



EFFECTS OF BREWERS' SPENT GRAIN ON FECUNDITY AND GONAD DEVELOPMENT OF *Clarias gariepinus*

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ABSTRACT

The rising cost of fish feed has made farmers to search for cheaper protein sources that could be used in feeding fish without negative impacts. This study was carried out for 120 days to evaluate the effects of brewers' spent grain (BSG) on the fecundity and gonad development of *Clarias gariepinus*. Six tarpaulin tanks (100 x 80 x 100 cm³) were stocked randomly with twenty (20) healthy post fingerlings of *C. gariepinus* with a mean initial bulk body weight of 300.20 ± 1.56 g and total length of 11.24 ± 0.18 cm. Two isonitrogenous diets (42% CP) were formulated and used to feed the fish. Diet A (control) was formulated with fishmeal as the protein source while Diet B was formulated with brewers' spent grain (BSG) as the protein source. Water parameters of the culture systems were monitored using standard procedure. Results showed that mean fecundity of fish fed Diet A (control) ($30,368 \pm 428.02$ eggs) was not significantly different ($p > 0.05$) from fecundity of fish fed Diet B ($30,296 \pm 275.28$ eggs). Also, mean GSI of fish fed the two diets were not significantly different ($p > 0.05$). Results obtained for mean egg diameter of fish fed Diet A (1.29 ± 0.02 mm) was not significantly different ($p > 0.05$) from that of fish fed Diet B (1.30 ± 0.01 mm). Inference from the present study revealed that brewers' spent grain (BSG) did not impact negatively on the fecundity, egg diameter and gonad development of *C. gariepinus* and is therefore recommended for possible use in aquafeed production.

Keywords: *Clarias gariepinus*, Protein source, Brewers' spent grain, Fecundity, Gonad Development

INTRODUCTION

In Nigeria, the cost of fish and fish products such as fresh, frozen and smoked farmed fish is on the increase due to so many factors including the rising cost of fish feed (Eriegha *et al.*, 2022). This is due to the fact that feed cost account for the majority of the total production cost (Eriegha and Ekokotu, 2017). With the removal of fuel subsidy in May 2023,

inflation has increased across all products including fish and aquaculture inputs such as feed products since fuel price has a direct influence on cost of transportation of goods and services. According to Eriegha *et al.*, (2022), one of the major impacts of inflation on aquaculture in Nigeria is high cost of fish feed which is exacerbated by competition for feed ingredients among fish farmers, operators of animal husbandry,

and human consumption. The African catfish, *Clarias gariepinus* is mostly cultured in Nigeria and other West African countries. The domination of Nigerian aquaculture by *C. gariepinus* is due to its size, growth rate, high fecundity, disease resistance, fast gonad growth and development, ease of artificial breeding, ability to tolerate a varying range of physicochemical conditions, acceptance of formulated feed, high quality meat good taste, and high market value (Eyo *et al.*, 2013; 2014; 2016; 2022). Fecundity which is the number of eggs carried by a gravid female fish or shrimp is a very crucial index used in fisheries and aquaculture for several biological evaluations. It is one of the important indices considered by aquaculture scientists during selection of a new species for aquaculture.

Fecundity also provides reliable information on the evaluation of a feed performance in aquaculture. According to Ekanem *et al.* (2012 and 2013), feed quality had been documented to affect fecundity and gonadal development in fishes. Due to the increasing inflation on the prices of fish feed in Nigeria, there is need to search for alternative ingredients and cheaper protein sources that would boost fish growth and fecundity without having any adverse effects on the fish development. According to Mussatto (2014), brewery waste known as brewers' spent grain (BSG) is a major by-product of beer production in the beer industry, representing about 85% of the total amount of generated by-products. Globally, beer has been reported to be the fifth most consumed beverage with an annual average of 39 million tonnes of BSG produced globally (Mussatto, 2014). During brewing, the soluble part of the grain is removed, thus allowing the

