Semen Quality And Spermatozoa Morphology Of *Clarias Gariepinus* Broodstock Fed Two Different Feed Levels

Dominic Olabode Odedeyi* and Abiodun Adeyemi Eniade**

*, **Adekunle Ajasin University, Department of Environmental Biology and Fisheries, P.M.B. 01, Akungba-Akoko, Ondo State, Nigeria.
E-mail:bodeyi@yahoo.com or millenium_success@yahoo.com

Abstract: The effect of two different feeding levels of feed fed to African catfish, *Clarias gariepinus* broodstocks on semen quality were investigated. Forty eight, 9 months old hatchery bred male (sub-adults), with mean weight (125 ± 0.90g) were acclimated in a concrete tank of 2m x 2m x 1m and fed commercial pellets (Durantee) for two weeks. Thereafter they were randomly distributed into two tanks 2m x 2m x 1m, each tank containing 24 units of fish. Treatment 1 were fed 2% body weight while Treatment 2 were fed 4% body weight for fourteen weeks to allow sexual maturation. Semen was collected individually from all members of the two treatments. Sperm concentration, motility, motility duration, number of sperm cells and volume of semen were measured in order to compare the semen parameters. Data collected were subjected to analysis of variance and means followed by Duncan's multiple range test using SPSS 17.0 Software on Windows 7.0 application. Results showed that broodstocks fed 4% bodyweight produced more spermatozoa, had higher concentration of sperm and more volume of semen. The duration of motility and rate of motility were not significantly different from each other ($P > 0.05$), thus feeding at 4% body weight is preferred. Morphology of the spermatozoa from the two feeding levels was discussed.
[Dominic Olabode Odedeyi and Abiodun Adeyemi Eniade. **Semen Quality And Spermatozoa Morphology Of *Clarias Gariepinus* Broodstock Fed Two Different Feed Levels.** *Researcher* 2014;6(1):28-32]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 5

Key words: Broodstock, Semen volume, Spermatozoa motility, Sperm cells, Sperm morphology, Feeding levels, *Clarias gariepinus,* Sperm concentration

1. Introduction

African catfish (*Clarias gariepinus)* is very important in the commercial fisheries in Nigeria because of its desirable qualities, such as ability to tolerate low dissolved oxygen, ability to reproduce in captivity and fast growth rate. It is also observed to have efficient feed conversion capacity especially in the males (Nweke and Ogwumba, 2005).

An adequate feeding with a well balanced diet is very important in aquaculture. An under-nourished fish is never able to maintain its health and attain its worth potentials, regardless of the quality of its environment (Zulkiph, 1993).

Male C*larias gariepinus* do not release semen under abdominal massage and need to be sacrificed in order to obtain semen for induced breeding. Though semen collection after killing is effective for artificial breeding purposes, but reduces the number of males in the population. This is a major problem in the African catfish farming sector. There is need for better management that would improve the quality of semen thereby reducing the number of males being sacrificed for induced breeding.

The capacity of spermatozoa to fertilize an egg is determined by the sperm motility, duration of contact and number of spermatozoa, and these are often used to estimate semen quality (Suquet *et al*., 1994; Cheregumi *et al.,* 1999 and Rurangwa *et al.* 2004). The use of high quality gametes from captive fish broodstock is of great importance for ensuring the production of valuable offspring for aquaculture (Bromage and Roberts, 1995).

The fish farming industry in Nigeria appear to be more focused towards the quality of eggs and larvae rather than that of sperm, even though the sperm quality of male broodstock impacts greatly on the production of healthy larvae. Nevertheless, in most commercial hatcheries where African Catfish seeds are being propagated, semen is often inadequate both in terms of quantity and quality and does not always give successful fertilization in the artificial insemination (Rurangwa *et al*., 2004). When male broodstock is limited, it is especially important to ensure that the sperm quality is good enough to achieve high percentage fertilization. The movement of fish spermatozoa begins at the moment of their contact with water (based on the sperm concentration). However, it is possible to directly or indirectly determine the correct or irregular functioning of male gonads (Krol *et al*., 2006). Sperm quality in farmed fish may be affected by different components of broodstock management, for example; it has been observed that gonad maturation in *Clarias gariepinus* could be significantly affected by their dietary energy intake (Cek and Yilmaz, 2009).

Artificial feeding of fish allows high stocking density, promotes fast growth, and stimulates rapid production of plankton (Ekelemu, 2010). Among fish farmers, there are divergent views as to what percentage body weight should broodstocks be fed to enhance good gonadal development and maturation putting cost of feed into consideration. This is as a result of the reported cases of broodstocks having very small testes, very little quantity of semen (even from some big testes) and low rate of fertilization. Therefore, it was the aim of this study to compare the effect of two different feeding levels on the semen quality of African catfish in order to determine the preferred one.

