

Original article

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Reproduction performance of climbing perch *Anabas testudineus* F1 and F2 broodstock with different dietary supplementation

Performa reproduksi induk ikan betok *Anabas testudineus* F1 dan F2 dengan suplementasi pakan yang berbeda

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ABSTRACT

Reproduction enhancement of climbing perch from reared inland water in the controlled environment has done successfully to generate its broodstocks and offsprings. This species is potential to create the sustainability production in the future because they offer a promising price, so it needs a further study for optimizing the quality of broodstock and its offsprings. This study aimed to analyze the reproduction of female broodstocks from various family F1 and F2 by adding multiple dietary supplements in the different dose. The research was conducted on Fish Hatchery Unit Mulia Plaju, Palembang for three months and biology laboratory in Agriculture Faculty, University of Muhammadiyah Palembang. The research method used completely randomized design with three replications, began by rearing the female broodstock (F1 and F2) with adding dietary supplement for three treatments, they were P1: vitamin E (250 mg/kg dietary weight), P2: vitamin C (125 mg/kg dietary weight), and P3: *Spirulina* sp. (10% / dietary weight). Then, for each female broodstock from F1 and F2 of every treatment was selected for breeding with ratio two females (♀) (F1 & F2) : male F1 (♂). The research showed that reproduction performance of F2 was better than F1 in broodstock growth, fecundity, egg diameter, and larvae growth parameter, but it had low fertility than F1. Adding supplement of vitamin E to female broodstock resulted in a better productivity performance than the vitamin C and *Spirulina* sp..

Keywords: climbing perch, family, dietary supplement, reproduction

ABSTRAK

Rekayasa reproduksi ikan betok dari perairan umum telah berhasil dilakukan di lingkungan budidaya secara terkontrol untuk menghasilkan calon induk dan keturunannya. Ikan ini berpotensi untuk diproduksi lebih lanjut di masa yang akan datang karena memiliki harga yang cukup menjanjikan sehingga butuh kajian optimasi kualitas induk dan keturunannya. Penelitian ini bertujuan untuk mengetahui performa reproduksi induk betina ikan betok dari keturunan famili yang berbeda (F1 dan F2) yang diberikan suplemen berupa vitamin E, vitamin C dan *Spirulina* sp. dengan dosis yang berbeda. Penelitian ini dilakukan di Unit Pembenihan Ikan Mulia Plaju Palembang selama 3 bulan dan di Laboratorium Biologi Fakultas Pertanian Universitas Muhammadiyah Palembang. Penelitian ini menggunakan metode rancangan acak lengkap tiga perlakuan dan tiga kali ulangan, yang diawali dengan pemeliharaan induk betina F1 dan F2 dengan menggunakan suplemen pakan berbeda pada setiap perlakuan, yaitu P1: vitamin E (250 mg/kg pakan), P2: vitamin C (125 mg/kg pakan), dan P3: *Spirulina* sp. (10% per berat pakan). Penelitian dilanjutkan dengan perkawinan induk betina (♀) F1 dan F2 dengan induk jantan (♂) F1 rasio perkawinan 2:1 pada masing-masing perlakuan. Hasil penelitian ini menunjukkan bahwa performa reproduksi Induk keturunan F2 lebih baik dari pada F1 dengan parameter reproduksi berupa pertumbuhan induk, fekunditas, diameter telur, dan pertumbuhan larva, namun fertilisasi lebih rendah dibandingkan F1. Penambahan suplemen vitamin E pada pakan induk betina ikan betok memiliki produktivitas reproduksi yang lebih baik dibandingkan dengan vitamin C dan *Spirulina* sp..

