



EFFECT OF DIETARY *VERNONIA AMYGDALINA* DEL.  
SUPPLEMENTATION ON SOME BIOLOGICAL PARAMETERS  
OF MILT OF MALE AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

O. O. AJALA & A. O. OWOYEMI

Department of Veterinary Surgery and Reproduction, Faculty of Veterinary  
Medicine, University of Ibadan, Nigeria

**Summary**

Ajala O. O. & A. O. Owoyemi, 2016. Effect of dietary *Vernonia amygdalina* Del. supplementation on some biological parameters of milt of male African catfish (*Clarias gariepinus*). *Bulg. J. Vet. Med.*, **19**, No 1, 30–39.

The effect of *Vernonia amygdalina* Del. leaves on the reproductive potential of male *Clarias gariepinus* was investigated using 56 fish (425±75.7 g). Four diets with crude protein level of 45% were formulated with different inclusion levels of *V. amygdalina* leaves. The leaves were added to the basal diet at 0% (control), 5%, 10% and 15% inclusion levels. The 56 male *C. gariepinus* were randomly distributed in duplicate into 4 concrete ponds and 4 plastic tanks at a stocking density of 7 fish per concrete pond and plastic tank. The fish were fed at 3% body weight twice daily for 45 days. The milt was then analysed for some biological parameters. Motility and liveability percentage values (88.33±14.720% and 91.83±6.824% respectively) were the lowest in the control (0%) group but the difference was not significant ( $P>0.05$ ) across the groups. Morphology studies showed that total sperm abnormalities were the lowest in the 10% group (45.67±2.73) and total normal sperm cell number was the highest in the 10% group (393.83±14.15). Total sperm counted in morphology study was the highest in controls (442.50±22.12). The differences in these values were not significant across the groups ( $P>0.05$ ). The results of the study show that studied inclusion levels of *V. amygdalina* did not have significant effects on sperm motility, liveability and morphology; hence it can be concluded that Bitter leaf may be included at the studied levels with no untoward effect in the diet of male *C. gariepinus*.

**Key words:** *Clarias gariepinus*, milt, motility, percentage liveability, morphology, *Vernonia amygdalina* Del.

INTRODUCTION

African catfish, *Clarias gariepinus* is one of the highly priced food fish in Nigeria and most parts of the world. An omnivorous scavenger that eats everything it

finds, the African catfish is particularly amenable to the farming practices of smallholders, who comprise the majority of farmers in developing countries (Musa

*et al.*, 2012). The availability of gametes throughout the year is important to ensure a constant supply of fish (Oteme *et al.*, 1996). This availability is dependent on gonadal development and fecundity, which are subsequently affected by dietary nutrients (Izquierdo *et al.*, 2001). The quality of sperm is highly variable and dependent on feeding regime, feed quality and the rearing temperature of the fish (Adewumi *et al.*, 2005). Other factors include stress, age of broodstocks, breeding season, diseases, hormonal induction and spermiation (Azlina *et al.*, 2011). Androgen synthesis and sperm quality are positively impacted by dietary lipid and fatty acids (Nyina-Wamwiza *et al.*, 2012). In the rainbow trout, ascorbic acid deficiency reduced both sperm concentration and motility, and thus fertility (Ciereszko & Dabrowski, 1995). Insufficient intake of energy and protein has been associated with sub-optimal reproductive performance (Bindari *et al.*, 2013). Sperm quality is determined by sperm motility and fertilisation potential (Adewumi *et al.*, 2005).

The use of plants as fertility enhancers is now receiving attention in aquaculture (Dada *et al.*, 2010). *V. amygdalina* Del., commonly called Bitter leaf, is a perennial shrub belonging to the family Asteraceae (Ijeh & Ejike, 2011). Indigenous edible vegetable leaves such as Bitter leaf are inexpensive sources of proteins, carbohydrates, minerals, vitamins and fibres. It is rich in beta-carotene, a precursor of vitamin A (Aregheore, 2012) and ascorbic acid (Ucheck, 2004). Phytochemicals present in the plant include anthraquinones, tannins, flavonoids, alkaloids, saponins, glycosides and terpenoids (Audu *et al.*, 2012). The vitamins A and C, and the phytochemicals are responsible for its anti-oxidant properties (Hamzah *et al.*, 2013). The A-series saponins have been

