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The Introduction of Pituitary Gland Extract of Crab-eater Frog (*Fejervarya Cancrivora*) to Accelerate Ovulation of Eggs and Spawning of Common Carp (*Cyprinus Carpio)*

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ABSTRACT: Induced breeding by hypophysation technique is widely known as an effort conducted to stimulate ripe fish breeders to induce ovulation of eggs and spawning through injection of pituitary gland. This research was aimed to determine the most effective dosage of frog (*F. cancrivora*) pituitary gland which best accelerates ovulation of eggs and spawning of common carp fish (*C. carpio*). The experimental method was performed with 4 different doses treatment levels (0, 0.3, 0.5, and 0.7 ml/kg) per female brood fish with 3 replications. All data collected from the research was then statistically analyzed using Completely Randomized Design (CRD) method. This research indicated that the most effective dosage was T3 treatment with a dose of 0.5 ml/kg of frogs' pituitary gland per female brood fish. This treatment showed the highest increase of estradiol-17ß concentration (25253.65 ± 1050.68) pg/ml, and the fastest spawning period (12.03 ± 0.44) hours. The result proves that pituitary gland of crab-eater frog (*F. cancrivora*) can be used for induced breeding of common carps (*C. carpio*) with competitive performance compare to other pituitaries.
Key words: Hypophysation, *Cyprinus Carpio*, *F. Cancrivora*, Pituitary Gland, Estradiol-17β, Vitellogenin, Ovulation and Spawning

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INTRODUCTION

Pituitary gland frog (*F. cancrivora*) has some endocrine glands which produce some hormones to manage and control body tasks, stimulate and also activate the reproductive systems [1]. The anterior part of pituitary gland secretes growth and reproductive hormones which stimulate gonad maturation and spawning of the parent fish [2].

Common carp (*C. carpio*) is one of the most important food fishes in Indonesia and has economic potential in aquaculture due to its tasty flesh and high market demand and price. Although common carps able to spawn naturally or conventionally but the success rate of their spawning are still quite low. Therefore, artificial spawning through hormonal application is needed by using hypophysation technique which induces, accelerates ovulation of eggs and spawning and also improves the seeds' quantity [3].

The objective of this research was to determine the most effective dosage of frog (*F. cancrivora*) pituitary gland which best accelerates the ovulation of eggs and spawning of common carp (*C. carpio*). Some researchers have used pituitary glands to promote fish ovulation and spawning such as: Epler and Sokolowska [2] used common carp pituitary gland with 0.5 ml/kg dosage to improve gonadal maturation of the common carp (*C. carpio*) brood fish with guaranteed ovulation success of 80%. Rusdi Hi [4] used broiler chicken pituitary gland with dose of 0.6 mg/kg per brood stock of goldfish which able to promote gonadal maturation and its spawning by 100%. Muchlisin and Arfandi [5] used chicken pituitary gland extract which gave 776 minutes (13 hours) of latency period with 82.33 % of fertilization, 66.66 % of hatching and 45.66 % of survival rates.

MATERIAL AND METHODS

The research was conducted from December 2014 to May 2015 at Fish Reproduction and Breeding Laboratory of Fishery and Marine Sciences Faculty, and Pathology Laboratory of Saiful Anwar Hospital, University of Brawijaya, Malang for estradiol-17β analysis.

The experimental method was performed with 4 different doses treatment levels (0, 0.3, 0.5, and 0.7 ml/kg) per female brood fish with 3 replications. The adult female frog (*F. cancrivora*) from Tumpang village, Malang,

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weighted 140 - 160 g or about 6 - 7 frogs/kg, aged 7 - 9 months old were used in this research for their pituitary glands.

The brood fishes used in this research were 25 female common carps (*C. carpio*) from Punten village, Batu Malang, weighted 1,000 – 1,800 g, with 32 – 38 cm length, and aged 15 – 18 months old.

Figure 1. Crab-eater Frog (*Fejervarya cancrivora*) from Tumpang Village, Indonesia

Figure 2. Common Carp (*Cyprinus carpio*) from Punten Village, Indonesia

The main parameters observed in this research were estradiol-17β concentration [6], and spawning period [3] of the brood fishes. In order to determine the concentration of estradiol-17β during the research, the blood samples of the brood fishes were taken and centrifuged before and after the injection of pituitary gland of the frog. Then the plasma was separated and stored at -20 ^{o}C in fridge before examined quantitatively using a coated tube Radio Immuno Assay (RAI) kit (diagnostic product corporation Los Angeles, USA). The research was conducted based on Completely Randomized Design (CRD) method. This method provides maximum degree of freedom for error since all factors are under control of researcher and the analysis of data very simple.

