



Role of Prebiotic, Probiotic and Symbiotic Diets on Bacterial proliferation in Feed and Intestine of African (*Clarias gariepinus*) Catfish

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ABSTRACT: The influence of prebiotic, probiotic and symbiotic diet on microbial proliferation was studied using an in vitro method. In the present trial, formulated diets were supplemented with prebiotic (*Sargassum muticum*), probiotic (*Parkia biglobosa*) and combination of *Parkia biglobosa* and *Sargassum muticum* (symbiotic diet). Bacteria proliferation in supplemented feeds, small and large intestine of the African catfish, *Clarias gariepinus* fed the formulated diets were evaluated. The feeding trial that lasted 12 weeks was conducted in plastic aquaria, with each treatment replicated three times. A control diet containing only the feed ingredients was also formulated and fed for the same period. The result showed bacteria proliferation was lowest in the control diet and highest in feed supplemented with prebiotic. Low bacteria proliferation was observed in the small intestine of fish fed symbiotic diet while highest proliferation was recorded in the fish fed prebiotic diet. The result also revealed the lowest bacteria proliferation in the large intestine of fish fed symbiotic diet and highest in fish fed probiotic diet. Using the cell morphology and biochemical characteristics of bacteria isolates in supplemented feed, *Clarias gariepinus* small and large intestine, the result indicated the presence of some Lactic Acid Bacteria (LAB) known to produce a variety of antimicrobial substances which are able to stop the development of foodborne diseases by inhibiting the growth of food spoilage and pathogenic organisms.

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Aquaculture is a vital means of employment, income, food, and recreation for humans around the world. Specifically, African countries have great influences for Fish rearing with 37% and 43% surface area for artisanal and commercial Fish rearing (Adewunmi and Olaleye, 2011). Though Africa adds approximately 2.7% to world aquaculture industry (Halwart, 2020) despite increased large scale investment in Egypt, Nigeria, Uganda and Ghana (FAO, 2018). This region had a twenty-fold increase in produce from 110,200 to 2,196,000 tons between 1995 to 2018 and

approximately 15.55% compound annual growth rate (CAGR) (FAO 2018, Halwart, 2020). Egypt, Nigeria and Uganda account for 90% of the region total aquaculture production (Soliman and Yacout, 2016). Table 1 shows the increase in aquaculture production in the region in tons. The major species reared in Nigeria include tilapias, African catfish and carp, but, the African catfish species (*Clarias gariepinus*) are more resistant and most accepted and highly valued fish that are reared in Nigeria (Orire and Sadiku, 2014). Fish is useful in diet of most Nigerians, with

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optimum nutritional value with complete wide range of amino acids, vitamins and minerals (Akinrotimi *et al.*, 2007), and it's a vital source of animal protein for both man and livestock in third world countries. Reports from FAO (2014) shows that fish contributes above 60% of the world's supply of protein, especially in third world countries. Despite these great potentials of natural resources and man-power availability to fish rearing in Nigeria, the country is currently unable to fill the gap in the short fall between total internal fish production and the total internal demand (Ozigbo *et al.*, 2013). Though aquaculture is the fastest growing food sector, diseases most especially bacterial infection tends to hold back the expansion of aquaculture (Pieters *et al.*, 2008; Abd El-rhman *et al.*, 2009). Bacterial, viral and other types of diseases are usually treated with antibiotics. A substantial dependence on the use of veterinary medicines has been on the increase of recent years. Application of antibiotics has led to a strong selection pressure on resistance on bacteria which adapted to this situation (Cabello, 2006; Yousefian and Amiri, 2009). The ban by regulatory bodies prohibiting the use of antibiotics in animal husbandry has made aquaprenuers seek for alternatives. The use of other measures like probiotics and prebiotics has been of great value as alternative therapy in fish culture, which appears to be strategic biological control and a necessary step for aquaculture practices for enhancing growth and disease resistance (Rombout *et al.*, 2010). Beneficial microbes manipulates the gut microbiota through dietary supplementation, a novel approach from both nutritional as well as immunological aspect. Application of Probiotics and Prebiotics in animal husbandry is in the increase to boost growth rate, decrease mortality, increase immune functions amongst others (Dimitroglou *et al.*, 2011). Hence, the objective of this study was to investigate the role of prebiotic, probiotic and symbiotic diets on bacterial proliferation in the feed and intestine of African (*Clarias gariepinus*) catfish.

