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Effect of vitamin E on the egg quality of Bonylip barb fish *Osteochilus vittatus* (Valenciennes, 1842)

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ABSTRACT

Bonylip barb, also called Osteochilus vittatus, is a local fish specie of significant economic value with vast potential in commercial freshwater fish culture. Traditional cultivation methods are currently employed depending on the seeds, although a low hatchery rate is assumed. The purpose of this study, therefore, was to determine the effect of dietary vitamin E on the quality of Bonylip barb eggs, with applied dosage of 0, 125, 250, and 375 mg kg^{-1}. Subsequent parameters measured include fecundity, drowned egg percentage, chemical composition of eggs, and total energy retention. The results showed the several vitamin E doses significantly (P<0.05) influenced these aforementioned constraints. Furthermore, the application of vitamin E at 375 mg kg^{-1} recorded the best dosage to improve egg quality. In addition, the fecundity was estimated at 23.484 eggs and sink egg percentage reached 92.66%, while protein and fat content were 21.43 mg kg^{-1} and 27.88% higher compared to the controlled sample. Therefore, it is concluded that the administration of vitamin E in the feed tend to increase the egg quality.

Introduction

Bonylip barb, *Osteochilus vittatus* (Valenciennes, 1842), is a local freshwater fish commodity of superior economic value feasible for development in commercial quantity (Muchlisin, 2013). This specie has been applied successfully as biocontrols to reduce phytoplankton blooms in waters (Syandri *et al.*, 2015). In addtion, the cultivated yields show high survival and reproduction rates with remarkable resistant to various diseases (Tarigan *et al.*, 2017). However, this activity is less-developed in Bonylip especially from Sumatra regions (Hermawan, 2013) due to traditional processes involved depending on seed and low hatchery rate. Therefore, efforts to enhance production is strongly influenced by reproductiion and the expected egg quality (Ibrahim *et al.*, 2018). This is achieved by enriching the feed composition, for instance, nutritional improvement is believed to be facilitated by increase in the amount of protein, fats and vitamins (Utomo, 2009). However, the feed is a significant component in vitelogenin formation (egg yolk), where the addition of certain nutrients is known to accelerate egg maturation and quality (Roy and Mollah, 2009). The use of vitamins are essentially required due to the inability to self-synthesize. Moreover, the fish has needs for these various materials based on species, size and age (Izquierdo *et al.*, 2001), and are vital to boost egg quality (Leroy *et al.*, 2014)

The role of vitamin E as an antioxidant help to protect unsaturated fatty acids in phospholipids contained in the cell membranes, hence the tendency to accelerate reproductive hormone secretion is possible (Napitu *et al.*, 2013). In addition, vitamin E defends the acids against oxidation, particularly in order to regulate the quality during steady

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embryogenesis (Yulfiperus *et al.*, 2003). These are digested in the small intestine and stored in some body parts, including adipose, liver, and other tissues (Pour *et al.*, 2011). Furthermore, vitamin E also acts as a coenzyme assisting the cytochrome P450 enzyme to synthesize cholesterol reproductive hormone formation. For this reason, the role is crucial in reproduction and egg quality improvement (Tahapari *et al.*, 2019). Previous reports showed that the addition of vitamin E in feed was successfully improved the egg production and quality of *Osteochilus kelabau* (Nasution and Nuraini, 2014), *Oreocromis niloticus* (Nacimento *et al.*, 2014), *Carrassius auratus* (Arfah *et al.*, 2013); *Osteochilus hasselti* (Tarigan *et al.*, 2017); and *Oxyeleotris mamorata* (Wahyudi *et al.*, 2016).

However, application of vitamin E on Bonylip barb to evaluate egg quality has not been presently reported. There, the objective of present study was determine to optimum dosage of vitamin E on the eeg quality of Bonylip barb *O. vittatus*.

Materials and Methods

Place and time

This study was conducted in the Laboratory of Aquatic Animal Physiology Department of Aquatic Resources Management, Faculty of Fisheries, Bogor Agricultural University, Indonesia between December 2015 to April 2016.

