POTENTIALS OF *Garcinia kola* ON THE HEALTH STATUS AND MATERNAL DERIVED IMMUNITY OF *Clarias gariepinus* FRY

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ABSTRACT

Low hatchlings survival rate has continued to bedevil successful fish seed production. The present study evaluated the effects of bitter kola (Garcinia kola) seed powder on hatchability and survival of Clarias gariepinus larva. Powdered G. kola seed was incorporated into formulated diets (40% C.P.) at 0, 5, 10, 15 and 20 g kg^{-1}making a total of five dietary treatments. Two gravid catfish broodstock that weighed between 1013-1059 g were selected each from a pool of 10 fish per treatment fed the five test diets over a period of three months and conditioned for artificial fish seed production. Haematology, blood serum chemistry, hatchability and survival rate of C. gariepinus were determined and significant mean difference was separated at 0.05 probability level. Red blood cell, haemoglobin count and packed cell volume all improved from their initial values and glucose level and total protein all increased in all the treatment. Hatchability rate increased with increase in bitter kola seed inclusion while fry survival rate ranged from 29% in the control to 93% in diet 4 and could be attributed to the G. kola seed powder inclusion in the diet. The present study suggests that dietary G. kola seed powder improved growth, hatchability and survival rates of C. gariepinus larvae and could be exploited for mass fish seed production. Inclusion of bitter kola at 15 g kg^{-1}was best as health and survival rate of the fish larvae improved.

Keywords: Fish seed, *Clarias gariepinus*, Hatchability rate, Survival rate, Bitter kola

INTRODUCTION

Garcinia kola also known as bitter kola (due to its bitter astringent taste when chewed) is a nut-bearing tropical tree native to Nigeria's coastal rainforest. The plant (*G. kola* Heckel) is extensively used in herbal medicine and as food. It is a medium-sized tree growing up to 12 m tall and 1.5 m wide usually found in the rain forest of Nigeria (Iwu, 1993). *Garcinia kola* belongs to the family of tropical plants known as Guthfera (Plowden, 1972). The seed is rich in flavonoids, which have been shown to have antibiotic property (Hong-Xi and Song, 2001) and anti-inflammatory attribute (Madubunyi, 1995) among others. The seed is used in traditional medicine for various therapeutic purposes based on pharmacological effect of the active component (flavonoid) in the seed and other parts of the plant (Huang and Song, 1999).

The quest for naturally occurring compounds of herbal or plant origin that could be of benefit as contraceptive and fertility control agents stimulated the interest in bitter kola seed which is widely consumed as a stimulant (Dada and Ikuorowo, 2009).The use of plant extract as immunostimulant in aquaculture has not received considerable attention just like some other plant material. It is now necessary to investigate whole bitter kola meal as an herbal based plant material for healthy fish production and successful hatchery management. The present study therefore investigated the antibacterial effects of *Garcinia kola* seed meal on the hatchability and survival of *Clarias gariepinus* fry to improve fish seed production.

MATERIALS AND METHODS

Study location

The experiment was carried out at the Fisheries Unit of the Experimental Farm of the Department of Forestry Wildlife and Fisheries of the Faculty of Agriculture, Nasarawa State University Keffi, Shabu- Lafia Campus, Nigeria.

Preparation of experimental materials

Garcinia kola seeds were obtained from the Central Market in Lafia, Nasarawa State and were oven dry at 65°C for 48 hrs in an open foil paper. The dried seeds were ground into fine powder with a grinding machine and added to fish feeds formulated at 42% C.P. defined for broodstock of catfish by Sotolu (2010). Ingredients used for feed formulations include fishmeal, soya bean meal, groundnut cake and noodle wastes. Test ingredient (Powdered *Garcinia kola*) was incorporated into the diets at five different levels of 0, 5, 10, 15 and 20 g kg^{-1}. The required quantity of the test ingredient was weighed using a sensitive scale (QE- 400 electronic kitchen scale) during feed preparation. Gross and proximate analysis of experimental diets were carried out according to AOAC (2000) and presented in Table 1. Catfish broodstocks used weighed between 1013-1059 g and they were selected from a pool of 10 fish per treatment the five test diets over a period of 3 months to ensured that the broodstocks developed viable eggs for the seeds production study.

Table 1: Gross ingredient and proximate composition of experimental diets

Ingredients	Dietary treatment (g/100g/DM)				
	0.0	**0.5**	**1.0**	**1.5**	**2.0**
Fishmeal	31.86	31.86	31.86	31.86	31.86
Soya bean meal	25.78	25.78	25.78	25.78	25.78
Groundnut cake	17.67	17.67	17.67	17.67	17.67
Noodle waste	20.19	20.19	20.19	20.19	20.19
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Fish oil	2.50	2.50	2.50	2.50	2.50
G. kola	0.00	0.50	1.00	1.50	2.00
Proximate composition					
Crude protein	42.06	42.06	42.06	42.06	42.06
Crude fibre	4.66	4.66	4.66	4.66	4.66
Fat	7.82	7.82	7.82	7.82	7.82
Ash	14.44	14.44	14.44	14.44	14.44
	31.02	31.02	31.02	31.02	31.02

