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Egg and Sperm Quality of the African catfish, *Clarias gariepinus* (Burchell) Broodstock Fed Differently Heated Soybean-based Diets

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Abstract: The effects of feeding *Clarias gariepinus* broodstock with differently heated soybean-based diets on the egg and sperm quality was investigated. Four hundred and eighty male and female *C. gariepinus* (182.02 ± 10g for females and 208.27 ± 5g for males) were distributed in groups into hapa nets. Iso-nitrogenous (31% crude protein) and iso-caloric diets (3.34kcal g^{-1}) prepared from raw soybean (D0) and soybean autoclaved for 5, 10, 15, 20, 25 and 30 minutes labeled D0, D5, D10 D15, D20, D25 and D30, were fed to the fish for 84 days. The fishmeal-based diet (DFM) served as control. The broodfish fed the fishmeal-based diet and diet D25 had significantly higher ($p<0.05$) oocyte diameter, milt density, milt volume, sperm motility and higher percentage of egg fertilization and hatching than the fish fed the other diets. The eggs of the specimens fed the control diet and diet D25 also had significantly higher ($p<0.05$) crude protein and lipid contents than those of the fish fed the other diets while the ash content was not significantly different ($p>0.05$). This study showed that diet D25 was found to be the best substitute for fishmeal that provided adequate nutrients required for the formation of virile genital products.

Key words: *Clarias gariepinus*, soybean, milt, sperm, oocytes, fertilization, hatching

INTRODUCTION

The culture of the African catfish, *Clarias gariepinus* in Nigeria is bedeviled by the problem of high mortality in the young stages and the resulting problem of seed scarcity. Unlike some other fish species, *Clarias gariepinus* does not show any parental care, and under pond conditions, the fry become cannibalistic. In addition, there is predation by frogs and other aquatic animals in ponds hence the need for induced spawning. To ensure high fry survival and reproductive success there is need to improve the sperm and egg quality through improved broodstock nutrition.

Little research has been done on sperm physiology and its interaction with the eggs in fertilization especially in tropical fish species. The quality of sperm is highly variable and depends on various external factors such as feeding regime, the quality of the feed and the rearing temperature of the fish [1]. Sperm quality is determined by the sperm motility and fertilization potential. Egg quality, defined as those characteristics of the egg which determine its capacity to survive, is a significant problem for many species of farmed fish and can be manipulated through good broodstock nutrition. Knowledge of the effects of broodstock nutrition on egg production and quality is important because good broodfish feeding leads to successful spawning and good growth and health of the progeny. Studies have shown that the depletion of minerals, omission of vitamins and size of ration affect the viability of eggs in fish [2]. Increased dietary protein content also resulted in increased ovarian size and gonad weight in the guppy, *Poecilia reticulate* [3]. For adequate consideration of the effects of nutrition on sperm and egg quality of *C. gariepinus*, the criteria considered are the oocyte diameter, chemical composition of eggs, milt volume, milt density, sperm motility, and percentage egg fertilization and hatching.

Fishmeal, a major protein source in aquaculture feed production is scarce and expensive, particularly in tropical Africa. The current focus of nutrition work is to reduce protein costs by replacement or supplementation of fishmeal with alternative cheaper protein source. In Nigeria, soybean is the most predominant and readily available plant protein source available for the replacement or supplementation of fishmeal in aquaculture. The suitability of soybean in feed manufacture was attributed to its amino acid profile and high digestibility [4]. Despite these advantages however, soybean has deficiencies of some essential amino acids and contains several anti-metabolites, the most common of which are the trypsin inhibitors. High activity of protease inhibitors in raw or inadequately heated soybean

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meals adversely affects growth and reproductive performance of fish[5]. Heat treatment reduces the level of the anti-nutritional factors. It is therefore pertinent to know how to adequately heat-process soybean to formulate diet for *C. gariepinus* broodstock. The ultimate aim of such exercise is to improve egg, sperm and larval quality and ensure reproductive success.

