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Effects of Substitution of Fishmeal with Bambaranut Meal on Growth and Intestinal Microbiota of African Catfish (*Clarias gariepinus*)

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Abstract

Substitution of fishmeal with plant protein is trending but there had been no study of its effects on catfish gut microbial communities. We made Five isonitrogenous (44.29±0.45%) and isoenergetic (2747.24±0.09 kcal) diets labelled as feed 1 (F1) to feed 5 (F5). The feeds varied in composition of fishmeal (FM) with bambaranut meal (BNM), as: F1, 65:0, F2, 45:5, F3, 25:25, F4, 5:45, and F5, 0:65. There was a control diet F6. The diets were fed to *Clarias gariepinus* distributed into three replicate aquaria at 15 fish per aquarium. Feed 4 (F4) had the best specific growth rate (SGR, 7.03±0.03% day^{-1}), which was better than F3, with SGR, 6.77±0.08% day^{-1} and 65% fishmeal diet F1, with SGR 6.67±0.06% day^{-1} ($P<0.05$). F2 and F4 fed catfish were used to analyze gut microbiota. The F2 catfish foregut had microbiota comprised Gram –ve rod *Citrobacter freundii*, Gram +ve cocci *Staphylococcus aureus spp* and Gram +ve straight rod *Bacillus spp, Citrobacter freundii* was the dominant. The F4 catfish had foregut dominated by Gram –ve rod *Citrobacter freundii* and Gram +ve cocci *Staphylococcus aureus aureus.* The midgut of catfish fed with F2 had microbiota dominated by *Citrobacter freundii* and *Bacillus subtilis.* The performance of catfish on diets seems to be enhanced by the gut microbiota. Most digestion takes place in midgut and the consortium of bacteria dominant in African catfish midgut are known to be enzyme producing and celluloselytic and seems to be reason for African catfish known ability to utilize high carbohydrate diets.

Introduction

Bambaranut (*Voandzea subterranea*) is a neglected proteinous herbaceous legume from the family Fabaceae. It is commonly called bambara groundnut, peanut, ground bean, Congo-goober or earth pea. It is called "Okpa" by Igbos of eastern Nigeria and "Gurujia" by Hausas of northern Nigeria. Bambaranut is supposedly of African origin (Obizoba & Egbuna, 1992; Basu, Roberts, Azam-Ali, & Mayes, 2007). However analyses of naturally occurring stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ of bambaranut, revealed that it falls within the C_3 plant groups instead of C_4, and has C:N ratio of 12.37 (Enyidi, 2012). Bambaranut is a secondary food crop grown all over the region of sub-Saharan Africa (Karunaratne, Azam-Ali, Sasey, Adu-dapaah, & Crout, 2008). The crude protein content of bambaranut is about 24-30% (Dakora & Muofhe, 1995; Basu *et al.* 2007; Enyidi, Pirhonen, Kettunen, & Vielma, 2017). Bambaranut is cheap, abundant and easily accessible. Moderate protein content, low phytic acid and low price makes bambaranut a plausible alternative to both fishmeal (Enyidi & Mgbenka, 2014) and soybean meal in the diets of African catfish (Enyidi *et al.* 2017). Plant proteins are used in substituting fishmeal due to the unstably supply and high prices of fishmeal (Hardy,

2010). Substitution of fishmeal with plant proteins is essential if aquaculture is to be net producer than consumer of fish (Hardy, 2010). Feed and changes in feed type influences the micorobiota of fish consequently newly hatched fish larvae seemingly tend to harbour few bacteria (Ringø, 1993, 1993a). The frequency of feeding has also been noted as a short term deciding factor on the gut microbiome of fish (Parris *et al.* 2018). Moreover, the use of alternative ingredients in feeds could affect gut microbial communities (Zhou, Ringø, Olsen, & Song, 2017; Gatesoupe, Fauconneau, & Deborde, 2018; Miao *et al.* 2018). According to literature, diets exert much influence in determining complexities of the gut microbial community starting from first feeding larval stages and its diversities (Blanch, Alsina, Simon, & Jofre,1997; Reid, Treasurer, Adam, & Birkbeck, 2009; Giatsis, Sipkema, Smidt, Verreth, & Verdegem, 2014). Gut microbial communities also differ within gut sections like the hind gut and foregut, due to feed intake and digestive activities within the gut sections (Ye *et al.* 2013). In a recent research on the nutrition of northern snake head, it was noted that different inclusions levels of soybean meal in their diets produced different gut microbial communities like *Firmicutes*, *Lactococcus*, *Proteobacteria*, *Bacteroidete*s and *Planctomycetes* (Miao *et al.*, 2018). *Firmicutes*, were also notably present in plant protein diet fed fish (Gatesoupe *et al.*, 2018). Several bacteria have been associated or commonly isolated from fish fed with fishmeal diets compared to plant based diets, for example, *Sphingomonas* sp. from rainbow trout given fish meal diet, (Heikkinen, Vielma, Kemiläinen, Tiirola, Eskelinen, Kiuru, Navia-Paldanius, & von Wright, 2006). Proteobacteria *Aeromonas* sp. OTU_23s (Gatesoupe *et al.*, 2018). Similarly, use of high inclusion of bioprocessed soybean meal in the diets of rainbow trout decreased the microbial richness indices taken from distal intestine of the fish when compared to fishmeal (Bruce, Neiger, & Brown, 2018). Inclusion of plant proteins in fish diets affects gut microbiota and these effects also depends on prior processing methods of the plant proteins (Zhou, Ringø, Olsen, & Song 2017).

The microbial communities have been associated with the digestion and absorption in fish (Sire & Vernier, 1992; Olsen & Ringø, 1997; Bakke, Glover, & Krogdahl, 2010). Gut microbiota contributes enzymes to help in digestion of complex food substances like cellulose, chitin, and collagen that may otherwise not be easily digested. The microbes also contribute in proteins, carbohydrates and lipids digestion (Austin, 2002)**.** The microbial communities have also been noted to influence host body functions such as larval development, disease resistance and immunity development of the mucosal system and angiogenesis (Midtvedt & Gordon, 2002; Ray, Ghosh, & Ringø, 2012; Vijayaram, Kannan, & Muthukumar, 2017). There are two major groups of gut microbial communities the autochthonous communities, referring those that colonize host fish epithelial linings and are associated with the microvilli. There is also the allochthonous community which are opportunistic, transient and present in the lumen and associated with the fish digesta (Kim, Brunt, & Austin, 2007; Ringø & Birkbeck, 1999; Ringø, Olsen, Mayhew, & Myklebust, 2003; Ringø, Zhou, Gonzalez-Vecino, Wadsworth, Romero, Krogdahl, Olsen, Dimitroglou, Foey, Davies, Owen, Lauzon, Løvmo, Martinsen, De Schryver, Bossier, Sperstad, & Merrifield, 2016). The allochthonous communities are not well established and changes regularly. It had been noted that inclusion of alternative protein ingredients in the diets of fish can affect the allochthonous or digesta community while it has little effects on the mucosa communities (Gajardo *et al.* 2017). The microbial community in the fish gut are also affected by developmental stages, adaptation to nutrition and environmental conditions (Nayak, 2010; Romero and Navarrete, 2006; Navarrete, Magne, Araneda, , Fuentes, Barros, Opazo, Espejo, & Romero, 2012; Li, Yu, Feng, Yan, & Gong, 2012). The gut microbiota of fish can be examined by conventional microbiological methods (Suau, Bonnet, Sutren, Godon, Gibson, Collins, & Dore, 1999), but this yields low fraction of bacteria.). However combination of bambaranut meal and corn meal as fish meal substitutes produced fast growth in first feeding African catfish (Enyidi, Kiljunen, Jones, Vielma, & Pirhonen 2013). Therefore, the substitution of fishmeal with solid state fermented bambaranut meal have been noted as growth promoter of African catfish *C. gariepinus* (Enyidi & Etim, 2018). There had been no research done on the effects of substituting fishmeal with bambaranut meal on the gut microbial communities of African catfish.

