The effect of the addition of cow brain powder in commercial feed on the gonadal maturity of comet goldfish (Carassius auratus auratus)

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Abstract. The aim of this research was to analyze the effect of addition bovine’s brain meal in artificial feed on gonad maturity and to find out the best time of gonad maturity in comet fish. This research was conducted at Fourth Building Hatchery Faculty of Fisheries and Marine Sciences Padjadjaran University on November 2014 until January 2015. Freeze drying of bovine brain was conducted at Research Center Inter University Bandung Institute of Technology. The research was using Completely Randomized Design (CRD) with four treatments and three replications. The treatment were 20 mg/kg, 35 mg/kg, 50 mg/kg and control. The parameters of this research are Gonado Somatic Index (GSI) and egg maturity level. Addition of bovine brain meal in feed with the dose of 50 mg/kg are giving the best result until 45 days of the care time against gonad maturity of comet fish with GSI result 12.93 %, egg maturity level ripe phase 21.115 and fecundity 1520 grain/g.

1. Introduction
Gonadal maturity and egg quality can be improved by good broodstock management. The external factors that need to be considered in broodstock management are, among others, cultivation container, water quality, temperature, broodstock density, and feed. Internally, the use of natural or synthetic hormones can accelerate the maturity of the gonads.

Cow brain is one of the natural ingredients that can be used to accelerate gonadal maturity. The brain is an organ that is extremely important in the reproductive system, because Gonadotropin-Releasing Hormone (GnRH), which consists of 10 amino acids (decapetide) that stimulate the release of gonadotropin (GTH) in the pituitary gland, is found in every part of it [1]. In a cultivation container, environmental signals are often weak or nonexistent that the reproduction process cannot run perfectly [2]. Stimuli from the environment, such as temperature, are processed through sensory receptors. The resulting neural signals, when reaching the hypothalamus in the brain, affect the pituitary gland through a chemical messenger which is referred to as releasing hormone. The releasing hormone stimulates the pituitary to produce a hormone with the gonads being the target organs. This hormone is called gonadotropin. This hormone affects the production of sex steroids in the gonads that eventually play a role in the maturation of gametes [3]. Therefore, hormonal preparations such as ovaprim, hCG (Human Chorionic Gonadotropin), and LHRH (Lutenizing Hormone Releasing Hormone) are used.

Cow brain that is processed into powder and mixed in feed can be used as an alternative ingredient for stimulating the gonadal development in comet goldfish. Comet goldfish (Carassius auratus auratus) is one of goldfish strains with physical beauty represented by a longer and more beautiful tail,
better endurance, and relatively lower price compared to the price of goldfish. There has been high demand for comet goldfish in the market that it is necessary to have a strategy to increase its production. One of the strategies that can be applied is having appropriate and efficient broodstock management. This study examined the extent to which cow brain powder mixed in feed affects the maturation time of gonads in comet goldfish.

2. Methodology
2.1. Tools and materials
This study was conducted in the Hatchery of the Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The freeze-drying of cow brain was conducted at the Inter-University Research Center of Institut Teknologi Bandung.

The materials used in the study were: female comet goldfish broodstocks weighing 9.67-12.22 g; cow brains from Caringin market, Bandung; fish pellets with 28 % protein content; carboxymethyl cellulose (CMC); sera solution made of 99 % alcohol solution; 40 % formaldehyde solution; and 100 % acetic acid solution at a ratio of 6:3:1 [4]. The tools used were: twelve 60 x 30 x 30 cm aquariums; aeration installation; DO meter from Lutron with a precision of 0.01 mg/L; pH Meter from Lutron; digital thermometer; blender; dissecting kit; thermostats for stabilizing the temperature of the aquarium water; analytical balance; microscope; and digital camera.

