Asian Journal of Biology

7(2): 1-13, 2018; Article no.AJOB.43150
ISSN: 2456-7124

Comparative Evaluation of the Reproductive Indices and Gonadal Development of *Clarias gariepinus* Fed Chicken Offal and Shrimp Based Diets

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Authors' contributions

This work was carried out in collaboration among all authors. Author APJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ROA and EMA managed the analyses of the study. Author EAI managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2018/v7i230047
Editor(s):
(1) Dr. Behrouz behrouzi-Rad, Associate Professor, Department of Environment, Ahvaz Branch, Islamic Azad University, Tehran, Iran.
Reviewers:
(1) Bruno Fiorelini Pereira, University of West of Bahia, Brazil.
(2) Ali Türker, Mugla Sıtkı Kocman University, Turkey.
(3) Emmanuel T. D. Mensah, CSIR-Water Research Institute, Ghana.
Complete Peer review History: http://www.sdiarticle3.com/review-history/43150

Original Research Article

Received 15 May 2018
Accepted 02 August 2018
Published 15 March 2019

ABSTRACT

The study on the reproductive indices and gonadal development of African catfish (*Clarias gariepinus*) fed shrimp based diet (SBD), chicken offal based diet (COBD) and coppens (commercial diet) was carried-out over a 22 weeks period in concrete tanks measuring 3.5 x 1.7 x 1.5 m^3 (8.9 m^3). Forty juveniles with average length of 9.15 ± 0.17 cm and weight of 20.00 ± 4.53 g were stored per group in triplicate, resulting in 360 juveniles in total. Fish were fed daily at 3% of their body weight through-out the duration of the experiment. The nutrient composition of the 3 nutrients differed significantly at $p<0.05$. The dissolved oxygen, temperature, pH and ammonia levels in the culture water with fish fed the 3 diets were within the required level for normal fish growth through-

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out the experiment. The gonadosomatic index (GSI) of the male *C. gariepinus* fed diet A (Coppens feed), diet B (SBD) and diet C (COBD) were 0.35 ± 0.03%, 0.41 ± 0.04% and 0.36 ± 0.02% respectively. Female *C. gariepinus* fed diet A, diet B and diet C had a mean GSI of 1.17 ± 0.26%, 0.88 ± 0.27% and 0.77 ± 0.06% respectively. The male gonad weight and GSI varied significantly between the treatment group fed coppens, shrimp based diet and chicken offal based diet ($p<0.05$), while female gonad weight and GSI did not vary significantly between treatment groups ($p>0.05$). The hepatosomatic index (HSI) of the male *C. gariepinus* fed diet A, diet B and diet C were 1.36 ± 0.07%, 1.18 ± 0.07% and 1.21 ± 0.06% respectively. Female *C. gariepinus* fed diet A, diet B and diet C had a mean HSI of 1.27 ± 0.09%, 1.20 ± 0.06% and 4.27 ± 0.38% respectively. The male and female HSI varied insignificantly between the treatment group at $p>0.05$. Fecundity was highest (3200 ± 717.90 eggs) in fish fed diet A, followed by fish fed diet B (2392 ± 749 eggs) and least in fish fed diet C (1973 ± 184 eggs). The mean fecundity varied significantly between the fish fed the 3 experimental diet at $p<0.05$. Normal arrangement of the oocytes, liver and testis was observed in fish fed COBD and SBD, just as in the case of the group fed coppens. Though coppens feed yielded better fecundity, the use of COBD and SBD is recommended for fish farmers in Nigeria. More researches should be carried out on using varying levels of chicken offals and shrimps in fish feed formulation.

Keywords: Reproductive indices; gonadal development; fecundity; hepatosomatic index; gonadosomatic index; Clarias gariepinus; coppens; chickel offal and shrimp based diet.

1. INTRODUCTION

Aquaculture possesses the capacity to become a sustained farming practice which can add to capture fisheries, and considerably help in feeding the growing population of the world [1]. Currently, majority of aquacultures are undertaken in Asia. In the year 2002, about 70% of global production of reared fish was recorded to have been in China alone [2]. Majority of the cultured fish including shellfish were raised in small-scale traditional mediums that benefits indigenous communities and reduces negative environmental effects. Using culture mediums that are simple and with small inputs, have been utilised for hundreds of decades. The indigenous methods of fish rearing can be significant and impactful as notable in the economic gains.

Presently, an estimated 16% of protein eaten globally is gotten from fish, and more than one billion people world-wide rely on fish as their major protein nourishment. Besides, fish has touched our lives in many ways, serving as a source of food for both humans and other animals [3]. *Clarias gariepinus* is among the largely produced catfish domestically in Belzoni, Mississippi. Economically, *C. gariepinus* has been extensively harvested and cultured for several years in Africa, Asia, Europe and North America as the most excellent food fish [4].

Nutritionally, this fish is rich in vitamin D, low in Omega-3 fatty acids and high in Omega-6 fatty acids which promote growth and brain development. *Clarias gariepinus* has a fast growth performance rate based on tolerance of adverse ecological conditions. African catfish, over the years in Nigeria, has been the species of fish with the highest prospect in fish production. In the year 2004, as stated by [5], it had approximately 32 percent of the overall production. This according to [6] is owing to the fact that the technologies involved is able to operate effectively in the rural set up. Chicken offal meal, also known as poultry by-product meal has been a very essential source of protein incorporated into feeds used in rearing domestic animals. It is used together with meat, bone meal, blood meal, feather meal and fish meal in formulating diets used in feeding fishes, poultry birds, and other intensively grown domestic animals [7].