RESULTS AND DISCUSSION

Estradiol-17β

Estradiol is a very important hormone produced by the ovary especially in female brood fish which is experiencing vitellogenesis (also known as yolk deposition). Estradiol is increasing gradually during yolk formation phase. Vitellogenin is stimulated by Estradiol-17ß to enter the blood circulatory systems and stimulate liver to start vitellogenesis and then promote the ovulation and spawning. Estradiol-17ß has an important role during the yolk formation process. Observation on estradiol-17β content is crucial and usually conducted by taking some blood samples as the material to be examined to determine the significant of liver synthesis to process estradiol-17β into vitellogenin which the original form of yolk.

The increasing of estradiol-17ß concentration in the blood will affect the acceleration of yolk maturation of common carp (*C. carpio*). Table 1, Table 2 and Figure 3 show the amount of estradiol-17β concentration before and after frog (*F. cancrivora*) pituitary gland injection in this research.

Table 1. Estradiol-17β concentration in common carp (*C. carpio*) before frog (*F. cancrivora*) pituitary gland injection

Treatment	Estradiol-17β Concentration (pg/ml)					
	Replication			Total	Average	STD
	1	2	3			
T1	169.4	465.27	346.7	981.37	327.12	148.9
T2	463	926.26	826.59	2215.85	738.62	243.84
T3	857.1	905.35	817.27	2579.72	859.91	44.11
T4	795.2	925.29	806.13	2526.62	842.21	72.16

Remarks: T1: Treatment with a dosage of 0 ml/kg of frog (*F. cancrivora*) pituitary gland; T2: Treatment with a dosage of 0.3 ml/kg of frog (*F. cancrivora*) pituitary gland; T3: Treatment with a dosage of 0.5 ml/kg of frog (*F. cancrivora*) pituitary gland; T4: Treatment with a dosage of 0.7 ml/kg of frog (*F. cancrivora*) pituitary gland; STD: standard deviation

Table 2. Estradiol-17β concentration in common carp (*C. carpio*) after frog (*F. cancrivora*) pituitary gland injection

Treatment	Estradiol-17β Concentration (pg/ml)					
	Replication			Total	Average	STD
	1	2	3			
T1	1898	5656.32	4274.71	11829.03	3943.01	1900.99
T2	2287	6161.51	5466.65	13915.16	4638.39	2065.79
T3	25630	26064.32	24066.63	75760.95	25253.65	1050.68
T4	11920	24463.1	18264.28	54647.38	18215.79	6271.69

Remarks: T1: Treatment with a dosage of 0 ml/kg of frog (*F. cancrivora*) pituitary gland; T2: Treatment with a dosage of 0.3 ml/kg of frog (*F. cancrivora*) pituitary gland; T3: Treatment with a dosage of 0.5 ml/kg of frog (*F. cancrivora*) pituitary gland; T4: Treatment with a dosage of 0.7 ml/kg of frog (*F. cancrivora*) pituitary gland; STD: standard deviation

