Effect of *Spirulina platensis* supplementation in the diet to sperm performance of silver rasbora (*Rasbora argyrotaenia*)

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Abstract. Silver rasbora (*Rasbora argyrotaenia*) is a fish that has high economic price, a relatively short maintenance period, and cultivation can be in a narrow area. The purpose of this research was to know the effect and optimum dosage of *S. platensis* supplementation in diet to sperm performance of silver rasbora. This study was conducted in February until March 2019 at Wet Laboratory and Instrument Laboratory Universitas Airlangga, Banyuwangi Campus, Indonesia. A total 24 male of silver rasbora with initial weight of 3.96 ± 0.41 g and length of 7.58 ± 0.41 cm was randomly distributed in 4 tanks (6 fish per tank) according to 4 different treatment doses of *S. platensis* in the diet namely 0% (P0), 1% (P1), 2% (P2), and 3% (P3). The parameters were observed in this study are milt volume, sperm concentration, sperm motility, and sperm viability. Supplementation of *S. platensis* in diet was increased sperm performance of silver rasbora and the optimum dosage is 3% with the amount of milt volume, sperm motility, sperm concentration, sperm viability are 9.11 ± 0.95 μL/g body weight, 76.07 ± 0.97 %, 82.70 ± 0.79 ×10⁴ cell/μL, and 73.90 ± 1.8 %.

1. Introduction
Silver rasbora (*Rasbora argyrotaenia*) is a new aquaculture commodity and has been ornamental and consumption fish in Indonesia and neighboring countries [1, 2]. Silver rasbora culture production is limited, so wild catchments are the main source to fulfillment the demand [3]. As a new aquaculture species, the silver rasbora farming has several problems and obstacles and still developing. The focus of aquaculture developed of a new commodity or as new domesticated species is about the breeding program as a way to increase fry production without relying on wild catches. Silver rasbora has been able to spawn in captive either by induction or naturally with male and female ratio 2:1 [4]. The higher number of males than females in spawning shows that the number of sperm produced from a male is insufficient for fertilized all of the eggs from a female. The previous study in zebrafish (*Danio rerio*) shows that the higher number of males than females in spawning has resulted in a higher fertilization rate than spawning with sex ratio 1:1 [5]. In addition, the amount of milt volume of a male silver rasbora is also very little [2]. The quantity and quality of silver rasbora sperm should be increased for the development of breeding programs such as artificial spawning and cryopreservation.
There are internal and external factors affecting gamete quality in fish. Nutrient content of the feed, such as fatty acids, amino acids, minerals, and vitamins are one of the external cues that influence reproduction in fish [6, 7]. *S. platensis* is microalga that can be used as a feed additive to increase the reproductive performance of the fish broodstock because it has a high nutrient value namely vitamins (B1 and E), essential amino acids (isoleucine, leucine, phenylalanine, methionine, valine and arginine), minerals, and essential fatty acids (linolenic and linoleic fatty acids) [8, 9]. Using *S. platensis* for supplementation in the diet has been shown to increased the gonadosomatic index in three-spot gourami (*Trichopodus trichopterus*) [10] and enhanced total egg production and hatching rate in yellowtail cichlid (*Pseudotropheus acei*) [11].

The study about the effect of *S. platensis* supplementation in the fish reproduction only conducted on females until now, meanwhile in male has never been. Though the use of spirulina in mammals has been shown to increase sperm productivity such as rat [12] and boar [13]. In the present study, we aim to assess the effect of *S. platensis* supplementation in the diet to sperm performance of silver rasbora (*Rasbora argyrotaenia*).

2. Materials and methods

2.1. Fish origin, rearing, and experimental design

This research was conducted in February until March 2019 at wet laboratory and instrument laboratory Universitas Airlangga, Banyuwangi Campus, Indonesia. A total of 24 mature males of silver rasbora with an initial weight of 3.96±0.41 g and length of 7.58±0.41 cm were obtained from Technical Implementation of Development Unit of Freshwater Aquaculture of Umbulan (Pasuruan, East Java, Indonesia) in early February 2019. The fish were adapted one week before starting of the experiment in the rearing container (70 x 50 x 50 cm³) to stress minimizing during treatment.

After one week, to achieve the same initial condition, all of the fish were stripped gently in the abdominal part of the body to remove sperm. The fish randomly distributed in 4 tanks (30 × 40 × 50 cm³; 6 fish per tank) according to 4 different treatment doses of *S. platensis* supplementation in the diet namely 0% (P0/control), 1% (P1), 2% (P2), and 3% (P3). The treatment was conducted for 14 days and fish feeding three times per day (at satiation; at 08.00 am, 12.00 am, and 04.00 pm). The gentle aeration was given continuously, and the water was siphoned every day in the afternoon for maintenance of the water quality.

