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Effects of Velvet Bean (*Mucuna pruriens*) on Sperm Quality of African Catfish, *Clarias gariepinus* (Burchell, 1822) Broodstock

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ABSTRACT

The ever-growing demand for the seed of *Clarias gariepinus* calls for the supply of fertile milt of high quality that would give high survival and growth rate. Studies have shown that *Mucuna pruriens* can influence fertility in men and animals. In this study, the effect of dietary velvet bean (*M. pruriens*) seed meal on reproductive performance was investigated in catfish *C. gariepinus*. Broodstocks of average weight of 275 ± 25 g were fed diets supplemented with four concentrations (900, 1200, 1800 and 3400 mg kg⁻¹) *M. pruriens* seed powder for eight weeks. Fish fed supplemented diets showed significantly improved reproductive performance over the control (0 mg kg⁻¹ *M. pruriens* seed meal) treatment. The highest motility duration was obtained in fish fed with 3400 mg kg⁻¹ of dietary *M. pruriens* seed meal and lowest in fish fed with 0.00 mg kg⁻¹ of dietary *M. pruriens* seed meal. There was no significant difference in the sperm density of the experimental fish fed with different inclusion levels of dietary *M. pruriens* seed meal. The milt volume increased in fish fed with the different inclusion levels of *M. pruriens* seed meal. The findings suggest that feeding *M. pruriens* seed powder improved the milt quality of *C. gariepinus*.

Key words: Fertility agent, hatchability, motility duration, milt volume, sperm density

INTRODUCTION

The use of medicinal plants as fertility enhancer in aquaculture has now received much attention (Dada and Ebhodaghe, 2011). With the shift away from synthetic drugs, the use of plants for enhancing growth and reproductive performance in animals and fishes is becoming acceptable (Farinu *et al.*, 2006; Adeparusi *et al.*, 2010; Dada and Oviawe, 2011). *Mucuna pruriens* commonly known as velvet beans contain high amounts of protein, carbohydrate and rich source of macro-and micro elements. The plant is used as food and herbal medicine and produces green pods containing the seeds (Siddhuraju *et al.*, 1996). The presence of prurienine and prurienidine compounds in *M. pruriens* seeds has been confirmed (Ahmad *et al.*, 2008). These compounds are potent pro-fertility enhancers which stimulate the secretion of testosterone to ensure greater availability to gonads in men thereby leading to increase in spermatozoa concentration and size of the testes and this may have similar effects in fish (Siddhuraju *et al.*, 1996). In addition to the nutritive value of the seeds, they are used for the management of several free radical-mediated diseases, such as male infertility (Tripathi *et al.*, 2001). The seed is also a good source of the non-protein amino acid L-3, 4- dihydroxyphenylalanine (L-dopa) (Tripathi *et al.*, 2001). *M. pruriens* belongs to the family

Fabaceae and the powder of the seed has been credited with aphrodisiac or fertility in men (Ahmad *et al.*, 2008).

M. pruriens seed had been used as one of the ingredients in fish feed preparation but there is paucity of information on its effect on the fertility in fish and the need to establish the efficacy of its seed as pro-fertility agents in fish prompted this study. This method of enhancing fertility in fish could be easier to be adopted by poor fish farmers since *M. pruriens* seeds are available all year round in the tropics and sub-tropical regions. The African catfish *Clarias gariepinus* fingerlings are widely produced in Nigeria under hatchery management (Dada *et al.*, 2002). The objective of this study was to investigate the effects of dietary concentration of *M. pruriens* seed meal on sperm quality in *C. gariepinus* broodstock.

