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Domestication of *Chrysichthys Nigrodigitatus* of the Cross River, as an Adaptation to Climate Change and a Boost for Aquaculture Expansion in Nigeria

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Abstract
Chrysichthys nigrodigitatus, commonly known as silver catfish, is a prized food fish in Nigeria. It occurs in several African rivers. In the Cross River system, it occurs all year round with peak in the rainy season, from April to October. Several studies have reported on some threatening factors to the species' survival, including overfishing, low genetic diversity and climate change. The proposed method to curb the depletion of the resource is domestication. Studies were conducted to determine the reproductive ecology of the species. Simulation of these ecological factors in the hatchery for possible domestication of the species was carried out. Results show that the species is a freshwater species with salinity range from 0.01-0.06 ppt. Their preponderance was directly correlated with turbidity and indirectly corelated with transparency, showing that they prefer dark and shaded conditions. Rainfall triggered reproductive activities. Spawning occurred within the rainy period in crevices and holes in the rocky substratum of the river bed. We simulated these conditions in the hatchery and fish farm by placing cylindrical pipes as holding receptacles in the ponds. This provided hiding place and shade for them. Spawning inside the receptacles was observed in the month of June through September, coinciding with the phenomenon in the wild. The species is shown to embark on parental care. The male parents carry the brood in their mouths until they were able to fend for themselves. Thus, understanding the reproductive ecology of *Chrysichthys nigrodigitatus* in the river, enabled us to develop protocol that assisted in its domestication. This is the first time *Chrysichthys nigrodigitatus* has been raised in the hatchery and made to spawn in captivity. Thus, our work has expanded the aquaculture range in Nigeria and Africa.

1.0 INTRODUCTION

Chrysichthys nigrodigitatus, commonly known as silver catfish, is a prized food fish in Nigeria. The species is widely distributed in freshwater of West Africa, where they are valued in human nutrition (Holden, 1991). According to Paugy *et al* (2003), *Chrysichthys* is found in Nile, West Africa and western coast of Central Africa. In the Cross River system, east of Niger Delta, the species occurs all year round with peak in the rainy season (Ama-Abasi *et al*, 2019); here the species forms the most commercially important single species fishery, providing employment opportunity for the teeming populace. In the Cross River estuary, the species is the third most important species after bonga and croaker (Holzloehner, *et al*).

However, the species is listed in the IUCN list of threatened species (Da Costa, 2010). Recent researches have also shown declining population of the species due to overfishing (Ama-Abasi and Uyoh, 2020) and possible impacts of climate change, Ama-Abasi, *et al* (2019). Adilieje *et al* (2020) and Uyoh *et al* (2020) demonstrated that *Chrysichthys nigrodigitatus* of the Cross River has low genetic diversity with the potential of spontaneous wiping out of the population in the face of environmental variability.

Such trends may predispose the species to decline. To shield the species from the vagaries of climate change and overfishing, there is need for domestication of the species. Domestication will not only shield the species from the vagaries of climate change but will lead to expansion of aquaculture in the continent. Hitherto, the aquaculture of the species was not considered because of the erroneous belief and misconception that the species is estuarine and so needs saltwater for its growth. Ordinary observation has it that the species is preponderant in the freshwater of the Cross River in the rainy season. So, the theory of being estuarine was an enigma (Ama-Abasi *et al* 2019). This study was conducted to establish the reproductive ecology of *Chrysichthys nigrodigitatus* and to also attempt its domestication, based on the ecological findings.

2.0 MATERIALS AND METHODS

Study Area: Studies on the reproductive ecology was conducted in the Cross River system. The Cross River is the main river in southeastern Nigeria. It originates from the Cameroon mountains and flows through the rainforest area westward before meandering southward through the mangrove vegetation and finally emptying its water in the Atlantic Ocean at the Bight of Biafra. The River is located between latitudes 4^0 $34.59^/$ and 5^0 $870^/$ N and longitudes $8^0 59.9^/$E and 7^0 $24.3^/$E. The river basin and its catchment are characterized by dry season from November to March and wet season from April to October. The main occupation of the people includes fishing, hunting, farming and boat making.

