Original article

DOI: 10.19027/jai.18.1.23-32

Female maturation and rematuration acceleration of Mutiara strain catfish *Clarias gariepinus* using combination of oocyte developer hormone and astaxanthin addition diet

Maturasi dan percepatan rematurasi ikan lele Mutiara *Clarias gariepinus* betina dengan kombinasi hormon Oodev dan suplementasi astaxanthin pada pakan

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(Received August 10, 2018; Accepted November 2, 2018)

ABSTRACT

Reproductive design for gonadal maturation process mostly related with some factors such as environmental signals, reproductive organs, hormonal and nutrition. This research was conducted on female Mutiara strain of North African catfish, *Clarias gariepinus* by combining two kinds of materials administered to broodstock diet, namely oocyte developer (Oodev) which contains of PMSG hormone and antidopamin, and astaxanthin carotenoid. Research designs were divided into C (Control), A50 (astaxanthin 50 mg/kg feed), A100 (astaxanthin 100 mg/kg feed), Od0.5 (Oodev 0.5 mL/kg fish for two weeks), Od1 (1 mL/kg fish for 2 weeks), Od0.5A50 (combined Od0.5 with A50), Od1A50 (combined Od1 with A50), Od0.5A100 (combined Od0.5 with A100), and Od1A100 (combined Od1 with A100). This research was performed during twelve weeks of feeding. The Od1A100 treatment showed the best reproduction performance result compared to other treatment with highest hepatosomatic (HSI) and gonadosomatic (HSI) indexes ($P<0.05$), also fastest increase in egg diameters ($P<0.05$), shorter rematuration periods and highest proportion of mature broodstock. These results indicated that Oodev and astaxanthin could accelerate gonadal maturity in female broodstock of Mutiara catfish.

Keywords: Broodstock, hormonal, reproduction, oocyte developer, astaxanthin

ABSTRAK

Rekayasa reproduksi untuk proses pematangan gonad sebagian besar terkait dengan beberapa faktor seperti sinyal lingkungan, organ reproduksi, hormonal dan nutrisi. Penelitian ini dilakukan terhadap strain ikan lele Mutiara *Clarias gariepinus* betina menggunakan dua bahan yang dicampur pada pakan induk, yaitu oocyte developer (Oodev) yang mengandung hormon PMSG dan antidopamin, dan karotenoid astaxanthin. Eksperimen yang dirancang adalah K (Kontrol), A50 (Astaxanthin 50 mg/kg pakan), A100 (Astaxanthin 100 mg/kg pakan), Od0.5 (Oodev 0,5 mL/kg induk untuk 2 minggu), Od1 (Oodev 1 mL/kg induk untuk 2 minggu), Od0.5A50 (kombinasi Od0.5 dan A50), Od1A50 (kombinasi Od1 dan A50), Od0.5A100 (kombinasi Od0.5 dan A100), dan Od1A100 (kombinasi Od1 dan A100). Penelitian ini dilakukan dengan memberi makan dua belas minggu. Performa reproduksi terbaik didapat pada perlakuan Od1A100. Od1A100 memiliki indeks hepatosomatik (HSI) dan gonadosomatik (HSI) tertinggi ($P<0,05$), juga diameter telur paling cepat besar ($P<0,05$), periode rematurasi terpendek, dan proporsi induk matang gonad tertinggi. Hasil ini menunjukkan bahwa Oodev dan astaxanthin dapat mempercepat kematangan gonad pada induk betina ikah lele Mutiara.

Kata kunci: Induk, hormon, reproduksi, oocyte developer, astaxanthin

INTRODUCTION

North African catfish *Clarias gariepinus* which is high economic value commodity, has been widely produced in Indonesia. According to Ministry of Marine Affairs and Fisheries (MMAF) of Indonesian Republic (2015), its production increased 37.13 % yearly. The North African catfish production continues to grow as the increases demand on local and international markets, from 144.744 tons at 2009 become 679.376 tons at 2014. However, North African catfish production has been marked by scarcity of the seeds problems, caused by the gap between increased market demands which was not followed by increased in seeds supply because the seeds production is still very low. This is because North African catfish are like other tropical fish that breeding seasonally (Saadony *et al.,* 2014), and only breeding during the rainy season (Dadebo *et al.,* 2011).

