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Effects of Different Sperm Extenders on the Fertility, Hatchability and Survival of *Clarobranchus*

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ABSTRACT
This study assessed the effects of natural extenders on the spermatozoa of African giant catfish (*Heterobranchus bidorsalis* x *Clarias gariepinus*) hybrids, with the intent of determining the effect of the different sperm extenders on fertility, hatchability, survival, and growth of *H. bidorsalis* x *C gariepinus*. Semen samples (milt) were extracted from mature broodstock males. Soybean milk, sugarcane and saline solution were used to preserve obtained semen at temperatures 4°C for 0, 3 and 6 hours respectively. The different trials were used to fertilize readily counted eggs. Fertility was evaluated 2-3hours after fertilization. Saline solution had the highest (57.50±7.5) after 6 hours of exposure, followed by Soya bean milk (55.00±5.00) and Sugar cane had the least (47.5±2.50). The eggs began to hatch from 19 hours, Soybean milk had the best results at 0 hour (52.50±2.50), followed by Sugarcane (42.50±2.50) but at 6 hours the control had the best (25.00±7.50). Percentage survival of the hatchlings were evaluated for two weeks and at the end of the 14th days, sugarcane solution had the highest percentage survival (94.00±5.00) with the extended semen fertilized at the sixth hour of exposure while saline solution had (85.00±5.00). From the results natural extenders could be used by hatchery managers as an alternative during fish breeding.

KEYWORDS: Saline water, sugarcane water, soybean milk, spermatozoa, fertilization, *Clarobranchus*

INTRODUCTION

World aquaculture production has been in the increasing order (FAO, 2013). The mean annual growth rate during the last decade (2001 – 2010) was 6.61% and it remained higher than that of other farming sectors. The production from aquaculture sector is being influenced by various factors like water quality, seed quality, and availability of feeds as well as the market price. The quality of the gametes assumes importance as it can decide the quality of the seeds and their growth potential as in the case of farm animals. In preserving the quality of the seeds and ensuring the use of quality gametes, cryopreservation has been considered as a viable tool. It has proved its worth in the animal

husbandry and milk production. In the same line, in fishes also for the quality seed production, cryo preserved gametes can be a useful tool in fish reproduction and mass fingerlings production. There is a big scope for research in this area**.** Cryo preservation is the process of freezing the cells or tissue slowly so as to preserve their current state for future use. While Tsai and Lin, (2012) reported that Cryopreservation is a long-term storage technique with very low temperatures to preserve the structurally intact living cells and tissues for extended period of time at a relatively low cost. Cryopreservation is to preserve and store the viable biological samples in a frozen state over extended periods of time. Development of fish seed production has been identified as a rational way of augmenting the dwindling fish supply from the capture fisheries (Dada and Fagbenro (2008). To provide fish in the required quantities at reasonable prices to Nigerians, there is a need for adequate broodstock management for fish seed production (Francis *et al.*, 2013).

African catfish *Clarias gariepinus*, *Clarias anguillaris* and *Heterobranchus bidorsalis*, is one of the widely farmed, consumed and suitable species for aquaculture in Nigeria. These species of fish have been widely accepted in Nigeria and are considered promising in fish farming system in Africa as a whole (Omitogun *et al.,* 2012). Catfish are advantageous because of its ability to adapt, withstand stress, have a high growth rate, resistance to disease, survive without water for period of 18 hours and can artificially be propagated through inducing with ovaprim, ovulin, and ovatide (Olaleye, 2005.). *Heterobranchus bidorsalis* grow to maturity after about two years unlike *Clarias gariepinus.* This affects the use of the fish for breeding because of the late maturity. The volume of milt in the fish is usually minimal. This is a challenge to fish hatchery manager and the way out depends on the use of sperm extenders. Extender is a medium to dilute sperm and to get a larger amount of diluted sperm for artificially induced breeding purposes (Muchlisin, 2005). In general, fish produce highly viscous sperm and in some cases only a small volume is produced. Hence, extenders are needed to increase the sperm volume, which will lead to increase in fertilization during artificial breeding (Ohta *et al.*, 2001).

Studies on extenders are important to determine the most suitable extenders, at concentrations optimal for a particular species (Scott and Baynes, 1980). Presently, chemical solutions are commonly used as extenders in artificial breeding and cryopreservation programmes of fish sperm. However, the use of chemical extender has been reported to be toxic to the fish sperm (Muchlisin and Siti-Azizah, 2009). Furthermore chemical extenders can be costly, require careful preparation and they are environmental unfriendly. Hence, it is considered relatively inefficient if more superior alternatives can be obtained. For a high production in both farmed and wild fish populations, there is a need for a quality eggs and sperm. Ochokwu *et al.* (2015) reported that fish populations which are farmed and wild are dependent on good quality sperm and eggs and ability of the sperm to effectively fertilize the eggs. This has necessitated the research on using natural and alternative environmental friendly, cost effective, easy to prepare extenders and their effect at different dilution levels on the fertility, hatchability and survival of *H. bidorsalis* hybrids. The natural extenders to be experimented include; coconut water, sugarcane water, soybean meal, and saline solution (general extender) as a control at three different dilution Ratio i.e. 1:20 sperm to extender. The objectives of this study are to evaluate the effects of natural extenders on the fertility, hatchability, survival and growth of *Heterobranchus bidorsalis* x *Clarias gariepinus* hybrids (*Clarobranchus*)

