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Ovarian Maturation in Asian Catfish (*Clarias sp.*) by Combination Oodev and Nutrition Addition *Spirulina plantesis*

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Abstract

This research aimed to accelerate maturation and increase the quantity and quality of catfish (*Clarias* sp.) eggs and larvae out of the spawning season (the dry season) through the combination between the supplementation using *Spirulina platensis* and the hormone Oodev. The dosage of the treatments were feed A_1: *Spirulina platensis* 0% combined with Oodev 0 IU; Feed A_2: *Spirulina platensis* 0% combined with Oodev 15 IU; Feed B_1: *Spirulina platensis* 1% combined with Oodev 0 IU; Feed B_2: *Spirulina platensis* 1% combined with Oodev 15 IU; Feed C_1: *Spirulina platensis* 2% combined with Oodev 0 IU; Feed C_2: *Spirulina platensis* 2% combined with Oodev 15 IU; Feed D_1: *Spirulina platensis* 2% combined with Oodev 1 IU; Feed D_2: *Spirulina platensis* 3% combined with Oodev 15 IU. The fish tested were 80 male broodstocks and each treatment used 10 fish. Every day the fish were fed the feed twice (morning and afternoon) *at satiation.*

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During the experiment, the parameters that were observed were GSI (Gonado Somatic Index), the concentration of estradiol-17β, fecundity, egg hatching rate, egg diameter, and larvae survival rate. The results of this study showed that the difference in the *Spirulina platensis* nutritional supplement content of the feed has an effect on the composition of fatty acids in the treatment feed. Treatment feed which was a combination between *Spirulina platensis* and Oodev 15 IU generally gave the fastest and best effect on the female catfish's physiological response (testing parameters) compared to treatment using Oodev or *Spirulina* supplementation alone, and even more so when compared to the control. The result of the 40 day experiment showed that treatment D_2 (*Spirulina platensis* 3% combined with Oodev 15 IU) gave a significant effect ($P<0.05$) on the acceleration of gonadal maturation and was able to increase the quantity and quality of the catfish's (*Clarias* sp) eggs and larvae. Thus, the combination dosage of *Spirulina* 3% and 15 IU Oodev (gonadotropin hormone) can accelerate oocyte growth and increase the viability of eggs and larvae.

Keywords: Catfish broodstock (*Clarias* sp); *Spirulina platensis*; Oodev; fatty acids.

1. Introduction

The production value of catfish (*Clarias* sp.) always increases from year to year because of its high economic value. However, it is not always followed by the increase in quality and quantity in line with the domestic and international market demands. This problem is yet to be solved. In addition, other hurdles faced are the lack of constantly available seed, the low egg fertilization rate, and the low egg hatching rate. One of the causes is the low quality of the feed given to the broodstocks.

One way to get optimal fish breeding is by improving the reproductive performance through nutritional supplementation in the broodstocks feed which is combined with hormone premix. This is meant to not only to accelerate gonadal maturation which is independent of the season but also to improve the quality and quantity of the larvae. The hormone premix used in this experiment is Oodev (Oocyte developer) which contains PMSG+ad (Pregnant Mare Serum Gonadotropin+anti dopamine). The principle and function of the use of this hormone premix are to stimulate a spike in the GnRH level which in turn will stimulate the pituitary to produce gonadotropin. Then, the gonadotropin will stimulate the ovaries to maturate the eggs in the fish [1]. The results of Sudrajat's (2011) study showed that the use of Oodev in Basa catfish broodstock (*Pangasius sp*) increased the reproductive performance [2]. In North African catfish (*Clarias gariepinus*), for instance, for the mass production scale, spawning was induced using a combination between the hormones PMSG and HCG [3].

The nutritional value of the feed given to the broodstocks is very important because it supports the development of the gonads and the quality of the eggs is the reflection of the egg yolk nutritional chemical build which is affected by the brooder's health and the nutrition received by the broodstocks. It also affects the synthesis or the release of hormones from the endocrinal glands [4]. Retarded gonad

development can also occur because the brooder's feed lacks some nutritional substances such as proteins, vitamins, essential amino acids, and minerals or the essential fatty acids are unsuitable or low or the dosage is unsuitable. These might cause low levels of gonadotropins produced by the adenohypophisal gland, a low ovary response, or probably the ovary's failure to produce adequate amounts of estrogen [5]. Fish broodstock which are fed with a diet low in essential fatty acids (EFA) will produce eggs with low hatching rates and most of the larvae produced will be abnormal [6].

Spirulina is a kind of micro algae which can be used as a feed supplement for broodstocks to improve reproductive performance and quality because of its high nutritional value. It contains 60-70% protein, vitamins (B1, B2, tocopherols), essential amino acids, minerals, and essential fatty acids such as g-linolenic acid (GLA) [7, 8, 9]. Studies about *Spirulina* as the main feed for Basa catfish (*Pangasius bocourti*) and the Nile tilapia reported that it could improve reproductive performance and the survival rate compared to conventional fish feed [10, 11, 12]. Other studies have also reported that Nile tilapia fed *Spirulina* alone could still reproduce normally for three generations [11].

Seeing the function and role of *Spirulina* and the hormone Ooedv in the gonadal maturation process, there needs to be an evaluation of the dosage of *Spirulina* in fish feed in combination with Oodev needed to accelerate the gonadal maturation and improve the quality and quantity of eggs produced. This study is aimed to study the effect of the combination between *Spirulina* in feed with hormone Oodev on the reproductive performance of catfish (*Clarias* sp.), especially gonadal maturation, and larvae and egg quality.

2. Material and Methods

2.1. The Experimental Fish

In this experiment, we use a good prospective broodstock of catfish (*Clarias sp*.) from the Freshwater Aquaculture Research Station (Balai Riset Perikanan Budidaya Air Tawar-BRPBAT) Sukabumi, West Java. Catfish prospective broodstocks used for dry season are about 18 months old, as much as 80 fish with an average weight of 350-400 g/fish. Male broodstocks used for artificial ovulation are about 40 fish with an average weight of 600 g/ fish. Prior to the study, the fish will be adapted for 12 days.

2.2. Experimental Feed

The experimental feed used was commercial fish pellets (protein 28 %, fat 5%, fiber 5%, ash 13% and moisture 12%) which were enriched with *Spirulina platensis*. The micro algae added to the feed was in the form of a ready-made powder from PT. Polaris Indonesia. The supplementation was done during re-pelleting according to the pre-determined dosages: 0% (control), 1%, 2%, and 3%. The feed was then baked in an oven at 60^0C for 12 hours. After that, it was analyzed to measure the nutrient contents: the protein, fat, and carbohydrate content [13]. The feed was also analyzed for the fatty acid content

using the gas chromatography method. During the maintenance, the catfish brooders (*Clarias* sp.) were fed at a feeding rate of 3% of their body weight twice a day, at 8.00 AM and 5.00 PM.

