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**A COMPARATIVE STUDY OF EGG AND SPERM QUALITY OF *Clarias gariepinus*
FED UNICAL AQUA FEED AND COPPENS COMMERCIAL FEED**

EYO VO¹, EKANEM, AP² AND OBIEKEZIE, AI²

1: Department of Zoology and Environmental Biology, University of Calabar, Nigeria

2: Institute of Oceanography, University of Calabar, Nigeria

Corresponding Author: E Mail: albertekanem@yahoo.com

ABSTRACT

A comparative study of egg and sperm quality of *Clarias gariepinus* fed Unical Aqua feed and Coppens commercial feed was conducted for six months in concrete tanks. Fifty post fingerlings with a mean weight of 0.50 ± 0.02 kg were stocked in six experimental units (A₁, A₂, A₃, B₁, B₂ and B₃). Fish in units A₁, A₂ and A₃ were fed Coppens feed while fish in units B₁, B₂ and B₃ were fed Unical Aqua feed twice daily at 3% of their body weight, in triplicates and controls. Proximate composition analysis of the dry matter of the two experimental feed showed that moisture, fat, crude fibre, ash, CH₂OH and Caloric contents were significantly different ($P < 0.05$) while crude protein contents, mean egg diameter (MED), mean sperm motility (MSM), mean milt density (MMD), mean sperm volume (MSV), and percentage hatchability of fish fed the two feed were not significantly different ($P < 0.05$). Water quality parameters were within the acceptable range for fresh water fish culture during the experiment. On the basis of the results obtained, it was concluded that the egg and sperm qualities of fish fed the two experimental feed were the same. Unical Aqua feed is recommended in place of Coopens commercial feed on the basis of affordability for the production of *C. gariepinus* brood fish.

Keywords: Unical Aqua Feed, Coopens Feed, Eggs and Sperm Quality, *Clarias gariepinus*

INTRODUCTION

The African catfish *C. gariepinus* is a very popular fresh water fish in Nigeria. It is regarded as one of the most important aquaculture candidates because of its ability

to tolerate a wide range of environmental conditions, high stocking densities under culture conditions, fast growth rate, high yield potential, high fecundity, air breathing characteristics and high market value [1, 2]. It is appreciated by consumers for its meat quality [3].

Good egg quality is defined by its ability to be fertilized and subsequently develop into a normal embryo while sperm quality is defined by its ability to successfully fertilize an egg and subsequently allow the development of a normal embryo [4].

The control of reproduction is a key issue in aquaculture and one of the limiting factors of the reproductive success is the quality of male and female gametes. Quality of fish gamete has received increasing attention and many studies have characterized the effect of specific factors on egg or sperm quality [4, 5, 6, 7]. Broodstock nutrition is an important factor on which gamete quality depends and can significantly impact reproductive performance of fish [8, 9, 10].

Fish feed alone accounts for at least 60% of the total cost of fish production [11] and has been rated as the highest recurring cost in catfish culture [12]. Availability, cost and supply of imported fish feed such as coppens, is one of the major challenges faced by catfish farmers in Nigeria. Efforts are being geared

towards providing a suitable and cheaper alternative catfish feed to Coppens without negotiating quality. The objective of this study is to determine and compare the egg and sperm quality of *C. gariepinus* fed Unical Aqua feed with fish fed Coppens commercial feed.

MATERIALS AND METHODS

Study Area

This research work was carried out in the Institute of Oceanography Fish Farm Hatchery complex, University of Calabar, geographically located within the historic peninsula between the Calabar River and the Great Kwa River with elevation of 41meters above sea level. This area has Latitude of 04°55.9"N and longitude 08°26"E respectively with a total surface area of three hectares.

Experimental Design

The experiment lasted for six months and was carried out in six circular concrete. The six units were labelled A₁, A₂, A₃, B₁, B₂ and B₃ to aid triplication of the experiment. Each unit measured 8.32m² in area. A total of 300 post fingerlings of *C. gariepinus*, average weight 0.50±0.02kg were stocked in the six experimental units (50 in each unit). Fish in units A₁, A₂ and A₃ were fed with Coppens feed while fish in units B₁, B₂ and B₃ were fed Unical Aqua feed. Feeding was carried out twice daily at 3% of their body weight.

Before stocking, the initial weight (g) of the fish was measured using a METLAR MT-5000D electronic balance and was repeated on monthly basis. Water quality parameters (dissolved oxygen (DO), pH, ammonia and temperature) were measured weekly with electronic balance.

