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The effect of vitamins AD$_3$E supplementation on the growth, reproductive performance and survival rates of climbing perch *Anabas testudineus* broodstock in cage culture environments

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Abstract

This study aims to compare three levels of vitamins AD$_3$E supplementation on the growth, reproductive performance and survival rates of climbing perch under cage culture conditions. The experiment was designed on a completely randomized model with one control group and three treatment groups. There were two replicates per treatment. Climbing perch broodstock of average body weight (32.65 ± 1.26 g) were acclimatized to experimental conditions for 15 days before they were randomly distributed in each group. The stocking density of each group was 10 fish m^{-2} in a cage (9 m^2) with the ratio of female to male set at 1 : 1. The control diet was a commercial diet consisting of 32% protein and 15% lipid with a lack of vitamins AD$_3$E. Three treatment diets were formulated containing the commercial diet supplemented with either 0.50, 2 or 5 ml of AD$_3$E kg^{-1}. During the experimental period, the fish were fed 5% of their body weight using each experimental diet at 09:00 and 17:00.

The results indicated that females fed a dietary supplementation of 5 ml of AD$_3$E kg^{-1} had the highest values of GSI (15.91 ± 1.03), total number of eggs (454 ± 20.18), fertilization rate (69.82 ± 11.42%), hatching rate (66.22 ± 9.26%) and survival rate of fish larvae 10 days after hatching (82.40 ± 7.25%). Average values of pH, dissolved oxygen, conductivity, total dissolved solids, water temperature, turbidity, salinity, and nitrates in the culture ponds ranged between 5.43 – 8.66, 5.89 – 6.40 mg l^{-1}, 0.128 – 0.301 ms cm^{-1}, 0.218 – 0.256 g l^{-1}, 29.45 – 31.11 ℃, 43.25 – 49.71 NTU, 0.10 – 0.10 ppt and 0.23 – 0.25 mg l^{-1}, respectively. This finding suggests that the supplementation of 5 ml of AD$_3$E in the diet improved the reproductive performance of female climbing perch broodstock. The knowledge obtained from this research can benefit climbing perch aquaculture broodstock management.

Keywords: Vitamins AD$_3$E; growth performance; reproductive performance; climbing perch

1. Introduction

The climbing perch, *Anabas testudineus* (Bloch) [1], belongs to the family: Anabantidae, Genus: *Anabas* and Species: *Anabas testudineus* [2]. This fish is economically significant and an important indigenous fish in Thailand because of the high demand for it in both local and foreign markets [3]. The value of total climbing perch fish production in Thailand is 16,400 tons per year (643.30 million) [4]. This fish has been reported as a fast growing species because it is omnivorous and consumes various kinds of food such as detritus, aquatic plants, crustaceans, worms, mollusks and insects [5 – 6]. Moreover, it can live out of water for an extended period of time because of a structure in the fish's head, which allows it to breathe atmospheric oxygen [7]. According to literature reported by Yakupitiyage et al. [8] climbing perch present as a carnivorous or insectivore species that guard their eggs, whilst living on a diet of water invertebrates and their larvae [9].

Today, climbing perch can be raised at a multitude of locations throughout Thailand by using smaller containment areas and less water than other aquaculture species [9 – 10]. However, larvae rearing of this species is still a problem for fish farming because of low survival rates. There are many reasons for this problem, such as nutritional quality, water temperature and environmental conditions. The nutritional requirements for climbing perch broodstock are similar to other freshwater carnivorous fish in respect of the quality of carbohydrates, amino acid vitamins, proteins, and minerals. The quantitative demands for these nutrients vary depending on the species, its growth and gonadal development stage, and environmental conditions. Vitamins are an important factor affecting the normal cellular metabolism, growth, maintenance and reproduction of aquatic animals [9 – 12]. A better understanding of the importance of fat-soluble vitamin requirements during the gonadal development of female climbing perch should allow us to establish productivity tools for controlling the seed quality production of females reared in cage cultures. Therefore, this study was conducted to investigate the effects of different levels of vitamins AD_3E on the growth and reproductive performance of climbing perch in their early maturation stages. It is hoped that this study will be helpful in developing an appropriate broodstock rearing technology for this species.

2. Materials and Methods

This study was carried out at the Faculty of Science and Agriculture Technology, Rajamangala University of Technology, Lanna, Nan, between April and May in 2015. This experiment was performed in cage cultures in an earthen pond for trial diets of climbing perch broodstock.

