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Substitution of fishmeal with bambaranut waste meal in diets of first feeding (*Clarias gariepinus* x *Heterobranchus bidorsalis*) "Heteroclarias"

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ABSTRACT
First feeding larvae of hybrid African catfish average weight, 1.85±0.79 g were fed five substituted diets of fish meal (FM) and bambaranut waste meal (BNWM) for 30d. Feeds were labeled feed 1 (F1) to feed 5 (F5). The percentage inclusion of Fish meal (FM) and bambaranut waste meal (BNWM), (FM: BNWM), per feed were; F1, (0: 94), F2, (5: 89), F3, (20: 74), F4, (35: 59), F5, (55: 39). The catfish specific growth rates were, F5, 6.48±1.71; F4, 6.40±1.56; F3, 6.32±1.70; F2, 5.02±1.87 and F1, 4.19±1.53 % day^{-1}. Food conversion ratios were as follows, F3, 1.45; F5, 1.45; F4, 1.89; F2, 2.85 and F1, 4.30. Catfish average weight gains were, F5, 6.98±0.42 g; F4, 6.83±0.67 g; F3, 6.66±0.14 g; F2, 4.51±0.98 g and F1, 3.52±0.17 g. The average length gain were as follows, F5, 2.0±0.65 ; F4, 2.0±0.37; F3, 2.0±0.46 ; F2, 1.0±0.15 and F1, 0.5±0.10 mm. Results indicates no significant difference between 55:39% (FM:BNWM) and 20:74 (FM:BNWM) diets. BNWM can reduce amount of FM in diets of heteroclarias to 20%.

Keywords: Specific growth rate, plant proteins, Amino acids, Fish meal supplementation.

1. Introduction

The subgenus *Heterobranchus* was indicated for aquaculture initially by [1]. *Heterobranchus* sp have higher parasitic resistance and firmer flesh quality than *Clarias gariepinus*. In addition to other general African catfish potentials, *Heterobranchus bidorsalis* also grows as big as clarias but possess stenohaline capabilities thereby inhabiting either fresh brackish water. The hybrid of heterobranchus and clarias was produced in 1985 at African regional aquaculture centre [2] and in South Africa [3].

The hybrids of *C. gariepinus* and *H.bidorsalis* have been reported to grow better and exhibit heterosis than parental [4, 5]. Heteroclarias F1 hybrids fry's have also been shown to have high survival rates, faster growth and more uniform growth than parental [2]. Male *C. gariepinus* have dress mass of 66% compared to female 61%, this was due to the higher gonadosomatic index (GSI) of the female compared to the male. However the mature hybrids were found to have lower GSI compared to female *C. gariepinus* [6]. The fillet yield of hybrids African catfish was 43.8% compared to that of *C. gariepinus* 38.9% [6]. The aquaculture potential of hybrid Heteroclarias is very bright and promising [2, 5]. In spite of all the better qualities of the hybrid the culture is still far below that of *Clarias gariepinus.*

However nutritional requirements of larval heteroclarias are not well documented. The larval food preference and effects of substitution of fish meal in feed preparation is also not well documented. Heteroclarias is a novel strain and may not respond exactly like *Clarias* or *Heterobranchus* sp to artificial feed. In a previous research, it was noted that fish seed production remains a major setback to aquaculture in Nigeria and mortality rates up to 65-95 % are very common in hatcheries [7]. The major causes of hatchery mortalities are lack of adequate feed and cost of necessary feed. Provision of quality and acceptable diet is imperative to catfish fry [8]. The African catfish hybrid has been reared on live feed like *artemia* and *moina* [2, 9].

The use of artemia in larviculture of African catfish is hampered by cost of artemia and the skill for production of both *artemia* and *moina* [10]. Preparation of artificial diet is faced by two major problems, the cost of fish meal and need for alternative ingredients to fish meal [11].