shown to be responsible for the bitter taste of *V. amygdalina* (Ijeh & Ejike, 2011). Apart from its dietary value, the leaf is also believed by many Africans to possess therapeutic properties on almost every system of the body. The leaf is anti-parasitic (Ucheck, 2004), hypoglycaemic and hypolipidaemic (Arhoghro *et al.*, 2009), hepatoprotective (Farombi & Owoeye, 2011), nephroprotective (Ebong, 2011), antidiarrhoeic (Hassan *et al.*, 2007), anti-cancer (Hamzah *et al.*, 2013), anti-febrile (Aregheore, 2012) and aids healing (Ucheck, 2004). In humans the effects of the plant on the reproductive system have been observed mainly in women but reports have been mostly conflicting. For example, in South Africa and Ethiopia, it is used traditionally for its anti-fertility effect. (Ayele, 2006) while in orthodox medicine, the plant has been used as fertility inducer in infertile women (Aregheore, 2012). Also, in Ivory Coast, the leaves are used to maintain pregnant women till childbirth (Nalule *et al.*, 2013) while its use in South Eastern Nigeria to encourage milk let-down implies that the extracts may be capable of hastening parturition or causing abortion if used in preterm pregnancy (Ijeh *et al.*, 2011). In animals, reports by researchers also vary widely. Oyeyemi *et al.* (2008) revealed that uncontrolled use of *Vernonia amygdalina* has an adverse effect on the spermiogram and spermatozoa morphology of the intact male Wistar rats. In same vein, the methanolic extract of the plant caused significant decrease in testosterone levels and sperm motility of treated rats, thus having a deleterious effect on the reproductive functions in male albino rats (Oyededeji *et al.*, 2013). The opposite was the case in fish where the leaves of the plant were reported to be very useful as fertility enhancer in male *Heterobranchus bidorsalis*

broodstock management (Francis *et al.*, 2013). The use of a combination of the extracts of *V. amygdalina* and *Ocimum gratissimum* was found to combat adverse effects of diabetes on the testes of Wistar rats (Asuquo *et al.*, 2010). In female mice the crude extract had anti-implantation and abortifacient effects making *V. amygdalina* a potentially anti-fertility agent (Ayele, 2006).

The demand for fish is continuously increasing and can only be confronted with correspondingly increasing fish yield. This increased demand is difficult for the average farmer to meet up with since to ensure continuous fish yield, there must be availability of gametes throughout the year (Oteme *et al.*, 1996) which is consequently hinged on feeding fish with essential nutrients (Izquierdo *et al.*, 2001). The cost of procuring conventional fish feed is high; it therefore becomes imperative to look into ways of improving fish production at affordable cost using locally available vegetables. Having an abundance of some of the nutrients essential for fish reproduction – carbohydrates, lipids, vitamins A and C, *V. amygdalina* Del. has considerable potential for use as a non-conventional feed resource in fish feed formulation and production. This study therefore aims to evaluate the effects of Bitter leaf inclusion in fish diet on the semen quality of male *Clarias gariepinus* with the objective of determining the suitability of Bitter leaf for fish consumption.

## MATERIALS AND METHODS

### *Experimental design*

The research was carried out at a vacant fish farm in Felele, Ibadan, Oyo State between April and May, 2013. Ibadan is located in the Southwestern part of Nigeria, latitude and longitude 7.23N, 3.52E

respectively (Balogun, 2000). Fifty-six mature *Clarias gariepinus* males (weight  $425 \pm 75.7$  g) were procured from a commercial fish farm in Ibadan. *Vernonia amygdalina* Del. (Bitter leaf) leaves were harvested from Orita-Challenge, Ibadan, Oyo State and were identified and authenticated at the Forest Research Institute of Nigeria, Ibadan, Oyo State with Authentication number FHI 109940. The leaves were soaked for 24 h in water and subsequently sundried till they were brittle. The dried leaves were later ground and added to the feed in graded proportions of 0 g/kg diet (control diet), 50 g/kg diet (5% inclusion level), 100 g/kg diet (10% inclusion level) and 150 g/kg diet (15% inclusion level) (Arhoghro *et al.*, 2009). The mixture was thoroughly homogenised and pelletised. Proximate and mineral analyses (Tables 1 and 2) were carried out on the leaves before and after processing to assess the nutrients and anti-nutritional factor, saponin lost to processing. Analyses were done using Association of Official Analytical Chemist, A.O.A.C., (2005) methods.

The fish were grouped into concrete ponds randomly at 7 fish per pond (1 m×1 m×1 m) with each treatment group having a duplicate in plastic containers (1 m×1 m×1.5 m). The experiment was conducted in a semi-intensive monoculture system with water renewal once a week. The four diets: 0%, 5%, 10% and 15% were randomly assigned to the fish. The fish were fed commercial feed containing 45% crude protein and varying inclusion levels of processed Bitter leaf for 45 days.

