The effects of turmeric flour *Curcuma longa* on fertilization, hatching and survival rates of seurukan fish *Osteochilus vittatus*

D Yusnita\textsuperscript{1}, Y Ibrahim\textsuperscript{2}\textsuperscript{*}, F Saputra\textsuperscript{2}

\textsuperscript{1}Department of Fishery, Faculty of Fisheries and Marine Science, University of Teuku Umar, Indonesia  
\textsuperscript{2}Department of Aquaculture, Department of Fishery, Faculty of Fisheries and Marine Science, University of Teuku Umar, Indonesia

\textsuperscript{*}Email: yusranibrahim@utu.ac.id

**Abstract.** Phytoestrogens, one of the biochemicals derived from turmeric plant, possessed a structural similarities to estradiol, which may possibly exert similar activities in stimulating liver to synthesize vitellogenin. This vitellogenesis is important part in follicular development. This study aimed to evaluate the effects of turmeric flour (*Curcuma longa*) on the fertilization, hatching and survival rate of Serukan fish (*Osteochilus vittatus*). Completely randomized design consisting of four treatments and three replications were used in this study. A total of 10 matured females were fed with turmeric flour for eight weeks at dose of 0, 0.25, 0.5, and 0.75 gr per kg feed. The results showed that turmeric flour could improve the survival rate of Serukan fish (Sig < 0.05). Specifically, administration of turmeric flour at level of 0.5 gr per kg feed could increase survival rate up to 95.78 ± 2.0 %, being much higher than control reaching up to 55.78 ± 1.9%. However, the fertilization and hatching rate seemed to be unaltered by the treatments (Sig>0.05). This study suggested that turmeric flour could significantly affect the survival of the early life of serukan fish.

1. Introduction
Domestification in fish significantly contributes to the growing fish production, and practically it covers many aspects such as acclimatization, feed, reproduction, fish health, and genetics. To date, production of serukan fish *Osteochilus vittatus* has mostly relied on fish seed from nature; while the seeding is still hampered by technological aspect such as low survival rate. For this reason, studies on factors affecting seeding process, gonad development, and growth performance of serukan fish are required. Technological upgrading of serukan fish is important since it has high economic value, with the price of Rp 30,000-35,000 per kg, being a potential candidate for aquaculture species [1]. Regarding to seed production, larval rearing including hatching stage shows a significant contribution to quality and quantity of the seed, in which the stage is remarkably altered by external and internal factors. The internal factors include hormone and yolk volume. Secretion of the hormone by hypophysis and thyroid serves key roles in promoting metamorphosis, while yolk sac is a vital component that regulates the embryonic stages. On the other hand, some external factors are temperature, pH, salinity [2], dissolved gasses such as oxygen, CO$_2$, ammonium, and light intensity[3]. In addition, feed quality is also an essential factor for development of fish gonad, reproduction
performance, fecundity, hatching rate, and survival rate [4,5]. In fish farming, nutrients in feed account for formation of vitellogenin which is a precursor protein of egg yolk. To enrich the quality of feed, incorporation of turmeric (Curcuma longa) on fish diets has been studied. It is well known as a medicinal herb that is reported to have advantages, such as improving productivity. The beneficial properties of the herb come from a variety of biochemicals including curcumin, essential oil, fat, carbohydrate, protein, vitamin C, mineral salts (iron, phosphor, calcium). Curcumin, an active compound in turmeric, serves as an antioxidant, anti-inflammatory, as well as acts in cellular recovery in liver and oviduct cells [3].

Phytoestrogen in turmeric was found to have similar characteristics to estradiol, capable of inducing biosynthesis of vitellogenin in liver [6]. Turmeric can increase liver activity to metabolize nutrients required for vitellogenin synthesis, which finally can increase follicle development. Besides, curcumin in turmeric has a hepatoprotective activity, preventing destruction of liver cells through acceleration of liver cell regeneration and protection of liver cells against hepatotoxic compounds[3]. Previous studies explained that turmeric can accelerate the process of gonadal development and growth performance serukan fish [7], Green terror [8], Nile tilapia [9] Common carp [10] and Clarias gariepinus [11]. In short, this present work was to investigate the effects of reproductive performance and survival rate of serukan fish fed with diets containing turmeric flour.