Mutiara strain of the North African catfish (*Clarias gariepinus*) which has various superior traits including growth, feed efficiency, size homogeneity, and resistance to disease and environmental conditions originated from The Ministry of Marine and Fisheries Affairs breeding result also requires long time to get mature. According to Iswanto *et al.* (2016) the female Mutiara catfish start mature on 10 months old and after spawning the rematuraion needs 1.5 months like other African catfish. The maturation processes of *C. gariepinus* kept in outdoor ponds are influenced by annual changes in water temperature, photoperiodicity, and the final triggering of spawning is usually caused by a raise in water level due to rainfall (De Graaf & Janseen, 1996).

Mature female *C. gariepinus* broodstock contains 15–20 % eggs of body weight if the water temperature is above 22°C, however the oocyte development drop if the temperature dropped below 22°C. At the peak of dry season (three months), water temperature dropped below 22°C, causes the ovaries produce only 5% eggs of female broodstock body weight. In addition the number of eggs produced dropped and the quality of eggs produced are decreased, seen from the decreased hatching percentage (De Graaf & Janseen, 1996). Therefore, even though North African catfish seeds can produced throughout the year, the optimal time of seed production is only nine months.

One way to get optimal fish breeding is by improving the reproductive performance through nutritional supplementation in the broodstock diet, which combined with hormonal rematuration induction (Nainggolan *et al.,* 2014). Nutrient quality improvements of broodstock diet and the ability to store energy can determine fish maturation time (Mañanós *et al.,* 2008), and the use of exogenous hormones is an effective way to induce maturation of eggs. Furthermore, hormonal manipulations can be used as management tools to enhance and synchronize egg maturation, spermiation and facilitate hatching operations in most fish culture (Mylonas *et al.,* 2010).

The fish female reproductive cycle is separated in the growth (gametogenesis) and maturation phase (oocyte maturation), controlled by the reproductive hormones of the brain, pituitary and gonad. Although the growth phase of reproductive development concluded, in captivity, most fishes oocyte maturation (OM) and ovulation in females may require exogenous hormonal therapies (Mylonas *et al.,* 2010). Maturation hormonal induction basically consists of two main principles: first by increasing the secretion of follicle stimulating hormone (FSH) of pituitary which will stimulate theca and granulose cells ovarian follicle to secrete sex steroid hormones estradiol-17β (E_2) that promotes oogonial proliferation and vitellogenesis, and progestogens that promotes initiation of germ cell meiosis and follicular maturation and ovulation (Reading & Sullivan, 2011). Secondly, by inhibiting the action of dopamine which acts as gonadotropin release inhibiting factor (GRIF) which can inhibit the release of FSH in oocyte maturation (Bryant *et al.,* 2016). According to Dufour *et al.* (2010) combined treatment with gonadotrophin releasing hormone (GnRH) agonist and anti-dopamine (AD) as a new method to induce spawning in aquaculture.

Hormonal induction to accelerate gonadal maturation has been widely applied to broodstock fish. One of them by using Oodev (oocyte developer), a premix hormone which contains PMSG (pregnant mare serum gonadotropin) and AD. The principle and function of this hormone premix are to stimulate a spike of GnRH level, which in turn will stimulate the pituitary to produce gonadotropin (Sudrajat *et al.,* 2016). The study results showed that the use of Oodev combined with *Spirulina platensis* supplementation in North African catfish broodstock diet can accelerate

gonadal maturation (Nainggolan *et al.,* 2014). Another study showed that Oodev combined with *Indigofera zollingeriana* plants able to accelerate gonadal maturation also increase protein levels and lower fat levels especially cholesterol in grass carp (*Ctenopharyngodon idella*) fish gonad (Mulyasih *et al.,* 2016).

Carotenoids suspected ingredient contained in *Spirulina platensis* and *Indigofera zollingeriana* that collaborate with Oodev hormones in accelerating gonadal maturation. Fish not only store carotenoids in integument but also accumulate in their gonads, which can improve ovarian development and fertilization. Polar carotenoids preferentially absorb more in many species of fish, particularly astaxanthin rather than canthaxanthin, zeaxanthin or carotenes (García-Chavarría & Lara-Flores, 2013). Carotenoids particularly astaxanthin are recognized to be involved in the reproductive processes of many organisms due to their accumulation within reproductive organs. It has been claimed that astaxanthin triggers a speedier oocyte maturation in rainbow trout (Lim *et al.,* 2017).