2.3. Hormones

The hormone used in this experiment was Oodev (Oocyte developer) which is a hormone product developed in the Reproduction and Genetics Laboratory, Department of Aquaculture, Bogor Agricultural University. Oodev contains PMSG and Antidopamine. The dosage of Oodev administered was 0 IU/kg fish and 15 IU/kg fish. In addition, Luteinizing Hormone Releasing Hormone (LHRH) + anti dopamine (Ovaprim, a product of Syndel Canada) was used at the end of the experiment to induce ovulation (spawning).

2.4. Holding Tanks

The brooders were kept in eight 3x2.5x1.5 meter ponds. For the incubation, hatching, and larvae raising, twenty-four 70 X 50 X 50 cm aquariums which were equipped with aeration pipes were used.

2.5. Methodology

The methodology used in this study was the experimental model using a 4x2 Factorial Design; therefore, there are 8 treatment interactions. The first factor was 4 different dosages of *Spirulina platensis* in the fish feed: 0%, 1%, 2%, and 3% which were given for the duration of the experiment. The second factor was 2 different dosages of the hormone Oodev, i.e. 0 IU/kg fish and 15 IU/kg fish which was injected 3 times in the posterior part of the dorsal fin intramuscularly at an interval of 10 days. After that, when the broodstocks gonads were mature, they were injected with Ovaprim in the same location at a dose of 0.5 ml/kg fish. The injection was meant to stimulate spawning in the broodstocks. The details about this treatment can be seen in Table 1.

The fish for each treatment were placed in one separate tank and each tank held 10 broodstocks. For the gonad histology observations, the Gonado Somatic Index (GSI), the estradiol hormone level, and the egg diameter were measured by taking one fish as a sample every 10 days. This was done 4 times and the rest had their fecundity and reproductive performance evaluated at the end of the experiment. Spawning was done artificially by stripping the broodstocks when the broodstocks had reached gonadal maturation, signified by distended abdomens and malleable anal regions.

After the stripping, the eggs were placed in a bowl and stirred for 10 seconds before the male sperm which had been prepared before was added to the eggs. The mixture was stirred again for 10 seconds and then placed in 70 X 50 X 50 cm hatching aquariums which were equipped with 24 aeration pipes (each treatment was done triplicate). Each aquarium contained 300 eggs which had been mixed with sperm and observed for the hatching rate and survival rate. Each aquarium was treated with methylene blue to prevent the growth of mould. In addition, the measurement of external factors such as the

analysis of the experimental feed (analysis of the nutritional value, protein and fatty acids in the feed) was also done. The data of the GSI, estradiol-17β hormone, egg diameter, egg hatching rate, larvae survival rate (SR), and fecundity were analyzed using analysis of variance (ANOVA) using the SPSS program software version 16 which used the Duncan test. The egg and larvae fatty acid content, GSI, and estradiole-17β hormone profile are presented descriptively in the form of tables and illustrations.

Table 1. Treatments using various combinations of *Spirulina platensis* supplement and the hormone Oodev injection dosages in catfish brooders

Dosis *Spirulina platensis*	Dosis hormon Oodev	
	0 IU/kg ikan (1)	15 IU/kg ikan (2)
0 % (A)	A1	A2
1 % (B)	B1	B2
2 % (C)	C1	C2
3 % (D)	D1	D2

Notes: A1= 0% *Spirulina platensis* supplementation, Oodev injection 0 IU/kg; A2= 0% *Spirulina platensis* supplementation, Oodev injection 15 IU/kg; B1= 1% *Spirulina platensis* supplementation, Oodev injection 0 IU/kg; B2= 1% *Spirulina platensis* supplementation, Oodev injection 15 IU/kg; C1= 2% *Spirulina platensis* supplementation, Oodev injection 0 IU/kg; C2= 2% *Spirulina platensis* supplementation, Oodev injection 15 IU/kg; D1= 3% *Spirulina platensis* supplementation, Oodev injection 0 IU/kg; D2= 3% *Spirulina platensis* supplementation, Oodev injection 15 IU/kg.

3. Result and Discussion

3.1. Results

Feeding the experimental feed supplemented with *Spirulina platensis* combined with the hormone Oodev to catfish broodstocks (*Clarias sp*) in aquaculture ponds for 40 days proved to be able to accelerate maturation and improve the quantity and quality of both the eggs and larvae out of the spawning season (the dry season). This can be seen from the observation results for all testing parameters.

3.1.1. Feed Protein and Fatty Acids

The micro algae *Spirulina* used in this experiment had a fairly high protein and fatty acid content. The supplementation of *S. platensis* to the feed caused an increase in the protein content of the feed and the decrease in coarse fiber content. The protein content of the feed without supplementation with *S.platensis* (control A) was 28.69%, whereas the feed which was supplemented with 1% of *S. platensis* (B) had a protein content of 29.54%, the feed with 2% *S. platensis* 2% (C) had 31.67 % protein, and the feed with 3% *S. platensis* (D) had 32.39% protein. The non-saturated *n-6* fatty acid content in the feed increased as the dosage of *S. platensis* supplement increased: 0% supplementation (control)

