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COMPARATIVE INFLUENCE OF *AZANZA GARCKEANA* ON THE SPERM QUALITY OF F_1 AND F_2 *CLARIAS GARIEPINUS* BROOD STOCK

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ABSTRACT

The study assessed the sperm quality of African mud catfish, Clarias gariepinus (Burchell, 1822), fed diets containing treated Azanza garckeana pulp meal. Sperm quality is an important consideration in the production of high-quality fish larvae and the efficient economic utilisation of hatchery facilities (Coban et al., 2011). The proximate composition of the experimental diets was determined following the procedures of the Association of Official Analytical Chemists (AOAC, 2010). The fish were randomly allocated to five treatments (T1, T2, T3, T4, and T5), with 12 fish assigned to each treatment and three replicates arranged in a completely randomized design using concrete tanks. The fish weighed between 750 and 850 g and were subjected to the experimental diets for 56 days at a feeding rate of 5% body weight, administered twice daily in the morning and evening. Treatment T5 recorded the highest proportion of active sperm cells (55%), followed by T4 (50%), whereas T3 had the lowest proportion (40%). The highest percentage of motile sperm cells was also observed in T5 (80%), followed by T4 (75%), while T3 recorded the lowest value (64%). Treatment T3 exhibited the highest proportion of sluggish sperm cells at 30%, whereas the greatest percentage of dead sperm cells was recorded in T1 (36%). In contrast, T5 recorded the lowest proportion of dead sperm cells (20%), indicating comparatively better sperm quality among the fish receiving this treatment.

Keywords: *Clarias gariepinus, sperm quality, Azanza garckeana.*

INTRODUCTION

Sperm quality plays a vital role in the production of high-quality fish larvae and economically efficient hatcheries (Coban et al., 2011). The quality of sperm in fish production is a significant parameter for reproductive success and performance, ensuring sustainability of fish production throughout the year that, in turn, satisfies the ever-increasing demand for fish and fish products. Enhanced fish production will also add value to the fortunes of those directly involved in the industry and the country's economy. According to Ochokwu et al. (2015), the essential dietary nutrients involved in the production of good-quality eggs and sperm are protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (<8.5%), phosphorus (<1.5%), water (<10%), and trace elements of vitamins and minerals. As such, fish reared in captivity in high stocking densities in indoor systems or confined in cages where they cannot forage need balanced diets to meet their nutritional requirements for sustaining their physiological functions.

Azanza garckeana is a local indigenous fruit tree species with a number of vernacular names. In Botswana, it is locally called "morajwa" or African chewing gum and is also called tree hibiscus, snot apple, and wild hibiscus (Orwa et al., 2009). In Northern Nigeria, specifically Gombe State, the locals know it as "Goron Tula" while in Tula, Kaltungo Local Government Area of Gombe State, Nigeria, the plant is an indigenous edible fruit tree. Being a local fruit tree, A. garckeana is a potential candidate for use

in feed formulation because of its availability in the local environment, palatability, and nutritional qualities.

The opportunity for using Azanza garckeana fruit to formulate diets is supported by the following considerations: chemical composition, low cost, availability in large quantities, and presence of few anti-nutritional factors. These qualities can enable the use of A. garckeana fruit pulp as a feed ingredient in fish diets, specifically when using locally available and affordable feed ingredients.

MATERIALS AND METHODS

Study Area

The study was conducted at the fish hatchery of the Department of Fisheries, Teaching and Research Farm, Modibbo Adama University, Yola, Adamawa State, Nigeria. The university lies between 9.20–9.33°N and 12.30–12.50°E in latitude and longitude respectively, at 185.9 m above sea level. The area has an average annual rainfall of about 759 mm and an average maximum temperature of about 39.7 °C The rainy season is usually from May to October while the dry season is from November to April. January and February are the driest months with a monthly mean relative humidity of about 13% (Can back Global Income Distribution Database 2014).

Experimental Design and Set-up.

Fish were randomly assigned to12 per treatment with 3 replicates in a completely randomized design in a concrete tank as T1, T2, T3, T4 and T5. Sizes of the fishes range from 750 – 850g.

