Assessment of bitter leaf (*Vernonia amygdalina*) as fertility enhancer in the giant African Catfish (*Heterobranchus bidorsalis*) broodstock

**Accepted 4th January, 2013**

**ABSTRACT**

Ninety six *Heterobranchus bidorsalis* broodstocks (1.2 kg ± 0.5) were fed four isonitrogenous diets with varying inclusion levels of bitter leaf (*Vernonia amygdalina*). The diets; Diet 1 (control), Diet 2 (50 g/kg), Diet 3 (100 g/kg) and Diet 4 (150 g/kg) were fed to the broodstocks for eighty-four days in outdoor concrete tanks (2 x4 x1 m) in triplicate treatments at a stocking density of 1 fish/m² in order to assess the efficacy of the plant as fertility enhancer. The broodstocks fed Diet 3 (100 g/kg inclusion level of the bitter leaf) had the highest milt volume (0.8 ml), while highest milt motility (83.2%), milt count (147,220), egg size (2.64 mm), percentage fertilization (92%) and hatchability (90%) were obtained in fishes fed Diet 4 which were significantly different (P < 0.05) from the other treatments except Diet 3. The study revealed that dietary inclusion of bitter leaf at 100-150 g/kg of feed improves fertility in *H. bidorsalis* broodstocks which invariably will improve the quantity of its seed production.

**Key words:** *Heterobranchus bidorsalis*, *Vernonia amygdalina*, broodstocks, fertility, medicinal plant.

**INTRODUCTION**

Development of fish seeds 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 price to Nigerians, there is the need for adequate broodstocks management for fish seeds production.

The use of plant extracts as fertility enhancer in animals is now in the increase because of the shifting attention from synthetic drugs to natural plants products. Fertility enhancing properties of some plants in some fishes in Nigeria has been ascertained. Some of these plants include *Garcinia kola* (Dada and Ajilore, 2009), *Kigelia Africana* (Adeparusi et al., 2010) and the use of these plants can easily be adopted by rural farmers to enhance fertility in fishes since the plants are available all year round in the tropics and subtropical region.

*Vernonia amygdalina* commonly called bitter leaf is a perennial shrub of 2-5 m in height that grows throughout tropical Africa. It belongs to the family Asteraceae and it’s a highly appreciated vegetable in west and central Africa where it’s commonly used in traditional medicine. Leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough as a laxative and as fertility inducer (Ijeh et al., 1996) In Zimbabwe the root infusion is used to treat sexually transmitted diseases (Akinpelu, 1999; Ijeh et al., 1996; Igile et al., 1994). Bark infusion are also taken to treat fever and diarrhoea, dried flower against stomach disorders (Moundipa et al., 2005; Huffman et al., 1996). The ash from burnt branches is used to control seed-borne fungi (*Curvulania, Aspergillus, Fusarium and Penicillium spp.*) thus ameliorating seed viability and germination capacity (Kabeh and Jalingo, 2007; Erasto et al., 2006). It has also been used for braving beer as a substitute for hop (Awua, 1989). Evaluation of this plant for its chemical and nutritional composition shows that it has fungitoxic properties, containing large amount of thiamine,
Eighteen months old sexually matured *H. bidorsalis* broodstocks (1.2 kg ± 0.5) which comprises of fifty females and fifty males each were collected from earthen broodstock ponds conditioned for two weeks in concrete holding tanks at the hatchery complex of Federal College of Freshwater Fisheries Technology, New Bussa, Niger State, Nigeria.

**Experimental diets**

Leaves of *V. amygdalina* were collected and sun dried and then pulverised. Four isonitrogenous diets with 40% crude protein based on the feed formulation defined for Clariid cat fishes (Fagbenro and Adebayo, 2005) were formulated from feed ingredients. The control diet (D1) was without *V. amygdalina* and the other diets; D2, D3 and D4 were supplemented with 50, 100 and 150 g of *V. amygdalina*/kg feed respectively as shown in Table 1. All dietary ingredients were weighed and ground into small particle size. In order to obtain a homogenous mass, cassava starch was added as a binder, pelleted and air dried at ambient temperature (27 – 30°C). The pelleted feed were stored in tight polythene bags at -18°C in a refrigerator.

**Experimental set up**

A total of 96 male and female brood stocks were randomly distributed into twelve outdoor concrete tanks (2 x 4 x 1 m) at a stocking density of 1 fish/m². Each treatment was in triplicates, which gives a total of 24 broodstocks per treatment. The water level was maintained at a constant water depth of 0.8 m. The fishes were fed at 3% of their biomass twice daily between 800 - 900 h and 1500 - 1600 h for a period of 84 days. All fishes were removed fortnightly and batch weighed using a Metler weigh balance in order to adjust their feed ration.

