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Protein profile of breeding discrepancies of African catfish *Clarias gariepinus* in aquaculture practices

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ABSTRACT

The protein analysis focused on sarcoplasmic muscle of protein constituents to improve heterogenous growth of *C. gariepinus.* Ninety juveniles were graded and selected for the top 10%, medium 10% and bottom 10% fish which represented fast, moderate and slow growing groups. The protein sample extracts were prepared by homogenizing 150 mg of fish muscle and electrophoretic run in Sodium Dodecyl Sulphate (SDS) polyacrylamide gel. Eleven bands were found in large fish, eight bands in the medium fish and seven bands were detected in the small fish class. The 2nd to 5th protein band having molecular weight (MW) ranging from 116,200 to 70 KDa was detected across all fish class sizes. The 6th and 7th band present in the large fish sample class but absent in the other two classes, having MW 54.52 KDa and 54.21 KDa respectively are assigned to Desmin protein. The 1st band was the heaviest (MW 118,600 KDa) band only found in the large sized fish. The results revealed that 1st 6th and 7th bands are the distinguishing protein bands and these proteins have contributed to the heterozygosity nature of the large size class which are most suitable for broodstock selection for future artificial propagation practices.

Keywords: Electrophoresis, molecular weight, broodstock, sarcoplasmic, heterozygosity.

INTRODUCTION

Clarias species is the most cultured fish species in the Nigerian Aquaculture industry today. The species dominating over 85% of the industry is plagued with the problem of unhealthy heterogeneous growth surprisingly from the early days of life (FAO, 2005). Hatchery experts have come up with grading systems for fingerlings resulting from differential growth patterns. Fish sizes obtained in breeding exercise are majorly grouped into three classes: large, medium and small classes. Consequently, aquaculture expansion has been in slow process as private sector fish farmers face constraints such as supply of quality fish seed (FAO, 2003). Discrepancies at the early stage of their life results to cannibalism amongst the species, with resultant effect in population decline, economic disadvantage and decreased profitability, other effects are reduction in production indices such as growth, feed conversion efficiency and adaptability (Nwafili and Gao, 2007). The desire of fish

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farmers is to produce table sized fish within the shortest possible time. Consequences of this simple problem have led to the fold up of many fish farms and the widening of the demand-supply gap. Agbebi et al. (2003) and Ataguba et al. (2010) suggested that efforts have to be geared towards producing more homogenous number of large sized class fish seeds which is profitable for grow out farmers.

This study was aimed at determining the protein profiles of each of these three classes of fish to improve on heterogeneous growth of the species.

MATERIALS AND METHODS

Broodstock selection

The broodstocks of *C. gariepinus* of known breeding records used for the experiment were obtained from Hepa Fish Farm, Asero, Abeokuta, Nigeria (Lat. $7^0$25"3.68 N to $7^0$4'13.06 "N and Long $3^0$0'9.20 "E to Long $3^0$24'10.92 "E). Gravid females of *C. gariepinus* were selected based on their swollen reddish genital papilla and a well-distended, swollen soft abdomen that oozed out eggs when gently pressed. Sexually matured males of *C. gariepinus* were selected based on reddish pointed and vascularised urogenital papillae. After selection, the broodstocks were immediately transferred in plastic troughs and taken into the hatchery.

Artificial fertilisation

Hypophysation of the specimens were carried out in the hatchery of Hepa Fish Farm, Asero, Abeokuta where hatchlings were reared. The selected broodstocks of *C. gariepinus* were kept separately in hatchery for two days without feeding to empty the alimentary tract at the time of stripping. Oocyte maturation and ovulation in the female broodstocks were induced by a single intramuscular injection of Ovaprim (SYNDEL, Canada) at a dosage of 0.5 ml/kg live weight as described by Viveen et al. (1985) and then left for 10-17 hours latency period to ensure high hatching rate and low proportion of deformed larvae.

The body of the female broodstock was carefully dried with a clean towel and tightly held at head and tail ends while the eggs were hand-stripped by pressing their abdomen into clean and dry bowl. The eggs were fertilized with milt obtained from lacerated testes by using feather to spread the mixture evenly for one min. The fertilized eggs from each mating combination were spread out in single layer on the screen nets (mesh size of 1 mm) placed in the hatching tanks at 27 °C to 28 °C.

Fertilized eggs were incubated separately in aerated water tanks with continuous flow through system. After eggs hatched, the larvae were allowed to absorb their yolks for three days, after which they were fed and reared for 8 weeks.