insoluble material in brewers' spent grain (BSG) to concentrate (Shen *et al.*, 2019). Nutritionally, brewers' spent grain (BSG) contains 15–26% crude protein and 35–60% crude fibre on dry basis (Ikram *et al.*, 2017). However, despite the nutritional qualities of brewers' spent grain (BSG) including significant amounts of valuable protein and fibre, it is commonly used in feeding animals, particularly cattle (Shen *et al.*, 2019). Due to the nutritional composition of brewers' spent grain (BSG), it could be used as a nonconventional protein source in fish feed production. Therefore, this study was conducted to evaluate the effects of brewers' spent grain (BSG) on the fecundity and gonadosomatic index of *C. gariepinus*

MATERIALS AND METHODS

Experimental Design

This study was conducted for 120 days using 6 tarpaulin tanks measuring 100 x 80 x 100 cm. The six tarpaulin tanks were labelled A₁, A₂, A₃, B₁, B₂ and B₃ to aid setting up the experiment in triplicate. A total of 120 healthy post fingerlings of *C. gariepinus* was procured from a private hatchery in Uyo, Akwa Ibom State and stocked in each of the six experimental units (20 in each unit). After stocking, the fish were acclimated for 14 days before the start of the feeding experiment. Within the acclimation period, fish were fed twice daily to satiation with Blue Crown feed. At the start of the experiment, the acclimated fish were starved for 24 hours. Thereafter, the initial wet body weight of the fish in each experimental unit was measured using a METTLAR MT-5000D electronic balance to the nearest gram (Eyo and Ekanem, 2011). Fish in units A₁, A₂ and A₃ were fed Diet A (control) and fish in units B₁, B₂ and B₃ were fed Diet B (BSG). The experimental fishes were fed twice daily by 9.00 am and 6.00 pm at 3 % of their body

weight. Total length (TL-cm) and total weight (TW-g) were measured fortnightly. Total length was measured from snout to the base of the caudal fin rays to the nearest 0.1 cm using measuring board while fish bulk weight was measured to the nearest 0.1 g using Metlar-2000D electronic weighing balance.

Experimental Feed Composition

In this study, two experimental diets (A and B) containing 42% crude protein were formulated and used as the experimental feed. Diet A was the control

diet whereas Diet B was formulated with brewery waste as the protein source. In formulating the experimental feed, ingredients used were bonga fishmeal, brewers' spent grain (BSG), corn meal, fish oil, corn flour, vitamin premix, lysine, methionine, calcium powder, and sodium chloride were used. The brewers' spent grain (BSG) was collected from Champion Breweries, Uyo, Akwa Ibom State and incorporated in the experimental diet. Each ingredient was measured in proportions to give a 42% crude protein diet (Table 1).

Table 1: Composition of the experimental feeds in gram per kilogram

Ingredient composition (g/kg)	Diet A (Control)	Diet B (BSG Waste)
Bonga fishmeal (BFM)	546.90	–
Brewers' spent grain (BSG)	–	601.6
Corn meal	393.1	338.4
Fish oil	10	10
Corn flour	10	10
Vitamin premix	10	10
Methionine	5	5
Lysine	5	5
Calcium powder	10	10
Sodium chloride	10	10
Proximate composition (%)		
Crude protein	41.24 ± 0.03 ^a	41.10 ± 0.02 ^a
Crude fat	7.54 ± 0.02 ^a	7.51 ± 0.01 ^a
Moisture	5.89 ± 0.02 ^a	5.78 ± 0.02 ^a
Ash	1.85 ± 0.02 ^a	1.88 ± 0.02 ^a
Lipid	2.68 ± 0.01 ^a	2.61 ± 0.02 ^a
Nitrogen free extract	40.78 ± 0.02 ^a	41.12 ± 0.02 ^a

Proximate Analysis of Experimental Diets

Proximate analysis of the two experimental diets was performed according to AOAC (2000) in the Department of Biochemistry Laboratory, University of Uyo, Akwa Ibom State. For, moisture content, 2g of the sample was weighed into a previously dried silica dish and dried in an electric oven at a temperature of 105°C for 30 hours to obtain a constant weight. **Percentage moisture** was then calculated:

Moisture (%) = Initial weight of sample – dry weight of sample/Initial weight of sample*100

Protein content was estimated by the kjeldahl method. Here, nitrogen content was used and this was converted to percentage protein by multiplying with a factor, 6.25 as followed:

Protein (%) = ((Va – Vb) × N × 14 × 100 × 6.25)/1000*Weight of sample

Where: Va = volume of HCL used in titration.