The fish were fed with the experimental diet for 56 days at 5% body weight twice daily, morning and evening.

At the end of brood stock treatment with the experimental diet for 56 days, a male and female brood stock was randomly collected from each treatment. They were used for artificial breeding and hatched the F1 generation.

Proximate composition of *Azanza garckeana*

The composition of *Azanza garckeana* was carried out in the department of fisheries, Modibbo Adama University, Adamawa state. The proximate composition was determined according to the Association of Official Analytic Chemists (AOAC, 2010).

Induced Breeding

The selected broodstock was maintained separately in designated tanks without feeding throughout the preparation period. Each fish was administered Ovaprim at a dosage of 0.5 mL per kilogram of body weight and subsequently allowed a latency period of 10–12 hours. This post-ovulatory maturation period was maintained to facilitate proper maturation and support high hatching rates while reducing the occurrence of deformed larvae. Following the completion of the latency period, the matured eggs were stripped for subsequent use in the breeding procedure.

Collection of Milt

Checking of ovulation usually starts 6 hours after injecting the fish with hormone and continued at one-hour intervals. Milt was obtained by sacrificing the males. Each male was dissected carefully and their testes obtained. The milts were collected into clean bowls labelled according to the treatments.

Taking of Readings

Readings such as milt volume, weight and of right lobe, weight and of left lobe, sperm count, sperm motility (active, sluggish and dead) and total motile sperm were taken and recorded carefully.

RESULTS

Proximate Percentage Composition of *Azanza garckeana* Pulp Meal in Experimental Diet.

The proximate percentage composition of *Azanza garckeana* pulp meal in experimental diet indicated highest of moisture in T3 with 11.70gm, Fats in T5 with 27gm, crude fibre in T5 with 7.5gm, Ash in T5 with 9.50gm and NFE in T2 with 18.00gm and ME cal/gm in T2 with 3,947.00.

Table 1: Proximate Percentage Composition of *Azanza garckeana*Pulp Meal in Experimental Diet.

Dietary Treatments	T1	T2	T3	T4	T5
Moisture content	10.2	8.7	11.7	9.6	9.6
Crude protein	40.0	40.0	40.0	40.0	40.0
Fat	24	24	25	26	27
Crude fibre	1.10	1.45	3.55	5.60	7.50
Ash	8.40	8.50	9.00	9.00	9.50
NFE	17.0	18.0	10.0	6.0	4.0
M. EKcak/g	3910.00	3947.00	3830.00	3840.00	3820.00

Nutrient Composition and percentage Dry Matter Basis of *Azanza garckeana* Pulp Meal

Table 2 showed the nutrient dry matter basis of *Azanza garckeana* pulp meal, with highest was crude fibre with 40%, while lowest was fats with 7%. The dry matter was sodium highest with 182mg/l and lowest magnesium with 5mg/l

Table 2: Nutrient Composition and percentage Dry Matter Basis of *Azanza garckeana* Pulp Meal

Nutrient and Mineral Composition	Values
Moisture content	14%
Crude Protein	12 %
Fat	7 %
Crude Fibre	40 %
Ash	11 %
NFE	16 %
Calcium	18mg/l
Iron	14mg/l
Sodium	182mg/l
Magnesium	5mg/l
Potassium	180mg/l
Phosphorus	60mg/l
PH	5
Ascorbic Acid	20mg/100g

Mean Sperm Quality of *Clarias gariepinus* Fed Different Inclusion of processed *Azanza garckeana* pulp Meal

shows the mean sperm quality of catfish fed different inclusion level of processed *Azanza garckeana* pulp mean. treatment 5 has the highest active sperm with 55% followed by Treatment (T4) with 50%and least with Treatment 3 with 40% active sperm cell, the total number of motile sperm cell is recoded in Treatment 5 with 80 % then 75% in T4 then while T3 had the least active sperm of 64%. T3 had the highest number of sluggish sperm with a value of 30. Highest dead sperms are recorded in T1 with 36% while T5 had the least number of dead sperm of 20.