MATERIALS AND METHODS

Study Area

The study was conducted in the fish hatchery of Department of Fisheries, Teaching and Research farm, Modibbo Adama University of Technology Yola, Adamawa State, Nigeria. Adamawa State is in North Eastern part of Nigeria; it is one of the largest states in Nigeria and occupies land size of about 36,917 square kilometres. It is bordered by the states of Borno to the northwest, Gombe to the west and Taraba to the southwest. Its eastern border forms the national eastern border with Cameroon. Adamawa is a mountainous land crossed by the large river valleys Benue and Gongola. It is located on latitude 9.20 –9.330N, longitude 12.30 – 12.500E and an altitude of 185.9m. It has an average annual rain fall of about 759mm with maximum temperature of 39.70C. The rainy season run from May through

October, while the dry season commences November and ends in April. The driest months of the year are January and February when the relative humidity drops to 13% (Canback Global Income Distribution Database, 2014).

Experimental Fish

The male broodstock of *C. gariepinus* and *Heterobranchus bidorsalis* (average weight of 800-2000g) and female (650-1800g) were collected from teaching and research farm Modibbo Adama University of Technology yola and Wisdom fish farm Jimeta Yola. The selection of the males was based on the weight and possession of well vascular genital papillae, while the female was selected based on the weight, by gently pressing the abdomen to ascertain if the eggs are gravid. The brood stocks were acclimatized for 3days in 30 m^2 earthen ponds. They were fed 35 % crude protein diet at 3% body weight twice a day before the commencement of the experiment.

Preparation of Soybean Milk

The soybean milk was prepared according to the method described by El-Keraby *et al.,* (2010). 20 grams of soybean were measured; stones and unwanted materials were removed. The clean soybean was soaked and mashed to remove the chaff, re-washed and grinded into smaller particles. The slurry was filtered using 50ml distilled water through a clean white cotton cloth and then boiled for 10 minutes. After boiling, it was allowed to cool down.

Preparation of Sugarcane Solution

The sugarcane was washed, pilled from the outer layer and rewashed. The washed sugarcane was mashed using mortar and pistol then grinded and filtered with 20ml of distilled water to collect the sugar cane juice.

Preparation of Saline solution

Saline solution was used as control which contained 1g of NaCl to 1 liter of water.

Hypophysation and Artificial Hybridization

The fishes were sexed and separated into males and females based on their genital papillae Viveen *et al.,* (1985); the weight and length of the gravid females were measured and induced with Ovulin at a dosage of 0.5 ml/kg body weight for female and 0.25 ml/kg body weight for male (Ochokwu *et al*., 2016). The brood fish were kept in well aerated plastic bowls of 120cm diameter × 50cm deep for 9 hours and covered with a net.

Fertilization and Hatchability

Fertilization and hatchability rate was determined using 100 eggs; the number of eggs was estimated using the gravimetric method (number of eggs/g). The translucent eggs containing embryonic eyes at the time of polar cap formation 10 - 20 minutes after fertilization were considered fertilized and counted to estimate fertilization rate. The number of hatchlings in each trough was recorded by direct counting of the hatchlings and un-hatched eggs for each female. The fertilization and hatching rates were estimated as follows:

Fertilization rate (%) = Number of fertilized eggs ÷ Total number of eggs x 100,

The parameters evaluated were

(i) % Fertility = (No. of fertilized eggs / No. of inseminated egg) X 100%

(ii)%Hatchability =

$$\frac{\text{No. of hatchlings}}{\text{No. of fertilized eggs}} \times 100\%$$

(iii)Survival rate (%) =

$$\frac{\text{Final number of fry}}{\text{Initial number of fry}} \times 100$$

(Richter *et al*., 1987; Viveen *et al*., 1985).

Statistical Analysis

Data obtained from the experiment were subject to a One-Way-Analysis of Variance (ANOVA). Duncan Multiple Range Test DMRT, Duncan (1985) was used to determine the differences between the means (P=0.05) using SPSS version 20.0.

RESULTS

Table 1, revealed the effects of using natural extender on the fertility of the *Clarobranchus*. There was a significant difference (P>0.05) at the initial fertility rate. Treatment with saline solution had the highest fertility rate (82.50±2.50) when compared with sugarcane solution (75.00±5.00). At sixth hours of fertilization it showed that fertility decreases with duration of extension. At the end of the fertility test, saline solution showed the best fertility rate while sugarcane solution showed the least.