This research seeks to find the effects of substituting fishmeal with graded levels of bambaranut meal on the gut microbial communities and growth of Africa catfish using conventional culture-based methods.

Materials and Methods

Feed Preparation

Five isonitrogenous diets of (44.29±0.45%) protein and isoenergetic diets of (2747.24±0.09 kcal) s labelled as feed 1 (F1) to feed 5 (F5) were produced to vary in composition of Nordic fishmeal (FM) with Nigerian bambaranut meal (BNM). The substitution percentages of the FM:BNM was as follows: F1, 65:0, F2, 45:5, F3, 25:25, F4, 5:45, and F5, 0:65. The diets had 10% pro rata inclusion levels of soybean meal. There was also inclusion of 10% poultry by products in all feeds except feed 4 that had 15% to beef up the protein content and maintain isonitrogenous form of the treatment diets. Similarly, white corn meal with <9% protein value was also included at 8.5% for feed F1 and F5 and between 23.5% to 18.5% in feed F2, F3 and F4 to balance the protein content of the diets (Table 1). Similar

percentages of essential amino acids like lysine and methionine were included to make up for deficiencies in ingredients. All other ingredients were similar in all the treatment the diets. All ingredients were mixed with electric mixer for 10 minutes and 1% of palm oil was added. The ingredients were preconditioned with warm water totally 30% moisture of the feed marsh at 100^{o}C. The dough was then pelleted with manual pelletizing machine. The pellets were dried in an electric oven at a constant temperature of 40^{o}C. Dried test diets were stored in airtight container till used.

Experimental Fish and Set Up

African catfish fingerlings of initial average biomass (±S.D) 9.07±0.05g, n=320, were purchased from Michael Okpara University of Agriculture Department of Fisheries and Aquatic Resources Management (FISHARM) fish farms. The catfish were stocked in four plastic aquarium of 40 l capacity at stocking density of 80 fish per aquarium and acclimated. Acclimation lasted for two weeks in the wet laboratory of FISHARM Department. During acclimatization period fish were fed between 8.00-9.00 am and 18.00-19.00 pm hours to apparent satiation with 35% protein commercial diet. After acclimation. The catfish were distributed at 15 fish per aquaria into separate 18 aquariums of dimension L=70cm, W=40cm, D=80cm, at three replicate aquaria per treatment feed, F1 to F5 and control commercial diet. The catfish were deprived of feed for a day to clear their gut and enhance faster intake of experimental feed. The aquaria were subjected to D18:6L photoperiod. The light intensity was 30 lux measured with (HD 9221 lux meter, Delta OHM, Padua, Italy). The experimental unit was shielded with a black nylon sheet to maintain the low light intensity. The fish rearing water was filtered water supplied from the university borehole water system. Average water temperature (mean+SD, n=18) was 25.32±1.5^{o}C. The average total gas pressure of the rearing was measured to be 100±2.07 (P4 Tracker Total Gas Pressure saturometer; Point Four Systems Inc., Richmond, Canada). Dissolved oxygen content was 5.20±0.09mg/L measured with (YSI oxygen meter model 550A, YSI Inc. Yellow Springs, Ohio, USA). The average water pH was 6.56. Ammonia concentration was 0.7±0.6 mg/l, measured with (Tetra ammonia kit, Malvern, PA, USA). Average water turbidity was 11.10NTU measured with Turbidometer HANNAH products equipment (Table 2).

Feeding of experimental fish

The fish were hand fed to apparent satiation three times daily. Feeding took place between hours of 8.00-9.00 am, 1.00-2.00pm and 18.00-19.00 pm. Feeding was done carefully to avoid over feeding and reduce uneaten feed. Any uneaten feed found in the aquaria were siphoned from the aquaria after 2h, dried, weighed and recorded.

Weighing of Fish

The catfish were fasted for 18 hours before weighing to allow clearing of the food in their gut. Subsequently, the catfish were weighed every fortnight till the end of the experiment. The weighing of the

Table 1. Feed composition and proximate composition of feeds used in feeding fingerlings of *Clarias gariepinus* fed with feeds varying level of bambaranut from 0-65% (F1-F5)

Ingredients	F1	F2	F3	F4	F5
Fish meal	65	45	25	5	0
Bambaranut meal	0	5	25	45	65
Soyabean meal	10	10	10	10	10
Poultry by-Product	10	15	15	15	10
Corn meal (White)	8.5	8.5	8.5	8.5	8.5
Lysine	1.5	1.5	1.5	1.5	1.5
Methionine	1.5	1.5	1.5	1.5	1.5
Vitamine C	1	1	1	1	1
Vit. Premix[a]	1.5	1.5	1.5	1.5	1.5
Palm oil	1	1	1	1	1
Cellulose	0	10	10	10	0
Crude Protein	44.29	43.29	43.85	44.65	43.21
Lipid	10.69	11.78	9.49	8.08	7.78
Starch	55.32	55.51	53.62	53.08	53.51
Crude fibre	0.7	0.77	2.7	2.5	2.77
Energy	2744.24±0.09	27472.24±0.09	2740.24±0.09	2741.24±0.09	2743.24±0.09
Ash	18.85	18.14	9.4	5.35	6.84
Moisture Content	12.39	26.46	14.25	15.1	14
Dry matter	87.61	73.54	85.75	84.9	85.96

[a] Vitamin premix. The following vitamins were added to supply the following Kg-1diet: cholecalciferol, 1300 IU; all-race-α-tocopheryl acetate, 140 IU; menadione sodium bisulfite, 12 mg; thiamin HCL, 8 mg; riboflavin, 16 mg; calcium d-pantothenate, 17 mg; biotin, 0.2 mg; folic acid, 5 mg; vitamin B12, 0.02, niacin, 40 mg; pyridoxine HCl, 16 mg; ascorbic acid (Stay C), 80 mg. magnesium phosphate, 5000 mg, potassium carbonate, 400 mg, manganous sulfate, 10; ferrous sulfate, 5 mg; zinc sulfate, 80 mg.

Table 2. The physio-chemical parameters of aquarium water used in rearing African catfish *C.gariepinus* raised on diets varying bambaranut meal with fish meal

Parameters	F1	F2	F3	F4	F5	F6
DO2 (mg/l)	5.20±0.11	5.20±0.02	5.11±0.09	5.41±0.23	5.20±0.09	5.20±0.07
pH	6.61±0.01	6.52±0.10	6.56±0.07	6.54±0.12	6.55±0.11	6.58±0.09
Temp. (ºC)	25.27±0.12	25.29±0.09	25.28±0.06	25.32±0.11	25.39±0.12	25.41±0.02
Turbidity (ntu)	12.09±0.04	10.21±0.06	11.01±0.08	10.91±0.21	10.40±0.09	12.01±0.07
Total gas pressure	100.12±0.03	100.09±0.10	100.23±0.08	100.12±0.07	100.56±0.02	100.35±0.05
Ammonia (mg/l)	0.71 ± 0.6	0.65±0.07	0.70±0.01	0.69±0.13	0.80±0.15	0.71±0.12

catfish was together per each of the three replicate aquaria per feed type. The initial weight of catfish in each replicate aquarium was recorded.

