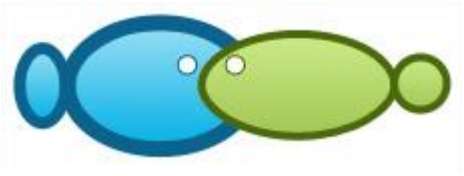

Improved vitellogenesis, reproductive performance and larval quality of striped catfish (*Pangasianodon hypophthalmus*) induced by the pregnant mare's serum gonadotrophin (PMSG) hormone and essential fatty acid diet in out-of-spawning season

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Abstract. This study was conducted to evaluate the effect of pregnant mare's serum gonadotrophin (PMSG) hormone combined with essential fatty acids diet as a new method to improve vitellogenesis, reproductive performance and larval quality of striped catfish (*Pangasianodon hypophthalmus*). This study used a completely randomized design with four treatments and three replicates. The treatments were: A) PMSG 20 IU kg^{-1} female + commercial feed, B) formulation feed, C) PMSG 20 IU kg^{-1} female + formulation feed, D) commercial feed. The fish specimens used were 15 females for each treatment, with a body weight ranging from 2.5 to 4 kg. Blood samples were collected on the 0th, 2nd, 4th, 6th and 8th weeks to measure the concentration of estradiol-17ß and vitellogenin. The reproductive parameters and larval quality of striped catfish were measured at the end of this study. The results showed that the combination of 20 IU kg^{-1} of PMSG and essential fatty acid diet produced a higher reproductive performance of the striped catfish with the gonad maturity rates of 93.33% and a highest larval production (160.013 larvae kg^{-1} female), with the highest larval survival rate (53.68%) compared to other treatments. Thus, the induction of PMSG at a dose of 20 IU kg-1 combined with formulated feed can improve vitellogenesis, reproductive performance and larval quality of striped catfish.
Key Words: essential fatty acid, larval quality, PMSG hormone, reproductive performance.

Introduction. The rapid cultivation of striped catfish has led to an increase of the production targets, which must be accompanied by adequate seed availability. However, the problem that occurs in striped catfish hatcheries is the limited availability of mature gonad broodstock, with adequate quantity and quality during the dry season. This causes the production of striped catfish seeds which are not available throughout the year. This happens because catfish breeding generally occurs during the rainy season, while in the dry season it is difficult to find adult female gonads (Moses et al 2016). This causes difficulties in obtaining seeds during the dry season and results in an unstable production of catfish seeds throughout the year. Therefore, efforts are still needed to overcome these problems through reproductive engineering.

Several factors are involved in the success of fish reproduction, including environmental signals, hormones, reproductive organs, nutrition of broodstock (Yousefian & Mousavi 2011; Pankhurst & Munday 2011; Izquierdo et al 2001). One of the efforts that can be made to increase the seed production is by accelerating the maturation time of the broodstock gonads after spawning (rematuration). Approaches that can be used to accelerate the gonad maturation are hormonal applications to stimulate the reproductive

process (Lieberman 1995) and nutritional support in the feed so that it will have a positive effect on the quality of eggs and larvae produced (Izquierdo et al 2001).

The PMSG hormone is a type of gonadotropin hormone that is very important for the reproductive process. PMSG is one of the steroid hormones that come from horse serum which contains gonadotropin as follicle-stimulating hormone (FSH) and the least amount of luteinizing hormone (LH). PMSG containing FSH activates gonads to synthesize estradiol-17β which in turn stimulates the liver to produce vitellogenin (Nagahama 1983; Swanson et al 2003). The previous research showed that the induction of PMSG at a dose of 20 IU kg^{-1} could be used to improve the reproductive performance of the striped catfish in the out-of-spawning season (Pamungkas et al 2019).

The gonadal maturation process is influenced by hormonal and nutritional factors that play a role in the maturation process of gonads, determining the quality of eggs and the larval production. The results of previous research indicated that the quantity and quality of feed given to the broodstock is an important factor that is closely related to the maturity of the gonads, the number of eggs produced and the quality of the eggs (Watanabe 1988). The energy source for the development of fish larvae is very dependent on the egg material that has been prepared by the broodstock.

