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Milt Quality of *Clarias gariepinus* (Male) Stimulated Using Different Commercial Hormones

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Abstract: ***The sperm production (milt volume and spermatozoa concentration) and sperm motility characteristics (percentage of motile spermatozoa and duration of motility) were measured in order to assess the milt quality between hormone A (sGnRH-a with domperidone) and hormone B (sGnRH-a with domperidone and Benzyl alcohol) stimulated African catfish, Clarias gariepinus. Six male broodstock were used, three were injected with hormone A and three with hormone B intramuscularly, eight hours after injection these fish were dissected to obtain their milt and the milt parameters were examined. Results showed that hormone A stimulated C. gariepinus produce more milt and the spermatozoa were more active than the hormone B simulated C. gariepinus.***

Keywords: Sperm concentration, sperm motility, *Clarias gariepinus*, commercial hormones

1. Introduction

World capture fisheries are in a state of crises due to stagnation of ocean captures fisheries, over-fishing and pollution concomitantly; world demand for fish and fish products is drastically increasing (FAO 2006). The increasing gap between the demand for quality fish and diminishing supply from ocean capture fisheries can be resolved by aquaculture which is the husbandry of aquatic organisms. Nigeria is a maritime country where fishing plays an important role in the national economy, and fish comprises an important, popular component of the diet. The demand for fish in Nigeria mostly outstrips the local production; Nigeria is the largest fish consumer in Africa and among the largest fish consumers in the world with over 1.5 million tons of fish consumed annually (Ozigbo *et al.*, 2014). Aquaculture is a fast growing sector in Nigeria contributing less than 5% of the total fish supply but a growth rate of about 2% per year (Moses, 2006). The culture of *Clarias gariepinus* increased in recent years due to their high market price, hardy nature and tolerance to adverse ecological condition enable its high density culture with a high production per unit area (Sherma *et al.*, 2010). During the last twenty years, one of the major developments in fish culture is captive breeding. Artificial reproduction under more controlled conditions including stripping of eggs, collection of sperms, followed by fertilization of eggs has been developed. Artificial reproduction by induced breeding through hormone treatment followed by artificial fertilization and incubation of fertilized eggs and subsequent rearing to fingerlings size has shown a better rate of fertilization and hatching, and better condition for growth and survival (Horvath *et al.*, 2003). Due to the location of the testes of *Clarias gariepinus*, it makes it impossible for stripping of milt from the males. There has been a rising application of modern and efficient techniques to induce final oocyte maturation, ovulation, spawning and spermiation. These methods engage the use of gonadotropin releasing hormone (GnRH) treatment which is gaining reception throughout the world, and surpass the hypophysation technique such as the pituitary treatment that has for a long time been used in the induced spawning of fish (Yaron, 1995). Commercially used hormones are (1) Ovopel, a mammalian analoque GnRH(D-Aa^{6}Pro^{9}Net-mGnRH) with a dopamine receptor antagonist i.e. metoclopramide (Cejko *et al*. 2010), (2) Ovaprim, a liquid that contains an analogue of salmon gonadotropin releasing hormone (sGnRH) and a brain neutransmitter (Dopamine) inhibitor (Goudie *et al.*, 1992). (3) Ovatide, a synthetic analogue of the peptide hormone of salmon gonadotropin releasing hormone (sGnRH) dissolved in a mixture of aqueous and organic solvents (Gupta *et al.*, 2002). The GnRH in these hormones elicits the release of stored gonadotropins steroid hormones. Fish farmers reported different results from the use of these hormones. This study was conducted to assess and discuss the effect of the active ingredients contained in the two most commonly used hormones on the milt quality of *Clarias gariepinus* in the South western, Nigeria.

2. Materials and Methods

A. Experimental Site

The experiment was carried out at the hatchery unit of the Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

B. Brooders Maintenance

Six (6) ripe broodstocks of *Clarias gariepinus* randomly selected with average weight of 0.8kg using Hanna top loader scale (0.05kg sensitive with number 2834024) were purchased from a reputable fish farm in Ayegunle Oka Akoko. The broodstocks were stocked in concrete tanks for a period of two weeks for acclimatization prior to induction.

C. Hormones

Hormone A contains Salmon Gonadotropin Releasing hormone Analogue and Domperidone BP.
Hormne B contains Gonadorelin A (sGnRHa), Domperidone BP and Benzyl alcohol IP

D. Induction of *Clarias gariepinus*

The males were selected on the basis of pointed and reddish genital papilla. Six (6) brooders were selected and weighed. These were placed in separate tanks and were not fed for 24

hours before hormonal injection, they were tagged and then divided into groups. The hormonal injections were given at 12 am, in such way that the fish were ready for examination at early morning and evening. These fish were injected muscularly in the region of the caudal peduncle above the lateral line with a 5 ml hypodermic syringe having 0.5 ml graduations. With hormone A or hormone B, the volume of hormone given was due to the weight of the fish. During the hormonal administration, each fish was held firmly by the head and injected with the appropriate amount of hormone. The site of injection was rubbed with the thumb to allow proper circulation of the hormone throughout the body. The brooders were examined and collected eight (8) hours after injection to obtain the milt.