The experiment was conducted under conditions of 12 h photoperiod and 12 h darkness. Rain water was used. The following water quality parameters were obtained using the methods described by

**Table 1.** Proximate analysis of *V. amygdalina* leaves

Content	Pre-soaked leaves	Post-soaked leaves
Total carbohydrates (%)	37.92	33.60
Lipids (%)	4.31	2.59
Saponins (%)	0.50	0.28
Vitamin A (µg/100g)	3728.50	3241.40
Vitamin C (mg/100g)	18.30	13.81

**Table 2.** Mineral analysis of *V. amygdalina* leaves

Content	Pre-soaked leaves	Post-soaked leaves
Calcium (%)	2.26	1.86
Magnesium (%)	0.19	0.32
Potassium (%)	0.22	1.21
Sodium (%)	0.01	0.02
Manganese (%)	0.004	0.004
Iron (%)	0.06	0.05
Copper (%)	0.001	0.001
Zinc (%)	0.007	0.003
Nitrogen (%)	2.93	3.18

American Public Health Association, APHA (1998); dissolved oxygen 8.7 mg/L, nitrate and ammonia levels – 2.12 mg/L and 0.43 mg/L respectively, pH 7.38. Temperature range was 25 °C – 30 °C.

The fish were allowed to acclimatise for 2 weeks before being introduced into the concrete ponds and plastic containers. The fish were fed pelletised feed at 3% body weight twice daily. After the experimental feeding period of 45 days, the fish were taken to the laboratory for laboratory procedures.

#### *Milt collection and analysis*

Fish were sacrificed by stunning (Adakole, 2012). The ventral surface of the body was incised to remove the testes. Milt was collected from the testes by dissecting the testes with a razor blade and squeezing out. Sperm activation was done at room temperature (Verma *et al.*, 2009). The milt was evaluated for mass activity,

motility, percentage liveability and morphology.

Macroscopic examination of the semen was done to observe mass activity (swirl motion) of the spermatozoa by putting a drop of milt on a warm slide and viewing at ×4 objective lens of the light microscope.

Motility was evaluated by subjectively assessing movement of sperm cells under the microscope (Viveiros *et al.*, 2001; Fauvel *et al.*, 2010). A drop of semen was put on a warm slide plus warm distilled water. A cover slip was put on the slide and the slide was viewed under the light microscope at a magnification of ×40 objective lens. Only sperm cells moving in a head forward unidirectional motion were included in the motility count while sperm cells moving in circles, in backward direction or showing pendulating movements were excluded.

Percentage liveability was evaluated by putting one drop of semen on a warm

slide. A drop of Eosin-Nigrosin stain was added and the slide was viewed under the light microscope at  $\times 40$  objective lens. The live/dead ratio was observed and based upon the principle of eosin penetrating and staining the dead sperm cells whereas viable sperm cells repel the stain (Zemjanis, 1970). Percentage liveability was evaluated by direct counting of sperm cells (Fauvel *et al.*, 2010). Hundred sperm cells were counted from different fields of the slide out of which sperm cells that did not pick the stain (the live ones) were counted and expressed as a percentage.

Morphology was used to determine the presence and incidence of morphologically defective spermatozoa through the use of Wells & Awa stain smear. A drop of semen was placed on a warm slide and with another slide; a smear was made and stained with Wells & Awa stain. The stained smear was air-dried and observed under the light microscope at  $\times 40$  objective lens. The spermatozoa abnormalities were classified into head, midpiece and tail abnormalities (Sekoni *et al.*, 1981) and expressed as percentages derived by dividing the mean value for a given parameter in a particular group by the mean value of the total sperm counted for that group multiplied by 100.

#### Statistical analysis

Data was subjected to one-way analysis of variance to compare variation across the treatment groups. Any test value with P

value less than 0.05 was considered significant (Orlu & Ogbalu, 2011). The data were presented as mean and standard deviation.

## RESULTS

Macroscopic examination revealed rapid (+++++) swirl motion in all the groups. There was no significant difference ( $P > 0.05$ ) among the treatment groups in the mean motility and percentage liveability values of spermatozoa (Table 3). The sperm motility in 0% (control), 5%, 10% and 15% groups was 83.33%, 87.17%, 89.17% and 86.25% respectively. The 10% group had the highest value of all. Mean liveability percentages in the four groups were 91.83% (0%), 93.50% (5%), 96.50% (10%) and 94.50% (15%). The highest value was observed in the 10% group just as seen in motility.