Linoleic and linolenic essential fatty acids in broodstock feed are needed, especially in the process of embryo development. Unsaturated fatty acids such as linoleic (18:2n-6) and linolenic (18:3n-3) in fish feed are the main determinants of reproductive success and larval survival (Izquierdo et al 2001). Therefore, the composition of the two fatty acids in the feed is expected to improve egg quality so that the production and survival of the striped catfish larvae increase. The previous research showed that the administration of 2% corn oil in the diet as a source of n-6 (linoleic acid) and 1.5% fish oil as a source of n-3 (linolenic acid) can improve the quality of eggs and larvae production of striped catfish in out-of-spawning season (Pamungkas et al 2020).

Based on the description above, the combination of PMSG hormone application and feed containing essential fatty acids is expected to accelerate the gonadal maturation of the striped catfish and to improve the quality of eggs and larvae produced. The combination of hormones and feed is expected to complement the shortcomings of the two in improving the reproductive performance of striped catfish in out-of-spawning season.

Material and Method. The study was conducted at The Research Institute for Fish Breeding (RIFB), Ministry of Marine Affairs and Fisheries, West Java, Indonesia. This research does not require ethical approval from any institution and there are no such applicable laws in Indonesia.

Striped catfish rearing. Sixty female striped catfish from the RIFB collection, weighing 2.5–4.0 kg, were used as experimental models. Fish were acclimated to experimental conditions for 2 weeks before treatment after being selected based on their gonad maturity (no eggs). Fish were kept in 12 net cages (3 × 5 × 1.5 m) located in the 6,000 m^2 of an earthen pond of the Research Institute for Fish Breeding (RIFB), Subang, West Java, Indonesia. Each net cage was stocked with 10 fish, consisting of five females and five males, cultured for 3 months. During the experiment period, the catfish were fed two times a day using the experimental diet at 3% of the total biomass per day. The experimental catfish were tagged using a microchip. Blood samples were collected from two catfish from each treatment on 0, 2, 4, 6 and 8 weeks to measure the plasma oestradiol and vitellogenin concentration. The gonadal maturity stage of the experimental catfish was observed every two weeks. The ovi somatic index, fecundity, fertilization rate, hatching rate, larval production and 4 days survival rate of larvae were measured at the end of the experiment.

Experimental design. The study used a completely randomized design with three replications. Four treatments were used in this study: A) commercial feed, B) induced PMSG hormone 20 IU kg^{-1} + commercial feed, C) formulated feed, and D) induced PMSG hormone 20 IU kg^{-1} + formulated feed. The doses of PMSG hormone were induced to the

females based on the previous research (Pamungkas et al 2019). The formulation feed used 2% corn oil as an n-6 fatty acid source and 1.5% fish oil as a source of n-3 fatty acid (Table 1) (Pamungkas et al 2020). The proximate composition of the experimental diets is presented in Table 2. The fatty acid composition of the diets is presented in Table 3.

Table 1

Feed formulation of the striped catfish diet

Ingredient	*%*
Fish meal	50.75
Soybean meal	18.61
Coconut oil	6.95
Fish oil	1.50
Corn oil	2.00
Premix	2.00
Choline chloride	0.50
Tapioca	5.70
CMC[1]	0.30
Wheat flour	11.69

[1)] CMC = carboxymethyl cellulose, [2)] NFE-Nitrogen Free Extract.

Table 2

Proximate composition of the striped catfish diet (%)

Proximate (%)	*Diet*	
	Commercial	*Formulated*
Protein	35.76±0.01[a]	36.82±0.59[b]
Fat	4.94±0.16[a]	12.72±0.01[b]
Fiber	2.46±0.48[a]	2.99±0.02[a]
Ash	7.82±0.05[a]	16.36±0.02[b]
NFE	49.04±0.70[a]	31.12±0.61[b]
Energy (Kcal kg^{-1} feed)	449.778	455.624
Energy/protein	12.579	12.374

Values (means ± S.D., n=3) in the same row with different superscript letters show significant differences (P<0.05). CMC-carboxymethyl cellulose; NFE-nitrogen-free extract.

