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# Effect of Protein Level on Gonadal Development of the African Catfish

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**Abstract**
A study was carried out to determine the effect of protein level, on the gonadal development of the African catfish, *Clarias gariepinus,* broodfish, using regularly recommended 31% and 40% crude protein (CP) diets. This was aimed at determining, the best level of crude protein inclusion in their diets, capable of enhancing gonadal recrudescence, maturity and spawning, and yet ensuring cost-effective feeding of pond-bred *Clarias gariepinus* broodstock. Juveniles weighing 5gms to 8gms with lengths ranging from 6cm to 9cm, were reared on the diets, for six months. Gonadal development (using Qualitative (Macro-morphological gonad examination) and Quantitative (Gonadosomatic index– GSI) methods), and Fish condition (using the Condition factor – K) were assessed at the third and sixth months. At the third month, qualitative gonad assessment revealed low gonadal development for all fishes though that, of the fishes (females and males) fed the 31% CP diets were 100% immature stage I gonads, whereas the 40% CP diet fed fishes were 70%immature stage I and 30%maturing stage II for males, and 80%immature stage I and 20%maturing stage II for females.. At the sixth month however, qualitative gonadal assessment revealed that, fishes fed 40% CP had 30% of the females spawning, and 30% of the male fishes attained full maturity, but amongst the fishes fed 31% crude protein, 30% of females and 20% of males were about attaining maturity. This was supported by higher GSI ranges of 25.5% - 39.1% for females and 0.17% - 0.22% for males fed 40% CP, as compared to lower GSI ranges of 20.5% - 28.6% for females and 0.11% - 0.14% for males fed 31%CP diet. The condition factor-K values were generally high at the third month (1.46 -1.90 and1.25 to1.68, for females and males fed 31%CP respectively; and 0.12 to2.16, and1.28 to1.95 for females and males respectively, fed 40%CP) but fell at the sixth month (0.57 - 0.75 and 0.59- 0.84, for females and males fed 31% CP; and 0.59 – 0.73 and 0.74 to 0.92 for females and males respectively, fed 40%CP). This down-ward trend indicates loss in condition related to gonadal development. However, the loss exhibited by fishes fed40% CP was less and their condition were better .The observed differences in GSI between 30% and 40% CP diets fed-fishes were significant at $P<0.05\%$ for females and $P<0.01\%$ for males. Also, the condition of females fishes fed 31% and 40% crude protein were not significantly different, but the males fed both experimental feeds were significantly different at $P<0.05\%$. It could thus be inferred that, the 40% CP diet gave better results than 31%CP diet. It is therefore recommended that, aquaculturist utilize 40% crude protein- inclusion for enhancing broodfish condition, gonadal recrudescence and spawning, for year-round seed production and cost effective *Clarias gariepinus* broodfish management.
**Keywords**: *Clarias gariepinus, C*rude protein, Gonadal development, Fish condition.

## 1. Introduction

Reproduction in the African catfish as in other animals involves a series of complicated, sequential processes, which proceeds with time in both males and females, and is influenced by several factors (Sundararaj, 1981). Baker (1938), cited in Sikoki (1987) reported that, reproductive cycles of teleosts are determined by both "ultimate" and "proximate" factors, and that, while the "proximate" control depends on environmental stimuli, the "ultimate" control is determined by food availability. Furthermore, Sundararaj (1981) reported that one of the major factors that determines success or failure of seed production programme is the choice of broodfish that are in right physiological state.

Nutrition is known to have a profound effect upon gonadal maturation and fecundity (Balogun *et al,* 1992). Specifically, in nutrition of broodfish, dietary protein is of fundamental importance as protein is required in the largest quantity for normal functions of the body (Solomon, Eyo, and Sikoki, 1999). It is the most important single nutrient required for growth, reproduction, and other body functions (Madu, 1989). Protein is also used by fish as a source of energy (Machiels and Henkel, 1987). This energy is required for development of yolky oocytes (Ayinla, 1991) and final ovarian development (Janssen *et al,* 1995). Thus the quantity and quality of the dietary protein must be correct in relation to the requirement of the fish species or sex. However, the capacity of fish to synthesize protein de-novo is limited and so most of the required protein must therefore be supplied through the fish's diet (Faturoti *et al,* 1992).