2.2. Method
This study used an experimental method with Completely Randomized Design (CRD), four treatments, and three replications. The treatments in this study were distinguished based on the administration of different doses of cow brain powder mixed with commercial feed as follows:
- Treatment A = Feed without cow brain powder (Control)
- Treatment B = Feed with cow brain powder, 20 mg/kg broodfish
- Treatment C = Feed with cow brain powder, 35 mg/kg broodfish
- Treatment D = Feed with cow brain powder, 50 mg/kg broodfish

2.3. Procedure
2.3.1. Test feed preparation
Fresh cow brains were cleaned and then cut/chopped into small pieces and inserted into a freeze-drying instrument. The freeze-dried brains were ground in a blender and the resulting powder was placed into a plastic container. This cow brain powder was then mixed with commercial feed according to the treatments. Adhesive (CMC binder) and 2 % of the commercial feed were added into the mixture. The mixed feed was then pelleted using a pellet mill. The pellets produced were sun dried and then stored in jars to be fed to the comet goldfish broodstocks until the gonads matured.

2.3.2. Comet goldfish broodstocks preparation
At the time of the study, the comet goldfish were in the initial maturation phase of gonads. The broodstocks used in the study were spawned first. Afterwards, stripping was performed on the comet goldfish broodstocks to remove eggs from the fish bodies.

2.3.3. Conduct of study
A total of 12 comet goldfish broodstocks were placed in individual aquariums. Feed was administered twice a day at 09.00 pm and 15.00 pm for 2 months of rearing. The amount of feed given was 3 % of the fish biomass weight. Container maintenance cleaning was performed every other day using siphon. The water quality parameters observed included temperature, pH, and dissolved oxygen content (DO). Observations were performed three times i.e., at the beginning, middle, and end of the study.

The comet goldfish gonadal maturity checks were conducted at 3 different times. The first check was performed after 1 month of rearing by examining 1 fish from every treatment to check the
development of the gonads. The second check was performed after 1 ½ months of rearing, while the third check was performed after 2 months of rearing using the same method i.e., examining 1 fish from every treatment to check the development of the gonads. The Gonad Maturity Index (GMI) was measured by deducting the weight of the dissected gonad from the weight of a comet goldfish broodstock. After the gonadal maturity examination was completed, the data obtained were further analyzed.

2.4. Observation Parameters
2.4.1. Gonad Maturity Index (GMI)
The GMI is calculated using the following formula of Effendi [5]:

\[ \text{GMI} = \frac{GW}{BW} \times 100 \% \]  \hspace{1cm} (1)

Notes:
GMI = Gonad Maturity Index
GW = Fish gonad weight in gram
BW = Body weight in gram

2.4.2. Percentage of Fish Egg Maturity Rate
The Egg Maturity Rate (EMR) was calculated based on the following formula [4]:

\[ \text{EMR of vitellogenic phase} = \frac{\text{Number of eggs centered nucleus} \times 100 \%}{\text{Number of eggs observed}} \]  \hspace{1cm} (2)

\[ \text{EMR of early maturity phase} = \frac{\text{Number of eggs with non-centered nucleus} \times 100 \%}{\text{Number of eggs observed}} \]  \hspace{1cm} (3)

\[ \text{EMR of mature phase} = \frac{\text{Number of eggs with fused nucleus} \times 100 \%}{\text{Number of eggs observed}} \]  \hspace{1cm} (4)

2.5. Data analysis
The data were analyzed using variance analysis with F-test to determine the effect of each treatment. If there were differences among the treatments, further analysis using Duncan’s multiple range test with a significance level of 5% was performed [6].

3. Results and Discussion
3.1. Gonad maturity index
Gonad maturation index (GMI) is an index that is used to determine the percentage of gonad weight in comparison to broodstock body weight. One of the factors influencing the maturity level of the gonads is the feed. Good quality feed contains compositions that support successful gonadal and spawning processes i.e., the compositions that include vitamin, 28-40 % protein content, and hormones, both synthetic and natural.