Table 3

The fatty acid composition of the experimental diets (% area)

Fatty acid	*Commercial feed*	*Formulated feed*
Σn−3 fatty acid (%)	10.05±0.02[a]	2.56±0.05[b]
Σn−6 fatty acid (%)	4.52±0.04[a]	13.75±0.08[b]
Σn−9 fatty acid (%)	47.81±0.04[a]	22.23±0.01[b]
ΣSaturated fatty acid (%)	53.44±0.03[a]	59.28±0.02[b]
ΣUnsaturated fatty acid (%)	46.76±0.04[a]	42.53±0.06[b]
n−6/n−3 ratio	0.45±0.00[a]	5.38±0.12[b]

Values (means ± S.D., n=3) in the same row with different superscript letters show significant differences (P<0.05).

Plasma oestradiol-17b and vitellogenin concentrations. Blood samples were collected every 2 weeks, that is on 0^{th}, 2^{nd}, 4^{th}, 6^{th} and 8^{th} weeks to measure plasma oestradiol-17b and vitellogenin concentrations. Blood was collected from the caudal artery using a syringe internally coated with heparin. Plasma was separated by centrifuging at 3,000 g for 15 min and stored at −20°C until further use. The oestradiol-17b concentrations were measured to determine the variations during the gonadal maturation process. The concentrations of oestradiol-17b in the plasma were measured by ELISA (EIA1561 DRG International Inc.). The plasma vitellogenin concentrations were

measured to determine the variations during the gonadal maturation process. Vitellogenin concentrations in the plasma were measured in two steps. The first step was the isolation of the vitellogenin from the plasma of experimental catfish by using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad) (Walker 2002). The second step was the quantification of the isolated vitellogenin using the Bradford method (Kruger 2002).

Five plasma samples from each experimental group were mixed and homogenized, and the mixed plasma was used to measure the plasma oestradiol-17b and vitellogenin concentrations. Because the data represent the averages of pooled samples for each group and do not account for the variation among individuals, they were not analyzed for statistical significance.

Water quality, rainfall, and air humidity. The water quality of the rearing pond was maintained under controlled conditions during the study period. The average water temperature ranged from 30 to 33.8°C, the pH ranged from 7.4 to 7.7 and the dissolved oxygen ranged from 4.0 to 5.0. The standard of water temperature, pH and dissolved oxygen range 28–30°C, 6.85–7.50 and 3–6, respectively. Rainfall and air humidity during the study ranged from 0.00 to 20.80 mm and from 63.30 to 94.20, respectively.

Reproductive performance. Reproductive performance of striped catfish female such as percentage of mature females, relative fecundity, fertilization rate, hatching rate, ovi somatic index, larval production, and the survival rate of larvae was observed at the end of the study. The following equations were used to calculate the reproductive performance:

Mature females (%) = (Number of mature females/Total number of females) × 100 (Wahyuningsih 2012);
Relative fecundity eggs kg^{-1} = Total number of eggs in the female ovary/Total weight of the females (Orlando et al 2017);
Fertilization rate (%) = (Number of fertilized eggs/Total number of eggs) × 100 (Tilahun et al 2016);
Hatching rate (%) = (Number of hatched eggs/Total number of fertilized eggs) × 100 (Hanjavanit et al 2008);
Ovi somatic index (OSI) (%) = (ovulation eggs weight/Body weight) × 100 (Hardjamulia 1987);
Larval production (larvae/kg of female body) = Total number of larvae per female/Bodyweight (Mimid et al 2009);
The survival rate of larvae (%) = (final number of larvae/initial number of larvae) × 100 (Effendie 1997).

Analysis of proximate and fatty acid composition. Analysis of proximate and fatty acid was carried out on an experimental diet, eggs and larvae. The proximate analysis was carried out according to the AOAC (2005), consisting of the analysis of protein, crude fiber, ash content and moisture content. Total lipid was determined using the method of Folch et al (1957). Analysis of fatty acid was carried out using gas–liquid chromatography (GLC). Experimental diet, eggs and larvae were stored in the freezer before their proximate and fatty acid was analyzed.

