22	20	18	27
9	25	23	20	35
10	28	25	22	41
Postlarvae	29	27	24	43

ments. The data obtained in the present investigation suggests that VA significantly influences the development of *M. acanthurus* larvae, particularly during stage 6.

CONCLUSIONS

VA is essential for normal development and survival during the larval development of *Macrobrachium acanthurus*. Enriching *Artemia* nauplii with 10 mg (or 18,000 IU) of VA resulted in higher survival rates and faster development to the postlarvae stage. Conversely, the inclusion of 20 mg of VA caused high mortality rates, suggesting that VA plays a role similar to that observed in vertebrates. However, the specific metabolic mechanisms remain to be elucidated. Enrichment with VA may help to overcome the bottleneck associated with larval rearing, thereby enabling continued culture supplementation of this species. Future research should include the analysis of the contents of different VA metabolites and the expression of genes of enzymes related to VA metabolism.

Credit author contribution

M. Castillo-Domínguez: conceptualization, feeding trial development, formal analysis; M.A. Fernández-Araiza: conceptualization, supervision, formal analysis, review and editing; S.A. Frías-Gómez: conceptualization, feeding trial development, formal analysis, review and editing; L.H. Hernández-Hernández: conceptualization, funding acquisition, supervision, formal analysis, writing-original draft, review, and editing. All authors have read and accepted the published version of manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data will be available on request.

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