

## POTENTIATION EFFECT OF VITAMIN E ON REDUCED DOSAGE OF OVAPRIM IN INDUCED SPAWNING OF *Clarias gariepinus*

Uka, A. and I. C. Obijiaku

Department of Fisheries and Aquatic Resources Management, College of Natural Resources and Environmental Management. Michael Okpara University of Agriculture Umudike, P. M. B. 7267 Umuahia, Abia State, Nigeria.

### ABSTRACT

A study was conducted to compare the fecundity, fertilization, hatchability and larval survival of *Clarias gariepinus* induced to spawn using ovaprim diluted by 20%, 30%, 40%, 50% and 0% with vitamin E. This was concomitant to administration of 0.4ml/kg, 0.35ml/kg, 0.30ml/kg, 0.25ml/kg and 0.5ml/kg ovaprim respectively to five groups of fish in three replicates. Water quality temperature, pH and dissolved oxygen (DO) were measured using digital metres during the study. There was significant difference ( $P < 0.05$ ) in egg production, fertilization, hatchability and larval survival among the treatments. Relative fecundity of 29.2g/kg, 36.8g/kg, 41.2g/kg, 43.4g/kg and 32.9g/kg was realized at 20%, 30%, 40%, 50% and 0% dilution respectively. Highest fertilization rate of 89.3% and best hatchability rate of 73.4% were recorded in fish induced at 50% dilution. Percentage survival of 75.4% and 73.4% recorded at 40% and 50% dilution respectively were significantly ( $P < 0.05$ ) higher than the rest including the control which recorded the lowest larval survival rate of 58.9%. There was no significant difference ( $P > 0.05$ ) in the water quality parameters among the experimental units. It was evident from the study that, adding vitamin E to generic ovaprim enhanced hormone potency of ovaprim and its efficiency in low dosage.

**Key words:** African catfish, alpha-tocopherol, gonadotropin, hypophysation, oocyte and larval quality.

### INTRODUCTION

The current demand for fish in Nigeria stands at about 3 million metric tons per annum, with production of only about 0.8 million metric tons, leaving a deficit of about 2.2 million metric tons that are yet to be bridged (FAO, 2008). This demand-supply gap can be closed by production from aquaculture, which has become one of the fastest growing food production sector (Bartley, 2006). Success of aquaculture is premised on high quality eggs for good quality fish seeds (Mylonas *et al.*, 2016). Dearth of quality fish seed has been a major problem in aquaculture. Availability of quality fish seed is therefore a pre-requisite to large scale production of fish (Olaniyi and Akinbola, 2013). Viveen *et al.* (1985) reported that artificial propagation of fish is the most promising and reliable way of ensuring all year round availability of good quality fish seed for sustainable aquaculture. It involves the use of natural or synthetic hormones to induce ovulation and spawning of fish. Ovaprim is one of the most widely accepted and readily available synthetic hormones. It is a combination of SGNRH $\alpha$  and domperidone (Sahoo *et al.*, 2008). It has been found to be very effective (Olubiyi *et al.*, 2005, Nwokoye *et al.*, 2007). However, it is very expensive. Olaniyi and Akinbola (2013) reported that one of the major constraints of fish breeders is the cost of procurement of this hormone.

Madu (2006) reported that about 50 – 60 % of the recurrent expenditure in catfish fingerling production is attributed to the cost of hormone procurement. In an attempt to reduce the cost of hormonal input in fingerling production, many researchers have used non-piscine pituitary hormones such as Bull frog (*Rana adspersa*)

(Salami *et al.*, 1993) and the Toad (*Bufo regularis*) (Fagbenro *et al.*, 1993). Some researchers have also tried diluting ovaprim with saline solution all in attempt to reduce cost (Olumiji and Mustapha, 2012; Ngueku, 2015). Techniques that may help reduce the cost of fish seed propagation without compromising quality and survival of the seeds is necessary. It is in view of this that this study was designed to investigate the outcome of dilution of ovaprim with vitamin E with the hope of enhancing hormone potency of ovaprim at reduced dosage with Vitamin E. Vitamin E is one of the primary non-enzymatic antioxidants in fish egg. Presence of Vitamin E in fish egg has been suggested to permit larger initial egg size which in turns has been correlated with larger larval size and better early survival (Lavens *et al.*, 1999). Vitamin E is by far the most important physiologically bioactive antioxidant in most vertebrates (Packer, 1991). It plays an important role in protecting eggs during early development. Vitamin E is by far cheaper than ovaprim. Hence, the successful incorporation of vitamin E as a spawning agent may not only reduce the cost of fish seed propagation, but may also improve the quality of the seeds.