Fatty acid methyl esters (FAME) were separated and quantified by gas–liquid chromatography (Automatic System XL, Perkin Elmer) equipped with a flame ionization detector (FID) and a 30 m × 0.32 mm fused silica capillary column (Omegawax 250, Supelco). Helium was used as the carrier gas and the temperature was programmed to rise from 50 to 220°C at 4°C min^{-1} and then kept at 220°C for 35 min. The injector and detector temperatures were set at 250°C and 260°C, respectively. Fatty acids were identified by comparing the retention times of FAME with the standard FAME component.

Data analysis. The data of the oestradiol and vitellogenin levels were presented descriptively to describe the changes in the egg development and in the oestradiol and

vitellogenin levels during the gonadal development. The reproductive parameters including the percentage of mature females, ovi somatic index, fecundity, fertilization rate, hatching rate, larval production and larval survival rate were analyzed statistically. The data were subjected to an analysis of variance using Microsoft Excel 2016 and SPSS program version 25. If the treatment effect was significant, the differences between the means were examined further using Duncan's test. Probabilities of <0.05 were considered significantly different. Data are presented as mean ± standard deviation.

Results

Oestradiol-17β concentration. Plasma oestradiol concentrations in the striped catfish increased during the gonadal maturation process. There was an increase in the concentration of oestradiol in all treatments in the second week. A significant increase in the concentration of oestradiol occurred in the catfish females, induced by PMSG. The highest oestradiol concentration was observed in the PMSG + commercial fed specimens on week 6 and began to decrease in week 8. The oestradiol concentration of fish induced by PMSG + formulated fed peaked in week 6 and decreased in week 8. In the other treatments, the changes of oestradiol concentration took place gradually (Figure 1.)

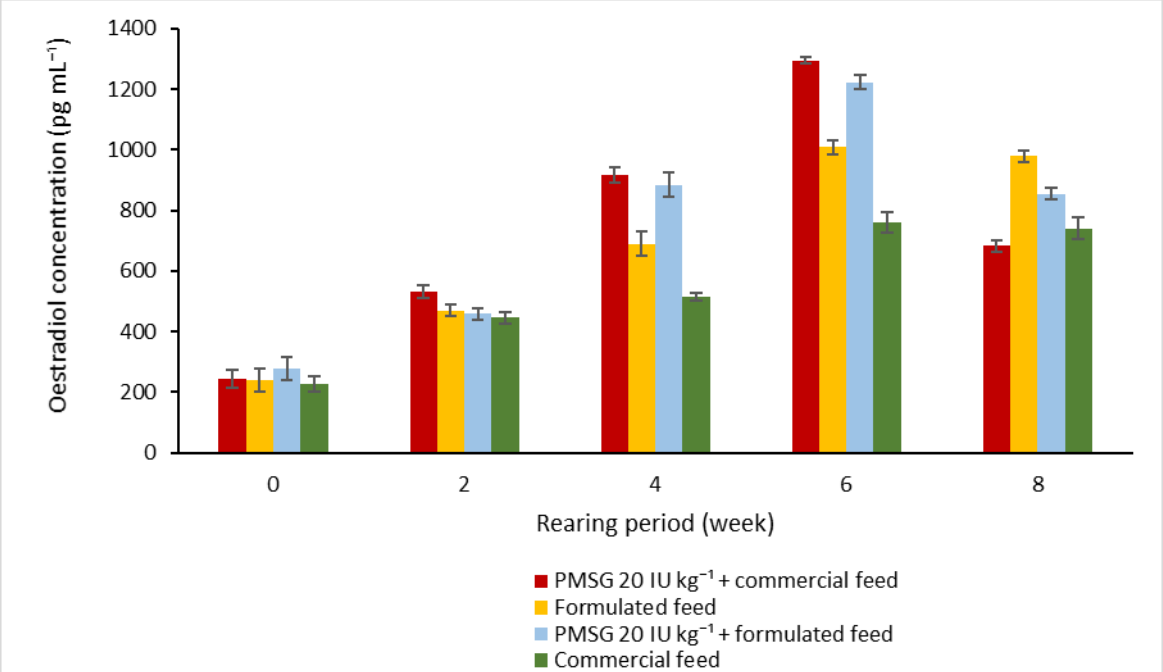

Figure 1. Oestradiol-17β concentrations in the striped catfish female.

