CYTOTOXICITY EFFECT AND UTILIZATION OF *Mytilus edulis* SHELL IN THE DIET OF *Clarias gariepinus*

Ayoola, S. O

Department of Marine Sciences, University of Lagos, Lagos State, Nigeria
Corresponding author:email:sayoola@unilag.edu.ng,soaayoola@yahoo.com,
Tel: 234(80)34650102

ABSTRACT

Compounded feed has been known to enhance high yield, promotion of growth and enables the fish farmers to produce table size fish within the shortest possible time. Five isonitrogenous diets (100% Dicalciumphosphate (DCP) compounded feed, 75% DCP Compounded feed and 25% Mytilus edulis shell, 50% DCP Compounded feed and 50% Mytilus edulis shell, 25% DCP compounded feed and 75% Mytilus edulis shell, 100% Mytilus edulis shell were formulated. The Mytilus edulis shell was washed, sun dried for 5 days and later ground and added to the feed and were fed to 150 juveniles of Clarias gariepinus which were distributed into 15 different plastic bowls. Ten specimens were stocked in each of the plastic bowls and they were fed twice daily for an experimental period of 12 weeks. In the cytotoxicity, five fishes were picked randomly from each triplicate and their blood collected carefully with syringe from the anal region and kept in sample bottles. The blood was smeared on slides and were air-dried for 24 hours, fixed in ethanol for 20 minutes and followed by 10% Giemsa staining. Each fish had 2000 erythrocytes examined and to detect micronuclei in erythrocytes, the slides were analysed using a 1000X oil-immersion lens. Result shows that specimens fed with 100% DCP compounded feed (control feed) had the highest Mean Weight Gain (MWG) (231.67±5.4g) and Specific Growth Rate (SGR) (1.2±0.3). The second best result was shown in specimens fed with 100% Mytilus edulis shell with the highest Average Daily Growth (ADG) (2.63±0.27), Protein Efficiency Ratio (PER) (5.54±0.59) and Average Final Weight (AFW) (353.33±7.63). . The lowest value Feed conversion ratio (FCR) of 2.21±.01 was obtained in T_4 (100% Mytilus edulis shell) hence, 100% Mytilus edulis shell recorded the best FCR. The poorest result was shown in specimens fed with 25% Mytilus edulis shell recording MWG (215±13.22g), SGR (1.15±0.09), ADG (2.50±15), PER (5.19±0.38) and AFW (341.67±10.0). In most cases, differences in fish weight or specific growth rate (SGR) were not significant. Results for micronucleus reveals that T_1 (25% MES) had the highest number of micronucleated cells while the rest (T_2, T_3, and T_4) including the control had no significant differences. Results for BN reveal that T_1 had the highest number of BN and T_0 while the control had the least number of BN. In this study the results showed Mytilus edulis shell can be used as subtitute for DCP in the diet of Clarias gariepinus.

Keywords: Cytotoxicity, Utilization, Mytilus edulis , Clarias gariepinus

INTRODUCTION

Aquaculture is regarded as being uniquely placed to reverse declining supplies from capture fisheries and the activity has notable potential for new livelihood opportunities, providing the mechanism for lower prices of fish, enhanced nutritional security and employment for poor communities by servicing urban markets (Jagger and Pender, 2001). The substantial expansion in aquaculture production is an indication that the sector could be a major player in augmenting the supply of fish protein for consumption and a major source of income for farmers and for foreign earnings. Recent trends all over the world, point to a decline in landing from capture fisheries, an indicator that fish stocks have approached or even exceeded the point of maximum sustainable yield. Omoregie and

Ogbemudia (1993) advised that it will be more economical to utilize plant protein and accept a reduced growth rate of aquatic animal than feeding fish meal at high cost. According to Ayinla (1991), the essential nutrient requirements of fish are protein, carbohydrates, oils, vitamins, minerals, salt and fibre. Rumsey (1993) reported that fish feed accounts for a major part (30-70%) of the total operational cost of an average fish farm. Warm water fishes require protein and calcium in their diets (Lim *et al.*,1998).

Artificial feeding of fish has many known advantages which include enhancement of high stocking density especially in polyculture system resulting in high yield, promotion of growth and enablement of the farmer to observe the behaviour of his fish during feeding in order to detect any abnormality (Schoonbee and Prinsloo, 1989). Unlike in the past when fish depends on natural food in the pond, the production of fish feed is becoming popular with each passing day in the country. The desire of fish farmers to produce table size fish within the shortest possible time has been on increase. Long term success in meeting this goal and having an all-year round supply of fish depends on the ability of the farmer to control the entire life cycle of the fish (Ekelemu and Ekokotu, 1999).This desire is met by the popular catfish of the genus *Clarias*. *Clarias gariepinus* occupies a unique and prominent position in the commercial fisheries in Nigeria because it is tasty, hardy, tolerating poor water quality conditions (Idodo-Umeh, 2003). It is also capable of reproducing in captivity and growing to a size of 7.0 kg (Idodo-Umeh, 2003), has an efficient feed conversion (Nweke and Ogwumba, 2005) and so attracts high market price. The objective of this study is to evaluate the level of replacement of *Mytilus edulis* shell in the diet of *Clarias gariepinus* and to determine the cytogenetic effect of the *Mytilus edulis* shell.

