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The Influence Of The Combination Of Palm Juice *(Phoenix dactylifera)* And Ringer Lactate To The Percentage Of Koi *(Cyprinus rubrofuscus)* Spermatozoa's Fertility

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Abstract

Koi fish (Cyprinus rubrofuscus) is included in freshwater ornamental fish commodity which has high economic value. As high of the market demand, it must be balanced with the increase of Koi fish production, especially in the hatchery sector. One effort that can be done is by preserving sperm with the addition of a solution of palm juice and ringer lactate. The purpose of this study was to determine the effect of the difference and the best value of the concentration of palm juice in ringer lactate during the storage period on the fertility power of Koi Fish sperm. The research method that used is the experimental method with the main parameters of motility, viability, fertilization, hatchability, survival rate and supporting parameters of macroscopic quality of fresh sperm and water quality. The results showed that the addition of palm juice and ringer lactate gave a very real effect on the level of motility with the best value on treatment B that is equal to 50.33%, the level of viability with the best value at treatment B that is equal to 64.67%, fertilization rate with the best value at treatment B that is equal to 84,91%, hatchability level with the best value at treatment B that is equal to 69,80%, but no significant effect to survival rate of the juvenile. The function of palm juice and ringer lactate is providing energy and keep the balance of the fluid in sperm that is useful to keep sperm still alive and still moving.

Keywords: ***Cyprinus rubrofuscus, fertilization, palm juice, sperm storage***

Introduction

Due to their color composition, freshwater ornaments are one of the leading goods that are in great demand by people in Germany and abroad. Koi Fish (Cyprinus rubrofuscus) is one of the most important raw materials that is still in demand today. Koi fish, including freshwater ornamental fish, have high economic value both on the national and international markets (Firdaus, 2010). Koi fish are characterized by attractive colors and different variations. In Indonesia, the area known for its high-quality Koi producers is currently in Blitar City and Yogyakarta. Koi fish are generally classified into 13 categories, namely Kohaku, Sanke, Showa, Bekko, Utsurimono, Asagi, Shusui, Tancho, Hikari, Koromo, Ogon, Kinginrin and Kawarimono. Fans of koi fish or hobbies in Indonesia not only cultivate koi fish in ponds, but also participate in the koi competition to increase the reputation of their owners (Triyanti and Maharani, 2012).

In order to meet the high market demand, effective artificial leaching techniques are required. Artificial spawning forms with the use of reproductive hormones on the parent fish ready for spawning (Sugiharto, 2014). Artificial spawning allows fish farmers to overcome the problem of lack of fish stocks. In artificial spawning, however, there are often obstacles, because the fertilization of the eggs is low. This is caused by too thick sperm. Therefore, we need diluents such as physiological Na and Linger rings. According to Nainggolan et al. (2015), high levels of sperm fluid can inhibit sperm activity, namely, impaired mobility. One way to overcome this problem is to add Ringer's lactate as a sperm thinner.

One ingredient that meets the criteria for inclusion in sperm dilution solutions is the date extract (P. dactylifera). According to Retnowati and Joni (2014), dates contain ingredients that mainly reduce sugar, namely glucose and fructose by 20 to 70% (dry weight), so that dates are easily digested and quickly replace lost body energy. Not only are dates easily digestible and energetic, they also contain high levels of antioxidants, are cancer and tumor therapeutics, and can prevent damage to spermatozoa due to pathogens and internal disorders.

According to Rahardianto et al. (2012), sperm storage with the addition of an extender solution aims to optimize the useful life of male spermatozoa for male parents to artificially fertilize similar female eggs and facilitate the transport of cement for other reproductive purposes. Essentially, the working principle of sperm preservation techniques is to compare the state of the sperm storage medium with conditions, e.g. When removed from the parent's body. In this case, ingredients are needed that can sustain life and reduce sperm activity. The energy requirement of spermatozoa to maintain fertilization is provided by simple sugars (monosaccharides) such as glucose and fructose. According to Salisbury and Van Denmark (1985), the energy required by spermatozoa is provided by simple sugars (monosaccharides) such as fructose and glucose. The addition of fructose or glucose in diluents is useful to aid in the process of forming adenosine triphosphate (ATP) and adenosine diphosphate (ADP), which must be continued.

Spermatozoa in sperm preservation techniques require energy to maintain their mobility. This energy requirement is covered by simple sugars (monosaccharides) such as glucose and fructose. In addition, cementitious material must be able to provide buffering properties and protect sperm from cold shock so that the condition of the sperm remains stable. The addition of energy sources to the seed can be done to prolong sperm storage. According to Adipu et al. (2011), the addition of NaCl and fructose solutions to dilute the sperm can maintain activity and extend sperm life so that they have plenty of time to find eggs. It is believed that the increase in motility time of spermatozoa is caused by variations in fructose dilution ratio, since fructose can be used as an energy source and nutrient for spermatozoa.