Vitellogenin concentration. The vitellogenin concentration in the blood plasma of the catfish female induced by PMSG began to increase at week 2 (Figure 2). The concentration of vitellogenin of the catfish female induced by PMSG in the fourth week was higher than other treatments. The highest vitellogenin concentration was detected in the striped catfish female induced by PMSG 20 IU kg^{-1} and fed with commercial feed on week 6 (89.75 mg mL^{-1}) and decreased rapidly on week 8 (30.80 mg mL^{-1}). The pattern of vitellogenin increase was also found in fish induced by PMSG and fed with formulated feed. The highest concentration of vitellogenin was observed on week 6 (70.50 mg mL^{-1}) and decreased rapidly on week 8 (28.15 mg mL^{-1}). In the other treatments, the vitellogenin concentration increased gradually in fish fed with formulated feed without PMSG induction and increased slowly in fish fed with commercial feed without PMSG induction. The increased vitellogenin concentration is in line with the increased oestradiol concentrations in the blood plasma.

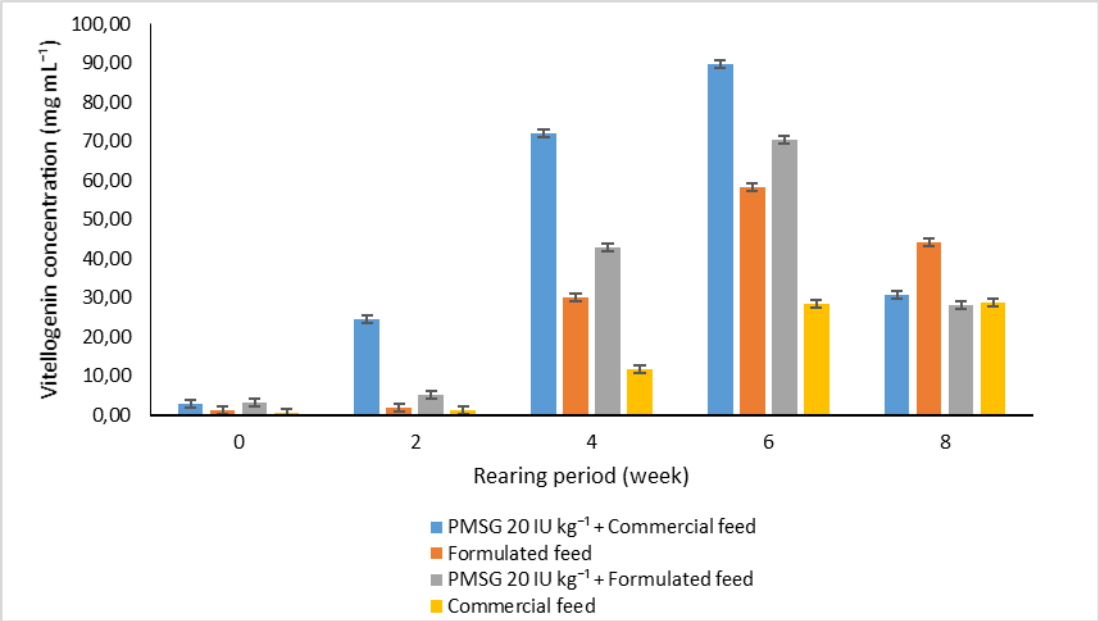

Figure 2. Vitellogenin concentrations in the striped catfish female.

Proximate and fatty acid compositions of the diets. The proximate compositions of the commercial and formulated feed showed significant differences in protein, fat, ash and NFE value (Table 2). The energy value of the formulated feed was higher than in the commercial feed. The fatty acid compositions of the commercial and formulated feed were significantly different (Table 3). The fatty acid composition of the formulated feed has a higher content of n-6 fatty acids (13.75% area) than the commercial feed (4.52% area) as well as a higher n-6/n-3 ratio (5.38) than the commercial feed (0.45).

Fatty acid composition in the eggs and larvae of striped catfish. The fatty acid composition of eggs in the four treatments is presented in Table 4. The fatty acid composition of eggs between the treatments was significantly different regarding the total saturated fatty acid, total unsaturated fatty acid and n6/n3 ratio. The n6/n3 ratio of eggs from the fish fed with the commercial feed was lower than in the fish fed with the formulated feed.

