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The Effects of Pineapple and Orange Juice as De-Adhesion Agents on *Clarias gariepinus* (African catfish) Eggs

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Abstract
The adhesive nature of eggs of Clarias gariepinus often results in the challenge of egg clusters during the induced breeding of this species. This study examined the possibility of using pineapple and orange extracts in the eradication of egg gluiness thus improving spawning in Clarias gariepinus. Three males and six female brood stocks were used for the study. Five treatments including pineapple and orange solution concentrations at 1% and 3% and the control (without juice) were used in the study. The highest fertilization rate (99% ± 0.1000), hatching rate (73.53% ± 4.3753) and survival rate (92.17% ± 2.9195) were found with the treatment of 1% orange juice solution followed by 1% pineapple juice and was significantly different from those obtained from other treatments (P<0.05). The fertilization and hatching rate decrease with a corresponding increase in the concentration of the juice (pineapple and orange). Orange juice and pineapple juice extract (1%) has a positive effect in reducing the stickiness of Clarias gariepinus eggs and improved the fertilization and hatching and survival rate. It is, therefore, recommended that 1% orange juice and pineapple juice should be used for de-sticking C. gariepinus eggs.

Keywords; de-adhesion agents, *Clarias gariepinus,* de-sticking, orange juice , pineapple juice

Introduction

In natural environment, the spawning of African catfish takes place during the night times in shallow waters with a temperature above 22^{o}C whereby the eggs stick to leaves and stems of vegetation (Sule, 2003). African catfish eggs are enclosed with a layer of mucus that results in adhesiveness. However, in artificial spawning, the adhesiveness of the eggs could reduce fertilization and hatching rates. This is due the agglutination of the eggs which covers the micropyles and hinders the sperms from fertilizing the eggs; the possibilities of sperm entering into contact with the eggs are reduced and hence the probabilities of the eggs getting fertilized are reduced (Sule, 2003). Also, when the eggs come in contact with water for a period of time, and clumped reducing the prospect of the eggs hatching.

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However, various solutions are adopted to manage reproduction in cultured fish species with the aim of achieving high fertilization of eggs and hence the production of an enormous number of fingerlings. Solutions used to reduce egg adhesiveness in fishes; especially in *C. gariepinus* includes powder milk as reported by El-Gamal *et al*., (2008). This proved to be the maximum suitable media for the elimination of egg adhesiveness in comparison with tannic acid and urea which can also be used. The use of powdered milk as organic dissolvent to reduce egg stickiness has also been reported by Linhart *et al.* (2003). Other authors like Żarski *et al.* (2015), were the first to report high hatching rates after using tannic acid to reduce adhesiveness. 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 low. The use of acylase enzyme (Linhart *et al*., 2003; Linhart *et al*., 2004) have been done as well. While milk powder is prohibitively expensive to acquire, tannic acid, acylase enzyme, besides being non readily available, are not very effective de-sticking agents. The removal of egg adhesiveness in *Heterobranchus bidorsalis* using pineapple juice (Paterson and Nwashi, 2014) has been done with great success. Nwachi and Igill-Iboi (2014) used pineapple juices only on *Clarias gariepinus*. There is paucity of knowledge on the most appropriate method on how to remove adhesiveness of the African catfish eggs in Nigeria using locally available de adhesive agents. Therefore, this study was designed to determine the possibility of using either of two common de sticking agents, pineapple and orange juice at different concentrations to remove the stickiness of the African catfish eggs for enhance fertilization and hatching rates

Materials and Methods

Experimental Site

The experiment was carried out at the Department of Aquaculture and Fisheries Management fish farm, Faculty of Agriculture, University of Benin, Benin City, Edo State.

Selection of experimental fish

Nine healthy brood stock of *C. gariepinus* (3 males, 6 females) were used for this study. They were procured from the Department of Aquaculture and Fisheries Management experimental fish Farm. Individual selection method was used to obtain the males, progeny of a different set of parents, weighing between 1.5-3kg each was used for the experiment. The male brooders were selected based on the elongated thick or swollen reddish urinogenital papilla extending to the beginning of the anal fin, and females were identified and selected by the presence of a well-rounded and soft abdomen with swollen and reddish genital opening, and uniform size of intra-ovarian oocytes or eyed eggs according to the method of Egwenomhe and Obi (2012).

