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EFFECT OF A BIODEGRADABLE SUBSTRATE SUGARCANE BAGASSE ON EGG AND SPERM QUALITY OF THE CATFISH, *CLARIAS BATRACHUS* (LINN.)

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ABSTRACT

The present investigation has been conducted to evaluate the egg and sperm quality of the catfish, *Clarias batrachus* (Linn.) cultured in sugarcane bagasse substrate medium. Adult *Clarias batrachus* (av. wt. 196 g for males and 215 g for females) were reared at 20 per tank. No feed was provided to the fish in 3 of the substrate-added tanks (T_1), while a pelleted diet was fed to the fish in the remaining 3 substrate-added tanks (T_2) and the other 3 tanks without substrate (T_3). Five pairs of matured male and female *Clarias batrachus* were bred by hypophysation using OVAPRIM. Spawning substrates made from cut nylon mosquito nets were spread inside the tank to a depth of 10cm for the purpose of incubation. Fertilized eggs obtained by mixing stripped eggs and spermatozoa from Ovaprim-induced brood fish were immediately spread thinly on the substrate for between 24-36 hours for incubation and hatching. The brood fish fed the fishmeal-based diet and diet with sugarcane bagasse substrate had significantly higher ($p<0.05$) oocyte diameter, milt density, milt volume, sperm motility, higher percentage of egg fertilization, hatching and crude protein and lipid contents. These results showed that sugarcane bagasse can effectively be use as a substrate for the culture of the catfish *Clarias batrachus.*

KEY WORDS: Sugarcane bagasse, artificial substrate, *Clarias batrachus.*

INTRODUCTION

Feeding is one of the major elements of cost of production in modern aquaculture. In most traditional aquaculture practices, herbivorous or omnivorous species have been preferred as they feed on natural food organisms in water, the growth of which can be enhanced through fertilization and water management (Pillay, 2001). But carnivorous species generally need a high protein diet and are therefore considered to be more expensive to produce, even though the costs will depend largely on local availability and price for the required feed stuffs. To compensate for feeding costs, most carnivorous species command higher market prices. Such species generally have greater export markets and therefore attract substantial investments. Species that are hardy and can tolerate unfavourable conditions will have the advantage of better survival in relatively poor environmental conditions that may occur occasionally in culture situations. In the present study *Clarias batrachus* has been selected because the ability to adapt to fresh and brackish waters with very low oxygen content and to grow under generally poor environmental conditions make these fish extremely valuable for small and large scale rural fish farming (Pillay, 2001). Substrate-based farming practices are considered viable low-cost technologies as they help in sustainable aquaculture production (Dharmaraj et al., 2002). Sugarcane bagasse is the fibrous residue remaining after sugarcane stalks are crushed to extract their juice, generated in large quantities and is currently used as a renewable resource in the manufacture of pulp and paper products and building materials. This investigation is to find out the effect of substrate (sugarcane bagasse) on egg and sperm quality of the catfish, *Clarias batrachus* (Linn.).

MATERIALS AND METHODS

Irrespective of sex, healthy fingerlings of *C. batrachus* (av.wt. 3.18 ± 2g) were collected locally from a single population and confined to large cement tanks in the laboratory. The experiment was conducted in nine 25 m^2 (5 X5 X1 m) cement tanks with 15-cm soil base following the method of Dharmaraj *et al.* (2002). In all the tanks initially added 0.25 kg of quick lime and 2.5 kg of poultry manure. Water was filled to the tanks from a perennial well and a depth of 90 ±2 cm was maintained throughout the experimental period. Subsequently, poultry manure was applied at 0.3 kg per tank every 15 days. Sugarcane bagasse, procured locally, was sun dried and bundles were made using nylon rope; they were introduced into 6 of the 9 tanks randomly at the rate of 5 kg each, by suspending the bundles at regular distances from bamboo poles kept across the tanks. After 45 days, once again 1.25 kg of the substrate was supplemented to each of the designated tanks. *C. batrachus* were stocked at 20 per tank two weeks after the addition of manure and substrate. No feed was provided to the fish in 3 of the substrate-added tanks (T_1), while a pelleted diet,