**Evaluation of milt quality**

The quality and quantity of milt were assessed at the end of the experiment. Nine male fishes randomly selected from each treatment was sacrificed and their testes removed.

**Milt volume**

A small incision was made in the lobes of the testes, the milt

<table>
<thead>
<tr>
<th>Feed Ingredients</th>
<th>D1 (Control)</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Soybean</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Yellow maize</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vegetable OIL</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Starch</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Vernonia amygdalina</em> (VA) leaves</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

**Proximate Composition (%DM)**

<table>
<thead>
<tr>
<th></th>
<th>D1 (Control)</th>
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</tr>
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<tbody>
<tr>
<td>Moisture</td>
<td>9.64</td>
<td>9.83</td>
<td>10.14</td>
<td>10.45</td>
</tr>
<tr>
<td>Crude protein</td>
<td>40.18</td>
<td>40.23</td>
<td>39.92</td>
<td>40.08</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>10.42</td>
<td>11.12</td>
<td>10.86</td>
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<tr>
<td>Crude fibre</td>
<td>7.06</td>
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</tr>
<tr>
<td>Ash</td>
<td>9.84</td>
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<tr>
<td>NFE</td>
<td>23.22</td>
<td>20.79</td>
<td>22.38</td>
<td>21.19</td>
</tr>
</tbody>
</table>

NFE: Nitrogen free extract; DM: Dry matter.

Despite its huge importance in traditional medicine purpose, there is not been any documented report on the effect of *Vernonia amygdalina* on fertility in fishes. This research was designed to assess the possibility of improving the reproductive performance of *H. bidorsalis* broodstocks using *V. amygdalina* by investigating the effects of varying dietary supplementation of *V. amygdalina* leaf meal on the milt, egg quality and fertility in *H. bidorsalis*.

**MATERIALS AND METHODS**

**Experimental fish**

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**Table 1.** Ingredient and proximate compositions (g/100 g ) of the experimental diets.

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NFE: Nitrogen free extract; DM: Dry matter.
was squeezed out into a Petri dish and this was measured with plastic syringe in millilitres (mL).

**Motility duration**

This was determined by placing 1µl of the milt collected using plastic syringe from each male on a Neubauer haemocytometer. A drop of distilled water was added and covered with a slip. The sperm activity was viewed under Olympus microscopic at 100x magnification to see when all the sperm get stopped (Mims, 1991).

**Percentage motility**

Each sample was estimated using light microscope at 600x magnifications after addition of 15 µl distilled water as an activating solution. During spermatzoa activation immotile sperm cells (ISC) were counted, and when the activation stopped, whole sperm cells (WSC) were counted. The motile sperm cell (MC) was then calculated using the method of Canyurt and Akhan (2008) as:

\[
MC = WSC - ISC
\]

\[
\% MC = \frac{MC}{WSC} \times 100
\]

**Milt count**

The concentration of sperm was determined by counting the number of spermatozoa in a sample diluted with distilled water in a Burker haemocytometer under 400x magnifications (Rainis et al., 2003).

**Egg size estimation**

Six female broodstocks were randomly collected from each treatment and used for egg size estimation and percentage fertilization.

Six eggs were randomly collected from females in egg treatment and viewed under a light microscope fitted with calibrated ocular micrometer to calculate the egg size.

This was calculated as described by Mollah and Tan (1997) as:

\[
\text{Mean egg size (mm)} = \frac{\text{length of long axis} + \text{length of short axis}}{2}
\]

**Artificial fertilization, incubation and hatching**

Three hundred eggs collected by hand stripping of female fishes from each dietary treatment were used for artificial fertilization with milt from the males. The numbers of eggs were estimated using gravimetric method has described by Akinwande et al. (2012). The eggs were then incubated in continuously aerated plastic trough. The translucent eggs containing embryonic eyes at the time of polar cap formation (about 20 minutes after fertilization prior to the 2-cell stage of first cleavage) were considered fertilized and counted to calculate percentage fertilization. Opaque eggs were considered unfertilized. Percentage fertilization was calculated as described by Britz and Hecht (1998) as:

\[
\text{Percentage fertilization (β) } = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100
\]

The mean number of hatchlings in each mating combination was obtained by direct counting of unhatched eggs as well as the number of hatchlings in the incubating troughs. Percentage hatchability was also calculated as described by Britz and Hecht (1998) as:

\[
\text{Percentage hatchability (β) } = \frac{\text{Number of hatchlings}}{\text{Total number of eggs fertilized}} \times 100
\]

**Statistical analysis**

All values were recorded as mean ± standard deviation and subjected to one-way analysis of variance (ANOVA) using SPSS 15 for window software package. Significant means were subjected to a multiple comparison test (Duncan) for post hoc comparison at α = 0.05 level.