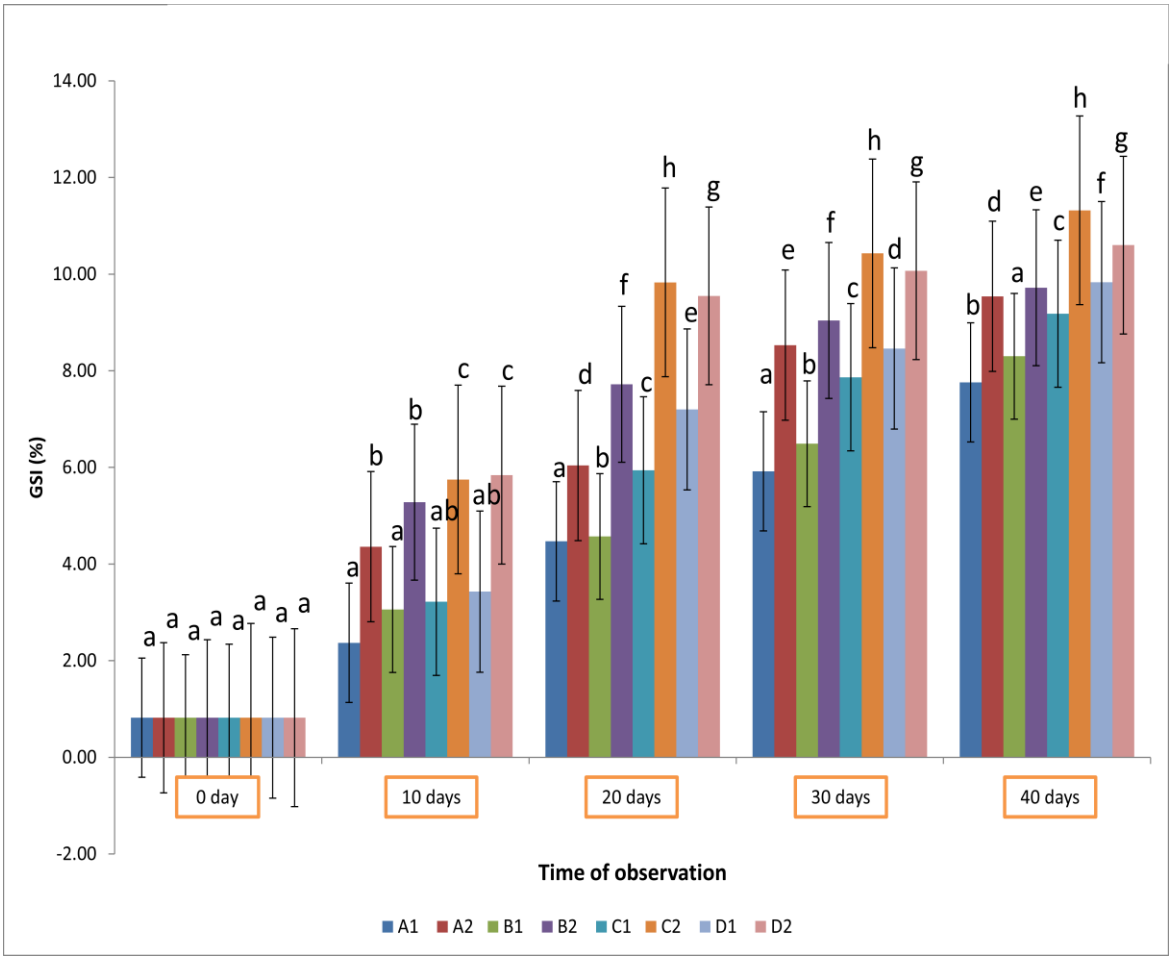
21.91%, 1% supplementation 23.07%, 2% supplementation 23.14% and 3% supplementation 23.28%. The content of non-saturated n-3 fatty acid in the feed also increased as the *S. platensis* supplementation dosage increased: 0% supplementation (control) 5.27%, 1% supplementation 5.43%, 2% supplementation 5.40%, and 3% supplementation 5.68%. The supplementation of the micro algae in combination with the hormone Oodev showed effect on the GSI, egg diameter, E2 profile and larvae SR.

3.1.2. Gonado Somatic Index (GSI)

The results of the different concentrations of *Spirulina platensis* in the feed combined with the hormone Oodev on the catfish broodstocks GSI value are presented in Fig. 1.

Fig.1 shows that the average GSI value at the commencement of the experiment (day 0) was 0.82% for all treatments; however, as the experiment progressed, the egg weight and diameter increased significantly. The highest GSI value observation results (%) were shown on the 10^{th} day by treatment D2 which was 5.84 %, followed by treatments C2 (5.75%), B2 (5.28%), A2 (4.36%), D1 (3.43%), C1 (3.22%), B1 (3.06%) and A1 (2.37%). On day 20, the value increased again with the highest results shown by treatment C2 which was 9.83%, followed by D2 (9.55%), B2 (7.72%), D1 (7.2%), A2 (6.04), C1 (5.94%), B1 (4.57%) and A1 (4.47%). On day 30, the value again increased; the highest was shown by treatment C2 which was 10.43 and followed by treatment D2 (10.07%), B2 (9.04%), A2 (8.53%), D1 (8.46%), C1 (7.87), B1 (6.49%) and A1 (5.92%). Day 40 was the peak of the GSI value. The highest result was shown again by treatment C2, 11.31%, which was followed by treatment D2 (10.59), D1 (9.83%), B2 (9.71%), A2 (29.53%), C1 (19.17%), B1 (8.29) and A1 (7.75%). The results of this analysis and the analysis of variance showed that there was a significant difference between treatments ($P<0.05$).

The increase in the Gonado Somatic Index or the development of the ovaries above was caused by the development in the oocyte stadia. During the development of the oocytes, there is a morphological change which characterizes the stadium. In certain kinds of fish, during the growth of the oocytes, there is a 1-20% increase in the Gonado Somatic Index (GSI) or more caused by the distribution of Estradiole 17β to the liver, entering the tissues through diffusion and its specifically stimulates the synthesis of vitelogenin [14]. The vitellogenetic activity causes the value of the fish's Gonado Somatic Index (GSI) to increase [15].

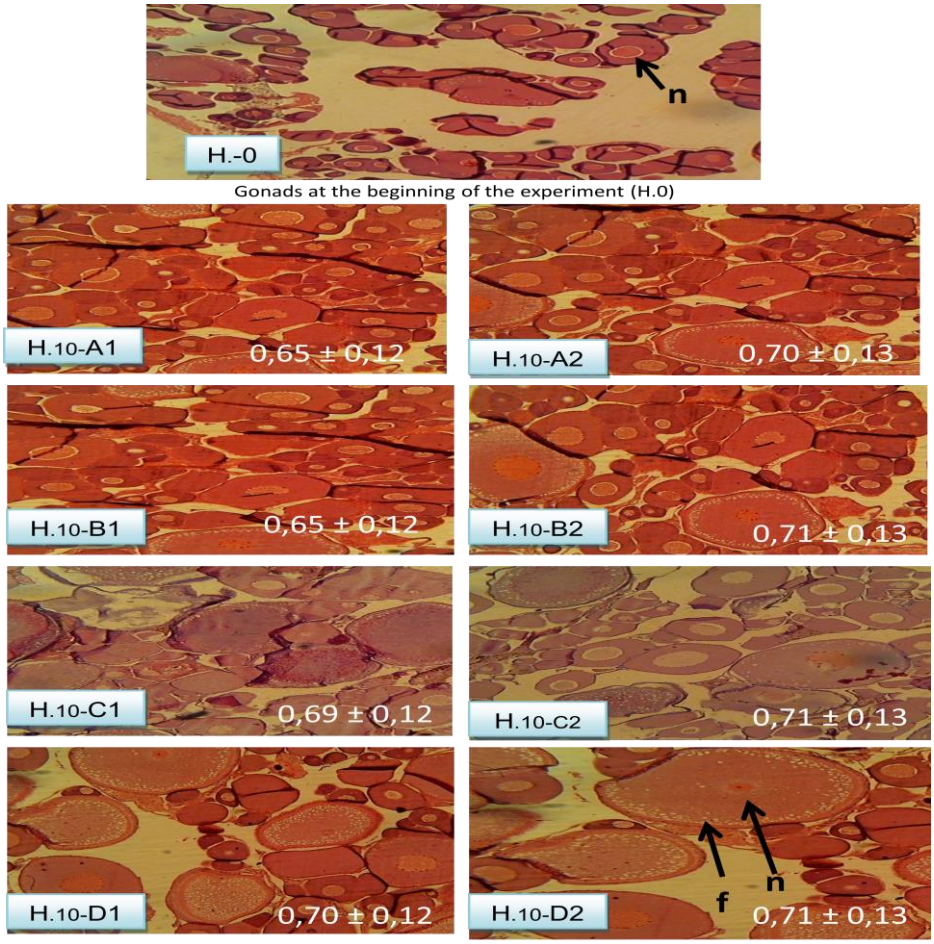


Note: Similar letters showa non-significant difference (P>0.05)