formulated according to Varghese et al. (1976) (Table 1) was fed to the fish in the remaining 3 substrate-added tanks (T_2) and the other 3 tanks without substrate (T_3) at 5% body weight for the first 30 days and 2% thereafter, in two equal rations daily. After 358 days, five pairs of matured male (av. wt. 196 g) and female (av. wt. 215 g) *C. batrachus* were selected for the present study and bred by hypophysation using OVAPRIM (0.02mg salmon gonadotropin-releasing hormone-sGnRHa + 10mg domperidone-Dom) in the hatchery. Spawning substrates made from cut nylon mosquito nets were spread inside the hatching troughs previously filled with properly aerated clean water to a depth of 10cm for the purpose of incubation. Fertilized eggs obtained by mixing stripped eggs and spermatozoa from Ovaprim-induced brood fish were immediately spread thinly on the substrate for between 24-36 hours for incubation and hatching. Five females randomly selected were weighed, killed and dissected to remove the ovaries. The ovaries were slit open and 10 fresh eggs were randomly selected for egg diameter (mm) measurement. For the pear-shaped eggs, the mean diameter of the long and short axes was taken as the diameter of the oocyte (Ayinla, 1988). The remaining eggs were oven-dried and subjected to proximate analysis. Data on egg diameter in conjunction with the proximate composition of the eggs were used to assess egg quality. Five male fish, randomly selected from each hapa, were killed and the testes were removed. Small incisions were made into the lobes of the testes, the milt was squeezed out into a petri dish and the volume of the milt was measured in (ml) with a plastic syringe. A drop of distilled water was quickly added to a drop of the milt (activation) on a clean slide and the sperm motility was observed under a microscope (x10) (Billard et al. 1995). The presence or absence of spermatozoa motility was then expressed as present (1) or absent (0). The density of the spermatozoa was rated on a scale of 1, 2 and 3 (representing low, medium and high density) respectively. The percentage number of eggs stripped from each fish, the percentage number of egg fertilized as well as the percentage number of egg hatched were computed according to the method described by Ayinla (1988).

(a) Number of eggs stripped (incubated) = Weight (g) of fish before stripping — wt (g) of fish after stripping x 66.6.

(b) % Egg fertilized =

$$\frac{\text{Number of eggs incubated - number of opaque eggs}}{\text{Total number of eggs incubated}} \times 100$$

(c) % Egg Hatching =

$$\frac{\text{Number of whitish broken eggs}}{\text{Number of eggs fertilized}} \times 100$$

Statistical analysis of the data included the one-way analysis of variance (ANOVA) using the SPSS version 10.0 for windows on PC (Statistical Graphics Corp, US). Significant mean differences were separated at 5% using the methods of Steel *et al.* (1997) whereas appropriate and values are expressed as means ± SE.

RESULTS AND DISCUSSION

In male brood fish the mean volume of milt collected from the testes in T_2 and T_3 group (0.84ml and 0.64ml) was significantly higher ($p<0.05$) than what was obtained from the testes of the fish treated in bagasse alone (T_1). There was a strong relationship between the milt volume and the percentage fertilization of the eggs (Fig.1). Under the microscope, the sperm could easily be seen sometimes in high density and sometimes scantily. The motility of the spermatozoa in the testes of the male fish in T_2 was higher but not significantly different ($p<0.05$) from those of T_1. It was also observed that motility increased with volume of milt and the spermatozoa were active for only 30-35 seconds. The milt collected from the brood fish in T_2 was significantly denser ($p<0.05$) than the milt collected from the fish in T_3. The fish in T_2 and T_3 had significantly different ($p<0.05$) oocyte diameter than those of the fish in T_1 (Fig. 1). In all the treatments, there was an increase in the crude protein content, lipid content of the oocytes. The oocytes of the specimens in T_2 and T_3 had significantly higher ($p<0.05$) crude protein content and lipid content than the oocytes of the specimens inT_1. Contrarily, the oocytes recorded significantly lower ($p>0.05$) moisture content respectively. The oocyte ash content of the fish in T_2 was not significantly different ($p>0.05$) from those of T_3(Fig.2). Significantly higher ($p<0.05$) egg fertilization and hatching was recorded for the fish in T_1 and T_2 than inT_3 (Fig.3).

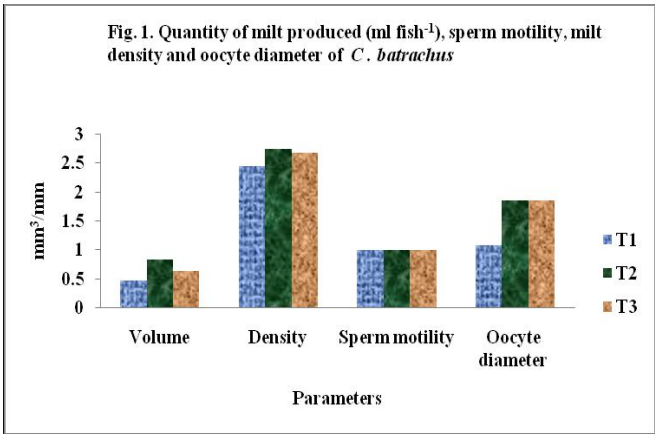
In the present study, the sperm motility increased with volume of milt and the strong relationship between milt volume and percentage egg fertilization and hatching in *C. batrachus* agree with the findings of Lamai (1996). Van de Waal and Polling (1984) and Lamai (1996) also observed that spermatozoa were active or motile for only 30 seconds. 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, as the fertilizing capacity is the most conclusive test of sperm quality (Rurangwa et al, 2003) and quality of eggs.