The research was carried out in March - July 2017 in the Breeding and Farming, Fish Breeding and Farming Fish Aquaculture Laboratory and the Aquatic Product Science Laboratory of the Department of Fishery Product Safety at the Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang.

Materials and Methods

Research instruments and materials

Tools used in this study include binocular microscopes, 2 ml Eppendorf tubes, Hg thermometers, 1 ml and 5 ml syringes, 250 ml beakers, handy meters, digital scales, refrigerators, drop Pipettes, hemocytometers, sesers, incubators, plastic bucket aerator, measuring cup, spray bottle, tray, heater, ruler, micropipette and clock cup.

The material was used in this study included koi fish, Sahara brand palm juice, Ringer's lactate, eosin, aquadest, 70% alcohol, aluminum foil, ovaprim, liquid soap, tissue, label paper, wet tissues, pH paper, and physiological NaCl.

Research methods and parameters

The research method was used the experimental method in which the data obtained in the form of numbers or data qualitative that were predicted. The main parameters were used in this study included motility, viability, fertilization, hatching rate, and survival rate. Supportive parameters were the macroscopic quality of fresh semen and water quality, including temperature, pH and dissolved oxygen.

Master preparation

The pond, which was served as a breeding container, was prepared with liquid soap, cleaned and rinsed with clear water and then dried. The serviceable pool was filled with clean water filled with water from the height of the pond. Then the heater, thermometer and ventilation set were installed. Selection of the male koifish occurs by sorting the abdomen into the urogenital hole when thick, thick, white fluid was presented. For the female parent, which was performed the same way, it can be seen if the mature egg comes out.

Sterilization with Eppendorf tubes

Eppendorf tubes with a capacity of 2 ml were prepared as experimental media containers. The Eppendorf tube was sterilized with 70% alcohol and then dried. Tubes placed on a tube rack were based on the randomized experiment.

Indigenous Koi males graze

After selecting the mature fish of the broodstock male gonads, fish was stripped off. The stripping of this brood was determined whether the broodstock has a gonad or not.

The fish was held back and the stomach was turned up with a damp cloth. The urogenital hole of the koi fish was cleaned with a cloth. The belly of the fish then sorted from the belly to the urogenital hole until the semen was released. The sperm was collected with a syringe of 5 ml.

Observation of mobility
The diluted fish sperm was removed with a drop of pipette and placed on a glass object, soaked with 1 drop of water to activate the sperm, and sealed with glass objects. Observed motility of spermatozoa at 400x magnification. Calculated the percentage of motile and immobile spermatozoa and obtains the results of the observations.

Observation of viability
The treated sperm sample was removed in 1 drop with a dropper pipette. Sperm samples fall on one end of the glass object. Sperm samples were dropped with a 1-drop eosin dye solution and homogenized using a glass cover. Sperm samples homogenized with a dye solution were made by swabbing, flattening the sample with another 45 ° tilted glass object as flat as possible and then venting to dryness. Prepared preparations were observed under a 400-fold microscope. The number of dead and live sperm was counted with a handy counter (dead sperm were red and the live sperm were transparent as they do not absorb eosin dye solution). Obtained observations of sperm ability. It clearly showed the transparent sperm and red. The sperm that dies on the sperm membrane was damaged so that it can absorb the eosin around it.

Fertilization
Eggs in each container were observed with the following provisions: clear white (fertile), cloudy white (infertile). Calculated fertile and infertile eggs.

Calculation of Hatching Power
The eggs, which were stored on the clock cup and fertilized, were placed in the incubator. Wait for the eggs to hatch. After hatching the eggs, the percentage of hatching eggs was calculated.

Larval life
The eggs, which hatched to be larvae, were stored in an incubator and fed with Artemia salina. After waited period of 2 weeks, the percentage of their living was calculated.

Data analysis
The data from this study has analyzed their effect on each treatment using diversity analysis or the F-test: if the calculated F-value was significant or very real, the analysis continues with the LSD test (Smallest Significant Difference) to determine the response that appeals to it. best with 5% and 1% confidence level. To find out the relationship between treatment and outcomes, an orthogonal polynomial test regression analysis was used.

