

# Reproductive Performance of Java Barb (*Punctius javanicus*) Injected sGNRH and Domperidone of Different Dosage

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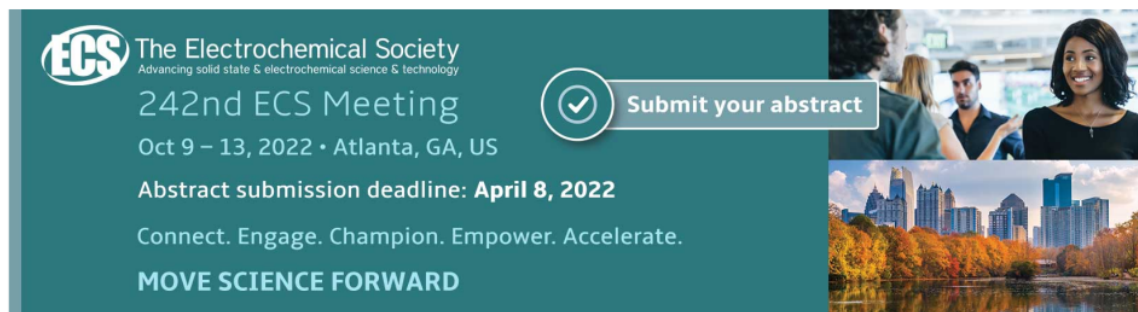
## Reproductive Performance of Java Barb (*Punctius javanicus*) Injected sGNRH and Domperidone of Different Dosage

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## Reproductive Performance of Java Barb (*Punctius javanicus*) Injected sGnRH and Domperidone of Different Dosage

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**Abstract.** Java barb (*P. javanicus*) is freshwater fish frequently found in Java and Sumatra waters of Indonesia. Java Barb has a seasonal spawning cycle. Accordingly, the production of java barb seeds is limited by season. Therefore, it is necessary to research on hormonal manipulating systems of reproduction to needs of seeds. This study aimed to determine the effect of sGnRH and domperidone on Java barb reproductive performance. There were 12 female and 12 male java barb broodstock, with 400-500 grams and 300-400 grams bodyweight respectively. The broodstock used on third stage of gonadal maturation. This study used an experimental method with a completely randomized design (CRD) consisting of 4 treatments and 3 replicates. Mixture of GnRH and domperidone was injected intramuscularly. The dosage treatments used were dosage A (control), treatment B (0.3 ml/kg), treatment C (0.4 ml/kg) and treatment D (0.5 ml/kg). The data gathered included latency period, fertilization rate (FR), hatching rate (HR), survival rate (SR) and water quality. The results showed that the injection of sGnRH and domperidone produced different latency periods, significantly affected ( $P < 0.05$ ) fertilization rate (FR) and hatching rate (HR), but had no significant effect on survival (SR). The best results were obtained at treatment B and treatment C with 0.3 ml/kg and 0.4 ml/kg dosage resulting in 9-12 hours latency period,  $80.50 \pm 5.57$  to  $84.33 \pm 5.51\%$  of Fertilization Rate (FR),  $68.83 \pm 6.81$  to  $71.17 \pm 5.35\%$  Hatching Rate (HR) and  $53.85 \pm 2.35$  to  $60.69 \pm 10.66\%$  of Survival Rate (SR).

### 1. Introduction

Java barb (*P. javanicus*), also popularly called silver barb and in Indonesia known as *tawes* is a type of freshwater fish found in Indonesia mostly in Java and Sumatra waters. Java barb is an endemic fish which usually lives in rivers and lakes in Sumatra and Java waters areas. Some of the Java barb advantages it has thick meat and high economic value [1]. Java barb has a seasonal spawning cycle, which occurs at the beginning of the rainy season. According to [2], the beginning of rainy season is the best time to cultivate Java barb since Java barb broodfish generally do not lay eggs in dry season.

The availability of high quality fish seeds with adequate and continuous quantity is one of production components which must be fulfilled in the efforts to develop in fish farming. The success of the hatchery really depends on the success of spawning. Obtaining good quality seeds in sufficient and continuous quantity can be done through controlled seeding, i.e. by conducting semi-artificial spawning.