The significantly higher ($p<0.05$) egg sizes, higher percentage fertilization and hatching observed in the fish T_2 and T_3 agrees with Cerd et al (1990) who reported that sea bass brood stock fed with commercial trout diets had smaller eggs and produced lower hatching rates and larval survivals than the control fish which were fed on trash fish. Richter et al (1995) and Sule and Adikwu (2004) also reported that species of *Clarias gariepinus* with larger eggs also have a higher viability and endurance to starvation than those with smaller eggs and that larger female catfish produce larger eggs. According to Carillo et al (1995) that egg diameter is not a good indicator of egg and larval quality. In this

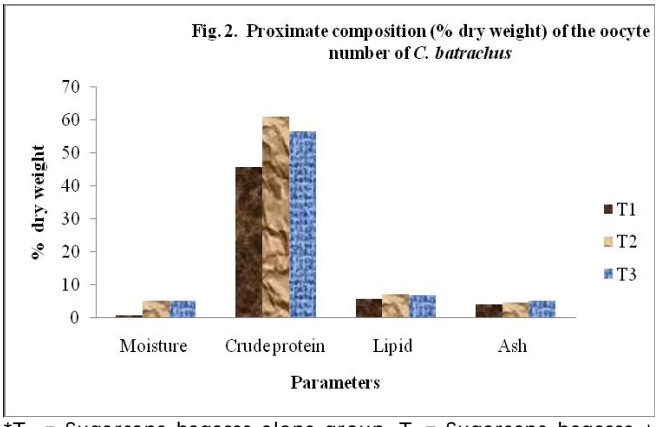
Table1. Ingredient proportion and proximate composition of feed (wet weight basis)

Ingredient	%	Proximate composition	% Fish
meal	25	Moisture	7.29 ± 0.13
Rice bran	40	Crude protein	28.17 ± 0.66
Groundnut			
oil cake	25	Crude fat	3.15 ± 0.08
Tapioca flour	10	Crude fibre	15. 90 ± 0.54
		Ash	13.80 ± 0.62
		NFE	31.69
		Energy content	12.50 (KJ^{-1})

* Average of three values ± S.E.

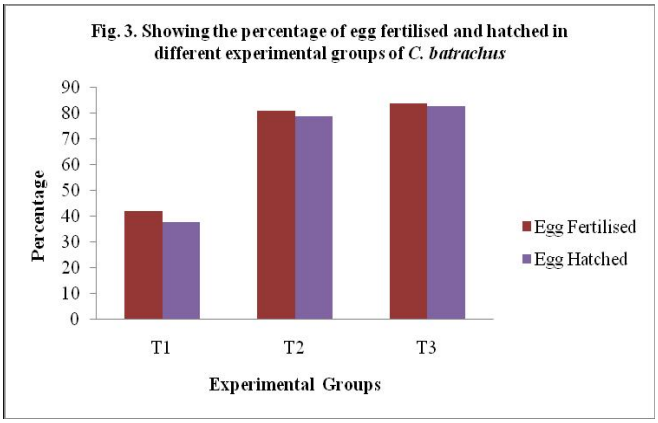


*T_1 = Sugarcane bagasse alone group ; T_2 = Sugarcane bagasse + supplemental feed group; T_3 = supplemental feed alone group.



*T_1 = Sugarcane bagasse alone group ; T_2 = Sugarcane bagasse + supplemental feed group; T_3 = supplemental feed alone group.

experiment, the high ovarian crude protein and lipid recorded for the fish in T_2 and T_3 coupled with the favourable size of eggs could be responsible for the good performance of the eggs. The viability of eggs is a reflection of the chemical composition of the yolk. Protein and lipids are the major components stored in egg yolk and play a major role in reproduction. The total lipid content of the eggs has also been correlated with egg and larval viability (Devauchelle, 1982). In this experiment, the progeny of the brood stocks T_2 and T_3



*T_1 = Sugarcane bagasse alone group ; T_2 = Sugarcane bagasse + supplemental feed group; T_3 = supplemental feed alone group.

survived better ($p<0.05$) than T_1. Compared to T_2 and T_3 the fish reared in sugarcane bagasse alone (T_1) also produced good quality egg and sperms. Hence, the utilization of sugarcane bagasse as a substrate would help for culturing the catfish *C. batrachus* in early life stages by providing additional food in terms of plankton.

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