Table 4

Fatty acid composition in eggs of striped catfish

Fatty acid	*Treatment*			
	PMSG 20 IU kg^{-1} +commercial feed	*Formulated diet*	*PMSG 20 IU kg^{-1} + formulated diet*	*Commercial feed*
Σn−3 fatty acid (% area)	5.08±0.52^{a}	5.14±0.80^{a}	5.17±0.29^{a}	7.88±0.86^{b}
Σn−6 fatty acid (% area)	7.47±0.47^{a}	9.17±0.40^{b}	10.69±0.86^{c}	10.23±0.59^{bc}
Σn−9 fatty acid (% area)	14.36±0.14^{a}	15.28±0.11^{a}	17.85±1.18^{b}	22.80±1.01^{c}
Σ Saturated fatty acid (% area)	29.35±0.51^{a}	33.09±1.71^{b}	38.42±0.59^{c}	52.36±1.05^{d}
Σ Unsaturated fatty acid (% area)	28.37±0.62^{a}	31.81±1.22^{b}	36.01±0.59^{c}	42.54±0.79^{d}
n−6/n−3 ratio	1.48±0.06^{a}	1.80±0.19^{b}	2.07±0.05^{c}	1.30±0.07^{a}

Values (means ± S.D., n=3) in the same row with different superscript letters show significant differences ($p<0.05$).

Fatty acid profile of 1-day post-hatching (DPH) larvae obtained from the broodstock fed with the experimental diets is shown in Table 5. There were changes in the total fatty acid percentage of the eggs and larvae. Eggs' total fatty acids as a whole are greater

than the total fatty acids in larvae. There was a decrease in the total value of fatty acids contained in the larvae. The sum of n-3, n-6, n-9 fatty acid has no significant difference between different treatments. The maximum (1.81) and minimum (1.33) values of the n-6/n-3 ratio were found in the formulated feed and in the commercial feed, respectively. The highest value of unsaturated fatty acid (19.27% area) was found in fish induced by PMSG and fed with formulated feed.

Table 5

Fatty acid composition in the larvae of striped catfish

Fatty acid	*Treatment*			
	PMSG 20 IU kg^{-1} female + Commercial feed	*Formulated diet*	*PMSG 20 IU kg^{-1} female+ Formulated diet*	*Commercial feed*
Σn−3 fatty acid (% area)	2.83±0.41^{a}	2.50±0.22^{a}	2.77±0.09^{a}	2.92±0.38^{a}
Σn−6 fatty acid (% area)	4.19±0.62^{a}	4.51±0.13^{a}	4.22±0.34^{a}	3.87±0.40^{a}
Σn−9 fatty acid (% area)	8.53±1.07^{a}	8.02±0.40^{a}	9.47±0.56^{a}	8.82±0.26^{a}
Σ Saturated fatty acid (% area)	16.63±2.09^{a}	15.87±0.71^{a}	19.65±1.21^{b}	18.95±0.55^{b}
Σ Unsaturated fatty acid (% area)	16.63±2.09^{a}	15.87±0.71^{a}	19.27±0.70^{b}	16.38±0.59^{a}
n−6/n−3 ratio	1.33±0.04^{a}	1.48±0.01^{a}	1.81±0.14^{b}	1.53±0.17^{a}

Values (means ± S.D., n=3) in the same row with different superscript letters show significant differences ($p<0.05$).

Reproductive performance of striped catfish. Reproductive parameters of the striped catfish with different treatments are presented in Table 6.

Table 6

Percentage of mature female (MF), ovi somatic index (OSI), fecundity, fertilization rate (FR), hatching rate (HR), larval production and larval survival rate (SR)