Considering biological function and role of Oodev hormone and astaxanthin in the reproductive cycle of fish, the objective of the present research was to investigate whether reproductive characteristics such gonadal maturation and percentage of maturation were correlated with the dietary of Oodev hormone and astaxanthin toward female North African catfish broodstock.

MATERIALS AND METHODS

The experiment was conducted at The Aquaculture Development of Pangasius and Clarias Catfish (ADPCC) Station of Marine and Fisheries Department of West Java Province (Subang, Indonesia) from August to November 2017. In order to compare diets from various amounts of Oodev hormone and astaxanthin with the control diet, nine treatments were designated for the study.

One-year-old of female Mutiara strain North African catfish from ADPCC with an average body weight of 550 ± 45.22 g were used as broodstock. The fish were tagged and randomly distributed into nine outdoor walled concrete ground based tanks (2×1.5×1.5 m³) with a total of 360 female distributed in nine tanks (40 fish in each tank). Before the treatment, the fish were adapted for two weeks and fed with control diet twice a day (09.00 am and 16.00 pm) for 2 % of biomass per day. Water quality status was daily examined as follow: temperature between 25–28.7ºC, pH between 6.6–7.8, dissolved oxygen between 4–6.5 mg/L, and ammonia between 0.01–0.09 mg/L.

The Method used in this study was the experimental model using completely randomize design (Table 1). Three different dosages of Oodev hormone in fish feed: 0, 0.5 and 1 mL/kg of broodstock for two weeks, and second was three different dosages of astaxanthin supplement in fish feed: 0, 50, and 100 mg/kg feed.

Table 1. Research experimental design*

Astaxanthin (mg/kg feed)	Oodev (mL/kg fish for 2 weeks)		
	0	0.5	1
0	C	Od0.5	Od1
50	A50	Od0.5A50	Od1A50
100	A100	Od0.5A100	Od1A100

Note: *C is Control with 0 mL/kg Oodev and 0 mg/kg Astaxanthin, Od0.5 consisted of 0.5 mL/kg Oodev and 0 mg/kg astaxanthin, Od1 consisted of 1 mL/kg Oodev and 0 mg/kg astaxanthin, A50 consist of 0 mL/kg Oodev and 50 mg/kg astaxanthin, Od0.5A50 consist of 0.5 mL/kg Oodev and 50 mg/kg astaxanthin, Od1A50 consisted of 1 mL/kg Oodev and 50 mg/kg astaxanthin, A100 consisted of 0 mL/kg Oodev and 100 mg/kg astaxanthin, Od0.5A100 consisted of 0.5 mL/kg Oodev and 100 mg/kg astaxanthin, Od1A100 consisted of 1 mL/kg Oodev and 100 mg/kg astaxanthin.

Preparation of experimental diet

The experimental diet ware performed by using commercial fish pellets (36 % protein, 5 % fat, 4 % fiber, 10 % ash, and 11 % moisture) which were enriched with Oodev hormone and astaxanthin. The hormone added to the feed was Oodev (oocyte developer) which is a developed hormone product from Reproduction and Genetics Laboratory of Aquaculture Department of Bogor Agricultural University. Oodev contains PMSG and anti-dopamine. The administered dosage of Oodev was 0, 0.5 and 1 mL/kg of fish for two weeks. Astaxanthin was as Carophyll ® pink 10 % DSM Nutrition and the administered dosage was 0, 50 and 100 mg/kg of feed. Astaxanthin obtained from The Research Institute for Ornamental Fish Aquaculture of Ministry of Marine Affairs and Fisheries of the Republic of Indonesia (BRPIH Depok).

The process of feed coating was started with the addition of water to designated amount of

Oodev and astaxanthin and sprayed to the commercial feed. Furthermore, carboxymethyl cellulose (CMC) powder was added as a binder 1 % of commercial feed to the commercial feed mix with Oodev and/ or astaxanthin on the surface layer of the feed. The feed was then dried indoor for two hours at 27 ºC. The prepared feeds were put into container and kept in room temperature. The feed preparation process was performed once in two weeks and given 2 % of biomass per day for 12 weeks.

