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Research Article

EFFECT OF MULBERRY LEAF MEAL ON GROWTH AND REPRODUCTIVE PERFORMANCE OF AFRICAN CATFISH

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ABSTRACT
The study was conducted to assess the effect of mulberry leaf on the growth, hatchability, fecundity, survival and sperm quality of African catfish (*Clarias gariepinus*). The mulberry leaf and fishes used were collected from a reputable farm in Aroje and Ibadan respectively. A total of thirty–two fishes were allotted to eight concrete tanks, four fishes in a tank at the ratio of 2 males: 2 females, replicated two times and acclimatized for two weeks. The fishes were randomly assigned to four dietary treatments (T1, T2, T3, T4) the treatments containing 0, 5, 10, 15% inclusion of mulberry leaf meal respectively as feed supplements. The parameters assessed were number of eggs released, number of hatched eggs, number of fries that survived and mortality rate, sperm count, percentage of motile and non-motile sperm and percentage of live and death rate. Data collected were subjected to analysis of variance (ANOVA) and level of significance ($p<0.05$) were determined. Generally, it was observed in this study that hatchability, fecundity, survival, sperm count, percentage of motile and non-motile sperm and percentage of live increased significantly as the inclusion level of mulberry in the diet increases. Fishes fed 15% inclusion of mulberry leaf meal (Treatment 4) performed excellently well and had the highest values (55.00, 86.80%, 97.15%) in terms of hatchability fecundity, survival sperm count, percentage of motile and of live sperm respectively increased significantly ($p<0.05$) as the inclusion level of mulberry inclusion increases in the diets of the fish. It was also observed that the sperm count, percentage of live and motile sperm of the African catfish fed diet 4 were higher compared to fishes fed other diets that contained mulberry leaf meal as well. However, the fish fed 5% of mulberry leaf meal (treatment 2) had the least hatchability fecundity, survival, sperm count, percentage of live sperm (18.95, 61.30%, 69.00%) respectively. Although, moderate necrosis, mild fatty degeneration and connective tissue edema were observed in the sperm histology and the progression of sperm movement was fair, forward and directional with African cat fish fed higher inclusion of mulberry in the diets of African cat fish. Therefore, enhancing better hatching success in induced breeding of *Clarias gariepinus,* high inclusion level of mulberry leaves are advised to be used.

Keyword**:** *Sperm, Mulberry Leaf, Hatchability, Live Motile Sperm, Fecundity*

INTRODUCTION
In Nigeria today, aquaculture is the fastest growing sector and recognized to bridge the gap between fish demand and supply because fish requirement by the consumers cannot be met from capture fisheries alone (Ayinla, 1988). Among the culturable fish in Nigeria, C*larias gariepinus* command a very good commercial value in Nigeria market (Ita, 1989) having a high growth rate, high resistance to handling and stress also considered to hold a great promise for fish farming (Agbebi *et al.*, 2012; Hengsawat *et al.*, 1997). However, the major constrain to its culture is the limited availability of quality fingerlings as fish seed (Sahoo *et al.*, 2007).
The report of united nation development program baseline study has shown that the total annual fingerlings requirement for Nigeria (250,000 million) is quite higher than the domestic production of 7.2 (Nwotoye *et al.*, 2007).
Availability of fish seeds depends on gamete production all year round (Conyurt and Akhan, 2008). This is also a function of gonadal development and fecundity (Izquiredo *et al.*, 2001).
The sperm quality is determined by sperm motility and fertilization potential (Adewumi *et al.*, 2005). It depends on feed quality and other factors such as stress, ages at breeding season, disease, hormonal

inclusion and spermiation (Azima *et al.*, 2001). Although, recent advocacy on organic farming has reduced the use of synthetic drugs as growth and reproductive enhancer in animal and fish.
Therefore, medicinal plant that were considered of no value are now investigated, evaluated and developed into drugs with no side effects (Adedeji *et al*., 2006).
Mulberry (*Morus alba*) belonging to the family moraceeae is mostly cultivated plant. Mulberry leaf is very rich in protein and minerals (Dada *et al*., 2007). The report has shown that incorporation of mulberry leaf in diets of poultry (Narayana and Sett, 1977) and rabbit (Bankole *et al*., 2005) resulted in better egg production and good growth. Mulberry plants are highly medicinal right from the leaf to the root. The fruits is used to treat premature, grey hair, to notify the blood, treat constipation and diabetes. The bark is use for treating cough, odema and to promote urination. Mulberry is used as protein supplements to partially replace fish meal (Mondal *et al.*, 2012). Morus alba leafs restore vascular reactivity in chronicle diabetic patients and rats (Majunda *et al.*, 1969), Edema haemorage and myonectotic activities were also neutralized effectively. This study therefore investigate the effects of mulberry leaf on the hatchability, fecundity, survival of fries, sperm and eggs quality of African cat fish (C*larias gariepinus*).