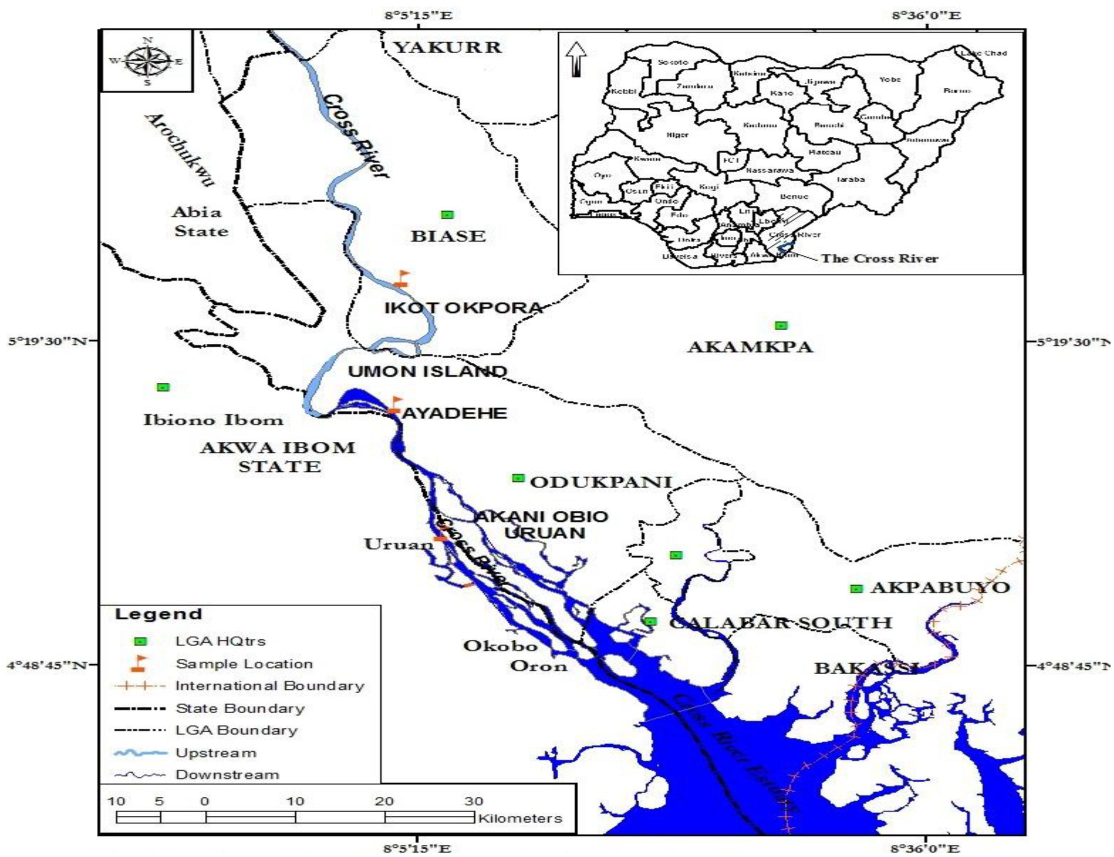

Fig 1: Map of Cross River System showing sampling stations

Sampling Procedure: The river was divided into the upstream and downstream sections for the purpose of this study (Fig 1). Surface water and fish samples were collected fortnightly at neap tide and spring tide from January 2017 to December 2017 at three locations along the length of the Cross River between Akani Obio Uruan (downstream) and Ikot Okpara (upstream) (Fig 1). The fishers used gillnet, drums and dragnet as their fishing gears. Fish catch were randomly sampled from five to ten boats and both the total catch and catch -per-unit effort were estimated from such samples.

In situ physico-chemical parameters such as surface temperature, conductivity and water depth were taken while water samples were collected in sterilized containers in iced cold boxes and transported to the analytical laboratory of Institute of Oceanography, University of Calabar for turbidity analysis using turbidimeter. Other parameters were analyzed using standard analytical methods for water analysis (Eaton and Franson, 2005). Temperature was measured using mercury in glass thermometer, transparency was measured using Secchi disk, electrical conductivity was obtained using VOLTCRAFT WA-100ATC. Rainfall data were collected from the meteorological unit of Geography and Environmental Science Department of the University of Calabar. River depth was measured by the use of echo sounder. Fish catch and abundance were measured using top-loaded sertoli balance in kg while the catch per unit effort was calculated as total catch per number of fishing boats. Reproductive behaviour was monitored by depending on the indigenous knowledge of the local fishers, majorly using their fishing gears to understand the species' reproductive strategies.

Once the reproductive strategies were understood, the conditions were simulated in fish pond of the Institute of Oceanography, University of Calabar. Spawning receptacles made of pvc pipes of 15cm diameter were placed in fish pond of Institute of Oceanography Fish Farms. The pond is made of subterranean water which increases in volume during the rainy season. The size of the pond was 24 m X 100 m. There is a control valve at one end which allows excess water to flow out from the pond. Water grass and other aquatic weeds like waterlily grow in the pond making it semi-natural. Fifty specimens of *Chrysichthys nigrodigitatus* comprising both males and females were introduced into the pond. The fish depended on *Tilapia* which formed part of the fish community as their staple and were not fed with any extraneous food. Critical water parameters of temperature, conductivity, pH and dissolved oxygen were monitored weekly. Temperature and dissolved oxygen were measured using dissolved oxygen meter, electrical conductivity was obtained using VOLTCRAFT WA-100ATC. pH of the water was measured using the pH meter with model PHT-01ATC. Fish weight measured using top-loaded Sertoli balance in grams. The spawning receptacles were monitored fortnightly in order to recover any spawning fish or hatchlings in the receptacles.

Once the hatchlings were recovered from the ponds, they were taken into the hatchery darkroom for further management and growth. In the darkroom there was a simulation of the critical parameters to enable *Chrysichthys nigrodigitatus* to survive and demonstrate the possibility of domestication. The hatchlings were raised in 1 X 1m plastic container with water volume of 1500 litres . Constant oxygen was supplied through by AquaOxy 2000 oxygen pump capable of supplying 2000 litres of oxygen per hour. The fry were fed with coppens and their growth monitored monthly by measurement of total length and weight. To maintain high hygienic condition and prevent disease infection, the water was changed every three day.

Statistical Analysis: All data obtained for the ecological studies were subjected to descriptive statistics for mean, ± standard error. Correlation analysis was used to test any relationship between the ecological variables and fish catch across months (January to December). Relationship between environmental/ecological variables and fish distribution and abundance were determined using simple linear correlation. Simple linear regression analysis was also done to standardize the relationship between rainfall and fish catch and the environmental variables. All statistical analyses were performed using the Prism GraphPad 5 (GraphPad software, La Jolla, USA).

3.0 RESULTS AND DISCUSSION

The ecological variables of rainfall, turbidity, transparency, temperature and conductivity shows some correlation with the fish catch/abundance (Fig 2).