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One of many factors influencing the spawning stimulation is the use of hormones and providing proper dosage. Inappropriate dosage will lead to unsatisfactory results [3]. Many types of hormones can stimulate spawning; each type of hormone nonetheless has different dosage. Therefore, it is necessary to research concerning the injection with a certain dose of combination of sGnRH and Domperidone in Java barb broodfish on reproductive performance, i.e. a continuous process in fish due to external stimulation or from within the fish's body itself. The research concerning injection of sGnRH and Domperidone hormones in Java barb broodstocks on reproductive performance. The parameter measured i.e. latency periode, fertilization rate, hatching rate, survival rate of larvae and water quality.

Hormone injection into Java barb body can accelerate the spawning process so that it can produce good quality and continuous or sustainable seeds. One of the hormones used is a combination of salmon Gonadotropin Releasing Hormone analog (sGnRH-a), anti-dopamine (domperidone) and propylene glycole. Hormone of sGnRH-a's role is to replace the role of natural GnRH to trigger pituitary gland to secrete gonadotropin hormone (GtH). In addition, anti-dopamine helps sGnRH-a's work by eliminating the effect of dopamine on GtH secretion [4].

## 2. Material and Methods

The test fish used in this study were 12 pairs of Java barb broodstock which were mature gonad, approximately 1 year of age, 400-500 grams female weight and 300-400 grams male weight. Female Java barb can be spawned after one year old and usually mature gonads weigh around 300-600 grams, while male Java barb can be spawned at 6-8 months age weighing around 300-400 grams. Java barb broodfish used were broodstocks originating from Laboratory of Fish and Environmental Health Testing, Muntilan, Central Java. The spawning container used was fish rearing tank (Indonesian term: *hapa*) with 1 m<sup>2</sup> area containing 1 pair of Java barb per container. The hormone used was salmon Gonadotropin Releasing Hormone analog (sGnRH-a) and anti-dopamine (domperidone) without dilution. The broodstock used were healthy and on the third stage of gonadal maturation. Combination of sGnRH and domperidone hormones solution used with brand name is Spawnprime. The broodstocks were injected with hormones in order to stimulate the mating and spawning process. Hormone injection was carried out before spawning. The injection on Java barb broodstock (*P. javanicus*) was done once. Broodstocks were transferred in to hapas, with 1 pair in each hapa. Parameters measured are latency period, fertilization rate, hatching rate, survival rate and water quality.

The method used in this research is the experimental method. The experimental design used a completely randomized design (CRD) with 4 treatments and three replicates. The broodstocks were injected. This study used the salmon hormone Gonadotropin Releasing Hormone analog (sGnRH-a) and anti-dopamine (domperidone) with different doses as follows:

Treatment A: Injection of 0.9% physiological NaCl (control)

Treatment B: Hormone injection at dose 0.3 ml/kg broodstock

Treatment C: Hormone injection at a dose of 0.4 ml/kg broodstock

Treatment D: Hormone injection at a dose of 0.5 ml/kg broodstock

### Data collection and analysis

#### a. Latency period

The latency period for Java barb spawning was calculated from the injection until the egg release or ovulation. It was conducted by checking the release of the eggs by checking the fish every 1 hour observation time interval.

#### b. Fertilized Rate (FR)

The eggs which have been ovulated by the broodfish were taken from the tank using hapas. Then, ± 200 eggs were taken as samples and incubated until the eggs hatched. The characteristic of fertilized eggs is they are transparent translucent, while unfertilized eggs are cloudy. Fertilized eggs were observed and counted manually by hand counter. The degree of fertilized rate can be calculated using the formula proposed by [5], i.e.:

$$FR (\%) = \frac{\text{Number of Fertilized Eggs}}{\text{Total Number of Eggs}} \times 100\%$$

**c. Hatching Rate (HR)**

The methods of collecting hatching rate data were by counting and writing/recording the number of hatched eggs. In addition, hatching rate was calculated using [6], i.e.:

$$HR (\%) = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs}} \times 100\%$$

**d. Survival Rate (SR)**

The value of survival rate was obtained by counting all larvae in the rearing container on day 7 after the eggs hatched. The percentage of survival rate (SR) was obtained by dividing the number of living larvae by the hatched eggs. The degree of survival rate was calculated using [6] as follows:

$$SR (\%) = \frac{\text{Number of seeds}}{\text{Number of hatched eggs}} \times 100\%$$

**e. Water Quality**

The quality of water in this study, in spawning ponds, hatching and larvae rearing containers, was measured using DO meters and pH meters. The parameters of water quality observed in this study were temperature (°C), degree of acidity (pH), and dissolved oxygen (DO). Water quality measurements were conducted three times a day at 08.00, 12.00 and 16.00 West Indonesia Time (GMT+7).