Experimental design

The study was conducted using a Completely Randomized Design (CRD). The outlay comprises of (15) plastic tanks which were assigned to two treatments (pineapple and orange juice) and two concentrations (1% and 3%) with three replications each and a control which was replicated three times.

Experimental Procedure

Administration of hormone (ovuline)

Female brooder (*C. gariepinus*) was injected with Ovuline hormone (0.5mL) per kilogram of body weight as recommended. The female brooder is artificially induced following the recommendations of Olanrewaju *et al.* (2009). The injection was done intramuscularly above the lateral line organ just under the dorsal fin. The injected area was rubbed with a finger in

other to distribute the ovuline evenly throughout the muscle and to stop backflow. The injected fish was placed in a bowl and covered with a netting piece to forestall it from jumping out. The temperature of the water holding the fish was measured with mercury in glass thermometer and the corresponding latency phase is additionally noted as recommended by Eric, (2002).

Procurement of milt

The milt was procured by sacrificing and dissecting the male (*C. gariepinus*) in order to remove the gonad (testis). Prior to this section the physiological solution was prepared by dissolving 9 grams of salt (NaCl) in one liter of water. Incisions were made into the creamy colored lobes of the testis and then squeezed and washed out of the testis sac with the physiological solution into a beaker.

Stripping of egg from female brood stock

The first step taken at some point of the stripping technique was to mop the frame of the female brooder with a towel, this was done to prevent the egg from coming in contact with water, which may consequently seal up the micropyle and prevent fertilization. Gentle pressure was applied on the abdomen of the female brooder and the ovulated eggs that will ooze out easily from the genital opening was collected in a stainless-steel bowl where the eggs were collected for experiments (Egwenomhe *et al*., 2017).

Pineapple and Orange juice preparation

Pineapple and Orange juice were prepared by squeezing peeled fresh fruits and made up to 1% by mixing 1mL of juice with 99ml of distilled water and 3% by mixing 3mL of juice with 97mL of distilled water respectively. A total of 2 Pineapple (ave.500 g) and Orange (ave.70 g each) juice solution were produced per trials and used soon after preparation.

Fertilization and pineapple juice application

The stripped egg was collected into a clean stainless bowl; milt solution was prepared from the male broodstock. The eggs were fertilized and divided into five equal part of 6 g each having 1191 eggs in triplicates (A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3 and E1, E2, E3) representing 1%, 3%, each for both orange juice and pineapple juice and control. A little volume of each of the mixture was first spread over the eggs and stirred continuously with a 5mL plastic spoon for 1 min. During stirring uninterruptedly sufficient solution was added to just cover the eggs, for a further 1 min and the supernatant decanted. The fertilization rate was obtained by counting physically the number of fertilized and unfertilized eggs for each treatment at about 3 min after fertilization. The fertilized eggs were greenish brown, and eyed whereas the unfertilized ones were whitish opaque in colour. The percentage of good eggs is then estimated as the fertilization rate according to Ataguba, Annune and Ogbe, (2010). Then, a subsample of the fertilized eggs were taken through capillary with the help of a glass tube (300 mm length and 2.5 mm diameter), and also the total numbers of the viable (live) and bad (dead) eggs are counted. The proportion of the viable eggs is then estimated to determine the fertilization rate. This method assumes that every one good eggs at this time (beyond 3 minutes after fertilization) are fertilized while the white/opaque (bad) eggs are unfertilized. The procedure was replicated with fresh juice and the eggs washed with clean hatchery water and maintained in hatchery water.

Incubation of fertilized eggs

Hatching is the mechanical and enzymatic process of breaking of the eggshell and release of larvae. The time of first hatch and completion of hatching was recorded against the temperature.