Experimental diets

The sample is a commercial type feed, while the powdered vitamin E was provided with 68% purity rate. In addition, proximate analysis was performed on the initial sample to determine the nutritional content (Table 1). The feed was equally mixed with vitamin E based on prescribed dosage of 0, 125, 250, and 375 mg kg^{-1}. Subsequently, the resulting material was restructured and sun-dried to obtain maximum moisture content of 10%.

Table 1. Proximate test feed used during the study

Proximate composition (% dry weight)	Doses vitamin E (mg kg^{-1} feed) 0	125	250	375
Water Content	10.09	10.09	10.09	10.09
Protein	40.65	40.65	40.65	40.65
Fat	14.08	14.08	14.08	14.08
Carbohydrates	18.69	18.69	18.69	18.69
Crude Fiber	4.25	4.25	4.25	4.25
Ash Content	12.24	12.24	12.24	12.24
GE (kkal/100 g)	436.21	436.21	436.21	436.21

Experimental fish and feeding

The healthy Bonlylip barb supplied observed the following criteria, including body length of 9-10 cm and weight of 10-11 g. In addition, 120 tails were withdrawn from the Freshwater Aquaculture Research Centre, Sempur - Bogor, West Java. The barb is in good condition with no spawning experienced and in a new phase with first level gonad maturity. An aquarium (30x30x40 cm) comprised of 12 individual of fish was used to maintain the fish for six weeks, and further treated with doses of Vitamin E such as 125 mg kg^{-1}, 250 mg kg^{-1}, 375 mg kg^{-1}. The feed was administered on three times a day at 08.00, 12.00, and 16.00 (Indonesian Time). Under these circumstances, about 85% of the water content is expected to be replaced once a week.

Fecundity

The fecundity were obtained by counting the number of eggs from the three sub-samples in fish ovaries located at the top, middle, and bottom position. Meanwhile, the data was recorded with 5% of the total gonad weight, and fecundity was calculated using the formula (Muchlisin *et al.*, 2011):

$$F = \frac{G}{Q} . X$$

Noted: F = Fecundity (egg/g total weight); G = total weight of gonad (g); X = value egg of gonad sub sample (egg); Q = total sub sample gonad of sample gonad (g).

Percentage of drowned eggs

The drowned egg percentages were appropriated with a fifty egg grains from separate ovaries and the remaining returned ito the aquarium. The drowned egg percentage was calculated using the following formula (Karina *et al.*, 2012):

$$\text{Egg sink (\%)} = \frac{\text{Number of drowned eggs}}{\text{Number of eggs observed}} \text{ x } 100$$

Chemical composition eggs analysis

Chemical composition analysis was performed using proximate approach to collect the fish ovaries from individual treatment of 15 g. Further examination was carried out to determine the protein, fat, and carbohydrate content using the AOAC methods (AOAC, 2005).

Energy retention

The energy retention was evaluated by comparing the difference in ovary energy at the beginning and end of the study divided by the energy values contained in the overall fish consumed. Therefore, the reproductive energy is calculated using the following formula (Watanabe *et al.*, 2001):

$$\text{Energy retention} = \frac{\Sigma \text{ generation in the early gonads of the study}}{\Sigma \text{ feed energy consumed during the study}} \text{ x } 100$$

Statistical analysis

The fecundity data, percentage drowned egg, chemical composition of egg, and energy retention were subjected to analysis of variance (ANOVA) with 95% confidence level. Subsenquently, the Duncan test was performed by observing siginificant differences among the treatments.