 

Though some work has been reported on the protein requirement for growth of the African catfish, there is paucity of information on optimum dietary protein level for gonadal development of farm-bred *Clarias gariepinus* broodfish. Ayinla (1988), recommended 40% crude protein diet for the broodfish. Faturoti *et al,* (1992), recommended 40% crude protein for the fry. Nwadukwe (1991), recommended a range of 35-40% crude protein. Ayinla (1991), reported that broodfish of this species will do well on a diet of 31% crude protein with 10% fish meal. Madu *et al*. (2003), reported that *C. gariepinus* required 40% crude protein level. However, non-specific or optional crude protein level in diets is not appropriate and not cost-efficient for gonadal development and eventual spawning of Clarias gariepinus brood fish. Therefore, a supplementary broodfish diet with an adequate and appropriate crude protein level cannot be over emphasized to, enhance gonadal development and ensure year-round and cost-effective seed production of the African catfish, *C. gariepinus.*

## 2. Material and Methods

2.1 Experimental Procedure

The study was carried using the hatchery facilities of the African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria, to rear 500 *Clarias gariepimus* fingerlings that were eight weeks old, of the same cohort, weighing between 5gm and 8gms, having total lengths ranging between 6cm and 9cm and secured from broodstock, reared in the ARAC.

**TABLE - 1**

| **NOS** | ***FEED INGREDIENTS*** | **DIET – "A" (31% PROTEIN)** | **DIET – "B" (40% PROTEIN)** |
|---|---|---|---|
| 1 | YELLOW MAIZE | 42.42 | 29.39 |
| 2 | GROUNDNUT CAKE | 16.87 | 23.38 |
| 3 | BLOOD MEAL | 16.87 | 23.38 |
| 4 | FISH MEAL | 10.00 | 10.00 |
| 5 | PALM OIL | 5.00 | 5.00 |
| 6 | COAGULATED STARCH OR GARRI | 5.00 | 5.00 |
| 7 | BONE MEAL | 2.50 | 2.50 |
| 8 | OYSTER SHELL | 0.50 | 0.50 |
| 9 | VITAMIN/MINERAL PREMIX | 0.60 | 0.60 |
| 10 | SALT | 0.25 | 0.25 |
| | **TOTAL** | **100%** | **100%** |

Two regularly recommended protein levels, (31% and 40%) for feeding the catfish were formulated into two experimental diets using locally available ingredients (Table 1.), and fed to acclimated fish that were randomly assigned to the duplicate treatment tanks and fed at 10% of their body weight for six months. Ten fish samples were randomly collected from the tanks and their body weights and body length were measured to the nearest 0.1gm and 0.1cmrespectively, and recorded bi-weekly.

2.2 Data Collection

2.2.1 Gonadal Development Determination: This was determined as follows: Qualitative (Macro-Morphological Gonadal Development) Method; External gonadal features(organs) of sampled fish were examined by mere sight. Subsequently, the gonads were extracted by incision and examined by sight and using a hand lens and gonadal development level determined using Macro-morphological maturity staging of Male fishes and fish gonads adapted and modified from Clay,1979,Legendre *et al.*,1996 and Yalcin *et al*.,2002; and female fishes and fish gonads adapted and modified from Gupta, 1974, Garcia – Diaz *et al*.,(1998; Garcia – Diaz and Gonzalez,2006, and Cek and Yilmaz,2007. Quantitative Determination (Gonadosomatic Index – GSI); was determined numerically as described by West, (1990, using the formula;

$$GSI\ (\%) = \frac{\text{weight of gonad (g)}}{\text{Weight of fish (g)}} \times 100$$

2.3 Fish condition

It was determined using the Condition Factor (K) as described by West (1990), using the formula; $K = (wgb/(L^3))100$

2.4 Data Analysis

Data obtained from the different treatments were subjected to Analysis of Variance (ANOVA) test and the treatment means were compared with each other at $P<0.05$. All statistical analysis was done using the statistical package, SAS 1999.