Fish feed remains one of the significant input in fish production. In Nigeria, the significant challenges confronting the development and growth of aquaculture is lack of feed, the technology of fish feed production is little developed in Africa and some developing Countries [8]. Gabriel, et al. [9] reported that nations such as Namibia, Malawi, Uganda, Kenya, Madagascar, Cote D' ivoire, Ghana and Nigeria are capable of producing their fish feed locally, but produce far below what is required for commercial aquaculture ventures and at inconsistent production rate. The global rate of aquaculture growth seems to be decreasing, it still remains one of the fastest growing animal producing sector that has continued to meet the market demands of the populace, accounting for almost half of the total food fish supply. The

growth rates of aquaculture production are slowing down, due to the impact of factors such as feed, and vary significantly among regions [10]. The study was aimed at assessing the fecundity and reproductive performance of *C. gariepinus* fed chicken offal and shrimp based diets.

2. MATERIALS AND METHODS

2.1 Study Area

This research took place at Andem and Sons Fish Farm Limited, Calabar South Local Government Area, Calabar, Cross River State, Nigeria.

2.2 Collection, Preservation and Preparation of Chicken Offal and Shrimp Waste

The chicken offal used in this study were purchased from the Watt Market and from Mr. Runyi broiler slaughtering farm at the Cross River State Water Board premises, all in Calabar, Cross River State.

The freshly collected offal was thoroughly washed in water carefully to remove the faecal content as much as possible before weighing. The Chicken offal were then par boiled for 30 minutes. It was allowed to cool and then sun dried. Shrimp waste was obtained from the Calabar beach market in the dry form, then packed in a sack bag and kept in a dry place until when needed.

2.3 Diet Ingredient and Formulation Method

Coppens feed produced by Coppens International in Netherlands is made up of good standard ingredients like calcium, methionine, copper sulphate (CuSo4), marine fish meal, phosphorus, lysine, selenium refined fish oil and several grains. Sizes of coppens feed used were 3 mm, 4 mm and 6 mm respectively.

Experimental diet were composed of Soyabean meal (SBM), Chicken offal (CO), Shrimp meal (SHM), Wheat offal (WO), Cassava starch, vitamin premix, bone ash/calcium, Sodium chloride (NaCl), vegetable oil, lysine and methionine.

Two diets were formulated for this experiment, adopting Pearson square system to arrive at a crude level of 42% protein. Various ingredients of feed were grinded and amalgamated properly in accordance with their percentages. After which the feed were pelletised using a Hand Cranker machine into small sizes and oven dried. Soon after drying, the feed were packed in bags and stored. The processes of the feed formulation were all carried out at Aqua Marvels Farms in Calabar. The farm is also a designated centre (Nigeria Markets II) used by the United State Agency for International Development (USAID) for Wet Field Demonstration on improved Aquacultural Practices, which was established in 2014.

2.4 Fish Stocking and Experimental Procedures

Prior to the commencement of the experiment, the tanks were treated with common salt (sodium chloride) (NaCl) for complete extermination of micro-organisms which can pose a threat to the juveniles. After which water was filled and allowed for two weeks then flushed out again before refilling and introduction of the juveniles.

Borehole water was pumped into the tanks by electrical pump and piped into the experimental tanks. Three hundred and sixty juveniles were obtained from the University of Calabar Fish Farm Hatchery complex, University of Calabar and transported to Andem and Sons fish farm in 50 liters water storage tank, where they were allowed to acclimate to the new environmental conditions for about 2 weeks. During this period, they were fed with coppens, twice a day between 7:00 and 8:00 am and 6:00 and 7:00 pm at 3% of their body weight. Forty fish juveniles with average length of 9.15±0.17 cm and average weight of 20.00±4.53 g were stocked per unit. The research was carried-out for 22 weeks (i.e June-November, 2016) using 9 concrete tanks measuring 3.5 x 1.7 x 1.5m^3 (8.9 m^3). The 9 concrete tanks were labelled A_1, A_2, A_3, B_1, B_2, B_3 and C_1, C_2, C_3. Two different types of experimental fish diet were formulated with the addition of Chicken offal (diet C) and shrimp waste (diet B), which were used to compare with Coppens commercial feed (control) (diet A). This scientific study was conducted in triplicates.

Before stocking, the initial length and weight of each fish was accurately measured using a measuring board (to the nearest centimeters) and electronic weighing balance (Metlar mt-5000 D version) to the nearest grams respectively. The culture water was changed every 48 hours, in order to maintain good water quality through-out the experiment.

The fish were sampled bi-weekly to determine their growth and survival (mean body weight, mean total length and mortality). The rations were always adjusted so as to correspond with the new body weight using the weighing balance. The sampling exercises were carried out between 7-8am to minimize heat stress [11]. The dissolved oxygen (DO), hydrogen ion concentration (pH), temperature (°C) and ammonia (NH_3) were monitored through-out the experiment duration. The DO and pH were monitored using Jenway meters; model 3050, England for DO in milligram per liter (mg/L) and model 9070 for pH. Mercury-in-glass thermometer was used to monitor water temperature (degrees celsius). Ice preserved collected water samples were analysed for Ammonia (NH_3) in physico-chemical laboratory of the Cross River State Water board, Calabar using a spectrophotometer in mg/L.