Kata kunci: ikan betok, famili, suplemen pakan, reproduksi

INTRODUCTION

Fish is one of the protein sources to fulfill the human needs, and it could be obtained through capture and aquaculture activity. Among various species of fishes, the climbing perch is one of the freshwater species with high nutrition content. During the past few years, the climbing perch is massively captured in its natural habitat. It is definitely dangerous because the aquaculture activity of climbing perch has not massively developed yet to compensate its captured in natural habitat. Along with the aquaculture technology development, the stock supply of climbing perch strives with domestication and reproduction engineering to obtain a high-quality offspring. The female climbing perch has a higher growth rate than the male so that the reproduction engineering can be conducted through feminization (Helmizuryani & Muslimin, 2016).

Domestication is expected to control fish growth and reproduction which selected from a certain morphological character to maximize the aquaculture output. The domestication of climbing perch has been successfully conducted on several experiments, started with the broodstock rearing through reproduction engineering (Zworykin, 2012), the climbing perch rearing in similar sex (Helmizuryani & Muslimin, 2016; Hidayat *et al.*, 2016), semi-artificial spawning using Wofa-FH hormone (Sarkar *et al.*, 2005), LHRHa (Zalina *et al.*, 2012), and the addition of AD3E vitamin towards growth and reproduction performance.

Domestication in several fish species produced a high quality of growth and reproduction offspring, such as Atlantic salmon, rainbow trout, coho salmon, Nile tilapia, and common carp (Trygve & Matthew, 2009). It was caused by an assumption that polymorphic structure changes adjusted to environmental changes which in theory correlated to phenotype (Dunham, 2004).

Beside domestication, the quality enhancement also conducted through reproduction engineering, especially the gonad maturation of broodstock. The climbing perch which fed using tubifex has a better gonad maturation level (TKG), TKG III & TKG IV, and the fecundity reached 1.269 eggs (Muslimin *et al.*, 2013). It is caused by the appropriate nutrient content to support the gonad maturation, especially for vitellogenesis process (Bobe & Labbe, 2010; Hajizadeh *et al.*, 2008; Getinet, 2008). Fecundity of females of the climbing perch was 2430–41600 (on

average, 21366) eggs (Zworykin, 2012) and it was potentially produced 35.000 eggs (Kiran *et al.*, 2013), while the climbing perch fecundity in natural habitat could reach 36.804 eggs (Marimuthu *et al.*, 2009). To obtain high-quality offspring, surely the broodstock reproduction performances, such as the egg quality, hatching rate, and the larvae growth, are essential.

Optimization of fish reproduction can be supported by adding a supplement for the female broodstock, one of them is *Spirulina* sp.. Several experiments indicated that *Spirulina* sp. was able to accelerate the reproduction mechanism in some different species, such as Nile tilapia, red swordtail, rainbow trout, and common carp (James *et al.*, 2009; Lu & Takeuchi, 2004; James *et al.*, 2008; Teimouri *et al.*, 2013). Therefore, a further study of reproduction performance supported with supplement addition is required. This study aimed to evaluate reproduction performance of female climbing perch from a different family (F1 and F2) which supplemented with vitamin E, vitamin C, and *Spirulina* sp..

MATERIALS AND METHODS

The experiment was conducted in Fish Breeding Unit Plaju and Biology Laboratory, Agriculture Faculty, Muhammadiyah University Palembang for three months from March until June 2017. The experimental fishes were the first and the second generation (F1 and F2) which have been domesticated. The total amount of experimental fish is 90 fishes consisted of 45 female broodstocks F1 and 45 female broodstock F2. The average length of the experimental fish is 11–14 cm and the average body weight is 30–35 g. The experimental fishes were reared in a net sized 50×50×130 cm³ as many of 36 units which placed inside a 15×15 m². The experimental fishes were fed using *Spirulina* sp., vitamin C, and vitamin E mixed with commercial feed with protein content 35%.

Experimental design

This study used complete randomized design with three different treatments, P1: vitamin E (250 mg/kg feed), P2: vitamin C (125 mg/kg feed), and P3: *Spirulina* sp. (10% of total feed amount), each of them was replicated three times. The experimental fishes were observed daily to record the survival and mortality rate, using this below equation by Khanzadeh *et al.* (2015):

$$SR (\%) = \frac{Nt}{No} \times 100$$

Note :

S= survival rate (%), Nt = final population, and No = initial population.