Fig 1. The histogram showing the relationship between the combination of the supplementation of *Spirulina* and the injection of Oodev and the changes in the catfish (*Clarias* sp.) brooders' GSI

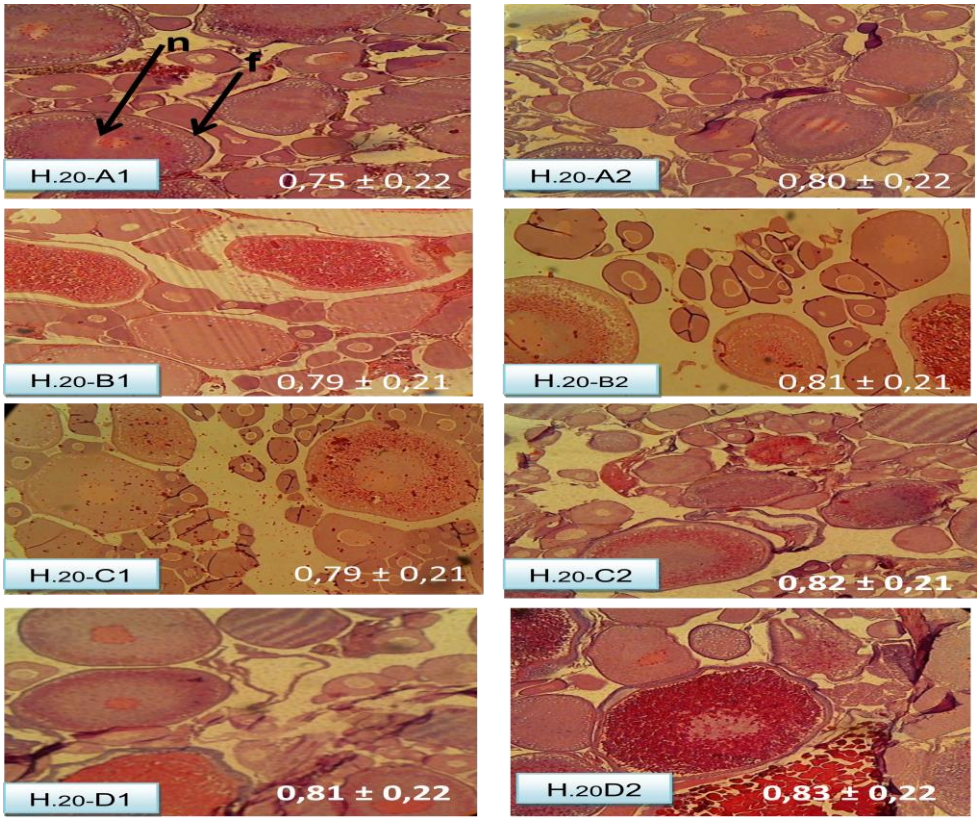
3.1.3. The Development of the Gonads and the Egg Diameter

The development of the gonads can be traced through the histological analysis because the vitellogenetic phases could be observed through histological analysis because the vitellogenetic stages can be seen as with oogenesis or egg development which starts with the development of oocytes as a result of the development of the characteristics of the germinative cells. Histological observations of the gonadal development conditions were done every ten days for each treatment. The stages in the development of the catfish's (*Clarias* sp) gonads in every treatment can be seen in Figure 2. From this figure, the development/maturation status and the gonadal tissue forms can be seen.

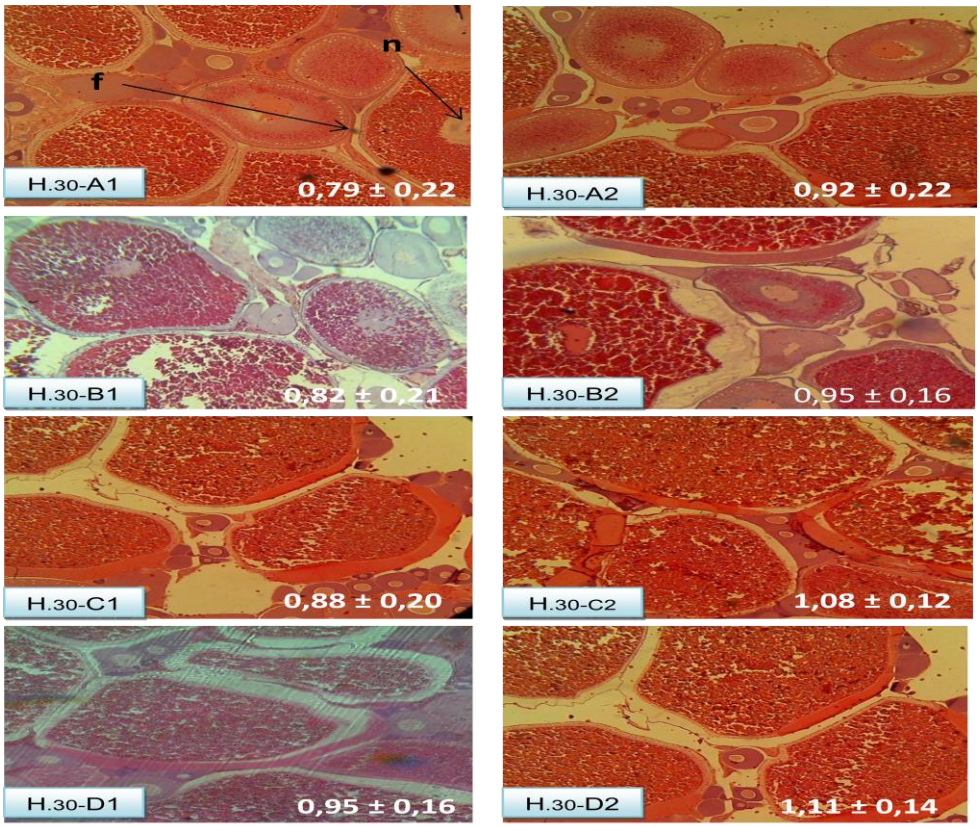


Gonads at the beginning of the experiment (H.0)