2. Materials And Methods

Forty eight, 9 month old hatchery bred sub adults with mean weight (125 $\pm$ 0.09g) of *Clarias gariepinus* were used for the study which lasted for 14 weeks. The fish were acclimated in a concrete tank measuring 2mx 2m x 1m indoors for two weeks and fed to satiation twice daily. Thereafter, each experimental treatment (24 units of broodstock males) were randomly distributed in a group of eight and replicated in triplicates into tanks of dimensions 1m x 1m x1m. The tanks were labeled T^1a, T^1b, T^1c and T^2a, T^2b, T^2c.

Fish brood stock in the different tanks were fed twice daily with commercially prepared (Durante) 3mm feed pellet at a rate of 2% and 4% total body weight per tank respectively. Proximate composition of feed used was crude protein 45%, crude fibre 2.3%, crude fat 14%, Ash 6.5%, calcium 1.6%, phosphorus 0.9%, methionine 0.9%, lysine 2.25%, Copper 3mg/kg, Vitamin A (i u/kg) 10,000, Vitamin D_3 (iu/kg) 750, Vitamin E (mg/kg) 150 and Vitamin C (mg/kg) 100.

Weights of broodstocks were measured weekly. At week fourteen, nine (9) sexually mature (reddish tip of genital papilla) fish from each treatment were surgically operated to remove the testes. The testes were placed into a petri –dish containing physiological saline solution (0.9% NaCL) and preserved in a refrigerator at 4°c. The surgery and semen collection were performed within an average interval of five (5) minutes on each specimen. Semen volume was measured in milliliters in graded glass tubes; semen volume was immediately recorded and stored under refrigeration at 4°c for later analysis.

Five uL of semen were placed under a light microscope (x10 objective lens power and x5 of eyepiece lens) on a slide, and 50 uL of physiological saliva solution were added as a dilutor. The tail whirling movement of the spermatozoa was considered, and the percentage of the field in movement was established according to an arbitrary scale of 0 to 100%, modified from Vuthriphandchai and Zohar (1999). The motility duration was counted until at least 5% of the spermatozoa were in motion – (modified from Tvedt *et al.*, 2001). Only the tail whirling straight-line movement of spermatozoa was noted. The movement of spermatozoa was studied using a digitalized Vista-vision Optika compound microscope (Model B-350) connected to a Toshiba Satellite M55-S141 Laptop Computer operating on Window 7.0 software. Spermatozoa were viewed as video image with a 1,500X final magnification on the screen.

The spermatozoa counts were determined with aNeubauer chamber. Physiological saline solution was used as a dilutor in a 1: 6 semen- saline solution ratio (5 uL of semen and 30 uL of NaCL solution) and mixed with a vortex. The Neubauer chamber was filled with the diluted semen, after one minute of rest two counts of $0.2mm^2$ were conducted under a microscope objective power of (x40). The spermatozoa concentration was calculated by the number of spermatozoa per ml of semen according to Rurangwa *et al.*, (2004).

Data for each parameter were evaluated and compared between treatments using one- way analysis of variance (ANOVA) to establish the statistical inferences, followed by Duncan 's multiple range test. For all statistical analysis SPSS 17.0 software was used. Broodstocks were measured to the nearest 0.1g and comparism on sperm morphology was performed using the digital image obtained from the Optika compound microscope (Model B-350).

Table 1: Quality of semen of *Clarias gariepinus* at different feeding levels

characteristics	Treatments	
	T^1 (n=9)	T^2 (n=9)
Weight of fish (g)	800.46±0.55[a]	800±0.31[a]
Sperm motility (%)	75.20±13[a]	75.40±11[a]
Sperm concentration (10^6 ml)	35.93±0.01[a]	45.95±0.03[b]
Volume of semen (ml^{-1})	1.03±0.02[a]	1.22±0.10[b]
Sperm duration (s)	62±0.20[a]	62.002[a]
Number of sperm (10^6)	37.01±0.06[a]	56.05±0.06[b]

Significant differences (P<0.005) are indicated by different superscript letters.
T^1: Fish broodstock feeding at 2% body weight; T^2: Fish broodstock feeding at 4% body weight

3. Results

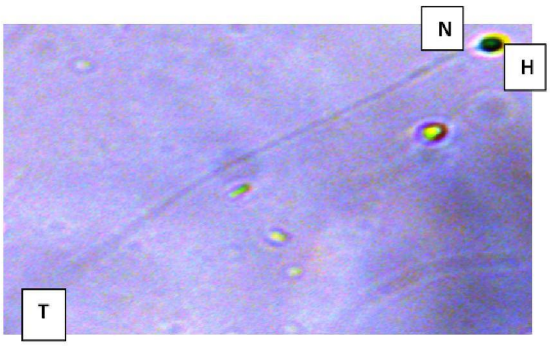
In both treatments (T^1 and T^2), sperm motility was comparatively high and amounted to more than 75%, equally the motility duration for both treatments was more than 60 seconds and was not significantly different (P>0.05) between the treatments (Table 1). The volume of the extracted semen and spermatozoa concentration were higher in Treatment 2 (T^2) and showed significant difference (P< 0.05) from Treatment 1 (T^1) (Table 1).