Proximate Analysis of Unical Aqua Feed and Coppens Feed

Proximate analysis of the dry matter of Unical Aqua feed and Coppens feed was performed according to [13], in the Department of Biochemistry, University of Calabar.

Determination of Egg Quality

Egg quality was assessed using data on egg diameter and proximate composition of the eggs [14]. At the end of the feeding trials, 10 females were randomly selected per dietary treatment in each of the triplicate unit. The selected female fish were weighed, killed and dissected to remove the ovaries. The ovaries were slit open and 10 fresh eggs were randomly selected for egg diameter (mm) measurement. For the pear-shaped eggs, the mean diameters of the long and short axes were taken as the diameter of the oocyte. The remaining eggs were oven-dried and subjected to proximate analysis [14].

Determination of Sperm Quality

Sperm quality was assessed using data on sperm motility, density and volume according

to protocols outlined in [14]. Ten male fish were randomly selected per dietary treatment in each of the triplicate unit. The selected fish were killed and the testes removed. Small incisions were made into the lobes of the testes, the milt was squeezed out into a petri dish and the volume of the milt was measured in ml with a plastic syringe. A drop of distilled water was quickly added to each drop of the milt (activation) on a clean slide and the sperm motility was observed under the microscope. The presence or absence of spermatozoa motility was expressed as (1) for present or (0) for absent. The density of the spermatozoa was rated on a scale of 1, 2 and 3 (representing low, medium and high density) respectively [14]. The percentage number of eggs stripped from each fish, the percentage number of egg fertilized as well as the percentage number of egg hatched was computed according to the method described by [15]:

(a) Number of eggs stripped =

$$\frac{\text{Weight (g) of fish before stripping} - \text{wt (g) of fish after stripping}}{66.6}$$

(b) % Egg fertilized=

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

(c) % Egg Hatching = $\frac{\text{Number of whitish broken eggs}}{\text{Number of eggs fertilized}} \times 100$

$$\text{Number of eggs fertilized}$$

Breeding Experiment

At the end of the feeding trials, three pairs of matured male and female *C. gariepinus* were selected from each of the triplicates for breeding by hypophysation with ovaprim (0.02mg salmon gonadotropin-releasing hormone-sGnRHa + 10mg domperidone-Dom) in the hatchery complex. Substrates for induced breeding made from nylon mosquito nets was spread inside two glass incubator filled with clean and aerated water to a depth of 20cm for incubation. Eggs were fertilized by mixing stripped eggs from hypophyzed female *C. gariepinus* obtained from each treatment with spermatozoa from male *C. gariepinus* obtained from the corresponding treatments. Fertilized eggs were immediately spread thinly on the substrate for between 24-36 hours for incubation and hatching. The fry were fed with artemia for 10 days, and subsequently fed *ad libitum* three times daily with 0.1mm coppens feed.

Statistical Analysis

Data obtained from the experiment were subjected to T-test analysis for significant difference using Predictive Analytical Software (PASW) for statistical analysis (version 18.0). Effects with a probability of ($P < 0.05$) were considered significant.

Statement of Hypothesis

1. Null hypothesis (H_0): There is no significant difference in data obtained in fish fed Unical pelletized feed and Coppens commercial feed.
2. Alternative hypothesis (H_1): There is significant difference in data obtained in fish fed Unical pelletized feed and Coppens commercial feed.

RESULTS

Mean Proximate Analysis of the Dry

Matter of Unical Aqua Feed and Coppens

Mean proximate analysis (**Table 1**) of the dry matter (mg/100g) of Unical and Coppens feed shows that moisture content was higher in Unical feed (13.53 ± 0.15) than Coppens (10.02 ± 0.02). Mean crude protein content was also higher in Unical feed (13.30 ± 0.23) than Coppens (11.67 ± 0.15). Mean fat content also followed the same trend with Unical feed showing a higher value (19.03 ± 0.02) than Coppens feed (12.02 ± 0.03). Mean crude fibre content was higher in Coppens feed (1.43 ± 0.21) than Unical feed (0.6 ± 0.1). Mean ash content was higher in Coppens feed (15.27 ± 0.21) than Unical feed (12.4 ± 0.2). Mean CH_2OH content was higher in Coppens feed (49.58 ± 0.29) than Unical feed (41.14 ± 0.27). Mean caloric value (C/V) was

also higher in Unical feed (388.97 ± 0.59) than Coppens feed (353.21 ± 1.51).

