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Growth Performance, Haematology and Biochemical Characteristics of *Clarias gariepinus* (Burchell, 1822) Juveniles fed Quail Eggshells as replacement for Dicalcium Phosphate

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ABSTRACT
A ten week feeding experiment was carried out in order to examine the effect of replacing dicalcium phosphate (DCP) with milled Quail Egg Shells (QES) on the growth performance, haematology and biochemical parameters of *Clarias gariepinus* juveniles. A control experiment was set up with 39% crude protein diet. One hundred and fifty juveniles of *Clarias gariepinus* with weight range 213.0 – 215.0 g (mean weight: 213.6 ± 0.55) was fed for ten weeks on, 0, 25, 50, 75 and 100% inclusion levels of QES as a substitute for dicalcium phosphate. The results show that, highest mean weight gain (MWG) was recorded in fish fed 50% QES-based diet (44.06±7.09 g), while the lowest weight gain was recorded on the fish fed control diet (25.62±10.16 g). In the haematological parameters, the fish fed 50% egg shell had the highest packed cell volume (PCV) (38.90±0.87 g/l) and the white blood cell count (WBC) (162.60±2.71 x 10^9 G), while the biochemical analysis showed that the fish fed 25% eggshell diet had the best values (13.88 ± 0.62 mg/gl; 2.00 ± 2.00 mg/gel) in urea and alkaline phosphatase (ALP) respectively. It is concluded, based on the growth performance and haematological parameters that the 50% dicalcium phosphate can be replaced by milled quail egg shells in the diet of *Clarias gariepinus* juveniles... Although it showed a slight decrease in the biochemical parameters, it had no negative impact on the health status of the species.

Key words: Dicalcium phosphate, Quail eggshell, replacement, *Clarias gariepinus*, fish feed

INTRODUCTION

Aquaculture started on a pilot scale in Nigeria since the mid-1950s. After a series of fluctuation, aquaculture has come to stay in Nigeria and is now passing through an exciting phase of evolutionary growth. Aquaculture has prospects in Nigeria with a population of over 140 million, the fish demand estimated at 1.6 million metric tons, while the current supply was 640,000 metric tons (FDF, 2005).
One of the major problems confronting aquaculture in Nigeria is the inadequate quality and quantity of feed supply (Fagbenro and Adebayo, 2002). Most fish farmers in Nigeria cannot make use of quality fish feeds due to its high cost (Eyo, 2002). It was noted that Nigeria produces large quantities of agricultural and agro-industrial byproducts, which serves as alternative feed sources to conventional feed (Ayoola, 2011). Dicalcium phosphate is a feed additive incorporated into fish feed to provide calcium and phosphorus, which are important minerals for growth. It is derived from bones of animals which undergo several chemical processes (in which a large amount of protein is lost) before it can be safe for usage. A breach in these chemical processes will complicate the end result, so the various steps must be strictly followed (Waldroup, 1997).

Quail is a collective name for genera of mid-sized birds generally of the family Phasianidae considered in the order Galliformes. The Quail eggshells are cream coloured with brownish speckles. They are very small in size compared to the everyday chicken eggshells readily available. In fact, about three quail eggs will make one chicken egg. They have been noted to contain a certain level of calcium (0.55 mg) and phosphorus (220 mg) (Shim Kim Fah (2005) cited by Onyewuchi *et al*, 2013)
Comparing the series of processes required to acquire dicalcium phosphate and the simple physical process of milling the eggshells of Quail, one would note that cost is being reduced, labour is minimal and nutrients are being preserved as no concentrated chemical is being added in the favour of quail eggshells, unlike in the case of dicalcium phosphate where concentrated hydrochloric acid (HCl) is

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added. This study was to investigate the effect of feeding milled Quail eggshell (QES) on the growth, haematological and biochemical parameters of *Clarias gariepinus,* with the view to determining the appropriate replacement level(s) with dicalcium phosphate.