Experimental fish

Climbing perch broodstock with an average age of 160 days and a body weight range of 32.43 ± 1.77 g to 32.13 ± 1.64 g.bw were obtained from a commercial freshwater fish farm located in Tak province. The fish were manually selected and transferred to a cage culture, where they were held and kept separately for 15 days for adaptation to the new environment before starting the experiment. A total number of 50 females and 50 males were counted, batch weighed and stocked in each cage culture (each group) at a rate of 100 fish per cage (10 fish m^{-2}).

At the beginning of the experiment, a random sample of 20 females and 20 males from each cage culture were individually weighed and released in each treatment group.

Experimental plan and diet

The study was assigned a completely randomized experimental design type which consisted of three treatment groups and one control group, with two replications in each group. The control diet was a commercial diet consisting of 32% protein and 15% lipid with a lack of vitamins AD_3E. Three treatment diets were formulated containing the commercial diet, supplemented with either 0.50 or 2 or 5 ml AD_3E kg^{-1}. During the adaptation period, the fish were fed 0.50% of their body weight (bw) with the commercial diet only. During the experimental period, the fish were fed 5% bw of each experimental diet. The diets were offered two times a day at 09:00 and 17:00.

Data collection and analytical methods

1) Assesment of the growth performance of climbing perch

Percentage of weight gain (PWG), average daily weight gain (ADG) and specific growth rate (SGR) were evaluated for 60 days. Before starting the experiment all fish from each group were batch weighed. Growth performance was examined at the end of the experiment. Feed intake was recorded daily and feed efficiency was calculated according to the method described by Zalina et al. [13] and Olvera-Novoa et al. [14] as follows:

Percentage weight gain:

$$PWG = [(\text{final weight} - \text{initial weight}) / \text{initial weight}] \times 100\% \quad (1)$$

Average daily growth:

$$ADG\ (g/day) = (\text{final weight} - \text{initial weight}) / \text{culture periods (days)} \quad (2)$$

Specific growth rate:

$$SGR\ (\%/day) = [(\text{final fish weight} - \text{initial fish weight}) / \text{culture period (days)}] \times 100 \quad (3)$$

Survival rate:

$$SR\ (\%) = [\text{final number of fish} \times 100] / \text{initial number of fish} \quad (4)$$

2) Assesment of the reproductive performance of climbing perch

In this study, two periods of close observation were used for assessing the reproductive performance of climbing perch; one at the beginning of the experiment and one at the end. On day 60 of the experimental period, 20 males and 20 females perch from each group were randomly selected and weighed in order to calculate the gonadosomatic index (GSI), fertilization rate and hatching rate. Subsequently, a total of 20 males and 20 females climbing perch from each group advanced to the maturation tank (1,500 liter $tank^{-1}$) for further reproductive evaluation. In each group, female fish were separated into two groups (10 fish $group^{-1}$) for GSI examination and

reproductive performance evaluation according to the method described by Morioka et al. [15] and Perera et al. [16]. After weighing the females individually, 10 fishs per group were randomly selected and dissected to remove the ovaries for the determination of the GSI. Moisture in the gonads was removed and the weight of the gonads was recorded in grams. The gonadosomatic index was calculated as per the following equation:

$$GSI = [\text{Gonad Weight (g)} / \text{Total fish body weight (g)}] \times 100 \quad (5)$$

Female climbing perch were induced using buserelin acetate (Luteinizing Hormone-Releasing Hormone Analog: LHRHa) at an intensity level of 20 and 5 micrograms kg^{-1} bw for females and males, respectively. The broodstock were injected one time; males at 17:00 and females at 21:00. They were then left to spawn in a maturation tank with the sex ratio between males and females fixed at 2 : 1. Twelve hours post fertilization, both the unfertilized and fertilized eggs in the upper water layer were collected and washed several times with fresh water to remove excess milt. Then the reproductive performance of the female climbing perch was evaluated. For the total number of eggs, the amount of unfertilized and fertilized eggs from each pair of broodstock was calculated three times over. Then, the fertilization rate (after 3 hours) and the hatching rate were evaluated as follows: The fertilization rate was determined 12 hours post fertilization in *triplicate* using the following equation:

$$\text{Fertilization rate (\%)} = (\text{number of fertilized eggs} / \text{total number of eggs released by the female}) \times 100\% \quad (6)$$

The hatching rate was determined, in triplicate, as the proportion of hatching eggs to total eggs as follows:

$$\text{Hatching rate (\%)} = (\text{number of fish larvae} / \text{total number of eggs}) \times 100\% \quad (7)$$

Water quality analysis

The Horiba U-50 Series water quality meter (Horiba, Japan) was used to monitor changes in water quality including pH, dissolved oxygen, conductivity, total dissolved solids, water temperature, turbidity, salinity, and nitrates. The concentration of nitrite and total ammonia in the water was measured weekly using the DR 3900 Benchtop Spectrophotometer (Horiba, Japan).