Sampling and water quality

The sampling used 50% of the population and observed once in 20 days. The sampling parameters were the total length and weight, and also the water quality parameters (temperature, dissolved oxygen, ammonia, and pH) as supporting data. The experimental fishes were weighed using digital scale (accuracy 0.1 g) and measured the body length using a ruler. The growth performance was calculated using Abdel-Tawwab *et al.* (2008) equation.

$$Wm = Wt - Wo$$

$$Lm = Lt - Lo$$

Note :

Wm = weight growth,
Wt = final weight (g)
Wo = initial weight (g)
Lm = length growth
Lt = final length (cm)
Lo = initial length (cm)

The water temperature parameter was measured using a thermometer. The dissolved oxygen was measured using DO meter, pH was measured using pH meter, and ammonia was measured using spectrophotometer every 20 days. The eggs condition changing was also observed through stripping method to obtain the egg and then observed it by a microscope.

Spawning and larval growth

After the female broodstocks were reared, the

female broodstock was selected and prepared to mate in a semi-natural way. The broodstocks were induced using ovaprim through intramuscularly injection, and after 15 hours, the broodstock would be spawned. The fecundity, fertility rate, and hatching rate were observed. The fecundity was counted volumetrically, the entire egg volume or partially egg sample \times total amount of egg sample (Effendi, 2004). The fertility percentage was calculated by dividing the fertilized egg and total amount of the eggs, multiplied by 100%, while the hatching rate was calculated by dividing the total amount of larvae and the total amount of eggs, multiplied by 100% (Duangjai *et al.*, 2017).

After reared for approximately 30 days (1 month), on the third day, the larvae were given live food *Artemia* with ad libitum method for 25 days, and then continued with silkworm until the 30th day. Furthermore, the growth parameters sampling (body weight, body length, and survival rate) were conducted using equation by Effendi (2004).

Data analysis

The entire data was analyzed in complete randomized design table (ANOVA) with confidence range of 95%. If the result was significantly differenced so that the analyzed would be continued using LSD (least significance different) test.

RESULT AND DISCUSSION

Result

Growth performance and survival rate of the broodstock

The statistical result of weight and length growth in all treatments showed a significant difference ($P < 0.05$). Both of the broodstocks (F1 and F2) showed increases in body size. However, the result was different with the highest average

Table 1. The growth performance and survival rate of female broodstock in F1 and F2

Broodstock	Feed	Broodstock weight (g)	Broodstock length (cm)	Survival rate (%)
F1	P1	17.73 ^b	0.72 ^b	80 ^a
	P2	5.67 ^a	0.41 ^a	80 ^a
	P3	10.4 ^a	1.06 ^c	93 ^a
F2	P1	18.29 ^b	1.42 ^a	87 ^a
	P2	9.86 ^a	1.04 ^a	80 ^a
	P3	11.54 ^a	1.61 ^a	100 ^a

Note: The numbers followed by the same superscript letter in the same column indicated not significantly different ($P > 0.05$)

Table 2. Reproduction productivity of the climbing perch female broodstock

Broodstock	Feed	Fecundity (eggs)	Hatching rate (%)	Fertilization rate (%)	Egg diameter (mm)
F1	P1	25.300 ^b	89 ^a	86 ^b	0.82 ^b
	P2	15.062 ^a	88 ^a	41 ^c	0.72 ^b
	P3	24.911 ^b	90 ^a	76 ^a	0.76 ^{ab}
F2	P1	33.957 ^b	90 ^a	85 ^c	0.87 ^b
	P2	28.101 ^a	92 ^a	71 ^b	0.76 ^a
	P3	20.753 ^a	91 ^a	51 ^a	0.92 ^b

Note: The numbers followed by the same superscript letter in the same column indicated not significantly different ($P>0.05$)

length in F1 treatment. 1.06 cm (P3), 0.72 cm (P1), and 0.41 cm (P2), while in F2 treatment the highest average length was in P3 (1.61 cm) and the lowest was in P2 (1.04 cm).

