

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

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 was carried-out for 22 weeks in concrete tanks measuring 3.5 x 1.7 x 1.5m³. Forty juveniles with average length of 9.15 ± 0.17cm and weight of 20.00 ± 0.00g 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-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 (Coppens feed), diet B (SBD) and diet C (COBD) had a mean GSI of 1.17 ± 0.26 %, 0.88 ± 0.27 % and 0.77 ± 0.06 % respectively. Statistically, the male and female GSI varied significantly between the group fed coppens, SBD and COBD at p<0.05. The hepatosomatic index (HSI) of the male *C. gariepinus* fed diet A (Coppens feed), diet B (SBD) and diet C (COBD) were 1.36 ± 0.07 %, 1.18 ± 0.07 % and 1.21 ± 0.06 % respectively. Female *C. gariepinus* fed diet A (Coppens feed), diet B (SBD) and diet C (COBD) 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 (Coppens feed), followed by fish fed feed B (SBD) (2392 ± 749 eggs) and least in fish fed Feed C (COBD) (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, chicken 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 utilized for hundreds of decades. The indigenous methods of fish rearing can be significant and impactful as

notable in the gains economically. 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. Beside, fish has touched our lives in many ways, serving as a source of food for both humans and other animals. Worldwide intake of fish as food has grown from 40 million tons in 1970 to 86 million tons in 1998 [3]. Globally, *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. *C. 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 remain one of the major input in fish production. In Nigeria, the major 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]. [9] reported that nations such as Namibia, Malawi, Uganda, Kenya, Madagascar, Cote D' ivoire, Ghana and Nigeria are capable of producing their own fish feed locally, but produce far below what is required for commercial aquaculture ventures, and the quality is poor and production rate inconsistent. 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 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, located in Calabar South Local Government Area, Calabar, Cross River State, Nigeria.

81 2.2 Collection, preservation and preparation of chicken offals and shrimp waste

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

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

90 2.3 Diet ingredient and formulation method

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

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

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

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)

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 refilled and allowed for two weeks then flushed out again before refilling and introduction of the juveniles.

Borehole water was the source of water, 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, 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 twice a day between 7:00 and 8:00am in the morning and 6:00 and 7:00pm in the evening at 3% of their body weight. Forty fish juveniles with average length of 9.15 ± 0.17 cm and average weight of 20.00 ± 0.00 g were stocked per unit. The research was undertaken for 22 weeks (i.e June-November, 2016). Nine concrete tanks measuring $3.5 \times 1.7 \times 1.5$ m³ were used. The 9 concrete tanks, labelled A₁, A₂, A₃, B₁, B₂, B₃ and C₁, C₂, C₃, making it nine experimental units to aid replication of the experiment. Two different types of experimental fish diet were formulated with the addition of Chicken offal (diet C), shrimp waste (diet B) and 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-5000D 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 survival (mean body weight, mean total length and mortality). The rations was always adjusted so as to correspond with the new body weight using the balance. The sampling exercises were carried out in the morning hours between 7-8am to minimize heat stress [11]. The dissolved oxygen (DO), hydrogen ion concentration (pH), temperature (°C) and ammonia (NH₃) 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 was analysed for Ammonia (NH₃) in physic-chemical laboratory of the Cross River State Water board, Calabar using 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, then weighed. The procedure was repeated until a constant weight (c) was reached. Same procedure was repeated three times for each sample. The percentage moisture level was mathematically calculated using the formula:

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

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, cooled then 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:

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

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. An extractor known as the soxhlet extractor into which the thimbles and its

content had been introduced, sooner became fitted into the spherical bottom flask and the condenser together with the extraction apparatus was set up with the flask sitting on the spaces provided on the hot plate. 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 it extracted the soluble ether contents into a round bottom flask. The process continued for 10 hours, the thimble was removed and dried in the air (later the fat from the extract was utilize for the determination of fibre). Then, 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 two days. The circular 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 obtained from the difference between the flask weights previously and later-on as shown below:

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

Crude fibre:

For acid digestion, the fat free material (8-10g) was weighed and transferred into a 400mL 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 H₂SO₄, 1 tablet of Kjeldahl catalyst and a pinch of anti-bumping chips were included. Same mixture sample was incinerated into a slowly boiling digestion rock, then subjected to strong heating till the digest appeared clear, and then heated for a further 3 hours. The digest at this point was removed and allowed to get cooled,

then certain amount of a known quantity was transferred into 100 mL volumetric flask up to a required mark or point. 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 till 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, exception that here, the digested sample was substituted by 0.1 mL of distilled H₂O. The sample inside the Erlenmeyer flask was subjected to titration with 0.1 NH₄Cl to arrive at pink end. Percentage protein was calculated as shown below:

$$\% \text{ protein} = (\text{MI HCl (test)} - \text{MI HCl (BLANK)}) \times \text{normality of acid} \times \frac{1.4}{1000} \times \frac{100}{10} \times 6.25 \times \frac{100}{0.1}$$

2.6 Fecundity and gonadosomatic index estimation

Fecundity estimation

According to [13], 1g 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:

$$\text{GSI} = (\text{Weight of gonad (g)} / \text{Full fish weight (g)}) \times 100 \text{ [14].}$$

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 was 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 was 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.

Photomicrographs 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 Proximate composition of experimental diet

The summary of the mean proximate composition of the experimental diet is shown in Table 1. Mean proximate analysis of the dry matter (mg/100g) of the three experimental diets showed that crude protein content was highest ($40.61 \pm 0.13\%$) in diet A (Coppens), followed by diet C (chicken offal based diet) with $38.15 \pm 0.16\%$ and least ($37.00 \pm 0.32\%$) in diet B (shrimp based diet). Mean ether extract was highest in diet A ($11.71 \pm 0.10\%$), followed by diet C ($10.00 \pm 0.30\%$) and least in diet B ($6.70 \pm 0.12\%$). Mean crude fibre was also highest in diet A ($7.43 \pm 0.01\%$), followed by diet B ($5.13 \pm 0.13\%$), and least in diet C ($4.30 \pm 0.33\%$).

266 **Table 1: 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

267 *SBD = Shrimp-based diet, COBD = Chicken Offal-based diet, NFE = Nitrogen Free Extract

268 Values are in mean ± standard deviation

269 Values with different superscript are significantly different at p<0.05

271 Also, mean ash content was maximum in diet A (9.10 ± 0.12 %), followed diet B (6.67 ± 0.33
272 %), and least in diet C (5.00 ± 0.00%). Mean moisture content was highest in diet B (17.37 ±
273 0.31%), followed by diet C (14.84 ± 0.14 %) and least diet A (8.50 ± 0.21%). Mean nitrogen
274 free extract (NFE) was also greater in diet C (27.76 ± 0.56%), followed by diet B (27.13 ±
275 0.23%) and least in diet A (22.68 ± 0.13%) (Table 1).

276 Statistically, the nutritional composition varied significantly between coppens, shrimp
277 based diet and chicken offal based diet at p<0.05 (Table 1).

278 3.2 Water quality

279 The dissolved oxygen, temperature, pH and ammonia levels in the culture water with
280 fish fed the 3 diets were within the required level for normal fish growth through-out the
281 experiment.

282 3.3 Gonadosomatic index (GSI) of fish fed experimental diets

283 The summary of the gonadosomatic index (GSI) of fish fed 3 different feeds is shown
284 in Table 2. Male *C. gariepinus* fed diet A (Coppens feed) had a mean total length of 41.37 ±
285 0.67 cm, weight of 457.94 ± 17.42 g, mean gonad weight of 6.19 ± 0.33 g and a mean GSI
286 value of 0.35 ± 0.03 %. Male fish juveniles administered diet B (shrimp based diet) had a
287 mean total length of 38.97 ± 0.58 cm, mean total weight of 384.94 ± 15.89 g, mean gonad
288 weight of 4.56 ± 0.35 g and a GSI mean value of 0.36 ± 0.02 %. Male fish juveniles fed diet
289 C (chicken offal based diet) had a mean total length of 38.91 ± 0.45cm, mean total weight of
290 389.79 ± 5.34 g, mean gonad weight of 4.74 ± 0.31g and a GSI mean value of 0.41 ± 0.04 %
291 (Table 2).