### MATERIALS AND METHOD

Eighteen healthy *C. gariepinus* of one year old comprising 15 females and 3 males were used for the study. *C. gariepinus* female was considered ripe for spawning when the abdomen was well distended and eggs oozed out freely when gently pressed antero-posteriorly. The male was considered ripe if the top of the genital papilla was reddish in colour (Olubiyi *et al.*, 2005). Five experimental treatments of serially diluted ovaprim with vitamin

E at 0%, 20%, 30%, 40% and 50%, corresponding to administration of 0.5ml/kg, 0.1ml/kg, 0.15ml/kg, 0.2ml/kg and 0.25ml/kg ovaprim respectively was conducted in three replicates in completely randomized designed experiment. The study was to determine level of success that can be achieved with reduced dosage of ovaprim made up with vitamin E in search of cost effective approach in hatchery propagation of *Clarias gariepinus*. Eleven hours after hormone administration, ovulated eggs were stripped into dry bowls from injected females.

Eggs generated per replicate was weighed and respective relative fecundity was estimated using the formula

$$: \frac{\text{weight of egg}}{\text{Weight of fish}} \times 100..$$

Milt collected from the three males were pooled together and used to fertilize all the eggs produced in all the treatments. Two grammes of egg from each replicate was incubated separately in 5 litre capacity container and observed to hatch for easy estimation of relevant parameters. Bults of the fertilized eggs were incubated to hatch outside the concern of this study in 15-liter plastic troughs containing about 10 liters of clean water.

## RESULTS

**Table 1: Egg production and relative fecundity of *C. gariepinus* induced to spawn with vitamin E diluted and undiluted ovaprim**

| Ovaprim (ml/kg)       | % dilution of ovaprim with vit. E | Vol, of E(ml) added to ovaprim | Weight. of Broodstock (g) | Mean weight of eggs (g) | Relative fecundity      |
|-----------------------|-----------------------------------|--------------------------------|---------------------------|-------------------------|-------------------------|
| 0.40                  | 20                                | 0.10                           | 400                       | 85.2                    | 29.2 <sup>c</sup>       |
| 0.35                  | 30                                | 0.15                           | 300                       | 119.0                   | 36.8 <sup>b</sup>       |
| 0.30                  | 40                                | 0.20                           | 300                       | 106.4                   | 41.2 <sup>a</sup>       |
| 0.25                  | 50                                | 0.25                           | 200                       | 113.4                   | 43.4 <sup>a</sup>       |
| <b>0.50 (control)</b> | <b>0 (control)</b>                | <b>0.00 (control)</b>          | <b>200</b>                | <b>65.7</b>             | <b>32.9<sup>b</sup></b> |

Values of Relative fecundity among treatments not followed with the same superscript letter are significantly different (P<0.05).

**Table 2: Effect of Vitamin E on reduced dosage of Ovaprim in artificial propagation of *C. gariepinus***

| Ovaprim (ml/kg) | %dilution with vit. E | Vol, of Vit E(ml) added | Weight. Of fish (g) | Mean weight of eggs (g) | Number of Fertilized eggs | Unfertilized eggs | Fertilization (%) | Hatchability (%)  | Survival (%)      |
|-----------------|-----------------------|-------------------------|---------------------|-------------------------|---------------------------|-------------------|-------------------|-------------------|-------------------|
| 0.40            | 20                    | 0.10                    | 400                 | 85.2                    | 135.0                     | 34.3              | 76.2 <sup>b</sup> | 59.8 <sup>d</sup> | 59.3 <sup>c</sup> |
| 0.35            | 30                    | 0.15                    | 300                 | 119.0                   | 133.0                     | 44.3              | 72.4 <sup>c</sup> | 64.1 <sup>c</sup> | 60.1 <sup>b</sup> |
| 0.30            | 40                    | 0.20                    | 300                 | 106.4                   | 198.7                     | 52.3              | 82.1 <sup>b</sup> | 70.3 <sup>a</sup> | 75.8 <sup>a</sup> |
| 0.25            | 50                    | 0.25                    | 200                 | 113.4                   | 254.0                     | 61.7              | 89.3 <sup>a</sup> | 73.4 <sup>a</sup> | 73.4 <sup>a</sup> |
| 0.50            | 0                     | 0.00                    | 200                 | 65.7                    | 98.3                      | 16.0              | 67.2 <sup>d</sup> | 67.8 <sup>b</sup> | 58.9 <sup>c</sup> |

Values with different superscripts letters within the same column are significantly different at (P<0.05)

Egg fertilization and hatchability were estimated from the experimental units using the formulae:

$$\text{Egg fertilization} = \frac{\text{Number of eggs fertilized}}{\text{Total eggs incubated}} \times 100.$$

$$\text{Egg hatchability} = \frac{\text{Total number of eggs hatched}}{\text{Total number of eggs fertilized}} \times 100.$$

Larval survival by day 4 post-hatch (4dph) was estimated from the formula:

$$\frac{\text{Number of larvae alive}}{\text{Number of hatched eggs}} \times 100$$

(Adebayo and Popoola (2008).