2.2. Feed supplementation

The feed in this research is commercial fish feed (PF-500, Prima Feed ltd.; Indonesia). The feed was mixed with *Spirulina* powder (Polaris ltd.; Indonesia) as supplement according to dosage and calcium ligno sulfonate (Progol™, Indosco ltd.; Indonesia) with a dose 5 g/kg feed as a binder. All three were mixed evenly, sprayed with distilled water (250 ml/kg feed), and shaken until *Spirulina* powder sticks evenly to the feed. Furthermore, the feed was stored in the oven at 60 °C for 24 hours.

2.3. Sperm analysis

Sperm analysis was conducted after 14 days treatment. Sperm analysis includes milt volume, sperm concentration, sperm motility, and sperm viability, which refers to the analysis procedure by Adawiyah et al. [2].

2.4. Data analysis

The data of parameters were analyzed with 95% confidence level using analysis of variance (ANOVA). Significance between doses applied was recognized using the Duncan’s multiple range test (DMRT) with SPPS version 7.0. statistical software.

3. Results and discussion
Based on the data, supplementation of *S. platensis* in the diet was affecting the sperm performance of silver rasbora showed in some parameters, namely milt volume, sperm motility, sperm concentration, and sperm viability (*P* < 0.05). All four parameters have the same tendency of value, which is directly proportional to the increase in supplementation dose, where the lowest value is at P0 (0% *S. platensis* supplementation dose), and the highest value is at P3 (3% *S. platensis* supplementation dose). The range of milt volume, sperm motility, sperm concentration, sperm viability are 6.70 ± 1.79 until 9.11±0.95 μL/g body weight, 68.68±0.55 until 76.07±0.97 %, 65.47±0.71 until 82.70±0.79×10^4 cell/μL, and 67.26±0.56 until 73.90±1.84 (Table 1).

### Table 1. Some parameters of sperm performance of silver rasbora (*Rasbora argyrotaenia*) fed with *Spirulina platensis* supplemented diet with different doses (n = 6, mean ± SD).

| Treatments | Milt volume (μL/g body weight) | Sperm motility (%) | Sperm concentration (x10^4 cell/μL) | Sperm viability (%) |
|------------|--------------------------------|--------------------|------------------------------------|--------------------|
| P0         | 6.70 ± 1.79ab                   | 68.68 ± 0.55c      | 65.47 ± 0.71d                      | 67.26 ± 0.56c      |
| P1         | 7.77 ± 1.11ab                   | 70.12 ± 1.46c      | 72.23 ± 1.17c                      | 70.58 ± 0.59b      |
| P2         | 8.22 ± 1.06ab                   | 73.93 ± 2.51b      | 74.85 ± 0.51b                      | 72.63 ± 1.63a      |
| P3         | 9.11 ± 0.95a                    | 76.07 ± 0.97a      | 82.70 ± 0.79a                      | 73.90 ± 1.84a      |

Note: P0, P1, P2, and P3 are treatment doses of *S. platensis* supplementation in the diet namely 0% (control), 1%, 2%, and 3%. The superscript in the same column indicated a significant difference (*P* < 0.05).

The water quality parameters were measured during the experiment can be seen in Table 2. The range of temperature, pH, dissolved oxygen, and total ammonia nitrogen are 25-28 ºC, 7.7-7.8, 6.8-6.9 ppm, and 0.00-0.002 ppm.

### Table 2. The water quality parameters during the experiment.

| Parameters                  | Range     |
|-----------------------------|-----------|
| Temperature (ºC)            | 25-28     |
| pH                          | 7.7-7.8   |
| Dissolved oxygen (ppm)      | 6.8-6.9   |
| Total ammoniac nitrogen (ppm)| 0.00-0.02 |

The results showed that *Spirulina platensis* supplementation was increased the sperm performance in silver rasbora. In previous study, using of *Spirulina platensis* increase the sperm performance in rats [12] and boar [13]. *S. platensis* has the high nutrient value for increase reproductive performance in the animal, namely vitamins (B1 and E), essential amino acids (isoleucine, leucine, phenylalanine, methionine, valine and arginine), minerals, and essential fatty acids (linolenic and linoleic fatty acids) [8,9]. Some parameters of sperm performance of silver rasbora that increase are milt volume, sperm motility, concentration, and viability. Based on results there are differences between treatments, and the best dose of *S. platensis* supplementation is 3%.

Finally, we recommend using *S. platensis* supplementation in the diet in rearing of male silver rasbora broodstock to increase sperm quality and production to support the increasing of hatchery production. The best dose for this application is 3%.

### 4. Conclusion

Supplementation of *S. platensis* in diet was increased sperm performance of silver rasbora and the optimum dosage is 3% with the amount of milt volume, sperm motility, sperm concentration, sperm viability are 9.11±0.95 μL/g body weight, 76.07±0.97 %, 82.70±0.79×10^4 cell/μL, and 73.90±1.84 %.

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