2.5 Proximate Examination of the Experimental Diets

The proximate examination for the 3 test diet was conducted in the Faculty of Agriculture Central Laboratory, University of Calabar following the procedures by [12]. The moisture content, crude protein level, lipid content, carbohydrate and ash content were analyze by the following methods:

Determination of moisture level: A neat crucible was subjected to drying in an oven to a constant weight: (a) before introducing a quantity of sample into a beaker, then weighed after the introduction (b). Next, the sample was dried inside a ventilated heated oven that was powered electrically at 75°C for 24 hours, then allowed to cool in a desiccator and weighed. The procedure was repeated until a constant weight (c) was reached. Same procedure was repeated 3 times for each sample. The percentage moisture level was mathematically calculated using the formula:

$$\% \text{ moisture content} = \frac{b-c}{b-a} x\ 100\%$$

Ash content: The crucible was ignited at 550°C for 3 hours, cooled and weighed. Five grams of the sample was placed in the crucible and weighed. It was burnt at 550°C for a day, then cooled and weighed. Same procedure was carried out over and over again until a constant weight was obtained. The calculation of percentage ash content followed the formula as shown below:

$$\% \text{ Ash content} = \frac{wt\ of\ ash}{wt\ of\ sample} x\ 100$$

Crude fat or ether extract: About five grams of the sample was weighed and put in a thimble. One hundred and twenty milliliters of petroleum ether was emptied into an earlier dried and weighed round bottom flask. Thimbles and its content were then introduced into an extractor known as the soxhlet extractor, and was later fitted into the spherical bottom flask. The condenser together with the extraction apparatus was set up with the flask sitting on the spaces provided on the hot plate and the hot plate was set to gentle heat. With tap on, the ether evaporated and as it condensed, it dropped into a thimble from where the soluble ether contents were extracted into a round bottom flask. The process continued for 10 hours, the thimble was removed and dried in the air and later the fat from the extract was utilize for the determination of fibre. Then, the petroleum ether present in the flask was distilled off and received in the soxhlet extractor tube. Drying of the flask was carried out in an air circulating desiccator for 2 days. The round bottom flask with the lipid extract inside was then weighed. The content inside the flask was dried and weighed to a constant weight. The lipid quantity that was then obtained from the difference between the flask weights previously and later-on as shown below:

$$\% \text{ Ether Extract} = \frac{wt\ of\ extract}{wt\ of\ sample} x\ 100$$

Crude fibre: For acid digestion, the fat free material (8-10 g) was weighed and transferred into a 400 mL beaker that had previously been marked at 200 mL level. Fifty milliliters of sulphuric acid (i:e 1.25%) was added and the mixture rose to 200 mL marked. The beaker together with the content was heated to a boiling point for half an hour. The content of the beaker was then filtered through a Buchner funnel with the aid of a suction pump. The residue was washed with hot water until it was acid-free. For base digestion, the residue left after acid digestion was transferred into 400 mL beaker. The mixture was again heated for 30 minutes with constant stirring. The content of the beaker was filtered through the Buchner funnel and washed several times with hot water until it was free from sodium hydroxide. Finally the residue was washed twice with 95% methanol, quantitatively transferred into a porcelain crucible and dried at 100°C. The weight of the dry residue was noted, and the residue ignited in a furnace at 550°C. The weight of the ash left after ignition

was also noted. The crude fibre content was determined from the loss in weight of crucible and its content after ignition.

Crude protein estimation (6.25 x N) micro kheljahl method: One grams of the sample powder was measured for weight into 50 mL digestive Kjeldahl flask. About 20 mL of concentrated H_2SO_4, 1 tablet of Kjeldahl catalyst and a pinch of anti-bumbing chips were included. Same mixture sample was incinerated into a slowly boiling digestion rock, then subjected to strong heating till the digest appeared clear before heating for a further 3 hours. The digest at this point was removed and allowed to cool, then certain amount of a known quantity was transferred into 100 mL volumetric flask up to the required mark. The Erlenmeyer flask with 100 mL of boric acid solution indicator was placed on the tip of the condenser unit of the distillation apparatus (which had been steam washed) so that the condenser tip extends below the upper layer of the solution. Then 10 mL of the digest sample was put into the dums sample tube and made to undergo steam heating. About 10 mL of NaOH solution at 40% was included in the digest and steam distilled into the Erlenmeyer flask, until the content increased more than double its original quantity. As the ammonia distilled into the boric acid indicator solution, it transformed into green. A black determination was conducted in the same manner as highlighted above, the exception that here, the digested sample was substituted by 0.1 mL of distilled H_2O. The sample inside the Erlenmeyer flask was subjected to titration with 0.1 NH_4Cl to arrive at pink end. Percentage protein was calculated as shown below:

% protein = (Ml HCl (test) – Ml HCl (BLANK)) X normality of acid x $\frac{1.4}{1000} x \frac{100}{10}$x 6.25 x $\frac{100}{0.1}$

2.6 Fecundity and Gonadosomatic Index Estimation

Fecundity estimation: According to [13], 1 g of *C.gariepinus* egg mass contains about 700 eggs. Therefore, the estimation of egg number carried or produced by a female gravid fish (fecundity) was undertaken by the multiplication of the weight of the egg mass by 700.

Gonadal development: Gonadal development was estimated using Gonadosomatic (GSI) index, and calculated according the formula below:

GSI = (Weight of gonad (g)/ Full fish weight (g) x 100 [14].

2.7 Histopathology of Tissues

This was conducted in the Histology Department, University of Calabar Teaching Hospital, Calabar. Tissues (female ovary, male testis and liver) extracted from fish reared with the 3 experimental diets, were subjected to manual tissue processing using the following procedures; fixation, dehydration, clearing, impregnation in wax, blocking out, sectioning, and photomicrography.