Selection of fishes into their different sizes

The fingerlings were selected into different sizes after breeding and feeding with the same feed type (Robinson et al., 1998). The fingerlings were separated after 4 weeks of rearing using their length and weight and were reared for 8 weeks. A transparent ruler was used to measure the length while weight was measured using a digital sensitive scale.

Fingerlings fish samples (comprising three samples from each fish sizes) were transported live in well open plastic rubber bowls to the Biotechnology Laboratory Centre of the Federal University of Agriculture, Abeokuta.

Muscle sample collection

Sarcoplasmic extracts were prepared by homogenizing 150 mg of muscle with the aid of a mortar and pestle (on ice) in 1.5 ml of rigor buffer containing10 mM Trismeleates, 60 mM KCl, 5 mM $MgCl_2$, 1

nM EDTA (Zapata et al., 2008). The extracts were centrifuged in a tube at 10,000 g for 5 min at 4 °C. Supernatant (sacroplasmic extract) was transferred to new microcentrifuge tubes. 500 µl of the sarcoplasmic fraction was then transferred to new tubes and 1 ml of urea/thiourea buffer was added to each tubes. All samples were mixed on vortex machine and incubated at room temperature for approximately 1 hour and then stored in a refrigerator at -20 °C for analysis.

Sample preparation for SDS-PAGE analysis and gel staining

Protein fractions were centrifuged for 5 minutes at 10,000 x g at room temperature prior to the electrophoretic analysis. Samples were loaded into 1 cm x 12 cm x 14 cm into 12.5% polyacrylamide gel with 3% stacking gel. First, gel capillary chamber was loaded with a broad range molecular weight standard (BioRad Laboratories, Hercules, CA). Proteins were separated by applying constant voltage of 300 Volts for 2 hours 30 minutes until the dye front reached the bottom of the gel. After electrophoretic separation, gels were stained overnight with gentle agitation on an orbital shaker. Staining buffer contains (400 ml methanol, 50 ml glacial ethanoic acid and Coomassie Brilliant Blue G250) and subsequently destained with 10% ethanoic acid.

Image was visually analysed into the three class sizes from the 12.5% SDS PAGE raw image produced.

RESULTS

In Figure 1, the electrophoregram resolved eleven bands found in large fish, eight bands in the medium fish and six bands were detected in the small fish class. The 2nd to 5th protein band having molecular weight (MW) ranging from 116,200 to 70 KDa was detected across all fish class sizes. Large sized and medium sized class observed a common band of MW 52 KDa which is absent in small class. The distinguishing bands 6th and 7th present in the large fish sample class but absent in the other two classes, having MW 54.52 KDa and 54.21 KDa respectively. The 1st band was the heaviest (MW 118,600 KDa) band only found in the large sized fish class but absent in the other fish classes.

In Table 1 a total number of thirteen protein bands were revealed by the protein electrophoregram. The Bio range standard was calibrated having molecular weight (MW) of 240,000 KDa to the minimum of 21,200 KDa.

No band was resolved for any of the class sizes between the highest MW ranges of 240,000 KDa and 230,000 KDa. The first largest protein band resolved was found in large size class with MW 118,600 KDa followed by 116,600 KDa. The MW of 116,600 KDa ran across the three class sizes followed by MWs of 110,000 KDa, 95,000 KDa and 70,000 KDa in the three class sizes.

The protein band size with MW of 66,200 KDa was resolved only in the large class size followed by MWs of 54,420 KDa and no band was resolved for medium and small class sizes. This was followed by MWs 52, 210 KDa in the large and medium category, at the eleventh reference number in Table 1, MW of 45,000 KDa was resolved for large, medium and small size classes. The lowest band in the bio-range standard 21,200 KDa was also resolved across the three class sizes.