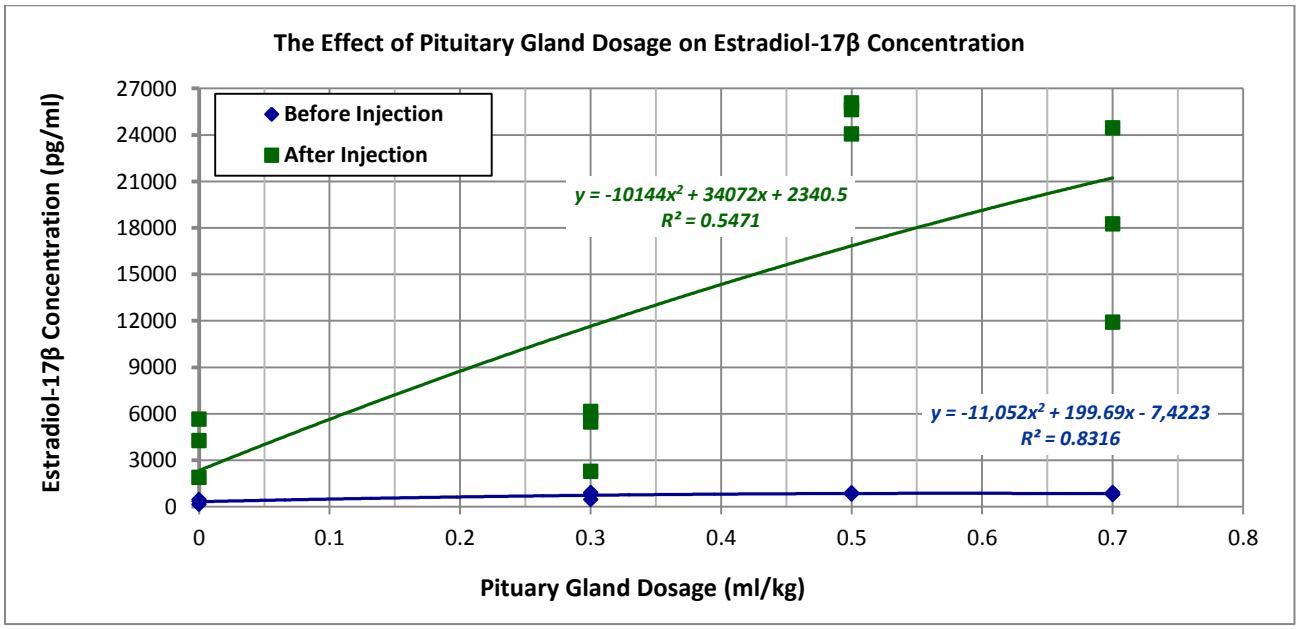

Figure 3. The comparison of estradiol-17β concentration in common carp (*C. carpio*) before and after the frog (*F. cancrivora*) pituitary gland injection

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Based on Table 2, it shows that T3 treatment yielded the highest increase of estradiol-17β with the average concentration of 25253.65 ± 1050.68 pg/ml after injection. It means that the use of pituitary gland from frog (*F. cancrivora*) with a dosage of 0.5 ml/kg gives the best performance of estradiol-17β. The intra and inter-assay coefficients of variation ranged from 2.9 to 9.7% and 2.3 to 10.2% respectively, over the entire dose response curve. The sensitivity of the assay was 5.4 pg/ml.

Figure 3 shows the mathematical correlation between the concentrations of Estradiol-17β in common carp (*C. carpio*) after the injection of pituitary gland with the pituitary gland dosage in a polynomial equation as: $y = -10144x^2 + 34072x + 2340.5$ with $R^2 = 0.5471$ and $r = 0.7396$. Where, correlation coefficient (r) stated the significant effect between independent variable and dependent variable which meant that the injection of pituitary gland of frog (*F. cancrivora*) significantly affected estradiol-17β concentration in common carp (*C. carpio*) by 73.96 %.

Theoretically, Estradiol-17β will stimulate liver to synthesize vitellogenin which is the original form of yolk [7]. And vitellogenin will be adsorbed selectively by oocyte follicle layers through blood veins [8]. Oocyte would grow to a maximum size, where the egg nucleus will moves to the edge side of cell which is indicated by the broken follicle layer and the movement of egg into the ovarian tube [6]. Higher dosage injection of frog (*F. cancrivora*) pituitary gland will decrease the growth rate of yolk but too low dosage administered will also decrease the growth rate of yolk. It occurs because lower administered dosage does not affect oocyte's growth while too high dosage would retard the growth process of oocytes. As results, ovulation migration (follicle damage) will be possibly occurs along the processes which causes degeneration of eggs and in the end will be adsorbed again by the ovary as the reaction of gonadotropin works [9].

Spawning Period

Spawning period is the time needed for the brood fish after injected with frog (*F. cancrivora*) pituitary gland to spawn. The observation time started from the time of injection up to the moment when ovulation happened and fish ready to spawn. Observation was conducted to each treatment to investigate the impact of pituitary gland injection on spawning period of the brood fishes as shown in Table 3 and plotted in Figure 4.

Table 3 shows that T3 treatment yielded the fastest spawning period with the average time was 12.03 ± 0.44 hours. It is indicated that the use of frog (*F. cancrivora*) pituitary gland with a dosage of 0.5 ml/kg per brood fish gave the best impact on acceleration of spawning period.

Figure 4 shows the mathematical correlation between the spawning period of common carp (*C. carpio*) after the injection of pituitary gland with the pituitary gland dosage in a polynomial equation as: $y = 8.5085x^2 - 9.3166x + 15.391$ with $R^2 = 0.6257$ and $r = 0.791$. Where, correlation coefficient (r) stated the significant effect between independent variable and dependent variable which meant that the injection of pituitary gland of frog (*F. cancrivora*) significantly affected the spawning period of common carp (*C. carpio*) by 70.10%.

Theoretically, deformation of follicle layer and the egg movement into the ovary tube are the signals which indicate that the brood fish ready to spawn. The research shows that different treatment level of will also affect the spawning period of brood fish at different time. Higher dosage injected of frog (*F. cancrivora*) pituitary gland will cause longer spawning period. While lower dosage level will also cause longer spawning period. It occurs because lower dosage does not affect oocyte's growth while higher dosage of frog (*F. cancrivora*) pituitary gland would retard the growth process of oocytes as the reaction of gonadotropin works.