Also, the morphology of the spermatozoa of *Clarias gariepinus* (figures 1a and 1b) for treatments 1 and 2 respectively, were similar sharing the same body features of a bullet-shaped head containing nucleus, a short neck or mid piece and a long tail or flagellum although the sperm vigour in Treatment 1 (T^1) appeared slimmer.

MG = X 1,500

H – Spermatozoan Head
N- Spermatozoan Neck
T – Spermatozoan Tail

Figure 1a: Spermatozoan of Clarias gariepinus fed at 2% body weight



MG = X 1,500

H – Spermatozoan Head
N- Spermatozoan Neck
T – Spermatozoan Tail

Figure 1b: Spermatozoan of Clarias gariepinus fed at 4% body weight

4. Discussion And Conclusion

Supply of fish seed and feeds are two major factors affecting artificial fish production in Nigeria. However, due to high cost of fish feed many fish farmers knowingly and unknowingly engage in improper feeding of their fish including broodstocks to reduce the cost of managing the fish on farm. This inadvertently constitutes a major nutrition based problem militating against normal gonadal development process including semen quality in male fish and hence inadequate fish seeds and table-fish production.

It is established that sperm quality is a very important factor determining the success of artificial spawning (Bromage and Roberts, 1995). Consequently, in the developed countries, the methods of artificial reproduction permit the use of cryo-preserved sperm (that is stored outside the male organism at ultra low temperature for a long time). However, in a developing country like Nigeria where constant electricity is a major problem and most farmers could not afford the cost of deployment of cryopreservative frizers on farm, adequate feeding not below 4% body weight for male brooders (according to the finding of this study) is recommended for good gonadal development (Table 1). Howbeit, to establish scientific facts and the cost benefit details of this finding for adoption by catfish farmers, more researches on the biology of its semen, its reproductive viability in relations to the dietary energy intake and the economic analysis of the benefits for farmers' are very important research options.

Sperm motility, motility duration, number of spermatozoa and volume of semen are parameters that are essential in determining the capacity of spermatozoa to fertilize (Billard *et al.,* 1995; Diyaware *et al.,* 2010; Hajirezace *et al.,* 2011). In this study, the

total number of spermatozoa, volume of semen and spermatozoa concentration, are significantly different (P<0.05) between treatments but the spermatozoa motility and motility duration did not (P> 0.05). This indicate that the treatment with 4% body weight, that is, increased feed level did not influence sperm motility (P>0.05). The 75% semen motility observed for *Clarias gariepinus* in this study is in line with the findings of Aas *et al.,* (1991) for *Salmo salar* who reported a semen motility range of between 35 and 95% as good for fertilization of eggs. Diyaware *et al.*, (2010) reported that testicular weight, seminal volume and gonadosomatic index (GSI) increased with an increase in the regeneration periods and attributed it to higher feed intake of the male broodstocks of *Clarias gariepinus.* The significant difference (P< 0.05) observed in the semen volume, number of spermatozoa and concentration of sperm between the treatments suggest that enhanced feeding rates and with it, enhanced protein intake had a positive impact on semen production. This observation is supported by Cek and Yilmaz (2009) who reported that fish fed on 12.73MJDE/Kg diet energy showed the best testis development in sharp-tooth catfish. This implies that adequate food supply is of foremost importance for catfish gonadal development and sperm production.

The spermatozoa morphology in the two treatments is undifferentiated although the sperm vigour in Treatment 1 (T^1) appeared slimmer than in Treatment 2 (T^2). Also, chances of spermatozoans morphological deformity were envisaged for low level of feed intake in (T^1) at the beginning of the study but was not, rather parameters such as sperm count, semen volume and concentration were affected at the end of the study (Fig.1a and 1b) which conform with the observation of Hajirezace *et al.,* (2010a) that the supply of balanced nutritional diets to the fish in adequate quantity determines the quality and quantity of fish seminal fluid in-terms of its organic and inorganic components which support the viability of spermatozoa.

In conclusion, results from this experiment confirmed that feed level has an influence on the quality of semen and that the low semen quantity and concentration observed for fish fed 2% body weight is not adequate for male *Clarias gariepinus* brooders. However, the effect of higher feed levels above 4% body weight on semen quality should be investigated.