Vb = volume of sodium hydroxide of known normality used in back titration.

0.014 = conversion factor of ammonium sulfate to nitrogen.

6.25 = conversion factor of nitrogen to protein.

Lipid content (ether extract) of the sample was determined using Soxhlet method. Here, two grams (2g) of the sample was used for lipid extraction for 5 hours at 100°C. Percentage lipid was calculated as followed:

$$\text{Lipid (\%)} = \left(\frac{\text{Extracted fat weight}}{\text{Initial weight}} \right) \times 100$$

Ash content was determined by heating 1g of the sample at 550 °C in a muffle furnace until a constant weight was obtained. The percentage of ash content was calculated as followed

$$\text{Ash (\%)} = \left(\frac{\text{Ash weight}}{\text{Sample weight}} \right) \times 100$$

The organic residue left after sequential extraction of sample with ether was used to determine the crude fibre content as followed:

Crude fibre (%) = (Dry weight of residue before ashing – weight of residue after ashing)/(weight of sample)*100

Carbohydrate was determined by subtracting the sum of percentages of all the nutrients already determined from 100.

$$\text{NFE (\%)} = 100 - (\% \text{ moisture} + \% \text{ crude fibre} + \% \text{ crude protein} + \% \text{ fat} + \% \text{ ash})$$

Estimation of Fecundity

Fecundity was estimated according to Viveen *et al.* (1985) by multiplying the number of eggs per gram of egg mass by the weight of the egg mass. All the females in the six tanks fed the two experimental diets were collected and used for fecundity estimation. Before egg counting, the total length (TL-cm), total weight (TW-g) and ovary weight (OW-g) were measured. The fishes were dissected carefully to remove

the ovary for ovary weight measurement using Metlar-2000D electronic scale. With a scissors, the sub-samples of the ovary weighing 1 g was cut off from three parts of the ovary including the posterior, middle and anterior sections and preserved in sample bottles with Gilson fluid to harden the eggs for easy separation and counting. Fifty (50) fresh eggs were randomly collected from the ovaries of each female fish to measure the egg diameter using a stereo microscope with ocular eye piece. Eggs with pear-shape were given two measurements and the mean diameter of the long and short axes was taken as the egg diameter.

Gonadosomatic index calculation (GSI-%)

The gonadosomatic index (GSI) of fish fed with the two diets was calculated according to methods of Bolger and Connolly (1989) as followed:

$$\text{GSI} = \left(\frac{\text{Weight of the gonad (g)}}{\text{Weight of the fish (g)}} \right) \times 100$$

Measurement of Water Quality Parameters

The water quality parameters of the culture systems including ammonia (mg/l), dissolved oxygen (mg/l), pH, and temperature (°C) were measured weekly throughout the experimental period. Ammonia was measured using ammonia test kit, dissolved oxygen was measured using DO9100 dissolved oxygen meter, pH was measured with pen type pH meter, and water temperature was measured with mercury in glass thermometer.

Statistical Analysis

Data obtained for fecundity and GSI were analyzed using Student's T-test to test for significance using Predictive Analytical Software windows (PASW), version 18.0. Effects with a probability of ($p < 0.05$) was considered significant.