Results and Disscusions
Quality of Koi fish sperm
The study and evaluation of fresh semen in this study was performed macroscopically and microscopically. The macroscopic examination involves the study of volume, color and viscosity. While the microscopic examination includes the concentration of spermatozoa, the percentage of motility and the percentage of viability (Partodihardjo, 1980). The results of the evaluation of fresh seed of Koi fish during the study yielded a white color such as milk, the concentration reached 9.58 x 10 9, volume 9 ml, thick like coconut milk, had a pH of 8 and the exercise was very active.
Based on the results of the macroscopic examination of fresh semen, the Koi fish still has a good quality for further processing. This was in line with Fujaya's (2004) opinion that the color of whitish liquid semen smelling of high viscosity sperm reaches the seed concentration of cyprinid fish at 3.00 x 109 cells / ml, according to Rustidja (2000). Sperm concentration was very important to know as it was one of the criteria for determining sperm quality.

Mobility of sperm
The results of the motility study suggest that the sperm motility of Koi fish (C. rubrofuscus) when adding date juice to the dilution solution resulted in a percentage of K treatment (0 ml date palm juice in 100 ml Ringer's lactate) of 37.00 % at 300% results in treatment A (0.5 ml date palm juice in 99.5 ml ringworm

lactate) of 46.33% and the highest yield was in treatment B (1 ml date palm juice in 99 ml ring lactate) of 50.33% in treatment C (1.5 ml of date palm juice in 98.5 ml Ringer's lactate), the value of motility dropped to 46.00% and in Treatment D (2 ml of palm juice in 98 ml of Ringer's lactate) it was 41.00%.

From the calculation of the smallest real difference test (BNT) it can be concluded that the treatment which gives the highest value is found in treatment B, namely by giving a dose of 1 ml of date juice in 99 ml of Ringer's lactate. Treatment B provides the highest results because it contains a mixture of extract dates and Ringer's lactate extract, which are suitable and contain substances that can prolong the movement of spermatozoa after leaving the fish body. This is corroborated by the opinion of Rustidja (2000), which is probably due to differences in the composition of liquid diluents with plasma medians and the degree of correspondence with the metabolic requirements of spermatozoa during their lifetime.

Viability of sperm

The results obtained from observations of viability can be concluded that the sperm viability of Koi fish (C. rubrofuscus) with the addition of dilution solution dates to a percentage of K treatment (0 ml of palm juice in 100 ml of Ringer's lactate) of 37.00% performs treatment A (0.5 ml of date juice in 99.5 ml of Ringer's lactate) of 53.00% and the highest yield was in treatment B (1 ml of date palm juice in 99 ml of Ringer's lactate) of 64.67% in treatment C (1.5 ml of palm juice in 98.5 ml of ringworm lactate) reduced the viability value to 54.67%, and in treatment D (2 ml of palm juice in 98 ml of ring lactate) the results were 44.00%.

From the calculation of the smallest real difference test (BNT) it can be concluded that the treatment which gives the highest value is found in treatment B, namely by giving a dose of 1 ml of date juice in 99 ml of Ringer's lactate. This is because the fructose solution contained in dates and Ringer's lactate contains a nutrient substrate for spermatozoa, glucose. Glucose is one of the seminal plasma compounds that serve as a source of energy for spermatozoa (Ridwan, 2009). The Palm juice solution also contains a glucose level that represents an energy exchange for fructose in plasma cement, which is needed for metabolic activity, so that the quality of the cement can be maintained (Danang et al., 2012).

Fertilization rate

The results of the fertilization observations suggest that the addition of date palm juice to the dilution solution gives a different average yield for fertility. In the treatment of K (0 ml palm juice in 100 ml Ringer's lactate) of 70.68%, Treatment A (0.5 ml of palm juice in 99.5 ml of ringworm lactate) was 81.39% and had the highest results in Treatment B (1 ml of palm juice in 99 ml of Ringer's lactate) was 84.91%, in treatment C (1.5 ml of palm juice in 98.5 ml of Ringer's lactate) there was a decrease in the fertilization value to 81.42% and in treatment D (2 ml of palm juice in 98 ml ringworm lactate) achieved results of 75.89%.

From the calculation of the smallest real difference test (BNT) it can be concluded that the treatment which gives the highest value was found in treatment B, namely by giving a dose of 1 ml of date juice in 99 ml of Ringer's lactate. The fertilization rate seems to follow what happens at the level of sperm quality, where high mobility also causes high fertilization. According to Meinawarti et al. (2011), high mobility will cause high fertility of the eggs. Low motility causes the spermatozoa to lose their ability to penetrate microphyll and lose the fertility of the egg. Another opinion suggests that a long viability does not necessarily lead to high fertilization, because if the sperm was alive, but can not move, it can not penetrate into the microfiltration hole, so that the egg can not fertilize. In this state, the sperm really need a lot of energy fertilizes the egg (Hidayaturrahmah, 2007).