Larval rearing

Larval rearing was carried out by placing the hatchlings, into 30 liters spawning bowls. In the first three days, the healthy larvae had endogenous feeding. From the fourth day the fry were fed with commercial feed (Coppens) of required sizes from 0.3mm to 0.5mm severally in a day to saturation. The duration of the study lasted for 1 week post yolk sac fry stage.

Hatchery management

The continuous flow of water (flow through) started immediately after hatching. Also, water quality parameter was regularly checked to ensure the survival of hatchlings.

Data collection

Data was collected at various stages of the study. These stages and data collected includes;
Data on Fertilization; Fertilization rate was determined after 3 minutes of incubation by taking count of the transparent embryos with respect to the white coloured unfertilized or dead eggs. The percentage fertilization was calculated (Florence and Harrison, 2012) thus:

$$Percentage\ of\ fertilization\ rate = \frac{Number\ of\ fertilized\ eggs\ (F2)\ \ x\ 100}{Total\ number\ of\ egg\ counted}$$

Data on Hatching rate; Hatching rates were observed after 24 hours of incubation, by counting the number of larvae produced. The hatchability percentage was estimated FAO (1996) as follows:

$$Hatching\ rate\ \ = \frac{Number\ of\ Hatchlings\ (Three\ days\ old)\ (H3)\ x\ 100}{Total\ number\ of\ fertilized\ eggs\ (F2)}$$

Data on survival rate; the survival rate of larva was monitored for one weeks for each treatment. The percentage survival rate was estimated (FAO 1996) as follows:

$$Percentage\ survival\ rate\ \ = \frac{Final\ number\ of\ hatchlings\ (H4)\ x\ 100}{Initial\ number\ of\ hatchlings\ (H3)}$$

Data analysis

Data were subject to analysis of variance (ANOVA), differences among treatments and the treatment means were separated by Duncan's Multiple Range Tests (DMRT) at 5% probability level. Computer analysis was carried out using the GenStat Package version for windows.

Results and Discussion

Results of the Mean (±SD) fertilization, hatching and survival rate of *Clarias gariepinus* egg treated using different concentration (1% and 3%) pineapple juice and orange juice and normal saline water which serve as the control, is discussed below.

From Table 1, Treatment D 1% orange juice had the highest fertilization percentage (99.00% ± 0.1000) and hatchability (73.53% ± 4.3753) and survival rate (92.17% ± 2.9195). Fertilization rate (98.33% ± 0.15275) and hatching rate (60.83% ± 0.92916) and survival rate (86.50% ± 9.15369) in the control (without orange and pineapple) were higher than those of treatment C (3% of orange juice) and treatment E (3% of pineapple juice). Treatment D with 1% orange juice had a significant difference ($P < 0.05$) from treatments A, C and E in fertilization rate and was significant at ($P < 0.05$) compared to other treatment in hatching rate and also had a significant difference ($P < 0.05$) from treatment B, C and E in survival rate.

Table 1: Mean (± SD) fertilization, hatching and survival rate of *C. gariepinus* egg treated using different concentration of pineapple and orange juice with a control

Parameter	Control (A)	Pineapple juice (1%) (B)	Pineapple juice (3%) (C)	Orange juice (1%) (D)	Orange juice (3%) (E)
Fertilization Rate (%)	98.33±0.15275^{ab}	98.67±0.2517^{a}	96.20±2.5239^{b}	99.00±0.1000^{a}	97.77±0.8387^{ab}
Hatching Rate (%)	60.83±0.92916^{c}	67.27±1.6042^{b}	51.97±4.7004^{d}	73.53±4.3753^{a}	56.57±3.3976^{cd}
Survival Rate (%)	86.50±9.15369^{a}	81.23±5.4857^{ab}	76.93±4.2853^{ab}	92.17±2.9195^{a}	68.53±15.3871^{b}

Mean with different superscripts along the row indicate significant differences (P< 0.05)

Fertilization rate

The result shows a significant difference ($p<0.05$) among the treatments. Highest percentage fertilization occurred in 1% orange juice and the lowest occurred in 3% pineapple juice. The fertilization rate decreased with further increase in concentration of the juice (pineapple and orange). This is in line with the findings of Nwachi and Igill-Iboi (2014) who stated that the application of pineapple juice for de sticking of *Clarias gariepinus* eggs will decrease fertilization rate with further increase in the concentration of the juice. The highest fertilization rate in 1% Orange and pineapple juice was probably due to the low concentration of juice, which did not cause osmosis problems in the eggs, this further increased fertilization rate as the sperm had more direct interaction with the eggs without the hindrance of the mucus layer on the egg surface.