MATERIALS AND METHODS

Formulation of experimental diets: *M. pruriens* seeds were obtained from the Federal University of Technology wildlife park, Akure Nigeria, the plants were sun-dried so that the outer coat could be removed and the seed sun-dried and milled to a fine powder. Amounts of 0 (control), 900, 1800, 2400 and 3000 mg kg⁻¹ of *M. pruriens* seed powder were taken and mixed with a basal feed (40% crude protein), comprising standard amounts of fish meal, yellow maize, soy bean meal, blood meal, fish oil, vegetable oil, vitamin premix and starch, formulated according to Fagbenro and Adebayo (2005). All dietary ingredients were milled to a 3 mm particle size. The ingredients were thoroughly mixed in a Hobart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogeneous mass and cassava starch was added as a binder. The resultant mash was then pressed without steam through a mixer with a 0.9 mm die. The pellets were dried at ambient temperature (27-30°C) and stored at -20°C in a refrigerator. The diets were analysed for proximate composition, including crude protein, crude lipid, crude fibre, ash and moisture (Table 1).

Table 1: Ingredients in and proximate composition (g) of experimental diets D1 to D5

Items	Dietary treatment				
	D1 (control)	D2	D3	D4	D5
Ingredient					
Fish meal (65% CP)	250	250	250	250	250
Yellow maize	100	100	100	100	100
Soy bean meal (45% CP)	350	350	350	350	350
Blood meal (85% CP)	100	100	100	100	100
Fish oil	90	90	90	90	90
Vegetable oil	60	60	60	60	60
Vitamin premix	30	30	30	30	30
Starch	20	20	20	20	20
<i>M. pruriens</i> (mg kg ⁻¹)	0.00	900	1800	2400	3000
Proximate composition (% DM)					
Moisture	12.31	13.05	14.12	3.73	12.71
Crude protein	39.14	40.68	41.64	41.68	40.39
Crude lipid	14.58	12.33	11.54	13.69	13.21
Crude fibre	5.11	5.15	5.14	5.11	5.13
Ash	10.33	10.79	12.64	11.11	11.27
NFE	18.53	17.53	14.92	14.68	17.29

CP: Crude protein, DM: Dry matter, NFE: Nitrogen free extract

Experimental procedure: One hundred and fifty farm-raised *C. gariepinus* broodstock (mean mass 275 ± 25 g) were acclimated to laboratory conditions for 14 days before being distributed randomly into the 15 outdoor concrete tanks ($2 \times 2 \times 1.25$ m) supplied with 400 L of fresh water (water temperature, 27°C ; pH, 7.3; alkalinity, 50 ppm; dissolved oxygen, $7.6 - 7.9 \text{ mg L}^{-1}$) (10 fish tank $^{-1}$) representing four dietary treatments (900, 1800, 2400 and 3000 mg kg^{-1} of *M. pruriens* seed powder) and a control (0 mg kg^{-1} of *M. pruriens* seed powder) in April 2010. Fish were fed at 3% of their body weight (bw) per day in three equal meals, every five hours between 08:00 and 18:00. All fish were weighed and counted fortnightly and feeding rates adjusted accordingly. The experiment lasted for 56 days.

Evaluation of milt quality: At the end of the feeding trial, 10 male fish, randomly selected per dietary treatment were killed and the testes were removed to determine the following milt quality indices: milt volume, motility duration, percentage motility and spermatozoa concentration.

Milt volume: Small incision was made into the lobes of the testes, the milt squeezed out into a Petri dish. This was measured with plastic syringe in mL.

Motility duration: These were determined placing 1 μL of milt from each male on a Neubauer hemocytometer, 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 got stopped (Mims, 1991).

Percentage motility: Each sample was estimated using light microscope at 400x magnification immediately after addition of 20 μL distilled water as an activating solution. During spermatozoa activation, Immotile Sperm Cell (ISC) was counted and when the activation stopped, Whole Sperm Cells (WSC) were counted (Canyurt and Akhan, 2008). The Motile Sperm Cells (MC) were calculated as:

$$\text{MC} = \text{WSC} - \text{ISC}$$

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

Spermatozoa concentration: Concentration of sperm was determined by counting the number of spermatozoa in sample dilute with distilled water (100x) in a Burkner haemocytometer, under 400x magnification (Rainis *et al.*, 2003).