RESULTS

The results of fecundity of *C. gariepinus* fed with the two experimental diets (Table 2) showed that *C. gariepinus* fed with diet A (control) had fecundity ranging between 24,200 eggs for fish with body weight of 387 g, total length (35.2 cm), ovary weight (34.6 g) and mean egg diameter (1.25 mm) to 34,510 eggs for fish with body weight of 452 g, total length (41.4 cm), ovary weight (49.3 g) and mean

egg diameter (1.33 mm) with a mean of 30,368 ± 428.02 eggs. In fish fed diet B (BSG), fecundity ranged between 23,870 eggs for fish with body weight of 379 g, total length (34.1 cm), ovary weight (34.10 g) and mean egg diameter (1.24 mm) to 34,230 eggs for fish with body weight of 448 g, total length (40.6 cm), ovary weight (48.9 g) and mean egg diameter (1.32 mm) with a mean of 30,296 ± 275.28eggs.

Table 2: Mean fecundity of *C. gariepinus* fed the experimental diets

Parameters	Feed A (Control)	Feed B (BSG)
Mean weight (g)	426.28 ± 20.38 ^a	425.32 ± 16.38 ^a
Mean length (cm)	39.23 ± 0.44 ^a	39.04 ± 0.12 ^a
Mean ovary weight (g)	43.42 ± 0.54 ^a	42.96 ± 0.68 ^a
Mean egg diameter (mm)	1.29 ± 0.02 ^a	1.30 ± 0.01 ^a
Mean fecundity (Thousands)	30,368 ± 428.02 ^a	30,296 ± 275.28 ^a

*Values represents the mean ±standard error of the triplicate experimental group. Means with the same superscript are not significantly different (P>0.05). BSG is brewer’s spent grain

The result of gonadosomatic index (GSI) of *C. gariepinus* fed with the two experimental diets is shown in Table 3. For female *C. gariepinus* fed with Diet A (control), GSI ranged between 8.94 % for fish with body weight of 387 g, total length (35.2 cm), and ovary weight (34.6 g) to 10.91 % for fish with body weight of 452 g, total length (41.4 cm), and ovary weight (49.3 g) with a mean of 9.42 ± 0.24 %. For female fish fed with Diet B (BSG), GSI ranged between 8.99 % for fish with body weight of 379 g, total length (34.1 cm), and ovary weight (34.10 g) to 10.92 % for fish with body weight of 448 g, total length

(40.6 cm), and ovary weight (48.9 g) with a mean of 9.46 ± 0.16 %. For male *C. gariepinus* fed with Diet A (control) GSI ranged between 0.57 % for fish with body weight of 385 g, total length (34.9 cm), and gonad weight (2.2 g) to 0.97 % for fish with body weight of 431 g, total length (40.5 cm), and gonad weight (4.2 g) with a mean of 0.79 ± 0.03 %. For male fish fed with Diet B (BSG), GSI ranged between 0.55 % for fish with body weight of 380 g, total length (34.1 cm), and gonad weight (2.1 g) to 0.94 % for fish with body weight of 427 g, total length (40.1 cm), and gonad weight (4.0 g) with a mean of 0.78 ± 0.04 %.

Table 3: Gonadosomatic Index (GSI) of *C. gariepinus* fed the experimental diets

Parameters	Feed A (Control)	Feed B (BSG)
Mean female body weight (g)	426.28 ± 20.38 ^a	425.32 ± 16.38 ^a
Mean female total length (cm)	39.23 ± 0.44 ^a	39.04 ± 0.12 ^a
Mean female gonad weight (g)	43.42 ± 0.54 ^a	42.96 ± 0.68 ^a
Mean female GSI (%)	9.42 ± 0.24 ^a	9.46 ± 0.16 ^a
Mean male body weight (g)	412.10 ± 6.26 ^a	408.18 ± 2.28 ^a
Mean male total length (cm)	39.25 ± 0.52 ^a	39.02 ± 0.42 ^a
Mean male gonad Weight (g)	3.68 ± 0.12 ^a	3.65 ± 0.20 ^a
Mean male GSI (%)	0.79 ± 0.03 ^a	0.78 ± 0.04 ^a

Values represents the mean ± standard error of the triplicate experimental group. Means with the same superscript are not significantly different (P>0.05). BSG is brewer’s spent grain.