Reproductive parameters measurement

Measurements of fish body, gonad and liver weights were performed at weeks 0, 2, 4, 6, 8, 10 and 12. The measurement of gonad and liver weights was to calculate the gonadosomatic (GSI) and hepatosomatic (HSI) indexes. Gonads and liver are obtained by dissections of four broodstock in every two weeks for each treatment. Gonads and hearts are then weighed in digital scales. The index were calculated according to formula by Tyor and Pahwa (2017) as following:

$$GSI\ (\%) = \frac{\text{Gonad weight}}{\text{Fish body weight}} \times 100$$

$$HSI\ (\%) = \frac{\text{Liver weight}}{\text{Fish body weight}} \times 100$$

Measurement of eggs diameter was performed by cannulation using catheter with 100 egg samples per treatment. Eggs samples were fixed in formaldehyde alcohol acetic acid (FAA) solution with ratio of 6:3:1, microscope and digital ocular micrometer of Olympus AnalySIS Image Processing v. 5.1 software were used to determine the eggs diameter. The measurement of eggs diameter has been performed at Research Institute for Fish Breeding, Sukamandi-West Java.

The rematuration period was measured after the broodstock that has been tagged mature then spawned until the same broodstock mature again. The length of rematuration time was observed weekly for 12 weeks during the raising 20 broodstocks for each treatment.

Data analysis

The obtained results were compared based on mean number of replications and with the inclusion of standard error of measurement. Periods and length of rematuration were described by descriptive statistic. Whereas, analysis of GSI, HSI, eggs diameter, and total fecundity were performed using one-way analysis of variance to compare the data. In order to find out whether or not there was any significant difference (if any) between the means, Tukey test was used, at $P<0.05$ level of significance.

RESULTS AND DISCUSSION

Results

The broodstock growth

The average body weight are presented on Table 2. The average body weight of matured female Mutiara strain catfish two-weekly showed an increasing trend on all treatments during 12 weeks of observation. However, the average body weight between treatments in the same week showed no significant difference ($P>0.05$).

Gonadal somatic and hepatosomatic indexes

Gonadal somatic index (GSI) is an indicator of broodstock gonadal and maturity level

Table 2. Average body weight of experimental fish two-weekly

Treatment	Average body weight (g) week of**						
	0	2nd	4th	6th	8th	10th	12th
C	568 ± 45.37a	699 ± 25.45a	817 ± 36.74a	949 ± 36.95a	1.10 ± 26.31a	1.24 ± 43.75a	1.35 ± 35.55a
A50	578 ± 42.66a	697 ± 36.37a	811 ± 36.63a	946 ± 41.37a	1.08 ± 47.42a	1.22 ± 35.61a	1.34 ± 28.43a
A100	572 ± 42.77a	705 ± 27.66a	807 ± 30.88a	942 ± 39.61a	1.08 ± 39.27a	1.23 ± 55.14a	1.34 ± 30.45a
Od0.5	582 ± 50.19a	704 ± 30.20a	834 ± 45.50a	965 ± 22.20a	1.08 ± 44.42a	1.22 ± 37.89a	1.34 ± 27.38a
Od1	559 ± 45.25a	717 ± 29.40a	811 ± 46.46a	947 ± 41.81a	1.06 ± 43.58a	1.23 ± 36.04a	1.33 ± 33.88a
Od0.5A50	563 ± 44.68a	702 ± 26.47a	821 ± 38.45a	954 ± 43.38a	1.08 ± 40.77a	1.23 ± 36.88a	1.34 ± 25.88a
Od0.5A100	559 ± 39.67a	710 ± 26.91a	814 ± 33.20a	943 ± 43.79a	1.08 ± 38.59a	1.22 ± 46.04a	1.35 ± 25.46a
Od1A50	578 ± 40.11a	708 ± 30.80a	814 ± 37.62a	930 ± 40.23a	1.08 ± 43.73a	1.22 ± 44.67a	1.35 ± 30.52a
Od1A100	575 ± 46.12a	705 ± 23.62a	809 ± 35.20a	950 ± 40.92a	1.08 ± 42.10a	1.22 ± 45.69a	1.34 ± 32.90a

*Note: C= Control, A= Astaxanthin, Od= Oodev. Number= dose; ** The same superscript letter in the same column indicate no significant differences between treatments in the same week ($P>0.05$).

development that will increase when the fish has matured and dropped drastically thereafter. The GSI is also used to study the spawning period. Whereas the hepatosomatic index (HSI) is an indicator of vitellogenesis process in the liver where it produces vitellogenin as yolk egg formers (Muddasir & Neelofar, 2017).