Table 3: Mean Sperm Quality of *Clarias gariepinus* Fed Different Inclusion of processed *Azanza garckeana* pulp Meal

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**
Weight of fish (g)	800	815	815	820	825
Length of fish (cm)	55	57	57	58	58
Weight of right lobe (g)	1.55	2.30	2.40	2.50	2.55
Weight of left lobe (g)	1.35	2.80	2.89	2.90	2.77
Length of right lobe (cm)	5.30	5.50	6.20	7.30	7.44
Length of left lobe (cm)	5.20	5.45	6.00	7.00	7.20
Milt volume (mls)	3.20	4.50	5.70	6.10	7.20
Sperm count 10^{X6}	100	330	340	350	390
Sperm motility					
Active	45	45	40	50	55
Sluggish	19	25	30	25	25
Dead	36	30	30	25	20
Total motile sperm (TMS)	64	70	70	75	80

Keys: T1: (Control basal diet) without processed *Azanza garckeana* pulp meal, T2: Processed *Azanza garckeana* pulp meal at 50g/kg, T3: Processed *Azanza garckeana* pulp meal at 100g/kg, T4: Processed *Azanza garckeana* pulp meal at 150g/kg, T5: Processed *Azanza garckeana* pulp meal at 200g/kg

Mean Sperm Quality of F1 *Clarias gariepinus* Fed with different Inclusion of Processed *Azanza garckeana* pulp meal for 2nd Breeding.

The mean sperm quality of catfish fed different inclusion level of processed ***Azanza garckeana* pulp meal** is shown in Table 4. Treatment 5 has the highest active sperms with 80 followed by T4 having 70 while T1 has the least active sperms T2 has the highest sluggish sperms with the value 25 while T5 has the lowest sluggish sperms with 15, T1 has highest dead rate of sperms cell with 40 while T5 has the lowest with 5%. The total motile sperm of 95 was the highest which was from T5 and T4 while other Treatments T2, T3 and T1 has 85, 80 and 60 respectively.

Table 4: Mean Sperm Quality of F_1 Clarias *gariepinus* fed different Inclusion of *Azanza garckeana* pulp meal for 2nd Breeding

Parameters	T1	T2	T3	T4	T 5
Weight of fish (g)	800^{c}	900^{a}	550^{c}	700^{b}	850^{c}
Length of fish (cm)	46.7^{b}	45.2^{a}	48.5^{b}	47.5^{a}	54.0^{c}
Weight of right lobe (g)	1.59^{d}	2.50^{b}	2.30^{c}	2.80^{a}	2.42^{b}
Weight of left lobe (g)	1.40^{d}	2.85^{b}	2.90^{a}	2.44^{c}	2.90^{a}
Length of right lobe (cm)	5.00^{a}	5.00^{a}	3.89^{c}	4.00^{c}	4.2^{b}
Length of left lobe (cm)	4.5^{b}	4.9^{a}	4.3^{c}	4.00^{d}	4.22^{c}
Milt volume (mls)	$1.82b^{d}$	3.30^{b}	3.10^{c}	3.40^{b}	3.60^{a}
Sperm count 10^{6}	110^{d}	340^{c}	341^{c}	352^{b}	394^{a}
Sperm motility					
Active	40^{b}	60^{a}	60^{a}	70^{c}	80^{b}
Sluggish	20^{b}	25^{b}	20^{c}	20^{a}	15^{b}
Dead	40^{a}	15^{b}	20^{a}	15^{a}	05^{a}
Total Motile Sperm (TMS)	60^{b}	85^{a}	80^{b}	95^{b}	95

Means of Data on the same row with different superscript are significantly different ($p<0.05$).