Table 4 shows the effects of treatment levels on the morphology of spermatozoa in the testes. There was no significant difference among the treatment groups ( $P > 0.05$ ) in the tailless head percentages of group 0% (1.36%), group 5% (1.40%), group 10% (1.18%) and group 15% (1.20%). The 10% group had the least value. Mean values for abaxial mid-piece were 2.00% (0%), 1.95% (5%), 1.75% (10%) and 1.83% (15%). The 10% group had the least value but without significant difference among the treatment groups ( $P > 0.05$ ). No significant difference was

**Table 3.** Effects of dietary *V. amygdalina* levels on motility and percent liveability of spermatozoa in the testes of African catfish

Treatments	Motility (%)	Percent liveability (%)
0%	83.33 $\pm$ 14.720	91.83 $\pm$ 6.824
5%	87.17 $\pm$ 8.727	93.50 $\pm$ 6.775
10%	89.17 $\pm$ 7.360	96.50 $\pm$ 1.643
15%	86.25 $\pm$ 11.087	94.50 $\pm$ 3.317

**Table 4.** Effects of dietary *V. amygdalina* levels on morphological parameters of spermatozoa in the testes of African catfish

Abnormality	Treatments			
	0%	5%	10%	15%
<i>Heads</i>				
Tail-less heads	6.00±10 (1.36%)	6.00±0.89 (1.40%)	5.17±0.75 (1.18%)	5.25±0.50 (1.20%)
<i>Middle piece</i>				
Abaxial (%)	8.83±0.75 (2.00%)	8.33±1.03 (1.95%)	7.67±1.21 (1.75%)	8.00±1.83 (1.83%)
Coiled (%)	9.00±0.89 (2.03%)	8.67±0.82 (1.96%)	8.67±0.52 (1.97%)	8.50±1.00 (1.94%)
<i>Tails</i>				
Simple bent (%)	8.33±0.82 (1.88%)	9.50±1.05 (2.22%)	8.00±0.89 (1.82%)	8.75±0.50 (2.00%)
Coiled (%)	8.50±1.38 (1.92%)	8.83±0.98 (2.00%)	8.50±0.84 (1.93%)	8.25±0.96 (1.89%)
Coiled around the head, %	1.33±0.52 (0.30%)	1.17±0.41 (0.27%)	1.33±0.52 (0.30%)	1.25±0.50 (0.29%)
Headless tail (%)	5.00±0.63 (1.13%)	5.83±0.75 (1.36%)	4.33±1.03 (0.99%)	4.25±1.26 (0.97%)
Rudimentary (%)	2.00±0.89 (0.45%)	2.83±0.41 (0.66%)	2.00±0.89 (0.46%)	2.25±0.96 (0.52%)
Total abnormalities (%)	49.00±3.79 (11.07%)	51.17±1.47 (11.96%)	45.67±2.73 (10.39%)	46.50±1.91 (10.64%)
Total normal (%)	393.50±23.87 (88.93%)	376.50±11.69 (88.04%)	393.83±14.15 (89.61%)	390.75±10.21 (89.37%)
Total sperm counted	442.50±22.12	427.67±12.16	439.50±16.20	437.25±9.03

seen among the groups for coiled mid-piece – 2.03%, 1.96%, 1.97% and 1.94% in groups supplemented with 0%, 5%, 10% and 15% *V. amygdalina* Del. leaves. The 15% group recorded the lowest value.

No significant difference in tail abnormalities ( $P>0.05$ ) occurred across the groups. The 5% group had the highest percentage of abnormalities among all the categories of tail abnormalities except for coiled around the head abnormality. Percentages for simple bent tail were 1.88% in control fish and 2.22% (5%), 1.82% (10%) and 2.00% (15%). Respective values obtained for coiled tail were 1.92%, 2.00%, 1.93% and 1.89%. Coiled around

the head tail prevalence was 0.03% (0%), 0.27% (5%), 0.30% (10%) and 0.29% (15%), whereas respective headless tail percentages were 1.13%, 1.36%, 0.99% and 0.97%. Percentages for rudimentary tail were 0.45% in the control group, 0.66% in the 5% group, 0.46% in 10% group and 0.52% in the 15% group.

Percentages of total sperm abnormalities, total normal spermatozoa and total number of sperm cells counted in morphology evaluation were not significantly different ( $P>0.05$ ) across the groups although the 10% group had the least percentage of abnormal sperm cells and highest percentage of normal sperm cells. The

control group had the highest total spermatozoa mean value ( $442.50 \pm 22.12$ ). Abnormal sperm prevalence was 11.07% (group 0%), 11.96% (group 5%), 10.39% (group 10%) and 10.64% in the 15% group.

