GROWTH AND BONE CALCIUM CONTENT OF *Clarias gariepinus* FINGERLINGS FED DIET ENRICHED WITH *Egeria radiatia* (CLASS: BIVALVIA) SHELLS

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Abstract: The shells of *Egeria radiata* were collected from DadinKowa reservoir in Gombe State. Eighty *Clarias gariepinus* fingerlings were bought from a fish farm in Samaru, Zaria, Kaduna State. The *Egeria radiata* shells were decarbonized (this was done by heating the shells to 400 – 600°C in a furnace) after which 200 g of it was used to formulate four experiment diets with different inclusion levels of the decarbonized shells viz: D_1(control diet), D_2 (3% shell and 2% bone meal), D_3(4% shell and 1% bone meal), D_4 (5% shell and 0% bone meal). The experimental diets were fed to the *Clarias* fingerlings for eight weeks. The effect of the experimental diets on the weight gain, specific growth rate length of fish, total and standard length were measured according to standard procedures. The best growth obtained with feed formulated from 4% snail shell and 1% bone meal inclusion levels (D1 = 84.87 g, D2 = 43.32 g, D3 = 147.86 g and D4 = 125.05 g). The highest bone calcium deposition was obtained with feed formulated from 3% *Egeria radiata* shell and 2% bone meal (457.488±6.55 ppm.). This shows that decarbonized shell from *Egeria radiata* (at 3% *Egeria radiata* shell and 2% bone meal) can be used as an alternative source of calcium in *Clarias gariepinus* feed for proper growth.

Keywords: Composition, *Clarias gariepinus,* fingerlings, bone content, diet, *Egeria radiata*

Introduction

Nigeria is endowed with abundant and enormous quantities of seashells (Ohimain *et al.*, 2009). Seashells are hard coated protective features as component of the body anatomy in small to medium size soft-bodied sea animals. These animals are found mostly on shores of the world's coastal regions. In West Africa, two of the dominant benthic organisms found along its coastal lagoons and mangrove swamp are *Egeria radiata* and *Thais coronate* (Ardovini and Cossignani, 2004). In Nigeria, shell fish are widely eaten for their proteineous and nutritious mineral content (Ogogo, 2004; Ohimain *et al.*, 2009) by the people of the Niger Delta region of Nigeria. However, after the soft flesh is eaten, the empty shells are repetitively discarded around settlements as refuse in spite of their ostensible economic value (Sidney and Young, 1981; Chang, 1991; Claude, 2002). Available evidence shows that these seashells contain a high proportion of calcium carbonate ($CaCO_3$) and trace amount of metal oxides which can be exploited and processed as a source of calcium supplement for our indigenous food industries (Malu and Bassey, 2003; Kocot *et al.*, 2016). One of the most important limiting factor in fish production especially in cultured fisheries is inadequate food supply that is balanced in energy, protein, minerals and vitamins for healthy growth and reproduction (Ovie, 1986). Fish was shown to be the primary source of calcium in the Filipino diet (Philippine Information Agency, 2012). Scarcity of high quality conventional feeds and the high competition between man and animals for cereals has necessitated the greater attention to be given into researching on unconventional feed resources in the developing nations including Nigeria. However, such unconventional feeds must be used with caution or backed up with published reports because some of them could contain toxic substances, the consumption of which could be harmful (Abowei and Ekubo, 2011; Soetan *et al.*, 2010).).

Calcium is one of the macro elements present in animal body including fish and are necessary for the maintenance of certain physicochemical processes in the tissues and fluids. They may be broadly classified into macro elements which includes; calcium, phosphorus, sodium, chloride, and micro elements such as iron, cobalt, potassium, iodine, zinc, magnesium, molybdenum, fluoride, chromium and Sulphur.