Table 1: Effect of Natural Sperm Extenders on Fertility of *Clarobranchus*

Duration/hour	Saline solution	Soybean	Sugarcane
0	82.50±2.50^{a}	81.00±1.00^{a}	75.00±5.00^{ab}
3	72.50±2.50^{c}	76.50±2.50^{b}	77.50±2.50^{a}
6	57.50±7.5^{a}	55.00±5.00^{b}	47.5±2.50^{c}

Means with the same superscript are not significantly different (P>0.05).

Table 2 revealed the effect of natural sperm extender on hatchability of *Clarobranchus*. There was a significant difference (P>0.05) at 0hour duration. At sixth hours of hatchability, it showed that hatchability decreased. The cause of decrease in hatchability rate of all the treatment is attributed to oxygen depletion, temperature and stocking density. At the end of the 24hours, saline solution showed the best hatchability rate while sugarcane solution showed the least.

Table 2: Effect of Natural Sperm Extenders on Hatchability of *Clarobranchus*

Duration/hour	Control	Soybean	Sugarcane
0	50.00±5.00^{a}	52.50±2.50^{a}	42.50±2.50^{ab}
3	35.50±2.50^{a}	25.00±5.00$^{bc`}$	22.50±2.50^{b}
6	25.50±7.5^{a}	15.00±5.00^{c}	13.00±2.50^{bc}

Means with the same superscript are not significantly different (P>0.05).

Table 3, showed the Survival of hatchlings on different type of extenders used. In the current study, at 0% all the treatment had high rate of survival. However reduced at 6 hour duration in the treatment that contains soybean solution. There was no significant difference in the survival of the hatchlings. This can be related to the water quality where the fish was stocked, the feeding rate and frequency, the nutritional content of the fish and management practices because all treatment should have excellent survival since the treatment has no negative effect on the survival of the hatchlings.

Table 3: Effects of sperm extenders on survival of *Clarobranchus*

Duration/hour	Control	Soybean	Sugarcane
0	97.50±2.50^{a}	95.00±5.00^{ab}	96.00±4.00^{ab}
3	85.00±5.00^{c}	92.50±5.00^{b}	95.00±5.00^{a}
6	85.00±5.00^{b}	60.00±1.00^{c}	94.50±2.50^{a}

Means with the same superscript are not significantly different (P>0.05).

DISCUSSION

In this research saline solution tends to show higher result. There was a significant difference (P>0.05) at the initial fertility rate. At sixth hours of fertilization it showed that fertility decreases with duration of extension. At the end of the fertility test, saline solution showed the best fertility

rate while sugarcane solution showed the least. The differences in fertility with control and extended semen tested may be due to the mild damage done to the spermatozoa during the process of lacerating the testes to extract the semen, and also due to effect of the osmolality of the extended semen to that of the sperm, the water quality of the incubated eggs and many other factors such as sperm motility, sperm count, volume, quality of the sperm etc. The higher the motility, sperm volume, the higher the fertility rate. Muchlisin *et al.,* (2004), studied the effects of coconut water, soybean and sugarcane solution as extenders on *Clarias gariepinus* and concluded that coconut water is a better extender. In addition Qureshi *et al.,* (2014) in effect of soybean based extenders on sperm parameters of Holstein reported that the viability of the sperm was higher in 25% soy milk solution than 50% soy extender. Mean while Papa *et al*., (2011) stated that extender containing soybean source is also an alternative conventional extender that contain skimmed milk and or egg yolk. Akhtar *et al.,* (2011) reported that sperm motility and viability were better in soya lecithin based extender when compared to milk, tris-citric egg yolk and egg yolk-citrate extender of buffalo sperms stored at 5ºC. Recently, De Paz *et al.* (2010) reported that lecithin soybean extender is equally effective in preserving the motility and viability of liquid ram sperm at 5 - 15ºC. Meanwhile Zhang *et al*., (2009) suggested that the enhanced improvement of sperm parameter when soybean is used as an extender is due to its low viscosity and less debris.

Fertility has positive relation with hatchability. The higher the fertility rate, the higher the hatchability. Fertility and hatchability was highest in sperm diluted with saline solution as compare to those treated with soybean milk, and sugarcane solution. In the study it showed that the conventional diluents are still the best in artificial fish breeding experiments as it yield higher percentage fertility and hatchability than natural sperm extenders. The study also showed a significant difference ($P>0.05$) within the same extender at different exposure period. Similar work was also carried out by Muchlisin *et al.,* (2004), on hatching rate of fertilized eggs with different natural extenders. His result is in agreement with this study though he used Ringer solution rather than saline solution which are both conventional

solutions frequently used in artificial breeding exercises.

CONCLUSION

From the study, it showed that sperm of African giant catfish (*Heterobranchus bidorsalis)* survived more and enhanced higher fertility in the control solution which also recorded higher hatchability, whereas, the sugarcane solution had the highest survival than other extenders. It can therefore be concluded that saline solution which is a conventional solution in artificial breeding exercises still remains the best extender. Nevertheless, sugarcane and soybean solution can still be useful in sperm extension programs.

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