## 3. Results

3.1 Gonadal Development Pattern:

Macroscopic gonadal assessment (table 2) of fish fed 31% CP diet to be undifferentiated thin and ribbon-like structures (100% immature stage I gonads) at the end of the third month, whereas the 40% CP diet fed fishes had 70%immature stage I and 30%maturing stage II for males, and 80%immature stage I and 20%maturing stage II for females. At the sixth month, gonads were noted to have developed to different degrees in both 31% and 40%

 

CP diet groups. The males of the fish group fed 31% crude protein exhibited, 10% stage I immature (ribbon-like) testis, 70% maturing tubular stage II testis (fairly big, smooth edged and grayish) and 20% stage III mature (large serrated and creamy) testis. The females of this group exhibited 15%immature stage I (small tubular) ovary, 55%maturing stage II ovary (small, thick membrane) and mature stage III (fairly large, thin-walled) ovary with visible ova, being the most developed stage was merely 30%. The male fish fed 40% crude protein also attained stage III mature testis in the sixth month (Table 2) that were serrated, large, creamy, and stage IV – fully ,mature testis, that were much larger and fluid filled. The stage IV testis also made up 30% of the testes observed. Female fishes fed 40% crude protein were observed to exhibit stages IV-fully matured ovaries (that were large and filled with ova and the stages V-ovaries that were running ripe (Table 2), making up to 30% of sampled fish ovaries

**TABLE – 2: Macro-morphological stages of male and female fish fed 31% and 40% protein diets**

| TREATMENT | MALE | STAGE | FEMALE | STAGE | MALE | STAGE | FEMALE | STAGE |
|---|---|---|---|---|---|---|---|---|
| | A 31% PROTEIN DIET | | | | B 40% PROTEIN DIET | | | |
| | M1 | III | F1 | I | M1 | IV | F1 | II |
| | M2 | I | F2 | II | M2 | III | F2 | IV |
| | M3 | II | F3 | II | M3 | III | F3 | IV |
| | M4 | II | F4 | II | M4 | IV | F4 | IV |
| | M5 | II | F5 | I | M5 | II | F5 | IV |
| | M6 | II | F6 | II | M6 | III | F6 | IV |
| | M7 | II | F7 | II | M7 | III | F7 | IV |
| | M8 | I | F8 | III | M8 | IV | F8 | V |
| | M9 | III | F9 | III | M9 | III | F9 | V |
| | M10 | II | F10 | III | M10 | II | F10 | IV |

**Table –3: Gonadosomatic index (gsi) of *C. gariepinus* fish fed 31% protein diet in treatment A**

| Treatment | Sex | Fish No. | Fish Weight (gm) | Gonad | GSI | GSI Mean | Sex | Fish No. | Fish Weight (gm) | Gonad | GSI | GSI Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Male | 1 | 300 | 0.39 | 0.132 | - | Female | 1 | 200 | 45.5 | 22.7 | - |
| | | 2 | 200 | 0.25 | 0.125 | - | | 2 | 200 | 49.5 | 24.8 | - |
| | | 3 | 220 | 0.26 | 0.118 | - | | 3 | 225 | 49.5 | 22 | - |
| | | 4 | 210 | 0.28 | 0.137 | - | | 4 | 195 | 41 | 21.07 | - |
| | | 5 | 220 | 0.35 | 0.114 | - | | 5 | 180 | 40 | 22.2 | - |
| | | 6 | 300 | 0.39 | 0.132 | - | | 6 | 260 | 88.9 | 35.4 | - |
| | | 7 | 200 | 0.25 | 0.125 | - | | 7 | 180 | 59.5 | 33 | - |
| | | 8 | 220 | 0.36 | 0.163 | - | | 8 | 332 | 95 | 28.57 | - |
| | | 9 | 210 | 0.31 | 0.147 | - | | 9 | 170 | 45 | 26.4 | - |
| | | 10 | 200 | 0.28 | 0.14 | 0.133 | | 10 | 200 | 41 | 20.5 | 25.66 |