Statistical Analysis

All data on the growth, reproductive performance and survival rates of climbing perch collected during the experiment were recorded and analyzed by one-way ANOVA. The Duncan's Multiple Range Test was used to determine the differences between the treatment means. Alphabetical notation was used to mark any differences at the significant level of an Alpha 0.05 [17].

3. Results

Effect of vitamins AD_3E on the growth performance of female climbing perch

The growth performances of climbing perch fed the control diet and three treatment diets are shown in Fig. 1. The average initial weights of climbing perch were 35.23 ± 1.22, 35.31 ± 0.85, 35.22 ± 0.62 and 35.26 ± 0.56 g $fish^{-1}$ for the control group and groups 1, 2, and 3 respectively. The mean initial weight of all experimental fish showed no significant differences ($P > 0.05$), however it increased the highest in group 3 which was fed with the treatment diet coated with vitamins AD_3E at 50 ml AD_3E kg^{-1}. The mean final weights of climbing perch in the control group and groups 1, 2 and 3 were 62.20 ± 4.70, 65.58 ± 2.69, 69.30 ± 2.08, and 74.40 ± 1.51 g $fish^{-1}$, respectively.

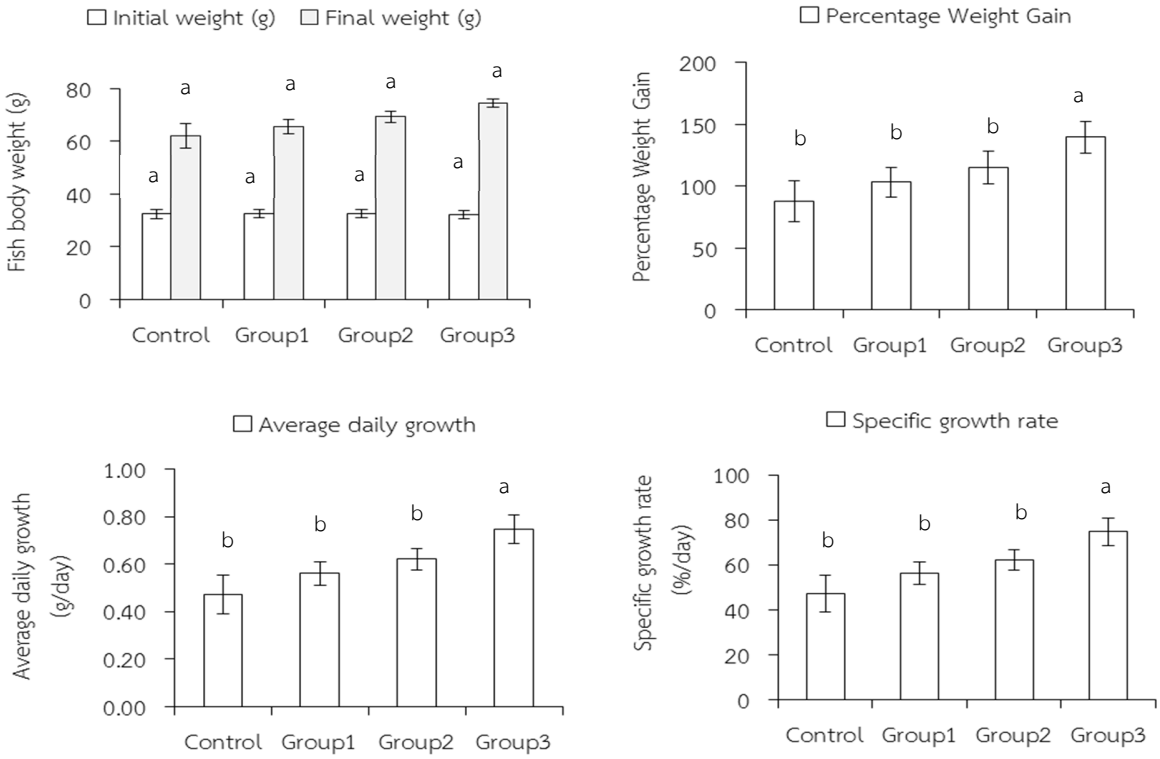

Fig. 1 Comparison of the mean (± S.D.) values of the growth performance parameters for climbing perch in three different treatment groups and a control group over 8 weeks**.** Means with different superscripts (a, b, c, d) in the same bars were significantly different ($P < 0.05$).