MATERIALS AND METHODS

Preparation of feed

Dried quail egg shells were bought from Lagos environs, and milled to powder using Hammer miller. The feed was formulated using Pearson's Square method to derive 39% CP (Table 1). A small quantity of the milled quail egg shell was taken to the Department of Chemistry, University of Lagos for analysis of the proximate composition. The feed was pelleted to 2mm size to enable the fishes feed on it.

Experimental procedure

The experiment was carried out in the Research Laboratory of the Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka. One hundred and fifty (15) juveniles of *Clarias gariepinus* were procured from a reputable source in Ogun state. The fishes were transported in an open plastic container to the location of the experiment and acclimatized for 14 days in the experimental medium. They were fed 2mm Coppens feed prior to the commencement of the experiment. The experiment was done in triplicates so each replacement level had three tanks (55 x 33 x 31 cm^3) allocated with ten fishes with a mean weight of 213.6 ± 0,55g. The tanks were labelled according to the inclusion level Control 1 (a, b, c), Treatment 2 (a, b, c), Treatment 3 (a, b, c), Treatment 4 (a, b, c) and Treatment 5 (a, b, c) as indicated in Table 1. The tanks were filled with de-chlorinated water, which was changed every two days to ensure good water quality management.

Water quality management

The physical and chemical parameters of de-chlorinated water used were recorded during the experiment. The air and surface water temperatures were recorded using a mercury-in-glass thermometer; pH was recorded by a Jenway Hanna pH-meter (Model: H1 991301), while the Dissolved Oxygen was measured using the Winkler method described by Boyd (1979).

Table 1: Feed composition of experimental diet

Feed ingredients (%)	**QES inclusion level (%)**				
	0	**25**	**50**	**75**	**100**
Fish meal	21.07	21.07	21.07	21.07	21.07
Groundnut cake	21.06	21.06	21.06	21.06	21.06
Noodle waste	16.40	16.40	16.40	16.40	16.40
Soybean meal	21.07	21.07	21.07	21.07	21.07
Maize	16.40	16.40	16.40	16.40	16.40
Palm oil	0.7	0.7	0.7	0.7	0.7
Salt	0.3	0.3	0.3	0.3	0.3
Fish premix	0.7	0.7	0.7	0.7	0.7
Vitamin C	0.3	0.3	0.3	0.3	0.3
D.C.P.	2.0	1.5	1.0	0.5	0
QES	0	0.5	1.0	1.5	2.0
% Total	100	100	100	100	100
Total Crude protein	39.38	39.38	39.38	39.38	39.38

QES = Quail Egg Shell

Growth and nutrient utilization parameters:

The mean weight of the fish in each tank was determined at the beginning of the experiment and at the end of each week. The weight of the fish was recorded using top loading electric weighing balance (OHAUS model Cs 5000). At the end of the growth trial, the followings were recorded: final weight, survival and feed consumed.

The following growth indices according to Efthimiou *et al*., (1994) were estimated using the following formulae:

i) Mean Weight Gain (g) $=W_f - W_i$/Number of days, Where W_f = final average weight (g), W_i = initial average weight (g).
ii) Relative Growth Rate (%) =Weight gain/ Initial body weight x 100
iii) Specific Growth Rate (%/day) = Log W_2 – Log W_1/ time (days) x 100
iv) FCR = Dry feed fed (g)/Live weight gained (g)

Haematological studies

The blood sample from each treatment were collected via caudal peduncle puncture as described by Stockopf (1993). The blood samples were emptied into the EDTA bottle for haematological analysis at the Department of Paediatrics Research Laboratory, College of Medicine, University of Lagos. The necessary parameters were analysed using Automated Mindray Haematological Machine (Model BC-2800).

Statistical analysis

All the data collected during the course of the experiment were subjected to one-way analysis of variance (ANOVA) and DMRT, (1950) was used to compare mean, among the treatments at the 95 % level of confidence using SPSS Version 10.