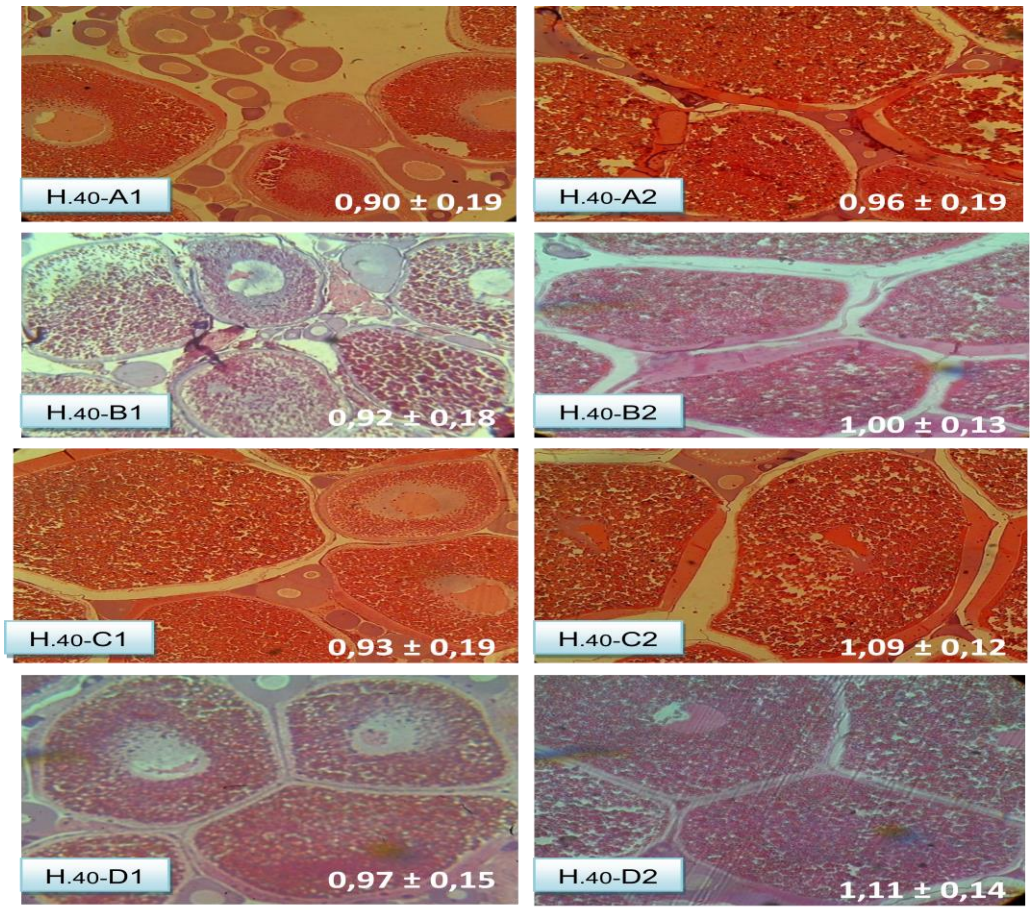
Gonads on day 10 (experiment A1-D2) with a diameter of 0.65 to 0.71 mm



Gonads on day 20 (experiment A1-D2) with a diameter of 0.75 to 0.83 mm



Gonads on day 30 (experiment A1-D2) with a diameter of 0.79 to 1.11 mm



Gonads on day 40 (experiment A1-D2) with a diameter of 0.90 to 1.11 mm

Fig. 2. The histological structure of catfish's (*Clarias sp)* gonads after treatment with the food supplement *Spirulina* combined with the hormone Oodev (histological profile of the medial part of the ovary, stained with Bouin's HE, 100x maginification); n = nucleoulus and f = follicle.

In Fig. 2, the first day (H-0) showed the initial stadium (the oogonia stadium) where the percentage of immature oocytes and germ cells was quite high. In the oogonia stadium, the cells are elliptical and small (7.5-10 µm) [16]. In the next stadium was seen on the 10th day (H-10). In this stadium, the euvitelline nucleus (oocyte) had developed and was near the nucleus membrane. This stadium is the beginning of vitellogenesis which is characterized by the presence of the yolk granules in the cytoplasm. The diameter of the eggs in the combination treatments (B2, C2, and D2) were 0.71 mm in average, larger than the non-combination treatments (*Spirulina* or Oodev only) and the control which were 0.65-0.70 mm in average. The next stadium was seen on the 20th day (H-20), showing the increased size of oocytes because they were filled with yolk. The yolk granules were larger and filled the cytoplasm. In this stadium, the gonadal development had entered stage III. The diameter of the eggs

in treatment D2 (*Spirulina* 3% combined with Oodev 15 IU) were 0.83 mm in average, larger than the other treatments and the control (A1) which were only 0.75 mm in average. On the 30th day, the stadium was still oocyte growth and yolk development. This stage is called stage III. The size of the eggs in all of the treatments was 0.79-1.11 mm in average. The largest diameter was also found in treatment D2. The next stage was the end of the experiment, Day 40. This stage was characterized by the nucleus in the periphery, signifying that the fish is ready to spawn (stage IV). The diameter of the eggs in all the treatment was 0.9-1.11 mm in average. The largest diameter in this stage was also found in treatment D2 with an average of 1.11 mm.

In general, it can be seen that the diameter of the eggs of the catfish (*Clarias* sp.) grew as the maintenance duration increased. The egg diameter grew as a result of the yolk accumulation, hydration, and the development of oil granules in the egg. The difference in size in every observation (H-10 to H-40) between treatments showed that there is a significant effect on the gonadal development. The results of the analysis of egg diameter variance show that the treatments which combine between *Spirulina* supplementation and Oodev 15 IU gave a significant effect on the egg diameter ($P<0.05$). The treatments which gave the best effect on egg diameter are treatment C2 (*Spirulina* 2% and Od. 15 IU) and D2 (*Spirulina* 2% and Od. 15 IU).