MATERIALS AND METHODS

EXPERIMENTAL PROCEDURE

This experiment was carried out in Department of Marine Sciences, Faculty of Science, University of Lagos. *Mytilus edulis* shell (mussel shell) was collected from Lagos lagoon, dried for 5 days, processsed by grinding and kept safe. And other ingredients such as maize, indomie, wheat offal, groundnut cake, soyabean meal, palm kernel cake, fishmeal, dicalcium phosphate, fish premix, vitamins were bought from fish feed market at Agege, Lagos State. *Mytilus edulis* shell was sun-dried for 5 days and ground and other ingredients such as maize, groundnut cake and soybean meal were ground separately. The ground shell meal and DCP were added to other feed ingredients at different inclusion levels. The first experimental diet contained 75% of DCP and 25% of *Mytilus edulis* shell, the second contained 50% of DCP and 50% shell meal, the third contained 75% of DCP and 25% of *Mytilus edulis* shell and the fourth contained 100% of *Mytilus edulis* shell. The control diet had 100% of DCP and no *Mytilus edulis* shell. There are 5 tanks altogether in triplicates. The crude protein value of the diets is 40% The fish were fed 5% of their body weight. The experimental diets are shown in table 1 with different levels of test ingredients. A fresh water fish species, *Clarias gariepinus* was used for this research. One hundred and fifty fingerlings were bought from a private fish farm, Faurd farm at Cele, Ikotun-Egbe, Lagos and plastic tanks were used to transfer it to the eco-toxicology laboratory of Department of Marine Sciences, University of Lagos. The fish were kept in plastic tanks where the experiment was being carried out to acclimatise them which lasted for 2 weeks. During acclimatisation period, the fish were fed with 2mm coppens after which they were randomly allocated on basis of body weight into fifteen plastic bowls. In general, there were 15 bowls, each treatment was in triplicate making it five treatments in all. They were covered with net of mesh size 3mm to prevent the fish from jumping out of the tanks. The dimension of the tanks were 50x33x34 centimetres. Ten fish of average weight 126.67± 2.81g were stocked in each tank and they were in triplicates.

Table 1: Feed composition of experimental diet in percentage

Ingredients	T_0	T_1	T_2	T_3	T_4
	100 % Composition each				
Maize	15	15	15	15	15
Indomie	8	8	8	8	8
Wheat	8	8	8	8	8
Groundnut cake (GNC)	20	20	20	20	20
Soy bean meal (SBM)	11	11	11	11	11
Palm kernel cake (PKC)	10	10	10	10	10
Fish meal	10	10	10	10	10
DCP	2	1.5	1.0	0.5	-
Mytilus edulis shell	-	0.5	1.0	1.5	2
Fish premix	0.5	0.5	0.5	0.5	0.5
Vitamin	0.25	0.25	0.25	0.25	0.25

T_0 (Tank 0) = (100% DCP compunded feed) (control).

T_1 (Tank 1) = (75% DCP compounded feed and 25% *Mytilus edulis* shell).

T_2 (Tank 2) = (50% DCP compounded feed and 50% *Mytilus edulis* shell).

T_3 (Tank 3) = (25% DCP compounded feed and 75% *Mytilus edulis* shell).

T_4 (Tank 4) = (100% *Mytilus edulis* shell).

The first tank which is the control has no *Mytilus edulis* shell as a calcium source. Each tank was labelled T_0-A,T_0-B,T_0-C,T_1-A,T_1-B,T_1-C,T_2-A,T_2-B,T_2-C,T_3-A,T_3-B,T_3-C,T_4-A,T_4-B,T_4-C. The T_0-A, T_0-B and T_0-C served as the control tanks.

Fish were fed by 5% body weight and the feeding regime was divided into two at 07:00am and 05:00pm every day. The fish were weighed on weekly basis, the length was taken fortnightly, water in the tanks were changed every 2 days to maintain good water quality of 6mg/L of dissolved oxygen and replaced with already dechlorinised water of pH between 6.5-7.0 and water temperature range of 27-30° C. Fish were monitored daily and the experiment lasted for a period of 12 weeks (84 days).Water was taken from the tap in the experimental unit when needed and was timely fetched into the experimental tanks.

After 14 days of acclimatization, 10 juveniles of *C. gariepinus* were transferred into each of the plastic experimental tanks using a scoop net. The physico-chemical parameters in the tanks were measured during the experiment and suitable conditions were maintained by cleaning the tanks and constant changing of the water which took place every day.