Proximate Analyses

At the end of the experiment five fish were taken from each aquarium for analyses of proximate composition, hepatosomatic index (HSI) and peritoneal fat somatic index (FSI). The fish were dissected and liver and visceral fat of the fish were removed and weighed (to 0.01 g). The five replicate aquarium were oven dried at 40^{o}C and used for analyzing proximate composition of the fish. Each of the five treatment feed were also subjected to proximate analyses. The protein content was analyzed by Kjeldahl method, using Tecator Kjeltech model 1002 (Tecator, Kjeltect, Höganäs, Sweden). Protein % was expressed as %N x 6.25. Total lipids were analyzed using a modified chloroform methanol method (Enyidi *et al.* 2013). Lipids in the samples were extracted twice with 2:1 chloroform: methanol mixture. Lipid extraction was after modified methods of Parrish (1999), Kainz, Arts, and Mazumder, 2004; and Enyidi *et al.* (2013). Ash content was determined by incineration samples in a muffle furnace at 550°C for 24 hrs. The ash % was weight of ash/weight of sample*100. Carbohydrtae content was measured by adding the other variables and deducting from 100. The proximate composition of the experimental feed are recorded in Table1.

Gut Microbial Analyses

Preparation of Culture Media and Sterilization

The bench area to be use for this analysis was cleaned with water mixed with detergent, disinfected. About 13.44 g of nutrient agar, (tryptic soy agar plates (TSA); Merck, Germany, and MacConkey agar and Eosin methylene blue agar) were prepared for use in inoculation. In analysing the gut microbial communities using conventional methods, two feed types were chosen, namely feed 2 (F2) containing 45% fishmeal and 5% bambaranut meal and the reciprocal feed, feed 4 (F4) with 45% bambaranut meal and 5% fishmeal. In measuring initial microbial load, three fish were sacrificed by hitting them gently on the head with a laboratory plastic pestle. The fish were neither starved nor deprived of food. The fingerlings were used in analysing for the initial microbiome communities of the fish. After the feeding experiment, 3 catfish per treatment feed (F2 and F4) were also sacrificed for final analysis to determine the effects of the feed on the gut microbial communities. The catfish were hit with a gentle blow on the head using a rubber rammer. The catfish was dissected and the whole gut was extracted. The gut was divided into foregut, mid gut and hind gut. There were at least three replicates per foregut, midgut and hindgut. The division of the gut followed subjective observation of the gut structure and stages in digested feed and evacuation through the anus. The foregut, began at the posterior edge of the gills, and included the oesophagus, stomach and pylorus. The midgut is the longest portion of the gut; it includes the intestine, pyloric ceacea that also provides additional surface area for absorption. Most digestive activities take place in the midgut. The midgut ends where there is an increase in tube diameter and the fecal matter becomes more distinct. Where the tubular diameter starts to increase is the beginning of the hindgut, which is the distal intestine extending to the anus. Each of the sections (foregut, midgut and hindgut) had three replicates analysed. Similar treatments were carried for the pre feeding and post feeding gut samples. This was to give room for apparent complete analysis of the whole gut sections. The gut was cut open horizontally and 5cm of the foregut piece was cut and minced in a test tube and distilled water was used in making it up to 1ml. The 1ml stock solution was mixed with 9mls of distilled water to give a 1:10 dilution. The mixture was vortex for 5mins. Same procedures were carried out for intestinal samples from mid gut and hind gut. The stock solution was diluted with sterile 0.1% peptone water up to 10^{-6} according to (Cheesbrough, 2000). Pour plate techniques were used in spreading 1m of the stock dilution, on two replicate plates of nutrient agar, tryptic soy agar plates (TSA); Merck, Germany and MacConkey agar and Eosin methylene blue agar, to determine the total bacterial counts, using sterile glass spreader. Agar plates were incubated in an incubator adjusted to constant temperature of 36^{o}C for 24hrs. After incubation the plates were read by considering and selecting those plates which had between 30-300 colonies. Colony counting were done using and illuminated colony counter.

CFU/mL=cfu/ml=(no. of colonies x dilution factor)/volume of culture plate

Bacteria Isolation and identification

Sub culturing of identified representative colonies were done on freshly prepared agar plates for purpose of isolation. The colonies were subculture in tryptic soy agar plates (TSA; Merck, Germany) to obtain pure cultures. The plates were incubated at 37^{o}C for 24 hours. Bacterial isolates were subjected to morphological and biochemical characterisation of the sub cultured based on Gram staining techniques according to the Bergey's manual of determinative bacteriology (Buchanan & Giboons, 1974). Morphological characteristics examined colour, edge, elevation, shape and arrangement of microorganisms. The examination of microorganisms under slide was made in oil immersion after Gram staining.

Gram Staining Technique and Microscopy

The Gram staining technique was used as the staining reaction to identify the different bacteria species by their Gram reaction (Gram +ve or Gram -ve) and their morphology. A loopful of the bacterial colonies isolated was emulsified in sterile distilled water and a thin preparation was made on a glass slide. The smear was air-dried completely and rapidly passed through the flame of a spirit lamp and allowed to cool. The fixed smear was flooded with crystal violet stain for 60 seconds, after which it was washed off with sterile water and air-dried. Lugol's iodine was applied on the smear and allowed for 60 seconds and later washed off with sterile water. The smear was decolourized with ethanol for 30 seconds and immediately washed off with sterile water. Safranin was used to flood the smear for about 2 minutes and later washed off with sterile water. The back of the slide was wiped clean and placed in a drying rack for the stained smear to air-dry. All Gram stained smears of different colonies from different cultures were examined using oil immersion objectives (x100) of a compound microscope to check the staining reaction and morphology of the bacteria species and then with the oil immersion objective (Beishir, 1987; Green berge, Clesceri, & Eaton, 1992; Cheesbrough, 2000).

Biochemical Tests

Several biochemical tests were carried out using suspensions of organisms and chemically-defined solutions. The biochemical tests use the preformed enzymes of the bacterial cells. Cautions were taken in carrying out the test so the results would not be complicated by side effects or by the multiple reactions that occur in cultures growing in a nutrient media that contained test substrate. Among the biochemical tests done were; catalase test, oxidase test, indole test, citrate test, coagulase test, urease test, manitol test, H2S test, nitrase reductase test, methyl red test and Voges Proskauer test. The results of the test are tabulated in Tables 4 through 9.

Coagulase test: To carry out the coagulase test, drops of distilled water contained in sterile 25ml amber coloured bottle was placed on different glass slides. A thick suspension of the test bacteria under examination was made by emulsifying a colony of the bacteria sample in each drop. A sterile loop was used in adding a loop of plasma to each emulsified thick suspension and it was gently mixed. There was a control suspensions made wherein no plasma was added. This control would differentiate true coagulase clumping from any granular appearance of the test bacteria. After 10 seconds of adding plasma observations were done to identify clumping the test bacteria. The observed results for clumping within 10 seconds were coagulase +ve while, no clumping within 10 seconds were noted recorded as coagulase -ve (Matsen, 1980).

Oxidase test: This was done using filter paper test method. A small piece of filter paper was dipped in 1% Kovács oxidase reagent and then allowed to dry. Using a sterile loop a well isolated colony on a bacterial plate from a fresh 18-24hr culture was picked. The colony was rubbed on the dried paper. The colour change was observed after the rubbing. The result was judged oxidase +ve when the colour changes to dark purple within 5 to 10 seconds. Conversely the result was considered oxidase -ve if the colour does not change or it takes longer than 2 minutes (Harley, 2005).