Fertilization ability: To calculate the percentage fertilization, a female *C. gariepinus* broodstock (weight, 850 g) was induced with 0.43 mL ovaprim (0.02 mg salmon gonadotropin-releasing hormone-sGnRHa+10 mg domperidone-Dom) in the hatchery and left for 12 h before stripping. The female was stripped and 30 fresh eggs were measured into fifteen circular plastic bowl of 2 L capacity and labeled according to the treatments. The eggs were fertilized with the 1 mL milt measured with a plastic syringe from each dietary treatment. Fertilization experiments were performed in triplicate.

The number of fertilized and unfertilized eggs were counted under a microscope (40x magnifications) and calculated as follows:

$$\% \text{ Egg fertilized} = \frac{\text{No. of eggs incubated} - \text{number of opaque eggs}}{\text{Total No. of eggs incubated}} \times 100$$

$$\% \text{ Hatchability} = \frac{\text{No. of eggs hatched}}{\text{Total No. of eggs in the batches}} \times 100$$

$$\% \text{ Survival rate} = \frac{\text{Total No. of hatchlings}}{\text{Total No. of eggs counted}} \times 100$$

Water quality parameters: Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored weekly using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model) as described by APHA (1987).

Statistical analyses: Analysis of Variance (ANOVA) was used at 95% significance level to test for significant differences between the various treatment means obtained for the % egg fertilized, % hatchability, % survival, motility duration, % motility sperm count and milt volume. Tukey's multiple range test was used to determine which pairs of the treatment means differed significantly.

RESULTS

Mean water quality parameters during the experiment were dissolved oxygen $6.35 \pm 0.25 \text{ mg L}^{-1}$, pH 9.70 ± 0.1 and $26.2 \pm 0.2^\circ\text{C}$. These were within the optimal limits for the production of African catfish (Viveen *et al.*, 1986). Data on reproductive performance of male *C. gariepinus* fed on *M. pruriens* seed meal are presented in Table 2. There were no significant different ($p > 0.05$) in the milt volume of the experimental fish. However, the highest milt volume was recorded in diet D5 while the lowest was in diet D1. It was observed that motility increased with volume of milt and the spermatozoa were active for only 12-30 seconds (Table 2). The highest motility duration was obtained in diet D5 followed by diets D4, D3, D2 and D1, respectively. However, there were significant differences in motility duration across the different *M. pruriens* concentrations. There were greater improvements in the sperm density of fish fed on *M. pruriens*

Table 2: Milt quality parameters of *C. gariepinus* fed experimental diets for 56 days

Parameters	Dietary treatment				
	D1 (control)	D2	D3	D4	D5
Fertilization (%)	48.04±0.86 ^a	49.18±8.47 ^a	49.61±3.44 ^a	51.53±2.32 ^a	51.71±2.48 ^a
Hatchability (%)	61.40±2.91 ^a	73.89±2.79 ^b	80.76±3.30 ^c	84.05±0.44 ^c	91.37±0.95 ^d
Survival (%)	86.00±9.29 ^a	90.05±2.62 ^{ab}	94.27±0.53 ^b	97.86±0.33 ^b	98.22±0.28 ^b
Motility duration (sec.)	12.53±3.96 ^a	22.91±0.23 ^b	26.12±2.06 ^{bc}	28.65±1.52 ^c	30.33±0.04 ^c
Motility (%)	59.00±1.00 ^a	70.50±1.50 ^b	75.13±3.13 ^b	79.55±0.75 ^{bc}	85.65±1.85 ^c
Sperm count ($\times 10^3$ spz mL)	180675±6611 ^a	203000±3889 ^a	241825±7945 ^a	248500±8485 ^a	348050±55154 ^a
Milt volume (mL)	0.59±0.16 ^a	0.68±0.21 ^a	0.70±0.11 ^a	0.85±0.21 ^a	0.91±0.16 ^a

Mean in a given column with the same letter were not significantly different at $p < 0.05$

meal than the control fish. The average sperm densities were 348050 ± 55154 , 248500 ± 8485 , 241825 ± 7945 , 203000 ± 3889 and 180675 ± 6611 , respectively for diets D5, D4, D3, D2 and D1 (Table 2).