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the fishery unit of teaching and research farm Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria.

Experimental Fish

A total number of 32 healthy adult size *Clarias gariepinus* of an average weight of 1kg was procured from a reputable farm in Ibadan, Oyo State, Nigeria. The fish were divided into four groups of two replicate and four fishes were allotted to a tank and acclimatized for two weeks during which the fish were fed floating feeds.

Collection of Mulberry Leaf/Formulation of Diets

Mulberry leaves were collected from Aroje town in Ogbomoso, Oyo State and identified. After collection, the leaves were air dried to maintain the nutrients, and grounded into fine powder and analyzed for crude protein, crude fiber, ether extract, ash, moisture using the method of A.O.A.C (2000).

Experimental Diets

Four experimental diets (D_I, D_2 D_3 and D_4) containing varying levels of 0, 5, 10, 15% of *Morus alba* respectively. The experimental diets were formulated to contain 25% of crude protein.
All dietary ingredients such as yellow maize, rice bran, soya bean fish meal, oyster shell, bone meal, honey, premix, vegetable oil, salt and lysine were procured from a reputable farm and weighed on a digital scale, grounded to a small particle, mixed thoroughly to obtain a homogenous mass before they were pelleted and sundry to obtain a constant weight and then packed into air tight nylon.

Experimental Procedure

Water was sourced from LAUTECH Farm and aerated for at least three days prior the use. The diets were assigned randomly to the fish and they were fed 3% body weight per day in two equal portion in the morning and evening (8.00hrs and 17:00hrs) which lasted for eight weeks.

Milt Quality and Histology

Milt production and quality were determined at the end of the experiment, one male of the fish, selected from each treatment, were sacrificed and the testes removed and observed under light microscope.

Milt Count

Concentration of sperm was determined by counting the number of spermatozoa in sample dilutes with distilled water (100x) in a burker heamocytometer, under 400x magnification (Rainis *et al.*, 2003).

Motility Percentage

Each sample was estimated using light microscope at 400x magnification immediately after addition of distilled water as an activating solution. During spermatozoa activation, immotile sperm cell (ISC) was counted, when the activation stopped, whole sperm cell (WSC) was counted (Canyurt *et al.*, 2008). The motile sperm cell (MC) was calculated.

Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA).

Table 1: Gross Composition of Experimental Diets

Ingredient	D_1 control	D_2 5% MB	D_3 10% MB	D_4 15% MB
YM	43.76	40.62	37.49	34.34
RH	14.59	13.54	12.50	11.45
SB	12.03	11.76	11.49	11.22
FM	24.07	23.53	22.97	22.45
MB	-	5.00	10.00	15.00
OYSTER SHELL	0.50	0.50	0.50	0.50
BONE MEAL	1.00	1.00	1.00	1.00
HONEY	2.00	2.00	2.00	2.00
PREMIX	1.00	1.00	1.00	1.00
VEG OIL	0.50	0.50	0.50	0.50
SALT	0.05	0.05	0.05	0.05
LYSINE	0.50	0.50	0.50	0.50

RESULTS AND DISCUSSION

Results

The proximate composition of mulberry leaf meal is shown in Table 2.

Table 2: Proximate Composition of Mulberry Leaf Meal (Test Ingredient)

Parameters	Percentage %
Crude Protein (CP)	15.75
Crude Fibre (CF)	10.48
Ether Extract (EE)	4.05
Ash	17.65
Moisture	9.47

The effect of mulberry on adult *Clarias gariepinus* is shown in Table 3. Increase in inclusion of M*orus alba* resulted to decrease in protein value. Which means *Morus alba* is a good alternative protein supplement in *Clarias gariepinus* diet and the acceptability of the feed is high. Treatment 4 recorded higher number of eggs released (85.50) while treatment 3 recorded the least number of egg released (63.81).

The number of eggs released in Treatment 2 (5% of mulberry) was found to be less than the eggs released in T1 (control). However, mulberry leaf was significantly higher in T3 which contained 10% of mulberry. Egg released was significantly higher than T2 but lesser than T1. T4 had the highest total egg content than T2 – T3 but lesser than T1.