**f. Data Analysis**

The results of the study covering such following variables as fertilized rate (FR), hatching rate (HR) and survival rate (SR) were analyzed using statistical analysis. In addition, the test analyses included normality test, homogeneity test, additivity test and anova test. When the results showed is a significant influence (P <0.05) or very true (P <0.01), Duncan multiple range test was done to determine the difference in the mean value between treatments and determine the best one [7]. On the other hand, latency period and water quality were analyzed descriptively.

**3. Results and Discussions**

**3.1. Results**

The results showed that the injection of sGnRH and domperidone was able to improve Java barb reproductive performance as evidenced by latency period, fertilized rate (FR), hatching rate (HR) and survival rate (SR) of Java barb (*P. javanicus*) seeds.

**Latency period**

The latency period data of Java barb after being injected with sGnRH and domperidone until ovulation are presented in this following Table 1.

**Table 1.** Latency period of Java barb (*P. javanicus*)

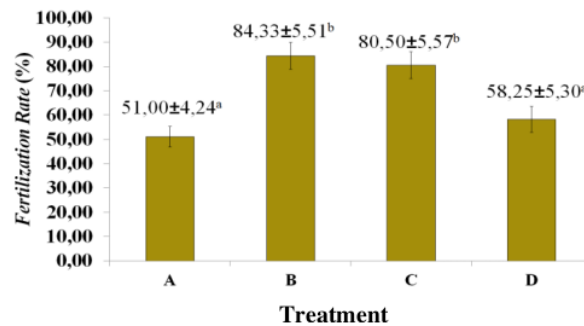
Repetition	Treatment (hours)			
	A	B	C	D
1	13	9	12	12
2	12	11	9	0
3	0	9	10	14
<b>Range</b>	12-13	9-11	9-12	12-14

The results of observation on Java barb (*P. javanicus*) latency period showed that after giving treatment, The fastest latency period occurred in treatment B at a dose of 0.3 ml/kg body weight with 9-

11 hours latency period. There was a pair of broodfish in each treatment A and treatment D which did not spawn until the end of observation.

**Fertilization Rate (FR)**

The following Figure 1 elaborates the fertilization rate (FR) of Java barb.

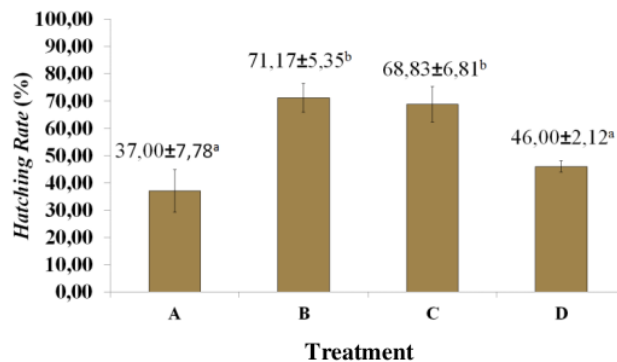


**Figure 1.** Fertilization Rate (FR)

The results of the study showed that the fertilization rate of Java barb eggs in treatment A (control) was (51.00 ± 4.24%), treatment B with a dose of 0.3 ml/kg was (84.33 ± 5.51%), treatment C with a dose of 0.4 ml/kg was (80.50 ± 5.57%), and treatment D at a dose of 0.5 ml/kg was (58.25 ± 5.30%). In addition, the results of variance analysis showed that the injection of sGNRH and domperidone hormones had a significant effect on the fertilization rate of Java barb (P < 0.05). Moreover, the results of Duncan test on fertilization rate (FR) of Java barb eggs showed that treatment B was not significantly different from treatment C, but it was significantly different from treatment A and D. Treatment C was not significantly different from treatment B, but it was significantly different from treatment A. At last, treatment D was not significantly different from treatment A.