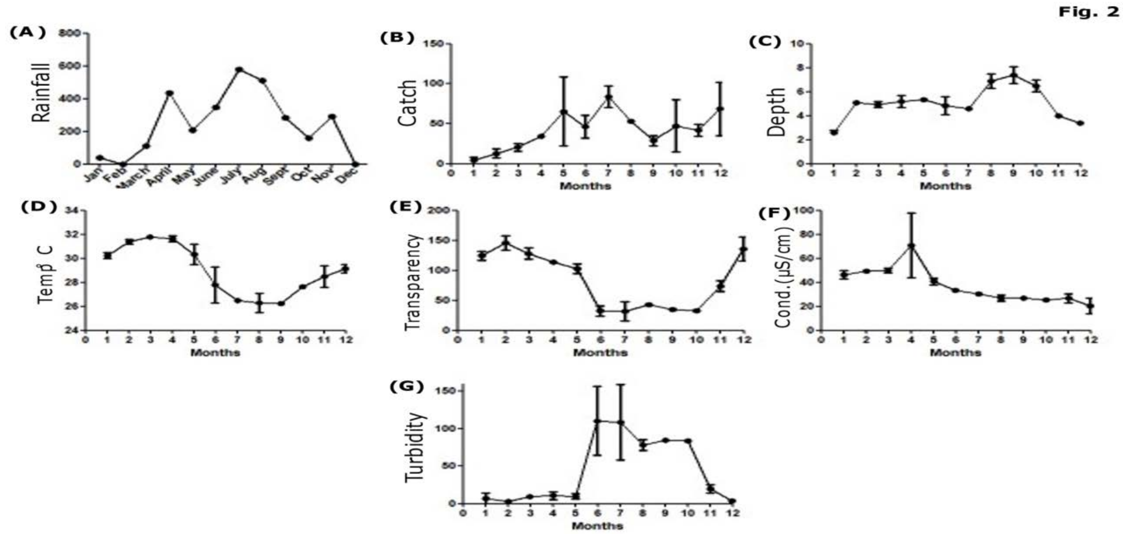

Fig 2: Time series of ecological parameters and *Chrysichthys* catch over the study period.

The salinity values ranged from 0.01 to 0.06.(Table 1). These values show that the *Chrysichthys nigrodigitatus* is a freshwater species. This is in corroboration with Holden (1991) that the species inhabits the freshwater of West Africa, Nile and Central Africa. This is in contrast to the view Sivalingam (1975) who opined that the species is brackish water. Consequently, the aquaculture of the species can be explored in the freshwater.

Table 1: Salinity values of the Cross River at the study Area.

Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Upstream	-	0.05	0.01	0.06	0.03	0.02	0.02	0.01	0.02	0.02	0.01	0.01
Downstream	0.01	0.04	0.03	0.05	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.01

Correlation analysis established a relationship between environmental variables and fish catch (Table 2). A positive and highly significant ($P<0.01$) relationship was observed between sampling months and fish catch (0.542); also, a negative and highly significant ($P<0.01$) relationship was observed between temperature and sampling months (-.610); fish catch and conductivity (-0.522). Linear regression analysis established the relationship between fish total catch and mean weekly rainfall with regression model of $Y=12.8+0.098x$, showing that there is a positive relationship between fish total catch and rainfall.

The highly significant correlation between the months of the year and fish abundance reveals that the species is seasonal. The highly significant negative correlation between temperature and sampling months also is an indication of negative influence of temperature on fish catch. A strong linear relationship between fish abundance and rainfall presents a lot of implications on fish abundance and distribution in relation to climate change.

Ama-Abasi *et al* (2019) and Ama-Abasi and Uyoh (2020) reported that rainfall initiates the reproductive events. Moses (2001) opined that reproduction during the rainy season makes use of the habitat expansion and food availability for the juveniles which also depends on allochthonous food source at this time. From Fig 2 A, B and D, the period of peak *Chrysichthys* abundance coincided with peak rainfall and lowest temperature.

Table 2. Correlational matrix of environmental variables and *C. nigrodigitatus* catch from the Cross River, Nigeria.

	Months	Catch	Trans	Tur	Temp	Depth	cond	rainfall
Months	1							
Catch	.542**	1						
Trans	-.473*	-0.404	1					
Tur	0.373	0.32	-.900**	1				
Temp	-.610**	-.427*	.769**	-.753**	1			
Depth	0.135	0.179	-.463*	.477*	-0.299	1		
cond	-.885**	-.522**	.528**	-.432*	.692**	-0.189	1	
rainfall	0.182	.488*	-.658**	.672**	-.482*	0.316	-0.169	1

Transp = transparency; Tur = turbidity; Temp=temperature, cond= conductivity. * Asterisks shows significant correlation. ** Highly significant correlation.

A relationship of fish catch to climatic factors like temperature and rainfall, means that in the face of climate change, *Chrysichthys nigrodigitatus* fishery can be adversely affected with decline in population and possible eradication of the source of livelihoods of millions of the riverine communities in Niger Delta and elsewhere in Nigeria and Africa, whose livelihood depends on the fishery.

The negative correlation between fish abundance and transparency implies that the species is sensitive to light and that is why they prefer turbid waters which provide some level of shading from light. This also is possible reason they hide in holes and crevices and the pipes which the fishers use as trap.

The reproductive strategy of the fish ensures that they spawn during the rainy season inside Indian bamboo and rock crevices (Plate 1). This ensures that sperms is not wasted and the male can easily pick the eggs into its mouth for brooding and safety.

We therefore adopted this techniques in the pond and provided a condition for the fish to spawn by placing the pipes in the ponds (Plates 2)

Plate 1: A species of *Chrysichthys* trapped inside Indian bamboo.(*Several pairs of Chrysichthys were recovered in Indian bamboos and drums which the fishers used as their trap).*

Plate 2: a) M.Sc. Student laying spawning receptacles in the pond.   b) Several spawning receptacles in the pond

Plate 3: Male *Chrysichthys* inside the spawning receptacle

Plate 4: Male and female *Chrysichthys* recovered from the spawning receptacle and their fry

With the supposition of potential climatic impacts on the fisheries, there is need to pursue the domestication of *Chrysichthys nigrodigitatus* as an adaptation to climate change impacts. This is why we attempted the domestication of the species. The simulations of the environmental conditions in the ponds including the use of pvc pipes as spawning receptacles (Plate 2) proved effective as the fish were able to grow and spawn in captivity in the ponds and hatchery (Plates 3 and 4). Within two months of rearing in the hatchery from hatchlings, the fry grew in length by 73 % and in weight by 400%. The ability of the species to survive in the ponds and reproduce is an innovation. It has not been reported anywhere. This is a breakthrough in aquaculture.