Indole test: In carrying out the indole test, the test organisms were inoculated in Bijou bottles that had 3ml of sterile tryptone water. This was incubated at 35^{o}-37^{o}C for 48 hours. After incubation 0.5ml of Kovac's reagent was added to test organism –tryptone water mixture and shaken gently. A change in colour of the system was used as indication of +ve or –ve Indole test. A red colour appearing in the surface layer of the tryptone water-Kovac's reagent mixture identified Indole +ve. The reverse was Indole -ve respectively (McFadden, 2000).

Citrate test: Citrate tests started with the preparation of Simmons citrate agar plate. The test organism was inoculated in it by streaking the agar slant and stabbing the butt using sterile wire needle. This was incubated at 37^{o}C and examined daily (24-72hrs) for possible growth. Citrate utilization requires oxygen. Consequently we placed screw caps loosely on the tube. Incubate at 35±2^{o}C for at least 48 hours. Some incubation was allowed to stay for 7 days due some organism limited rate of growth on citrate medium. The results were based on observation of colour change. Colour change from pale green to blue indicated a +ve result. Citrate +ve: growth was visible on the slant surface and the medium colour was intense Prussian blue. The result was Citrate –ve if mere trace or no growth was visible. Moreover no colour change will occur. The media remained the deep forest green colour originally of the uninoculated agar (McFadden, 2000).

Mannitol test: In making the mannitol test we added 1.0 mannitol to nutrient broth. Phenol red is added and the test organism is inoculated. Mannitol is a sugar that some bacteria can use because of an enzyme

that breaks down the compound. The test is +ve if the colour turn from usual red to yellow. The reverse is the –ve mannitol (McFadden, 2000).

H2S (Sulphate reduction test): In making the H2S test we made a sulphate agar and allowed it to solidify. The test organism was inoculated in the agar by stabbing of the Sulphate agar and the tubes incubated at 37^{o}C for 24 hrs. The result was judged +ve based on observation of black colouration at the point of stab. The reverse case was –ve (McFadden, 2000).

Catalase test: Catalase test started from making a hydrogen peroxide solution. Then several colonies of the test organism were collected using a glass rod and immersed in the hydrogen peroxide solution. Results were based on observation of immediate active bubbling in the test tubes. Results is +ve catalase test if there is active bubbling in the test tube and -ve catalase test if there are no bubbles in the test tubes (Matsen,1980).

Urease test: For the Urease test we prepared Urea agar and dispensed it into bijou bottles. The test organism was inoculated and the urea slants were incubated at 37^{o}C for 24hrs. Results were judged based on development of a bright pink colour indicating a +ve reaction. The reverse was -ve (McFadden, 2000).

Methyl red test: We prepared glucose phosphate media and inoculated it with the test organism. This was then incubated at 37^{o}C for 48hrs. After incubation, 0.04% methyl red indicator was added. Result was judged +ve at the formation of a red colour. The result was considered –ve reaction if there were yellow colour instead (McFadden, 2000).

Voges-Proskauer test: We prepared glucose phosphate media. This media was inoculated with test organism. After inoculation 3ml of creatine was added followed by 1mL of KOH and mixed thoroughly. The result was judged +ve at the appearance of a pink colour after 24hrs indicated a +ve result (McFadden, 2000).

Calculations and statistical analyses

The following calculations were made for each aquarium, which was the experimental unit in the calculations:

Colony forming unit (CFU)/ml = Number of colonies* dilution factor **/** inoculums volume

Specific growth rate (SGR, % day^{-1}) was calculated as 100 * (Ln w2– Ln w1) / t, where w1 and w2 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 (Gil Martens, Fjelldal, Lock, Wargelius, Wergeland, Witten, Hansen, Waagbø, & Ørnsrud, 2012).

Food conversion ratio (FCR) was calculated as (feed consumed in g)/(change in tank biomass in g) (Enyidi, 2012).

Daily feed intake (DFI) (% of final biomass) = 100 * (Feed fed in g)* (W2-W1)/ t, where, W1 and W2 were initial and final aquarium biomass in g. (Helland, Grisdale-Helland, & Nerland, 1996; Thodesen, Grisdale-Helland, Helland, & Gjerde, 1999). Condition factor = 100 x total weight (g)/total lenght^{3} (cm) (Enyidi 2012)

Protein efficiency ratio (PER)=(w2-w1) (g)/protein fed (g) (Enyidi *et al.*, 2013).

Hepatosomatic index (HSI)=100 * liver weight (g)/ fish weight (g) (Enyidi, 2012). Survival=100 * final number of individuals / initial number of individuals (Enyidi *et al.*, 2017)

Fish meal ratio (FMR)=FCR * % dietary fish meal inclusion/ 100 (Boyd 2007)

Results were analyzed using one way ANOVA in SPSS 14.0 software and least significant difference (LSD) 0.05 was used in separating possible differences of treatment means.

Results

The results of proximate composition of the fish after experiment are recorded in Table 3. There were no differences in the protein and lipid content of the catfish irrespective of treatment feed used. The treatment diets produced fast growth in the catfish. The catfish fed with feed F4 had the highest final weight 225.00±0.09g after the feeding trial. There was significant difference ($P<0.05$) in the final weight of the F4 fed catfish and those fed with F3 with final weight of 178±0.05g. The final weight of the catfish fed with F3 was much higher and significantly different from those fed with F1 (165.23±0.07g) ($P>0.05$). We did not notice any significant differences between the final weight of the catfish fed with either feeds F1 and F2 ($P>0.05$) (Table 4). The lowest final weight of the catfish (154.77±0.06g) was obtained from catfish fed with 0% fishmeal diet feed F5. The specific growth of the catfish in this experiment was high and those fed with feed F4, grew with the highest specific growth rate (SGR) of 7.03±0.03 % day^{-1}.

Table 3. Proximate composition of African catfish *C. gariepinus* fed for 70d with diets varying in the percentage inclusion levels of bambaranut meal as substitute of fishmeal

Feed Type	Moisture	Protein	Lipid	Ash
Feed 1	77.91±0.04^{ns}	15.04±0.14^{ns}	4.99±0.15^{ns}	1.06±0.01^{a}
Feed 2	77.74±0.01^{ns}	15.97±0.12^{ns}	4.76±0.08^{ns}	0.68±0.06^{b}
Feed 3	77.20±0.07^{ns}	16.6±0.23^{ns}	5.46±0.12^{ns}	0.23±0.08^{d}
Feed 4	77.70±0.08^{ns}	16.23±0.16^{ns}	5.01±0.11^{ns}	0.52±0.02^{c}
Feed 5	76.84±0.11^{ns}	16.82±0.60^{ns}	5.46±0.04^{ns}	0.37±0.05^{d}
Feed 6	77.532±0.12^{ns}	16.85±0.09^{ns}	5.31±0.02^{ns}	0.39±0.03^{d}