**Table –4: Gonadosomatic index (gsi) of *C. gariepinus* fish fed 40% protein diet in treatment B**

| Treatment | Sex | Fish No. | Fish Weight (gm) | Gonad | GSI | GSI Mean | Sex | Fish No. | Fish Weight (gm) | Gonad | GSI | GSI Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | Male | 1 | 330 | 0.651 | 0.186 | - | Female | 1 | 200 | 99.5 | 25.5 | - |
| | | 2 | 300 | 0.6 | 0.2 | - | | 2 | 200 | 93.5 | 26 | - |
| | | 3 | 290 | 0.555 | 0.19 | - | | 3 | 225 | 99.3 | 28.4 | - |
| | | 4 | 360 | 0.605 | 0.17 | - | | 4 | 195 | 95.5 | 38.2 | - |
| | | 5 | 295 | 0.5 | 0.17 | - | | 5 | 180 | 96 | 32 | - |
| | | 6 | 350 | 0.65 | 0.19 | - | | 6 | 260 | 93.5 | 38.9 | - |
| | | 7 | 290 | 0.55 | 0.193 | - | | 7 | 180 | 78 | 39 | - |
| | | 8 | 290 | 0.56 | 0.195 | - | | 8 | 332 | 79.3 | 39.1 | - |
| | | 9 | 360 | 0.61 | 0.171 | - | | 9 | 170 | 89.5 | 37.1 | - |
| | | 10 | 250 | 0.55 | 0.22 | 0.189 | | 10 | 200 | 75 | 30 | 33.42 |

Based on the gonadosomatic index- GSI, the fish fed 40% crude protein diet had higher gonadal development with higher GSI than those fed 31% crude protein diet, as the gonadosomatic index (GSI) for the fish fed 31% crude protein diet ranged between 0.114% to 0.137% and between 20.5% to 28.6% for the males and females respectively (Table 3); whereas the fishes fed 40% crude protein, had GSI ranges between 0.17% to 0.22% and between 25.5% and 39.1% for males and females respectively(Table 4).

 

**Table –5: Condition factor – k values at 3rd and 6th months for male and female *Clarias gariepinus* fed 31% crude protein diet (treatment A)**

| Treatment | Period (months) | Fish No. | Sex | K-Value | Perid (Month) | Fish No. | Sex | K-Value |
|---|---|---|---|---|---|---|---|---|
| A | 3 | 1 | Male | 1.25 | 6 | 1 | Male | 0.762 |
| | | 2 | Male | 1.50 | | 2 | Male | 0.59 |
| | | 3 | Male | 1.35 | | 3 | Male | 0.72 |
| | | 4 | Male | 1.42 | | 4 | Male | 0.84 |
| | | 5 | Male | 1.68 | | 5 | Male | 0.67 |
| | | 6 | Female | 1.46 | | 6 | Female | 0.75 |
| | | 7 | Female | 1.49 | | 7 | Female | 0.66 |
| | | 8 | Female | 1.9 | | 8 | Female | 0.57 |
| | | 9 | Female | 1.74 | | 9 | Female | 0.64 |
| | | 10 | Female | 1.74 | | 10 | Female | 0.69 |

**Table –6; Condition factor – k values at 3rd and 6th months for male and female *Clarias gariepinus* fed 40% crude protein diet (treatment B)**

| Treatment | Period (Month) | Fish No. | Sex | K-Value | Treatment | Period (Month) | Fish No. | Sex | K-Value |
|---|---|---|---|---|---|---|---|---|---|
| B | 3 | 1 | Male | 1.28 | A | 6 | 1 | Male | 0.83 |
| | | 2 | Male | 1.76 | | | 2 | Male | 0.78 |
| | | 3 | Male | 1.25 | | | 3 | Male | 0.74 |
| | | 4 | Male | 1.95 | | | 4 | Male | 0.92 |
| | | 5 | Male | 1.67 | | | 5 | Male | 0.81 |
| | | 6 | Female | 1.66 | | | 6 | Female | 0,65 |
| | | 7 | Female | 1.54 | | | 7 | Female | 0.71 |
| | | 8 | Female | 1.44 | | | 8 | Female | 0.73 |
| | | 9 | Female | 0.12 | | | 9 | Female | 0.60 |
| | | 10 | Female | 2.16 | | | 10 | Female | 0.59 |

3.2 Fish Condition

At the third month, the condition factor- K for all fish were similar but the 40%CP diet group were slightly better with male and female fish fed 31% crude protein diet having K ranges between 1.25 and 1.68, and 1.46 and 1.90 respectively (Tables 5); whilst the K ranges for males and females fed 40% crude protein diet were 1.28 to 1.95 and 0.12 to 2.16 respectively (Table 6). By the sixth month, K ranges for fish fed 31% crude protein were 0.59 to 0.84 and 0.57 to 0.75 for males and females respectively (Table 5) whilst the K ranges for fish fed 40% crude protein 0.74 to 0.92 and 0.59 to 0.73 for males and females respectively (table 6), indicating a general drop in condition for all fish-groups. However, the drop for the 40% diet fed fishes was less, and not significant between the female fishes (Tables 5 and 6) but significant for male fishes (at $P<0.05$**).**