The proximate composition of *Mytilus edulis* shell was analysed at Department of Animal Science University of Ibadan (UI). Crude protein fibre was determined using the Kjedahl distillation method (A.O.A.C, 2000).

CYTOTOXICITY

Micronucleus

Five fishes were picked randomly from each triplicate and their blood collected carefully with syringe from the anal region and kept in sample

bottles. The blood was smeared on slides and were air-dried for 24 hours, fixed in ethanol for 20 minutes and followed by 10% Giemsa (v/v) staining. Each fish had 2000 erythrocytes examined and to detect micronuclei in erythrocytes, the slides were analysed using a 1000 X oil-immersion lens.

GROWTH AND NUTRIENT UTILIZATION PARAMETERS

Growth and nutrient utilisation parameters were calculated as stated below:

Protein Efficiency Ratio (PER)

The PER was calculated using the formula below:

$$PER = \frac{\text{Mean weight}}{\text{Protein Intake}}$$

Mean Weight Gain (MWG)

$$MWG = \frac{\text{average weight gain}}{\text{Number of days}}$$

Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{feed intake}}{\text{Weight gain}}$$

Specific Growth Rate (SGR)

$$SGW = \frac{(\text{Log e } W_1 - \text{Log e } W_2) \times 100}{T_1 - T_2}$$

Where, e = natural logarithm

$T_2 - T_1$ = experimental period

W_1 = initial weight

W_2 = final weight

Average Daily Growth (ADG)

$$ADG = \frac{\text{Average weight gain}}{\text{Number of days}}$$

Voluntary Feed Intake (VFI)

$$VFI = \frac{100 \times \text{Average Feed Intake}}{\text{Initial weight} + \text{Final weigh}} \times \text{days}$$

Relative Growth Rate (RGR)

$$RGR = \frac{\text{Average Weight Gain}}{\text{Initial weight}} \times 100\%$$

Relative Weight Gain (RWG)

$$\text{Relative Weight Gain (RWG)} = \frac{W_2 - W_1 \times 100}{W1}\%$$

Where; W_1 = initial weight

W_2 = final weight

Average Feed Intake (AFI)

$$AFI = \frac{\text{Total feed}}{\text{Number of days}}$$

Protein Intake (PI)

PI = Feed Intake x % of protein in diet

STATISTICALANALYSIS

All the data collected throughout the experimental period were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests.

RESULTS

Table 2 shows the growth performance and nutrient utilization parameters of *Clarias gariepinus* fish fed with *Mytilus edulis* shell.The physico-chemical parameters measurement for all the treatment are shown in table 3 while the proximate analysis of *Mytilus edulis* shell was shown in table 4.

Growth and nutirent utilization parameters

Weight gain increased over the experimental period and the highest weight gain was recorded in T_0 (0% *Mytilus edulis* shell). It also recorded significant weight gain but the least weight gain was recorded in T_1 (25% *Mytilus edulis* shell). The average initial weights of the fish are not significantly different throughout the experimental tanks. Both Feed Conversion Ratio (FCR) and the Specific Growth Rate (SGR) were significantly different as T_0 recorded the best and Specific Growth Rate (SGR) (1.2±.03) and T_4 (100% *Mytilus edulis* shell) recorded the best in Feed Conversion Ratio (FCR) with almost no significant difference from T_0.(T_4 = 2.21±.23, T_0 = 2.21±.02 respectively).The use of the test ingredient did not significantly affect crude protein or replacement of Dicalciumphosphate, hence DCP can still be replaced but T_4 recorded the best FCR.

Feed Conversion Ratio (FCR) shows the amount of unit weight of feed that specimens were able to convert into unit weight of muscle. The higher the FCR, the worse it is while the lower the FCR the better it is. The lowest value of

Table 3: Physico-chemical parameters of the experimental tanks

Tanks	Temp ^{0}C	pH	Dissolved oxygen(mg/l)
T_0	28.4±0.51^d	7.4±0.25^d	7.6±0.62^b
T_1	28.4±0.39^b	7.0±0.23^c	7.6±0.58^a
T_2	28.2±0.46^c	6.9±0.21^b	7.4±0.57^a
T_3	28.3±0.40^c	7.1±0.15^a	7.4±0.59^a
T_4	28.3±0.20^a	7.0±0.25^d	7.8±0.66^b

**Mean values followed by the superscript in each row are not significantly different ($p < 0.05$)*

Table 4: Proximate analysis of *Mytilus edulis* shell

Sample	Crude protein	Fat	Fibre	Ash	Moisture	Calcium	Energy
Mytilus edulis shell	6.76	0.50	15.69	61.58	29.05	33.39	291

CYTOTOXICITY

The obtained results for micronucleus reveals that T_1 (25% MES) had the highest number of micronulcleated cells while the rest (T_2, T_3, and T_4) including the control had no significant differences (table 5). Results reveal that there were significant differences with T_1 having the highest number of binucleated cells (BN) and T_0 (control) having the least number of BN. Results show there were no significant differences between all treatments from control to T_1 to T_4 of lobbed cells (LB) while the blobbed cell(BL) shows no significant differences among the treatments. Notch cells reveal that there were no significant differences among the treatments using Duncan Analysis.