**Hatching Rate (HR)**

The following figure 2 presents observation data of hatching rate (HR).



**Figure 2.** Java barb Hatching Rate (HR)

The results of Java barb's eggs hatching rate obtained are as follow: treatment A ( $37.00 \pm 7.78\%$ ), treatment B ( $71.17 \pm 5.35\%$ ), treatment C ( $68.83 \pm 6.81\%$ ) and treatment D ( $40.00 \pm 2.12\%$ ). Thus, it can be seen from the results that the highest hatching rate was in treatment B with a dose of 0.3 ml/kg body weight ( $71.17 \pm 0.05\%$ ). The results of the analysis of variance showed that the injection of sGnRH and domperidone hormones with different doses had a significant effect ( $P < 0.05$ ) on the hatching rate of Java barb (*P. javanicus*). The results of the Duncan further test at the hatching degree (HR) of Java barb eggs showed that treatment B was not significantly different from treatment C, yet it was significantly different from treatment A and treatment D. Furthermore, Treatment C was significantly different from treatment A and D. Meanwhile, treatment D was not significantly different from treatment A.

### Survival Rate (SR)

The result of Java barb survival rate is described in Figure 3 below.

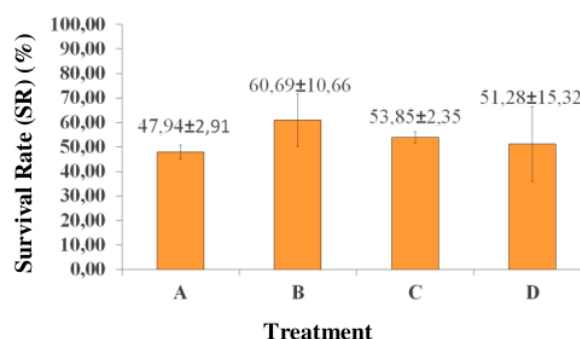


Figure 3. Java barb Survival Rate (SR)

Observation on the survival rate of larvae reared for 7 days resulted in treatment A (control) ( $47.94 \pm 2.91\%$ ), treatment B with a dose of 0.3 ml/kg ( $60.69 \pm 10.66\%$ ), treatment C with a dose of 0.4 ml/kg ( $53.85 \pm 2.35\%$ ) and treatment D with a dose of 0.5 ml/kg ( $51.28 \pm 15.32\%$ ). The results of variance analysis on sGnRH and domperidone hormones injections showed that these injections did not significantly influence ( $P > 0.05$ ) the survival rate of Java barb (*P. javanicus*).

### Water Quality

Based on the results of water quality observation, several parameters were categorized in optimal range. In addition, water quality parameters which were measured included temperature, pH, and dissolved oxygen (DO). The results of water quality measurements are presented in Table 2.

Table 2. The Results of Water Quality Parameters of Java barb (*P. javanicus*) Spawning

Parameter	Water Quality	Reference
DO	3,1 – 5,9 ppm	3,6 – 6,0 ppm **
Temperature	25,1 <sup>0</sup> C – 28,8 <sup>0</sup> C	25 <sup>0</sup> C – 30 <sup>0</sup> C*
pH	7 – 8	6,5 – 8,5*

Description : \*[8]  
 \*\*[9]

### 3.2. Discussion

#### a. Latency Period

The results of this study focusing on the reproductive performance of Java barb (*P. javanicus*) which was injected with different doses of sGnRH and domperidone showed different latency period. The fastest latency period was shown in treatment B, i.e. 9-11 hours, and followed respectively by treatment C for 9-12 hours, treatment A 12-13 hours and treatment D for 12-14 hours. The shortest latency period (9-11 hours), was achieved using dose B, 0.3 ml/kg body weight of fish. This scientifically proved that a dose of 0.3 ml/kg fish body weight can lead to an increase in the concentration of gonadotropin hormones in the blood. Accordingly, it can stimulate ovulation and spawning within 9-11 hours. According to [10], sGnRH + domperidone plays a significant role in stimulating ovulation and spawning during gonad maturation where analog sGnRH functions in stimulating the pituitary to release gonadotropins, in which gonadotropin secretion under natural conditions is inhibited by dopamine. Consequently, if dopamine is blocked by its antagonist, dopamine will stop and gonadotropin secretion will increase. Gonadotropin hormone contains FSH (Follicle Stimulating Hormone) that functions to stimulate vitellogenesis and LH (Luteinizing Hormone) which stimulate the maturation-ovulation process.

