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IN VITRO HYBRIDIZATION OF CLARIAS GARIEPINUS X HETEROBRANCHUS LONGIFILIS AND REARING OF LARVAE WITH FORMULATED DIETS FOR SELECTION OF DESIRABLE HYBRID.

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Abstract

A study was conducted on the *in-vitro* cross-breeding and larval rearing of African Catfish, *Clarias gariepinus x Heterobranchus longifilis* with formulated diets to produce hybrid with good survival, desirable growth rate and other metric characters. The larvae fed with live and/or artificial formulated diets for 21 days in an indoor hatchery were closely monitored. The brooders *Heterobranchus longifilis* -Av. wt of female 180 ± 0.50 g; versus the male C. *gariepinus*- Av. wt of 120g ± 0.75 g) were procured from kerma farms, Onna, Akwa Ibom State, Nigeria and stocked in Akwa Ibom state University Aquarium near the experimental site, 2-weeks prior to spawning for acclimatization. The fish were successfully induced spawning using ovaprim hormone at 0.5 –1.0 ml/g body weight to the female brooder. The milt was extracted from the male catfish *C. gariepinus*, and mix with 0.65% saline to inactivate and reduce its metabolic activities. The milt was added to the eggs with gentle shaking for 5 min and distilled water was later added to the mixture to activate fertilization. The results indicated that fertilization occurred and the eggs hatched within 24-30 hours. Fertilization, hatchability and survival percentages were respectively recorded as 87.35%, 92.40% and 80.7%. After yolk-sac absorption, fry of 7 days old were subjected to 3 types of formulated feed trials including *Artemia nauplii* as control for 21 days. The larvae fed on diets II and Ill showed significantly ($P<0.05$) better length and weight gain as compared to control than those of the larvae fed on diet I. The larvae fed on diet Ill showed the best survival rate (83%). However, the condition factor and SGR of the larvae fed on diet 11 was significantly better than those of the larvae fed on other two diets.

Keywords: Hybridization, *Clarias gariepinus x Heterobranchus longifilis*, fertilization.

Introduction

Aquaculture is still the fastest growing food producing sector, compared to other food commodities (FAO, 2009) and is a sector that is likely to benefit from the application of appropriate genetics and reproductive biotechnologies to increase food production. According to CGIAR (2006), aquaculture contribute to the increase in supplies of crustaceans, mollusks and some other aquatic animals, total by weight 3.9% in 1970, 27.1.% in 2000 and 32.4% in 2004. Unfortunately, its growth is impeded due to lack of adequate attention to selective breeding and Larval rearing knowhow which is considered as the most sensitive phase of its productive Iife for improve yield (Saha et. al., 1998).

The African catfish, (family clariidea) is one of the most popular food fish in sub-Sahara countries. Principally, *Clarias spp* are very popular in Nigeria. The hardy nature and tolerance to adverse environmental conditions particularly low oxygen levels in water enable its cultivation for intensive production in most countries (Sitasit, 1968). The high growth rate of the fish, as it can grow to a size of about 10kg made it very attractive to consumers, but raising fish seeds of the right quality and quantity remain a major challenge facing aquaculture industry in the region, particularly in Nigeria (Owodeinde, et. al., 2012). This also constitute a major constraint for wide spread culture of the species. Furthermore, this is manifested in the non availability of fingerlings from both indoor hatcheries and natural resources due to depletion of natural stocks by wild fisheries. However, several attempts have been made in past to induced bred *Clarias spp.* using various hormonal techniques (Mollah and Tan 1983). The commercially available synthetic hormonal preparation Ovaprim® has been found to be most effective inducing agent for the captive breeding of the species (Mollah, 1987). Rearing larvae with high growth rate and survival to fingerlings size is another challenge for the hatchery operators as no standardized protocol of larvae rearing have been established. Therefore, it becomes imperative to undertake this study with the objective of evaluating the potentials for crossing breeding *Clarias gariepinus* ♂ *x Heterobranchus longifilis* ♀ and rearing of the early stages of the hybrid *Heteroclarias* obtained from the crossing on live and artificially formulated diets in an indoor aquarium, as there is a dearth of African catfish fingerlings in the country.