The SGR of catfish fed with F4 was significantly different from that of all other feeds (P<0.05). The nearest catfish SGR to F4 was that of those fed with feed F3, 6.77±0.08 % day^{-1} and this was significant different (P<0.05) from SGR of all other catfish. We noticed that SGR was similar for catfish fed with control diet F6 (6.69±0.13% day^{-1}) and those fed diets having highest inclusion of fish meal, Feeds F1 (6.67±0.06% day^{-1}) and F2 (6.61±0.02 % day^{-1}) (P>0.05). The lowest SGR of the catfish was obtained from those catfish fed with 0% fishmeal diet feed F5, (6.58±0.01 % day^{-1}). Food conversion ratio (FCR) of the catfish was lowest for those fed with treatment feed F4, (FCR 1.05±0.07). The FCR of F4 was significantly lower than all other feeds (P<0.05) (Table 4). The next lower FCR to F4 was that of feed F3 (1.41±0.06) and it was also significantly different from FCR all other feeds (P<0.05). The FCR of catfish fed with high fish meal diets F1 (1.61±0.2) and F2 (1.62±0.06) was similar to that those fed with commercial diets F6 (1.66±0.10). However the 0% fishmeal diets F5 had the highest FCR 1.Weight gain of the catfish followed similar pattern as the FCR. The catfish fed with F4 had the highest weight gain of 219.10±0.08g which was significantly higher than all other feeds. Next to catfish fed with 4 were weight gains from those fed with feeds F3 (172.6±0.04g), control feed F6 (162.03±0.09g) and F1 (159.33±08g). Although weight gain of catfish fed with F3 was significantly higher than F6 and F1 (P<0.05).The lowest weight gain was by those fish fed with 0% fishmeal diet F5 (148.9±0.12g) (Table 3).The protein efficiency ratio was highest for catfish fed with F4 (4.68±0.09) followed by those fed with F3 (3.93±0.06). The PER of F4 was significantly (P<0.05) different from F3. Protein efficiency ratio of the 0% fishmeal diet F5 (3.70±0.08) was higher than that of 65% fishmeal diet (P<0.05). Hepatosomatic index of the fish (HSI) was decreasing with increasing inclusion of fishmeal. The catfish fed with 0% fishmeal diet had the highest HSI of 1.22±0.08. There was significant decreases in the HSI of the catfish as the fishmeal content of the diets were increased. Consequently the HSI of the catfish appeared as follows: F5 (1.22±0.08)>F4 (0.67±0.03)>F3 (0.49±0.06)>F2 (0.36±0.56)>F1 (0.14±0.08) (P<0.05) (Table 4). The HSI of the control feed F6 was higher than all other diets except F5 (Table 3). Condition factor (CF) of the fish did not follow any defined pattern. However the catfish fed with feed 1 had

Table 4. Growth and nutritional performances of African catfish fed with diet varying bambaranut meal with fishmeal for 76 days

	F1	F2	F3	F4	F5	F6
Initial	5.90±0.02^{ns}	5.73±.03^{ns}	5.40±0.08^{ns}	5.90±0.04^{ns}	5.87±0.07^{ns}	5.13±0.01^{ns}
Final	165.23±0.07^{c}	158.09±0.03^{d}	178.1±0.05^{b}	225.00±0.09^{a}	154.77±0.02^{d}	167.16±0.09^{c}
AWG	159.33±08^{cd}	152.36±0.07^{d}	172.6±0.04^{b}	219.10±0.08^{a}	148.9±0.12^{d}	162.03±0.09^{c}
SGR	6.67±0.06^{c}	6.61±0.02^{c}	6.77±0.08^{b}	7.03±0.03^{a}	6.58±0.01^{d}	6.69±0.13^{c}
FCR	1.61±0.2^{c}	1.62±0.06^{c}	1.41±0.06^{b}	1.05±0.07^{a}	1.71±0.04^{d}	1.66±0.10^{c}
FMR	1.18±0.05^{d}	0.98±0.12^{c}	0.39±0.34^{b}	0.04±0.54^{a}	0.00	n/a
PER	3.59±0.06^{d}	3.60±0.10^{d}	3.93±0.06^{b}	4.68±0.09^{a}	3.70±0.08^{c}	3.68±0.10^{d}
DFI	3.39±0.05^{a}	3.25±0.08^{b}	3.22±0.06^{b}	3.03±0.01^{c}	3.35±0.02^{ab}	2.59±0.05^{d}
CF	2.54±0.05^{a}	2.26±0.06^{b}	1.92±0.01^{b}	2.00±0.04^{b}	1.94±0.08^{b}	1.67±0.03^{c}
HSI	0.14±0.08^{f}	0.36±0.56^{e}	0.49±0.06^{d}	0.67±0.03^{c}	1.22±0.08^{a}	0.80±0.07^{b}
Survival	100^{a}	89.24^{b}	100^{a}	100^{a}	91.36^{b}	100^{a}

Where AWG is average weight gain in (g), SGR specific growth rate (% day^{-1}), FCR is feed conversion ratio, PER is protein efficiency ratio, FMR is fish meal ratio, HSI is hepatosomatic index, CF is condition CF, (g/ cm^{3}). DFI is daily feed intake in g. Survival in %. Data are presented as mean ± SD. (n-3). Different superscript within the same row are statistically significantly different (P<0.5)

Table 5. Results of colonies of foregut, midgut and hindgut after 48hrs incubation pre feeding experiment of the catfish

Fish sample	Inoculums Volume	Dilution Factor	No Colonies	Total No of Organism (CFU/ml)
Foregut	1	0.1	34	3.4x10^{-3}
F4	1	0.3	45	13x10^{-3}
	1	0.5	30	15x10^{-3}
Foregut	1	0.1	50	5x10^{-3}
F2	1	0.3	40	12x10^{-3}
	1	0.5	60	30x10^{-3}
Midgut	1	0.1	230	23.3x10^{-3}
F4	1	0.3	120	36x10^{-3}
	1	0.5	90	45x10^{-3}
Midgut	1	0.1	300	30x10^{-3}
F2	1	0.3	320	96x10^{-3}
	1	0.5	230	115x10^{-3}
Hindgut	1	0.1	420	42x10^{-3}
F4	1	0.3	320	96x10^{-3}
	1	0.5	210	105x10^{-3}
Hindgut	1	0.1	452	45x10^{-3}
F2	1	0.3	360	108x10^{-3}
	1	0.5	280	140x10^{-3}

F2 is gut from African catfish *C.gariepinus* that was fed with feed 2 while F4 is for those catfish fed with feed 4

highest CF 2.54±0.05 (P<0.05). There was no significant differences in the condition factors of the catfish fed with F2, F3, F4 and F5 (P>0.05). The fish fed with control diet had the lowest condition factor. The DFI was highest for the catfish fed with F1 and this was significantly different from all other diets (P<0.05). Conversely, DFI of the commercial diet was least of all treatment diets (P<0.05). The daily feed intake (DFI) of the catfish was reducing with reduction in inclusion of fishmeal in the diets. Similarly, fishmeal ratio (FMR) which measures the ratio of fishmeal needed to produce a gram of the fish was reducing significantly from F1 (1.18±0.05) to F4 (0.04±0.54) (P<0.05). The FMR of feed 5 was 0.0 because there was no fishmeal in the diets. The survival of the catfish was similar for all treatment diets except for feed F2 and feed F5. The survival of the catfish was high (Table 4).

The gut microbial communities of the catfish prior to the feeding experiment were different between catfish groups and gut sections analyzed. The foregut of the catfish to be fed with feed 2 (F2) had between $5x10^{-3}$ to $15x10^{-3}$ colony forming units per ml, (CFU/ml). The colonies increased with increasing inoculums volume (Table 5). Analysis of the streaked colonies by conventional biochemical tests showed that the colonies were dominated by *Staphylococcus aureus* and Gram -ve rod *Salmonella spp* (Table 6). The initial microbial communities in the foregut of the catfish to be fed with feed 4 (F4) had from $3.4x10^{-3}$ to $15x10^{-3}$ CFU /ml (Table 5). The CFU increased with increasing inoculums volume. Biochemical analysis of the colonies showed that the microbiome was dominated by the Gram -ve rod *Citrobacter freundii* and Gram +ve cocci *Staphylococcus aureus* (Table 6).