294 **TABLE 2: 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 ^b	2.81±0.26 ^c
Female GSI (%)	1.17±0.26 ^a	0.88±0.27 ^b	0.77±0.06 ^c

295 Values are in mean ± standard deviation

296 Values with different superscript are significantly different (P<0.05).

297 SBD = Shrimp-based diet, COBD = Chicken Offal-based diet

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300 Female *C. gariepinus* fed diet A (Coppens feed) had a mean total length of 37.40 ±
301 0.55 cm, mean weight of 382.42 ± 13.59 g, mean gonad weight of 4.57 ± 1.03 g and a GSI
302 mean value of 1.17 ± 0.26 %. Female fish fed diet B had a mean total length of 38.35 ± 0.56
303 cm, mean weight of 390.50 ± 7.39 g, mean gonad weight of 3.41 ± 1.07 g and a GSI value of
304 0.88 ± 0.27 %. Female fish fed diet C had a mean total length of 37.29 ± 0.53cm, mean
305 weight of 364.18 ± 8.46 g, mean gonad weight of 2.81 ± 0.26 g and a GSI value of 0.77 ±
306 0.06 % (Table 2).

307 Statistically, the male gonad weight, female gonad weight, male and female GSI all
308 varied significantly between the treatment group fed coppens, shrimp based diet and chicken
309 offal based diet at p<0.05 (Table 2).

310 **3.4 Hepatosomatic index (HSI) of *C. gariepinus* fed experimental diets**

311 The summary of the hepatosomatic index (GSI) of fish fed 3 different feeds is shown
312 in Table 3. Mean hepatosomatic index of *C. gariepinus* juveniles cultured with the
313 experimental diets showed that in male, highest liver weight (6.19 ± 0.33 g) and HSI (1.36 ±
314 0.07 %) was obtained in juveniles fed diet A (Coppens feed), followed by fish fed diet C
315 (chicken offal based diet) which had a mean liver weight of 4.74 ± 0.31g with a mean HSI
316 figure of 1.21 ± 0.06 %. The reverse was the case in fish fed diet B (shrimp based diet) had
317 the least mean liver weight of 4.56 ± 0.35g, and a mean HSI value of 1.18 ± 0.07 % (Table
318 3).

In female *C. gariepinus*, mean liver weight (g) 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 % and least in fish fed diet C (chicken offal based diet) with a mean liver weight of 4.27 ± 0.38 g and a mean HSI value of 4.27 ± 0.38 % (Table 3).

TABLE 3: 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

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 3).

3.5 Fecundity of fish fed with the experimental diets

The summary of the fecundity of fish fed the 3 different diets is shown in Table 4. Fecundity of fish reared with the experimental diets was highest (3200 ± 717.90 eggs) in fish fed the control feed (Coppens feed), followed by fish fed feed B (SBD) with 2392 ± 749 eggs and least in fish fed Feed C (COBD) with 1973 ± 184 eggs. The mean fecundity varied significantly between the fish fed the 3 experimental diet at $p < 0.05$ (Table 4).

TABLE 4: Fecundity of fish fed 3 different experimental diets

Indices	Feed A (Coppens)	Feed B (SBD)	Feed 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

3.6 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).