The GSI values are shown in Figure 1. From figure 1 above, it can be seen that the GSI values of all treatments were not significantly different (P>0.05) before the treatment was given (week 0). However, GSI values were significantly different (P<0.05) on the 2nd week to the 12th week. The variation of GSI value began to be seen since the 2nd week with the highest peak starting at the 4th week on Od1A100 (1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin) treatment of 20.00±0.22 %, while the lowest GSI peak value at the 4th week of the control was 7.62 ± 0.33 %. The combination of the Od1A100 (1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin) treatment resulted the highest GSI value, with three peaks, at the 4th, 8th and 12th weeks, with the highest values peak at the 4th week, while the highest peak of the control only occurred at the 6th week by 12.62 ± 0.15 %.

The HSI values are shown in Figure 2. From figure 2 above, it can be seen that the HSI values of all treatments were not significantly different (P> 0.05) before the treatment was given (week 0), t. However, the values of HSI were significantly different (P<0.05) from the 2nd week to the 12th week.

The variations of HSI value also began to be seen since the 2nd week with the highest level on the Od1A100 (1 mL/kg Broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin) treatment of 1.88 ± 0.06 % and the lowest HSI value at the 2nd week on the control was 1.02 ± 0.07 %. The combination of Oodev and astaxanthin on the Od1A100 treatment also made the highest HSI value with three peaks, namely at the 2nd, 8th, and 12th weeks, with the highest value of the peak at the 12th week of 2.00 ± 0.10 %, while in control, the highest peak at the 6th week of 1.5 ± 0.02 %. The increase of HSI value is due to the synthesis and secretion of vitellogenin in the liver.

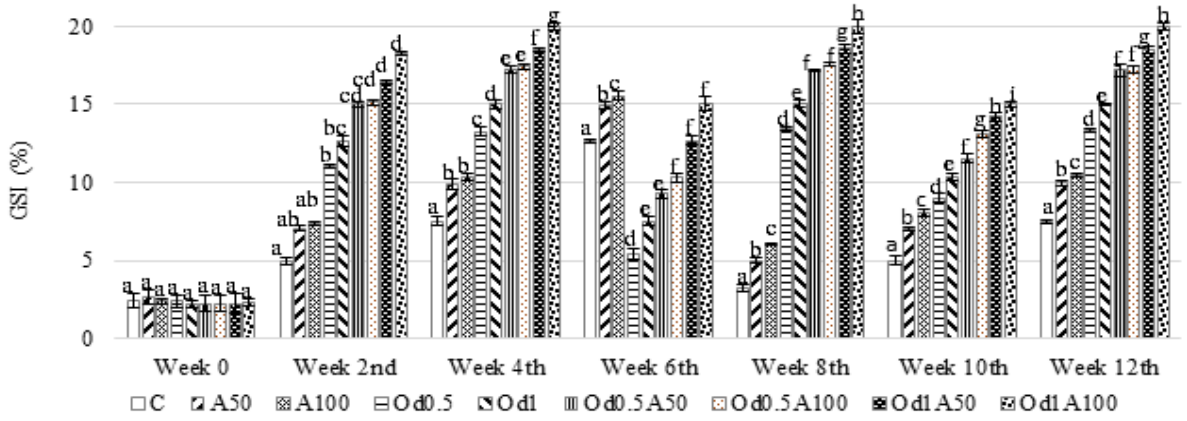

Figure 1. Two-weekly gonadal somatic index (GSI) result of Oodev and astaxanthin feed induction. The treatment code: C= control, A= astaxanthin, Od= oodev and number= dose. The different letters on top of the bar indicate significant differences between treatments in the same week (P<0.05).