The best growth performance, with a mean final weight of 74.40 ± 1.51 g $fish^{-1}$, a percentage weight gain of 139.75 ± 12.70%, an average daily weight gain of 0.75 ± 0.06 g and a specific growth rate of 74.67 ± 5.96 g was obtained in group 3. Group 2 followed with a mean final weight, percentage weight gain, average daily weight gain, and specific growth rate of 69.30 ± 2.08, 115 ± 03.27, 0.62 ± 0.05 and 62.08 ± 4.57, respectively. Next came group 1, with a mean final weight, percentage weight gain, average daily weight gain, and specific growth rate of 65.58 ± 2.69, 103.38 ± 11.78, 0.56 ± 0.05, and 56.11 ± 4.97, respectively. The poorest growth performance was observed in the

control group, which showed a mean final weight, percentage weight gain, average daily weight gain, and specific growth rate of 62.20 ± 4.70, 87.82 ± 16.65, 0.47 ± 0.08, and 47.20 ± 8.06, respectively. The larvae survival rates of climbing perch throughout the control and treatment groups showed no significant difference ($P > 0.05$). The larvae survival rate ranged from 92 – 98%.

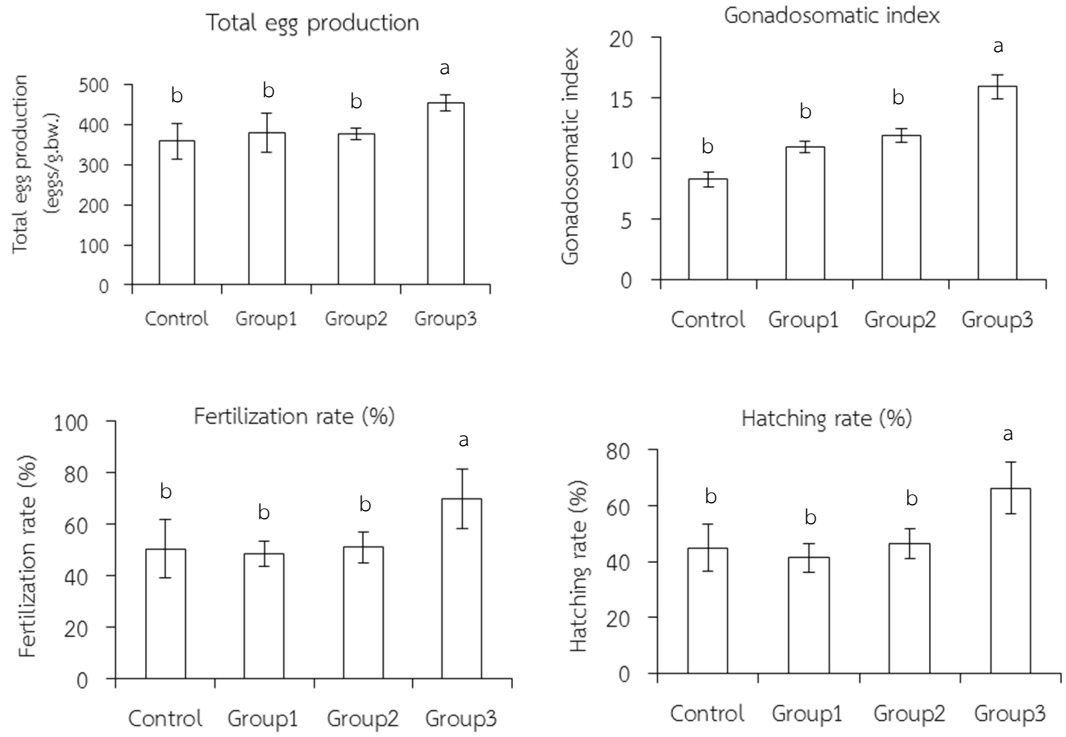

Fig. 2 Comparison of the mean (± S.D.) values of the reproductive performance parameters for climbing perch in three different treatment groups and a control group over 8 weeks**.** Means with different superscripts (a, b, c, d) in the same bars were significantly different ($P < 0.05$).

