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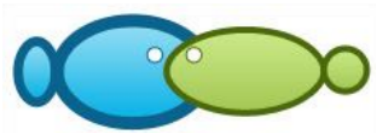
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The gonadal maturation of climbing perch, *Anabas testudineus* (Bloch, 1792) with dietary supplement add on feed

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Abstract. Fish use the food supply for energy and gonadal maturation, which can be optimised by addition of supplement in the form of vitamin C, vitamin E and *Spirulina* sp. This research was carried out to examine the impact of additional supplement on the level and index of gonadal maturation, diameter of ovary, fecundity and the successfulness of hatching on female parents of climbing perch (*Anabas testudineus*). There were 27 fish parents cultivated in nine ponds for three months from April to June 2017. The broodstocks were fed with the commercial pellets as much as 3% of fish biomass. For treatments, the pellets were added with the supplement of vitamin E and vitamin C i.e 0.1% each, as well as *Spirulina* sp. i.e. 3% in dosage. The experiment was set on completely randomized design with three replications. The results showed that the addition of *Spirulina* sp. had a significant impact on the index of gonadal maturation as well as the increase of body and gonadal weights. Meanwhile, vitamin E significantly affected the fecundity and the ovary diameter. There was no significant effect of vitamin C on all parameters.

Key Words: gonadal maturation, supplement, food, climbing perch, vitamin.

Introduction. Climbing perch (*Anabas testudineus*) is potential to be cultivated in pond or in any controlled aquatic environment (Ahmadi et al 2018). The increasing interest of fish farmers in cultivating the climbing perch has enhanced the increase of need for its juveniles (Uddin et al 2016; Izmaniar et al 2018). In fact, its fry can be obtained from nature or through hatching in fish hatchery unit (Mustakim et al 2018). The natural juveniles vary in size and quality, depending on the availability and season (Zworykin 2018). Therefore, the artificial hatching is required to produce juveniles in uniform size and quality (Kader et al 2019).

Some factors contributing on the successfulness of artificial hatching are quality of feed and parents as well as season (Kostow 2009). Feed quality can influence the level and index of gonadal maturation, diameter of ovary, fecundity, the percentage of hatching and the formation of yolk (Valdebenito et al 2015).

The use of feed supplements has various effects on different fish species. The additional of feed nutrients (i.e. AD3E vitamins) has been reported stimulating the egg maturation on catfish (*Clarias* spp.) (Duangjai et al 2017). Vitamins and *Spirulina* are beneficial for growth, sex maturity and gonadal maturation of tilapia (*Oreochromis niloticus*) (Lu & Takeuchi 2004) and gourami fish (*Trichopodus trichopterus*) (Khanzadeh et al 2015).

The gonadal maturation can be affected by addition of vitamin C on feed of milkfish (*Chanos chanos*) (Tan et al 2007) and goldfish (*Carassius auratus*) (James et al 2009). However, there is no much information, so far, about the use of vitamins as an additional supplement for gonadal maturation of parent on climbing fish. Therefore, this study was conducted to figure out the effect of the use of vitamin C, vitamin E and

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Spirulina sp. on the level and index of gonadal maturation, diameter of ovary, and fecundity on female parents of climbing fish.

Material and Method

The broodstocks culture. The parent fish was collected from flood swamps in Regency of Ogan Komering Ilir, Province of South Sumatra (Indonesia) and started to be domesticated on 2016. Fish was cultured in nine net-bordered ponds at the Fish Hatchery Unit "Mulia" in District of Plaju, Palembang City, Province of South Sumatra (Indonesia) for 2 months (May-June 2017). The net was 50×50×130 cm³ in volume. The culture period was determined by considering the formation of fish ovary, namely more than 1 month (Singh & Lal 2008).