Table 1. Average of gonad maturity index.
The results of the study showed that the average GMI on the 30th day of rearing ranged from 7 to 12.44% (table 1). The highest GMI (12.44 %) after 30 days of rearing was found in Treatment D, and the lowest (7 %) was found in Treatment C. The average GMI on the 45th day of rearing ranged from 6.93 to 12.93 %. The highest GMI (12.93 %) at this point of time was seen in Treatment D, and the lowest (6.93 %) was found in Treatment B. The average GMI on the 60th day of rearing ranged from 10.90 to 12.13 %. Based on the analysis results that showed different GMIs, it was revealed that the addition of cow brain powder into the feed did not significantly affect the GMI of comet goldfish broodstocks. Overall, the GMIs in this study were still within the normal GMI range for comet goldfish, i.e., 7.28 to 19.11 % [7].

The GMI in Treatment D was the largest, and an increase was seen between the 30th day and 45th day of rearing (table 1). This is assumed to be the effect of GnRH in cow brain powder mixed with the feed that increased the weight of the test fish gonads. The GMI in Treatment C was the lowest on the 30th day of rearing, and the GMI in Treatment B was the lowest on the 45th day of rearing. This may have been caused by lower GnRH content in these treatment compared to that in Treatment D. According to Hartika [8], ovaprim induction (GnRH) needs a long pathway to be able to affect gonadal maturation. GnRH will cause hypophysis to secrete Gonadotropin (GtH), which is then passed into the blood and only at a certain level stimulates the final gonadal maturity through stimuli to synthesize the steroid hormone maturation by follicles in the ovaries. Based on this statement, it can be concluded that higher doses of GnRH will be more effective in stimulating gonadal maturation.

The feed that is mixed with GnRH-containing cow brain powder is consumed by the fish and then enters the intestine to be digested. GnRH in the feed is absorbed by the intestine and bound by a receptor through the blood and then brought to the target organ, which is the hypophysis. GnRH stimulates the pituitary gland to release gonadotropin, i.e. the follicle-stimulating hormone (FSH). This gonadotropin hormone is then carried by the receptor through the blood into the gonadal theca cells to stimulate the formation of testosterone (figure 1). The testosterone formed then enters the granulosa cell to be converted by the aromatase enzyme into estradiol-17β hormone. Estradiol-17β is a steroid hormone derivative of cholesterol that plays an important role in the vitellogenesis [9]. Some of the estradiol-17β will go to the liver to form vitellogenin, which is the main component of egg yolks. The resulting vitellogenin is then carried by the blood into the gonads to be absorbed by the oocytes, leading to egg diameter expansion. The process of vitellogenin will continue in the fish body. According to Effendie [5], changes in GMI are closely related to the stages of egg development.

| Treatment | Gonad Maturity Index (%) |
|-----------|-------------------------|
|           | Day 30 | Day 45 | Day 60 |
| A (control) | 10.54  | 9.66   | 12.13  |
| B (20 mg/kg broodfish) | 10.44  | 6.93   | 10.90  |
| C (35 mg/kg broodfish) | 7.00   | 10.94  | 11.81  |
| D (50 mg/kg broodfish) | 12.44  | 12.93  | 11.54  |
Fish
Feed consumed by fish

Intestines
Digest in the intestine, GnRh is absorbed by the receptor through the blood to the target organs of the hypothalamus

Brain
GnRh will simulate the hypothalamus to release the gonadotropin

Gonads
Gonadotropin stimulated the formation of testosterone, which is then converted to estradiol-17β

Eggs
estradiol-17β play the role in vitelogenesis process

Figure 1. Flowchart of GnRH effect in feed.

The GMI value in Treatment D on the 60th day of rearing decreased when compared with the values of other treatments which increased. This is allegedly due to the process of atresia, which is the absorption of eggs that have reached the maximum development but not ovulated [10]. In figure 2, it is depicted that after going through the dormant phase (the nucleus of the cell is located in the centre), if the level of gonadotropin is adequate, the egg will progress to the next stage which is marked by the shifting of the egg nucleus to the edge. Atresia will occur if the eggs that are ready to ovulate are ignored for too long or not fertilized.
Figure 2. Egg development process.