Hatching

The results obtained from the observation of the hatching rate can be concluded that the addition of date juice to the diluting solution gives different average hatchability yields. In the treatment of K (0 ml palm juice in 100 ml Ringer's lactate), 59.10% was achieved, in treatment A (0.5 ml of date juice in 99.5 ml of Ringer's lactate) 65.69% and the highest results were achieved in treatment B (69 ml, in Treatment C (1.5 ml of date juice in 98.5 ml of Ringer's lactate) there was a decrease in the

hatching value to 67.42% and in Treatment D (2 ml of palm juice in 98 ml ringworm lactate) achieved results of 63.87%.

From the calculation of the smallest real difference test (BNT) it can be concluded that the treatment which gives the highest value is found in treatment B, namely by giving a dose of 1 ml of date juice in 99 ml of Ringer's lactate. Apart from the decrease in sperm motility caused by the preservation of the sperm, the low hatching of the eggs may also be influenced by the egg quality and water quality of the maintenance media. When handling eggs before fertilization, the female parent occasionally makes aggressive movements during sequencing, causing water to enter the egg container. Contamination of the water in the egg may cause the microfilm holes to close before fertilization. According to Rahardianto et al. (2011), fish eggs expand after contact with water, as water penetrates into the perivitelin, the space between shell and core. These conditions will close the microfilm hole faster and reduce the percentage of fertilization since the delay that the sperm reaches the microfilm hole will be lower in motion, especially as the sperm cools.

Livelihood

The results obtained from Table 14 can conclude that the survival of koi fish larvae (C. rubrofuscus) with the addition of palm juice in the dilution solution resulted in a percentage of K treatment (0 ml date palm juice in 100 ml ring lactate) of 79.56 % at 80%, treatment A (0.5 ml of date palm juice in 99.5 ml ringworm lactate) was 81.51% and the highest yield was in treatment B (1 ml palm juice in 99 ml ringworm lactate) of 82.22% in treatment C (1.5 ml of date palm juice in 98.5 ml Ringer's lactate), the livelihood decreased to 81.84%, and for treatment D (2 ml of date juice in 98 ml of Ringer's lactate), it was 80.84%.

The results of the above variance calculation suggest that the F number is less than F 5% and F 1%, so it can be concluded that the given treatment did not significantly affect the survival of Koi larvae after 2 weeks of maintenance Has. This shows that when the fish is in the larval stage, the treatment of the addition of extender in the form of Ringer's lactate and palm juice no longer affects the survival of the fish, but the life of the fish is strongly influenced by the environment and the availability of food which supports the life of the organism. In accordance with Effendie's (1997) Opinion, it states that the critical phase is from the beginning of the life cycle of the fish larvae before and after the egg yolk absorption, and the transitional period begins when food is taken in from outside. The movement of larvae or the behavior of larvae to maintain food and good food supply are factors that influence the survival of the larvae. The factors that influence the survival of fish larvae are also good water quality and good feed quality.

Water quality

During the research, water quality measurements were made including temperature, dissolved oxygen (DO) and pH in each media maintenance vessel. These factors were taken into account in the study because water can affect the survival of the fish itself. The results of the water quality measurements during the study can be found in the table 1 below.

The above table shows that water as a maintenance medium and live media of Koi fish still meets the requirements, so that the decline in physiological conditions is not affected. Partosuwiryo and Warseno (2011) argue that the optimal temperature for maintaining koi fish is in the range of 25 to 30 ° C, the pH of 6.5 to 8.5 and the DO value of 3 to 7 ppm. During the measurement of water quality during the study, temperatures in the range of 26 to 28 ° C, a pH of 6.75 to 8.38, and dissolved oxygen in the range of 4.38 to 7.41 ppm were found.

Conclusions

Based on the results of the study, sperm motility, sperm viability, sperm fertility on eggs and the rate of hatching of koi fish larvae (C. carpio) with a maximum dose of spermatozoa (P. dactilyfera) and ring lactate may result from the extraction of dates 1 ml of palm juice in 99 ml ringworm lactate has no significant effect on the survival of Koi fish larvae. When incubated, the water quality was observed, the temperature was between 26 and 28 °C, the pH between 6.75 and 8.38 and the dissolved oxygen between 4.38 and 7.41 ppm.

Based on the results of the study, it is recommended that Koi fish breeders (C. rubrofuscus) be able to store sperm with date

juice at a dose of 1 ml in 99 ml of Ringer's lactate. In addition, there is a need for further investigations using fillers with other ingredients.

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