Materials and Methods

Procurement of fish

The adults African Catfish, *Clarias gariepinus and Heterobranchus longifilis* were procured from Kerma farms, Onna, Akwa Ibom State, Nigeria and stocked in Akwa Ibom state University Aquarium near the experimental site. Both males and females were reared in an indoor hatchery for 2-weeks for acclimatization prior to spawning.

Induced breeding and hatching of larvae

After two weeks of acclimatization, the female brooders were identified as having round and bulging abdomen and button-shape genital papilla, and were prepared for undertaking hormonal induction. They were given Ovaprim injections (Manufacturer Syndel, Canada) at the rate of 0.5 to 1.0 ml 100 g^{-1} body weight for 10 hrs, before the eggs from all the females were stripped out following a standard method of dry stripping of eggs. The male abdominal part was surgically opened using a surgical blade and the testes removed and dissected to release the milt and immediately diluted with 0.65% saline (NaCl solution) to inactivate and reduce its metabolic activities. After the preparation of milt suspension in a clean tray, it was mixed with the stripped eggs with gentle shaking for 5 min and later diluted with distilled water to activate fertilization.

The fertilized eggs were incubated in rectangular sieve trays kept in a tank having provision of flow-through water system of (0.1-0.5 l/min). The eggs hatched out within 24-30 hours and yolk sac was absorbed in 7 days. The larvae were then stocked in the plastic cages (capacity 200 L) and given live *Artemia* as feed for subsequent feeding trials experiment. The experiment was carried out in three replicates.

Larval rearing /feeding trials

Two hundred and seventy *240* larvae of age 7 day old were collected from the plastic cages and used for feeding trials of larvae. The larvae were split into 4 treatment groups. Each treatment group contained 60 larvae, replicated three times. Larvae fish were reared for 21 days, all in plastic cages (capacity 200 L) arranged in completely randomized triplicate design. They were fed to satiation thrice a day. The initial length and weight of larvae was measured with a measuring scale and analytical

balance respectively, prior to stocking. The 7 day old larvae were splits into treatment group I, II and Ill and fed with laboratory formulated diets I, II and Ill respectively for 21 days and *Artemia nauplii* were used as standard (control). The feeding experiment continued thrice daily to satiation till the end of the experiment. The indoor rearing cages were cleansed every other day and about one half of the water was replaced with fresh water every day to reduce the nitrogenous waste accumulated. At the end of the 21 days of the trial, 10 surviving larvae from each replicate were collected and analyzed. The initial and final weight and length parameters were recorded. The percentage survival and Specific Growth Rate *SGR* were calculated according to Srivastava et al., (2012) as:

i. Survival (%) = (Number of larvae stocked − Number of dead larvae) / Number of larvae stocked × 100;

ii. Specific growth rate *SGR* = (*ln* final weight − *ln* initial weight) / Days of experiment × 100.

iii. Condition factor (K): condition factor (K) was calculated according to Ayo-Olalusi, (2014) with modification as shown below:

$$K = W \times 100 / L^b$$

Where, W=weight of fish (mg), L=Length of fish (mm) and b is exponent of the length-weight relationship.

Preparation of Laboratory formulated larval feed

The formulated diet was prepared by mixing ingredients listed in Table 3. The ingredients were cooked in a pressure cooker and after cooling they were hand-grated to fine particles of size 150-200 μ and stored in a plastic bottle with cover until used.

Physico-chemical parameters of water

The water quality indices of all the experimental pools for temperature, pH, DO and total alkalinity were monitored on every alternate day during the feeding trial following the standard methods (APHA, 2000)

Statistical analysis

Statistical analysis of the data for all experiments were done by using one-way ANOVA (Analysis of Variance) and DNMRT (Duncan's New Multiple Range Test) to determine differences between the means taking at 1 and 5 percent significance levels using SPSS version 14.0.