Fixation: Tissues were put inside a buffered formalin of 10% for 48 hours, thereafter, washed thoroughly in water to take out excess fixatives.

Dehydration and clearing: Fixed and washed tissues were dehydrated in descending grades of ethanol (30%, 50%, 70%, 90%, and 100%) for at least 2 hours in each change. Alcohol-filled tissues was cleared in xylene to enhance microscopic examination of tissues. Clearing of tissues was done in equal mixtures of choloform and xylene (1/1) and then in pure xylene.

Impregnation in wax and blocking out: Tissues were impregnated in paraffin wax to enhance sectioning with the microtome. The wax was dissolved under 60°C and when melted, tissues were left to be infiltrated for 2 hours. Tissues were embedded in an embedding mold and blocked out on wooden blocks to aid microtomy.

Sectioning and photomicrography of sections: Processed tissues were sectioned in a rotary microtome at 10µm and stained using haematoxylin together with eosin methods.

Photomicrograhs of the stained tissues mounted on glass slides were made digitally with Motic image capture.

2.8 Statistical Analysis

Data obtained were subjected to descriptive analysis (mean and standard deviation). Analysis of variance (ANOVA) was also used to test for the significance of difference between the nutrient composition, mean gonadosomatic and hepatosomatic indices of fish fed 3 different diets. ANOVA was also used to test for the significance of difference in proximate composition between the 3 treatments diets

using Version 20 of predictive Analytical Software (PASW) and Ms Excel 2013 at 0.05 level of significance and at their relevant degree of freedom.

3. RESULTS

3.1 Component and Proportion of Formulated Diets

The summary of the final component and proportion of the formulated shrimp-based diet (SBD) (diet B) and chicken offals based diet (COBD) (diet C) is shown in Table 1. Diet B contained 360 g (36%) of shrimp meal, 360g (36%) of soybean meal, 120 g (12%) of yellow maize, 120 g (12%) of wheat offal, 2.5 g (0.25%) of methionine, 2.5 g (0.25) of lysine, 5 g (0.5%) of bone ash/calcium, 15g (1.25%) of vitamin premix, 5 g (0.5%) of sodium chloride, 5 g (0.5%) of cassava starch and 5 g (0.5%) of palm oil (Table 1).

Diet C contained 370 g (37%) of chicken offal, 370 g (37%) of soybean meal, 110 g (11%) of yellow maize, 110 g (11%) of wheat offal, 2.5 g (0.25%) of methionine, 2.5 g (0.25) of lysine, 5 g (0.5%) of bone ash/calcium, 15 g (1.25%) of vitamin premix, 5 g (0.5%) of sodium chloride, 5 g (0.5%) of cassava starch and 5 g (0.5%) of palm oil (Table 1).

3.2 Proximate Composition of Experimental Diet

The summary of the mean proximate composition of the experimental diet is shown in Table 2. Mean proximate analysis of the dry matter (mg/100 g) of the three experimental diets showed that crude protein content was highest (40.61 ± 0.13%) in diet A (Coppens), followed by diet C (chicken offal based diet) with 38.15 ± 0.16% and least (37.00 ± 0.32%) in diet B (shrimp based diet). Mean ether extract was highest in diet A (11.71 ± 0.10%), followed by diet C (10.00 ± 0.30%) and least in diet B (6.70 ± 0.12%). Mean crude fibre was also highest in diet A (7.43 ± 0.01%), followed by diet B (5.13 ± 0.13%), and least in diet C (4.30 ± 0.33%).

Table 1. Final component and proportion of formulated diets

Ingredients	Diet B		Diet C	
	Amount in g	(%)	Amount in g	(%)
Chicken offal (CO)	---	---	370	37
Shrimps meal (SHM)	360	36	---	---
Soybeans meal (SBM)	360	36	370	37
Yellow maize (YM)	120	12	110	11
Wheat offal (WO)	120	12	110	11
Methionine	2.5	0.25	2.5	0.25
Lysine	2.5	0.25	2.5	0.25
Bone ash/calcium	5	0.5	5	0.5
Vitamin premix	15	1.25	15	1.25
Sodium chloride (NaCl)	5	0.5	5	0.5
Cassava Starch	5	0.5	5	0.5
Palm oil	5	0.5	5	0.5
Total in g/kg	1kg		1kg	

Diet B= Shrimp-based diet (SBD), Diet C = Chicken Offal-based diet (COBD)

Table 2. Mean proximate composition of the experimental diets

Indices	Diet A (Control) (coppens)	Diet B (SBD)	Diet C (COBD)
Crude protein (%)	40.61 ± 0.13^{a}	37.00 ± 0.32^{b}	38.15 ± 0.16^{c}
Ether extract (%)	11.71 ± 0.10^{a}	6.70 ± 0.12^{b}	10.00 ± 0.30^{c}
Crude fibre (%)	7.43 ± 0.01^{a}	5.13 ± 0.13^{b}	4.30 ± 0.33^{c}
Ash (%)	9.10 ± 0.12^{a}	6.67 ± 0.33^{b}	5.00 ± 0.00^{c}
Moisture (%)	8.50 ± 0.21^{a}	17.37 ± 0.3^{b}	14.84 ± 0.14^{c}
NFE (%)	22.68 ± 0.13^{a}	27.13 ± 0.23^{b}	27.76 ± 0.56^{c}

**SBD = Shrimp-based diet, COBD = Chicken Offal-based diet, NFE = Nitrogen Free Extract*
Values are in mean ± standard deviation
Values with different superscript are significantly different at $p<0.05$

Also, mean ash content was maximum in diet A (9.10 ± 0.12 %), followed diet B (6.67 ± 0.33 %), and least in diet C (5.00 ± 0.00%). Mean moisture content was highest in diet B (17.37 ± 0.31%), followed by diet C (14.84 ± 0.14 %) and least diet A (8.50 ± 0.21%). Mean nitrogen free extract (NFE) was also greater in diet C (27.76 ± 0.56%), followed by diet B (27.13 ± 0.23%) and least in diet A (22.68 ± 0.13%) (Table 2). Statistically, the nutritional composition varied significantly between coppens, shrimp based diet and chicken offal based diet at $p<0.05$ (Table 2).