MATERIALS AND METHODS

Feed formulation and analysis: Portions of full-fatted, dried soybean seeds (SAMSOY 2, TGX 636-02D) were broken into small granules by the mill, winnowed and then autoclaved at 116^0C (pressure 1.2 kg/cm^2 or 16.5 lb/in^2) for 5, 10, 15, 20, 25 or 30 minutes respectively. A portion of the soybean seeds was left un-autoclaved. The differently autoclaved soybean portions an un-autoclaved portion were sun-dried and milled separately to be used along with Herring fishmeal, yellow maize and brewery wastes to compound seven iso-nitrogenous (31%) and iso-caloric (33.4 Kcal kg^{-1}) diets marked D0, D5, D10, D15, D20, D25, D30 (Table 1) to correspond to soybean processing duration. Herring fishmeal was used as the sole protein source for the control diet (DFM). The feedstuffs were properly mixed together and extruded through a 8mm die using a Hobart A-200T mixer. The resulting pellets, which were air-dried, were kept in labeled polythene bags and stored in a deep freezer at 10°C until feeding trial commenced. The proximate composition of the feedstuffs, diets and the eggs of the fish, was determined according to AOAC [6] to confirm the intended computational values. The gross energy was determined by oxygen bomb calorimetry [7].

The Feeding trials: A year old, adult male and female *C. gariepinus* fish (182 ± 10g for females and 208.27 ± 5g for males) were obtained from a fish farm in Osun State of Nigeria. They were kept together in rectangular, cot-like hapas (4mx3mx1m) made from fine mesh nylon mosquito nets. The nets were placed in concrete tanks (6mx5mx1.5m) already filled with untreated water at the Aquaculture Development Centre of the Obafemi Awolowo University, Ile-Ife for two weeks acclimatization.

Thirty healthy and matured specimens of the fish (sex ratio 1:1) all of fairly equal sizes were distributed into each of sixteen labeled fine-meshed nylon hapas designated for the feeding trials at a stocking density of 3 fish per m^2. The fish which were stocked in duplicates for each dietary treatment were then fed the allotted experimental diets at 3% of their body weight calculated on a dry matter basis twice a day between 8.00-9.00 a.m and 6.00-7.00 p.m for a period of 84 days. Renewal of water, cleaning of hapas, ration adjustment and length-weight measurements were done every two weeks.

Breeding: At the end of the feeding trial, five pairs of matured male and female *C. gariepinus* were 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 stipped eggs and spermatozoa from Ovaprim-induced broodfish were immediately spread thinly on the substrate for between 24-36 hours for incubation and hatching.

The fry, weaned on artemia for 10 days, were subsequently fed *ad libitum* three times daily with milled dry prawns (*Parapenaeopsis atlantica*) irrespective of the additional natural planktonic population available to the fry.

Data collection and analysis: At the end of the feeding trials, five females randomly selected per dietary treatment 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 [8]. 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) [1]. 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[8]:

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

(b) % Egg fertilized =

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

(c) %Egg Hatching =

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

Data generated were subjected to a non-parametric t-test (Mann-Whitney U-Wilcoxon Rank Sum W-test) for comparison of means within treatments while the Tukey-HSD one-way ANOVA was used for between treatments comparison at the 5% level of significance[9].

RESULTS AND DISCUSSIONS

The mean volume of milt collected from the testes of the male broodfish fed diets DFM and D25 (0.62ml, 0.55ml) was significantly higher ($p<0.05$) than what was obtained from the testes of the fish fed the other diets (Table 2). There was a strong relationship between the milt volume and the percentage fertilization of the eggs of the fish fed the control diet and D25 respectively ($R^2 = 0.974$, $R^2 = 0.722$). 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 fed DFM, D20 and D25 was higher but not significantly different ($p>0.05$) from those of the other diets.

Table 1: Experimental diet formulations (g/100g), the proximate composition and the gross energy content of the experimental diets.