Corresponding Author:
Dominic Olabode **Odedeyi**
Department of Environmental Biology and Fisheries,
Adekunle Ajasin University, P.M.B. 01, Akungba-Akoko, Ondo State, Nigeria.
E-mail: bodeyi@yahoo.com

References

1. Aas G.H, Refstie T, Gjerde B.1991. Evaluation of milt quality of Atlantic salmon. *Aquaculture 95:125-132*
2. Billard R., cosson J., Perchec G. and Linhart O. 1995. Biology of sperm and artificial reproduction in carp. *Aquaculture 12a: 95-112*
3. Bromage N.R., Roberts R.J. (1995b). *Broodstock management and larval Quality*. Oxford: Blackwell science Ltd. Pp424
4. Cek.S. and yilmaz, E. 2009. The effect of varying dietary energy on gonad development at first sexual maturity of the sharp tooth catfish (Clarias gariepinus Burchell, 1822). *Aquaculture international 17:553-563*
5. Chereguini, O., Garcia dela Banda, I., Fernandez, A. 1999. Artificial fertilization in turbot, *Scophthalmus maximum*: different methods and determination of the optimal sperm-egg ratio. *Aquatic Resources 30:319-324*
6. Diyaware M.Y., A.B. Haruna and K.A Abubarka 2010. Determination of testes Regeneration period for African catfish (*Clarias anguillaris*) after milt (Semen) collection through Ablation. *Current Research Journal of Biological sciences 2 (6):375-379*
7. Ekelemu, J.K. 2010. Differential growth patterns of *Clarias gariepinus,Heterobranchus bidorsalis* and *Hybrid Heteroclarias* fed commercially prepared diets. *Agriculture and biology journal of North America 1(4):658-661*
8. Haji rezaee S, Mojazi Amiri B, Mirvaghefi A.R. 2010a. Fish milt quality and major factors influencing the milt quality parameters. *A review of African Journal of Biotechnology 9(54):9148-9154*
9. Haji rezaee S., H.Jafaryan, M. Asghari, A. Golpour and B.M amiri. 2011. Comparative analysis of milt quality in the cultured and wild stocks of endangered Caspian brown trout, *Salmon trutta caspius. African Journal of Biotechnology 10(14):2762-2765*
10. Igorsvi K. E., Mangor-Jansen A., Holmofjoid I. 1990. Egg quality in fishes. In Blaxter JHS southward A.J.eds. *Advance marine Biology 26:71-113*
11. Kowalski R.K., Hliwa P., Andronowska A., Krol J., Dietrich G.J, Wojtczak M., Stabinski R., Ciereszko A. 2006. Semen biology and stimulation of milt production in the European smelt *(Osmerus eperlanusL) Aquaculture 261:760-770*
12. Krol J, Glogowski J, Demska-Zaka K, Hliwa P. 2006. Quality of semen and histological analysis of testes in European perch *perca luviatilis* during

a spawning period. *Czechslovakia journal of Animal science 51:220-226*

13. Nweke, S.I. and Ugwumba, A.A. 2005. Effect of Replacement of Fishmeal with Duckweed (Lemna paucicostata) meal on the growth of *Clarias gariepinus* (Burchell, 1882) fingerlings. In proceedings of the 20th Annual conference of the Fisheries Society of Nigeria (FISON) Port Harcourt, 14th-18th November, 2005 pp 163-167.
14. Poupard G.P., Paxion C, Cosson J, Jeuclin C, Fieruille F. and Billard R. 1998. Invitation of carp spermatozoa motility and early ATP reduction after milt contamination by urine. *Aquaculture 160:317-328*
15. Rurangwa E., Kime D.E., F. Ollevier and Nash J.P. 2004. The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture 234:1-28*
16. Suquet M, Billard R, Cosson J, Dovange G, Chauvaud L, Mugnier C. Fauvel c. 1994. Sperm features in turbot (*Scophthalmus maximum*): a comparison with other freshwater and marine fish species. *Aquatic living Resources 7:283-294.*
17. Tvedt, H.B., Benfey, T.J., Martin-Robichaud, D.J., Power, J., 2001.The relationship between sperm density, spermatocrit, sperm motility and fertilization success in Atlantic habitat, *Hippoglossus hippoglossus. Aqualculture 194, 191-200.*
18. Vuthiphandchai V. and zohar. 1999. Age-related sperm quality of captive striped bass, Morone saxatilis. *Journal on world Aquaculture society 30:65-72.*
19. Tahib Zulkiph, 1993. Fish Nutrition in Malaysia-Status and problems. In proceedings of the Aquaculture Workshop for SEAFDEC/AQD Training Alumni,8-11 September, 1992. Iloilo, Philppines.

1/6/2014