RESULTS

The growth performance of *C. gariepinus* juvenile fed with QES are shown in Table 2.
The final weigh (FW), mean weight gain (WG), specific growth rate (SGR) and food conversion ration (FCR) were higher in juveniles fed 50% QES based diet. There were no significant differences ($p>0.05$) between the FW, WG, SGR and FCR of fish feed 50% QES compared to the entire treatments. However, feed intake was significantly lower in fish fed 100% QES based diet.

Table 2: Mean (± SEM) growth performance of *C. gariepinus* fed with Quail egg shell

Growth parameters	**QES inclusion levels (%)**				
	0	**25**	**50**	**75**	**100**
Initial average weight (g)	214.00	213.00	214.00	213.00	214.00
Final average weight (g)	1004.49	1141.72	1203.98	1096.11	819.18
Mean weight gained (g)	25.62±10.16^a	35.70±13.35^a	44.06±7.09^a	38.81±9.07^a	25.89±7.95^a
Specific growth rate	2.36±0.27^a	2.44±0.28^a	2.45±0.29^a	2.43±0.14^a	2.01±0.36^a
Relative growth rate	119.86±48.10^a	167.46±62.89^a	185.31±33.39^a	181.84±41.99^a	121.25±37.54^a
Food conversion ratio	3.70±1.24^a	2.84±1.23^a	4.09±1.29^a	2.19±0.65^a	2.54±0.75^a
Average feed intake	1.24±0.04^{bc}	1.29±0.04^{bc}	1.32±0.05^c	1.16±0.13^b	0.89±0.09^a

Means in the same column having similar superscript are not significantly different ($p>0.05$)

Table 3 showed the haematological parameters of *Clarias gariepinus* fed with QES based diet
The white blood cell was significantly in fish fed 50 % QES based diet, followed by those fed 0 % (control) based diet. The higher Hb were also observed in fish fed 50% QES, followed by those feed with 25% QES. There was significant differences between the Hb values of fish 50% QES compare to those of fed 25%. Similarly, there were significant difference between the Hb value of fish fed 75 and 100 % QES.

The RBC and PCV were signed ($p<0.05$) higher in juvenile fed with 50% QES followed by those fed control diets. Lower RBC and PCV were recorded in fish treated with 100% QES.
Fish fed 75% QES had the highest MCV and MCH. No significant variation ($p>0.05$) was observed in the MCV values of the fish fed 75% QES compared to those fed with 25% QES based diet. However, significant differences ($p<0.05$) were notice between the MCV values of fish fed 0, 50 and 100% QES based diet. No significant variation was observed in the MCH values among the entire treatments.
MCHC values were slightly higher in fish fed 100% QES based diet. The MCHC values of the entire treatment were statistically the same.

Table 3: Haematological parameters of *Clarias gariepinus* fed with Quail eggshell-based diet

Haematological parameters	**QES inclusion levels (%)**				
	0	**25**	**50**	**75**	**100**
WBC ($x10^9$(g)	160.60±5.81^c	144.70±4.03^b	162.60±2.71^c	141.60±1.56^b	133.80±1.80^a
Hb/l	11.70±0.20^b	10.67±0.21^a	11.90±0.20^b	10.50±0.20^a	10.20±0.50^a
RBC ($x10^{12}$(t)	2.60±0.20^{ab}	2.35±0.20^{ab}	2.69±0.23^b	2.30±0.27^{ab}	2.25±0.50^a
PCV (g/l)	37.50±1.87^{bc}	35.50±1.74^b	38.90±0.87^c	34.90±0.95^a	32.40±1.40^a
MCV ($x10^{-15}$ (Fe)	143.90±1.40^a	149.30±2.50^b	144.70±1.00^a	152.00±3.61^b	144.07±2.80^a
MCH ($x10^{-12}$ (pg)	44.80±1.18^a	44.90±1.18^a	44.20±1.13^a	45.60±0.90^a	45.30±3.15^a
MCHC (g/l)	31.20±3.10^a	30.10±2.56^a	30.50±1.90^a	30.13±1.31^a	31.50±2.25^a

Means in the same column having similar superscript are not significantly different (p>0.05).