Results of the water quality parameters of the experimental tarpaulin tanks (Table 4) showed that in tarpaulin tank A, (containing fish fed with the control diet) the values obtained for mean water temperature was 29.67 ± 0.33°C, mean dissolved oxygen (4.65 ± 0.25 mg/l), pH (6.92 ± 0.06) and mean ammonia (0.15 ± 0.01 mg/l). In tarpaulin tank B fed with diet B (BSG), mean water temperature ranged from 28.67 ± 0.36°C, mean dissolved oxygen (4.58 ± 0.16 mg/l), pH (6.94 ± 0.04) and mean ammonia (0.15 ± 0.01 mg/l).

Table 4: Water quality parameters of the tarpaulin tanks

Parameters	Tarpaulin A (Control)	Tarpaulin B (BSG)
Mean water temperature (°C)	29.67 ± 0.33 ^a	28.67 ± 0.36 ^a
Mean dissolved oxygen (mg/l)	4.65 ± 0.25 ^a	4.58 ± 0.16 ^a
Mean pH	6.92 ± 0.06 ^a	6.94 ± 0.04 ^a
Mean ammonia (mg/l)	0.15 ± 0.01 ^a	0.15 ± 0.01 ^a

Values represents the mean ± standard error of the triplicate experimental group. Means with the same superscript are not significantly different (P>0.05). BSG is brewer’s spent grain.

DISCUSSION

The fecundity of *C. gariepinus* has been reported by different authors to be influenced by several factors including feed type and quality (Ekanem *et al.*, 2013; Eyo *et al.*, 2016). In this study, there was no significant difference between fecundity of *C. gariepinus* fed the two experimental diets. This indicated that brewers’ spent grain (BSG) waste used as a protein source in *C. gariepinus* diet did not negatively impact fecundity. Shim *et al.*, (1989) opined that the composition and quantity of dietary protein may affect the fecundity of fish. This implies that brewers’ spent grain (BSG) could be a good nonconventional

feed ingredient that can be used as a protein source in catfish feed production. Results of the proximate analysis of the two experimental diets indicates that the two diets were complete in nutrients required for the growth and egg development in *C. gariepinus* such as crude protein, ash, moisture, crude fibre crude fat and nitrogen free extract. Additionally, no significant difference was observed between the two diets in terms on their percentage proximate composition. Gonadosomatic index (GSI) is an important tool used by fisheries and aquaculture scientists to evaluate the reproductive status and condition of fish (Opeh *et al.*, 2018; Eyo *et al.*, 2020).

According to Eyo *et al.*, (2014), gonad development and GSI in *C. gariepinus* could be influenced by feed quality.

However, findings of this study showed that the gonad weight and GSI in both male and female *C. gariepinus* fed with the two diets were not significantly different. The findings of the present study which could be related with the diet quality corroborated with the studies of Ekanem *et al.* (2012) and Eyo *et al.* (2014) who worked on gonad weight and GSI of *C. gariepinus* fed plant and animal-based diets and Coppens and Unical Aqua feed respectively. Egg quality is an important index used in evaluating the quality of feed in fish culture system. According to Izquierdo *et al.* (2001), many factors such as fish size, nutritional state, age, physical condition, and reproductive history have been known to influence the fecundity and egg size in fish. In this study, the mean egg diameter of fish fed with the two experimental diets were not significantly different, indicating that brewer's spent grain did not affect the egg size negatively. The results of water quality parameters were within the recommended range for optimal growth and good health of freshwater fishes (Boyd, 1979).

CONCLUSION

The utilization of brewers' spent grain (BSG) as an alternative source of protein in *C. gariepinus* feed did not impact negatively on the fecundity and gonad development in the present study. This may imply that brewers' spent grain (BSG) can successfully be used as a non-conventional feed ingredient in feeding *C. gariepinus* broodfish.

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