Bambaranut meal is a major food source in Nigeria [12]. Bambaranut meal is a good source of essential amino acids like lysine, and tryptophan, these are often limited in cereals [13]. The protein content of bambaranut meal is 24%. Bambaranut meal is readily available and a very promising aqua diet ingredient. Fish meal is costly and scarce in sub Saharan Africa [11]. Prohibitive cost of fishmeal has made search for alternative like soybean imperative [14]. However soybean has become costly due to excessive multi usages as biofuel, oil production, animal feed and demands by countries like China [15]. However, soybean have become expensive and had to be imported to meet local demands in sub Saharan countries like Nigeria [16, 17]. There is serious need to source other local agriculture by product as fishmeal alternatives.
Although culture of African catfish *C. gariepinus* is increasing and hybrid catfish was produced as far back as 1985, nutritional requirements and effects of fishmeal substitution diets on the larval hybrids are not well documented. The culture of hybrid catfish is also lagging behind that of *C. gariepinus.* This paper aims at finding primary growth and nutritional effects, of partially substituting fish meal with bambaranut waste meal on the first exogenous feeding larvae of hybrid African catfish "heteroclarias".

2. Materials and methods
2.1. Hybrid larvae and experimental set up.
First feeding larvae of hybrid *Clarias gariepinus X Heterobranchus bidorsalis* were obtained from African regional aquaculture center Aluu Port Harcourt Nigeria. The fish were transported in oxygen bags immersed in iced cold water to reduce stress resulting from the long journey. After four hours journey the hybrid catfish larvae average weight 1.85±0.79 g were stocked at density of 13 fish^{-1} glass aquaria. The glass aquaria measured 84 cm length, 40cm width and 40 cm height. There were three replicate aquaria per experimental feed type. Aquaria water was aerated for 24 h prior to stocking from a central aerator, aquatic eco-system inc. Florida 32703 Model no.s-41, SN 0.38. Water supply was from University of Nigeria Nsukka water works. The experiment was conducted during the cold harmattan periods in Nigeria (November to January). Harmattan period in Nigeria and Nsukka region is characterized by cold early morning and evening temperatures. A maximum and minimum thermometer was inserted into an aquarium to monitor temperature fluctuations. Temperature fluctuations were recorded morning and evening and fish behavior observed. Fish were acclimated for 10 days. During acclimation they were fed with 35% protein diet compounded in our laboratory.

2.2. Diet preparation
Fresh bambaranut seeds were bought from open market at Nsukka and milled. The initial endosperm was removed and the remnant bambaranut waste meal (BNWM) was used for feed preparation. Fish meal was also bought from the dealers of locally made fishmeal.
Feed formulation was done using the square methods [18]. Five feed types, Feed 1 to Feed 5 were made to vary in percentage of Fishmeal: bambaranut waste meal (FM:BNWM) as follows, F1, 0:94, F2, 5:89, F3, 20:74, F4, 35:59, F5, 55:39. Feed ingredients were measured with electronic balance and blended with Ken wood chef mixer (Thorn Eni Harrant England). The mixture was preconditioned and pressure cooked for 30 min after which vitamin c and vitamin premix were added. The dough was then pulverized using with 3mm Kenwood chef meat mincer (Thorn Eni Harrant England). Pellets were oven dried at 60 ^{o}C for 24 hrs and stored. Samples of each prepared feed (approximately 20 g) were placed in plastic bags and stored in the refrigerator until analysis for proximate composition. Moisture content was analyzed by weighing 4 gm of sample feed in a pre-weighed aluminum tray and drying the feed to a constant weight at 100 ^{o}C in oven. Moisture content was determined by; Weight of fresh sample weight of dry sample. Analysis for crude protein NFE, crude protein, crude fiber and ash followed standard of [19] methods. All proximate analysis of the feed was done at Leuven analytical Laboratory University of Nigeria Nsukka campus.

2.3. Larval feeding and experimental set up
Fish were fed two times in a day and feeding was done very early hours 6.30-8 am and late in the evening 18-19 hour GMT. Feeding was done after removal of waste products from aquaria. The fish were fed at 3% of their body weight. The feeding was adjusted according to growth rate of fish. The feed were ground to dust before administration due to size of the fish. Care was taken during feeding to avoid drifting of feed with water.
Water supply was from university water works. The water was stored in a settling aquarium and exposed for about 2 h before supplying to aquaria. The quality of aquarium water was maintained by constant changing of aquarium water on daily basis. Rubber siphon was used in water changes. Water was changed between 6.0-6.15 am early in the morning to avoid stress on the fish. Uneaten foods were removed and this was always done prior to feeding in the morning. Aquarium was constantly aerated using central aerator. Temperature measurements were done by the use of maximum and minimum thermometer inserted in the aquarium tanks.