**RESULTS**

The results obtained from the fertility indices of *H. bidorsalis* broodstocks fed *Vernonia amygdalina* (VA) leaves at varying inclusion levels is shown in Table 2. The milt volume of the experimental fish ranged from 0.5 ml in Diet 1 to 0.8 ml in Diet 3. The highest milt volume (0.8 ml) was obtained in Diet 3 while the least (0.5 ml) was obtained in Diet 1. Milt volume increases (0.5 l- 0.8 ml) with increasing inclusion levels of *V. amygdalina* meal up to Diet 3 of feed and then there was a reduction in Diet 4 (0.6 ml). There was however no significant difference (P > 0.05) in the milt volume among the treatments.

Milt motility duration varied between 44 and 47s with the highest duration obtained in broodstocks fed Diet 3 (100 g/kg of *V. amygdalina* diet). There was also no significant difference (P>0.05) in the motility duration of milt with increasing inclusion levels of *V. amygdalina*.

Milt motility from an initial level of 57.3% in the control (Diet 1) increased with increasing inclusion levels of the plant leaves to 83.2% in those fed 150 g/kg (Diet 4) and
Adeparusi et al. (1992). Moreover fertilizing capacity still remains the most conclusive way of testing sperm quality (Billard et al., 1995). Spermatozoon motility is the most commonly used criterion to evaluate semen quality (Bozkurt et al., 2006), however spermatozoon motility varies in rigor and duration not only among male but also within an individual male depending on the ripeness, age and time of sampling. In this study, fish fed on 100 g/kg dietary inclusion of *Vernonia amygdalina* had the highest milt volume (0.8 ml) and milt motility duration (47 s) while milt motility (83.2%) was highest in those fed on 150 g/kg of the diet which thus reveals that bitter leaf improves the milt quality in *H. bidorsalis* broodstock. However at the same 150 g/kg inclusion level, the milt volume decreased as shown in Diet 4 on Table 2. Furthermore, there was no significant difference in the milt volume of all the Dietary treatments which suggests that the bitter leaf plant do not improve the milt volume in this fish. The milt count, milt motility, percentage fertilization and hatchability were significantly higher (P<0.05) in fish fed the bitter leaf Diets compared to the control diet. In *Clarias gariepinus* broodstocks, milt volume and sperm density were reported to also increase with increasing inclusion levels of *Vernonia amygdalina* (Billard and Ogundiyile 2010). Similarly, this finding agrees with Oluym et al. (2007) who reported an increased in sperm counts of rats treated with extracts of *Garcinia cambogia*, while Sharma et al. (2008) also observed increase in sperm counts with extract of *Anacystis pyrethrum*. The milt count in this study increases with increased inclusion level of *Vernonia amygdalina*. The highest milt count (147,220/ml) was obtained in fish fed 150 g/kg inclusion level of the diet and was significantly different from the control diet. The higher sperm count obtained in treated groups may be attributed to the presence of androgen in *Vernonia amygdalina* since androgen is most effective as a direct stimulator for spermatogenesis (Ogbeche et al., 2002). This agrees with the findings of Adewumi et al. (2005) on the effect of heated soybean on sperm quality of *Clarias gariepinus* who reported increased in motility and sperm counts with volume of milt. The milt volume however did not increase proportionally to the other milt quality