Results

Breeding performance

Table 1 showed that the hormonal induction with (0.5 ml/ 100 g^{-1}) dose of Ovaprim to the female fish were effective for the maturation of ova to complete ripeness. Freshly fertilized eggs were hazy brown in colour. The number of fertilized eggs was found significantly higher ($p > 0.05$). On the other hand, the percentage fertilization and survival of larvae after yolk-sac absorption were insignificantly ($p < 0.05$) high. The percentage fertilization was found to be 87.35 ±1.3, hatching percentage 92.40 and percentage survival of fry after yolk-sac absorption was found 80 ± 7. The quality of hatchery water was found suitable with respect to temperature, pH, total alkalinity and dissolved oxygen levels which were recorded in the range of 25 ± 2°C, 6.8 – 7.4, 126 – 130 mg L^{-1} and 6.4 – 7.9 mg L^{-1}, respectively during the entire rearing period.

Survival and growth study for 21 days

The survival and growth performance of the hybrid *Heteroclarias* larvae in response to different formulated diets and standard/control diet have been shown in Table 3. The trend of growth performance as indicated by total length gain and weight gain at the time of rearing/feeding trial was interestingly high for groups fed diets 11, 111 which shows no significant different with the standard/Control feed group except for group fed diet 1. The final weight samplings indicated that there were no significant ($P < 0.05$) differences between larvae groups fed with diet 11 and 111 which were higher at 48.54mg and 49.32mg respectively with the group fed with standard/control diet (50.20mg).

Superior length increment was found in group fed with diet 111 having 5.26 mm compared to standard fed group with 5. 76 mm. Larvae maintained significant variations in weight between the groups fed diet 1 and the other two groups including the standard/ control as shown in table 3. SGR also showed interesting performance in the three diet groups with highest in group fed diet 111, followed by diet 11 and then diet 1 as indicated in table 3. The rate of percentage survival was found to be high in larvae group fed with diet 111, followed by group with diet 1 and group fed diet 11 as compared to larvae group fed with standard feed (see table 3).

Physico-chemical parameters of larval rearing tank water

The physico-chemical parameters of larval rearing tank water for temperature, pH, total alkalinity and dissolved oxygen were found in the range of 25 ± 2°C, 6.8 – 7.4, 126 – 130 mg L^{-1} and 6.4 - 7.9 mgL^{-1}, respectively during the entire rearing period.

Table 1: Hybridization performance of African Catfish, *Clarias gariepinus x Heterobranchus longifilis* induced bred with Ovaprim hormone.

Male Avg. Weight (g)	Female Avg. Weight (g)	Latency period (hr)	Total number of Eggs stripped out	Fertilized eggs	Fertiliza-tion (%)	Number of Hatchlings	Hatching(%)	Survival %
120 ± 0.75	180 ± 0.5	10 ± 1	1265 ±1.6	1105±2.2	87.35 ±1	1021 ± 2.1	92.40	80 ± 7

The data are based on the means (± SE) of pools in each group.

Table 2. Formulation and proximate composition of the test diets (% dry matter basis)

	DIETS			
INGREDIENTS	DIET 1	DIET 11	DIET 111	*STANDARD/CONTROL
Fish meal	15.00	27.0	20.0	
Baker's yeast	-	20	20	
Powdered milk	20.	-	-	
Boiled chicken egg yolk	15	20	-	
Whole egg (albumin + yolk)	-	-	35	
Wheat flour	10	15	12	
Cod liver oil	2	1	1	
Vitamin premix	3	2	1.5	
Mineral premix	2	1	2	

Vitamin and Mineral Composition (Per 100 g): Manufacturer: Sunder Chemical. Ltd., Chennai, India. Vitamin A, 70000 IU; D_3, 7000 IU; E, 25mg; Nicotinamide, 100 mg; Cobalt, 15 mg; Copper, 120 mg; Iodine, 32.5 mg; Iron, 150 mg; Magnesium, 600 mg; Manganese, 150 mg; Potassium, 10 mg;

Selenium, 1 mg; Sodium, 0.59 mg; Sulphur, 0.72%; Zinc, 960 mg; Calcium, 25.50%, Phosphorus 12.75%) , from Glaxo SmithKline Pharmaceuticals Ltd

*Standard/Control Feed: Live feed - *Artemia nauplii* was used.