2.4. Growth parameter measurements
Weight measurement was done every week end per replicate using electronic balance sensitive to 0.01 g. Average weight were pooled length measurement was done using meter rule. Length measurement was done after weighing the larvae in the mornings. Experiment was carried out during harmattan period of December which is characterized by early morning cold weather as low as 23 to 26 °C. At cold temperature African catfish larvae were easier to handle. Length was measured per individual and average length increase pooled.

2.5. Calculations and statistical analyses
The following calculations were made for each tank, which was the experimental unit in the calculations: Specific growth rate (SGR, % day^{-1}) was calculated as 100 (Ln w_2 – Ln w_1) x t^{-1}, where w_1 and w_2 were average weights in g at the start and the end of the experiment, respectively, and t was the length of the experiment in days.
Food conversion ratio (FCR) was calculated as (feed consumed in g) * (change in tank biomass in g)$^{-1}$.
Average weight gain was calculated as Average final sum of biomass-average initial biomass in g
Average length gain was calculated as Average final sum of length –average sum of initial length of fish in mm. The results were analyzed using Oneway ANOVA. Fisher's least significant difference 0.05 was used in separating possible

treatment means differences. SPSS v.14 was used in the statistical analyses.

3. Results

3.1. Fish larvae

There was no mortality as result of the stress due to long distance 240 km transporting of the fish larvae. The larvae acclimatized well and readily received prepared feed 17 h after arrival and for five days before experiment began. The hybrid catfish were not affected by temperature fluctuations. At low early morning and evening temperatures the fish were still feeding but where staying mainly at the bottom of the aquaria.

3.2. Specific growth rate (SGR)

The first feeding larvae of heteroclarias readily accepted experimental dry diets and grew with maximum average SGR of 6.40±0.08% day^{-1} for those fed with F3-F5 (Table 2). Catfish SGR seems to increase with FM inclusion but it was insignificant. There was no significant differences in the SGR of larvae fed diets with F5, 6.48±1.71% day^{-1} (FM20%: BNM 39%), F4, 6.40±1.56% day^{-1} (FM 35%: BNM 59%) and F3, 6.32±1.70% day^{-1} (FM 20%: BNM 74%), (P>0.05, FLSD $_{0.05}$ 1.45). However the larvae fed with F2, (5% FM: 89% BNM) had higher SGR, 5.02±1.87% day^{-1}, than those on F1, 4.19±1.53% day^{-1}, (FM 0%: BNM 94%) (P<0.05). There was no case of mortality throughout the research.

3.3. Food Conversion ratio, (FCR)

Food conversion ratios (FCR) were lowest for the catfish fed with Feed 3 (1.44±0.38) to Feed 51.44±0.28). There was no significant differences in the FCR of catfish fed with either Feed 5 to Feed 3, average FCR 1.4 (P>0.05, FLSD $_{0.05}$ 1.24). The hybrid larvae fed with diet with 5% FM inclusion F2, increased FCR to 2.80±0.24. Complete substitution of FM with plant protein as in F1 (0% FM) increased FCR even further to 4.30±0.78 (Table 2). There was significant difference, (P<0.05, FLSD $_{0.05}$ 1.20), in the FCR of fish fed with feed F1 and F2.

Table 1: Composition of experimental diets used in feeding larval Hybrids of *C. gariepinus* x *H. bidorsalis* "heteroclarias" for 30d

Ingredients		Feed types			
	F1	F2	F3	F4	F5
Fishmeal	0	5	20	35	55
Bambaranut waste meal	94	89	74	59	39
Palm oil	2.0	2	2	2	2
Vit. C	0.2	0.2	0.2	0.2	0.2
Vitamin premix.	3.8	3.8	3.8	3.8	3.8
Total	100	100	100	100	100
Proximate analyses					
Estimated crude protein	17.9	19.9	26.1	31.5	40.4
Crude fat	4.0	4.3	6.1	6.3	8.4
Crude ash	4.0	4.0	4.7	4.3	6.4
Crude fiber	7.0	6.9	6.2	6.4	5.0
Moisture	10.2	10.5	11.0	10.1	12.0

[c] Vitamin and mineral premix added for supplying following mineral and vitamins (mg kg^{-1} diet): zinc, 180; manganese, 60, iodine, 4, retinol acetate, 300 IU; menadione sodium bisulfite, 10 mg; thiamine-HCl, 20 mg, riboflavin, 30 mg, calcium d-pantothenate, 90 mg; biotin, 0.3 mg; folic acid, 6 mg; vitamin B12,0.04 mg; niacin, 120 mg; pyridoxine-HCL, 20 mg; ascorbic acid,315mg; inositol, 200 mg.