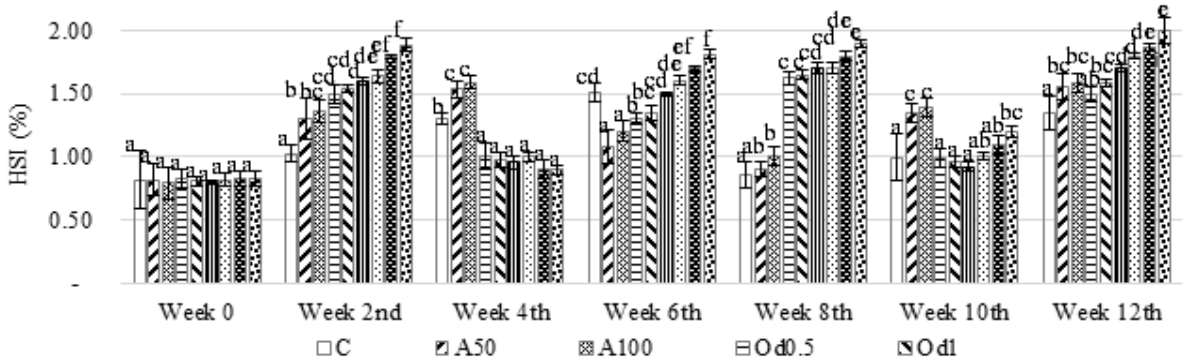

Figure 2 Two-weekly hepatosomatic index (HSI) result of Oodev and astaxanthin feed induction. The treatment code: C=Control, A=Astaxanthin, Od=Oodev and number=dose. The different letters on top of the bar indicate significant differences between treatments in the same week (P<0.05).

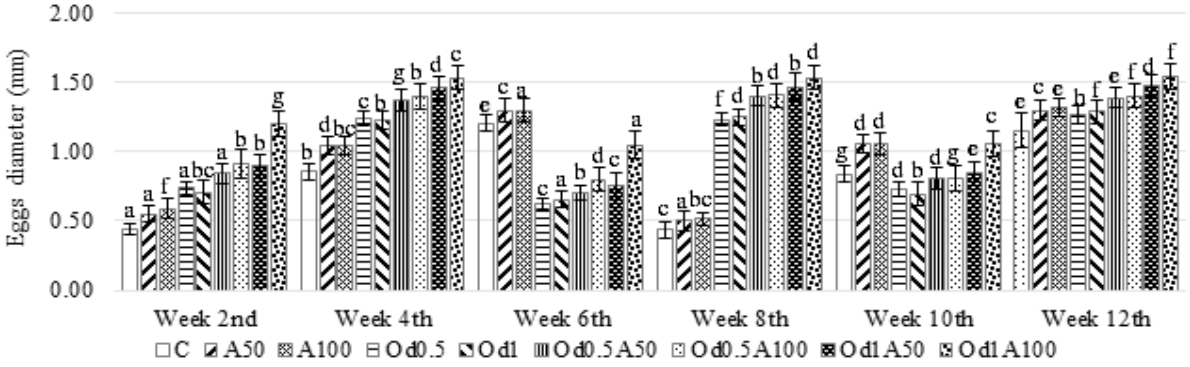

Figure 3. Two-weekly eggs diameter result of Oodev and astaxanthin feed induction. The treatment code: C= control, A= astaxanthin, Od= oodev and number= dose. The different letters on top of the bar indicate significant differences between treatments in the same week (P<0.05).

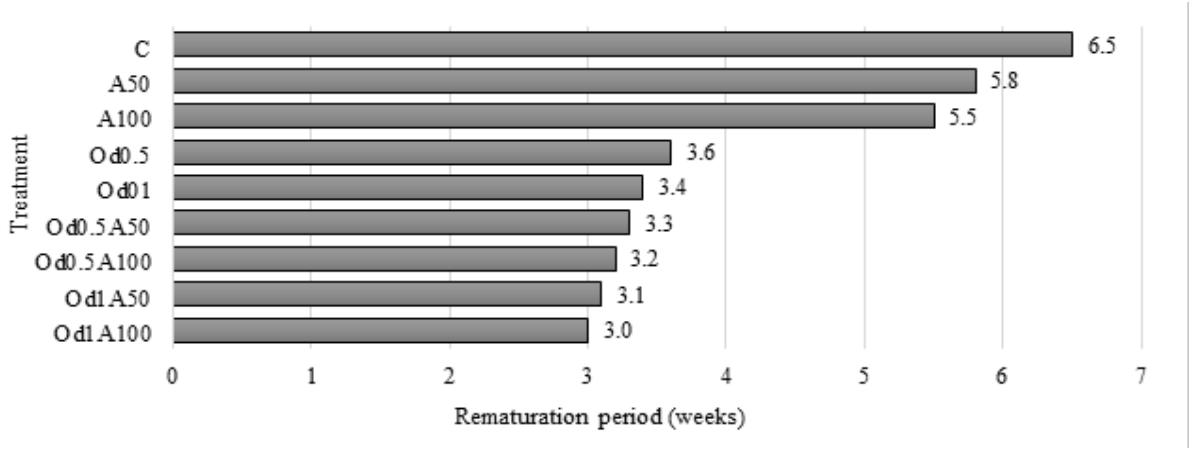

Figure 4. The rematuration average period of Oodev and astaxanthin feed induction. The treatment code: C= control, A=astaxanthin, Od=oodev and number= dose. The observed amount was 20 fishes each treatment