The midgut microbiome of the feed 2 (F2) catfishes had colonies between $30x10^{-3}$ to $115x10^{-3}$ CFU /ml. The dominant bacteria as defined by the biochemical tests are mainly the Gram -ve rod *Salmonella spp* followed by the Gram +ve straight rod *Bacillus subtilis.* The midgut of catfish to be fed with F4 had between $23.3x10^{-3}$ to $45x10^{-3}$ CFU/ml. Conversely the midgut microbiome of feed 4 groups of catfish had *Bacillus subtilis* as the main dominant bacteria followed by Gram +ve cocci *Staphylococcus aureus.* Results of analysis of the hindgut of the catfish to be fed with F2 showed that there were more colony forming units than in the midgut. There were bacteria in the hindgut of the F2 and F4 catfishes than in other gut sections. The hindgut microbiomes of F2 groups of fishes had $45x10^{-3}$ to $140x10^{-3}$ CFU/ml (Table 5), made up of a consortium of Gram –ve rod *Salmonella spp*, Gram -ve rod *Pseudomonas spp*, Gram +ve cocci *Staphylococcus aureus* and Gram –ve rod *Escherichia spp* (Table 6, 7). Catfish hindgut microbiomes within the F4 group had between $42x10^{-3}$ to $105x10^{-3}$ CFU/ml. The Gram -ve rod *Pseudomonas spp* was more abundant followed by Gram –ve rod *Escherichia spp* and *Bacillus subtilis.* There were more bacteria colony forming units for the F2 group than the F4 group.

The gut microbiomes of the Post feeding catfish showed reduction in CFU but increased bacteria diversity for the catfish fed with F2. The foregut of the F2 fed catfish had colonies numbering between $4x10^{-3}$ to $19x10^{-3}$ CFU/ml (Table 8). The Gram –ve rod *Citrobacter freundii* was the dominant species followed by *Staphylococcus aureus* and *Bacillus sp* (Tables 9, 10). The microbial composition of foregut microbiome of the catfish fed with feed F4 showed increased diversity in bacteria composition. The bacteria colonies of foregut of catfish fed with F4 were between $3x10^{-3}$ to$19x10^{-3}$ CFU/ml (Table 8). The dominant species were majorly the Gram –ve rod *Citrobacter freundii* and the some

Table 6. Results of Gram staining of streaked colonies of bacteria extracted from foregut, midgut and hindgut, after 48h of incubation and plausible organism's. (These results are from catfish before commencement of feeding experiment)

Fish sample	Dilution factor	Color	Gram stain colonies	Cell type	Shape	Cell Arrangement	Probable organism
Foregut	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
(F4)	0.1	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
	0.3	Red	-ve	Rod	Straight	Single	*C. freundii*
	0.5	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
Foregut	0.1	Yellow	+ve	Cocci	Circular	Cluster	*S. aureus*
(F2)	0.1	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
	0.3	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
	0.5	Brown	-ve	Rod	Straight	Single	*S. enteritidis*
Midgut	0.1	Yellow	+ve	Cocci	Circular	Cluster	*S. aureus*
(F4)	0.1	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.3	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.5	Cream	+ve	Rod	Straight	Single	*B.subtilis*
Midgut	0.1	Brown	-ve	Rod	Straight	Single	*S. enteritidis*
(F2)	0.1	Brown	-ve	Rod	Straight	Single	*S. enteritidis*
	0.3	Cream	-ve	Rod	Straight	Single	*S. enteritidis*
	0.5	Cream	+ve	Rod	Straight	Single	*B.subtilis*
Hindgut	0.1	Cream	-ve	Rod	Straight	Cluster	*P. aeruginosa*
(F4)	0.1	Cream	-ve	Rod	Straight	Single	*P. aeruginosa*
	0.3	Light red	-ve	Rod	Straight	Single	*E. coli*
	0.5	Cream	+ve	Rod	Straight	Single	*B.subtilis*
Hindgut	0.1	Brown	-ve	Rod	Straight	Single	*S.enteritidis*
(F2)	0.3	Cream	-ve	Rod	Straight	Single	*P. aeruginosa*
	0.5	Yellow	+ve	Coccci	Circular	Cluster	*S. aureus*
	0.5	Light red	-ve	Rod	Straight	Single	*E.coli*

Gram +ve cocci *Staphylococcus aureus* (Tables 9, 10). The midgut of the post-feeding catfish showed different composition from that the catfish pre-feeding. The microbiome of the midgut of African catfish fed with F2 had between 25.4x10^{-3} to 100x10^{-3} CFU/ml. The bacteria communities were dominated by almost same concentration of *Citrobacter freundii* and *Bacillus subtilis.* Similarly, the midgut of catfish fed with F4 had 19x10^{-3} to 30x10^{-3} CFU/ml. The biochemical analysis of the colonies showed that they comprised mainly of Gram +ve rod *Bacillus subtilis.* There was also some Gram-ve rod *Citrobacter freundii* (Tables 9, 10). The hindgut of the catfish had reduced CFU/ml compared to the midgut, for both the F2 and F4 fed catfishes. There were between 30x10^{-3} to 99.5x10^{-3} CFU/ml in the hindgut of catfish fed with F2 diets. The dominant flora were analyzed to be consortium of Gram –ve rod *Salmonella spp,* brown Gram –ve rod *Pseudomonas spp*, Gram +ve cocci *Staphylococcus aureus* which was more abundant and light red Gram –ve rod *Escherichia coli*. Hindgut microbiome of the catfish fed with F4 had 32x10^{-3} to 106x10^{-3} CFU/ml (Table 8). Conventional biochemical analyses showed that the dominant flora was the Gram –ve rod *Citrobacter freundii*. The microbiome was consortium of *Citrobacter freundii, Bacillus subtilis* and *Escherichia coli* (Tables 9, 10).

Discussions

African catfish *C. gariepinus* grew fast on the treatment diets substituting fishmeal with bambara nut meal. This is in line with findings of previous researches (Enyidi, 2012; Enyidi *et al.* 2017). An evaluation of the growth and nutritional performances of catfish fed with Feed 1, (F1, 65%FM:0%BNM), feed 4 (F4, 5%FM:45%BNM) and feed 2 (F2, 45%FM:5%BNM) suggests that African catfish does not need as much 45% fishmeal inclusion in its diet. Consequently F4 (45% BNM) fed catfish grew better than those fed with F2 (45% FM), proving that 45% FM inclusion was not necessary in African catfish *C. gariepinus* diets. This is in line with previous findings (Enyidi and Mgbenka, 2014). Complete substitution of FM produced lowest growth performances even with the *pro rata* inclusion of poultry by products. Poor growth effects of complete substitution of FM by plant proteins in catfish diets had been noted by (Imorou Toko, Fiogbe, & Kestemont, 2007; Enyidi & Mgbenka 2014, Enyidi *et al.*, 2017). Bambaranut is known to contain high amount of essential amino acids lysine, cystine and methionine (Dakora & Muofhe, 1995). Bambaranut meal is also high in carbohydrates (Minka & Bruneteau, 2000; Sirivongpaisal, 2008; Enyidi, 2012). Bambaranut has over 50% carbohydrates (Sirivongpaisal, 2008) and is estimated to contain about 30% neutral sugars identified as glucose and Galactose (Minka & Bruneteau, 2000). There are also oligosaccharides in the meal and bambara nut meal has very high oil absorbance of 1.30±0.06 ml g^{-1} (Sirivongpaisal, 2008). Nutritional value and availability makes BNM a good candidate for supplementing fishmeal in aquafeeds. Bambaranut meal inclusion at high percentage in these experimental diets did lead to reduced growth but not necessarily poor growth. Although there is about 50% carbohydrate in BNM, African catfish has been noted to utilize the carbohydrate in plant ingredients for growth (Ali & Jauncey, 2004). They reasons for this ability of the catfish has not been fully discovered or have not been given serious attention.