Table 2: Food conversion ratio (FCR), Specific growth rate (SGR), Average weight gain (AWG) and average length gain of first feeding African catfish larvae fed experimental diets varying bambaranut waste (BNWM) with fish meal for 30d

FEED	FCR	SGR	AWG	ALG	Ini Wt.	Final Wt.
F1	$4.30±0.78^{c}$	$4.19±1.53^{c}$	$3.52±0.17^{c}$	$0.5±0.10^{c}$	$1.85±0.79^{ns}$	$5.42±0.02^{c}$
F2	$2.80±0.24^{b}$	$5.02±1.87^{b}$	$4.51±0.98^{b}$	$1.0±0.15^{b}$	$1.85±0.79^{ns}$	$6.41±0.12^{b}$
F3	$1.44±0.38^{a}$	$6.32±1.70^{a}$	$6.66±0.14^{a}$	$2.0±0.46^{a}$	$1.85±0.79^{ns}$	$8.56±0.05^{a}$
F4	$1.80±0.40^{a}$	$6.40±1.56^{a}$	$6.83±0.67^{a}$	$2.0±0.37^{a}$	$1.85±0.79^{ns}$	$8.73±0.02^{a}$
F5	$1.44±0.28^{a}$	$6.48±1.71^{a}$	$6.98±0.42^{a}$	$2.0±0.65^{a}$	$1.85±0.79^{ns}$	$8.88±0.04^{a}$
Flsd $_{0.05}$	1.24	1.45	1.0	0.62		

Means not followed by the same superscript are significantly different P<0.05, SGR= 100 (In W_f- W_i) T_2-T_1, FCR = Food intake/ weight gain, AWG = W_f-W_i/number of fish, ALG = L_f-L_i/number of fish, where $_f$ =final, $_i$= initial.

Table 3: Temperature ranges of aquarium culture water of larval African catfish *C. gariepinus* fed diets varying bambaranut waste meal and fish meal

Weeks	Morning Afternoon.	Morning Afternoon	Average
W I	25-29 ºC	27-31 ºC	
	27±0.09 ºC	29±0.40 ºC	Average
W II	21-23 ºC	22-24 ºC	
	21±0.01 ºC	23±0.01 ºC	Average
W III	22-25 ºC	22-24 ºC	
	23±0.07 ºC	25±0.06 ºC	Average
W IV	26-28 ºC	28-32 ºC	
	27±0.04 ºC	30±0.06 ºC	Average

3.4. Final weight and Average weight gain (AWG)

The final weights of the hybrid were similar for those fed with

F5, (8.88±0.04 g), F4, (8.73±0.02 g) and F3, (8.56±0.05 g). There was no statistical differences between the final weight of the hybrids fed with F5, F4 or F3 (P>0.05) (Table 2). Reduction of FM contents from 20% in F3 to 5% (and increase in BNWM from 74 to 89%) in F2, significantly reduced hybrids weight from 8.56±0.05 g to 6.41±0.12 g. There was however significant differences between the final weight of catfish fed with F2 (6.41±0.12 g) and F1 (5.42±0.02 g) (P<0.05). The hybrid catfish average weight gains were similar for the fish fed with F5, AWG (6.98±0.42 g) to F3, AWG (6.66±0.14 g). Although weight gain seemed to increase with increase in FM content it was not significant. However there was higher weight gain for the catfish fed with F2 AWG, (4.51±0.98 g) compared to those fed with F1 AWG, (3.52±0.17 g) (P>0.05, FLSD $_{0.05}$ 1.016), (Table 2).