The diameter of the eggs

Eggs diameter of all treatments in the same week was significantly different (P<0.05) and increased with the increasing of rearing times until spawned. The largest rapid growing eggs diameter occurred in Od1A100 (1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin) treatment which the peak started since the 4th week (1.54 ± 0.087 mm) and then rose again in the 6th week (1.53 ± 0.097 mm) and the highest at the 12th week (1.55 ± 0.090 mm). While the slowest and the smallest growing eggs diameter occurred in the control with the peak at the 6th week of 1.21 ± 0.058 mm. According to Iswanto *et al.* (2016) the eggs diameter of ready-to-breed Mutiara strain catfish is between 1.20–1.54 mm. The eggs diameter observation result is shown in Figure 3.

Rematuration period and proportion of mature broodstock

The result of average rematuration period and proportion of mature broodstock over 12 weeks of observation are presented in Figure 4 and Table 3, respectively. The fastest average rematuration period occurred in Od1A100 (1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin) treatment which only takes three weeks to remature after breeding with the high proportion of mature broodstock (90 %). While the longest average rematuration period occurred in controls which take 6.5 weeks to remature after breeding with the average proportion of mature broodstock at the 6th week only 55 %.

Discussion

The results observations of Mutiara catfish body weight on all treatments showed every two weeks (Table 2). However, body weight increased was not significantly different in all treatments at the same week. Thus, indicating that all of Mutiara strain catfish in each treatment were fulfilled their energy needs through feeding evenly distributed. Nevertheless, the results showed that the reproduction performance between treatments were significantly different. This indicates that

Table 3. Weekly proportion of mature broodstock

Treatment	Proportion of mature broodstock (%) week of									
	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
C	-	-	-	55	45	-	-	-	-	-
A50	-	-	35	55	10	-	-	-	-	-
A100	-	-	55	35	10	-	-	-	-	-
Od0.5	40	30	10	15	65	10	5	45	40	15
Od01	65	35	-	25	65	5	5	70	25	-
Od0.5A50	80	20	-	50	40	10	15	70	15	-
Od0.5A100	80	20	-	70	25	5	65	25	10	-
Od1A50	85	10	5	70	25	5	70	25	5	-
Od1A100	90	10	-	90	10	-	90	10	-	-

Notes: C= control, A= astaxanthin, Od= oodev. Number= dose.

the energy derived from the feed were used differently in each treatment. The energy will be utilized for growth or reproduction. The increase in weight also influenced by the process of gonadal development affecting energy consumption, thus requiring more energy for gamete formation on the broodstock.

The value of GSI in Mutiara strain catfish appears to be different in each treatment. GSI values for 12 weeks of observation provide a fluctuating pattern, which tends to increase more rapidly than controls in all treatments using astaxanthin, Oodev or the combinations of both (Figure 2). The combination of 1 mL/kg broodstock of Oodev for 2 weeks and 100 mg/kg astaxanthin on feed showed the highest GSI value compared to all other treatments with three peaks occurred in the 4th, 8th and 12th week, with the highest peak values on the 4th week (20.00 ± 0.22 %). These results is higher than common *C. gariepinus*, which were between 11.73–17.27% (AlDeghayem *et al.,* 2017). This suggests that the higher doses of Oodev combined with astaxanthin will further increase the value of GSI. This result is also higher when compared with other reports that also use Oodev and spirulina that contained carotenoids, the peak of *Clarias sp.* GSI value is between 7.75–11.31 % on day 40 or week six after spawn (Nainggolan *et al.,* 2014).

The lower doses of Oodev and astaxanthin combined resulted in the lower GSI value obtained, although it still appears to have three peaks. While in the control appear to have the lowest GSI value and only one peak that occurs in the 6th week with 12.62 ± 0.15 %. The rapid increase in the GSI especially on Oodev induced treatment was caused by the influence of PMSG and AD contained in Oodev can increase the expression of FSH and aromatase enzymes as well as GSI (Rafiuddin, 2014).