The effect of vitamins AD$_3$E on the reproductive performance of climbing perch

The reproductive performance of the climbing perch fed with a control diet versus the treatment diets is presented in Fig. 2. The mean total egg production of climbing perch broodstock was 358.67 ± 44.94, 380.20 ± 49.78, 377.38 ± 14.96, and 454.00 ± 20.18 eggs g^{-1} bw for the control group and treatment groups 1, 2 and 3, respectively. There was no significant effect of the treatment diet on total egg production values for the climbing perch observed in groups 1 and 2. The values of the fertilization rates and hatching rates were significantly different ($P < 0.05$) among all treatments. Female fish in group 3 had significantly higher ($P < 0.05$) values for the fertilization rates and hatching rates when compared with groups 1 and 2. The values for the fertilization rates of fish in the control group and groups 1, 2 and 3 were 50.37 ± 11.26, 48.48 ± 4.73, 50.91 ± 5.88, and 69.82 ± 11.42, respectively. The values for the hatching rates (%) of fish larvae in the control group and groups 1, 2 and 3 were 44.92 ± 8.37, 41.38 ± 5.12, 46.36 ± 5.52, and 66.22 ± 9.26, respectively. The gonadosomatic index of fish in group 3 was significantly higher

(15.91 ± 1.03, $P < 0.05$) compared to the control group and the fish in groups 1 and 2, (8.27 ± 0.60, 10.98 ± 0.48, 11.87 ± 0.55, respectively).

The changes of water quality in the culture ponds

The study results showed that there were minor changes in some of the parameters of water quality in the earthen pond, such as: total dissolved solids (which ranged between 0.218 – 0.256 g l^{-1}), and turbidity (which ranged between 43.25 – 49.71 NTU), but salinity remained a constant (0.1 ppt) from the beginning to the end of the experiment. The results mean that the factors of feed composition and feeding frequency had no significant influence on these parameters of water quality. The dissolved oxygen concentration ranged from 5.89 ± 0.30 mg l^{-1} to 6.40 ± 0.08 mg l^{-1}, while the pH in the earthen pond ranged from 5.43 ± 0.18 to 8.66 ± 0.24. Salts dissolved in water are separated into cations and anions. Such a solution is called an electrolytic solution. The levels of conductivity in the water displayed minor changes from the beginning to the end of the experiment. The levels ranged from 0.128 – 0.301 ms cm^{-1}. Water temperature was measured two times a day; once in the morning between 8:00 – 9:00 when it ranged between 29.45 – 29.69 ℃, and once in the afternoon between 17:00 – 18.00, when it ranged between 30.21 – 31.11 ℃. At the latter daily observation, the levels of ammonia and nitrate concentration were lowest in the first month and later increased in the culture during the second month.

4. Discussion

In these experiments, the fish that were fed diets containing 5 ml AD_3E kg^{-1} showed the best growth rates and significantly higher percentages of weight gain than noted for the fish that were fed any of the other experimental diets or the control diet. Following behind these top rates in decreasing order, were the fish that were fed diets containing 0.50 and 2 ml AD_3E kg^{-1} feed and the control diet, respectively. These results indicate that growth performance was higher when the fish were fed trial diets containing more vitamins AD_3E than the control diet. These results agree with the literature reported by Kraisurasre et al. [10] pertaining to glass catfish, whereupon a study on the effect of vitamin E on ovarian development indicated that 200 mg kg^{-1} of vitamin E encouraged gonad development in the mature stage. The gonadosomatic indexes were not significantly different but higher than fish fed with 0 mg kg^{-1} vitamin E.

Pitaksong et al. [11] reported that hybrid catfish fed with trial diets containing a co- supplementation of 500 mg kg^{-1} vitamin C and 125 mg kg^{-1} vitamin E, or 1,000 mg kg^{-1} vitamin C alone had beneficial effects on growth under stressful conditions. These results above could be related to the palatability, digestibility and absorption of diets by fish during the gonadal development stages. According to the National Research Council [18], vitamin requirements depend upon size, stage of sexual maturity, growth rate, environmental conditions and dietary nutrient interrelations, and they function independently of enzymes or, in some cases such as vitamin K, may have coenzyme roles. Based on the results gathered from the experiments, the increase in growth performance with increasing levels of vitamins AD_3E in the trial diets may have been caused by an improvement in the absorption efficiency of the digestive tract of the fish.