2. Materials and Method
The 6 month-old broodfish (average weight of 60-80 g) was used. Completely randomized design consisting of 4 treatments was made and performed at triplicates. The diet was supplemented with turmeric flour at various levels: 0, 0.25, 0.5 and 0.75 g per kg feed. The fish was maintained at a density of 10 fish per container and treated for 14 days. Restricted feeding was carried out at feeding rate (FR) of 3% per day, given two times a day (08.00 am, 06.00 pm). Prior to spawning, the broodfish was selected for injection, enabling to gain the best-growing spawners (disease- and defects-free fish). A female with the best gonadal maturity has a well-rounded belly, slower swimming speed, and a swollen and reddish genital papilla. The sexual maturity of female was also indicated by emission of thick and white fluid (sperm) upon hand pressure onto the abdomen. The spawning was induced by ovaprim at the dose of 0.5 ml/kg body weight.

Parameters
Fertilization Rate (FR)
FR was used to calculate how many oocytes become fertilized by sperm, expressed as percentage. The viable eggs were transparent in appearance. The measurement was carried out to observe the fertility of the eggs sampled one hour after spawning. FR was calculated based on Muchlisin et al [12].

\[
FR = \frac{\text{the number of fertilized eggs}}{\text{the number of incubated eggs}} \times 100
\]

Hatching Rate (HR)
HR was defined as the percentage of eggs hatched to eggs fertilized within a period of time (%), calculated based on Muchlisin et al [12].

\[
HR = \frac{\text{the number of hatched eggs}}{\text{the number of incubated eggs}} \times 100
\]

Survival Rate (SR)
SR demonstrated the viability of organisms within a particular period of time, and calculated based on Muchlisin et al [13].

\[
SR = \frac{N_0 - N_t}{N_0} \times 100
\]
where \( N_t \) is the number of fish dead during the experiment, while \( N_0 \) is the number of fish at the start of the experiment.

3. Results and Discussion

The results of our experiment were presented in Table 1. Based on statistical analysis, we could argue that administration of turmeric flour into feed did not affect fertilization rate (FR) and hatching rate (HR); however, the treatments showed a significant effect on survival rate (SR) of serukan fish.

Table 1. Reproductive and survival performance of serukan fish fed with diets containing turmeric flour.

| Parameters          | 0 g/kg  | 0.25 g/kg | 0.5 g/kg  | 0.75 g/kg |
|---------------------|---------|-----------|-----------|-----------|
| Fertilization Rate  | 95±1.7  | 95±1.0    | 95.3±1.5  | 95.3±1.2  |
| Hatching Rate (%)   | 94.3±0.6 | 95±1.0   | 94.7±0.6  | 94.7±0.6  |
| Survival Rate (%)   | 55.78±1.9 | 89.33±2.1 | 95.78±2.0 | 80.11±2.3 |

Different superscript letter on the line indicate significant differences between treatments (sig < 0.05)

Fertilization Rate
The results indicated that the pattern between parameters tended to be similar. In this case, FR was found to have the highest percentage (95.3%) at doses of 0.5 g/kg and 0.75 g/kg, being slightly higher than treatment of 0.25 g per kg and control, reaching up to 95%. Under normal conditions, HR of serukan fish was found to be good, thus the addition of turmeric flour had no effect. FR represents the ability of sperm cells and oocytes to combine in cytoplasm for generating zygotes [14]; and then the fertilization occurred in the body. The fertilized eggs appeared clear and transparent, which was not observed in unfertilized eggs with white in color. Failure of egg fertilization occurred due to low quality of the eggs, which was caused by many factors such as oxygen supply to the egg. During fertilization, genetic materials of both sperm cells and oocytes were combined. Fish fertilization was often carried out in the out of the fish body (external). After released, the eggs were highly sticky due to presence of an adhesive layer activated after in contact with water. The egg attachment may be very strong, which make it difficult to harvest. However, the adhesiveness gradually reduced with the progression of egg maturity[15].