The biochemical parameters of *Clarias gariepinus* fed QES is presented in table 4. The higher TP and ALB were each recorded in the fed control diet, followed by those fish fed 75 and 100% QES based diet. Significant differences were observed between the TP values of the control diet compared to the rest of the QES based diet. However, there were no significant differences between the ALB values of the control diet compared to rest of the treatment. Fish fed 0% QES were significantly higher, followed by those fed 100% QES based diet.

Table 4: Biochemical parameters of *Clarias gariepinus* fed with Quail eggshell for 10 weeks

Biochemical parameters	**QES inclusion levels (%)**				
	0	**25**	**50**	**75**	**100**
TP g/l	5.00±0.20^b	4.00±0.40^a	4.20±0.30^a	4.90±0.20^a	4.48±0.47^b
ALB mg/dl	2.00±0.40^a	1.30±0.40^a	1.60±0.40^a	1.80±0.30^a	1.60±0.39^a
TRIG mg/gl	228.00±6.00^b	154.00±3.00^a	148.00±8.00^a	140.00±2.00^a	180.00±14.00^a
UREA mg/gl	14.82±0.55^b	13.88±0.62^a	16.77±0.51^d	18.81±0.54^c	18.88±0.26^d
ALP mg/gl	28.33±3.51^b	2.00±2.00^a	69.00±4.00^c	22.00±3.00^a	31.00±3.00^b
Creat mg/dl	0.50±0.10^c	0.30±0.10^{ab}	0.20±0.10^a	0.50±0.10^c	0.40±0.10^{bc}
GLB mg/dl	3.00±1.00^a	2.70±0.60^a	2.70±0.80^a	2.70±0.17^a	3.10±0.36^a

Means in the same column having similar superscript are not significantly different (p>0.05)

The UREA level increased with an increase in the QES level in the diet of the juvenile *C. gariepinus*. The higher UREA was observed in fish fed 100% QES based diet, those fed 75 and 50 % QES based diet. Significant variation (p<0.05) was observed in all the UREA values among the entire treatments. The ALP values were higher in juveniles *C. gariepinus* fed 50% based diet followed by those fed 31% QES based diet. Low ALP was recorded in fish fed 25% QES based diet. There was not significant differences between the ALP values from the fish fed 25, 75 and 100 % QES based diet.

Creat level were significantly higher in fish fed 0 and 75 % QES based diet, followed by those fed 100, 25 and 50% QES with 0.40±0.10, 0.30±0.10 and 0.20±0.10, respectively. No significant difference (p>0.05) was observed between the Creat values of 0, 75 and 100% QES based diet. Similarly the Creat values of fish fed 25 and 50% were statistically the same (p>0.05).

The highest GLB still observed in the fish fed 100% QES followed by the control diet. However, fish fed 25, 50, and 75 % has similar levels (2.70±0.60-0.8 mg/dl) of GLB. There was no significant difference between the GLB levels among the entire treatments.

DISCUSSION

The feeding trials revealed that *Clarias gariepinus* responded to all the experimental diets, irrespective of the composition of the QES. *Clarias gariepinus* was able to effectively utilize the Quail eggshell for growth. It is important to note that better growth and nutrient utilization were achieved at relatively low inclusion levels of quail egg shell compared to high incorporation of the test ingredient. The fish showed a good appetite to all the treatment diets, attested to by the increases in body weight and feed intake except the 100% QES replacement level diet, which had the lowest average feed intake (AFI) of (0.89±0.09) as seen in Table 5. This might be due to the fact that, the increase in the inclusion level of quail egg shells probably reduces palatability, thereby reducing the intake of feed. A similar

trend was reported by Adikwu (1992) and Haruna (1997). However the greatest mean weight gain was achieved in the fish fed 50% eggshell-based diet (44.06±7.09) which was also a trend in the other parameters. The values of the minerals such as calcium (10.41 mg) and phosphorus (0.49 mg) obtained in the mineral composition analysis of the Quail eggshell for this study were higher than those reported by Shim Kim Fah (2005) cited by Onyewuchi *et al,* (2013) who reported calcium (0.55 mg) and phosphorus (220 mg) values in their findings.