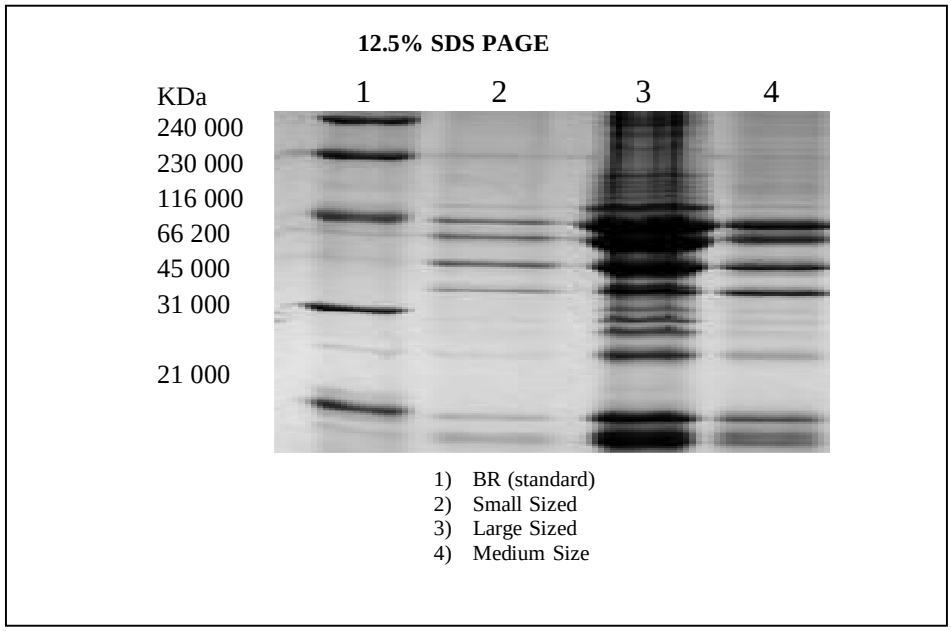

Figure 1: Raw image of 12.5% SDS Whole Muscle Protein of *C. gariepinus.*

Table 1: Molecular weights of bands detected for Calibrated Image of 12.5% SDS Sarcoplasmic Muscle Protein of *C. gariepinus,*

Ref Band	Bio Range	Large	Medium	Small
1	240,000	-	-	-
2	230,000	-	-	-
3	-	118,600	-	-
4	116,600	116,600	116,600	116,600
5	-	110,000	110,000	110,000
6		95,000	95,000	95,000
7		70,000	70,000	70,000
8	66,200	66,200	-	-
9		54.420	-	-
10		54.210	54,210	-
11	45,000	45,000	45,000	45,000
12		40,000	40,000	40,000
13	31,000	-	-	-
14	21,200	21,200	21,200	21,200

DISCUSSION

The ratio of large sized to medium sized and small sized fingerlings obtained in this research show a remarkable need for more research and development of large sized fingerlings. Electrophoresis separation by SDS-PAGE muscle sarcoplasmic proteins revealed protein variations among same species of fish. This corroborated the findings of Agbebi et al. (2013) where the protein fingerprint of fish size variation in yellow perch was identified. Protein variation among the same batch of fish species connote that there are proteomic differences amongst them therefore amino acid improvement in their diets could be the possible solution. The problem resulting from unwholesome hatchery practice of mixed broodstock crossing should be addressed (Nwafili and Gao, 2007) so that aquaculture practitioners exploiting the fertility of the intraspecific F_1 and F_2 *Clarias* (*gariepinus* and *anguilaris*) hybrids as breeders for further propagation.

This result has revealed that 1st, 6th and 7th bands were the distinguishing protein bands. The first band was the heaviest MW (118,600 KDa) band and was identified as myosin breakdown protein product, the sixth and the seventh bands with MW of 54 KDa were assigned as Desmin protein (Albert et al., 1994) which helps in muscle contraction, plays significant roles in maintenance and structural built up muscles. The 2nd to 5th bands of Molecular weight ranging from 116,200 to 70 KDa correspond to protein Nebulin and Actin. The combinations of these proteins have contributed to the heterozygosity nature of the large size class which are most suitable for broodstock selection for future artificial propagation practices.

Conclusion

This work has been able to prove that the challenge of size variation in fish breeding or artificial propagation of the same species fed with the same feed and at the same time can be tackled by aquaculturist using the right brooders. The protein profile of the fish in this study suggests that the heterozygosity in large fish class might be as a result of the presence of the protein Desmin which is the influential protein in this fish class. This study also suggests that the higher the band, the greater the heterozygosity in fish sizes as the large fish size had the highest number of bands compared to the medium and small fish sizes. Desmin play a critical role in the maintenance of structural and mechanical integrity of the contractile apparatus in the muscle tissues. This large fish should be jealously guided for future use as it will be excellent brooders for breeding exercise although the medium class can also be used.

This study displaces the hypothesis that discrepancies in fish class size is as a result of environmental or management problems, but could be concluded as a result of the protein profiles of the various class sizes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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