Hatching Rate
The hatching rate (HR) between treatments showed a slight difference. The highest HR was attributed to treatment of 0.25 g/kg, i.e. 95%, followed by treatment of 0.5 and 0.75 g/kg reaching up to 94.7%, and control reaching up to 94.3%. Statistical analysis also showed that the treatments did not influence HR significantly. HR represents the percentage of larval to eggs fertilized. This is a critical parameter since production of high quality seed is affected by physical and chemical features, and genetics. Absence of one or more the factors, the egg could not grow optimally in various stadia. Egg quality was highly dictated by fertilization, morphology, and egg size [16]. Essential fatty acids served important roles in developing egg morphology, particularly in formation of cellular membrane, as well as in synthesizing precursor prostaglandin, which make it less susceptible towards defects. In short, the chemical improved HR [17].

Survival Rate
Our experimental data demonstrated that survival rate (SR) reached maximum level at treatment of 0.5 g/kg (95.78%), then followed by treatment of 0.25 g/kg (95.78%), 0.75 g/kg (80.11%), and control (55.78%). Evaluation by ANOVA revealed that the treatment showed significant effect on SR. As stated above, diet containing turmeric flour at 0.5 g/kg exhibited the highest level of SR. At this treatment, the fish may have the highest supply of fatty acids from turmeric flour. The fatty acids were deposited in larvae, which were then used for nutritional source in further larval stages. At initial stage, larvae cannot utilized nutritional source from feed, thus they depend highly on yolk as the main energy source.
source. The presence of yolk is a determinant factor for SR of fish seed, while SR is also dictated by external factor such as feed availability. The early stage larvae incapable of utilizing nutrients from feed still relied on endogenous feeding, in which egg yolk acts as a stored energy that is important for survival [15]. In addition, yolk absorption rate is influenced by composition of turmeric flour incorporated to feed [17].

4. Conclusion
This study suggested that turmeric flour could significantly affect the survival of the early life of Serukan fish.

References
[1] Muchlisin Z A 2013 *Jurnal Iktiologi Indonesia* 13 91-96
[2] Kamler 1992 *Early Life History of Fish An Energetic Approach* (London: Chapman and Hill)
[3] Sukendi 2003 *Vitellogenesis and Fertilization Manipulation* Riau University Pekanbaru
[4] Muchlisin Z A 2005 *Biologi* 4(6) 411-427
[5] Abidin M Z, Hashim R and Chien A C S 2006 *Aquaculture Research* 37 416-418
[6] Somchit M N, Zuraini A, Bustaman A A, Sulaiman M R and Noratunlina R *International Journal of Pharmacology*
[7] Ravindar P N, Babu K N and Silverman K 2007 *Turmeric. The Genus Curcuma.* (London: CRC Press)
[8] Ibrahim Y *Journal of Akuakultura* 2(2)
[9] Mooraki N, Batmany Y, Zoriejahra S J and Kakoolaki S H 2018 *Journal of Survey in Fisheries Sciences*. 5(2) 37-47
[10] Mahmoud M A, El-Lamie M M, Dessouki A A and Yusuf M F 2014 *Global Research Journal of Fishery Science and Aquaculture* 1(12) 026-033
[11] Tawwab A, Mohsen, Abbas and Fayza E 2016 *Journal of the World Aquaculture Society*
[12] Muchlisin Z A, G Arfandi, M Adlim, N Fadli, S Sugianto 2014 *AACL Bioflux* 7(5): 412-418.
[13] Muchlisin Z A, A A Arisa, A A Muhammadar, N Fadli, I I Arisa, M N Siti Azizah 2016 *Archives of Polish Fisheries* 24: 47-52.
[14] Adeshina I, Adewale Y A and Tiamiyu L O 2017 *West African Journal of Applied Ecology* 25(2) 87-99
[15] Nainggolan 2014 *Improving Reproduction Quality of Catfish Clarias sp Broodstock Using a Combination of Diet with Supplement Spirulina platensis and the Oodev* (Indonesia: IPB University)
[16] Darwisito S 2006 *Reproductive Performance of Nile Tilapia Oreochromis niloticus Supplemented with Fish Oil and Vitamin E in Diets and Reared at Different Salinity* (Indonesia: IPB University)
[17] Utiah A, Zairin M J, Mokoginta I, Affandi R and Sumantadinata K 2007 *Jurnal Akukultur Indonesia* 6(1)