The microbial communities associated with African catfish could be reason for the ability of the catfish to utilize bambaranut in these diets even though it has high carbohydrate content. The results of this research exhibited that administration of the diets caused a

Table 7. Result of biochemical test of streaked colonies of bacteria extracted from foregut, midgut and hindgut, after 48h of incubation and plausible organism's. (These results are from catfish before commencement of feeding experiment)

	Dilution factor	Catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H_2S	Nitrase reductase	Methyl red	Voges Proskauer	Probable organism
Foregut	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
Section 1	0.1	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.3	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
	0.3	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
Foregut	0.1	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
Section 2	0.1	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.3	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.3	Positive	Negative	Negative	Negative		Negative	Positive	Positive	Positive	Positive	Negative	*S. enteritidis*
Midgut	0.1	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
Section 1	0.1	Positive	variable	Negative	Positive		Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.3	Positive	variable	Negative	Positive		Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.5	Positive	variable	Negative	Positive		Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
Midgut	0.1	Positive	Negative	Negative	Negative		Negative	Positive	Positive	Positive	Positive	Negative	*S. enteritidis*
Section 2	0.3	Positive	Negative	Negative	Negative		Negative	Positive	Positive	Positive	Positive	Negative	*S. enteritidis*
	0.5	Positive	Negative	Negative	Negative		Negative	Positive	Positive	Positive	Positive	Negative	*S. enteritidis*
	0.5	Positive	variable	Negative	Positive		Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
Hindgut	0.1	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative	*P. aeruginosa*
Section 1	0.3	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative	*P. aeruginosa*
	0.5	Positive	Negative	Positive	Negative	N/A	Negative	Positive	Negative	Positive	Positive	Negative	*E. coli*
	0.5	Positive	variable	Negative	Positive		Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
Hindgut	0.1	Positive	Negative	Negative	Negative		Negative	Positive	Positive	Positive	Positive	Negative	*S.enteritidis*
Section 2	0.3	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative	*P. aeruginosa*
	0.5	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.5	Positive	Negative	Positive	Negative	N/A	Negative	Positive	Negative	Positive	Positive	Negative	*E. coli*

change in the dominance of bacteria communities in the catfish gut. This is inline with previous findings of Ye *et al.* 2014 who noted significant difference in the microbiota of silver carp and gizzard shad due to their diets. In a more recent research Viana *et al.* (2018) also noted changes in the gut microbiota of carnivorous fish *Totoaba macdonaidi* fed extruded diets. There were differences in the pre feeding catfish microbiota for the foregut, the midgut and the hindgut. There were also similar differences in gut microbiota of the analysed catfishes from treatments F2 and F4. The foregut of F2 catfish shows a dominance of *Staphylococcus aureus* and few *Salmonella spp* while that of F4 catfish shows consortium of *Citrobacter freundii* and *Staphylococcus aureus.* The differences in the catfish microbiota could be due to the feed used (Lauzon *et al.*, 2010, Ye *et al.* 2014). The change in the microbial communities can as well be as result of feeding or together with the culture system. Some of the dominant microbiota in the catfish gut prior to administration of experimental diets lost dominance after the feeding experiment like *Staphyloccoccus aureus.* while others like *Citobacter freundii* and *Bacillus subtilis* continued in dominance of the microbiota of the African catfish. The feeding of the fish created changes due to treatment diets and their composition. The effects of diets on fish microbiota had been studied by several workers like (Muroga *et al.*, 1987; Tanasomwang & Muroga, 1988; Munro *et al.*, 1993; 1994; Bergh *et al.*, 1994; Bergh, 1995; Griez *et al.*, 1997, Merrifield *et al.*, 2011).

The feeding experiment produced specific changes in the catfish gut microbial communities. Bearing in mind that our diets varied in inclusion of fishmeal and bambara nut meal it seems plausible that the quantities of the animal protein and plant protein in the diets could have caused the changes in gut microbiota. Previously Dimitroglou *et al.* (2010), noted that fingerling sea bream fed soybean meal showed different microbiota compared to those fed fishmeal diets. The feed composition of F2 is opposite of F4 in terms of fishmeal and bambaranut meal content. While F2 contained 45% fishmeal and 5% bambaranut meal F4 contained 5% fishmeal and 45% bambaranut meal. Since bambaranut has about 50% carbohydrate the two feeds are at opposite ends of the feed formulation continuum. These feed compositions must have influenced the microbiota composition of the foregut, midgut and hindgut. Most digestion takes place in midgut and the consortium of bacteria dominant in African catfish midgut (both for F2 and F4 fed catfish) are known to be enzyme producing and celluloselytic and seems to be reason for African catfish known ability to utilize high carbohydrate diets. Carbohydrate composition of feed has been noted to influence gut microbiota (Gatesoupe *et al.*, 2014; Pedrotti *et al.* 2015; & Ringø *et al.*, 2016). It seems that the feed composition and the gut microbiomes changes could as well have contributed to the feed utilisation and growth performances of the fish. Nutritional composition and feed had been noted as pivotal in determining fish gut microbiome (Scott *et al.*, 2013; Geurden *et al.*, 2014; Ringø *et al.*, 2016). Consequently in the catfish fed with F2 the foregut was a consortium of *Citrobacter freundii*, *Staphylocchoccus aureus* and *Bacillus subtilis.* The foregut of catfish fed with F2 experimental diet was different from the pre fed microbiota. This could be due to the treatment feed effect. Similar microbiota changes were seen for foregut of catfish fed with F4. While the hindgut of the catfish fed F2 showed consortium of *Staphylococcus aureus aureus*, *Pseudomonas spp, Salmonella spp* and *E.coli*; that of F4 fed catfish were mainly a consortium of *Citrobacter freundii*, *Bacillus subtilis* and *E. Coli*. These

Table 8. Results of number of colonies of bacteria extracted from foregut, midgut and hindgut of African catfish *C. gariepinus* after 48hrs incubation These results are from catfish after feeding experiment

Fish sample	Inoculums Volume	Dilution Factor	No colonies	Total no of organism (CFU/ml)
Foregut	1	0.1	30	$3x10^{-3}$
F4	1	0.3	32	$9.6x10^{-3}$
	1	0.5	38	$19x10^{-3}$
Foregut	1	0.1	40	$4x10^{-3}$
F2	1	0.3	34	$10x10^{-3}$
	1	0.5	50	$25x10^{-3}$
Midgut	1	0.1	190	$19x10^{-3}$
F4	1	0.3	100	$30x10^{-3}$
	1	0.5	60	$30x10^{-3}$
Midgut	1	0.1	254	$25.4x10^{-3}$
F2	1	0.3	287	$86.1x10^{-3}$
	1	0.5	200	$100x10^{-3}$
Hindgut	1	0.1	326	$32x10^{-3}$
F4	1	0.3	300	$90x10^{-3}$
	1	0.5	212	$106x10^{-3}$
Hindgut	1	0.1	300	$30x10^{-3}$
F2	1	0.3	228	$68x10^{-3}$
	1	0.5	199	$99.5x10^{-3}$

F2 is the gut of fish fed with feed 2 and F4 is for gut of fish fed with feed 4

consortiums seem to be autochthonous and indigenous to the African catfish. Accordingly, Egerton *et al.*, (2018) noted that the autochthonous microbiota of fish is established from feed. These compositions could be based on the fishmeal of F2 and bambaranut meal of F4. The F4 fed catfish dominant bacteria were *Citrobacter freundii* and *Bacillus subtilis.* These have been identified previously as enzyme producing. In previous researches isolated bacteria from the gut of fish belonging to *Aeromona*s, *Enterobacter*, *Citrobacter*, *Bacillus*, and *Pseudomonas* were identified as the cellulolytic enzyme-producing bacterial community (Ray *et al.*, 2012; Li *et al.*, 2016). The growth effects of feed 4, F4 on the catfish could be attributed at least in part to the contribution of the microbiota. *Bacillus spp* and *Citrobacter freundii* are known to utilise carbohydrate. *Bacillus subtilis* has also being successfully used as first feed of African catfish (Enyidi & Onuoha, 2016). African catfish *Clarias gariepinus* has been known to utilize carbohydrate very well (Ali & Jauncey, 2004). There are no documented reasons so far, at least to the best of our knowledge, as to why the African catfish is so good in utilizing carbohydrates. Some researchers have noted that bacteria contribute in enhancing fish nutrient utilization.