## DISCUSSION

Sperm quality can be quantified by evaluation of sperm motility and fertilisation rate but the former is a faster approach than the latter (Fauvel *et al.*, 2010). In this study, sperm motility values increased parallelly to dietary content of *V. amygdalina*. This shows that Bitter leaf supports sperm motility and agrees with the report of Francis *et al.*, (2013) that motility increased with inclusion levels of *V. amygdalina* in the diet of the giant African catfish (*Heterobranchus bidorsalis*) broodstock. It also agrees with Dada & Ogunduyile (2011) that motility was the lowest in the control group and increased proportionally to levels of *Mucuna pruriens* fed to African catfish broodstock. This is contrary to the report of Orlu & Ogbalu, (2011) that the exposure of *C. gariepinus* to increasing extract concentrations of *L. alopecuroides* (Vahl) caused a decrease in sperm motility. The increased motility observed in treated groups may be due to the carbohydrate, lipid and vitamin C contents of the leaves. Vitamin C-deficient diet is known to decrease sperm motility (Ciereszko & Dabrowski, 1995). In fish, glucose is the major substance utilised for energy used in motility (Ayoola, 2009). Carbohydrate content of *V. amygdalina* is a rich source of this energy much needed for motility.

Percentage liveability has a direct correlation with sperm motility since only live cells can be motile hence the higher the motility the higher the percent live-

ability and vice-versa. That was the case in this study. The results obtained in this study showed higher percentage liveability values in the treated groups as was observed for sperm motility although the difference was not significant. This suggests that the leaves increase sperm viability opposite to the report of Oyeyemi *et al.* (2011) that male *C. gariepinus* treated with *Aloe vera* gel extract had decreased sperm viability. The higher liveability percentages obtained in groups fed Bitter leaf though not significant may be associated with the supplementary lipid, vitamin A and vitamin C derived from *V. amygdalina* in their diets.

Sperm defect is attributable to the activities of free radicals (Ligha *et al.*, 2013). These radicals cause conditions like lipid peroxidation, protein oxidation, DNA damage and cellular degeneration. The activities of these free radicals can be checked by anti-oxidants such as present in the Bitter leaf (Hamzah *et al.*, 2013). Yan *et al.* (2013) reported that increased dietary fatty acid intake also decreased sperm deformity rate in male Sprague Dawley rats. Increased dietary lipid and anti-oxidant in the feed of groups fed higher levels of *V. amygdalina* (10% and 15%) might have contributed to lower though not significant sperm morphology abnormalities. These groups not surprisingly had also the highest values of spermatozoa with normal morphology.

High milt production is of vital importance in artificial reproduction of fish, as large amount of spermatozoa gets wasted due to external factors such as poor handling of milt and short motility time of spermatozoa (Verma *et al.*, 2009). The high sperm count seen in the control group and the decreased sperm count in groups fed with *V. amygdalina* though not significantly different might be suggestive

that the plant is not androgenic. Lower sperm counts observed in treated groups agrees with the report of Orlu & Ogbalu (2011) that the exposure of *C. gariepinus* to extract concentrations of *L. alopecuroides* (Vahl) caused a decrease in sperm output. It also agrees with the report of Oyeyemi *et al.* (2011) that *C. gariepinus* groups treated with *Aloe vera* gel extract had decreased total sperm count than the control. The findings are contrary to the results of Adeparusi *et al.*, (2010) that male *C. gariepinus* fed *Kigelia Africana* had significantly higher sperm count than the control.

In conclusion, the non-significant effects of the *V. amygdalina* leaves on semen parameters such as motility, liveability percentage and morphology makes the plant relatively safe at tested inclusion levels for use as a feed supplement in the diet of male *C. gariepinus*. However, further studies should be carried out to harness the potentials inherent in the Bitter leaf for reproduction by isolating the aforementioned essential nutrients needed for improved sperm quality from the leaves and conducting feeding trials.

#### ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the staff of the Reproduction Laboratory, Department of Veterinary Surgery and Reproduction, University of Ibadan, Nigeria, as well as Engr. Odunfa and Adeniyi Aderibigbe for their assistance during the conduct of the study.

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Paper received 17.11.2014; accepted for publication 13.03.2015

#### Correspondence:

Dr. O. O. Ajala  
Department of Veterinary Surgery and Reproduction,  
Faculty of Veterinary Medicine,  
University of Ibadan, Nigeria.  
tel: +2348023415281  
e-mail: oluwatoyinajala63@yahoo.com