Dietary calcium plays a crucial role in the regulation of energy metabolism in aquatic organisms as such fish rely entirely on calcium that is present in water (Hossain and Yoshimatsu, 2014). The nature and quality of nutrients in fish is dependent upon their food types. Also, feed habit of an individual fish species has a great effect on its body nutrient composition. The principal proximate components of fish are water, protein, lipid and carbohydrate (Waterman, 1980; Odiko and Obirenfoju, 2017) while the following minerals viz: sodium, potassium, calcium, magnesium, phosphorus, sulphur, iron, chlorine, silicon, manganese, zinc, copper, arsenic and iodine are commonly found in fish, Odiko and Obirenfoju (2017).

Seashells are known to be useful natural calcium sources for food as well as in animal feed application (Palma *et al.*, 2017). Kocot *et al.* (2016) reported adult mollusks shell to be rich inorgano-mineral in which the calcium carbonate mineral makes up 95–99%. In the oyster shells, the high calcium carbonate content (80-95%) gives it the potential of been used as raw material for several products (Alvarenga, 2012). Although there are other sources of obtaining calcium which includes; from bone meal of fish and bones of land animals. The seashell of *Egeria radiata* is soft, cheap and easy to process compared to those of other species. The extraction and processing of seashells are more environmentally friendly than those of quarrying and mining of bone meal which comes with pollution of the neighboring environment (Jag, *et al.*, 2014). The knowledge of fish composition is essential so as to enable maximum utilization of needed mineral constituents for increased productivity in fish industry as well as reducing cost of production (Silva and Chamul, 2000). There is dearth of knowledge regarding the use of *E. radiata* shells as potential calcium source for fish production and hence the need for this study.

Material and Methods

Study area

DadinKowa reservoir is located in Yamaltu Deba Local Government Area of Gombe State in the Northern part of Nigeria. The dam is located about 35 kilometers from the metropolis. It is an important source of water for domestic activities for the inhabitants of the study area. The bivalve

Egeria radiata were collected from DadinKowa reservoir (11˚19'N and 11˚28'54'E) in Gombe State.

Collection of Egeria radiate and fingerlings

A random collection of 363 g of the snail shell was made. They were collected into prewashed transparent sample bottles. The shells were taken to the Hydrobiology Laboratory of the of the Biology, ABU Zaria for digestion and further analysis. Eighty *Clarias gariepinus* fingerlings were purchased from a fish farm in Kaduna for the experiment.

Diet formulation

Four different diets were prepared with varying calcium supplement; which was 0, 3, 4 and 5% shell and represented as D1, D2, D3 and D3, respectively. The basal supplements were: yellow maize, wheat grain and rice bran while the protein supplement are fish meal and groundnut cake. The experiment lasted for eight weeks. The quantity of feed administered to the fingerlings per day at 5% body weight was 16.5 g.

Ashing of Egeria radiata shell

Excess sand particles were removed from the snail shell using a fine brush after which the shells were wrapped in foil paper and heated to 400 – 600^{o}C in a furnace. This was done to remove the carbon content and to obtain pure calcium from the shell.

Experimental fish

Feeding of Clarias gariepinus fingerlings

Eight experimental plastic tanks were used. Ten (10) of *Clarias gariepinus* fingerlings were stocked in each tank, containing 20 liters of water. The fish were fed ad libitum 16.5 g of 1 h body weight in the morning and evening each day for eight weeks. The water quality was maintained using the flow through system which was used to change the water.

Morphometric indices of the fish

After feeding for two weeks, the fish were weighed using a sartorial weighing balance. The length (total and standard) were also measured using a meter rule. The following growth parameters were determined:

Weight gain = initial body weight – final body weight

Specific growth rate was expressed asThe specific growth rate was calculated as described by Aderolu and Sogbesan (2010) as follows:

$$SGR = \frac{\log e\ wt - \log e\ w0}{t - t0}\ (\%days)$$

Where SGR = Specific growth rate, *wt* = weight at time of observation, *w0*= initial weight, *t-t0* = duration of experiment and *e*= the base of natural logarithm (10).

The first one week of the experiment was used to acclimatize fish to experimental diet. Thereafter morphometric indices were measured fortnightly. Each diet was allotted 15 *Clarias* fingerlings: with the control having 10 *Clarias* fingerlings in group of 5 fish each. Three replications of five fish each.