There were improvements in the percentage fertilization of fish fed on *M. pruriens* seed meal. The percentage fertilization was 51.71 ± 2.48 , 51.53 ± 2.32 , 49.61 ± 3.44 , 49.18 ± 8.47 and 48.04 ± 0.86 for the fish fed diets D5, D4, D3, D2 and D1, respectively (Table 2). Similarly, there were significant differences in the percentage fertilization across the different *M. pruriens* concentrations. The results of percentage hatchability followed the same trends as sperm density and percentage fertilization which was found to be 91.37 ± 0.95 , 84.05 ± 20.44 , 80.76 ± 3.30 , 73.89 ± 2.79 and 61.40 ± 2.91 for fish fed diets D5, D4, D3, D2 and D1. Fish fed on *M. pruriens* seed meal had significantly ($p < 0.05$) higher percentage hatchability than fish fed the control diet (Table 2). There was a significant difference ($p < 0.05$) in percentage survival of hatchlings among the treatments. Fish fed on diets supplemented with *M. pruriens* seed meal had significantly higher ($p < 0.05$) percentage survival of hatchlings.

DISCUSSION

The results of this study showed that *M. pruriens* seed affects the milt quality of *C. gariepinus* such as sperm density, motility duration and milt volume (Table 2). The improvements in reproductive performance obtained in this study could be ascribed to the presence of Levodopa (L-dopa) in the plant. This L-dopa contains natural secretagogues which support the body's ability to stimulate the natural release of growth hormone (Ahmad *et al.*, 2008). The blood carries the dopamine into the brain where it naturally increases growth hormone production from the pituitary gland. The increased dopamine levels also optimize the production of other hormones including testosterone, leading to increased sex drive. Also it could be due to the presence of total alkaloids from *M. pruriens* which comprises of prurienine and prurienidine which are found to stimulate the secretion of testosterone to ensure greater availability to gonad.

The observation that sperm motility increased with volume of milt and the strong relationship between milt volume and percentage egg fertilization and hatching in *C. gariepinus* agrees with the findings of Lamai (1996) but disagree with the findings of Pardo-Carrasco *et al.* (2006) who evaluated the semen of *Brycon amazonicus* under induction with Carp Pituitary Extract (CPE) and reported that volume increased without increasing the sperm counts. The researchers also observed that spermatozoa of *C. gariepinus* were active or motile for only 30 sec. Motility of the spermatozoans is the most commonly used indicator of sperm quality since high motility is a prerequisite for fertilization and correlates strongly with fertilization success (Rurangwa *et al.*, 2004). According to this author, the fertilizing capacity is the most conclusive test of sperm quality.

The significantly higher ($p < 0.05$) sperm density, higher percentage fertilization and hatching observed in the fish fed the *M. pruriens* seed meal agrees with Adeparusi *et al.* (2010) who reported that *C. gariepinus* broodstock fed *Kigelia africana* seed meal had higher sperm density and produced higher hatching rates and larval survivals than the control fish (Adewumi *et al.*, 2005) also reported that soybean increases the sperm quality in *C. gariepinus* broodstock. Other studies in man and rat have shown that *M. pruriens* improved fertility in male, especially sperm characteristics (Salman and Adesokan, 2008).

CONCLUSIONS

In conclusion, dietary *M. pruriens* seed meal, which improves the milt quality of cultured African catfish, *C. gariepinus* is useful and reliable method for propagating seedling production and rearing

strategy. This study established the efficacy of *M. pruriens* seed powder as fertility enhancer in male *C. gariepinus* broodstock and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents. Therefore, future research should focus on the improvement of seedling production technology for different fish by *M. pruriens*, since the main aim of aquaculture is to maximize fish production and this plant has promising pro-fertility property which can be exploited in aquaculture.

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