Mean proximate Analysis of the Oocytes of *C. gariepinus* Fed Unical and Coppens Feed

Mean proximate analysis (**Table 2**) of the dry matter of the oocytes of *C. gariepinus* fed Unical and Coppens feed shows that moisture content was higher in Unical feed (8.46 ± 0.02) than Coppens (8.01 ± 0.23). Mean crude protein content was lower in Unical feed (48.30 ± 0.81) than Coppens (49.62 ± 0.21). Mean fat also followed the same trend with Unical feed showing a lower value (5.60 ± 0.20) than Coppens feed (6.01 ± 0.10). Mean crude fibre content was lower in Coppens feed (1.03 ± 0.20) than Unical feed (1.23 ± 0.15). Mean ash content was higher in Coppens feed (5.26 ± 0.12) than Unical feed (5.0 ± 0.3). Mean CH_2OH content was lower in Coppens feed (30.06 ± 0.15) than Unical feed (31.41 ± 1.04).

Mean Hatchability of *C. gariepinus* Fed Unical and Coppens Feed

Female *C. gariepinus* fed Unical feed showed a mean length of 38.66 ± 12.04 cm, mean Weight of (563.8 ± 286.68) before stripping, mean Weight of (496.8 ± 247.68 g) after stripping, mean ovary weight of 67 ± 51.43 and mean number of eggs incubated was 46900 ± 36004.10 eggs. Female *C. gariepinus* fed Coppens feed showed a mean length of

46.16 ± 3.05 , mean Weight of (724.2 ± 57.40 g) before stripping, mean Weight of (651.2 ± 67.59 g) after stripping, mean ovary weight of 77 ± 8.75 and mean number of eggs incubated was 53900 ± 6122.50 eggs. Percentage number of eggs fertilized in fish fed Coppens feed (97.79%) was higher than 97.12% obtained in fish fed Unical feed. Percentage number of eggs hatched in fish fed Coppens feed (94.23%) was lower than 96.42% obtained in fish fed Unical feed. Oocyte diameter of *C. gariepinus* fed Unical feed had a mean value of 1.39 ± 0.08 mm while *C. gariepinus* fed Coppens feed had a mean of 1.40 ± 0.09 mm. Percentage hatchability of *C. gariepinus* eggs fed Unical and Coppens feed is shown in **Table 3**.

Mean Sperm Volume, Motility and Density of *C. gariepinus* Fed Unical and Coppens Feed

For *C. gariepinus* fed Unical feed, mean sperm volume was $2.29 \text{ ml} \pm 0.61$, mean sperm motility (MSM) was 0.89 ± 0.33 and mean milt density (MMD) 2.11 ± 1.05 . For *C. gariepinus* fed Coppens feed, mean sperm volume was $2.78 \text{ ml} \pm 1.05$, mean sperm motility (MSM) was 0.78 ± 0.44 and mean milt density (MMD) was 2.0 ± 1.23 . Mean sperm volume, motility and density of *C. gariepinus* fed Unical and Coppens feed is shown in **Table 4**.

Table 1: Mean Proximate Analysis of the Dry Matter of Unical Aqua Feed and Coppens

	UNICAL FEED	COPPENS FEED
Mean Moisture Content	13.53±0.15	10.02±0.02
Mean Crude Protein Content	13.30±0.23	11.67±0.15
Mean fat Content	19.03±0.02	12.02±0.03
Mean Crude Fibre Content	0.6±0.1	1.43±0.21
Mean ash Content	12.4±0.2	15.27±0.21
Mean CH ₂ OH	41.14±0.27	49.58±0.29
Mean Caloric Value (C/V)	388.97±0.59	353.21±1.51

Table 2: Mean Proximate Analysis of the Oocytes of *C. gariepinus* Fed Unical and Coppens Feed

	UNICAL FEED	COPPENS FEED
Mean Moisture Content	8.46±0.02	8.01±0.23
Mean Crude Protein Content	48.30±0.81	49.62±0.21
Mean Fat Content	5.60±0.20	6.01±0.10
Mean Crude Fibre Content	1.23±0.15	1.03±0.20
Mean Ash Content	5.0±0.3	5.26±0.12
Mean CH ₂ OH	31.41±1.04	30.06±0.15

Table 3: Percentage Hatchability of *C. gariepinus* Fed Unical and Coppens Feed

	UNICAL FEED	COPPENS FEED
Length (cm)	46.16±3.05	46.16±3.05
Weight Before Stripping (g)	563.8±286.68	724.2±57.40
Weight After Stripping (g)	496.8±247.68	651.2±67.59
Ovary Weight (g)	67±51.43	77±8.75
Oocyte Diameter (mm)	1.39± 0.08	1.40± 0.09
No. of Eggs Incubated	46900±36004.10	53900±6122.50
% No. of Eggs fertilized (%)	97.12	97.79
% No. of Eggs hatched (%)	96.42	94.23