Parameter	*PMSG 20 IU kg^{-1} female +Commercial feed*	*Formulated diet*	*PMSG 20 IU kg^{-1} female+ Formulated diet*	*Commercial feed*
MF (%)	100.00±0.00^{a}	80.00±00.00^{b}	93.33±11.55^{ab}	53.33±0.00^{c}
OSI (%)	11.01±2.32^{a}	11.69±1.09^{a}	12.04±0.80^{a}	9.01±0.81^{a}
Fecundity (egg kg^{-1})	171,800±6,662^{a}	203,232±10,377^{b}	214,280±28,286^{b}	133,027±6,406^{c}
FR (%)	85.24±0.23^{a}	86.66±8.27^{a}	85.09±2.16^{a}	76.11±7.98^{a}
HR (%)	77.25±3.37^{a}	87.46±7.44^{a}	88.08±2.00^{a}	57.58±12.75^{b}
Larval production (larvae kg^{-1} female)	113,001±1,986^{a}	155,333±32,742^{a}	160,013±14,224^{a}	58,676±16,044^{b}
SR (%)	23.89±7.52^{b}	40.93±7.86^{c}	53.68±2.83^{d}	11.21±4.12^{a}

Values (means±S.D., n=3) in the same row with different superscript letters show significant differences ($p<0.05$).

The lowest value of mature female percentage (53.33%), ovi somatic index (9.01%), fecundity (133.027 egg kg^{-1}), fertilization rate (76.11%), hatching rate (57.58%), larval production (58,676 larvae kg^{-1} female) and survival rate of larvae (11.21%) was obtained from fish fed with commercial feed, without induction of PMSG. The highest percentage of mature females were observed in fish induced by PMSG and fed with commercial feed (100%), followed by fish induced by PMSG and fed with formulated feed (93.33%) and by fish fed with a formulated diet without PMSG induction (80.00%). The fecundity of the eggs was significantly different between fish fed with formulated feed and commercial feed. The survival rate of larvae was significantly different between the

different treatments. The highest value of survival rate was observed in fish fed with formulated feed and induced by PMSG (53.68%). No significant differences in the ovi somatic index and fertilization rate were recorded in fish fed with the different treatments.

Discussion. The study of the effect of the combination of PMSG hormone induction with essential fatty acid feed on the striped catfish female is an effort to produce striped catfish seeds throughout the year, with good larval quality. Hormonal application is intended to stimulate the reproductive process, by inducing maturation of the gonads (Lieberman 1995), while the nutrients in the broodstock diet play a role in the synthesis and release of hormones from the endocrine glands to the target organs (Watanabe 1988). The nutritional content of the diet also has a positive effect on the quality of eggs and larvae (Izquierdo et al 2001).

The gonadal maturation process of the striped catfish is characterized by an increase of oestradiol-17β and vitellogenin concentrations in the blood plasma and the development of oocytes during the maturation process. Oestradiol levels peak towards the end of the vitellogenesis and they decline rapidly during the maturation stage of the gonads. This study described that the increase of the oestradiol levels was in line with an elevation in the vitellogenin levels during the vitellogenesis. This result was similar to the previous research in Caspian Kutum (Heidari et al 2010), sea bass (Mananos et al 1997) and *Prochilodus lineatus* (Hainfellner et al 2012). The concentrations of oestradiol and vitellogenin were high during the vitellogenesis in fish induced by PMSG. The hormonal application can accelerate the gonadal maturation process of the striped catfish.

Oestradiol-17β is the main estrogen in female fish (Wang et al 2008; Cabrita et al 2009), which plays a role in the gonadal maturation process (Klinge 2000; Nilsson et al 2001). The concentration of oestradiol in plasma increases during vitellogenesis and the concentration level remains high during the process (Lubzens et al 2010). Some previous studies showed that the variations in the steroid hormone levels correlated with reproductive cycles (Liu et al 2008; Yan et al 2011; Ni et al 2013). Liver was stimulated by oestradiol to synthesize and secrete vitellogenin, which is concentrated in oocytes. Correlations between gonadal steroid levels and oocyte development during the gonadal maturation process have been observed in marine fish species including gilthead seabream (*Sparus aurata*) (Pozo et al 2008), pejerrey (*Odontesthes bonariensis*) (Elisio et al 2014) and several freshwater species including cutum (*Rutilus frisii*) (Sabet et al 2009), mahseer (*Tor tambroides*) (Ismail et al 2011), Shad India (*Tenualosa ilisha*) (Pramanick et al 2013) and Asian Redtail catfish (*Hemibagrus nemurus*) (Adebiyi et al 2013). The elevation in oestradiol and vitellogenin levels is related to the gonadal maturation process in the striped catfish female. The success of the gonadal maturation process will affect the reproductive performance of the striped catfish.