Table 3. Various growth parameters and survival rate of hybrid larvae fed with three different artificial diets for 21 days experimental period.

Treatments/ parameters	Diet 1	Diet 11	Diet 111	Control
Initial length (mm	5.47c ± 0.12	5.47c ± 0.12	5.47c ± 0.12	5.47c ± 0.12
Initial weight (mg	3.16b ± 0.1	3.16b ± 0.1	3.16b ± 0.1	3.16b ± 0.1
Final length (mm)	8.73b	10.55 a	10.73 a	11. 23 a
Final weight (mg)	40.76 b	48.54 a	49.32a	50.20a
Length gain (mm)	3.26	5.08	5.26	5.76
Weight gain (mg)	37.60	45.38	46.16	47.04
Specific growth rate (SGR)	179.05	216.10	219.81	224.00
Percentage Survival (%)	80	75	83.33	76.66
Condition factor	1.56	1.53	1.53	1,49

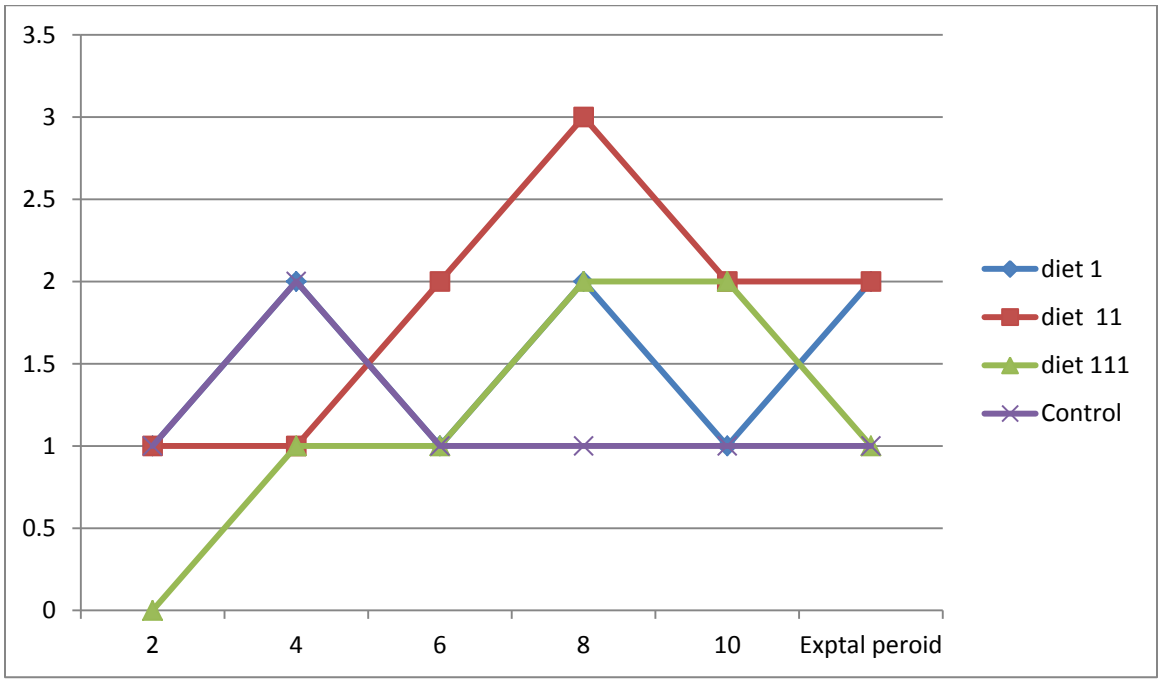

Fig. 1. Mortality of larvae during 21 days feeding trial of hybrid larvae fed on different formulated diets.