Results

Table 2 shows the results of fecundity and the parameters of drowned egg percentage observed during maintenance. Also, Table 2 indicated larger presence of vitamin E resulted to more fecundity. Subsequently, the average values produced in the treatment of vitamin E at 125 mg kg^{-1}, 250 mg kg^{-1} and 375 mg kg^{-1} were 7.02, 15.34 and 23.48 eggs per gram respectively, estimated to be higher compared to the control sample of 6.72 eggs per gram. The drowned egg measurement is one of the key factors in determining egg quality. Based on statistical analysis, vitamin E varied insignificantly ($p>0.05$), hence the result of the drowned egg improved with further dosage. Furthermore, Table 2 presents the mean drowned egg percentage by vitamin E at 125 mg kg^{-1}, 250 mg kg^{-1}, 375 mg kg^{-1} were 82.66%, 89.33%, 92.66%, respectively, estimated to be greater compared with control. This was influenced by high protein and fat content, and therefore tend to expand in egg weight potentially resulting to a higher drowned egg percentage. This inclination is due to more addition of vitamin E to the feed.

The egg quality was also determined by chemical composition (Table 3), as the components contained protein, fat, carbohydrates, crude fiber, ash content and moisture. The results on the chemical components of eg show the chemical components such as water content, ash, and crude fiber content declined as more vitamin E were added (Table 3). Conversely, increase in vitamin E results to inclined chemical components such as fat, protein and carbohydrate. The average fat and protein values in Bonylip barb ranged between 18.5 - 21.43 % and 25.45 - 27.88%, respectively. This showed a relative effect compared to the control after the treatment

Table 2. The effects of vitamin E in feed against fecundity and percentage drowned of the egg of six weeks of the experiment.

Parameters	Dose of vitamin E (mg kg^{-1} feed)			
	0	125	250	375
Fecundity (egg/female)	6.717 ^{a}	7.027^{b}	15.34^{b}	23.48^{c}
Drowned egg (%)	80.00±3.5^{a}	82.66±5.8^{ab}	89.33±3.1^{bc}	92.66±2.0^{c}

Note: The values followed by the different superscript letter in the same line significantly different (P<0.05).

The purpose of the energy retention analysis is to determine the amount of energy contained in the ovary in respect to the fat content in the egg. The results showed the initial ovary energy before the addition of vitamin E was 402.72 Kcal g^{-1} in all the treatments. However, the amount of ovary energy on the 42nd day has increased approximately from 1024.11 - 2279.75 Kcal g^{-1} along with the increasing dose of vitamin E compared with the control specified at 468.07 Kcal g^{-1}. Table 4 highlights the results of the total energy measurement.

Table 3. The chemical composition of Bonylip barb egg at the initial and end of research

Chemical composition (%)	Egg first stage	Dosage of vitamin E (mg kg^{-1})			
		0	125	250	375
Water content	58.01	57.52± 0.89^{a}	51.21± 0.95^{b}	49.39± 0.66^{c}	45.49± 1.25^{d}
Protein	21.93	23.9± 1.03^{a}	25.45± 0.46^{ab}	26.47± 1.23^{bc}	27.88± 0.74^{c}
Fat	13.52	13.52± 0.55^{a}	18.54± 1.07^{b}	18.60± 0.68^{b}	21.43± 1.09^{c}
Carbohydrate	2.02	1.84± 0.36^{a}	1.70± 0.56^{a}	2.22± 1.36^{a}	2.77± 0.66^{a}
Crude fiber	2.72	1.35± 0.07^{a}	1.70± 0.08^{b}	1.72± 0.02^{b}	1.46± 0.39^{b}
Ash content	1.91	1.87± 0.07^{a}	1.57±0.23^{b}	1.69± 0.36^{c}	1.44± 0.20^{d}
Total	100	100	100	100	100

Note: The values followed by the different superscript letter in the same line significantly different (P<0.05)

Table 4. The average value of energy retention in Bonylip barb ovary at the initial and end of research

Energy changes	Dosage of vitamin E (mg kg^{-1})			
	0	125	250	375
Initial of ovary energy (Kcal g^{-1})	402.72±1.12^{a}	402.72±1.12^{a}	402.72±1.12^{a}	402.72±1.12^{a}
End of ovary energy (Kcal g^{-1})	468.07±0.12^{a}	1024.11± 0.19^{b}	1519.02± 0.32^{c}	2279.75± 0.21^{d}