DISCUSSION

Percentage Proximate Composition of *Azanza garckeana* Pulp Meal

The proximate composition of the processed Azanza garckeana pulp meal diets used in the present study was generally within the expected range as reported by Craig et al. (2002). The latter authors stated that prepared fish diets may either be complete or supplementary feeds which are formulated to satisfy the nutritional requirement of the target species. In this study, the moisture contents of the formulated diets ranged from 9.6 to 11.7% which falls within the theoretical moisture contents of low moisture foods as reported by Bradley (1994). None of the formulated diets had more than 75% moisture contents. According to Crickenberger and Carawan (1996), feeds and feedstuff materials with less than 75% moisture contents are fit for human and animal consumption as exemplified by the catfish feeds. With this observation, it could be argued that the A. garckeana pulp meal diets used in this study are low moisture biological materials which are less prone to biological deterioration and hence less likely to develop aflatoxin (Sogbesan & Ekundayo, 2014). The crude protein content of the formulated diets was 40% which is within the recommended level for prepared fish feeds (Balogun et al., 2016). The crude lipid contents of the diets ranged from 24 to 27%, which also fall within the recommended lipid levels for most tropical fish species (Adikwu, 2003). On the contrary, the crude fibre contents of the diets ranged from 1.10 to 7.50%, which is far higher than the 3.5-5% recommended by Olomu (1995) for animal diets. However, it is noteworthy that diets with more than 10-15% crude fibre contents are less desirable as feed ingredients because of the increased volume of the diet which may lead to decreased voluntary feed intake (Braude, 1967).

Reproductive Performance of *Clarias gariepinus* fed different Inclusion Levels of Processed *Azanza garckeana* Pulp Meal

The fish fed with *Azanza garckeana* Pulp Meal showed high active sperm motility value compared to the control, and also in the aspect of egg quality. It was observed that there was an improvement in the fertilization and hatchability rate from F_2 when compared to the breeding done with the F_1 fed with *Azanza garckeana* Pulp Meal inoculated feed. This agrees with the study of Sahoo *et al*., (2003) which showed a significant improvement in the reproductive performance of the offspring (F_2) when compared with the parent's (F_1) reproductive performance. This showed that inclusion of *Azanza garckeana* Pulp Meal have more effect on the female reproductive performance compared to the males. In this study, the parent broodstock for first breeding tend to have the highest values in milt volume and sperm count when compared to the breeders for the 2nd breeding, this disagrees with the study of Sahoo*et al*., (2003) that observed a significant increase in the reproductive performance and quality of the male broodstock fed with formulated feed inoculated with plant-based supplements.

A relatively high percentage of total motile sperm cells was observed in some fish fed diets containing processed Azanza garckeana pulp meal, indicating an improvement in sperm viability and possibly enhanced fertilizing potential. The sperm concentration observed in the present study was higher than the values reported by Abu et al. (2013) for fish fed varying dietary inclusion levels of Moringa oleifera leaf meal. This might suggest that the incorporation of A. garckeana pulp meal, which was previously associated in this study with an increase in testosterone production, may have had a positive effect on the quality of sperm and egg. Testosterone is the principal androgen produced by the

male gonads, and it plays an important role in normal male development and in maintaining such characteristics as muscle mass and strength, bone mass, libido, sexual potency, and spermatogenesis (Sanni et al., 2012). These functions are essential for the production of viable sperm cells, and they may therefore serve to enhance the reproductive success of fish. In this respect, Ebong et al. (2014) found that Moringa oleifera extract was able to normalize serum testosterone levels, further supporting the association between diet, testosterone regulation, and reproductive performance.

CONCLUSION

The inclusion of processed Azanza garckeana in the diets of Clarias gariepinus was proven to be beneficial to the reproductive performance of broodstock. The study established the potential of A. garckeana as reliable feed ingredient that can be utilized in the propagation and production of C. gariepinus fingerlings. The A. garckeana can be used as a potential fertility-enhancing feed additive for C. gariepinus broodstock and its use could reduce the reliance on synthetic fertility enhancing agents. Feeding the male fish with A. garckeana pulp meal produced better-quality sperm compared to the control diet with the highest inclusion level performing best in terms of gonadal development, fertility and hatchability. The groups that were fed the experimental diets processed compared to control group performed better. Thus, the addition of A. garckeana pulp meal in the diets of C. gariepinus broodstock could be beneficial to their reproductive performance as well as sperm quality.

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