3.5 Average length gain (ALG)
The average length gain of the fish was best for fish fed with feed F5, (2.0±0.65 mm), F3, (2.0±0.46 mm) and F4, (2.0±0.37 mm). There was no significant difference (P>0.05, FLSD 0.05, 0.62), in the ALG of the fish fed with F3, F4 or F5. The ALG of the catfish fed with feeds F1, (0.5±0.10 mm) and F2, (1.0±0.15 mm) were poorest. The ALG of Feed 1 fed fish was however significantly different (P<0.05, FLSD 0.05 0.6188), from the ALG of the catfish fed 2 (Table 2).

4. Discussions
First feeding fry's of African catfish hybrid readily accepted prepared diets. Hybrids catfish were observed to feed during early morning and late evening feeding periods, although aquarium water temperature was colder. The maximum temperature for culture of African catfish larvae is 30 ºC [20]. There was no special preference to diet type. African catfish hybrid quickly accepted experimental diet immediately they were fed. The acceptance of bambaranut waste meal diet could be attributed to the organoleptic qualities of BNWM [13] and also FM. Bambaranut is known to have 30% neutral sugars present as glucose and galactose [21]. The immediate acceptance of the diets by the hybrid catfish could as well be affected by the neutral sugars in the diets. Effects of organoleptic status of ingredients are important factor in considering aquaculture feed additive [22]. However the nutritional performance of the fish based on treatment feed was very distinct. The performance of the fish on F5, F4 and F3, shows that bambaranut meal could be included in hybrid diet up to 74%. Very high percentage inclusion of fish meal (up to 55%) may not be necessary for production of heteroclarias feed. Since fish meal is costly and influences the cost of production [23], lower inclusion levels of between 20-30% seems to be advisable for heteroclarias larvae.

The feed conversion ratio of the hybrids for F5, F4, and F3, shows that BNWM can be effectively converted by hybrids African catfish. Feed utilization could also be due to our mode of feed processing. The starch content of our feeds was initially conditioned before pelleting. Conditioning of starch enhances its gelatinization and dough expansion, which in turn enhances utilization by recipient fish. FCR ratios were within range of findings of previous works of [24] for hybrid *Clarias gariepinus X C. macrocephalus* who had FCR of 1.41. The inclusion of 10% palm oil to the feed must have also accounted for the acceptance of the feed and improved growth of fish. The growth improvement of palm oil inclusion was also noticed by [25], who noted that inclusion of at least 8% palm oil improved growth performance, protein retention and fillet vitamin E concentration of African catfish *Clarias gareipinus*.

The inclusion of 20% fish meal in F3 gave similar growth rate result compared to F4 and F5 that has higher FM content. This suggests that it may not be necessary to include up to 50% FM in the diets of larval hybrid African catfish. Bambaranut nut has high content of protein 24% [26, 27], and essential amino acids like lysine and methionine [27]. Low levels of lysine and methionine negatively affects growth and feed utilization African catfish [28]. The high Specific growth rates (SGR) of the catfish larvae fed with high BNWM could as well be due to the high amino acids from FM and BNWM which could be why SGR were also not significantly different for F3, F4 and F5. This suggests that bambaranut waste meal could be plausible substitute of fish meal. The preparatory methods of the feed like boiling and steaming may have accounted for the nutritional effects of BNM [13]. Steam conditioning gelatinizes starch enhancing utilization and assimilation. The quality of the diets depends so much also on preparatory mechanisms of the sieviate. Well ground and conditioned sieviate would reduce chaff that otherwise would be indigestible by the hybrids.

The average weight gain of the fish was not significantly different for feed 3, feed 4 and feed 5. The poor performance of the fish on feeds 1 and 2 is indicative of the need to supplement bambaranut meal diet with fishmeal. It is also important to note that we utilized BNWM which is a sieviate of bambaranut meal. All plant protein are limiting in methionine [29] but bambaranut is rich in methionine [26].

5. Conclusion
Based on results of this research, BNWM is a good alternative plant protein to FM in hybrid catfish diet. High inclusion of FM (50%) produced similar growth rate as 20% and is therefore not necessary in larval hybrid catfish diet. Although the hybrid catfish larvae grew fairly well on our diets, the anti nutritional effects of bambaranut waste meal should be characterized. The cheap cost of bamabaranut waste meal is an attraction to its plausible use in aqua feed.

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