Note: The values followed by the different superscript letter in the same line significantly different (P<0.05)

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Discussion

Table 2 showed the improvement of vitamin E doses resulting to increased fecundity. The average value due to vitamin E at 125 mg kg^{-1}, 250 mg kg^{-1} and 375 mg kg^{-1} were 7.02, 15.34 and 23.48 eggs per gram, respectively. This observed a higher value compared to the control. However, previous report showed the administration of vitamin E to fish feed in the amount of 300 mg kg^{-1} provides the best fecundity value compared to comet fish (James *et al.*, 2008). Meanwhile, the administration of vitamin E at 375 mg kg^{-1} produced fecundity value of 56 eggs per gram compared to gold fish *Carassius auratus* (Arfah *et al.*, 2013). In addition, the similar reports were also reported several researcher, for instance Izquierdo *et al.* (2001), and Aryani and Hamdan (2014). This was caused by the role of vitamin E in the feed content, by acting as an antioxidant in the oxidation prevention of fatty acids. In support, previous study proved the administration of vitamin E plays a significant a role as an antioxidant, as more addition of vitamin E lowered oxidation the fatty acids (Tarigan *et al.*, 2017).

The existence of high doses of vitamin E in the feed causes an optimal increase in vitelogenin accumulation in the ovaries. This subsequently triggered egg formation with the tendency to influence fish fecundity value. In addition, reports showed the projection in the fecundity instigates the increase in the potential number of egg formation.

Furthermore, drowned egg percentage also intensified. This was due to highly unoxidized protein and fat contents contributing to expand the egg weight. Vitamin E acts as an antioxidant in preventing the oxidation of fatty acids in the egg. The percentage of drowned egg showed an improvement ranged from 82.66 - 92.66%. Previous study reports the vitamin E of 2000 mg kg^{-1} tend to boost the egg buoyancy rate by 96% in *red seabream* fish (Watanabe *et al.*, 1991). In addition, increased protein and fat also influenced the percentage of eggs to sink in water, hence leading to weight gain.

The dispensation of vitamin E tends to enhance the fat, protein and carbohydrate contents in egg cells possibly due to increasing vitelogenin during development. Also, the accumulation of vitelogenin was able to reserve fatty acid content. Meanwhile, the average fat and protein contents after treated by vitamin E are assigned relatively effects compared to control. This resulted to average values ranged between 18.5-21.43% and 25.45-27.88%, respectively, where the vitamin E acts as an antioxidant in an effort to prevent the oxidation of fatty acids during vitelogenesis. Research reported by the vitamin E potentially prevents fat oxidation during vitelogenin (Arfah *et al.*, 2013). The fatty acid content was continuously allocated to the egg during gonad development later used as energy reserves for subsequent application. This was supported by reports stating fat content increased along with vitamin E dose introduced (Mokoginta *et al.*, 2000) (Yulfiperius, 2003). Thus the protein and fat stored in the egg is used as an energy reserve.

The function of vitamin E in the feed role as an antioxidant increases the fatty acid content in the egg, believed to be converted into a major source of energy to triger development and maturation. In addition, the total energy allocated to the ovary tend to increase as egg development progress (Chansela *et al.*, 2012; Zudaire *et al.*, 2014). Also, a portion of the energy derived from the feed is allocated into egg development (Fernandes, 2012). Therefore, beside effect on egg quality, the vitamin E is also playing important role in fish growing (Muchlisin *et al.*, 2016).

Conclusions

Based on results and discussions, vitamin E dose at 375 mg kg^{-1} is the best dosage to increase the egg quality of Bonylip barb in terms of fecundity, percentage of sink egg, protein and fat content, and energy retention, where the values recorded were

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23.44, 92.66, 21.43, 27.88, and 2279.75 Kcal g^{-1}, respectively.

Declarations of interest

The author(s) declare that there is no conflict of interest with regards to the research, authorship and/ or publication of this article.

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