The observation of gonad. The female fish were separated from the male ones to prevent any mating during the treatment. A hundred and fifty eggs were sampled from the genital orifice of five fishes (30 eggs per fish) on each net-bordered pond using catheter hose and then brought to the laboratory for the observation of ovary diameter under optical microscope (10X magnification). The ovary diameter was digitally captured for measurement using the Image J program (Elliot 1979; Pavlov 2006). The initial weight of mother fish was recorded about 27-31 g per fish in average. The fecundity was observed through the gonadal surgery at the end of the study and counted with hand counter from the collected ovaries using the volumetric sampling technique.

The application of additional supplement. The feed was pellet with a composition of 35% of protein. It was about 3% of fish biomass. There were three treatments, i.e. P1 (addition of 0.1% vitamin E of the pellet weight) (Mehrad & Sudagar 2010), P2 (addition of 0.1% vitamin C of the pellet weight) (Emata et al 2000), and P3 (addition of 3% *Spirulina* of the pellet weight) (James et al 2009), while the feed without any additional supplement was considered as control. Those supplements were dissolved into 150 mL of water and then stirred for becoming homogenous. The mixture was evenly sprayed into the tested feed and then air-dried. The pellets were applied to the test fish three times a day, i.e. every morning, afternoon and evening.

Growth measurement. Observation on fish growth and maturation was conducted during the cultivation. Every 10 days, three fish were randomly sampled from each treatment for measurement of their growth using digital balance. We used digital balance trade mark A&D technology series GF-6100 (A&D Company, Ltd., Oxford, England) and caliper to measure total length of fish with unit of cm.

Data collection. The correlation level of fecundity and ovarian diameter, gonadal maturity index (GMI), gonadal weight, total length and weight as well as hatching rate were measured. Such correlation was useful for assessing the effect of additional supplements on reproduction.

Data analysis. The specific growth was calculated using the following formula:

$$\text{Specific growth rate} = \frac{\text{final weight} - \text{initial weight (g)}}{\text{period of cultivation (day)}} \times 100$$

Whereas the GMI was measured using formula of James et al (2006):

$$\text{GMI} = \frac{\text{gonadal weight (g)}}{\text{fish weight (g)}} \times 100$$

The measurement of fecundity was conducted with below formula:

$$\text{Fecundity} = \frac{\text{weight of sampled eggs (g)}}{\text{weight of total eggs (g)}} \times \text{number of sampled eggs}$$

Those data were statistically analysed with analysis of variance (ANOVA) using SPSS program to determine the effect of supplementation on hatching rate, ovarian fecundity and egg diameter. The analysis of correlation and regression were performed to

fecundity, gonadal weight, total body length, total body weight, and GSI. Furthermore, the data were tested for its accuracy using F-test with a probability level of 0.05.

Results

Specific growth. The growth in length and weight from this research results showed that *Spirulina* procures the best results than another supplement. Figure 1(A) and 1(B) is a mean of growth length and weight. *Spirulina* increasing weight was significantly higher ($p < 0.05$) than vitamin E, C, and control. *Spirulina*-fed fish in this research had the length and weight approximately 1.66 cm and 12.28 g in average, respectively. Meanwhile, the the mean of shortest length and lightest weight was found on control and treatment of vitamin E, respectively.

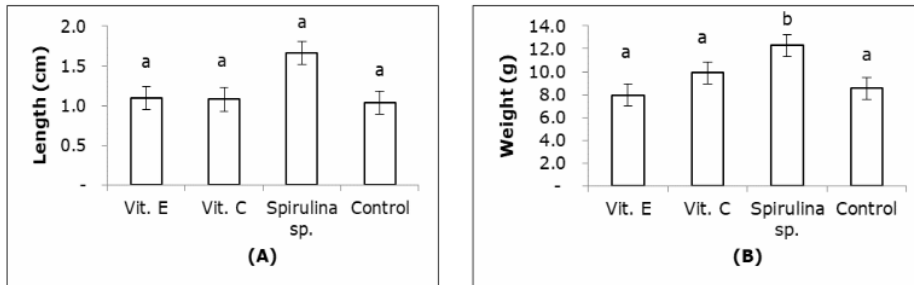


Figure 1. The growth rate of climbing perch of A) length and B) weight during research. Different superscript indicates significant differences at $\alpha = 0.05$. Error bars represent standard error.