Calcium content in the bone of the experimental fish was determined by digesting the bones through wet chemical digestion, after which calcium content analysis was done using Atomic Absorption Spectrophotometer at the Mathieson Laboratory of Ahmadu Bello University, Zaria.

Statistical analysis

A two way analysis of variance was employed to determine the variation between the period of exposure relative to the different diets.

Results and Discussion

The result obtained within the four experimental weeks indicates that there was a general higher calcium bio-concentration in the bones of the fingerlings when exposed to diet D_2 (3% shell and 2% bone meal) with 442.647, 450.657, 465.750 and 470.900 ppm at week two, four, six and eight respectively (Figs. 1 – 4) with mean calcium content of 457.488±6.55 ppm.

The present experiment showed that calcium supplement from the shell of *Egeria radiate* have significantly improved growth in *Clarias gariepenus* fingerlings. The high calcium concentration observed across the weeks upon exposure to D_2 indicates that the fingerlings had the best utilization of the mineral content of calcium in the diet. This is because calcium is important in the formation and stability of cell walls and in maintenance of membrane structure and permeability. It has also been reported that calcium activates some enzymes, regulates many responses of cells to stimuli in animals (Houtkooper *et al.*, 2017). Ogino *et al.* (1976) observed whole body calcium content of rainbow trout increased from 0.084 to 0.146% when fed on diets supplemented with increasing levels of calcium from 0.046 to 0.779%.

The mean calcium content of D_1 was observed to be 45.305±0.72 ppm. However, lower values of calcium content was observed in fingerlings within the period of study when exposed to D_1 (Control diet) with 44.213, 44.303, 45.401 and 47.301 ppm at weeks two, four, six and eight, respectively. D_1 had the lowest performance (calcium utilization), and it is attributed to the absence of the inclusion of *Eigeria radiata* shell. The variation observed across other inclusion levels in the calcium uptake of *C. gariepinus* fingerlings could be related to differences in preferential bioaccumulations. The study has shown that fish fed with 1 and 4% inclusion levels gave the best growth performance relative to the other inclusion levels. This could be attributed to palatability and digestibility efficiencies that is probably associated with the inclusion level. There was significant variation ($p<0.05$) in the concentration of calcium across the diets (Figs. 1 – 4).