Table 3. The effect of frog (*F. cancrivora*) pituitary gland on spawning period of common carp (*C. carpio*)

Treatment	Spawning Period (hour)					STD
	Replication			Total	Average	
	1	2	3			
T1	14.30	16.35	15.05	45.70	15.23	1.04
T2	13.25	14.55	14.35	42.15	14.05	0.70
T3	11.55	12.15	12.40	36.10	12.03	0.44
T4	13.15	13.35	13.50	40.00	13.33	0.18

Remarks: T1: Treatment with a dosage of 0 ml/kg of frog (*F. cancrivora*) pituitary gland; T2: Treatment with a dosage of 0.3 ml/kg of frog (*F. cancrivora*) pituitary gland; T3: Treatment with a dosage of 0.5 ml/kg of frog (*F. cancrivora*) pituitary gland; T4: Treatment with a dosage of 0.7 ml/kg of frog (*F. cancrivora*) pituitary gland; STD: standard deviation

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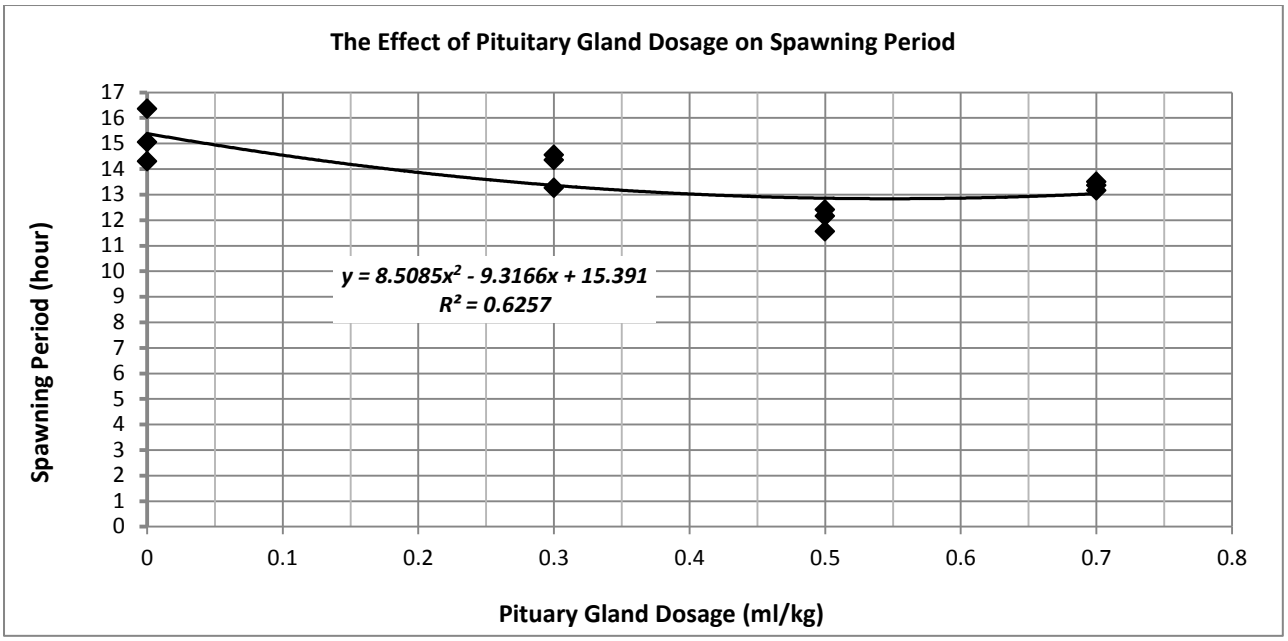

Figure 4. Regression curve of frog (*F. cancrivora*) pituitary gland usage on spawning period of common carp (*C. carpio*)

CONCLUSION

The research indicated that the most effective dosage for this hypophysation technique was T3 treatment with the dosage of 0.5 ml/kg frog (*F. cancrivora*) pituitary gland which yielded the highest estradiol-17ß concentration (25253.65 ± 1050.68) pg/ml, and the fastest spawning period (12.03 ± 0.44) hours. This result proves that pituitary gland of crab-eater frog (*F. cancrivora*) can be used for induced breeding of common carps (*C. carpio*) with competitive performance compare to other pituitaries. Since the crab-eater frog (*F. cancrivora*) is less cost than broiler chicken or common carp fish, thus the idea of using pituitary gland of crab-eater frog (*F. cancrivora*) for hypophysation technique is considered to have a good prospect in the future.

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