Gonadal maturation index, gonadal weight, ovary diameter and fecundity. The data of gonadal weight, fecundity and GMI of climbing perch fish at the end of the study are presented in Table 1. The highest percentage of GMI in Figure 2A and the highest gonadal weight in Figure 2B were resulted from the vitamin E treatment. However, the *Spirulina* treatment had a significant effect compared to other treatments. The highest percentage of GMI was generated from the vitamin E treatment with a value of 11.5% and the lowest one was found in the control treatment at 4.3%. The best gonadal weight was also produced from vitamin E at 2.44 g and the lowest one was found in vitamin C treatment at 1.18 g. Similarly, the highest fecundity value was found in the vitamin E treatment with 5,719 fish eggs and the lowest one was found in the control treatment with 1,166 eggs.

Table 1

The gonad development data is taken a base on gonad weight, final weight and GSI

Treatment	Gonad weight (g)	Final weight of broodstock (g)	GSI (%)
Vitamin E	2.4±0.10	38.1±0.6	6.4±0.3
Vitamin C	1.2±0.25	37.8±2.1	3.1±0.7
Spirulina	2.0±0.19	42.6±2.1	4.8±0.3
Control	1.2±0.12	34.0±1.2	3.6±0.5

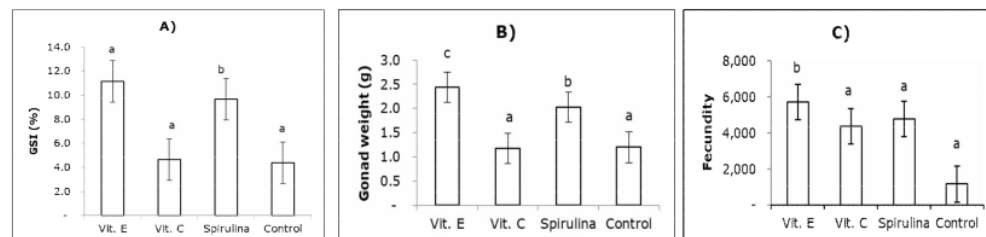


Figure 2. Gonadal development for A) GSI, b) gonad weight, and c) fecundity. Different superscript indicate significant differences at $\alpha = 0.05$. Error bars represent standard error.

The eggs of the climbing perch fish sampled every 20 days increased in size as shown in the data in Figure 3 and Figure 4. The changes in climbing perch eggs occurred on day 60 in which it began to be clearly shown by yolk eggs. These changes (Figure 4) were the result by observing fish eggs with vitamin E. The observations was conducted using a microscope with a 10X magnification lens.

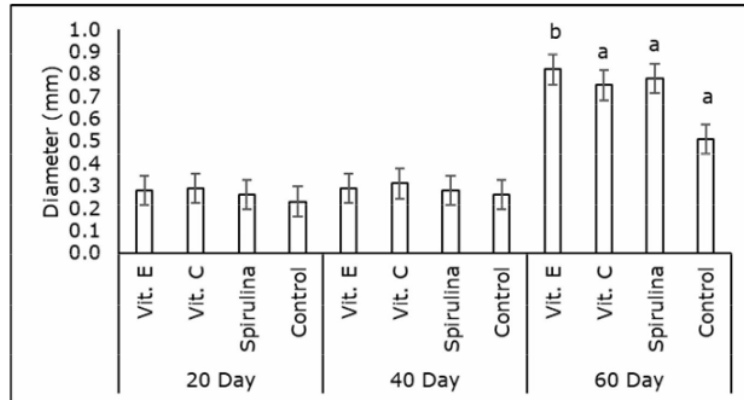


Figure 3. The ovarium diameter. The average measure on day 20, 40, and 60. Different superscript indicates significant differences at $\alpha = 0.05$. Error bars represent standard error.