Table 5: Mean frequencies of different nucleated cells in erythrocytes of *Clarias gariepinus* fed with *Mytilus edulis* shell.

CELLS/DIETS	T_0 (CONTROL)	T_1 (25% MES)	T_2 (50% MES)	T_3 (75% MES)	T_4 (100% MES)
MN	2.29±0.68^b	4.14±4.02^a	2.17±1.06^b	2.15±0.67^b	2.21±.66^b
BN	7.30±7.79^c	45.13±33.71^a	31±30.34^{ab}	13.20±11.71^{bc}	15.60±8.95^{bc}
LB	3.80±1.03^a	5.20±5.07^a	3.73±0.79^a	3.60±1.05^a	3.60±0.82^a
BL	2.22±0.42^a	2.57±2.17^a	2.33±0.09^a	2.24±0.35^a	2.38±0.56^a
NT	1.51±0.26^a	1.58±0.50^a	1.75±0.35^a	1.64±0.00^a	1.66±0.09^a

**Mean values followed by the superscript in each row are not significantly different ($p < 0.05$)*
MES = Mytilus edulis shell, MN = Micronucleus, BN = Binucleus, LB = Lobe, BL = Bleb, NT = Notch.

DISCUSSION

The feeding trials revealed that *C. gariepinus* responded to all the diets, irrespective of their composition. *C. gariepinus* was able to effectively utilize the *Mytilus edulis* shell in the feed. Calcium has been a major constituent of inorganic portion of feeds, they make up 70% (including phosphorus) of the mineral elements in the body and are essential for the formation of bone, energy transfer through ATP (Adenosine triphosphate) and an essential component of buffer system in the blood (Ayoola, 2010). In the present investigation, all the experimental diets were accepted by *Clarias gariepinus* juveniles, indicating that the levels of incorporation of *Mytilus edulis* shell did not affect the palatability of the diets. There were no significant difference in the growth and weight especially in the control diet (T_0) and this might be attributed to acceptance of *Mytilus edulis* shell. Schwarz (1995) considered that calcium requirements of fish are usually adequately supplied from the water and the calcium in feed can thus be minimised. Under practical farming conditions, mineral deficient signs often arise from a dietary imbalance of calcium due to the antagonistic effect of excess dietary calcium on absorpton when there is excess of calcium (Nkamura, 1982). *Clarias gariepinus* is commonly produced in Nigeria because of its fast growth rate and profitability. Efficient production and growth of fish depend on feeding the best possible diets at levels not exceeding the dietary needs (Charles *et al.*, 1984). In fish culture practices, studies on the amount and efficacy of *Mytilus edulis* shell are aimed at identifying the optimum inclusion levels. This experiment was done to determine the effect of *Mytilus edulis* shell as a source of calcium in the diet of *Clarias gariepinus*.

Hayashi *et al.*, (1998) evaluated monitoring systems that use aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. In a field study, micronucleus assay was shown to be applicable to freshwater and marine fishes and that gill cells and hematopoietic cells are sensitive to micronucleus (MN) inducing agents. Bolognesi *et al* (2006) evaluated the centromeric labeling to distinguish micronuclei induced by chromosomal loss and breakage *in vitro*. While Rodriguez-Cea *et al.* (2003) determined the sensitivity of micronucleus test in freshwater fish species for application in field surveys. Mutagenic studies with native fish species like *C. gariepinus* represent an important effort in determining the potential effects of *Mytilus edulis* shell (MES) to check if toxic agents are present. This study was carried out to evaluate the use of the micronucleus test (MN) and other cell aberations for the estimation of haematopoietic damage using different fish genotypes under laboratory conditions. Fish serve as useful genetic models for the evaluation of pollution in aquatic ecosystems (Cavaş and Ergene-Gozukara (2005a). The erythrocyte micronucleus test has been used with *Clarias gariepinus* to check the extent of mutagenic features caused by using MES in diets of fish. The obtained results support the fact demonstrated by Fagr *et al.*, (2008) that fish inhabiting polluted waters have greater frequencies of micronuclei.

CONCLUSION

The micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. Hence, the more the toxins the more micronucleus. But from the results, it is shown that the *Mytilus edulis* shell (MES) has no toxins since there are less micronucleus and other aberrations in the haematopoietic cells. Hence, *Mytilus edulis* shell can be included at all levels.

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