3.3 Water Quality of Culture Water

The summary of the mean water quality of culture water in tank with fish fed 3 diets is shown in Table 3. For the fish group fed Diet A, the temperature of the water ranged from 27.27 – 32.83°C, with a mean and standard deviation of 29.975 ± 0.291°C, while pH ranged from 6.87 – 7.40, with a mean and standard deviation of 7.082 ± 0.144. The dissolved oxygen (DO) ranged from 3.33 – 5.33 mg/L, with a mean and standard deviation of 4.648 ± 0.603 mg/L, while the ammonia level ranged from 0.00 – 0.17 mg/L, with a mean and standard deviation of 0.133 ± 0.048 mg/L (Table 3).

For the fish group fed Diet B, the temperature of the water ranged from 27.33 – 33.46°C, with a mean and standard deviation of 30.099 ± 0.380°C, while pH ranged from 6.91 – 7.19, with a mean and standard deviation of 7.085 ± 0.088. The dissolved oxygen (DO) ranged from 3.37 – 5.34 mg/L, with a mean and standard deviation of 4.188 ± 1.370 mg/L, while the ammonia level ranged from 0.00 – 0.20 mg/L, with a mean and standard deviation of 0.130 ± 0.052 mg/L (Table 3).

For fish fed Diet C, the temperature of the water ranged from 27.30 – 33.10°C, with a mean and standard deviation of 30.111 ± 0.278°C, while pH ranged from 6.93 – 7.27, with a mean and standard deviation of 7.089 ± 0.119. The dissolved oxygen (DO) ranged from 3.76 – 5.34 mg/L, with a mean and standard deviation of 4.574 ± 0.559 mg/L, while the ammonia level ranged from 0.00 – 0.17 mg/L, with a mean and standard deviation of 0.126 ± 0.045 mg/L (Table 3).

The water temperature, pH, DO and ammonia level of culture water with fish fed the 3 diets were all within the range suitable for a healthy living of fish. Statistically, the temperature, pH, DO and ammonia levels varied significantly between the culture water with fish fed the 3 diets at $p<0.05$ (Table 3).

3.4 Gonadosomatic Index (GSI) of Fish Fed Experimental Diets

The summary of the Gonadosomatic Index (GSI) of fish fed 3 different feeds is shown in Table 4. Male *C. gariepinus* fed diet A had a mean total length of 41.37 ± 0.67 cm, weight of 457.94 ± 17.42 g, mean gonad weight of 6.19 ± 0.33 g and a mean GSI value of 0.35 ± 0.03%. Male fish juveniles administered diet B had a mean total length of 38.97 ± 0.58 cm, mean total weight of 384.94 ± 15.89 g, mean gonad weight of 4.56 ± 0.35 g and a GSI mean value of 0.36 ± 0.02%. Male fish juveniles fed diet C had a mean total length of 38.91 ± 0.45 cm, mean total weight of 389.79 ± 5.34 g, mean gonad weight of 4.74 ± 0.31g and a GSI mean value of 0.41 ± 0.04 % (Table 4).

Table 3. Mean physico-chemical parameters of water in each treatment tank

Water parameters	Tank A (control) (coppens)	Tank B (SDB)	Tank C (COBD)	FAO limit
Temperature (°C)	29.975 ± 0.291^{a} (27.27 – 32.83)	30.099 ± 0.380^{b} (27.33 – 33.46)	30.111 ± 0.287^{c} (27.30 – 33.10)	<40
Ph	7.082 ± 0.144^{a} (6.87 – 7.40)	7.085 ± 0.088^{b} (6.91 – 7.19)	7.089 ± 0.119^{c} (6.93 – 7.27)	6 – 9
Dissolved oxygen (mg/L)	4.648 ± 0.603^{a} (3.33 – 5.33)	4.188 ± 1.370^{b} (3.37 – 5.34)	4.574 ± 0.559^{c} (3.76 – 5.34)	>4
Ammonia (NH_3) (mg/L)	0.133 ± 0.048^{a} (0.00 – 0.17)	0.130 ± 0.052^{b} (0.00 – 0.20)	0.126 ± 0.045^{c} (0.00 – 0.17)	<1

**SBD = Shrimp-based diet, COBD = Chicken Offal-based diet*
Values are in mean ± standard deviation
Ranges are in parenthesis ( )
Values with different superscript are significantly different at p<0.05