Hatching rate

There was significant difference ($p<0.05$) among the treatments. The highest percentage hatchability occurred in 1% orange juice and the lowest occurred in 3% pineapple juice. The hatching rate decrease with further increase in concentration of the juices (pineapple and orange), this is in agreement with the result obtained by Paterson *et al.,* (2014) in *Heterobranchus bidorsalis.* Decrease in stickiness of eggs is an important procedure under controlled artificial reproduction in fresh water aquaculture which help in improving hatchability of fish under hatchery conditions (Rottmann *et al*., 1991). The use of salt, Urea and tannin have been reported to be very effective in eliminating egg stickiness in fin fishes traditionally. These traditional methods require some time lagged during treatment compare to the use of pineapple juice and orange juice which works immediately on application. However, chemicals such as tannin have been found to be very toxic to eggs if not well used, mostly at a contact over a few seconds (Horvath *et al*., 2002). Billard (1999) suggested the use of egg and milk mixtures in Zoug Jars with the aid of bubbled air, despite the merit of this procedure it is of note that most of the area that need this technology might not have the capacity to power aerating system at a profitable level. Orange and Pineapple fruits have a high concentration of ascorbic acid (Vitamin C) with orange juice having the highest of about 124.0mg 149% while pineapple juice is about 9.5mg 11% in their composition. Vitamin C contributes a significant role in fish reproductive biology such as antioxidant effect on gametes, with ability to prevent DNA damage, recovery in multiple spawners, endocrine regulations, maturation and fertility as reported by (Dabrowski and Ciereszko, (2001). Pineapple juice contains a mixture of enzymes called bromelain, which has strong anti-inflammatory properties while orange juice naturally contains large amounts of pectin esterase - an enzyme that strips methoxyl groups from the pectin molecules. This is thought to have mucolytic properties that help break up and expel mucus layer in the eggs (Balsamo *et al*., 2010).

"The juice is extracted and mixed with water; the solution (juice and water) is added to the egg which reacts with the egg shell, thereby reducing the blood vessel and the hardness (thickness) of the egg shell". This helps to improve hatchability. Desticking of egg using pineapple is quick and simple and requires three (3) minutes only instead of the one hour (1hr) for conventional de-sticking".

Survival rate

There was significant difference ($p<0.05$) among the treatments. The highest percentage hatchability occurred in 1% orange juice and the lowest occurred in 3% orange juice. Orange juice and pineapple juice have little effect on the survival rate, this is in line with the findings of Kareem *et al*., (2017) who stated that the high survival rate observed under this treatment indicated that, at this optimum concentration, the traditional method of de-sticking eggs and juice concentration do not have any negative effect on the survival of hatchlings. Paterson and Nwachi (2014) reported that the use of organic products such as orange and pineapple can help reduce danger of death when applied in excess and increases the hatchability rate of incubated.

Conclusion

In conclusion, orange juice and pineapple juice solution effectively reduce stickiness of *Clarias gariepinus* eggs and improved the fertilization and hatching and survival rate. The results obtained from this study showed that 1% orange juice is an ideal concentration for optimal fertilization of eggs, hatching and subsequent survival of the larvae. However, 3% pineapple juice showed the lowest fertilization rate and hatching rate while the lowest survival rate was 3% orange juice. Therefore, increase in the concentration of the juices will decrease the fertilization rate and hatching rate. It is there for recommendations that 1% orange juice and pineapple juice be used for de-sticking *C. gariepinus* and higher concentrations of these juices should be discouraged. Further research and analysis should be conducted using other fruit juice such as lemon, grape as de-sticking agent on Catfish egg.

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