Since African business opportunities are projected to be in $3.5trillion in the nearest future, and since these business opportunities are in agriculture, and natural resources among others, expansion of aquaculture through *Chrysichthys* fish farming perfectly fits into this business plan and projection. This will increase food production and ensure food security. With the forecast that if the GDP grows at the same rate till 2030, the poverty rate will decline to 23%, *Chrysichthys nigrodigitatus* aquaculture as an innovation will reduce poverty rate and increase diversity of diets. When the *Chrysichthys* fish farming is taken up, more women and youths will go into aquaculture, leading to access to job availability, and improvement in living standard as such expansion will create more job for them. This will reduce insecurity and violence in the continent.

4.0 CONCLUSION

Chrysichthys nigrodigitatus population is significantly influenced by rainfall and temperature, two climatic factors that can have adverse effects on the fish and its fisheries in the face of climate change. Domestication of the species has been achieved with high prospect for aquaculture expansion in African. To shield the species from the vagaries of climate change and overfishing, the aquaculture of the species should be vigorously pursued. This has the potential of increasing African business and reducing poverty, thus fulfilling the main thrust of ASRIC.

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Abstract

Palm bunch ash electrolyte solutions (0.25, 0.50, 0.75, and 1.00 g/100ml water) were used to blanch cocoyam (*Xanthosoma mafafa*) leaf meal at 100ºC for 3 minutes and its proximate, phytochemical, and mineral compositions were determined for each of the electrolyte concentration. Moisture content range was 9.72 – 11.02%, while the crude protein content ranged from 27.74 – 28.72%. Crude fat, crude fiber, and NFE, ranges were 9.72 – 17.5%, 2.37 – 3.54%, and 35.34 – 38.62% respectively. The phytochemical composition of the samples showed that the values of tannin, phenol, cyanide, trypsin inhibitor and alkaloids were reduced significantly ($p<0.05$) by the ash electrolyte blanching. Samples treated at 0.75% concentration showed better results in terms of reduction of anti-nutrients present in the leaf meal. Carotenoid, flavonoid and phytates values were increased by increase concentrations of the electrolyte solution and peaked at 0.75% concentration after which they decreased at 1.00% concentrations. The macro mineral contents result further showed that calcium, Magnesium, Potassium, phosphorus, and Sodium concentrations increased with electrolyte blanching treatments across L2 – L5 treatments. The micro mineral profile showed that iron, copper, and Zinc concentrations decreased significantly ($p<0.05\%$) from the control L1 and increased across the blanching treatments L2 –L5. Lead content was found to be zero in all the samples. The feeding trial showed that birds fed the 0.75% ash concentration blanched cocoyam leaf meal-based diets supplemented with betaine showed better daily feed intake, growth performance, final weight gains and feed conversion ratio at seventh day of life. Birds fed betaine supplemented leaf meal based diets (LT6), recorded significantly ($p<0.05$) higher GIT, large intestine, and liver weights than all the other groups. The GIT segments pH measurement did not follow definite pattern among the treatment groups, but the values generally increase towards neutrality to alkanity from the small intestine to the large intestine. Plain diets group (LT1) without betaine and cocoyam products recorded least performance in almost all the parameters measured.

Keywords: Cocoyam, ash solution, phytochemicals, betaine.

1.0 INTRODUCTION

Among the alternative feed materials, cocoyam (*Xanthosoma mafafa)* leaves are generally common, wasted, and cheaper in many localities in sub-saharan Africa where they are used as vegetables in some communities. The leaf meals are rich in protein, carbohydrate, vitamin A and K among other nutrients (Okoli, 2020a). Cocoyam (*Xanthosoma mafafa)* leaf meal contains 16.41- 25.71% crude protein and could therefore, substitute costly protein ingredients in animal diets (Olaleye *et al*., 2013). The use of *X. maffafa* as food and feed without processing is limited by the presence of unacceptable levels of anti-nutritional factors such as phytates, tannins, saponins, phenols, trypsin inhibitors and oxalates (Agwunobi *et al*., 2002). Recent studies have shown the values of blanching agents such as heat applied through boiling water, roasting and frying in the reduction of such endogenous anti-nutritional factors of cocoyam leaves (McEwan *et al.,* 2014). Ani *et al.* (2015) also reported the effectiveness of debittering agents such as plantain peel ash in reducing the levels of such anti-nutritional factors in leafy vegetables indicating that such a technology could also be applied to cocoyam leaf meal. Since the palm bunch ash solution is a local food tenderizer (Narisu *et al.*, 2011), it could be used as a blanching agent to improve the nutrient value of cocoyam leaves. The objective of this study is therefore to determine the values of proximate, fiber fraction, mineral and phyto-chemical characteristics of *X. mafafa* leaf meal blanched in an electrolyte solution of palm bunch ash and partial replacement of wheat offal in broiler chicken diets with the blanched cocoyam leaf meal (BCLM) with an option of betaine hydrochloride supplementation. Since betaine have been demonstated by several authors (Zhang *et al*., 2012; Zhang and Kim, 2014) to contribute positively to poultry nutrition chiefly in two ways (methyl group donation and non-ionic osmotic regulation), it's supplementation in cocoyam leaf-based broiler diets are expected to have synergistic effects on the performance improvement of the birds, and may ameliorate the additional ionic burdens from the electrolyte blanched leaf meal in broiler chicken diets.

2.0 MATERIALS AND METHODS

The palm bunch sample used in the research was collected and ashed in Owerri according to the method described by Ohanaka (2016).

The ash solution for the blanching process was prepared by adding 0.25, 0.50, 0.75, and 1.00 g of the palm bunch ash (PBA) into 100 ml of distilled water in glass beakers to achieve concentration of 0.25, 0.50, 0.75, and 1.00% PBA solutions. The required PBA was weighed out using an electronic scale (Diamond scale model 14), and the required volume of water also measured out with a measuring cylinder. The solutions were stirred thoroughly and allowed to stand until needed for the blanching experiment which was within two hours.