Table 4. Mean gonadosomatic indices of *C. gariepinus* fed experimental diets

Gonad Indices	Diet A (Control) (Coppens)	Diet B (SBD)	Diet C (COBD)
Male total length (cm)	41.37±0.67	38.97±0.58	38.91±0.45
Male total weight (g)	457.94±17.42	384.94±15.89	389.79±5.34
Male gonad weight (g)	6.19±0.33[a]	4.56±0.35[b]	4.74±0.31[c]
Male GSI (%)	0.35±0.03[a]	0.36±0.02[b]	0.41±0.04[c]
Female total length (cm)	37.40±0.55	38.35±0.56	37.29±0.53
Female total weight (g)	382.42±13.59	390.50±7.39	364.18±8.46
Female gonad weight (g)	4.57±1.03[a]	3.41±1.07[a]	2.81±0.26[a]
Female GSI (%)	1.17±0.26[a]	0.88±0.27[a]	0.77±0.06[a]

Values are in mean ± standard deviation
Values with different superscript are significantly different (P<0.05).
SBD = Shrimp-based diet, COBD = Chicken Offal-based diet

Female *C. gariepinus* fed diet A had a mean total length of 37.40 ± 0.55 cm, mean weight of 382.42 ± 13.59 g, mean gonad weight of 4.57 ± 1.03 g and a GSI mean value of 1.17 ± 0.26%. Female fish fed diet B had a mean total length of 38.35 ± 0.56 cm, mean weight of 390.50 ± 7.39 g, mean gonad weight of 3.41 ± 1.07 g and a GSI value of 0.88 ± 0.27%. Female fish fed diet C had a mean total length of 37.29 ± 0.53 cm, mean weight of 364.18 ± 8.46 g, mean gonad weight of 2.81 ± 0.26 g and a GSI value of 0.77 ± 0.06 % (Table 4).

Statistically, the male gonad weight and male GSI varied significantly between the treatment group fed coppens, shrimp based diet and chicken offal based diet at $p<0.05$, while female gonad weight and female GSI did not vary significantly between treatment groups at $p>0.05$ (Table 4).

3.5 Hepatosomatic Index (HSI) of *C. gariepinus* Fed Experimental Diets

The summary of the hepatosomatic index (GSI) of fish fed 3 different feeds is shown in Table 5. Mean hepatosomatic index of *C. gariepinus* juveniles cultured with the experimental diets showed that in male, highest liver weight (6.19 ± 0.33 g) and HSI (1.36 ± 0.07%) was obtained in juveniles fed diet A, followed by fish fed diet C which had a mean liver weight of 4.74 ± 0.31 g and a HSI figure of 1.21 ± 0.06%. The reverse was the case in fish fed diet B, which had the least mean liver weight of 4.56 ± 0.35 g and a mean HSI value of 1.18 ± 0.07 % (Table 5).

In female *C. gariepinus*, mean liver weight and HSI was highest (4.92 ± 0.31 g and 1.27 ± 0.09 % respectively) in fish fed diet B, followed by fish fed diet A which had a mean liver weight of 4.57 ± 0.25 g and mean HSI value of 1.20 ± 0.06%. It was least in fish fed diet C with a mean liver weight of 4.27 ± 0.38 g and a mean HSI value of 4.27 ± 0.38 % (Table 5).

Statistically, the male liver weight varied significantly ($p<0.05$), while female liver weight, male hepatosomatic index and female hepatosomatic index varied insignificantly between the treatment group fed coppens, shrimp based diet and chicken offal based diet at $p>0.05$ (Table 5).

Table 5. Mean hepatosomatic index indices of *C. gariepinus* fed experimental diets

Gonad Indices	Diet A (Control)	Diet B (SBD)	Diet C (COBD)
Male total length (cm)	41.37±0.67	38.97±0.58	38.91±0.45
Male total weight (g)	457.94±17.42	384.94±15.89	389.79±5.34
Male liver weight (g)	6.20±0.33[a]	4.56±0.35[b]	4.74±0.31[c]
Male HSI (%)	1.36±0.07[a]	1.18±0.07[a]	1.21±0.06[a]
Female total length (cm)	37.40±0.55	38.35±0.56	37.29±0.53
Female total weight (g)	382.42±13.59	390.50±7.39	364.18±8.46
Female liver weight (g)	4.57±0.25[a]	4.92±0.31[a]	4.27±0.38[a]
Female HSI (%)	1.20 ±0.06[a]	1.27±0.09[a]	1.16±0.08[a]

Values are in mean ± standard deviation
Values with different superscript are significantly different (P<0.05).
SBD = Shrimp-based diet, COBD = Chicken Offal-based diet

3.6 Fecundity of Fish Fed with the Experimental Diets

The summary of the fecundity of fish fed the 3 different diets is shown in Table 6. Fecundity of fish reared with the experimental diets was highest (3200 ± 717.90 eggs) in fish fed the control feed (diet A), followed by fish fed feed B with 2392 ± 749 eggs and least in fish fed Feed C with 1973 ± 184 eggs. The mean fecundity varied significantly between the fish fed the 3 experimental diet at $p<0.05$ (Table 6).

3.7 Histological Sections of *C. gariepinus* Tissues Fed Experimental Feeds

The histological representation of the tissues of fish fed the 3 experimental diets is shown in Plates 1 – 4. The result for the histology of ovaries, testes and liver of fish fed diet A (Coppens feed), diet B (shrimp based diet) and diet C (chicken offal based diet) showed normal changes in their developments. The oocytes were fully matured in *C. gariepinus* fed with the three test diets (Plate 1). Similarly, the interstitial cells of the testes of *C. gariepinus* fed the three experimental feeds showed normal testicular cells (Plate 2). Plate 3 shows the testes and ovaries of *C. gariepinus* reared with the 3 test diets. The result of the histology of the liver of fish fed with the three experimental feed also revealed normal distribution of the liver cell (hepatocytes) with reduced vacuolization (Plate 4).