Feedstuffs	DFM (Control)	D0	D5	D10	D15	D20	D25	D30
Soybean	00.00	33.35	34.43	34.43	34.43	34.43	34.20	34.58
Fishmeal	37.77	04.42	03.34	03.34	03.34	03.34	03.57	03.19
Brewery wastes	37.78	37.78	37.78	37.78	37.78	37.78	37.78	37.78
Yellow Maize	22.45	22.45	22.45	22.45	22.45	22.45	22.45	22.45
Vitamin premix[a]	01.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00
Vegetable oil	01.00	01.00	01.00	01.00	01.00	01.00	01.00	01.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition								
Crude protein	31.17	30.52	30.88	30.45	29.51	30.55	31.20	30.99
Moisture	03.71	06.98	06.97	06.59	05.49	05.06	04.94	04.46
Lipid	05.83	03.44	03.51	04.02	04.65	04.68	04.81	03.82
Ash	11.68	08.10	07.70	06.81	07.22	07.41	07.85	09.33
Crude fibre	01.51	06.05	06.92	07.91	07.81	05.73	05.86	06.22
NFE[b]	46.10	44.91	44.02	44.22	45.32	45.90	46.34	45.18
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ME (Kcal/g)[c]	04.28	03.38	03.43	03.46	03.37	03.86	03.93	03.06

[a]Vitamin premix - A Pfizer livestock product containing the following per kg of feed:
Vit. A, 4 000 000i.u; Vit. D3, 800 000i.u; Vit.E, 10 000mg; Vit. K3; 1 200mg;
Vit.B1, 1 000mg; Vit. B2, 1 500mg; Vit. B6, 1 500mg; Niacin, 10 000mg; Panthothenic acd, 3 500; Biotin 15mg; Vit. B12, 10mg; Folic acid, 200mg; Chlorine chloride, 120 000mg; Manganese, 60 000mg; Iron, 15 000mg; Zinc, 15 000; Copper, 800mg; Iodine, 400mg; cobalt, 80mg; Selenium 40mg; anti-oxidant, 40 000mg.

[b]NFE = Nitrogen-free Extract = 100 – (Crude protein + Crude fibre + Lipid content + Moisture content + Ash)
[c]ME = Metabolizable energy

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 broodfish fed DFM, D20 and D25 was significantly denser ($p>0.05$) than the milt collected from the fish fed the other diets. The fish fed the DFM, D20 and D25 diets had significantly different ($p<0.05$) oocyte diameter than those of the fish fed the other diets (Table 2).

Significantly higher ($p<0.05$) egg fertilization and hatching was recorded for the fish fed diets DFM, D25 than for the fish fed the other diets (Figure 1).

The results showed that as the duration of heating of the soybean component of the diet increased, the percentage egg fertilization and hatching and survival of progeny increased except when the fish were fed diet SB30 where a sharp decrease was recorded for all the parameters.

In all the treatments, there was an increase in the crude protein content, lipid content of the oocytes after feeding (Table 3). The ooocytes of the specimens fed diets DFM and D25 had significantly higher ($p<0.05$) crude protein content and lipid content than the oocytes of the specimens fed the other diets. Contrarily, the oocytes recorded significantly lower ($p>0.05$) moisture content respectively. The oocyte ash content of the fish fed the fishmeal-based diet (DFM) was not significantly different ($p>0.05$) from those of the soybean-based diets.

The observation that sperm motility increased with volume of milt and the strong relationship between milt volume and percentage egg fertilization and hatching

Table 2: Quantity of milt produced (ml fish^{-1}), estimates of the sperm motility, mean milt density and oocyte diameter of *C. gariepinus* fed the different experimental diets.

Diet	Mean milt volume	Mean milt density	Mean sperm motility	Mean oocyte diameter (mm)
DFM	00.62^{a}	02.60^{a}	01.00^{a}	1.80 ± 0.50^{a}
D0	00.33^{bc}	01.40^{b}	00.60^{b}	1.08 ± 0.41^{c}
D5	00.34^{bc}	01.80^{b}	00.80^{b}	1.10 ± 0.43^{c}
D10	00.37^{b}	01.60^{b}	01.00^{a}	1.26 ± 0.41^{c}
D15	00.42^{b}	02.00^{a}	01.00^{a}	1.44 ± 0.27$^{b\,c}$
D20	00.48^{ab}	02.40^{a}	01.00^{a}	1.63 ± 0.13^{ab}
D25	00.55^{a}	02.40^{a}	01.00^{a}	1.65 ± 0.22^{ab}
D30	00.38^{b}	01.60^{b}	00.60^{b}	1.16 ± 0.24^{c}

Values with the same superscript in each column are not significantly different from each other (p>0.05)

Table 3: The proximate composition (% dry matter content) of the oocytes of *C. gariepinus* fed the different experimental diets