Blanching of the cocoyam leaf meal was carried out by first boiling 1 litter of different concentrations of the PBA water solutions in beaker at 100^{o}C before adding 200 g of the fresh leaf meal. The mixtures were allowed to boil for another 3 minutes before cooling. After cooling, the electrolyte solution was filtered off through a sieve, leaving the blanched materials which were designated as blanched cocoyam leaf meal (BCLM). The BCLM were thereafter dried on a local mat for 7 days, and subsequently stored in plastic bags. The method described above was adopted for the preparation of blanched cocoyam product for laboratory analysis. To up-scale the production, an aluminum cooking pot containing 20 liters of the desired PBA solution by adding either 50, 100, 150, or 200 g of PBA before cooking on a gas cooker at 100^{o}C, and addition of 4 kg of the cocoyam leaf meals to be blanched, and the processes reported above repeated in each case.

The proximate of composition of cocoyam leaf meal was determined according to the methods of AOAC (2010), where their moisture contents (MC), dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), Ash (AS) and nitrogen free extract (NFE) values were determined. Phytochemical compositions were also carried out to determine the tannins, alkaloids, phenols, trypsin inhibitor, carotenoids, antioxidants, flevonoids, phytate, and cyanide content of the BCLM.

Laboratory analysis on the blanched cocoyam leaf meals were done with an atomic absorption spectrophotometer (AAS) bulk scientific model 210 VGB, to determine the following minerals: Calcium (Ca), phosphorus (P), sodium (Na), potassium (K) and magnesium (Mg) according to the methods described by Sofowora, (1980). The treatment group with best anti-nutrients profile (0.75%) was adopted for further treatment of cocoyam leaf meals, to be used in broiler feeding trial.

Feeding trial was conducted with 126 broiler birds, where the wheat offals in the broiler diets were partially replaced with the BCLM at 5 and 10% levels of replacement. The chicks were divided into six groups of 21 birds per group, and replicated three times with 7 birds per replicate, in a completely randomized design (CRD). Treatments L1 and L2 served as the control for the trial, with control L2 containing an addition of 0.05% betaine HCL as its difference from control L1. Group L3 and L4 contained 5, and 10% BCLM as partial replacement of wheat offal respectively, while groups L5, and L6 equally contained 5 and 10% BCLM as partial replacement of wheat offal respectively in addition to similar quantities (0.05%) of betaine HCL. Since the cocoyam leaf meal and betaine supplementations are novel alternative feedstuffs, the seventh day performance evaluation of the experimental birds was carried out to determine their early effects on the experimental birds. The seventh day performance parameters evaluated included, growth performance, gastro intestinal (GIT) development, and pH of the GIT contents according to the method described by Ukwu (2013).

3.0 RESULTS AND DISCUSSIONS

Proximate composition of PBA solution treated *X. maffafa* leaf meals

Table 1 shows the proximate composition of blanched *X. maffafa* leaf meal. The moisture content of the ash solution treated *X. maffafa* leaf meal (9.72 – 11.02%) indicated proper drying. Although there were significant treatment effects ($p<0.05$) across groups, there was no particular trend to these effects. Oladeji *et al*. (2013) reported moisture content values of 88.5 and 76.8% for raw, and blanched cocoyam leaves respectively on fresh weight basis, indicating again the need for proper drying of the feedstuff, when intended for storage.

Table1. Proximate compositions of BCLM

Parameter (%)	L1	L2	L3	L4	L5	SEM
Moisture	9.72^{d}	11.02^{a}	10.70^{c}	10.73^{bc}	10.87^{ab}	0.12
Crude protein	28.47^{b}	28.54^{ab}	28.72^{a}	28.07^{c}	27.74^{d}	0.10
Crude fat	17.15^{a}	14.36^{a}	11.50^{b}	10.22^{b}	9.73^{b}	0.82
Crude fiber	3.54^{a}	3.06^{b}	2.77^{c}	2.43^{d}	2.37^{d}	0.12
Total ash	7.80^{e}	8.18^{d}	8.91^{c}	10.18^{b}	10.68^{a}	0.30
NFE	35.34^{c}	35.18^{c}	37.41^{b}	38.38^{a}	38.62^{a}	0.40

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: Treatment 1 = 0.00% concentration of palm bunch ash solution
Treatment 2 = 0.25% concentration of palm bunch ash solution
Treatment 3 = 0.50% concentration of palm bunch ash solution
Treatment 4 = 0.75% concentration of palm bunch ash solution
Treatment 5 = 1.00% concentration of palm bunch ash solution

The crude protein (CP) content of the leaf meals increased significantly ($p<0.05$) up to the 0.5% electrolyte treatment before dropping progressively, such that L4 and L5 values were significantly lower ($p<0.05$) than the values recorded in the other groups. The CP contents of the leaf meals at 27.74 - 28.72% were higher than most leaf meals, indicating that they could serve as protein feedstuff in livestock production. The present results were similar to the 29.7% reported by Saephoom *et al*. (2016) in *C.esculenta* leaf meal. Other studies by Rodriguez (2010) reported a range of 16.0 - 26.0% as influenced by the rate of cutting.

The crude fat content of the leaf meal decreased progressively with the increasing strength of the electrolyte solutions, such that L1, and L2 recorded significantly higher ($p<0.05$) crude fat values than the rest. These results reflect the emulsification properties of the PBA electrolyte solution and agree with the results of Igbabul *et al*., (2014), and Duruanyim (2017) who reported similar trends in fat content of beef treated with PBA solution. The crude fat content of the leaf meal was higher than the 4.3% reported by Saenphoon *et al*. (2016) in *C. esculenta* but similar to the 13.13 and 14.05% reported by Ogukwe *et al*. (2017) in *X. sagittifolium* and *C. asculenta* flowers.

The crude fiber content of the samples also decreased progressively with increase in the levels of the electrolyte concentrations, such that the control samples (L1) recorded significantly higher ($p<0.05$) values than the electrolyte treated samples. Rodriguez (2010), reported a similar crude fiber value of 14.2% in *X. sagittifolium* leaf meal, while other studies have reported 12.4% in *C. esculenta* leaf meals (Okoli, 2020b). Odedeji *et al*. (2014), however reported much lower values for blanched *colocassia spp* leaf meal.