The deposition of protein in the whole body was significantly higher in fish fed T2 (5% MB) but CP decreased in fish fed T3 (10% MB) and T4 (15% MB) treatment as compared with those fed Treatment 1 (0% MB).

Table 3: Effect of Mulberry Leaf on Hatchability, Fecundity and Survival on Brood Stock Catfish (*Clariasgariepinus)*

Parameters	T1	T2	T3	T4	SEM
No of Egg Released	142^{a}	62.81^{d}	79.50^{c}	85.50^{b}	6.23
No of Hatched Eggs	7875.00^{a}	656.00^{d}	4835.50^{c}	5393.00^{b}	541.06
No of Fries Survived	5333.50^{a}	565.50^{d}	3959.50^{c}	4331.50^{b}	23.04
Mortality Rate	2341.50^{a}	73.50^{d}	775.00^{c}	1987.00^{b}	71.64

The effect of *morus alba* on milt motility and histology is shown in Table 4. The percentage sperm motility in *C. gariepinus* fed with *Morus alba* ranged from 61.30 in diet 2 to 87.10 in diet 3. The highest sperm motility obtained is 87.10 in diet 3 and least is 61.30 in diet 2 and milt volume differed significantly among the treatments.

The result of percentage sperm motility showed inter specific differences and mean values ranged from 61.30 to 87.10 the levels of inclusion of *Morus alba* leaf affect sperm motility. There was increased in motile percentage in diet 1, diet 3 and diet 4 and reduction in percentage motility was observed in treatment 2. Higher sperm counts and live sperm percentage were obtained in treatment- T_1, T_3 and T_4, even though the highest values was observed in treatment 4 while the least value were recorded in treatment 2.

It was also observed that, as the percentage of mulberry inclusion in diets increases the progressive movement of the fish sperm shifted from being excellent and good forward directional to fair forward directional. However, no progressive movement was observed in treatment four.

Table 4: Effect of Mulberry Leaf on Sperm Motility of African Catfish

Parameters	T_1	T_2	T_3	T_4	SEM
Sperm Count	56.150^{a}	18.950^{d}	53.000^{c}	55.000^{b}	3.24
Motile Percentage	87.000^{ab}	61.300^{c}	87.100^{a}	86.800^{b}	2.32
Non-Motile Percentage	13.000^{b}	38.700^{a}	12.900^{c}	13.200^{bc}	2.32
Live Percentage	89.650^{c}	69.000^{d}	92.700^{b}	97.150^{a}	2.25
Death Percentage	10.350^{b}	31.000^{a}	7.300^{c}	2.850^{d}	2.25

Progressive movement
A – Excellent forward directional movement
B – Good forward directional movement
C – Fair forward directional movement

Discussion

The present study confirmed that dietary inclusion of *Morus alba* is essential for *Clarias gariepinus* higher inclusion of mulberry in the diets of the fish favoured the hatchability, fecundity and survival rate of African catfish *(Clarias gariepinus).* Also, the sperm motility, including some parameters such as sperm count, motile percentage were positively affected by the dietary inclusion of *Morus alba*. Moreover reproductive capacity is the most conclusive way of testing sperm motility (Billard *et al.,* 1995). It was observed that fish fed 15% dietary inclusion of *Morus alba* T_4 has the highest live sperm percentage (97.15) while lower value (89.65) was obtained in treatment 1 (contol). The result shows a strong relationship between motility and reproduction. An increased motility results into increased reproduction. However, cellular infiltration and connective tissue edema observed in the sperm cell and tissues showed that higher inclusion of mulberry in the diet had negative influence on the male African cat fish because it can lead to a serious problem in the organ.

Conclusion

The results from this study have proofed that African catfish performed better on 15% inclusion of mulberry compared with control diet. Meaning that, the higher the inclusion of mulberry, the higher the hatchability, fecundity and survival rate of African catfish *(Clarias gariepinus).* In the same vein, 15% inclusion of mulberry leaf gave the best results in terms of sperm quality such as the highest sperm count and live percentage. Although, excess fat was deposited in the sperm cells and connective tissues of fish fed D4 which may cause serious damages to the organs such as cellular infiltration and connective tissue edema.

Recommendation

Therefore, 15% inclusion of mulberry in the diet of African Catfish *(Clarias gariepinus)* could be suggested because it supports high fecundity, hatchability, and survival in female fish. However, inclusion of mulberry in the diets of male *Clarias gariepinus* should be either partial or very low because

higher level mulberry in the diets of male African catfish will lead to serious damages of the sperm cells and tissues.

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