On the contrary, in weight growth, F1 broodstock with the highest weight was P1 (17.73 g), and then P3 (10.40 g) and the lowest was P2 (5.67 g). However, broodstock with the highest weight was P1 (18.29 g), and the lowest was P2 (9.86 g). The treatments on the broodstocks did not affect the survival rate significantly ($P>0.05$).

Reproduction

The highest fecundity in F2 broodstock was P1 (33.957 eggs), and the lowest was P3 (20.753 eggs) which showed a significant difference in variance analysis table ($P<0.05$), while the F1 broodstock was lower than F2. The P1 treatment in F1 broodstock has 25.300 eggs, and the lowest fecundity was P2 treatment with 15.062 eggs.

The highest hatching rate in F1 progeny was in P3 treatment (90%), while in F2 progeny was in P2 (92%). It indicated no significant difference ($P>0.05$), so that the treatments didn't affect the hatching rate significantly ($P>0.05$). The highest fertilization rate in F1 was in P1 treatment (86%), while in F2 was P1 (85%), so that the treatment affected the fertilization rate significantly ($P<0.05$). The highest egg diameter in F2 was in

P3 treatment (0.92 mm), while in F1 was in P1 treatment (0.82 mm).

The growth performance and survival rate of larvae

The highest weight growth in F2 progeny was in P1 treatment (0.35 g) and the lowest was in P3 treatment (0.23 g). The highest weight growth in F1 progeny was in P2 treatment (0.22 g) and the lowest was in P1 treatment (0.12 g). According to the variance analysis, the treatment significantly affected the weight growth in both progenies (F1 and F2).

The highest length in F2 progeny was in P1 treatment (2.31 cm), while in F1 progeny was in P3 treatment (1.87 cm). The result of variance analysis in F1 progeny was not significantly different, while in F2 progeny was significantly different. During the rearing period, the highest survival rate in F1 progeny was in P1 treatment (61%), while in F2 progeny was P2 (58%) and the lowest was in P1 (48%). The variance analysis in both progenies showed not significantly different ($P>0.05$).

Discussion

Growth performance and survival rate of the broodstock

The growth performance result was quite

Table 3. The weight growth of climbing perch post-larvae in F1 and F2 progeny

Broodstock	Feed	Weight (g)	Length (cm)	Survival rate (%)
F1	P1	0.12 ^a	1.55 ^a	4 ^a
	P2	0.22 ^c	1.76 ^b	58 ^a
	P3	0.17 ^b	1.87 ^b	47 ^a
F2	P1	0.35 ^b	2.31 ^a	61 ^a
	P2	0.31 ^b	2.27 ^a	51 ^a
	P3	0.23 ^a	2.23 ^a	40 ^a

Note: The numbers followed by the same superscript letter in the same column indicated not significantly different ($P>0.05$).

varied. The F1 broodstock had higher average length, while the higher average weight was obtained from the F2 broodstock. It was assumed that the reason was that of a genetical factor which affected the various result on each progeny, in the assumption that each progeny would have a better growth performance than its broodstock (Lucas & Southgate, 2014). The weight growth was also affected by an environmental factor, one of them was feed, which contributed in the gonad weight gain in climbing perch (Bernal *et al.*, 2015) and Nile tilapia (Lu & Takeuchi, 2004).

According to Peyghan *et al.* (2012), the gonad weight gain and the broodstock weight towards the feed quality were positively correlated each other. The feed quality affected thyroid gland performance to conduct steroid and estradiol biosynthesis. The biosynthesis produced albumin and yolk as compositions to produce an egg. The survival rate of F1 broodstock was ranged from 80–93%, while the F2 broodstock was ranged from 80–100%. The survival rate result was quite similar to the earlier study using pellet without any supplement by Helmizuryani and Muslimin (2016) which the survival rate ranged from 75–100%.