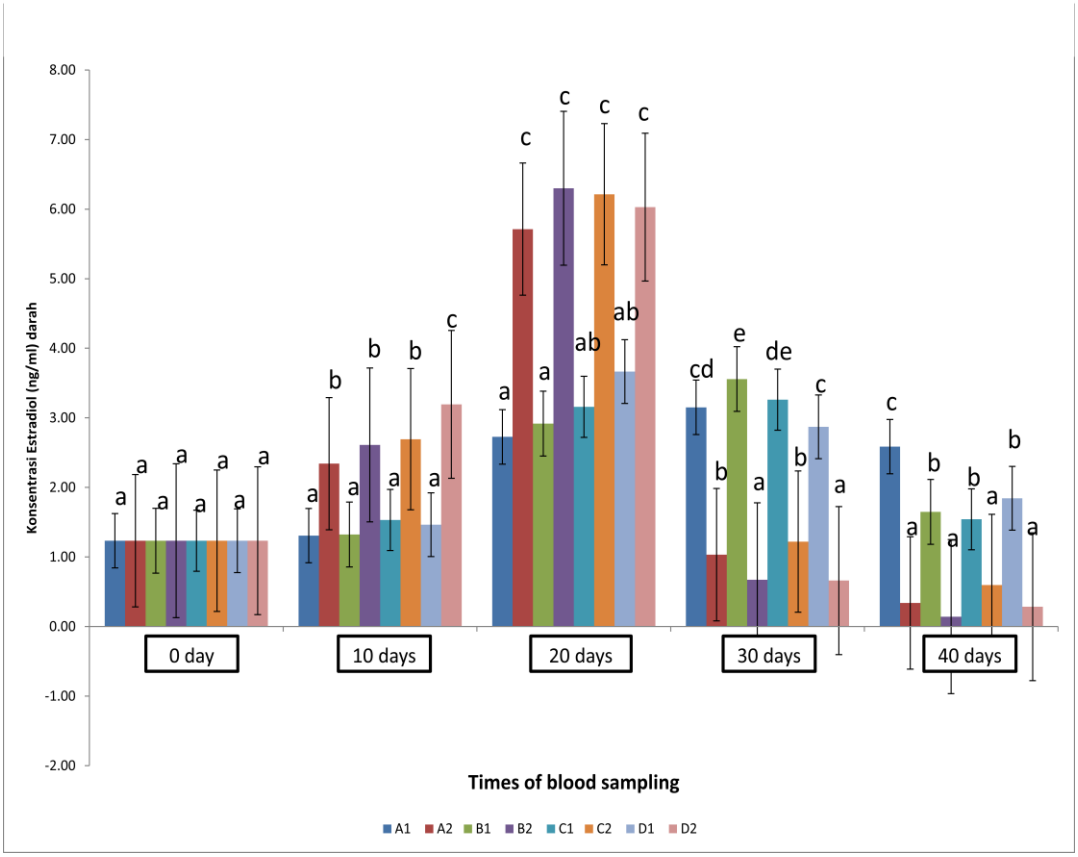
3.1.4. Estradiol -17β (E2) Profile

The catfish brooder blood estradiol profile during the experiment showed an increase every time samples were taken. The measurements of Estradiol-17β (ng/ml) or E_2 concentration in blood which were done during the experiment on catfish brooders can be seen in Fig. 3.

Fig. 3 shows that in the beginning (Day 0), the average E2 concentration in all treatments was 1.23 ng/ml. As the vitellogenesis commenced, it started to increase on Day 10 and Day 20 and decreased on Day 30 and Day 40. The increase and decrease of blood E2 concentration in all treatments were different. Treatments by adding *Spirulina* to the feed (Sp. 1%, Sp. 2% and Sp. 3%) and Oodev injections of 15 IU/kg brooder on Day 10 and Day 20 showed the highest increase in blood estradiol profiles compared to treatments using *Spirulina* supplementation or Oodev injections alone. On Day 10, the highest E2 concentration was found in treatment D2 (Sp.3 %; Od.15 IU) i.e. 3.19 ng/ml and and the lowest in treatment A1 (Sp.0 %; Od. 0 IU) i.e. 1.31 ng/ml. On Day 20, the highest concentration was in treatment C2 (Sp.2 %; Od.15 IU) i.e. 6.21 ng/ml and the lowest in treatment A1 (Sp.0 %; Od. 0 IU) i.e. 2.73 ng/ml.

Spirulina supplementation or Oodev injections alone did not have a significant effect on estradiol concentration ($P<0.05$). The decrease in the concentration of blood E2 in catfish broodstock during the maintenance in all treatment happened on Day 30 and Day 40. However, the decrease differed between treatments. The most rapid decrease (the highest concentration) was shown by treatments which combined between *Spirulina* and Oodev 15 IU compared to treatments with *Spirulina* or Oodev alone. On Day 30, the largest decrease in the concentration was found in treatment D2 (Sp.3%; Od.15 IU) at

0.66 and lowest in treatment C1 (Sp.2%; Od. 0 IU) and A1 (Sp.0%; Od. 0 IU) at 3.26 ng/ml and 3.15 ng/ml, respectively. On Day 40, the decrease in E2 concentration was quite rapid in all the treatments except for the control which had a slower decrease. The largest decrease was found in treatment D2 (Sp.3%; Od.15 IU) at 0.28 ng/ml and lowest in treatment A1 (Sp.0%; Od. 0 IU) at 2.59 ng/ml. The decrease in E2 on Day 30 in all treatments showed that all the broodstock had mature gonads.



Note: Similar letters showa non-significant difference (P>0.05)

Fig.3. The histogram depicting the effect of combinations between *Spirulina* feed supplementation and Oodev injections on the changes in the concentration of Estradiol-17β in catfish (*Clarias sp.*) brooders

3.1.5. Fecundity, Hatching Rate (HR) and Larvae Survival Rate (SR)

The results of the observation of fecundity (number of eggs), hatching rate, and larvae survival rate can be seen in Table 2.

Table 2. The development of gonadal maturation and reproductive performance in catfish (*Clarias* sp) treated with a combination of *Spirulina platensis* supplementation and injections of the hormone Oodev.

Treatment	Fecundity (eggs / kg broodstock)	HR (%)	SR (%)
A1 (Sp. 0% ; Od.0 IU)	30040 ± 59,37[a]	75,16 ± 1,9021[a]	75,01 ± 2,6729[a]
A2 (Sp. 0% ; Od.15 IU)	43185 ± 1036,28[d]	75,75 ± 1,9728[a]	75,46 ± 1,7819[b]
B1 (Sp. 1% ; Od.0 IU)	33155 ± 1596,59[b]	84,00 ± 1,7819[b]	86,45 ± 1,4991[bc]
B2 (Sp. 1% ; Od.15 IU)	49262 ± 183,45[e]	83,99 ± 1,7183[b]	87,18 ± 1,8173[c]
C1 (Sp. 2% ; Od.0 IU)	40435 ± 141,53[c]	88,25 ± 2,2274[c]	89,43 ± 0,8132[d]
C2 (Sp. 2% ; Od.15 IU)	47764 ± 478,68[e]	88,76 ± 2,2415[c]	89,95 ± 0,80,61[d]
D1 (Sp. 3% ; Od.0 IU)	42502 ± 911,63[d]	90,61 ± 1,2657[c]	92,65 ± 0,5020[d]
D2 (Sp. 3% ; Od.15 IU)	48953 ± 8,94[e]	91,10 ± 1,0465[c]	92,94 ± 0,0636[d]