Such as the GSI value, the HSI values also fluctuate from the beginning to the end of the treatment, but the HSI value tends to increase and reach the peak faster than the GSI value, and its value falls as the GSI value reaching its peak in all treatments. Consistent with the GSI value, the highest HSI value also occurred in the combination of 1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin treatment with three peaks at the 2nd, 8th and 12th week. Increased in HSI level indicates the growth of liver during maturation. The liver was induced by the increased of FSH due to Oodev hormone stimulation where the liver is a place to synthesize vitellogenin as the yolk eggs forming (Marina *et al.,* 2008).

The increased treatment affects not only GSI and HSI values, but also occurs in rapidly growing of egg diameter (Figure 3). The study showed consistent results from several test parameters, including the egg diameter parameters obtained from the combination of 1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin, which delivered the best results that can make faster and larger growth in egg diameter. Aside from being influenced by hormonal stimulation, this cannot be separated from the role of astaxanthin. As can be seen that the best results are not only due to hormonal stimulation, but it is a combination of hormonal stimulation from Oodev hormone and astaxanthin. This was similar as described by Lim *et al.* (2017) that

astaxanthin triggers a speedier oocyte maturation. The same thing is reported to occur in goldfish *Carassius auratus* with bigger egg diameter when compared with those without astaxanthin (Tizkar *et al.,* 2013).

Fish cannot synthesize carotenoids such as astaxanthin, so it is essential to be incorporated into the diet (García-Chavarría & Lara-Flores, 2013). Carotenoids are hydrophobic compounds that are not easily solubilized in the aqueous environment of the gastrointestinal tract of fish. Therefore, digestion, absorption and transport processes are associated to lipids (Das & Biswas, 2016). Fish carotenoids are mostly transported to peripheral tissues by high density lipoproteins (HDL) and to a limited extent by low density lipoproteins (LDL). In rainbow trout and other *Oncorhynchus* species, astaxanthin and were found to be present in all serum lipoprotein fractions. In mature female fish, significant amounts of carotenoids also bind to vitellogenin, a female specific serum lipoprotein. During sexual maturation of *Oncorhynchus keta*, HDL and vitellogenin were associated with carotenoid transport during redistribution of carotenoids from muscle to the integument, and from muscle to ovaries, respectively (García-Chavarría & Lara-Flores, 2013).

There are two sources of astaxanthin production, which is natural and synthetic sources. The natural sources of astaxanthin are algae, yeast, salmon, trout, krill, shrimp and crayfish. Whereas synthetic astaxanthin is produced through chemical synthesis (Ambati *et al.*, 2014). The synthetic form is used predominantly for animal feed (Nguyen, 2013). the use of synthetic astaxanthin (100 mg astaxanthin/kg fish feed) does not pose a significant additional risk to the environment compared with natural astaxanthin (EFSA, 2014). Similar to astaxanthin, PMSG hormone is also harmless because it is an organic compound from pregnant mare's serum (Rensis & López-Gatius, 2014).

The improvement of reproductive performance that seen from GSI and HSI value also the egg diameter of Mutiara strain catfish broodstock due to administration of Oodev hormone and astaxanthin. Consistent with other result, the fastest average period of rematuration occurred in Od1A100 (1 mL/kg broodstock of Oodev for two weeks and 100 mg/kg feed of astaxanthin) treatment, which only take three weeks to remature after breeding with the proportion of mature broodstock is reaching 90% This is twice faster than common Mutiara strain catfish rematuration period which usually takes 1.5 months or 6.5 weeks after spawning (Iswanto *et al.,* 2016). These results are also faster than those reported using PMSG hormones and spirulina in *C. gariepinus* that can only accelerate the rematuration period to four weeks with the proportion of mature broodstock of 80 % (Mayasari *et al.,* 2012).

CONCLUSION

Combination of 1 mL/kg broodstock of Oodev hormone for two weeks and 100 mg/kg feed of astaxanthin diet showed the best result in accelerating rematuration period of the Mutiara catfish, up to three times faster and increased the proportion of mature broodstock up to 35 %, compared to control. The reproductive performance improvement of this combination is characterized by faster and higher increases in HSI and GSI values, the fastest growth of eggs diameter, accelerating the rematuration period, as well as the increased proportion of mature broodstock.

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