Figure 2 shows that the vitellogenin concentration in fish induced by PMSG increases rapidly during the vitellogenesis. The concentrations of oestradiol and vitellogenin were higher in fish induced by PMSG and fed with a commercial or formulated diet than in the other treatments. Vitellogenin levels decreased at the 8th week, which may be related to a decrease in the rate of vitellogenin synthesis in the liver. In other treatments, the changes in oestradiol and vitellogenin levels were slower than in the fish induced by PMSG hormone (Figure 1 and Figure 2).

Hormone induction is performed to replace the environmental signals, as a signal for the maturation process of the gonads. PMSG that contains a lot of FSH will activate the gonads to synthesize estradiol-17β, which in turn stimulates the liver to produce vitellogenin (Nagahama 1983). The PMSG hormone plays a very important role in the process of vitellogenesis and maturation of the gonads. In the reproductive process, nutrition plays an important role in improving the reproductive performance of fish and in producing good quality eggs and larvae. Nutrients in broodstock diets are the main elements that affect their reproductive performance. In the gonadal maturation phase, nutrients will be used for reproductive purposes, namely for determining the quality and quantity of eggs. The quality and quantity of broodstock nutrients are also associated with the spawning and fecundity (Watanabe 1988).

The reproductive performance of striped catfish female induced by PMSG gave a physiological response, seen from the percentage of the female reaching maturity: 100% in PMSG treatment with 20 IU kg^{-1} + commercial feed and 93.33% in PMSG treatment 20 IU kg^{-1} + formulated feed. The combination of PMSG hormone induction and essential fatty acid feed resulted in a higher ovi somatic index (12.04%), fecundity (214,280 eggs kg^{-1} female), larval production (160,013 kg^{-1} female) and a higher larval survival rate (53.68%) higher than in the other treatments. These results show that PMSG induction and an essential fatty acid diet can improve the reproductive performance of striped catfish.

The presence and composition of nutrients in the form of fatty acids in broodstock feed are the main factors that play an important role in the reproductive success and larval survival (Meinelt et al 2004). Essential fatty acids are known to be precursors of prostaglandins. In fish, prostaglandins have a functional role in accelerating the ovulation and in regulating the synchronization of the spawning behavior (Shilo & Sarig 1989). Essential fatty acids play a role in the formation of prostaglandins and prostaglandins act as hormones that help in ovulation, which is the breakdown of the follicular cells. So it can be said that the presence of prostaglandins formed from essential fatty acids determines the success of the oocyte maturation which is related to the degree of egg fertilization. The success of the embryogenesis process can also show the quality of the eggs.

The addition of n-3 and n-6 fatty acid levels in the feed, to a certain extent, will affect the success of the embryogenesis process as shown by the value of the degree of eggs fertilization and the high degree of hatching. The previous study reported that linoleic fatty acids with levels of 1.56% can increase the fecundity, egg diameter, egg hatching rate and survival rate of larvae in baung fish, *Hemibagrus nemurus* (Utiah et al 2007). Mokoginta et al (2000) reported that catfish require n-3 and n-6 fatty acids to produce a good quality of eggs and for the reproductive process. Further investigations revealed that the catfish required n-3 (0.9%) and n-6 (2.2%) fatty acids in the total level of 12.87% of fish lipids, in order to produce good quality eggs. An increase in the composition of essential fatty acids in the feed raised the hatching rate and larvae survival rate on day 14 of rabbit fish. An increase in the essential fatty acids in feed can also increase the weight of fish larvae and the resistance to changes in the osmotic pressure, so that their survival is higher (Izquierdo et al 2001). The availability of adequate nutrients and hormonal signals causes the vitellogenesis process to work faster and results in an accelerated maturation of the gonad and in the oocyte development.

Conclusions. The results of the present study indicated that the induction of PMSG hormone and essential fatty acids in the diet have a positive effect on estradiol levels, vitellogenin profile, gonadal maturity level, quality of eggs and larvae of striped catfish. The combination of PMSG hormone induction and essential fatty acid feed (n-6 and n-3) can accelerate maturation and improve the reproductive quality of striped catfish in out-of-spawning season.

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