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A preliminary study to evaluate the effects of powder milk solution on the eggs adhesiveness and fertilization rates of African catfish, *Clarias gariepinus*

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Abstract. The effect of different full cream powdered cow's milk concentration and rinsing time on adhesiveness and fertilization of African catfish, *Clarias gariepinus* eggs were evaluated. The objective of the study was to determine the optimum concentration and rinsing time of powdered milk to remove the adhesiveness of African catfish eggs and hence to investigate its effect on fertility. Four different concentrations of full cream powdered cow's milk were tested, namely 10 g L^{-1}, 12 g L^{-1}, 14 L^{-1} and 16L^{-1}. Then 1 g NaCl L^{-1} was added into each diluted milk solution, whilst the control was 1 g NaCl L^{-1} water without milk. Each concentration of milk solution was rinsed at three different times i.e. 10 min, 20 min and 25 min before being incubated. The results showed that 14 g L^{-1} powdered milk displayed highest non-stickiness among eggs (79.27%) while, the highest fertilization rate was found in 12 g L^{-1} powdered milk (91.75%). In addition, the optimum rinsing time was 25 minutes which resulted in 70.24% de-adhesion among eggs and fertilization rate of 84.50%.
Key Words: fertilization, stickiness, catfish, urea.

Introduction. African catfish *Clarias gariepinus* is considered as an important food source not only for low income families but also for rich families over the world. The African catfish is a native species to Africa; its native range extends from South Africa through central, west and North of Africa into the Middle East and eastern of Europe. It is indigenous to many rivers from the Nile to the Orange River (Teugels 1986). This species is very popular for aquaculture in Southeast Asia countries including Indonesia, Malaysia, Thailand and Vietnam (Muchlisin et al 2010). Moreover, it is a very suitable species for aquaculture as it grows fast and it feeds on a large variety of food. It can also tolerate a wide range of water quality conditions and it is relatively easy to reproduce in captivity. It also can be raised in high density resulting in high yield production (Okechi 2004). However, the development of African catfish culture is still in infancy. The main limitation of the African catfish culture was the inadequate supply of larva (Hogendoorn 1979). However, in the past two decades, methods of fish larva production and pond rearing of *C. gariepinus* have well been developed (Hogendoorn 1979; Richter 1979; Brzuska 2003).

Eggs of African catfish are covered with a layer of mucus that gives them adhesiveness. In natural condition, egg adhesiveness is a reproductive strategy of most teleost fishes in order to protect the eggs from water drifting. In the wild, African catfish spawning takes place at night in shallow water with temperatures above 22°C and the eggs stick to the leaves and stems of vegetation (Little et al 1994). However, in artificial spawning, the adhesiveness of the eggs could reduce fertilization and hatching rates. This is because the adhesiveness covers the microphiles and hinder the sperms from

fertilizing the eggs (Prinsloo et al 1987); the chances of sperm to get in contact with the eggs are reduced and hence the chances of the eggs to be fertilized are reduced as well. Moreover, when the eggs come into contact with water during the incubation period, the eggs become clumped and this reduces the chance of the eggs to hatch.

Some materials have been used to remove egg adhesiveness of fishes, for example milk for common carp, *Cyprinus carpio* (El-Gamal et al 2008), tannic acid for pikerpich, *Sander lucioperca* (Demska et al 2005), kaolin for shishamo smelt, *Spirinchus lanceolatus* (Mizuno et al 2004), mud solution in Japanese dace, *Tribolodon hakonensis* (Nakamura 1966) and urea solution in carp (Rothbard 1978). The removal of eggs adhesiveness of African catfish using urea solution has been reported by Asraf et al (2013), however, the fertilization and hatching rates were lower. Probably the urea solution is not suitable for African catfish eggs. Herein, we evaluate the powdered milk solution at various concentrations as alternative rinsing solution for African catfish eggs.

Material and Method. The study was conducted at the Aquaculture Research Complex Universiti Sains Malaysia, Penang, Malaysia on July to September 2011.

Brood stock and rinsing agent. Two males and three females donors of African catfish weighing 800-1200 grams were obtained from the Aquaculture Research Center, Universiti Sains Malaysia; they were injected with 0.3 mL of ovaprim per kg body weight. The male and female fishes were kept in separated tanks (75 L) with aeration at room temperature (28-29^{o}C) for 12 hours.