The percentage total ash recorded in the meals expectedly increased progressively with the increase in the concentration of the electrolyte solutions, such that L2 - L5 recorded significantly higher values than the control samples. This is understandable since the PBA electrolyte solution will progressively add to the ash content of the test materials. The ash content is an index of mineral content of a feedstuff, thus higher ash content represents high mineral content (Esonu, 2009). The present results are in agreement with other reports (Sefa-Dedeh and Se Kofi-Agyir, 2002; Davies *et al*., 2008), of high mineral contents of cocoyam leaves. The ash electrolyte blanching resulted in progressive increase in the nitrogen free extract (NFE) values of the leaf meals. Odedeji *et al*. (2014) also reported that hot water blanching increases NFE content of cocoyam leaf meal.

Phytochemical characteristics of PBA solution treated *X. maffafa* leaf meals

The common anti-nutrients found in cocoyam include, phytates, oxalates, tannins, saponins, hydrogen cyanide, trypsin, and alpha amylase inhibitors (McEwan *et al*., 2014; Adeyanju *et al*., 2019). The common approaches to overcoming these nutritional limitations in cocoyam in human, and animal food applications have been peeling, heat applications, pounding, and ethanolic extraction (Onu and Madubuike, 2006;

McEwan *et al*., 2014; Odedeji *et al*., 2014; Adeyanju *et al*., 2019). These treatments are able to some degree eliminate or reduce the anti-nutrients in the cocoyam to levels that result in the improvements in its palatability, nutrient digestibility and utilization. The blanching of the *X. maffafa* tuber, and leaf meals with ash electrolyte solutions was therefore employed to reduce the levels of their anti-nutrient contents, and improve their nutritional value.

Table 2 shows the phytochemical characteristics of the ash electrolyte blanched *X. maffafa* tuber, and leaf meals. The results show that the tannin, phenol, cyanide, and trypsin inhibitor levels were significantly reduced ($p<0.05$) below the control levels due to the ash electrolyte treatments. In most cases the lowest value of these phytochemicals were recorded at the 0.75% ash solution treatment, except for cyanide that recorded the lowest value at the 0.25% (L2). The L2 also recorded the lowest alkaloid values, while L4 returned significantly lower value ($p>0.05$) blend and trypsin inhibitor values than the other leaf meals samples. The carotenoids, and flavonoids which are essentially beneficial phytochemicals increased progressively in their values with increasing ash electrolyte concentration treatment, and peaked at the L4 treatment levels so that they were significantly higher ($p>0.05$). The phytate values followed the same trend and peaked at the L5 treatment levels so that L4 recorded the second highest values.

Table 2 Phytochemical characteristics of *X. maffafa* leaf meals

	L1	L2	L3	L4	L5	SEM
Tannin (mg/kg)	1610.27^{a}	1486.70^{b}	1335.66^{e}	1272.20^{d}	1340.77^{c}	32.91
Phenol (mg/kg)	860.56^{a}	708.90^{c}	604.54^{d}	589.94^{e}	842.34^{b}	30.50
Cyanide (mg/kg)	6.36^{a}	3.24^{e}	3.54^{d}	4.17^{c}	4.43^{b}	0.29
Trypsin inhibitor (mg/kg)	1109.02^{b}	1013.55^{c}	1001.47^{d}	979.92^{e}	1243.21^{a}	26.07
Alkaloids (mg/kg)	2.61^{b}	1.63^{e}	2.02^{d}	2.36^{c}	3.40^{a}	0.16
Carotenoids (mg/kg)	17.56^{e}	32.42^{d}	35.81^{c}	52.35^{a}	43.83^{b}	3.12
Flavonoids (mg/kg)	5283.15^{c}	5393.09^{d}	6006.77^{b}	7095.90^{a}	5676.19c	174.42
Phytate (mg/kg)	91.92^{e}	99.13^{d}	101.26^{c}	107.63^{b}	111.26^{a}	1.81

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: AS in table 1

These results therefore show that L4 recorded the lowest levels of most of the anti-nutrients, and the highest levels of most of the beneficial phytochemicals, thus pointing to the fact that this might be the optimal ash electrolyte treatment level. The untreated leaf meal was exceptionally high in carotenoids, and flavonoids, and also moderately high in phenols, and phytate. These results indicate that the leaf meal may exhibit greater anti-nutritional effects on the livestock (Okoli, 2020).

Boiling of *C. esculenta* leaf at 80^{o}C for 5 minutes was reported by Lewu *et al*. (2009) to substantially reduce its oxalate (16 - 78%), phytate (17 - 41%), and tannin (28 - 61%) contents, while improving the crude protein, and the carbohydrate contents. In the present study, most of the phytochemicals either reduced or increase in their values due to the ash electrolyte solution treatment. For example, at the 0.75% ash electrolyte solution treatment, the values of tannin, phenols, cyanide, and trypsin inhibitors were reduced in the leaf meals. The levels of tannin, and trypsin inhibitors, were particularly high in the leaf meals. Odedeji *et al*. (2014) reported a lower value of 34.5, and 25.5 mg/kg for raw, and blanched cocoyam leaves respectively.

Oxalate has been reported as the most critical anti-nutrient in the cocoyam. This is because the presence of calcium oxalate crystals in both the tuber, and foliage is chiefly responsible for the acridity, scratchiness, and off-taste problems associated with their consumption (Okoli, 2020a). Although the oxalate contents of *X. maffafa* tuber, and leaf meals were not determined in this study, Odedeji *et al*. (2014) also reported an oxalic acid value of 55.20 mg/kg in *C. esculenta* leaves in Nigeria, while the oxalate content of *C. esculenta* foliage grown in Fiji ranged from 278 - 574 mg/100g wet matter. Blanching of cocoyam leaves at 80^{o}C for 5 minutes has been shown to substantially reduce the oxalate content (Okoli, 2020c). Lewu *et al*. (2009) reported 16-78% reduction after 5 minutes of boiling. It is therefore assumed again that ash electrolyte solution blanching for 3 minutes may have yielded similar oxalate reduction results in the *X. maffafa* leaf meals.