Note: The number followed by a superscript letter in the same column shows there is no difference (P>0.05); HR; Hatching Rate, SR; Survival Rate

The results of the analysis of the data in Table 2 showed that in general, the supplementation of *Spirulina* or injections of Oodev alone and the combination of the two significantly increased fecundity (number of eggs), hatching rate and survival rate (P>0.05) compared to treatment A1 (control). The highest fecundity rate was found in treatment B_2 at 49,262 eggs, D_2 48,953 eggs and C_2 47,764 eggs. Even though there is a small difference in the numbers, the variance analysis showed there is no significant difference between treatments B2, D2 and C2. This means that treatments combining *Spirulina* supplementation and Oodev 15 IU are better than treatments using *Spirulina* supplementation or Oodev 15 IU alone or the control (A1) for fecundity. For the hatching rate parameter, both supplementation of *Spirulina* or injections of Oodev alone and combinations of the two had better hatching rates than the control. The highest hatching rate was shown by treatment D2 (*Spirulina* 3% combined with Oodev 15 IU) at 91.10, whereas the lowest was shown by treatment A1 (control) at 75.01%. As for the larvae survival rate larvae (SR), the highest was shown by treatment D2 at 92.94 and the lowest by treatment A2 (control) at 75.01 %.

3.2. Discussion

Based on the results of this study, supplementation of *Spirulina platensis* in feed combined with the hormons Oodev in Asian catfish (*Clarias* sp.) broodstock improved the broodstock reproductive performance and increased the quantity and quality of the eggs and larvae.

The fastest gonad maturation was shown by the combination between *Spirulina* 3% and Oodev 15 IU with 88.89% of the brooders gravid on Day 20, whereas for the combination between *Spirulina* 0% and Oodev 15 IU the percentage of gravid broodstock was only 33.33%. The data showed that the development and maturation of gonads can be accelerated by *Spirulina* supplementation in combination with the hormone Oodev. Injection of Oodev at a dose of 15 IU in brooders caused an increased concentration of estradiol in the blood. Increased concentration of estradiol in the fish's blood will stimulate the liver to perform the process of vitellogenesiswhich will in turn accelerate the gonadal maturation process because estradiol is a stimulant for vitellogenin biosynthesis in the liver. In addition, the hormone PMSG in Oodev plays a role in stimulating the formation of follicles and in the process of vitellogenesis because it contains a lot of FSH activity and just a small amount of LH activity. Follicle Stimulating Hormone (FSH) or GTH I will stimulate a spike in GnRH concentration which will stimulate the pituitary produce to gonadotropin [1]. The supplementation of *Spirulina*, which is rich in protein, *n-6* and n-3 fatty acids, to the feed in different amounts played a role in the hydroxylation process in the biosynthesis of steroid hormones, which affect the acceleration of gonadal maturation. Fatty acids also play an important role in the biosynthesis of the hormone estradiol as an electron donor for the hydroxylase enzyme which converts testosterone to estrogen. Then, together with vitamin A (which functions as an antioxidant), it will increase the PUFA (polyunsaturated fatty acid) function which is needed in the hormone formation process. This hormone is synthesized and secreted by the layer of granulosa cells and thecal cells in the oocyte follicles under the influence of FSH [17].

The Gonado Somatic Index (GSI) in all treatments (A1, A2, B1, B2, C1, C2, D1, and D2) showed an increase as the vitellogenesis commenced (Figure 1). Vitellogenetic activities cause the GSI to increase [15]. The observations results of the GSI showed that the highest value was shown by treatment D2 (*Spirulina* 3%; Oodev 15 IU) and the lowest was shown by treatment A1 (*Spirulina* 0%; Oodev 0 IU). This combination between the hormone and the nutritional supplementation resulted in the highest value in all treatments. The combination treatment is very beneficial because the hormone Oodev and *Spirulina* supplementation work in sync, meaning that Oodev directly stimulates the GnRH spike which will stimulate the pituitary to produce gonadotropin, whereas the nutrition contained in the micro algae, i.e. protein, n-3 and n-6, are directly used in supporting the growth and development of the gonads. The nutrition is immediately distributed to and absorbed by the gonads. The ω-6 and ω-3 essential fatty acids contained in *Spirulina* could also affect the fluidity of the cell membrane. The changes in membrane fluidity caused by the changes in fatty acid composition will affect the cell metabolism through changes in the activity of enzymes found in the cell membrane [18]. The improved membrane fluidity due to the effect of essential fatty acids will cause the vitellogenin to easily enter the oocyte and be absorbed by the oocyte; therefore making the egg diameter to grow larger (Figure 2) which in turn will automatically cause a higher GSI.

The effect of the combination on the development of the GSI can be seen from the value of each the GSI in each treatment. The better the nutritional intake combined with Oodev 15 IU, the stronger the effect on the GSI value. This shows that the combination between the two can affect the speed of

gonadal maturation. This result is supported by Watanabe's (1988) opinion that nutrition in the brooder's feed in very important in supporting the development of the gonads because the quality of the egg reflects the chemical build of the egg yolk [4]. In some kinds of fish, during the oocyte growth there is an increase in the Gonado Somatic Indexup to 20% or even more caused by the Estradiol (E2) which is distributed to the liver, enters tissue by diffusion and specifically stimulates the synthesis of vitellogenin [14]. In addition, during vitellogenesis, the egg yolk granules increase in number and size, increasing the volume of the oocyte [16]. During the process, most of the results of the metabolism are directed to gonadal development. This can cause changes within the gonad itself. In general, the growth of the gonads in a female fish is between 10-25% of its body weight [19].

The egg diameter which was measured every 10 days increased as maintenance time elapsed with different results. Based on the statistical test, both the injections of Oodev and the supplementation of *Spirulina platensis* in the fed had significant effects on the catfish egg diameter. There is also effect of the interaction between the two factors on the egg diameter. The best egg diameter was shown by treatment C2 (Oodev 15 IU/ kg and *S. platensis* 2%), D2 (Oodev 15 IU/ kg and *S. platensis* 3%) and B2 (Oodev 15 IU/ kg and *S. platensis* 1%). This proved that the combination between Oodev and *Spirulina platensis* would produce a larger egg diameter. Egg diameter indicates the amount of energy stored in the egg which will be used for embryonic development. The larger egg diameter is the result of yolk accumulation, hydration, and the formation of oil granules in the egg.