## Data Analysis:

Data generated from the study were analyzed using one way analysis of variance (ANOVA) to compare the relative fecundity, fertilization, hatchability and survival rates of the fish receiving different hormonal treatments. Significant differences were further tested using Duncan's multiple range test at 5% level of probability. The analysis was facilitated with the use of Statistical Package for Social Science (SPSS version 17.0).

Egg spawning and hatching were observed in *C. gariepinus* treated with undiluted ovaprim at inducing dosage of 0.5ml/kg and with ovaprim diluted by 20%, 30%, 40% and 50% with vitamin E resulting to administration of 0.4ml/kg, 0.35ml/kg, 0.30ml/kg and 0.25ml/kg ovaprim (Table 1). The weight of spawners used in the experiment ranged from 0.2kg – 0.4kg. All the spawners irrespective of their weight responded well to the hormone injections (Table 1). There was significant difference ( $P<0.05$ ) in egg production among the treatments. Relative fecundity of 29.2g/kg, 36.8g/kg, 41.2g/kg, 43.4g/kg and 32.9g/kg was realized at 20%, 30%, 40%, 50% and 0% dilution respectively (Table 1). Dilution of ovaprim by 50% with vitamin E gave the best result in terms of egg production. Highest fertilization rate of 89.3% and best hatchability rate (73.4%) were recorded in fish induced at 50% dilution. Percentage survival of *C. gariepinus* injected with diluted ovaprim at 40% (75.4%) and at 50% (73.4%) were observed to be significantly higher ( $P<0.05$ ) than the rest of the treatments including the control which recorded the least survival rate of 58.9% (Table 2).

## DISCUSSION

The performance of ovaprim as a reproductive hormone was not hindered but enhanced in a solution that contained vitamin E. Undiluted ovaprim at 0.5m/kg used as control in this study had been successfully used to achieve completion of oocyte maturation and ovulation in African catfish (Olubiyi *et al.*, 2005). The better performance of ovaprim when administered with vitamin E may be due to the synergistic effect between vitamin E and ovaprim. Lavens *et al.*, (1999) reported that the availability of vitamins E and A as well as pro-vitamin A in fish egg permit larger initial size which in turns has a correlation with larger larval size and better early survival.

Lavens *et al.* (1999) also reported that vitamin E plays an important role in protecting eggs during early development. Watanabe *et al.* (1985) found that increased levels of dietary vitamin E up to 2000 mg/kg in sea bream diets improved percentage of buoyant eggs, hatching rates and percentage of normal larvae. King (1985) reported that the presence of vitamin E in the diet of rainbow trout had a significant effect on the final levels of alpha-tocopherol in eggs than fish deprived of vitamin E. King (1985) opined that during egg development, alpha-tocopherol was slowly, but efficiently transferred from the yolk to the developing embryo and that mortalities during egg development were inversely related to the alpha-tocopherol content of the eggs. These facts may explain the better performance of fish treated with vitamin E diluted ovaprim than generic undiluted ovaprim. The size of the brood fish used for this study substantiated the report of Viveen *et al.* (1985)

that *C. gariepinus* from 200 g body weight can reproduce.

## CONCLUSION

The responses of *C. gariepinus* to undiluted and diluted ovaprim with vitamin E was found to be good considering the breeding success achieved in this study. From this study 50 % dilution of generic ovaprim gave the best result. This implies that incorporating vitamin E in ovaprim will increase functionality of ovaprim as hormonal agent in hypophysation exercise and larval survival for enhanced hatchery management of *Clarias gariepinus*.