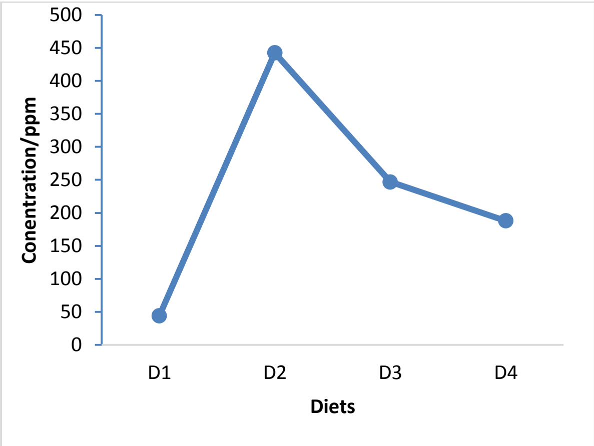

Fig. 1: Calcium concentration in fingerlings at two weeks

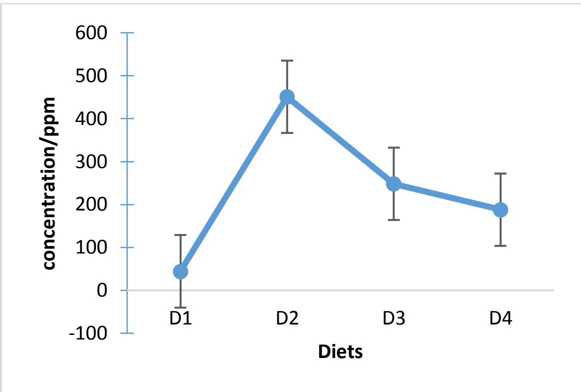

Fig. 2: Calcium concentration in fingerlings at four weeks

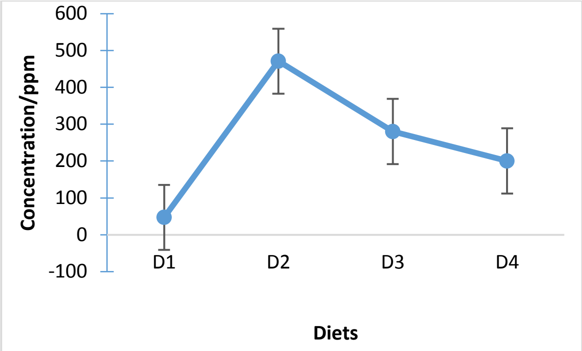

Fig. 3: Calcium concentration in fingerlings at six weeks

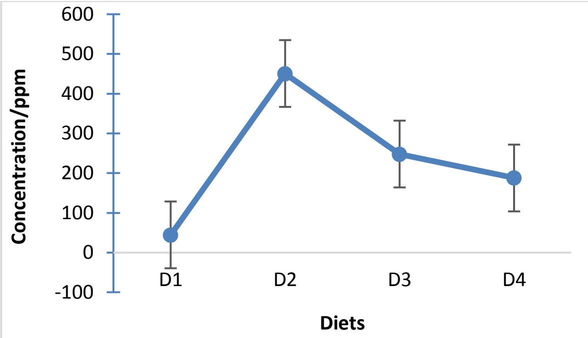

Fig. 4: Calcium concentration in fingerlings at eight weeks

Table 1: Weight gain and specific growth rate of *C. gariepinus* fingerlings after eight weeks exposure

Diet	Initial weight	Final weight	Weight gain	Specific growth rate
D_1	5.085	33.52	28.43	**0.0149**
D_2	5.21	11.33	6.12	**0.0056**
D_3	5.37	3.91	-1.46	**0.0196**
D_4	4.86	57.09	52.23	**0.0194**

Table 2: Average length and weight of fish after feeding with the formulated diet

Growth parameter	Diet (1)	Diet (2)	Diet (3)	Diet (4)
Weigh (g)	84.872	43.317	147.858	**125.052**
Total length (cm)	64.61	56.786	75.456	**73.007**
Standard length (cm)	57.633	50.137	66.451	**64.463**

The present experiment showed that calcium supplement from snails' shell, *Egeria radiata* have significantly improved growth in *Clarias gariepinus* fingerlings (Tables 1 – 2) indicating that it had higher feed conversion ratio. When the fish were fed with diet three containing 4% shell, significant average length and weight was observed (Table 2), which is very low compared to when the fish were fed with diet one (0%) which does not have the snail shell supplement. Calcium and phosphorus are closely related, phosphorus has also been found to significantly improve growth and feed efficiency in juvenile bighead carp at 1.59% inclusion level (Liang *et al.*, 2018), rainbow trout (Ketola and Richmond, I994). Kandeepan and Poongulali (2009) reported highest growth rate of 30.164 mg/g live fish/day in *Oreochromismos sambicus* when fed with diet supplemented with 1.097% calcium. A very high percentage of phosphorus is found in fish meal about 50%, combine with the percentage of calcium in *Egeria radiata* which has 95% calcium oxide has been reported to induce rapid increase in growth and weight gain of the rabbit (Houndonougbo *et al*., 2012).

Conclusion

The best growth obtained with feed formulated from 4% snail shell and 1% bone meal inclusion levels (D1 = 84.87 g, D2 = 43.32 g, D3 = 147.86 g and D4 = 125.05 g). The highest bone calcium deposition was obtained with feed formulated from 3% *Egeria radiata* shell and 2% bone meal (457.488±6.55 ppm.). This shows that decarbonized shell from *Egeria radiata* (at 3% *Egeria radiata* shell and 2% bone meal) can be used as an alternative source of calcium in *Clarias gariepinus* feed for proper growth. Therefore, the shells of *Eigeria radiate* have great benefits in aquaculture productions.

Conflicts of interest

The authors declare that there were no conflicts of interest.

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