In a previous experiment Wu *et al.*, (2012) noted that in foregut of grass carp, fed 3.0 $X10^{-9}$ probiotic *B. subtilis,* amylase activity increased significantly from 14 to 56 days. The author added that in same experiment of probiotic treatment amylase activity increased significantly in the midgut and hindgut from day 14 to 56 day when the grass carp were fed the probiotic diet. Probiotics have also been used in combination with beta glucans in fish feed Ringø, & Song (2015). The carbohydrate utilization effects of fish fed *Bacillus spp* probiotic was also suggested by Hamza, *et al.*, (2016). The authors fed sea bass lavae with rotifers *Brachionus plicatilis* enriched with *B. mojavensis* and a mixture of *B. mojavensis* and *Virgibacillus proomii* and noted improved amylase activity. A bacterium *Cetobacterium somerae* that inhabit the gut of some fishes like *Oreochromis niloticus* (Tsuchiya *et al.*, 2008) is known to produce vitamin B12 (cobalamin) in large quantities within gut of the fish. It is also present in other fishes like rainbow trout (Kim *et al.*, 2007, *Oreochromis niloticus*, Tsuchiya *et al.*, 2008, and zebrafish (Roeselers *et al.*, 2011). Consequently there is no nutrient requirement for vitamin B12 for *O. niloticus*. This may be because of the constant provision from the gut *Cetobacterium somerae* microbiota. The provision vitamin B12 through this source for fish has been suggested by Sugita *et al.* (1991). From all indications more bacteria were detected in the hindgut than the rest of the guts. This suggests the effects of fecal wastes and presence of bacteria associated with excretory products like *Staphylococcus aureus* and *E.coli* and *Salmonella* that seems to be allochthonous spp. in the catfish. In as much as we have detected these few bacteria in the African

Table 9. Results of Gram stain of streaked colonies of bacteria extracted from foregut, midgut and hindgut of African catfish *C. gariepinus* after 48hrs incubation. These results are from catfish after feeding experiment

Fish sample	Dilution factor	Colour	Gram stain colonies	Cell type	Shape	Cell Arrangement	Probable organism
Foregut	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
F4	0.1	Cream	+ve	Cocci	Circular	Cluster	*S.aureus*
	0.3	Red	-ve	Rod	Straight	Single	*C. freundii*
	0.3	Red	-ve	Rod	Straight	Single	*C. freundii*
Foregut	0.1	Red	-ve	Rod	Straight	Single	*C.freundii*
F2	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
	0.3	Cream	+ve	Cocci	Circular	Cluster	*S. aureus*
	0.3	Cream	+ve	Rod	Straight	Single	*B.subtilis*
Midgut	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
F4	0.1	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.3	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.3	Cream	+ve	Rod	Straight	Single	*B.subtilis*
Midgut	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
F2	0.1	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.3	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.3	Red	-ve	Rod	Straight	Single	*C. freundii*
Hindgut	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
F4	0.1	Red	-ve	Rod	Straight	Single	*C. freundii*
	0.3	Cream	+ve	Rod	Straight	Single	*B.subtilis*
	0.5	Light red	-ve	Rod	Straight	Single	*E. coli*
	0.5	Red	-ve	Rod	Straight	Single	*C. freundii*
Hindgut	0.1	Brown	-ve	Rod	Straight	Single	*S.enteritidis*
F2	0.1	Cream	-ve	Rod	Straight	Single	*P. aeruginosa*
	0.3	Yellow	+ve	Cocci	Circular	Cluster	*S.aureus*
	0.5	Cream	+ve	Cocci	Circular	Cluster	*S.aureus*
	0.5	Light red	-ve	Rod	Straight	Single	*E. coli*

F2 is the gut of fish fed with feed 2 and F4 is for gut of fish fed with feed 4

Table 10. Results of biochemical tests of streaked colonies of microbial communities in foregut, midgut and hindgut, after feeding experiment

	Dilution factor	Catalase	Oxidase	Indole	Citrate	Coagulase	Urease	Mannitol	H_2S	Nitrase reductase	Methyl red	Voges Proskauer	Probable organism
Foregut	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
F4	0.1	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.3	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
	0.3	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
Foregut	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
F2	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
	0.3	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.3	Positive	variable	Negative	Positive		Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
Midgut	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
F4	0.1	Positive	variable	Negative	Positive	N/A	Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.3	Positive	variable	Negative	Positive	N/A	Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.3	Positive	variable	Negative	Positive	N/A	Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
Midgut	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
F2	0.1	Positive	variable	Negative	Positive	N/A	Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.3	Positive	variable	Negative	Positive	N/A	Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.5	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
Hindgut	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
F4	0.1	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
	0.3	Positive	variable	Negative	Positive	N/A	Negative	Positive	N/A	Positive	Negative	Positive	*B.subtilis*
	0.5	Positive	Negative	Positive	Negative	N/A	Negative	Positive	Negative	Positive	Positive	Negative	*E. coli*
	0.5	Positive	Negative	Negative	Positive	N/A	Variable	Positive	Positive	Positive	Positive	Negative	*C. freundii*
Hindgut	0.1	Positive	Negative	Negative	Negative	N/A	Negative	Positive	Positive	Positive	Positive	Negative	*S.enteritidis*
F2	0.1	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative	*P. aeruginosa*
	0.3	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.5	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	*S. aureus*
	0.5	Positive	Negative	Positive	Negative	N/A	Negative	Positive	Negative	Positive	Positive	Negative	*E. coli*

F2 is the gut of fish fed with feed 2 and F4 is for gut of fish fed with feed 4

catfish, more would certainly be detected using more modern means. These findings would however serve as baseline knowledge to future results using molecular biology techniques. It's noteworthy that conventional means yields fewer bacteria compared to molecular biology techniques.

Conclusions

The African catfish *C. gariepinus* utilised the diets made from bambaranut meal substitution of fishmeal. *C. gariepinus* does not need high inclusion of fishmeal up to 45% with bambaranut meal substitution. There were notable differences in the gut microbiomes of African catfish fed the fishmeal substituted diets. It seems that performances of the fish were enhanced by the microbiota. The dominance of carbohydrate utilizing bacteria like *Bacillus spp* and *Citrobacter freundii* in the African catfish gut microbiome, seems to be responsible for the catfish ability to utilize high carbohydrate diets. Moreover, the dominant bacteria are celluloselytic and enzyme producing which could have aided the catfish utilization of bambaranut meal diets that has up to 50% carbohydrate. Feed and nutrition are pivotal in determining gut microbiota of African catfish. More researches are needed in these areas using molecular biology techniques.

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