Minerals compositions of PBA solution treated *X. maffafa* leaf meals

The results in table 3 show that generally, the macro-mineral contents of the leaf meal increased with increasing ash electrolyte concentration treatment. Expectedly, therefore the values recorded in the ash solution treated samples were significantly higher ($p<0.05$) than the control values. The exception is the P content in which the control value was significantly higher ($p<0.05$) than the ash solution treated groups. Based on these trends, the mineral values recorded in 1.0% ash electrolyte solution treated group (L5) with exception of the phosphorus value in the L5, were significantly higher ($p<0.05$) than the values recorded in the other treatment groups.

At the highest ash electrolyte solution treatment levels (L4, and L5) the leaf meal recorded highest values of minerals. Rodriguez (2010) reported approximately 18,000, 2000, 32000 and 2000 mg/kg of calcium, phosphorus, potassium and magnesium respectively in *X. sagittifolium* leaf meal, which with the exception of potassium value, were within the range reported in this study. comparatively higher levels of minerals in the treated cocoyam meals could be attributed to the high mineral content of the palm bunch ash used in the blanching process.

Table 3 Macro-mineral concentrations in ash solution treated *X. maffafa* leaf meals

	L1	L2	L3	L4	L5	SEM
Calcium (mg/kg)	17255.00^{e}	17735.00^{d}	17850.00^{c}	17950.00^{b}	18772.50^{a}	131.52
Magnesium (mg/kg)	2263.00^{e}	1978.50^{d}	2268.50^{c}	2491.50^{b}	2750.00^{a}	68.90
Potassium (mg/kg)	13090.00^{d}	13002.00^{e}	16499.50^{c}	18765.00^{b}	22005.00^{a}	919.27
Phosphorus (mg/kg)	2460.00^{a}	560.50^{e}	868.00^{d}	1058.00^{c}	1425.00^{b}	175.26
Sodium (mg/kg)	1252.50^{e}	1382.00^{d}	1507.50^{c}	1599.00^{b}	1799.00^{a}	49.89

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: As in table 1

Micro minerals concentrations in the *X. maffafa* leaf meals

The micro-mineral values in the leaf meals decreased significantly ($p<0.05$) in response to the ash electrolyte solution treatment (Table 4). In all cases, values increased progressively in all the electrolyte treated groups so that L5 recorded the highest values among these groups. Oladeji *et al*. (2013) recorded a lower iron level of 240.11 mg/kg in cocoyam flour, while Eyasu *et al*. (2019) reported values of 80.20 and 90.88 mg/kg in green, and purple cocoyam respectively in Ethiopia. They also recorded lower copper content in their work.

Table 4 Micro mineral concentrations in ash treated *X.maffafa* tuber and leaf meals

	L1	L2	L3	L4	L5	SEM
Iron (mg/kg)	1664.50^{e}	781.00^{e}	972.00^{d}	1028.00^{c}	1144.50^{e}	79.51
Copper (mg/kg)	110.56^{a}	47.95^{e}	57.13^{d}	67.08^{c}	77.49^{b}	5.78
Zinc (mg/kg)	42.43^{a}	35.68^{e}	37.11^{d}	37.91^{c}	40.49^{b}	0.65
Lead (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: As in table 1

Seventh day performance characteristics of broiler chicks fed cocoyam leaf meal supplemented with betaine

The growth performance, gastro-intestinal tract (GIT) development and pH of the GIT contents of the birds determined after seven days of feeding are shown in tables 5 – 7.

Table 5: The 7th day growth performance of chicks fed blanched cocoyam tuber, and leaf meal-based diets supplemented with betaine

Parameters	LT1	LT2	LT3	LT4	LT5	LT6	SEM
Initial weight	41.00	39.71	41.39	40.43	39.98	40.53	0.28
Final weight	111.65^{b}	118.84^{a}	113.87^{b}	112.14^{b}	118.21^{a}	119.80^{a}	0.84
Weight gain	71.66^{b}	79.12^{a}	72.48^{b}	71.71^{b}	78.23^{a}	79.27^{a}	0.88
Ave daily wt gain	10.23^{b}	11.30^{a}	10.35^{b}	10.24^{b}	11.17^{a}	11.32^{a}	0.13
Ave daily feed intake	17.05^{c}	18.26^{b}	17.78^{bc}	18.32^{b}	20.07^{a}	19.41^{a}	0.27
FCR	1.66^{c}	1.61^{c}	1.72^{ab}	1.78^{a}	1.79^{a}	1.71^{ab}	0.02

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: LT1=control (plain feed), LT2=control + betaine, LT3=LT1+5% BCLM, LT4=LT1+10%BCLM, LT5=LT3+ betaine, LT6=LT4+ betaine.

Growth performance

In the present studies, the seventh day parameters considered were 7th day final weight, weight gain, daily weight gain, daily feed intake, and the feed conversion ratio (FCR). At the end of 7 days of feeding, the growth performance results showed statistical treatment effects across all parameters (Table 5). The results of the cocoyam leaf meal experiment shows that the birds fed the LT2 (LT1+betaine), LT5 (5% leaf meal+ betaine), and LT6 (10% leaf meal + betaine) diets recorded significantly higher ($p<0.05$) final body weights, weight gains, and daily feed intake than the birds fed the LT1 (control), LT3 (5% leaf meal), and LT4 (10% leaf meal) diets. The LT1, and LT2 birds however recorded better FCR values than the other groups. These results show that the best growth performance indices were recorded in birds fed the betaine supplemented leaf meal-based diets (LT2, LT5, and LT6). The superior performance results of the betaine supplemented diets suggest the overall advantage of betaine inclusion in the diets of the chicks at the early stages of development. Carvalho *et al*. (2020) evaluated the effects of betaine supplementation on post-hatch chicks aged 7 days, and reported that betaine supplementation of about 0.15% increased feed intake, and interfered with intestinal villi of the chicks. Final body weight, and weight gains were also improved similar to the results obtained in the present study. Other studies reported that betaine supplementation did not interfere with feed intake at 7 days (Pereira *et al*., 2010), but improved live weight up to 14 days (El Shinnawy, 2015). Similar lower weight results recorded in this study were also reported by Ukwu (2013), and Ohanaka (2016) and has been attributed to several stress factors in the study environment, especially the quality of the experimental diets. It is however interesting that partial replacement of wheat offal with blanched cocoyam leaf meal resulted in better growth performance than whole maize-based diet at the end of 7 days of feeding. This implies that the nutrient values of such ingredient combinations are superior.