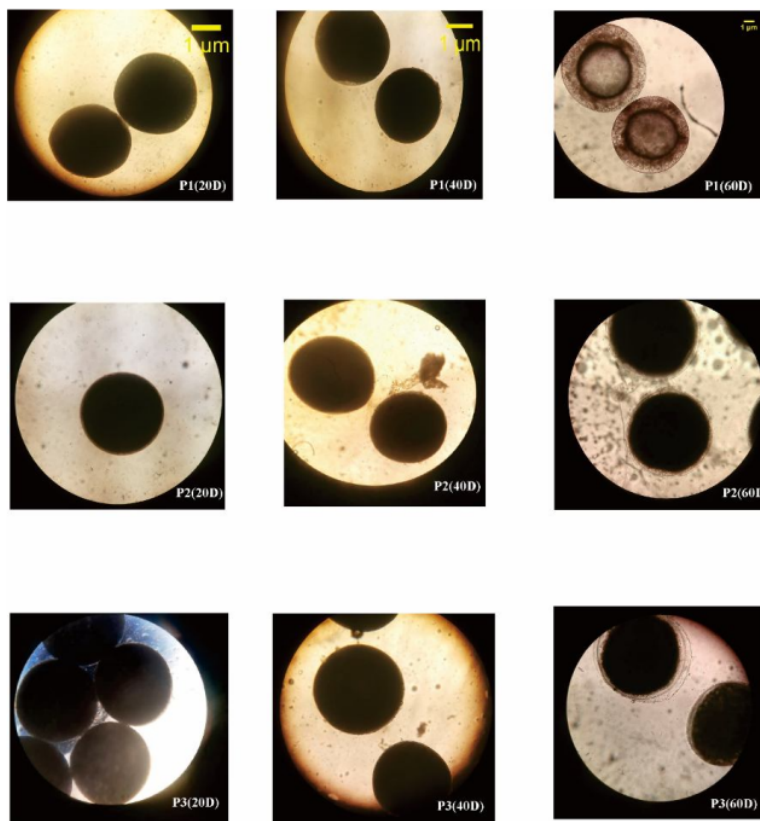


Figure 4. The vitellogenin form on P1, P2, and P3 (20D; 40D; 60D). The ovary observed during cultivation of 20th, 40th, and 60th day.

Discussion. The results of the research on the growth of climbing perch given with the supplements showed that provision of *Spirulina* can increase the growth. This result resembles to Lu & Takeuchi (2004) study finding that the use of *Spirulina* in the larvae of tilapia *O. niloticus* showed the different growth rates (lower) within 150 days after hatching (HSM)/Day Post Hatching from F1 breeding parent. It is in line with the results of the study of Teimouri et al (2013). Rainbow trout (*Oncorhynchus mykiss*) uses *Spirulina* as a dietary supplement to increase the growth performance at an estimated 10% dose of body weight. The results of the study by James et al (2006) showed that 8% *Spirulina* provision in food for red swordtail (*Xiphophorus helleri*) could give some significant results on nutritional intake and growth.

The high level of growth in treatment 3 was caused by the large amount of protein contained in *Spirulina* sp. extract, i.e. 55-70% (Vonshak 2002). *Spirulina platensis* is a blue-green algae rich in protein, vitamins, minerals and other nutrients. It can be used as a dietary supplement for food and medical treatment (Barus et al 2014). The protein contained in *Spirulina* is an energy source for fish and absolute protein needed by fish. Protein can be useful to repair the damaged cells, as one of the cell membrane forming. It can also be an energy source for fish (James et al 2006) in which this energy can increase the palatability of fish so that it can increase the growth of fish weight.

The process of ovary maturation was seen from the vitellogeny on Figure 4. The vitellogeny of climbing perch treated with vitamin E was obviously formed at 60 days after treatment compared to other treatments. Previous research of Reidel et al (2010) reported that the vitellogeny of teleostei fishes like *Rhamdia quelen* needed 90 days. This can be caused by the nutritional quality which can affect the quality of egg, fingerlings, and juvenile (Hasan et al 2007). Especially, the process of cell membrane formation required the nutrition from vitamin E such as non-saturated amino acid (linoleic fatty acid, linolenic, and phospholipid) (Aryani et al 2014).