4. DISCUSSION

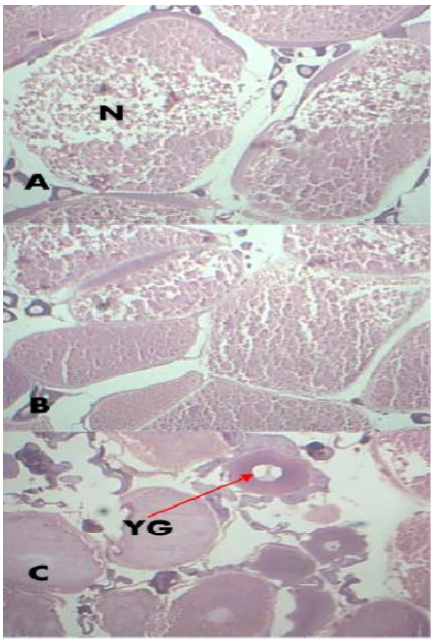
Aquaculture possesses the capacity to become a sustained farming practice which can add to capture fisheries, and considerably help in the feeding the growing population of the World [1]. The nutritional composition of the 3 feeds used differed in this study, with coppens being the best from the nutritional point of view, but all 3 feeds were well formulated to have a balanced diet. The differences in the nutritional levels of the 3 diets could be due to the difference in the components of the 3 diets. This corroborated with the findings of [15,16], who both purported that, the nutritional constituents of fish meal can vary depending on the part being processed.

The study revealed that utilization of good quality feeds plays a vital role in gonad development of *C. gariepinus*. Gonad form the micro environment enabling the germ cell to differentiate into ripe male and female gametes. [17] reported that in adult fish spermatogonia and oogonia differentiate into mature spermatozoa and sperm cell respectively. Before sex differentiation in fish, the undifferentiated gonad contains all the cell types required to make it capable of developing into either a testis or an ovary [18]. The study revealed that sub-adults of *C. gariepinus* had developed matured gonad under standard outdoor circular tank condition for the 3 treatment groups, with the phyico-chemical characteristics such as, pH, dissolved oxygen, water temperature being within the normal level as recommended by [19] for fresh water fish culture. The factors of the environment, namely: temperature, photoperiod, nutrient supply, dissolved oxygen, disease (parasites) etc. are observed to influence gametogenesis (process by which gamete (sperm and egg) are produced from the gonia of matured gonad during reproductive cycle) in fish [20]. Thus their effective management is important for sustainable aquaculture especially in relation to management of broodstock egg and larval quality [21,22].

Table 6. Fecundity of fish fed 3 different experimental diets

Indices	Diet A (Coppens)	Diet B (SBD)	Diet C (COBD)
Female total length (cm)	37.40±0.55	38.35±0.56	37.29±0.53
Female total weight (g)	382.42±13.59	390.50±7.39	364.18±8.46
Female ovary weight (g)	$4.57±1.03^{a}$	$3.41±1.07^{b}$	$2.81±0.26^{c}$
No. of females	14	12	11
Mean fecundity	$3200 ± 717.90^{a}$	$2392 ± 749^{a}$	$1973 ± 184^{a}$

Values are in mean ± standard deviation
Values with different superscript are significantly different (P<0.05).
SBD = Shrimp-Based Diet
COBD = Chicken Offal-Based Diet



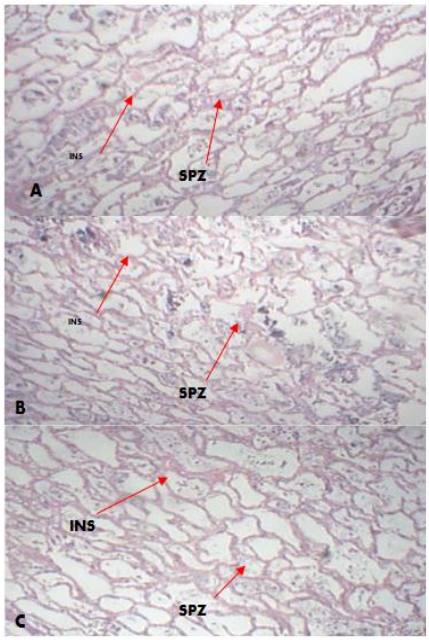


Plate 1 **Plate 2**

Histological sections of oocytes (Plate 1) and testes (Plate 2) of *C. gariepinus* reared with the experimental diets. Diet A, Diet B and Diet C showing normal structure of ovarian lamellae, which contains oocytes. N: nucleus and YG: Yolk globules (plate 1) as well as showing normal structure of testicular cell (spermatozoa) (plate 2) (X 40, H&E stains)

Plate 3. Ovaries (up) and testes (down) of *C. gariepinus* reared with the three test feeds. From left to right: Fish fed Diet A, Diet B and Diet C

In the present study, maturation in the male and female *C. gariepinus* (development of genital papilla and spermatozoa) was visibly noticed early. According to [23], the early maturation in fish can be achieved chiefly by better nutrition or genetic selection. The male gonad weight and GSI varied significantly between the treatment group fed coppens, shrimp based diet and chicken offal based diet at $p<0.05$, while female gonad weight and GSI did not vary significantly between treatment groups at $p>0.05$. Also, the male liver weight varied significantly ($p<0.05$), while female liver weight, male and female HSI varied insignificantly between the treatment group fed coppens, shrimp based diet and chicken offal based diet at $p>0.05$, and these variations could be due to the difference in the nutrient composition of the 3 diets. Female *C. gariepinus* given diet A, had the highest GSI value followed by Female fish fed diet B and then Female fish administered diet C. Body size did not affect gonad development as observed in both male and female fish fed the three experimental diets, and these findings are similar to that of [23] who opined that maturity is link to age in *C. gariepinus* but disagrees with [24] whose observation was the reverse (i.e maturity is linked to size rather than age).