3.2. Egg maturity level

Based on the nucleus positions, the egg maturity rate (EMR) is divided into three according to different phases of development, namely before the egg is ovulated—which is called vitellogenic phase, initial maturation phase, and mature phase. The vitellogenic phase is characterized by the position of the egg nucleus at the center. The nucleus subsequently migrates from the centre to the edge during the initial maturation phase, and then fuses in the mature phase (figure 3). Gonadotropin and steroid hormonal actions cause the nucleus that is originally centered to move to the edge of the egg, near the microphyllid, and to fuse just before the ovulation [11]. This process is known as GVBD (Germinal Vesicle Breakdown) which indicates that the egg has entered the final maturation process and it may ovulate.

Figure 3. Nucleus movement phases of comet fish eggs (A) Edged nucleus; (B) Centered nucleus; (C) Fused nucleus (GVBD).

According the results of the observation on the 30th day, overall increases consistent with the increased doses of cow brain powder in feed are presented in figure 4. The average EMRs in
Treatment A were 90.56 %, 9.44 %, and 0 % for the vitellogenic phase, initial maturation phase, and mature phase, respectively. The average EMRs in Treatment B were 59.44 % for the vitellogenic phase, 33.89 % for initial maturation phase, and 6.67 % for the mature phase. The average EMRs in Treatment C were 82.22 %, 13.33 %, and 4.44 % in the vitellogenic phase, initial maturation phase, and mature phases, respectively. The average EMRs in Treatment D were 38.89 % for the vitellogenic phase, 38.89 % for the initial maturation phase, and 22.22 % for mature phase. Based on the analysis of variance on the maturity level of the eggs on the 30th day of rearing, it is apparent that there was a significant difference in the mature phase between treatments.

![Figure 4. Egg maturity rate on the 30th day of maintenance.](image)

Based on the results of the Duncan's follow-up test, Treatment A was not significantly different from Treatment B and Treatment C but significantly different from Treatment D (table 2). Treatment D had higher values than those of other treatment groups, which was caused by the high level of GnRH contained in the feed that put more trigger on the mature eggs. Murtejo [11] stated that gonadotropin and steroid hormone actions cause the nucleus that is initially located in the centre to move to the edge, near the microphyllid, and then it fuses just before ovulation. This process is known as GVBD (Germinal Vesicle Breakdown) which indicates that the egg has entered the final maturation process and can ovulate. When eggs are in the GVBD, the process of vitellogenesis or absorption of the vitellogenin in oocyte will be stopped and the egg diameter reaches the maximum size.

| Cow Brain Meal (mg per kg broodfish) | Vitellogenic Phase (%) | Initial Maturation Phase (%) | Mature Phase (%) |
|-------------------------------------|------------------------|-----------------------------|------------------|
| A (Control)                         | 90.56 ± 3.47           | 9.44 ± 3.47                 | 0 ± 0 a           |
| B (20 mg/kg broodfish)              | 59.44 ± 37.28          | 33.89 ± 34.17               | 6.67 ± 7.64 a     |
| C (35 mg/kg broodfish)              | 82.22 ± 10.05          | 13.33 ± 8.82                | 4.45 ± 2.55 a     |
| D (50 mg/kg broodfish)              | 38.89 ± 16.68          | 38.89 ± 3.47                | 22.23 ± 13.47 b   |

Notes: Figures followed by the same lowercase letters mean no significant difference at the significance level of 95%

The results of the observation on the 45th day still showed that Treatment D presented better results than those of other treatments in terms of egg maturity rate (figure 5). The average EMRs in Treatment A were 73.33 %, 19.44 %, and 7.22 % for the vitellogenic, initial maturation, and mature phases, respectively. Meanwhile, for Treatment B, the average EMRs were 78.33 % for the vitellogenic phase,
1.11 % for the initial maturation phase, and 0.56 % for the mature phase. The average EMRs for Treatment C were 71.67 %, 27.78 %, and 0.56 % for the vitellogenic, initial maturation, and mature phases, respectively. For Treatment D, the values were 38.33 %, 40.56 %, and 21.11 % for the vitellogenic, initial maturation, and mature phases, respectively.