**DISCUSSION**

The use of medicinal plants as fertility enhancer in aquaculture has being receiving some attention. Dada and Ajilore, (2009) used extracts of *Garcina kola* seed to enhance fertility in *Clarias gariepinus*. Adeparusi et al. (2010) also used *Kigelia Africana* fruit to enhance milt production in *Clarias gariepinus*. Apart from the medicinal use of *Vernonia amygdalina*, there has not been any documented report on the use of this plant as fertility enhancement in *Clariid* catfishes. The result of this study reveals that the leaves of the plant is very useful as fertility enhancer in *H. bidorsalis* broodstocks management since all the fertility variables in this present study increased with increasing level of the bitter could be ascribed to the presence of steroid in the bitter leaf (Eik et al., 1965) which ensures greater availability of oestrogen and androgens to the fish gonads. *Mucuna pruriens* (velvet beans) has also been reported to improve sperm quality of the African catfish (*C. gariepinus*). The length of time and intensity of spermatozoa motility, percentage motile sperm and sperm density are all parameters that have been measured in an attempt to assess sperm quality in human, mammals and fish (Billard and Cosson 1992). Moreover fertilizing capacity still remains the most conclusive way of testing sperm quality (Billard et al., 1995). Spermatozoon motility is the most commonly used criterion to evaluate semen quality (Bozkurt et al., 2006), however spermatozoon motility varies in rigor and duration not only among male but also within an individual male depending on the ripeness, age and time of sampling. In this study, fish fed on 100 g/kg dietary inclusion of *Vernonia amygdalina* had the highest milt volume (0.8 ml) and milt motility duration (47 s) while milt motility (83.2%) was highest in those fed on 150 g/kg of the diet which thus reveals that bitter leaf improves the milt quality in *H. bidorsalis* broodstock. However at the same 150 g/kg inclusion level, the milt volume decreased as shown in Diet 4 on Table 2. Furthermore, there was no significant difference in the milt volume of all the Dietary treatments which suggests that the bitter leaf plant do not improve the milt volume in this fish. The milt count, milt motility, percentage fertilization and hatchability were significantly higher (P<0.05) in fish fed the bitter leaf Diets compared to the control diet. In *Clarias gariepinus* broodstocks, milt volume and sperm density were reported to also increase with increasing inclusion levels of *M. pruriens* (Velvet beans) (Dada and Ogundiyile 2010). Similarly, this finding agrees with Oluym et al. (2007) who reported an increased in sperm counts of rats treated with extracts of *Garcinia cambogia*, while Sharma et al. (2008) also observed increase in sperm counts with extract of *Anacystis pyrethrum*. The milt count in this study increases with increased inclusion level of *Vernonia amygdalina*. The highest milt count (147,220/ml) was obtained in fish fed 150 g/kg inclusion level of the diet and was significantly different from the control diet. The higher sperm count obtained in treated groups may be attributed to the presence of androgen in *Vernonia amygdalina* since androgen is most effective as a direct stimulator for spermatogenesis (Ogbeche et al., 2002). This agrees with the findings of Adewumi et al. (2005) on the effect of heated soybean on sperm quality of *Clarias gariepinus* who reported increased in motility and sperm counts with volume of milt. The milt volume however did not increase proportionally to the other milt quality

### Table 2. Milt and egg quality of *H. bidorsalis* broodstock fed *Vernonia amygdalina* (VA) leaves at varying inclusion levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milt volume (ml)</td>
<td>0.5±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Motility duration (s)</td>
<td>44±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47±1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44±0.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milt motility (%)</td>
<td>57.3±3.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.6±5.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.4±8.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.2±9.66&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milt count (×10⁶spm/ml)</td>
<td>102,112±17426&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116,400±12224&lt;sup&gt;b&lt;/sup&gt;</td>
<td>126,140±7542&lt;sup&gt;c&lt;/sup&gt;</td>
<td>147,220±10016&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Egg size (mm)</td>
<td>2.18±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.26±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.64±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fertilization (%)</td>
<td>80±8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82±8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85±14.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92±10.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hatchability (%)</td>
<td>76±15.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82±9.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>90±4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90±6.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different superscripts in each row are significantly different from each other (P < 0.05).
indicators as observed. There was a drop in the milt volume at 200g/kg of the bitter leaf inclusion (Diet 4). It however differs from results by Adeparusi et al. (2010) on the effect of dietary inclusion of *Kigelia Africana* on the sperm quality of *C. gariepinus*, who observed that the milt volume increases without increasing the sperm count. Percentage fertilization and hatchability were also observed to increase with dietary inclusion levels and there was a strong correlation between milt count and percentage fertilization. This is in line with the report of Rurangwa et al. (2001) who also observed a high correlation between sperm fertility and spermatozoa motility.

**Conclusion**

Viable sperm and egg is an essential component of any successful animal production operation and the success of reproduction process is dependent on a supply of high quality gametes. This study shows that dietary inclusion of *V. amygdalina* can enhance the fertility of *H. bidorsalis* since broodstocks. Bitter leaf plant is very much in abundance in West African countries and in Nigeria in particular. As such the leaves can be incorporated in *H. bidorsalis* broodstock diet as fertility enhancer instead of using synthetic drugs that may not be easily affordable and it may also have residual side effect on the organism. Management of *H. bidorsalis* broodstocks with *V. amygdalina* inclusion in the diet at the rate of 100-150 g/kg of diet will enhance both the quality and quantity of *H. bidorsalis* fish seed production by fish breeders in the tropics.

**ACKNOWLEDGMENTS**

The authors wish to thank the management of Federal College of Freshwater Fisheries Technology, New- Bussa, Niger State, Nigeria for making their facilities available to carry out both the field and laboratory experiments. The efforts of Mr Shaba Alhassan of the College hatchery is also appreciated for feeding of the experiment fishes throughout the duration of the experiment.

**REFERENCES**