The seventh day gastro-intestinal development characteristics

Feeding particularly accelerates the morphological development of the small intestine (Noy and Sklan, 1998; Ukwu, 2013), in readiness for the heavy nutritional demand involved in the rapid growth of modern broilers.

Table 6 shows that the LT6 birds recorded significantly higher ($p<0.05$) GIT, large intestine, and liver weights than all the other groups. The LT1 birds however recorded significantly higher ($p<0.05$) small intestine weight than the other groups and was followed by the LT4 - LT6 groups, representing the 10% BCLM, and the betaine supplemented BCLM diets. Again, the LT4 birds fed the 10% BCLM containing diet, returned significantly higher ($p<0.05$) gizzard, and proventriculus weights than the other groups, except LT2 that recorded similar proventriculus weight ($p>0.05$). The LT1 group (non-BCLM diets) recorded the most inferior values for all parameters except in its small intestine value.

These GIT development results support the earlier observations in the table 6 that birds fed the diets containing the cocoyam meals or supplemented with betaine performed better than the control group fed the maize-based diet for 7 days. This implies better GIT development in the cocoyam leaf meals fed birds, and the potential for more efficient utilization of the dietary nutrients for growth.

The reports of Uni *et al*. (2003) agree with the present report of significant increase in GIT weight due to BCLM and betaine supplementation. Calvalho *et al*. (2020) attributed this to the tissue osmolytic effects of betaine on the intestinal cells especially through the jejunum villus development.

Table 6: The 7th day GIT characteristics of chicks fed blanched cocoyam leaf meal-based diets supplemented with betaine

Parameters	LT1	LT2	LT3	LT4	LT5	LT6	SEM
GIT	26.28^{d}	26.24^{d}	27.77^{c}	28.64^{b}	28.23^{bc}	29.88^{a}	0.32
Crop	2.08	2.69	2.04	2.59	2.56	2.51	0.09
Gizzard	9.73^{e}	8.78^{f}	9.97^{d}	10.95^{a}	10.57^{b}	10.29^{c}	0.18
Proventriculus	3.12^{c}	3.74^{a}	3.21^{b}	3.73^{a}	2.35^{e}	2.94^{d}	0.12
Small intestine	7.29^{a}	6.25^{e}	6.68^{d}	6.84^{bc}	6.88^{b}	6.80^{c}	0.08
Large intestine	1.04^{f}	1.25^{e}	2.22^{d}	1.36^{c}	2.35^{b}	2.94^{a}	0.17
Liver	3.38^{b}	3.50^{b}	3.33^{b}	3.72^{b}	3.52^{b}	4.40^{a}	0.10

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: As in table 5.

The pH of the GIT of contents

Table 7 presents the data representing the pH of GIT content of the experimental chicks after seven days of feeding. The crop, and proventriculus of all the treatment groups were statistically similar ($p>0.05$), and also acidic, with the crop content of the leaf meal fed birds being more acidic than other parameters. The small intestine contents were mildly acidic, while the large intestine was mildly basic.

As the bird matures, the crop microflora becomes predominantly acidogenic with lactobacilli becoming the most common bacterial species. The increase in lactobacilli population results in a decrease in the pH of the digesta in the crop (Rynsburger, 2009). The gizzards pH in this study are higher than the values published by Rynsburger (2009) who reported a pH value of 3.24 and 3.27 at 6 and 15 days of age respectively in broilers. Ohanaka (2016) also reported much lower gizzard content pH (3.16 – 3.17) after 7 days of feeding palm kernel ash supplemented diets.

Rynsburger (2009) reported the small intestinal pH to be 6.42 at 15 days of age, which is in agreement with the findings of this study. The pH values of the GIT increases towards neutrality to alkanity from the small intestine to the large intestine.

Table 7: The 7th day pH of GIT contents of birds fed blanched cocoyam meal-based diets supplemented with betaine

Parameters	LT1	LT2	LT3	LT4	LT5	LT6	SEM
Crop	5.50	5.10	5.17	5.50	5.17	5.37	0.08
Prov	5.93	6.07	5.93	6.03	6.00	6.00	0.02
Gizzard	4.00	4.07	4.07	4.00	4.13	4.00	0.02
Small intestine	6.60	6.80	6.60	6.50	6.63	6.70	0.04
Large intestine	6.87^{b}	7.00^{ab}	7.20^{a}	7.07^{ab}	7.13^{ab}	7.00^{ab}	0.04

Means with different superscript on the same horizontal row are significantly different at ($p<0.05$)
Legend: As in table 5.

4.0 CONCLUSION

The nutritional profile of the cocoyam (*X. maffafa*) leaf meal was improved by the palm bunch ash electrolyte blanching. The chemical analysis shows that most anti-nutritional factors in the blanched cocoyam produced are within the tolerable values for the birds. However, the minerals contents appear to be higher than safe values in some instances. This ought to be taken into consideration when formulating the ration of monogastric animals.

The blanching of cocoyam tuber and leaf meal with 0.75% palm bunch ash electrolyte solution resulted in appreciable reduction in the essential anti-nutrients in the meals such as tannin, trypsin-inhibitor, phytate and cyanide. It seemed however, from the results of the feeding trial that the blanching duration was not sufficient to completely eliminate these anti-nutrients.

The birds fed blanched cocoyam leaf meal with betaine supplementation show remarkable performance at 7th day. The birds fed on betaine included diets also recorded higher GIT weights. The superior performance results of the betaine supplemented diets suggest the overall advantage of betaine supplementation in cocoyam leaf meal-based diets at early stages of broiler chicks development.

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