The egg diameter of climbing perch changed from the beginning to the end of the study. The averaged egg diameter of climbing perch fish on day 20 was 0.28 mm, and on day 40 it became 0.29 mm with the increase of diameter into 0.82 mm. One of criteria for gonad maturity of female climbing perch was egg diameter of 0.6-0.8 mm (Zalina et al 2012). Thus, the average of egg diameter at the end of research under all treatments, excluding control, could be categorized into mature.

Duangjai et al (2017) reported that a significant increase of GMI in climbing perch fish ($p < 0.05$) using vitamin AD3E at a dose of 5 mL kg⁻¹ and with the value of GMI at 16%. *Spirulina* acts to increase the weight of the gonads into 350 mg and *Spirulina* mixed with vitamin E could reach a weight of 470 mg. On the other hand, the control treatment or without treatment only reached 100 mg. *Spirulina* plays a role of increasing the ovarian diameter of goldfish between 0.71 to 0.8 mm (James et al 2009). However, this study found that treatment of vitamin E had significant impact on egg diameter of climbing perch. This is because vitamin E has an antioxidant enzyme, called glutathione peroxidase (GSH-Px) that is active in several organs such as the hepatopancreas and ovaries (Barim 2009).

The ovum diameter of the climbing perch was between 0.6 and 0.84 mm in the study of Sarkar et al (2005). In the same study, fecundity was between 52,000 and 130,000 eggs with a female parent of 65.3 g in weight. The lower yield for fecundity was between 358 and 454 eggs per g (Duangjai et al 2017). Previous studies measured the fecundity of climbing perch fish obtained from nature reaching a range of 3,120 to 84,690 eggs (Marimuthu et al 2009).

Spirulina was used as the main food for *O. niloticus* fish which was under a stressful condition (Ibrahim & Ibrahim 2014). Another study explained that pellets with 2% *Spirulina* powder did not change the quality or quantity of eggs in striped jack fish (*Pseudocaranx dentex*) (Lu & Takeuchi 2004). However, it is different in the cases with freshwater fish with a positive effect on gonads. The use of *Spirulina* can be helpful in the gonadal development and maturation as, for some species, it has sufficient protein and nutrients to support the sexual maturity, egg yolk, so that it has a higher rate of fertilization (Lu & Takeuchi 2004). For gourami fish (*T. trichopterus*), *Spirulina* can significantly increase the growth and reproductive performance (Khanzadeh et al 2015).

However, growth and sexuality can be different in each generation (F1 and F2) in tilapia, because of the very plastic growth and determining factors (Lu & Takeuchi 2004).

Vitamin C and vitamin E are reported to be able to increase the specific antibodies to several species, i.e. *Monipterus albus*, rainbow trout, and catfish (*Clarias gariepinus*). However, vitamin C in milkfish (*Chanos chanos*) can produce more eggs, and vitamin E can improve the egg quality, ovaries and hatchery rate (Tan et al 2007). The addition of vitamin E supplement on female brood of climbing perch in this research had an effect on ovary diameter and fecundity. However, another environmental factors such as quality and quantity of feed as well as physical and chemical properties of water affecting the optimization of gonad maturity on climbing perch are required to be investigated in the future. Previously Marimuthu et al (2009) reported that there was positive correlation between gonadal maturation and water environment.

Conclusions. *Spirulina*, vitamin C, and vitamin E as the supplement have a significant impact on GMI, gonadal weight, fecundity, and ovarian diameter. The best values for GMI, gonadal weight, fecundity, and ovarian diameter were obtained in the use of vitamin E supplement. Further studies on gonadal maturation of climbing perch (*Anabas testudineus*) may be required using other factors in optimising its reproduction.

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