Four different concentrations of full cream powdered cow's milk were tested, namely 10 g L^{-1}, 12 g L^{-1}, 14 L^{-1} and 16 L^{-1}. Then 1 g NaCl L^{-1} was added into diluted milk, whilst the control was 1 g NaCl L^{-1} water without milk.

Sperm and eggs collection. Nine hours after injection, the male fishes were anesthesized with star anise oil extract (three drops of star anise oil were dissolved in 10 liters of tap water) prior to sacrifice by spinal transaction. This was necessary as catfish sperms can not be released by abdominal pressing. Testes were removed by dissection and semen was gently squeezed onto a Petri dish which was placed on crushed ice (4°C). The female abdomen was gently squeezed onto a Petri dish to collect the eggs (Richter et al 1987).

Fertilization procedure. The sperm and eggs were mixed and two drops of saline water were added to activate the sperm, then agitated with soft feather and then left for five minutes to allow the eggs to make sufficient contact with the sperm. The ratio of sperm and eggs was 1:4.

Approximately after five minutes of fertilization, 1 mL of eggs were randomly taken and rinsed for each concentration of full cream powdered cow's milk solution for 15, 20 and 25 minutes, respectively. All experiments were conducted in three replicates.

Incubation procedure. After the rinsing procedure, all the treatments were rinsed with tap water prior to transfer of eggs into the 200 mL plastic jar at room temperature. The incubation containers were set up with continuous aeration. Each treatment was incubated in a different container.

Evaluation of non-stickiness eggs and fertilization rate. The number of completely free (non-stickiness) eggs and aggregated eggs in each container were counted after 15 minutes of incubation. Unfertilized eggs that became opaque were counted and removed from the container after 30 minutes of incubation. The rate of non-stickiness eggs (R_r) was calculated as number of non-stickiness eggs (R_1) from the initial numbers of eggs (R_2) as follow: $R_r = R_1/R_2 \times 100$. The percentage of fertilization rate (F_r) was calculated as the number of fertilized eggs (F_2) from the initial numbers of incubated eggs (F_1) as follow: $F_r = F_2/F_1 \times 100$. The hatching rate was calculated as the number of hatched eggs (H_3) divided by the number of fertilized eggs (F_2) as follow: $H_r = F_3/F_2$.

Statistical analysis. The raw data were tested by multivariate general linear model to exam the main effect of milk concentrations at different times as well as the interaction on free, fertilized and hatching rates. A Duncan's multiple range tests were used to determine if there were significant differences among treatments.

Results and Discussion. The multivariate test showed that the rinsing agent concentrations had significant effect, but the rinsing time and interaction between rinsing agent and rinsing time were not showing any significant effects. Test of between subjects effects demonstrated that the rinsing agent showed significant effect on the reduction of egg adhesiveness, while the rinsing time did not have significant effect on the hatching rate. However, interaction effect between rinsing agent and rinsing time did not significantly affect the eggs adhesiveness and fertilization rate.

The results revealed that 14 g powdered milk + 1 g NaCl L^{-1} gave the highest quantity of non-stickiness eggs (79.27%), but it was not significantly different with other treatments ($p > 0.05$) except for 0 g powdered milk + 1 g NaCl L^{-1} (49.28%) ($p < 0.05$, Table 1). The fertilization rate was higher in 12 g powdered milk + 1 g NaCl L^{-1}, but it was not significantly different with other treatment ($p > 0.05$) except for the control ($p < 0.05$). The rinsing time showed that the rate of non-stickiness eggs was increased with increasing of rinsing time. The highest rate of non-stickiness eggs was found at 25 minutes (70.24%). However, this value was not significantly different to other treatments ($p > 0.05$). The highest fertilization rate was found at 20 minutes (86.90%), but it was no significantly different to other treatments ($p > 0.05$, Table 2). The interaction effects between rinsing agents and rinsing times for non-stickiness eggs and fertilization rate were higher at 14 g powdered milk with 15 minutes rinsing time, 16 g powdered milk with 20 minutes rinsing time and 0 g powdered milk with 15 minutes rinsing time, respectively. However, there was no significant difference among values for non-stickiness eggs (Table 3).