In treatment D with a hormone dose of 0.5 ml/kg fish body weight, there was 1 pair of Java barb which could not spawn and the latency period of treatment D showed the longest one compared to other treatments. The result may cause negative feedback effect of hormones which inhibiting of producing internal hormones from pituitary gland. Meanwhile, treatment A (0.9% NaCl injection) also showed 1 pair of Java barb which could not spawn since NaCl does not contain any hormones that could stimulate ovulation. This confirmed the study conducted by [11] who stated that the use of stimulants to shorten latency period depends on the dose of the stimulant used. According to [12] besides hormones, latency period is influenced by several factors such as the quality of the broodstock (age, size and frequency of spawning), egg quality and male broodstock quality.

#### b. Fertilization Rate (FR)

The result of the experiments showed that the injection of sGnRH and domperidone hormones at different doses had a significant effect ( $P < 0.05$ ) on the fertilization rate (FR). Treatment B with a dose of 0.3ml/kg and treatment C with a dose of 0.4 ml/kg body weight showed no different results but they were higher than treatment A and treatment D (dose 0.5). The high average percentage of fertilization rate obtained at a dose of 0.3 ml/kg and 0.4 ml/kg body weight of fish is due to the fact that this dose is the optimal dose in affecting egg cell development. The optimal dose will bring advantages on the quality of the eggs produced by Java barb fish. This result is confirmed by [13] stating that the use of the right or maximum dose to fish will cause the fish to ovulate completely and make the egg quality better.

Injecting 0.5 ml/kg body weight doses of sGnRH and domperidone in treatment D and control (injection of 0.9% NaCl) in treatment A resulted in the average fertilization was below 50%. This is confirmed by [14] suggesting that an injection with a higher dose results in early ovulation and the egg remains in the ovary lumen for a longer time and this leads to decreased egg quality. Fertilized java barb eggs are transparent in color, while unfertilized eggs are cloudy white. The fertilization rate is influenced by egg quality and sperm quality. According to [15] damaged eggs can occur if the sperm is highly concentrated so that there is an intense competition among sperm cells to enter the microfil hole.

#### c. Hatching Rate (HR)

The results showed that the injection of sGnRH and domperidone with different doses had a significant effect ( $P < 0.05$ ) on the value of hatching rate (HR). In addition, the hatching rate (HR) of Java barb eggs shows the percentage of hatching from the highest value to the lowest one as follows: treatment B was  $71.17 \pm 5.35\%$ , and respectively treatment C was  $68.83 \pm 6.81\%$ , treatment D was  $40.00 \pm 2.12\%$  and treatment A was  $37.00 \pm 7.78\%$ . The increase in hatchability of eggs given with hormones is due to the increased content of Follicle Stimulating Hormone (FSH) in the blood. Thus, the follicles



develop and the hatchability of eggs also increases. According to [16] follicular development is influenced by FSH activity in the pituitary which will stimulate the secretion of estrogen in the follicle.

Java barb (*P. javanicus*) broodfish that were injected with hormones at a dose of 0.3 ml/kg and 0.4 ml/kg body weight showed a high hatching rate. According to [13], sGnRH + Domperidone not only encourage the broodstock to ovulate but also relate to fertilization. The optimal dosage can improve the biological performance on its target. Providing hormone at a dose of 0.5 ml/kg fish body weight led to a decreased hatching rate. This is reinforced by [3] stating that the working mechanism of the hormone will work normally (optimally) at certain levels. Moreover, the decrease or increase is assumed to reduce biological potential of the hormone toward its target. According to [17], the percentage of egg hatchability is always determined by the percentage of egg fertilization, of which the higher the percentage of egg fertilization, the higher the percentage of egg hatchability, unless there are environmental factors which influence it such as sudden changes in temperature, oxygen and pH.