Discussion

The feasibility and suitability of laboratory formulated feed for rearing of hybrid larvae was investigated in the present study. Three diets were tested during the period of 21 days feeding trial. At the end of the feeding trial, it was observed that the larvae fed with diet 111 showed better growth and survival rate followed by the larvae fed with diet II. The larvae fed diet I11 showed an average weight gain of 46.16 mg after 21 days feeding trial, followed by larvae fed diet I1 with weight gain of 45.38mg. These figures were comparable to 47.04mg obtained from larvae fed standard /control diets.

The specific growth rates *SGR* of the larvae fed diets II and Ill which showed 216.10 and 219. 81 respectively were also comparable to the larvae fed standard /control diets with 224.00. Higher survival rate which portray the feed acceptance and assimilation by larvae was obtained from larvae fed diet II1 followed by diet 1. The larvae fed diet Ill and diet 1 showed survival rate of 83.33% and 80.00 % respectively. This was in agreement with the findings of Dabrowski *et. al,*. (1984), who successfully uses dry and live feed to reared larvae.

Ovaprim has been optimally employed for inducing spawning of fish used in a number of commercially important food as well as ornamental and threatened species (Lakra et.al., 1996; Pandey et. al., 1999). The hormone has been reported to be an efficient inducing agent for oocyte maturation and ovulation in *Clarias spp.* In the present study, the latency period of 10-15 hours after the injection of Ovaprim, dose 0.5 ml/100 g^{-1} body weight to female fish was shown to be suitable for the maturation and ovulation of this species. Similar findings were also reported by Sahoo et al., (2005), in the same species while using gonadotrophin releasing hormone *GnRH* in combination with *domperidone* (14 to 23 hours). However, according to Sahoo et al. (2005), the suitable latency period for final maturation of ova is also dose dependent when using GnRH and domperidone combinations on spawning performances. Thus our findings on spawning and larval production are in support with the reports of Sahoo et al. (2005) in case of *Clarias spp*.

Many authors have attested to the fact that suitable feed is the basic requirement for growth and survival of fish larvae (Srivastava et al., 2012, Mollah et al. 1987 & Mollah and Nurullah, 1988).

Furthermore, most fish larvae are known to feed best on zoo planktons or live feeds, before the later stage of their life which needs nutritionally balanced feed. This is well demonstrated in the present findings of 21-day feeding trials with *artemia nauplii* as standard feed compared to other 3 different formulated diets. Here, group of hybrid larvae fed with *Artemia nauplii* showed high performance in all parameters including percentage survival (76.00%) and SGR (224) in comparison to other formulated diets (see table 3). This could be attributed to the nutritive richness of *artemia nauplii* as live feed. Secondly, larvae being small in size needed such minute live agent (*Artemia nauplii*) in contrast to the formulated diet. This study outcome is also in line with Mollah et al. (1987) & Mollah and Nurullah (1988) who have reported high percentage survival of 97.6 -99.6% while feeding tubifid worms to *C. batrachus* fry (size 88.1mg). Our findings of improved length size, weight increment also find support with the study of Thakur, (1976), who reported that *Clarias* fry attained 3-7 cm size within 20-25 days feeding trials..

Therefore, the present study demonstrated that the hybrid larvae of *heteroclarias* can be reared with formulated artificial diets for 21-days as with live feed of *artemia nauplii* for enhanced growth as well as high survival performance of larvae. Principally, these laboratory formulated diets can be use as alternative to artemia nauplii thereby reducing cost of production in term of foreign exchange reduction, since artemia live feed is an imported feed.

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