Table 4: Mean Sperm Volume, Motility and Density of *C. gariepinus* Fed Unical and Coppens Feed

	UNICAL FEED	COPPENS FEED
Mean Sperm Volume (mls)	2.29± 0.61	2.78± 1.05
Mean Sperm Motility (MSM)	0.89±0.33	0.78±0.44
Mean Milt Density (MMD)	2.11±1.05	2.0±1.23

DISCUSSION

Broodstock nutrition is an important factor affecting not only fecundity and gametogenesis but also gamete quality [8, 9, 10]. For adequate consideration of the effects of nutrition on sperm and egg quality of *C. gariepinus*, the criteria considered are the

oocyte diameter, chemical composition of eggs, milt volume, milt density, sperm motility, and percentage egg fertilization and hatching [14]. In the present study, sperm volume of *C. gariepinus* fed Unical feed showed a mean of 2.29± 0.61ml which was not significantly different ($P>0.05$) from

sperm volume of *C. gariepinus* fed Coppens feed with a mean of 2.78 ± 1.05 .

Apart from fertilization ability, sperm motility is an integrative quality parameter which will combine the quality of several cellular compartments responsible for motility activation and progressive movement sustainability. Among these, plasma membrane mediates the ionic motility activation signal and maintains the permeability barrier in order to prevent leakage of important intracellular components [4]. Mitochondria activity results in adequate energy stores, and axoneme structure and composition is responsible for the efficiency of spermatozoa movement [16].

In the present study, mean sperm motility (MSM) of fish fed Unical feed (0.89 ± 0.33) was not significantly different ($P > 0.05$) from fish fed Coppens feed (0.78 ± 0.44). This indicates that the two experimental feed did not impose a negative effect on the sperm quality with regards to sperm motility. Motility analysis is very useful to compare different experimental conditions such as collection procedures, sperm dilution medium, and sperm storage conditions [4].

In the present study, it was also observed that sperm motility increased with sperm (milt) volume in fish fed Unical and Coppens feed

and the spermatozoa were only active for 30-36 seconds. This observation is similar to [14] who reported similar findings in *C. gariepinus* broodstock fed different heated soybean-based diets. Mean milt density (MMD) of fish fed Unical feed (2.11 ± 1.05) showed no significant difference ($P > 0.05$) with fish fed Coppens feed (2.0 ± 1.23) which also confirms the high quality of Unical feed when compared to imported Coppens commercial feed.

From a biological standpoint, the quality of a gamete can be defined as its ability to fertilize or to be fertilized, and subsequently develop into a normal embryo [4]. However, egg quality defined as those characteristics of the egg which determine its capacity to survive, is a problem of many species of farmed fish and can be manipulated through good broodstock nutrition [14]. In the present study, mean oocyte diameter of *C. gariepinus* fed Unical feed was 1.39 ± 0.08 mm while *C. gariepinus* fed Coppens feed showed a mean oocyte diameter of 1.40 ± 0.09 . According to [17], egg diameter is not a good indicator of egg and larval quality. The size and appearance of unfertilized eggs can tentatively be used to evaluate or estimate the overall developmental potential of an egg after fertilization [4]. However, large variations in egg weight and size attributed to extrinsic

factors such as feed can be obtained in fish within the same species as observed in the present study.

Another important criterion used in evaluating the egg quality of fish species is proximate composition of the eggs (oocytes). However, results obtained in this study indicated that there was no significantly difference ($P>0.05$) in the proximate composition (moisture content, mean crude protein content, mean fat content, mean crude fibre content, mean ash content and mean CH_2OH) of the dry matter of the oocytes of *C. gariepinus* fed Unical and Coppens feed. According to [14], the viability of eggs is a reflection of the chemical composition of the yolk. Protein and lipids are the major components stored in egg yolk and play a major role in reproduction.