#### d. Survival Rate (SR)

The results of the experimental study showed that injecting sGnRH and domperidone hormones with different doses in Java barb had no significant effect ( $P < 0.05$ ) on survival rate of larvae (SR). In this study, survival rate ranged from  $47.94 \pm 2.91$  -  $60.69 \pm 10.66\%$ .

Fish survival rate is not directly affected by hormone administration, but it is also influenced by external factors including water quality, stocking density and disease. According to [18], fish survival rate is influenced by biotic and abiotic factors. Biotic factors influencing SR are population density, adaptability of animals and human handling. Meanwhile, abiotic factors that can affect include physical and chemical properties of waters.

The decline in Java barb survival rate is assumed to be due to a decrease in water quality in the rearing containers which eventually affected the survival rate of the fish. Seeds mortality is also related to the remaining eggs which do not hatch so they became rotten. This result confirmed [19] stating that eggs which do not undergo fertilization will rot. Environmental condition is one of the crucial factors in larval rearing since these organisms are still very vulnerable and still do not have complete body organs.

#### e. Water Quality

The results of water quality observation carried out during the study included the quality of water for spawning, hatching and larval rearing. The water quality observations carried out included temperature, DO, pH in spawning ponds and hatcheries and larvae rearing containers.

Water temperature is very important for the survival rate of fish. This is because temperature helps the metabolic processes occurring within fish's body. The results of temperature measurements in the spawning pond, hatchery and rearing containers were  $25.1^{\circ}\text{C}$  -  $28.8^{\circ}\text{C}$ . Such temperature obtained during the research is categorized as feasible. According to [20], Java barb can live with an optimum temperature between  $25^{\circ}\text{C}$  -  $33^{\circ}\text{C}$  and a minimum temperature of  $1^{\circ}\text{C}$ . According to [21], an important factor affecting the growth and survival of fish in addition to feed is water quality, especially temperature. Temperature can affect such important fish activities as respiration, growth and reproduction. High temperatures can also reduce dissolved oxygen.

Dissolved oxygen is one of the most critical water quality parameters in fish culture. Aquaculture water which contains low dissolved oxygen concentration will affect fish growth. The value of dissolved oxygen was not really good, i.e. around  $3.1 - 5.9$  ppm. According to [9], dissolved oxygen concentration is influenced by stocking density of cultured fish. The higher the stocking density in a pond, the greater the oxygen needed. In addition, dissolved oxygen is also influenced by climatic factors and seasonal changes. Furthermore, DO in Java barb fish culture is  $3.6 - 6.0$  ppm.

The degree of acidity (pH) in the research including in spawning ponds, hatching and seeds rearing containers, was quite good which was in the range of  $7 - 8$ . This is reinforced by [8] suggesting that the value of acidity degree (pH) which is good for Cyprinidae family fish is  $6.5 - 8.5$ . The value of

pH that is too high can affect the metabolic performance of the fish body so that the fish will not grow properly or will be 'stunted'.

Hormonal roles in the environment (temperature, pH and dissolved oxygen) are also able to influence the spawning of Java barb (*P. javanicus*). Environmental signals such as rain and temperature changes will be received by central nervous cells and transmitted to the hypothalamus. The hypothalamus will respond by releasing GnRH hormone to act on the pituitary gland. Furthermore, the pituitary will release FSH hormone which acts on the theca layer of the oocyte. If the environmental conditions do not support, the oocyte will degrade or the ovulation will fail. Moreover, if the environmental conditions support, there will be a process of pre-ovulation and ovulation.

#### 4. Conclusion

Based on the results of the study, it can be concluded that:

1. The sGnRH and domperidone hormones had a significant effect ( $P < 0.05$ ) on fertilization rate, hatching rate and latency period but they had no effect on the survival rate of Java barb (*Puntius javanicus*) seeds.
2. The best doses of sGnRH and domperidone in this study were 0.3 ml/kg and 0.4 ml/kg body weight of fish resulting in 9-12 hours latency period,  $80.50 \pm 5.57$  to  $84.33 \pm 5.51\%$  fertilization rate (FR),  $68.83 \pm 6.81$  to  $71.17 \pm 5.35\%$  hatching rate (HR) and  $53.85 \pm 2.35$  to  $60.96 \pm 10.66\%$  survival rate (SR).

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