## REFERENCES

- Adebayo, O. T. and Popoola, O. M. (2008). Storage Period; its effect on efficacy of non-piscine (frog) Hormone used in inducing ovulation in African Catfish (*Clarias gariepinus* Burchell, 1822). *International Journal of Zoological Research*, 4: 124 – 128
- Aquaculture in the Tropics*, 9: 25 – 33.
- Bartley, D. M. (2006). Status of world's fishery genetic resources. *Proceeding of the International Workshop on the Role of Biotechnology*, Villa Gualino, Turin, Italy, pp: 7 – 16.
- Fagbenro, O. A., Salami, A. A. and Sydenham, D. H. J. (1993). Induced ovulation and spawning in the catfish, *Clarias isheriensis*, using pituitary extracts from non-piscine Sources. *Journal of Applied Aquaculture*, 4: 15 - 20.
- FAO (2008): In Shinkafi, B, A and Ilesanmi, B. D (2014): Effect of varying doses of ovatide on the Breeding performance of African Catfish (*Clarias gariepinus*, Burchell, 1822) in Sokoto, North-Western Nigeria. *Asian Journal of Animal Science*. 8(2):56 – 64.
- King, I. B. (1985). Influence of vitamin E in reproduction in rainbow trout (*Salmo gairdner*). Ph.D. Thesis, University of Washington, USA
- Lavens, P. E., Lebegue, H., Jaunet, A., Brunnel, P. D. and Sorgeloos, P. (1999). Effect of dietary essential fatty acid and vitamins on egg quality in turbot broodstocks. *Aquaculture International*, 7: 224 – 240.
- Madu, C. T. (2006). The effects of brood stock size on the economy of catfish (*Clarias anguillaris*) fry production using the hormone induced natural breeding technique. *Journal of Aquatic Science*, 21: 19 - 22.
- Mylonas, C. C., Duncan, N. J. and Asturiano, J. F. (2016). Hormonal manipulations for the enhancement of sperm production in

- cultured fish and evaluation of sperm quality. *Aquaculture*, <http://dx.doi.org/10.1016/j.aquaculture.2016.04.021>
- Ngueku, B. B. (2015) The Efficacy of Synthetic and Non-Synthetic Hormones in the Induced Spawning of the African Catfish (*Clarias gariepinus* Burchell, 1822). *International Journal of Fisheries and Aquatic Studies*, 3(1): 34 - 37.
- Nwokoye, C. O., Nwuba, L. A. and Eyo, J. E. (2007) Induced propagation of African Clariid Catfish *Heterbranchus bidorsalis* (Gerffrey Saint Hillaries, 1809) using synthetic and homoplastic hormones. *African Journal of Biotechnology*, 6: 2687-2693.
- Olaniyi, C. O and Akinbola, D. O. (2013). Comparative studies on the hatchability, performance and survival rate of African catfish (*Clarias gariepinus*) larval produced: using ovaprim and catfish pituitary extract hormones. *Journal of Biology, Agriculture and Healthcare*, 3(9): 57 - 62.
- Olubiyi, O. A., Ayinla, O. A. and Adeyemo, A. A. (2005). The effects of various doses of ovaprim on reproductive performance of the African catfish *Clarias gariepinus* (Burchell) and *Heterobranchus longifilis* (Valenciennes). *African Journal of Applied Zoology and Environmental Biology*, 7: 101 - 105.
- Olumuji, O. K. and Mustapha, M. K. (2012). Induced breeding in african mud catfish, *Clarias gariepinus* (Burchell, 1822) using different doses of normal saline diluted ovaprim. *Journal of Aquaculture Research Development*, 3:133 doi:10.4172/2155-9546.1000133.
- Packer, L. (1991). Protective role of vitamin E in biological systems. *American Journal of Clinical Nutrition*, 53: 1050S – 1055S.
- Sahoo, S. K., Giri, S. S. and Chandra, S. (2008). Induced spawning of *Clarias batrachus* (Linn): effect of ovaprim doses and latency periods on the weight of stripped eggs and ovary. *Asian Fisheries Science* 21: 333 - 338.
- Salami, A. A., Balogun, A. M., Fagbemi, S. and Edibite, L. (1993). Utilization of Non-piscine pituitary extract in the breeding of *Clarias gariepinus*. *Proceedings of the 10th Annual Conference of Fisheries Society of Nigeria (FISON), UNAAB, Abeokuta, Nigeria*.
- Viveen, W. J. A. R., Ritcher, C. J. J., Van Oordt, P. G. W. J., Janseen, J. A. L. and Huisman, E. A. (1985). *Practical manual for the culture of the African Catfish Clarias gariepinus Section for Research and Development Co-operation*, The Hague, Netherlands.
- Watanabe, T; Koizumi, T; Suzuki, H; Satoh, S; Takeuchi, T; Yoshid. N.; Kitada. T and Tsukshima, Y (1985): Improvement of quality of Red Sea Bream Eggs by feeding Broodstock on a Diet containing cuttlefish meal or on Raw Krill shortly before spawning. *Bulletin of the Japanese Society of Scientific Fisheries* 51(9):1511 – 1521.