Table 3. Average egg nucleus movement of comet fish eggs on the 45th Day.

| Cow Brain Meal (mg per kg broodfish) | Vitellogenic Phase (%) | Initial Phase (%) | Mature Phase (%) |
|-------------------------------------|------------------------|-------------------|------------------|
| A (Control)                         | 73.33 ± 3.33           | 19.44 ± 8.22      | 7.22 ± 5.36      |
| B (20 mg/kg broodfish)              | 78.33 ± 5.77           | 21.11 ± 4.81      | 0.56 ± 0.96      |
| C (35 mg/kg broodfish)              | 71.67 ± 1.67           | 27.78 ± 0.96      | 0.56 ± 0.96      |
| D (50 mg/kg broodfish)              | 38.33 ± 12.58          | 40.56 ± 20.02     | 21.11 ± 16.69    |

Notes: Figures followed by the same lowercase letters mean no significant difference at the confidence level of 95%
The results from the observation on the 60\textsuperscript{th} day of rearing were different from the previous days of rearing (figure 6). Treatment C had the highest EMR in the mature phase compared to other treatments. The average EMRs for Treatment A were 52.22\%, 31.67\%, and 16.11\% in the vitellogenic, initial maturation, and mature phases, respectively. In Treatment B, the EMRs for the vitellogenic phase were 62.22\%, while the values for the initial maturation phase and mature phase were 29.44\% and 13.89\%, respectively. In Treatment C, the EMRs were 22.78\% for vitellogenic phase, 40.56\% for initial maturation phase, and 36.67\% for mature phase. The average EMRs in Treatment D were 37.22\%, 50\%, and 23.78\% for vitellogenic, initial maturation, and mature phases, respectively.

![Figure 6. Egg maturity rate on the 60\textsuperscript{th} day of maintenance](image)

The results of the analysis of variance revealed that the egg maturity levels on the 60\textsuperscript{th} day in the initial maturation and maturation phases were not significantly different, but they were significantly different from that of the vitellogenic phase. Based on the results of Duncan’s follow-up test in vitellogenic phase, Treatment A was not significantly different from Treatment B, but it was significantly different from Treatment C and Treatment D (table 4).

| Cow Brain Meal (mg per kg broodfish) | Vitellogenic Phase (%) | Initial Phase (%) | Mature Phase (%) |
|-------------------------------------|------------------------|------------------|------------------|
| A (Control)                         | 52.22±11.71\(^a\)      | 31.67±16.07      | 16.11±11.10      |
| B (20 mg/kg broodfish)              | 62.22±13.37\(^a\)      | 29.44±8.39       | 13.89±14.17      |
| C (35 mg/kg broodfish)              | 22.78±13.47\(^b\)      | 40.56±14.17      | 36.67±25.22      |
| D (50 mg/kg broodfish)              | 37.22±19.32\(^b\)      | 50.00±16.41      | 12.78±7.52       |

Overall, the EMR of comet goldfish on the 60\textsuperscript{th} day of rearing showed an increased egg maturity level in the vitellogenic phase compared to the EMR on the 45\textsuperscript{th} day, which experienced a decrease. Decreased EMR in the mature phase of Treatment D was caused by atresia, which is the re-absorption of eggs that have reached the maximum development but not ovulated [10].
4. Conclusions
The addition of 50 mg/kg broodfish cow brain powder to the feed leads to the best effect on comet goldfish gonadal maturity on the 45th day of rearing with EMRs of 12.93 % and 21.11 % in the initial maturation phase and mature phase, respectively.

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