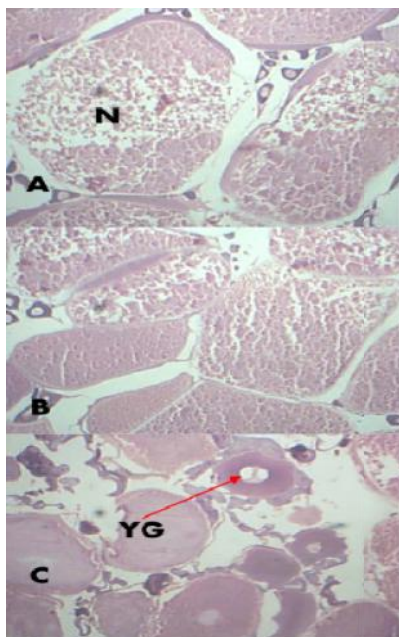


PLATE 1

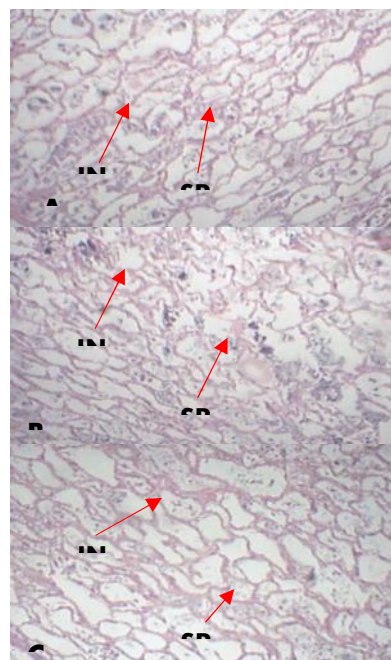
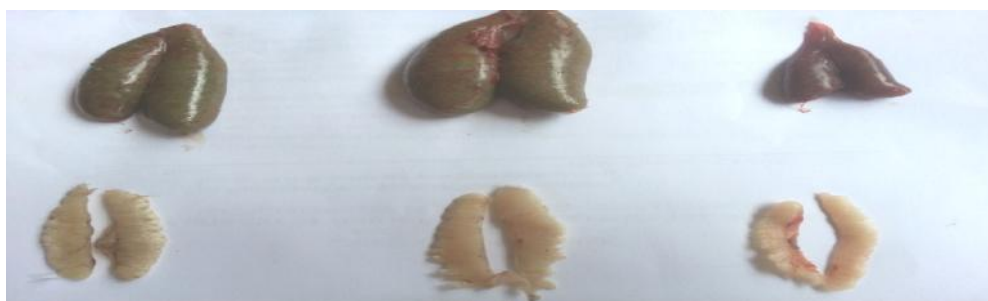