The mean survival rates of all the experimental fish were not significantly different ($P > 0.05$). These results may be related to the behavioral and physiological attributes of climbing perch. Similarly, Graham [19] reported that this species can thrive in oxygen depleted water using their special auxiliary air breathing organ, which facilitates the utilization of atmospheric air for their respiration. In these experiments, reproductive performance in terms of GSI, mean total egg production, fertilization rates and hatching rates of climbing perch which were fed diets containing 5.0 ml AD_3E kg^{-1} diets, was significantly highest among the treatments ($P < 0.05$). These agreed with James et al. [20] who showed that increasing the level of vitamin E in the goldfish diet produced the best feeding rates, weight gain, and specific growth rates. Also, females fed the 300 mgE kg^{-1} diet had significantly ($p < 0.01$) heavier gonads and higher numbers of eggs with better hatchability than those fed other diets. This was probably correlated with the effect of fat-soluble vitamins on gonadal development activity, especially vitellogenesis in females and active spermatogenesis in males. These agreed with the research of Tan et al. [20] on rice field eels, where increasing dietary vitamins A, D_3, E and C supplementation levels significantly increased the GSI and lowered the serum content of malondialdehyde in the eels [20].

At the end of this current study, the values of total egg production, fertilization rate and hatching rate showed a tendency to increase with the extra levels of vitamins AD_3E in the trial diets. These results indicated that the values of total egg production, fertilization rate and hatching rate of the climbing perch broodstock was higher in group 3 followed by group 2, group 1 and the control group, respectively. The results presented in this study imply that vitamins AD_3E might be necessary to encourage vitellogenesis of female climbing perch in cage cultures.

In general, fat-soluble vitamins are absorbed from the digestive tract and the function is independent of enzymes or, in some cases such as with vitamin K, may have coenzyme roles [12, 21]. These results agreed with literature reported by Bilguven on rainbow trout, which when fed with fat-soluble vitamins A and E it was found that diets containing increasing amounts of vitamins E and A had a significant ($p < 0.05$) effect on the hatching rate of fertilized eggs and the survival of larvae during the incubation period [22]. Some water quality parameters such as pH, dissolved oxygen, conductivity, total dissolved solids, water temperature and turbidity, nitrites and ammonia have been widely acknowledged as some of the most important rearing conditions that might be controlled to promote growth and reproductive performance and survival rates of climbing perch [23 – 24]. According to the results of the present study, the changes of all water quality parameters in the rearing ponds were not significantly different ($P > 0.05$) because all of the cages were positioned in the same big reservoir. Boyd et al. [25] reported that growth is fastest and healthiest for several species of aquatic animals when dissolved oxygen concentrations are above 3 mg l^{-1}. Therefore, the water quality parameters which were monitored in this study were within the desirable ranges for climbing perch, *Anabas testudineus* reared in captivity.

5. Conclusion

According to the results and discussion of this present study, a conclusion can be drawn as follows: A significant effect of vitamins AD_3E in climbing perch diets was observed on the growth performance including the percentage of weight gain, average daily weight gain and specific growth rate, respectively. The survival rates were observed to

be relatively high for all treatments except for fish fed with the commercial diet that lacked vitamin supplementation (control group). There was also a significant effect of the vitamins AD_3E on the gonadosomatic index, fertilization rate and hatching rate. The best growth and reproductive performances of climbing perch occurred in group 3, which was fed a commercial diet coated with 5 ml AD_3E kg^{-1}, followed by climbing perch fed with a commercial diet coated with 2 and 0.50 ml AD_3E kg^{-1} diets and the control diet, respectively. Water quality parameters which included PH, dissolved oxygen, conductivity, total dissolved solids, water temperature, turbidity, salinity, and nitrates were all within an acceptable tolerance range for climbing perch cultures. In conclusion, it was clearly shown that vitamins AD_3E played an important role in the growth and reproductive performance of climbing perch. It might be necessary therefore to include sufficient amount of vitamins AD_3E in diets for climbing perch for a successful seed production outcome. Further studies are required to investigate the effects of vitamins AD_3E on female climbing perch during previtellogenesis and vitellogenesis.

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