	Moisture	Crude Protein	Lipid	Ash
Pre-cultured fish	07.33^{b}	44.52^{b}	02.33^{d}	04.88^{ab}
DFM	02.69^{a}	57.88^{a}	06.20^{a}	05.66^{ab}
D0	12.35^{c}	51.93^{b}	04.04^{c}	05.00^{b}
D5	12.35^{c}	53.38^{b}	03.20^{b}	05.80^{ab}
D10	12.40^{c}	52.29^{b}	03.50^{b}	05.95^{ab}
D15	12.55^{c}	51.93^{b}	03.80^{b}	05.65^{ab}
D20	12.20^{c}	51.7^{b}	04.05^{c}	05.80^{a}
D25	05.27^{ab}	56.59^{a}	05.20^{ac}	05.19^{a}
D30	12.45^{c}	47.93^{b}	03.85^{b}	05.50^{b}

Values with the same superscript in each column are not significantly different from each other (P>0.05)

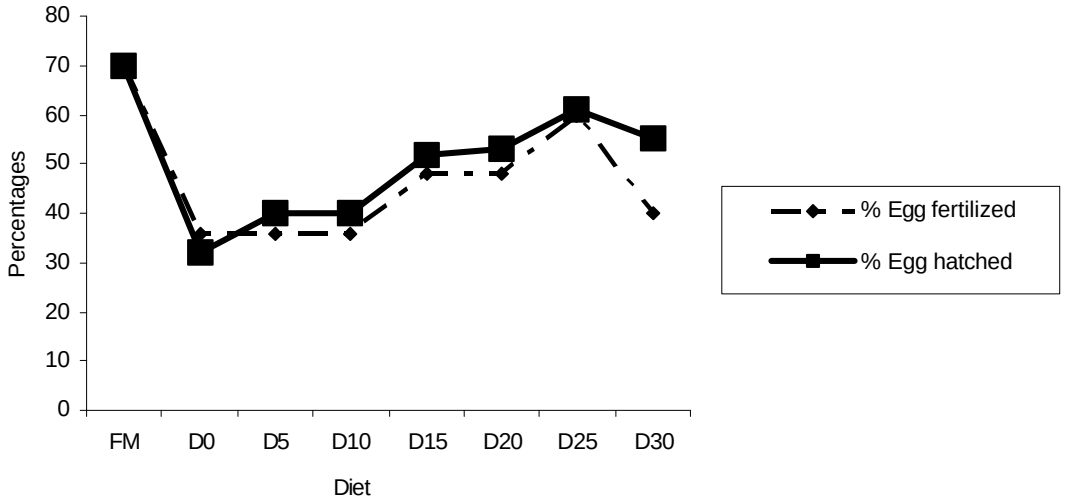

Fig. 1: Percentages of fertilized eggs, hatched eggs and survival of the progeny of *C. gariepinus* fed the different diets.

in *C. gariepinus* agree with the findings of Lamai [10]. Van de Waal and Polling [11] and Lamai [10] also observed that spermatozoa of *C. gariepinus* 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 [1; 12]. According to these authors, the fertilizing capacity is the most conclusive test of sperm quality. However, fertilizing capacity integrates an independent factor, that is, the 'quality' of eggs. The interactions between gametes and between seminal and ovarian fluids also have their own impacts on fertilization.

The significantly higher (p<0.05) egg sizes, higher percentage fertilization and hatching observed in the fish fed the DFM and D25 diets agrees with Cerd© *et al* [13] who reported that sea bass broodstock 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* [14] and Sule and Adikwu [15] also reported that species of the genus *Clarias* 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.

Some authors however opined that egg diameter is not a good indicator of egg and larval quality [16]. In this experiment, the high ovarian crude protein and lipid

recorded for the fish fed the control diet and diet D25 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 following alterations in spawning time [17]. In this experiment, the progeny of the broodstocks fed on the DFM and D25 diets survived better ($p<0.05$) than the ones placed on other diets [18]. Since most of the losses in hatchery are recorded at the critical transitional period of moving from endogenous feeding to exogenous feeding [19], any effort made to improve the quality of the sperm and egg will surely equip the fry for survival.

The poor egg and milt quality performance of the fish fed diets containing either raw, under-heated or overheated soybean corroborates the findings of a number of authors [20; 21], that improperly heated soybean is detrimental to fish.

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