Table 1

The main effect of the rinsing agent on the reduction of adhesiveness and fertilization rates of African catfish eggs (±SD) in various rinsing concentration

Rinsing agents	*Non-stickiness eggs (%)*	*Fertilized (%)*
0 g powdered milk + 1 gm NaCl L^{-1} (control)	49.283 ± 7.05^{a}	71.085 ± 5.63^{a}
10 g powdered milk + 1 gm NaCl L^{-1}	73.182 ± 7.05^{b}	88.791 ± 5.63^{b}
12 g powdered milk + 1 gm NaCl L^{-1}	77.650 ± 7.05^{b}	91.748 ± 5.63^{b}
14 g powdered milk + 1 gm NaCl L^{-1}	79.268 ± 7.05^{b}	87.849 ± 5.63^{b}
16 g powdered milk + 1 gm NaCl L^{-1}	66.188 ± 7.05^{b}	88.791 ± 5.63^{b}

The different superscript in the same column denotes significant differences ($p < 0.05$).

Table 2

The main effect of rinsing time on the free, fertilization and hatching rates of African catfish eggs at various rinsing times

Rinsing time (min)	*Non-stickiness eggs (%)*	*Fertilization rate (%)*
15	69.857 ± 5.46^{a}	85.556 ± 4.36^{a}
20	67.251 ± 5.46^{a}	86.901 ± 4.36^{a}
25	70.235 ± 5.46^{a}	84.503 ± 4.36^{a}

The different superscript in the same column denotes significant differences ($p < 0.05$).

The study revealed that powdered cow's milk had increased the quantity of non-stickiness eggs and fertilization rate compared to the control. This is because the milk reduces the rate of aggregating eggs by coating them with the milk particles. The coating prevents the eggs from sticking to each other. The fertilization rate also increased because during the fertilization the sperm can go directly towards the eggs without any obstacle of the mucus layer in egg surface. The rinsing time also has a significant effect on the rate of de-adhesion, fertilization and hatching of eggs.

Table 3

Effect of different concentrations of full cream powdered cow's milk at different time intervals on the percentage of de-adhesion, fertilized and hatching rate of African catfish eggs

Rinsing agents	*Rinsing time (min)*	*Non-stickiness eggs (%)*	*Fertilization (%)*
0 g powdered milk + 1 gm NaCl/L	15	44.0546± 25.71[a]	79.4347±27.74[ab]
	20	50.2924±32.49[a]	73.5867±20.39[ab]
	25	53.5088±46.46[a]	60.2339±33.66[a]
10 g powdered milk + 1 gm NaCl/L	15	77.5828±4.77[a]	87.3294±13.22[ab]
	20	74.1715±8.99[a]	85.6725±12.22[ab]
	25	68.1287±34.93[a]	93.3723±6.86[ab]
12 g powdered milk + 1 gm NaCl/L	15	82.0663±8.46[a]	87.9142±16.90[ab]
	20	75.7310±7.04[a]	93.1774±9.28[ab]
	25	75.2437±5.25[a]	94.1520±4.05[b]
14 g powdered milk + 1 gm NaCl/L	15	85.0877±15.97[a]	87.2320±15.67[ab]
	20	69.2008±6.35[a]	87.5244±16.04[ab]
	25	83.4308±6.96[a]	88.7914±12.32[ab]
16 g powdered milk + 1 gm NaCl/L	15	60.4288±19.75[a]	85.8674±19.97[ab]
	20	67.3489±10.50[a]	94.5419±3.70[b]
	25	70.9552±17.66[a]	85.9649±11.60[ab]

The different superscript in the same column denotes significant differences ($p < 0.05$).

Many factors contribute to successful egg adhesiveness removal including the type and concentration of rinsing agent, fertilization protocols and incubation protocols. The results obtained from the present study indicate that the use of powdered cow's milk to rinse the eggs can reduce the clumping of African catfish eggs and hence enhance the fertilization rate. This finding is very useful because African catfish eggs usually clump together when they are released into the water because of the adhesiveness. By increasing the rate of free eggs, the chance of the eggs to become fertilized also increased. However, the fertilization rate of eggs was not observed in this study, this is because of technical error has been occurred during the experiment where the oxygen supply into incubation jars have stopped due to electrical problem in our lab. Therefore, further study is needed to evaluate the hatching and survival rate of the larvae.

Conclusion. The present study revealed that the concentration of 14 g powdered milk + 1 g NaCl L^{-1} of solution with 25 minutes rinsing time is a optimum concentration and rinsing time for African catfish eggs. Further study is needed to evaluate the eggs hatching rate and survival of African catfish larvae.

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