E. Collection of Milt

The testis of the African catfish *Clarias gariepinus* are situated in the dorsal part of the abdominal cavity. They are covered by the intestine in such a way that application of pressure cannot easily release milt (Viverios *et al.,* 2002). They do not release milt under abdominal massage in captivity and need to be killed in order to obtain milt. The fish were dissected at the lateral part using a dissecting scissors and the testis were taken, squeezed into Petri dishes and weighed with the aid of electronic weighing scale (OHAUS Cs 5000).

G. Determination of Sperm Quality Milt Volume

Small incision was made into the lobes of the testes, the milt squeezed into petri dish. This was measured with a plastic syringe in ml (Oguntuase *et al.,* 2014)

Motility Duration

These were determined by adding 1µl of normal saline to the sample for activation of sperm. The sperm activity was viewed under a microscope at magnification x 100 to see when all the sperm got stopped (Mims, 1991).

Sperm Motility

Each sample was estimated using light microscope at magnification x400 immediately after addition of saline solution. During spermatozoa activation immotile sperm cell (ISC) was counted and when the activation stopped, whole sperm cells (WSC) were counted (Canyurt *et al.,* 2008). The Motile Sperm Cells (MC) was calculated as:

$$MC = WSC - ISC$$

$$\%MC = \frac{MC}{WSC} \times 100$$

Spermatozoa Concentration

Concentration of sperm was determined by counting the number of spermatozoa in sample, diluted with saline and examined using a microscope at magnification x400 (Rainis *et al.,* 2003).

H. Statistical Analysis

Statistical of variance (ANOVA) was used at 95% significant level of test for significant differences between the various treatments means obtained for the motility duration, percentage of sperm in milt, milt volume, concentration of sperm, sperm motility. Thereafter, the treatment was subjected to t-test. so as to determine the better hormone between hormones A and B for artificial propagation of *Clarias gariepinus.*

3. Results

Data analysis of milt parameters of hormone A stimulated *Clarias gariepinus* is presented in table 1, while that of hormone B stimulated *Clarias gariepinus* is presented in table 2, the comparison of milt parameters between hormones A and B stimulated *Clarias gariepinus* is presented in table 3 respectively. The mean milt volume obtained in hormone A stimulated *Clarias gariepnus* was 1.14 ± 0.02, while that obtained for hormone B treatment was 1.6 ± 0.04. There was a significant difference ($p < 0.05$) in values of milt volume between the two hormones stimulated *Clarias gariepinus* (table 3). Differences in average number of spermatozoa in milt of hormone A stimulated was significant ($p < 0.05$) from those of hormone B,, the mean number of spermatozoa in milt of hormone A stimulated fish was 96.50 ± 0.03 while that obtained for hormone B stimulated fish was 84.05 ± 0.05 (table 3). The average concentration of sperm obtained in milt of hormone A stimulated *Clarias gariepinus* was 61.13 ± 0.15, while that for hormone B treatment was 41.86 ± 0.15, this showed a significant difference in values between the two hormones stimulated *Claria gariepinus* (table 3). Values of sperm motility obtained in milt of hormone A stimulated fish was 62.50 ± 0.10, while 50.07± 0.06 was obtained from hormone B stimulated ones. There was a significant difference ($p < 0.05$) in values between hormones A and B stimulated *Clarias gariepinus* (table 3). The mean duration obtained in motility in milt of hormone A stimulated fish was 60.50 ± 0.10, while 58.58 ± 0.38 was obtained for hormone B. There was a significant difference ($p < 0.05$) in the duration of motility in milt between hormones A and B stimulated *Clarias gariepinus* (table 3).