During the study, the different supplement addition in pellet did not significantly affect the survival rate of the F1 broodstock. The highest survival rate was in P1 treatment. It was assumed that the vitamin E (250 g/feed amount) and its protein content were contributed in increasing the survival rate, compared to the study by Duangjai *et al.* (2017) which reared hybrid catfish (*Clarias microcephalus* × *Clarias gariepinus*) using vitamin C (500 mg/kg) and vitamin E addition (125 mg/kg). The nutrient feed content was the main factor which is necessary to grow and increase the survival rate. Helmizuryani and Muslimin (2016) explained that when the feed energy was extremely high, it would decrease feed consumption. Furthermore, the nutrient supply to the experimental fish would be low to survive.

Reproduction

The vitamin E addition in both broodstock (F1 and F2) showed a better result in the fecundity (33.957 eggs) and fertilization rate (86%). The fecundity was quite high when compared with the other study without any supplement addition. The results of the earlier study were quite varied. Perera *et al.* (2013) showed as many of 40.220 eggs, while Sarkar *et al.* (2005) showed as many of 36.447 eggs. Despite those results, the fecundity

of climbing perch was estimated to range from 3.120–84.690 eggs.

It was in accordance with the fact that vitamin E contributes in sex maturity and enzymatic activity, increases average growth and egg maturity, and also increases fertilization rate (James *et al.*, 2009; Zegin *et al.*, 2015; Duangjai *et al.*, 2017). Meanwhile, vitamin C contributes to hatching rate until 92%. Furthermore, vitamin C also contributes to increasing body weight, feed efficiency, and assist the digestion process (James *et al.*, 2009). Several species of *Spirulina* sp. even produce more intense egg colour because of its content, for instance, zeaxanthin, astaxanthin, and myxoxanthopyll, yet it does not affect the quality of the egg (Lu & Takeuchi, 2004).

The productivity of vitamin E in this study was by several results in some species. The fertilization rate of climbing perch reached up to 70% with 5 mL/kg vitamin E addition in the fish feed (Duangjai *et al.*, 2017). Vitamin E addition as many of 270 mg/kg of feed produced gonad maturity index until 4.53% (Tan *et al.*, 2007). The hatching rate of common carp would reach up to 95% when it was given 300 mg/kg of feed vitamin E addition (James *et al.*, 2009). The climbing perch which given a higher concentration of vitamin E (1200 mg/kg) would result in a better reproduction performance compared with spirulina and vitamin C addition, however for the upcoming study, it is necessary to discover the optimal vitamin E dosage for climbing perch.

Post-larvae growth

The post-larvae size in this study was quite excellent, especially the growth compared with previous studies, 5-day post-larvae grew up to 7.2 mm (Sarkar *et al.*, 2005), post-flexion (juvenile) length in the 6th day reached up to 7.2 mm (Morioka *et al.*, 2009). While in this study, the length of post-larvae from F1 broodstock was 1.87 cm and the post-larvae from F2 broodstock was 2.31 cm (Table 3). The best growth performance was in vitamin C addition treatment. It was in accordance with Darias *et al.* (2011) that vitamin C contributed to fish growth, especially in fish bone. It was because vitamin C contained hydroxylase and oxygenase enzyme which induces pro-collagen and carnitine biosynthesis in bone forming. According to Darias *et al.* (2011), although vitamin C was not an energy source, it was also required as catalysator in metabolism to support excellent growth.

CONCLUSION

The F2 broodstock showed greater reproduction performance consisted of the broodstock growth, fecundity, egg diameter, and post-larvae growth. However, the hatching rate was lower than the F1 broodstock. Vitamin E addition in the climbing perch female broodstock feed performed higher reproduction performance, compared with the vitamin C and *Spirulina* sp. addition. The quality of broodstock of climbing perch is potential to improve on the next progeny using male and female broodstock ratio 1:2, respectively. However, a further study to observe the next progeny (F3) which selected through phenotype and genetically approach are necessary.

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