**TABLE –7; Analysis of variance (anova) of condition factor k at 3rd month and 6th month, and gonadosomatic index (gsi) at 6th month of male *Clarias gariepinus* fed 31% crude protein diet and 40% protein diet**

| VARIABLES | PERCENT | CRUDE PROTEIN IN DIET | MEAN | SIGNIFICANCE |
|---|---|---|---|---|
| **Condition Factor K at 3rd Month** | 31% (A) 1.44±0.16 | 40% (B) 1.582±0.31 | 1.511 | NS |
| **Condition Factor K at 6th month** | $0.716^{b}$±0.09 | $0.816^{a}$±0.06 | 0.766 | * |
| **Gonadosomatic Index (GSI) at 6th Month** | $0.125^{b}$ | $0.183^{a}$ | 0.154 | ** |

Ns – non-significant, F-test significant level; *P<0.05, **P<0.01, a, b; means with different superscript are significantly different.

 

**TABLE –8; Analysis of variance (anova) of condition factor k at 3rd month and 6th month, and gonadosomatic index (gsi) at 6th month of male *Clarias gariepinus* fed 31% crude protein diet and 40% protein diet**

| VARIABLES | PERCENT | CRUDE PROTEIN IN DIET | MEAN | SIGNIFICANCE |
|---|---|---|---|---|
| **Condition Factor K at 3rd Month** | 31% (A) 1.67±0.16 | 40% (B) 1.38±0.31 | 1.53 | NS |
| **Condition Factor K at 6th month** | 0.66±22.55 | 0.66 | 0.66 | NS |
| **Gonadosomatic Index (GSI) at 6th Month** | 0.125±1.39 | 30.02±5.25 | 26.29 | * |

Ns – non-significant, F-test significant level; *P<0.05, **P<0.01, ***P<0.001, a, b; means with different superscript are significantly different means.

## 4. Discussion

The third month undifferentiated to slightly-differentiated gonads for fish fed 31% and 40% crude protein respectively, were similar to what happens in nature. Arockiaraj *et al*. (2004), reported similar findings on cyclical changes in gonadal maturation of Catfish. However, the 40% CP fed fishes still developed their gonads slightly faster than the 31% fed fishes.

At the sixth month, all fish gonads were maturing at different stages as was similar to the findings of Cek and Yilmaz (2007) in their study on *Clarias species*. Secondly, the 40%CP diet fed fish group exhibited higher gonadal maturation when compared to the fish group fed 31% crude protein diet. Madu *et al.*, (2003) reported similarly that, 40% crude protein diet gave better reproductive potentials to *Heterobranchus longifilis.* Richter *et al.,* (1982), achieved faster sexual maturity for *C. gariepinus* using improved high quality diets(40% CP). Hogendoorn and Vismans (1980), working on *Heterobranchus longifilis* reported better development using quality trout feed. Clay (1977), reported that the African catfish require high protein food as they are carnivorous in nature. According to Madu *et al.* (2003), 40% crude protein is considered as optimum dietary protein level for gonadal maturation of brood fish of the African catfish. The differences between the groups of fish fed 31% and 40% were significant at P<0.01% among males and P<0.05% among females indicating that, the 40% crude protein diet resulted in enhanced gonadal development above that of the 31% crude protein diet.

As witnessed at the third month of the experimental fishes and similar to the findings of Arockiaraj *et a*l.,(2004), fish condition for all fish groups were generally high at the early stage of their lives, as no energy was being used up at this age for reproduction. However, the condition progressively decreased as the fish grew older and maturation began, as evidenced at the sixth month. Thome et al, (2005) also reported that as gonad maturation and egg formation progresses, the condition of the fish depreciates as they tend to use up stored fat/energy for reproduction. However, the condition of adult fishes fed 40% crude protein were better and even significantly for male fishes than those fed 31% crude protein.

It was therefore concluded that 40% crude protein was the ideal for enhancing gonadal development and spawning of *Clarias gariepinus,* and recommended to be used for cost-efficient broodfish management and gonadal development for year-round seed production.\

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