Fertilization success is probably one of the earliest factors that can be use to accurately estimate egg quality and it is the most integrative estimator of sperm quality. Indeed, the ability to fertilize or be fertilized is one of the key components of gamete quality. In some species, recording of fertilization rates is relatively easy. This is especially true for species with transparent eggs such as cod (*Gadus morua*), turbot and yellowtail flounder (*Limanda ferruginea*). Percentage number of eggs fertilized in fish fed Coppens feed was 97.79% while in fish fed Unical,

97.12% was fertilized. Percentage number of eggs hatched did not increase with percentage fertilization. In fish fed Coppens feed, 94.23% eggs hatched out of 97.79% eggs fertilized while in fish fed Unical feed 96.42% eggs hatched out of 97.12 eggs fertilized. It is also obvious that fertilization success is not necessarily reflective of further developmental success as shown in several marine species [18]. The shape of the first embryonic cells and the occurrence of abnormal cell division patterns have also been used as quality estimators in species with transparent eggs [4].

Unical Aqua feed competed favourably with Coppens commercial feed in the egg and sperm quality of *C. gariepinus*. The cost of Unical Aqua feed is about 50% of Coppens commercial feed and is hereby recommended for theproduction of *C. gariepinus*.

REFERENCES

- [1] Hecht T, Oellermann L and Verheust L, Perspectives on clariid catfish culture in Africa, Aquatic Living Resources, 9, 1996, 197-206.
- [2] Babalola TOO and Apata DF, Effects of Dietary Protein and Lipid Levels on Growth Performance and Body Composition of African catfish *Heterobranchus longifilis* (Valenciennes, 1840) Fingerlings, J.

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- Animal and Veterinary Advances, 5(12), 2006, 1073-1079.
- [3] Pruszyński T, Effects of feeding on ammonium excretion and growth of the African catfish (*Clarias gariepinus*) fry. Czech J. Animal Sci., 48 (3), 2003, 106-112.
- [4] Bobe J and Labbe C, Egg and sperm quality in fish General and Comparative *Endocrinology*, 165, 2010, 535-548
- [5] Howley C and Ho RK, MRNA localization patterns in zebrafish oocytes, Mech. Dev., 92, 2000, 305-309.
- [6] Pelegri F, Maternal factors in zebrafish development, Dev. Dyn., 228, 2003, 535-554.
- [7] Lahnsteiner F and Patzner RA, Sperm morphology and ultrastructure in fish. In: Alavi SMH, Cosson JJ, Coward K, Rafiee G, (Eds.), Fish Spermatology Alpha Sci. Int. Ltd., Oxford, 2008, 2-61.
- [8] Kjorsvik E, Mangor-Jensen A and Homefjord I, Egg quality in marine fishes, Advances in Marine Biol., 26, 1990, 71-113.
- [9] Brooks S, Tyler CR and Sumpter JP, Egg quality in fish: what makes a good egg? Revi. Fish Biol. Fish., 7, 1997, 387-416.
- [10] Izquierdo MS, Ndez-Palacios H and Tacon AGJ, Effect of broodstock nutrition on reproductive performance of fish, Aquaculture, 197, 2001, 25-42.
- [11] Jamu DM and Ayinla OA, Potential for the development of aquaculture in Africa NAGA, 26, 2003, 9-13.
- [12] Agokei EO, Oparah CA and Apapa U, Growth of *Clarias gariepinus* Juveniles fed five commercial feed, Continental J. Fisheries and Aquatic Sci., 5 (3), 2011, 1-5.
- [13] Association of Official Analytical Chemists International (AOAC), Official methods of analysis, 17th Edition, AOAC International, Gaithersburg, MD, USA, 2000.
- [14] Adewumi AA, Olaleye VF and Adesulu EA, Egg and Sperm Quality of the African catfish, *Clarias gariepinus* (Burchell) Broodstock Fed Differently Heated Soybean-based Diets. Res. J. Agri. and Biol. Sci., 1(1), 2005, 17-22.
- [15] Ayinla OA, Nutritive and Reproductive Performance of *Clarias gariepinus* (Burchell 1822),
-

Unpublished Ph. D Thesis, University of Ibadan, Nigeria, 1988, 433.

- [16] Cosson J, The motility apparatus of fish spermatozoa, In: Alavi SMH, Cosson JJ, Coward K, Rafiee G, (Eds.), Fish Spermatology, Alpha Science Int. Ltd., Oxford, 2008, 281-316.
- [17] Carillo M, Sanuy S, Prat F, Cerda J, Ramos J, Mananos E and Bromage N, Sea Bass (*Dicentrarchus labrax* L). In: Broodstock Management and Egg and Larval Quality, (eds: Bromage, N. and J. Roberts), Blackwell Sci., Osmead, Oxford, 1995, 424.
- [18] Shields RJ, Brown NP and Bromage NR, Blastomere morphology as a predictive measure of fish egg viability, Aquaculture, 1997, 155, 1-12.