Fingerling production

The hatchery was prepared for the study with three plastic tanks per treatment for incubating the eggs. Two gravid females were selected per treatment and artificial hormone (Ovaprim©) was administered on them based on the manufacturer's recommendation. A fish was kept in a separate tank over night to attain latency period as they were all ready to release their eggs within 11 hrs of injection with Ovaprim©. Stripping of the brood fish was done and were all fertilized by homogenous mixture of milt from three males that were equally developed for the study under the same condition. Incubation took place under indoor hatchery system with aeration and the eggs started to hatch after 23 hrs. Water in culture media was monitored to conform to APHA (1995) standard for sustainable fresh water fish culture. Fertilization and hatchability rate were determined. The percentage of egg fertilized as well as the percentage number of egg hatched and percentage survival were determined according to the method of Ayinla (1988) as follows:

$$\text{Percent egg fertilized} = \frac{\text{Number of eggs incubated} - \text{Number of opaque eggs}}{\text{Total number of eggs incubated}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad i$$

$$\text{Percent egg hatched} = \frac{\text{Number of whitish broken eggs}}{\text{Number of eggs fertilized}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots ii$$

$$\text{Percent survival} = \frac{\text{Number of hatchling alive up to larvae stage}}{\text{Total number of hatchlings}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots iii$$

Determination of fry survival rate

After fifth day of hatchlings survival, 300 fry from each treatment were stocked in glass aquaria of 0.5 x 0.35 x 0.3 m in triplicates for 28 days. Fry were fed *Artemia* for 10 days followed by 0.3 mm and 0.5

mm of Coppens© feed before fry survival rate was determined at the end of study by the 28th day.

Blood Collection and Analysis

Health status of the broodstock was however monitored through haematology and blood serum analysis. Blood sample was collected from the experimental fish before the three month feeding (initial) and at the end (final) before preparing the fish for artificial spawning. Blood was collected by puncturing the caudal artery at the pedicle by using micro-capillary and sampling tubes treated with anticoagulant. Haemoglobin concentration was estimated by cyanomethemoglobin and white blood cells (WBC) were counted by Neubaurs improved hemocytometer as described by Svobodova *et al*. (1993). Red blood cell (RBC) and packed cell volume (PCV) were estimated as described by Blaxhall and Daisley (1973).

Statistical analysis

Data obtained from the study were subjected to descriptive analysis and one way analysis of variance (ANOVA) while significant mean differences were separated at 0.05 probability level as described by Steel *et al*. (1997).

RESULTS

Table 2 revealed that diet 2 had the highest Haemoglobin concentration with (7.6 g/dl) while the least haemoglobin concentration (Hb) was obtained in fish fed diet 4 (6.7 g/dl). This value was less than the Hb (g/dl) of the initial and the same variation was recorded for red blood cell (RBC) count per litre with the least value (3.5) recorded in fish fed diet 4. The MCV is lowest in the initial value (135.33) while diet 1 and diet 3 are the highest with equal value of (759.5) as presented in Table 2.

Table 3 showed that all serum parameters considered increased in all treatments from their initial values. Treatment 5 had the highest glucose level (8.4) and least in T3 (7.3) while Total protein was highest in treatment 2 (62.6) and least in T5 (57.4).

Changes in the weight of experimental fish after stripping of eggs for incubation are presented in Table 4and that there was no mortality recorded after stripping while egg weights incubated ranged between 126 and 152 g and percent hatchability and survival rates as shown in Table 5. Fry survival rate is shown in Fig. 1 which translates to 29%, 76%, 74%, 93% and 86% from treatment 1 to 5 respectively.

Table 2: Haematological parameters of *C. gariepinus* fed diet containing bitter kola seed

Parameters	Initial	Final				
		T1	T2	T3	T4	T5
PCV (%)	23.17	24.02	26.30	24.02	23.60	23.41
Hb (g/dl)	6.82	7.30	7.60	7.20	7.70	7.90
RBC ($x10^{12}$/1)	1.66	3.16	3.06	3.16	3.50	3.14
WBC ($x10^{9}$/l)	150.60	166.13	146.65	145.76	158.22	156.30
MCV (fl)	135.33	759.5	691.5	759.5	730.2	732.5
MCHC (g/dl)	28.67	3.041	2.923	3.00	2.913	3.00
MCH (pg)	37.33	23.10	20.21	22.78	21.27	21.97

Table 3: Serum biochemistry of *Clarias gariepinus* fed diet containing bitter kola seed

Parameters		Final				
	Initial	T1	T2	T3	T4	T5
Glucose	7.23	7.60	7.40	7.30	7.70	8.40
Cholesterol	4.16	4.30	4.50	4.41	4.34	4.72
Total protein	54.6	60.0	62.6	58.6	48.9	57.4
Urea	3.26	3.91	4.50	3.73	3.34	4.82