The effects of feed quality on egg characteristics such as the size and composition have often been reported [20]. Females that were fed pellets alone without any nutrition supplements produced less and smaller eggs than fish fed pellets and nutritional supplements. Other nutritional components such as fat are a very important factor in fulfilling the fish's energy needs and the surplus is stored in the embryos. Oodev has a significant effect on the diameter because GTH I which is contained in Oodev is able to give a quicker signal to the gonads which will then command the liver to start vitellogenesis. The availability of adequate nutrition and signals will accelerate viltellogenesis. When the diameter of the eggs has reached its maximum size, the pituitary will secrete LH (Luteinizing hormone) which will initiate the final gonadal maturation or ovulation. The faster the diameter reaches its maximum size, the faster the final gonadal maturation phase is reached, and then it simply needs to wait for the signal to ovulate.

Injections of Oodev in combination with *Spirulina* supplementation could increase the Estradiol (E2) levels in blood plasma. The concentration of E2 increased significantly on Day 10 and Day 20, and then decreased on Day 30 and Day 40 (Figure 3). This is because the fish which were given a combination of *S. platensis* and Oodev went through the final vitellogenetic phase before Day 30. The decrease in estradiol concentration causes steroidogenesis which ends in ovulation. This proves that induction using *S. platensis* and Oodev has a role in the initial gonadal development process. This is supported by Thomas and Rahman (2009) who stated the importance of the presence of fatty acid transport which is the oocyte filling material in follicles [21]. Regulation by Oodev in the gonadal development could increase the process of testosterone conversion to estradiol through aromatase

activity. In the following process, the estradiol is used as the main ingredient to fill the oocytes in the egg follicles [22].

It is indicated that Oodev's mechanism works with forskolin and dbcAMP in the aromatization process as a response to the secretion of GtH [23, 24, 25]. The results of this study show that antidopamine in Oodev could have a role in the development of gonads in a small amount. It is suggested that this might be caused by the difference in the antidopamine's working system in gonadal development; therefore, the role in the brain is not yet known. The E2 profile showed an increase in all treatments on Day10 and was significantly affected by the administration of Oodev ($P<0.05$). The highest value was showed by treatments A2, B2, C2, and D2, in which all these treatments were accompanied by the injection of Oodev 15 IU/kg. This shows that the injection of Oodev is very effective in stimulating early gonadal maturation, proven by treatment A2 which had Oodev 15 IU/kg injections alone without supplementation using *S. platensis* but showed similar estradiol levels as treatment with the combination between Oodev injections and *Spirulina* supplementation (B2, C2, and D2).

The increase in estradiol concentration in the blood will stimulate the liver to perform vitellogenesis and will in turn accelerate the gonadal maturation process. Therefore, the blood plasma estradiol level can be used as an indicator for gonadal maturation [26]. As a result of the increase in E2 concentration, vitellogenesis in the blood plasma will also increase. In this process is found the yolk material accumulation by the oocyte; and during the process of vitellogenesisthere will be an increase in the corona radiata, granulosa cells, and thecal cells. These thecal cells will be responsible for the synthesis of 17alpha-hydroxyprogesterone and testosterone. The granulose cells will change the hormone into 17alpha, 20beta-dihydroxy-4-pregnen-3-one (17.20-P) and estradiol-17beta. The circulation of estradiol-17beta controls the development of several vitellogenin genes (Vg) [27]. Vitellogenin is a glycofosfoprotein which is approximately 20% fat, especially fosfolipid, triglycerides, lipoprotein and cholesterol. These molecules will become the energy source for the oocyte formation process which will be extremely important in the development of the embryo and the survival of the larvae [19, 28].

Another parameter which is related in fecundity, the number of eggs produced (eggs/kg brooder). If compared between treatments, the highest fecundity was shown by treatment B2 (Oodev 15 IU/ kg and *S. platensis* 1%) and D2 (Oodev 15 IU/ kg and *S. platensis* 3%) (Table 2). This shows that treatment using a combination is very good for the broodstock fecundity. The results of this study are supported by Kamler's. (1992) statement that all animals fed good quality feed will have higher fecundity; in contrast, low quality feed reduces the number of eggs [29]. In turn, if the signals from the hormone do not work well, it will affect the production of hormones which form follicles. In other words, the factor which has the strongest effect on fecundity is the interaction between nutrition and hormones.

The *S. platensis* factor had a significant effect ($P<0.05$) on the hatching rate, while the Oodev factor did not have a significant effect ($P>0.05$) and there is no interaction between the two factors. The best hatching rate (HR) was shown by treatment D2 (Oodev 15 IU/ kg and *S. platensis* 3%) at 91.10 %. Treatments D1, C2, C1, B2, B1 and A2 also showed higher percentages which were significantly

different from A1 (control). The fatty acids from *Spirulina* which were available in the feed are beneficial in the development of egg morphology such as the development or the building of the cell membranes and as a prostaglandin precursor so that the eggs were not easily damaged and the hatching rate improved. According to Mokoginta (1992), essential fatty acids in the eggs affect initial embryogenesis, determining whether the embryo develops or not [30]. Essential fatty acids function as a precursor for the prostaglandin compound which functions as a hormone. The recognition process between cells in the egg is influenced by prostaglandin. If the egg is deficient in essential fatty acids, the embryogenesis will fail.

The eggs that hatched were then kept until the larvae were 4 days old. The results of the observation of the larvae's survival rate without feed showed the higher the dosage of *Spirulina*, the higher the survival rate. Based on the statistical analysis, the *S. platensis* factor had a significantly different effect ($P<0.05$) on the larvae survival rate. The Oodev factor did not have a significant effect ($P>0.05$) and there were no interactions discovered between the two factors on the survival rate. The best survival rate was shown by treatment D2 (Oodev 15 IU/kg and *S. platensis* 3%) i.e. 92.94% (Table 2). The larvae's survival rate is strongly related to the inherent energy reserves in the egg yolk. The development of inherent energy reserves is strongly affected by the brooder's nutrition. The larvae's initial survival rate is very much determined by the energy reserves which were prepared by the brooder from the moment vitellogenesis commences until the egg matures (is ready to be spawned). This result is supported by the opinions of Tang and Affandi (2001), Mokoginta (1992) and Utomo (2009), who state that the content of egg fatty acids could increase larvae survival rate [19, 30, 31] .

4. Conclusion

- The experimental feed which was combined with the hormone Oodev which was best for accelerating gonadal maturation and improving egg and larvae quality and quantity was treatment D2 (*Spirulina* 3% ; Oodev 15 IU).
- The supplementation using *Spirulina platensis* to the feed which was combined with 15 IU of the hormone Oodev could accelerate the brooder's gonadal maturation, and stimulate the reproductive performance, especially the gonad development, egg and larvae quality, and the larvae survival rate.

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