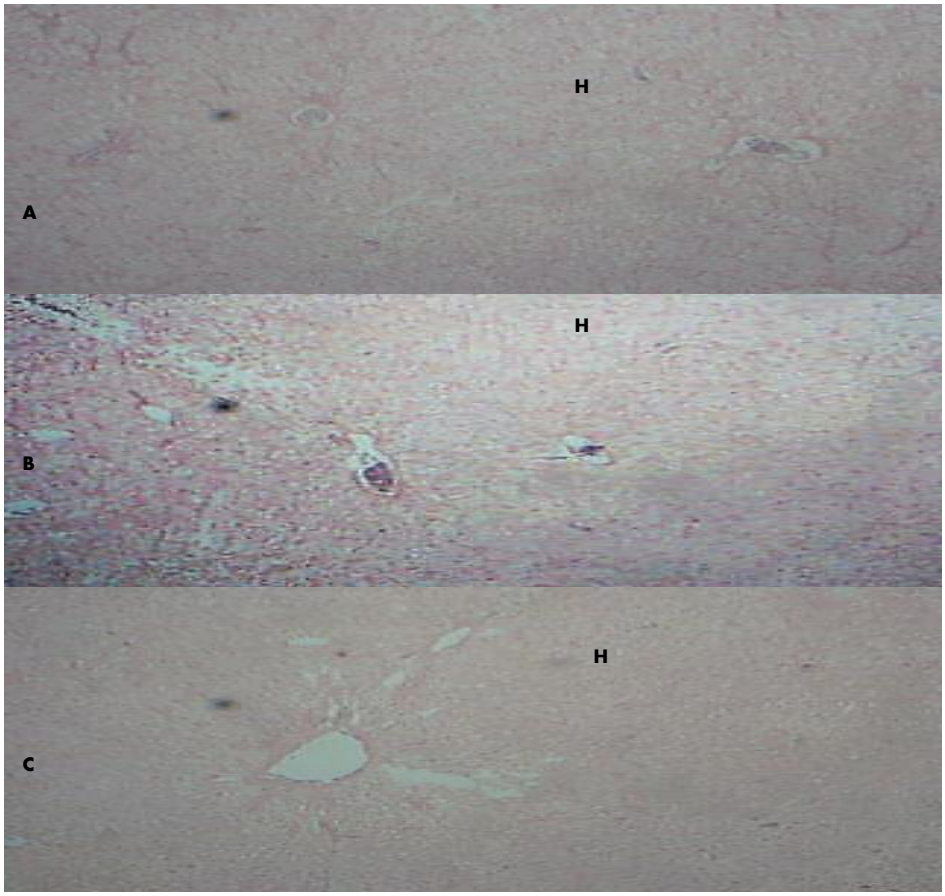


Plate 4. Histological sections of *C. gariepinus* Liver reared with the experimental diets. Diets A, B and C showing normal distribution of the liver cell (hepatocytes) with reduced vacoulization (X 40, H&E stains)

According to [25], for good gonadal development, the dietary protein level must be stepped up to 40% in catfish diets, which concurred with that of [26], who pointed out that catfish broodstock performance can be influenced by dietary protein level. [27] also reported that increasing dietary crude protein content resulted in high values of fish egg weight. Utilization of experimental feeds by the experimental fish stimulated an early maturation as observed in their early gonadal development. This corresponds with the findings of [28], who reported that nutrients serve as a cornerstone for fish growth and therefore should be able to be digested and absorbed in a form that makes them available to provide energy. The study revealed the normal arrangement of the oocytes, liver and testis in fish fed Diet B and C, just as in the case of group fed coppens.

Fecundity, which is the amount of eggs conveyed by a female gravid fish is an essential area of farming that specifically deals with the reproductive potentials of fish. In the present research work, it was observed that the amount of eggs of *C. gariepinus* fed Diet A, B and C was not significantly ($P>0.05$) different. This indicates that Diet B and C was as good as Diet A (coppens) and should be used in aquaculture since the fecundity and fish gonad development assessment helps in evaluating the reproductive potentials of fish species individually [29].

Although the fish fed coppens was the best feed, as indicated by a better fecundity, the 2 formulated feeds (COBD and SBD) also ensured remarkable gonadosomatic index, hepatosomatic index and fecundity in the fish, and as such should be used in aquaculture.

5. CONCLUSION

Diet B and C competed positively with Diet A (coppens) regarding nutritional composition,

fecundity and gonadal development of *C. gariepinus*. Despite the fact that coppens yielded better nutritional richness, fecundity and gonadal development, fish fed SBD and COBD to maturity can be used as a reliable broodstock. Though the use of coppens feed for catfish farming is more productive, it is more expensive and as a result, a formulated diet using SBD and COBD should be used by fish farmers in Nigeria. More researches should be carried out on using SBD and COBD in fish feed formulation.

ETHICAL APPROVAL

The authors ensured that all the ethical, other basic principles underlying behavior and advancing welfare for the use of animals in research including; handling, relevant laws and regulations were considered before proceeding with the research. Permission was also received from the relevant bodies for the use of fish for this experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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