Table 1: Milt parameters of hormne A stimulated male *Clarias gariepinus*

Parameters	1	2	3	Mean	Standard deviation
Milt volume	1.15	1.12	1.14	1.14	0.02
Number of spermatozoa in milt	96.50	96.55	96.55	96.53	0.03
Sperm concentration	61.10	61.30	61.00	61.13	0.15
Sperm motility	62.50	62.40	62.60	62.50	0.10
Duration of motility	60.50	60.60	60.40	62.50	0.10

Table 2: Milt parameters of hormone B stimulated male *Clarias gariepinus*

Parameters	1	2	3	Mean	Standard deviation
Milt volume	1.03	1.05	1.10	1.06	0.04
Number of spermatozoa in milt	84.10	84.00	84.05	84.05	0.00
Sperm concentration	41.85	41.70	42.00	41.86	0.15
Sperm motility	50.10	50.00	50.10	50.07	0.06
Duration of motility	59.00	58.25	58.50	58.58	0.38

Table 3: Comparison of milt parameters between hormones A and B stimulated *Clarias gariepinus*

Parameters	Hormone A	Hormone B
Milt volume	1.14 ± 0.02^a	1.06 ± 0.03^b
Number of spermatozoa in milt	96.53 ± 0.03^a	84.05 ± 0.00^b
Sperm concentration	61.13 ± 0.15^a	41.86 ± 0.15^b
Sperm motility	62.50 ± 0.10^a	50.07 ± 0.05^b
Duration of motility	60.50 ± 0.10^a	58.58 ± 0.38^b

Means with different superscript along same row are significantly different ($p<0.05$)

4. Discussion

The hormonal stimulation of *Clarias gariepinus* in the present work confirmed the effectiveness and usefulness of hormones A and B for spemiation in *Clarias gariepinus* and was similar to that of Marta *et al.* (2008) who reported that ovaprim is used as a spawning aid for inducing ovulation and spemiation of *Clarias gariepinus.* It is noteworthy that the results obtained in the current work following stimulation of male *Clarias gariepinus* with hormone A were higher than that of the hormone B, this observation was similar to results reported by Marta *et al.* (2008). The milt volume of *Clarias gariepinus* injected with hormone A was higher and significantly different ($p < 0.05$) from that of hormone B. This finding was the same with that observed by Breata *et al.*(2007) who found that milt volume of ovaprim administered *Aspius aspius* were higher than that of ovopel treatment, and this also affirmed the report of Marta *et al.* (2008) that semen parameters were higher in ovaprim stimulated ide, *Leuciscus idius* than with the application of ovopel. Though, Aril *et al.* (2011) reported higher values of milt volume in *Clarias gariepinus* which was not treated with hormone, and Oguntuase *et al.*(2014) found out that milt was highest in males *C. gariepinus* treated with ovatide and was significantly different from other treatments($P < 0.05$). Values obtained for the number of spermatozoa in milt for hormone A stimulated *Clarias gariepinus* was higher than that of hormone B stimulated ones and this is confirmed by the report of Beata *et al.*(2007) and Kucharczyk *et al.*(2007) who reported higher values of spermatozoa in milt for ovaprim than ovopel stimulated ide, *Leuciscus idus.* Motility of spermatozoa is the most commonly used indication of sperm quality since high motility is a prerequisite for fertilization and correlates strongly with fertilization success (Rurangwa *et al.*, 2004). This was also affirmed by Lahnsteiner *et al.* (1998) who reported that milt with an increased percentage of motile spermatozoa would have a better chance to successfully fertilize larger numbers of eggs. According to these authors, the test of sperm quality is a higher percentage of motile spermatozoa as this will increase the chance of fertilization which is the major purpose of spemiation. Sperm motility was found to be higher in *Clarias gariepinus* injected with hormone A than that of Hormone B fish and this was in line with the observation of Marta *et al.* (2008) who reported that sperm motility was higher in ovaprim stimulated ide, when compared with the ovopel treatment, and this was also in line with the report of Beata *et al.* (2007) who noted better motility in *Aspius aspius* semen after the administration of ovaprim than the ovopel treatment. The sperm concentration was higher in the hormne A stimulated *Clarias gariepinus* than that of the hormone B treatment. Comparism of values of sperm concentration between hormone A and B stimulated *Clarias gariepinus* showed a significant difference ($p < 0.05$) which was affirmed by Beata *et al.*(2007) who reported that mean concentration of sperm for *Aspius aspius* was higher in ovaprim stimulated fish with the value of 11 million ml^{-1} than ovopel stimulated ones with the value of 7 million ml^{-1}. Duration in motility of *Clarias gariepinus* stimulated with hormone A showed higher value and was significantly different ($p < 0.05$) when compared with that of the hormone B stimulated *Clarias gariepinus.* This finding was confirmed by Marta *et al.* (2008) who reported higher values of semen parameters of ovaprim stimulated ide than that of ovopel stimulated ide, *Leuciscus idus.*

5. Conclusion

Hormones A and B are both effective for the spermiation of *Clarias gariepinus* but the values obtained for hormone B might be due to the inclusion of Benzyl alcohol which may be acting as a depressant to the gonadotropin releasing hormones of the fish.

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