Table 4: Weight of experimental fish before and after stripping

Diet (Treatment)	Weight before stripping (g)	Weight after stripping (g)	Number of fish injected	Number of fish stripped
1	1013±0.02	897±0.01	02	02
2	1059±0.01	919±0.04	02	02
3	1020±0.02	894±0.10	02	02
4	1052±0.01	880±0.13	02	02
5	1034±0.02	890±0.13	02	02

Table 5: Hatchability and Survival rates of fish larval at 10th day

Diet	Mean weight of eggs (g) incubated	Hatchability rate (%)		
		Survival	Deformed	Dead
1	126	85.0±0.12[c]	9.70±0.17[a]	5.30±0.21[a]
2	140	89.3±0.03[b]	5.05±0.06[b]	5.65±0.14[a]
3	126	91.4±0.01[b]	6.46±0.05[b]	2.14±0.20[b]
4	152	94.3±0.44[a]	3.67±0.35[c]	2.03±0.10[b]
5	144	93.9±0.13 [a]	3.59±0.35[c]	2.51±0.10[b]

Mean values with the same letter along the same row are not significantly different ($p > 0.05$).

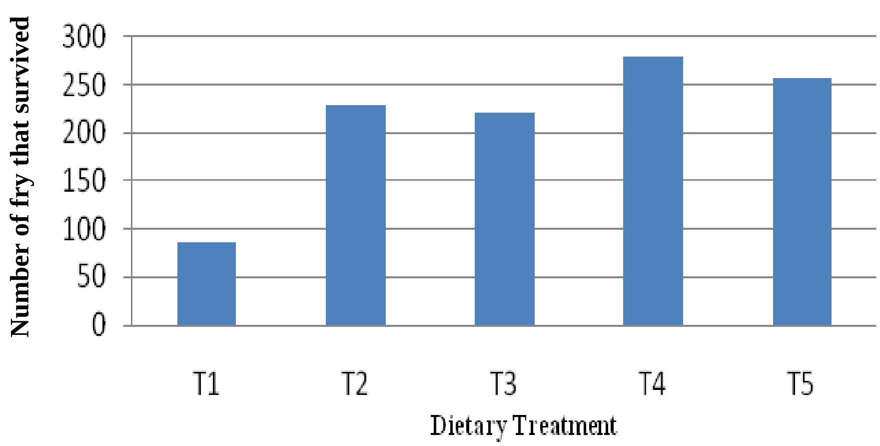

Fig. 1: Fry Survival rates at 28th Day of hatching

DISCUSSION

The final haematological values of fish fed graded levels of dietary *G. kola* whole seed were affected by the test ingredient. The PCV, Hb and RBC values increased in the fish fed all the diets. Aletor and Egberonge (1998) reported that red blood cell count and pack cell volume are mostly affected by dietary treatments and an increase in their values is better for the affected animal. Differences in blood parameters of fish in this study could therefore be ascribed to differences in dietary inclusions of *G. kola* whole seed powder in feed. This observation is similar to those of Mamman *et al*. (2013) who reported good health status of fish fed calabash seed meal due to increase in the RBC, Hb and PCV of the experimental fish. The range of values recorded in the present study are also in line with the reports of Ayoola and Maduekwe (2012)on catfish fed diets containing *Mytilus edulis* shell at varying level. Decrease in the quantity of PCV and erythrocyte count (RBC) indicated unhealthy state of fish which could become anaemic if the situation persists as observed by Adeyemo (2007). A change in white blood cells (WBC) is an indication that fish responded to certain antimicrobial properties of bitter kola included in the diets Hussain *et al.* (1982). The serum biochemistry profile is one of the most important initial tests that are commonly performed in assessing the functions of various organ and body system. All the parameters improved from the initial rate while the dietary bitter kola treatments are superior to the control diet. Maternal immunity is of paramount importance for protection of young ones at early stage of life since the immune factors of an immune-competent female are transferred transplacentally or through colostrums, milk or yolk to an immunologically naive neonate. Both innate and adaptive types of immunity are transferred from mother to offspring in fishes (Swain and Nayak, 2009). The maintenance of the broodstock immunity at high level during vitellogenesis and oogenesis, is utmost important for reducing mortalities at larval/post larval stages through maximum/optimum transfer of maternal immunity as emphasized by Mulero *et al*. (2007). Most hatchery diseases have been confirmed to be due to bacterial infection which bitter kola has proven to be effective in its control against (Dada and Ikuorowo, 2009). It is suggested that the high larval survival rates recorded during the present study is as a result of immune-competence of the broodstock and subsequent maternal transfer of immunity to fish larval.

CONCLUSION

Results generally suggest that dietary *G. kola* seed improve hatchability and survival rate of *C. gariepinus* eggs and improve health status of fish. The inclusion of *G. kola* at 15gkg^{-1} is most suited in fish feed preparation when preparing broodstock for high hatchability and larvae survival rates. The use of *G. kola* whole seed in fish feed formulation is therefore recommended for aquaculture especially as immunostimulant for quality fish seed production and ensuring profitability of fish seed production business in hatcheries. Further studies may be carried out for assessing the suitability of fish fed dietary bitter kola meal for human consumption.

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