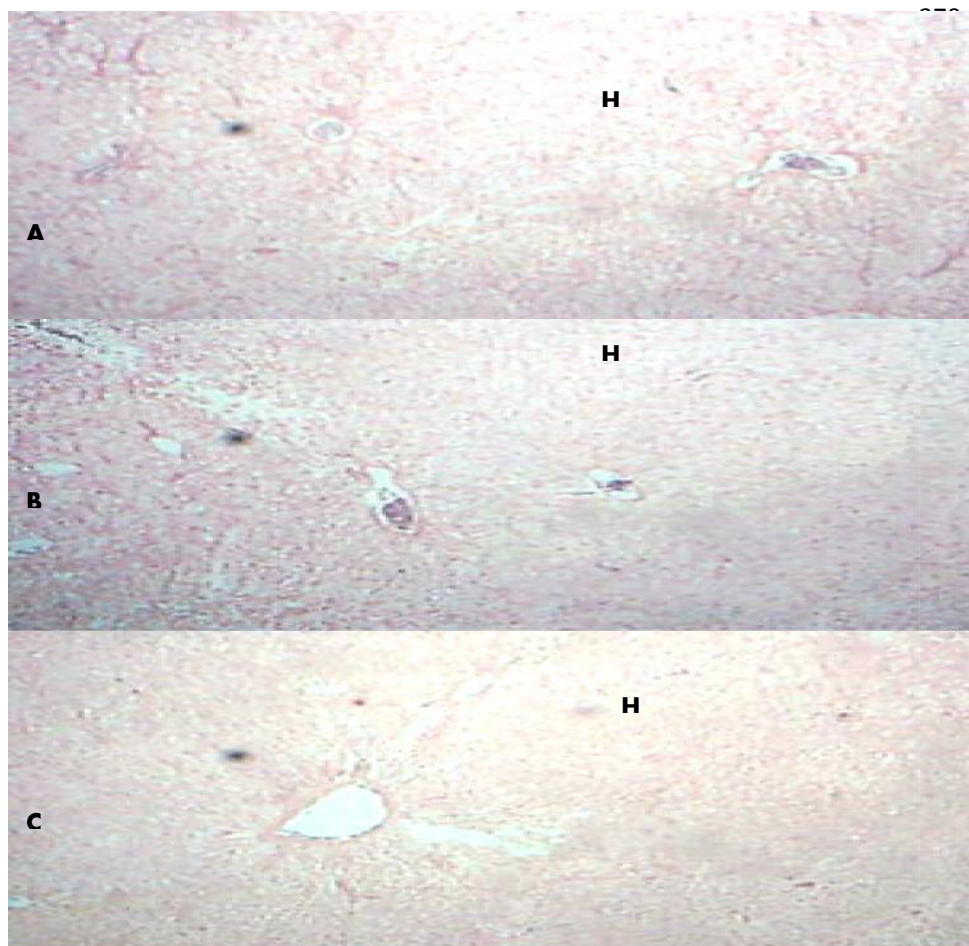
PLATE 2

Histological sections of oocytes (plate 1) and testes (plate 2) of *C. gariepinus* reared with the experimental diets. Diet A (Control Diet), Diet B (Shrimp-Based Diet) and Diet C (Chicken Offal-Based Diet) 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).



364

365 **PLATE 3:** Ovaries (up) and testes (down) of *C. gariepinus* reared with the three test
 366 feeds. From left to right: fish fed diet A (Coppens feed), diet B (shrimp based diet) and
 367 diet C (chicken offal based diet)



382

383 **PLATE 4:** Histological sections of *C. gariepinus* Liver reared with the experimental diets. A
 384 (Control Diet), B (Shrimp-Based Diet) and C (Chicken Offal-Based Diet) showing normal
 385 distribution of the liver cell (hepatocytes) with reduced vacuolization (X 40, H&E stains).

386

387

388 4 DISCUSSION

389 Aquaculture possesses the capacity to become a sustained farming practice which can
 390 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 coppers 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] and [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 physico-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 gonad 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].

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 mean gonadosomatic index for male *C. gariepinus* fed experimental diets differed significantly ($P < 0.05$), while that of the female fish was not significantly different ($p > 0.05$), and this 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 (shrimp based diet) and then Female fish administered diet C (chicken offal based diet), which were significant ($P < 0.05$). Body size did not affect gonad development as observed in both male and female fish fed the three experimental diets, these findings are similar to that of [23] who opined that maturity is link to age in *C. gariepinus* and disagrees with [24] whose observation reflected was the reverse (i.e maturity is linked to size rather than age).

[25], reported that for good gonadal development, the dietary protein level be stepped up to 40% in catfish diets, their opinion 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 normal arrangement of the oocytes, liver and testis in fish fed chicken offal based diet (COBD) and shrimp based diet (SBD), 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 coppens, chicken offal based diet (COBD) and shrimp based diet (SBD) was not significantly ($P>0.05$) different. This indicates that the chicken offal based diet (COBD) and shrimp based diet (SBD) was as good as 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

The shrimp based diet (SBD) and chicken offal based diet (COBD) competed positively with coppens in terms of nutritional composition, fecundity and gonadal development of *C. gariepinus*. Despite the fact that coppens yielded better nutritional composition, 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, 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 CONSIDERATION

The authors ensured that all the ethical and 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.

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