Since most of the haematological values obtained in this study were seen to be highest in the fish fed 50% quail egg shell diet, it could be suggested that in the case of the other diets, the replacement levels tested had a major physiological stress on the health status of the fish studied. Previous haematological studies of nutritional effects brought the knowledge that erythrocytes, PCV and Hb are major and reliable indicators of various sources of stress (Rainza-Paiva *et al*, 2000) and these parameters decrease in the presence of anti-nutritional factors (Osuigwe *et al.*, 2007). Reduction in Packed Cell Volume (PCV) and Haemoglobin (Hb) value was also observed by Osuigwe *et al.*, (2007) when raw and boiled jack bean were fed at different dietary levels to juvenile *Heterobranchus longifilis*. Low Hb value was associated with the low active fishes (Satheeshkumer, *et al*, 2011). Increased RBC indicates higher oxygen requirement at higher metabolic rates in tropical region (Engel and Davis, 1964). The result of the findings on the RBC in the present study is similar to that reported by Satheeshkumer, *et al*, (2011). However, Das (1965) pointed that RBC and Hb concentrations tend to increase with length and age of fishes. The fishes fed the control diet recorded values which were within the ranges for healthy juvenile catfish (Oyelese et al 1999; Omoniyi et al 2002). The values closest to the values in the control were those in the 50% inclusion diet.

The biochemical analyses showed advantages for different replacement levels. The juveniles fed 25% eggshell-based diet was seen to have the best values in urea and alkaline phosphatase (ALP). A high level of alkaline phosphatase (ALP) would mean there is a blockage in the bile system and high level of urea is indicative of acute renal dysfunction (Basten, 2010). In the triglyceride (TRIG) values in fish fed 50% and 75% QES based diet had the least value. This is an advantage because the normal level of triglyceride is less than 150 mg/DL (Semenkovich, 2011). While significantly lower ($p<0.05$) levels of potassium, bi-carbonate and triglycerides observed in fish fed poultry litter is an indication of lower immunity level of fish Omitoyin (2007). High values in Creatinine are indicative of chronic renal dysfunction (Basten, 2010). The lowest value for creatinine was seen in the juvenile fed 50% eggshell-based diet. The globulin and albumin values are related, in that the two are combined to know the level of protein in the blood stream (Uyanik *et al.*, 2001). Values, which are lower or higher than the normal ranges indicate serious health conditions. According to Omitoyin (2007), a significant increase ($p<0.05$) in the values of plasma electrolyte of sodium, chloride, phosphate: excretory products of urea and creatinine; globulin and albumin/globulin ratio compared with initial values and fish fed control diet are suggestible of abnormal physiological function of metabolism in fish. Adams, *et al.,* (1993) recorded the normal range for total protein to be between 3 g – 6 g/100 ml and Adeyemo *et al*. (2003) recorded the reference value to be 3.8 mg ± 0.11 mg/100 ml. The values obtained in the present study are within the normal ranges for *C. gariepinus*.

Conclusion

In conclusion, 50% replacement level of dicalcium phosphate with Quail eggshell can be incorporated into the diet of *Clarias gariepinus* without any adverse effect on growth and disastrous effect on the health of the fish. It is an indication that the Quail eggshell which is considered as domestic waste may help reduce dependence on dicalcium phosphate in the diet of juvenile *C. gariepinus*, and invariably reduce a large proportion of the production cost of the conventional additive.

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