MATERIALS AND METHODS

Experimental diets: The feed formulation had fishmeal serve as major source of protein whereas corn starch served as energy for all diets. Tapioca been the binder, other ingredients mineral premix and Vitamic C serving as mineral and vitamins, vegetable oil serving as lipid source. The ingredients were weighed (OHAUS) and mixed in appropriate proportions. Four experimental diets were formulated at varying percentage of inclusion of *S. muticum* (prebiotic 0.5%), *P. biglobosa* (probiotic 2%), symbiotic (prebiotic and probiotic 2.5%) and control with no inclusion. Adopted feed formulation calculated indicated for every 100g of feed contained

approximately 46% of cornstarch, 40% fishmeal, 7% vegetable oil, 4% mineral mix, 1% Vitamin C and 2% tapioca (binder).

Feeding, fish rearing condition: A total of 180 African Catfish (fingerlings) with average mean weight 2.53g was purchased from a commercial fish farm (Mallam Fisheries) at Minna and used for the study. Experimental tanks used for the study were twelve (12) 20L capacity plastic aquaria filled with water. The fish were stocked at a rate of 15 per tank using the 20L plastic aquaria. The aquaria were kept in a complete randomized design (CRD), fish were acclimated to the experimental facility conditions and fed control feed for one week. The fingerlings were fed at 5% body weight, twice a day at 7:00am and 5:00pm except o sampling days following the methods of Aliyu-Paiko *et al.*, (2010). The trial lasted for 12 weeks.

Microbial evaluation

Reagents and culture media: The culture media used were prepared based on the standard laboratory methods prescribed by Cheese Brough (2002) with little modification. The media and reagents used included Nutrient Agar (NA), Simon Citrate Agar (SCA), peptone water, oxidase reagent, glucose, sucrose, lactose, galactose, fructose, crystal violet, 80% alcohol, safranin, Kovac's reagent.

Isolation of bacteria: One gram (1g) of the sample was transferred into a test-tube differently, 9ml of Peptone water was added to obtain 1:10 dilution of sample. The samples were serially diluted 1ml of the serially diluted sample was poured onto a petri dish and the NA cooled to below 45°C was poured into the same petri dishes and allowed to gel and was incubated at 37°C for 24-48hours. After 24 hours, colonies were tallied and pure cultures was attained by repeated sub-culturing of the isolates on new media. Pure cultures were maintained on slants for further characterization and identification (Mohammed and Ijah, 2013).

Identification and characterization of probiotic bacteria:

Microbial isolates were characterized based on cell morphology and biochemical tests (Oyeleke and Manga, 2008; CheeseBrough, 2003). The biochemical tests included gram's staining, catalase test, coagulase test, oxidase test, citrate test, indole test, glucose, sucrose, lactose, galactose and fructose test. The microbial isolates were identified conventionally with the scheme of Bergey's manual of systematic Bacteriology (1984).

Data analysis: Results are presented as mean \pm SEM of three replicate determinations. Mean values for all monitored parameters were analyzed by one-way

analysis of variance (ANOVA). *P* values <0.05 were considered significant when compared by Turkey's test. All statistical analyses were carried out using SPSS software, version 21.

RESULTS AND DISCUSSION

Table 1 show the bacteria population of African catfish fed formulated diet supplemented with prebiotic, probiotic and symbiotic. Bacteria

proliferation was lowest in control diet and highest in feed supplemented with prebiotic. Low bacteria (2.17±1.11 cfu/mL × 10⁴) proliferation was observed in small intestine of the fish fed symbiotic diet and highest (6.26±1.16 cfu/mL× 10⁴) in fish fed prebiotic diet. The result also revealed the lowest bacteria proliferation in the large intestine of the fish fed symbiotic diet and highest in the large intestine of the fish fed probiotic diet.

Table 1: Bacteria population in formulated fish feed supplemented with prebiotic, probiotic and symbiotic and gut of African Catfish (*Clarias gariepinus*) fed the supplemented diets

Treatment	Feed (cfu/mL× 10 ⁴)	Small intestine (cfu/mL× 10 ⁴)	Large intestine (cfu/mL× 10 ⁴)
Control	3.46±1.16 ^a	5.46±0.58 ^b	5.46±0.26 ^b
Probiotic	5.86±0.58 ^a	6.26±1.16 ^b	7.06±0.53 ^c
Prebiotic	6.24±2.60 ^a	6.66±0.35 ^b	5.60±0.46 ^b
Symbiotic	5.28±5.61 ^a	2.17±1.11 ^a	5.13±5.44 ^a

Cell morphology and Biochemical characteristics of bacteria isolates from supplemented feed and intestines of African Catfish (Clarias gariepinus): Table 2 shows the result obtained during the identification and characterization of isolates from the formulated diet supplemented with prebiotic, probiotic and symbiotic. Cell morphology revealed most bacteria isolates were cocci or rod shape in both control and probiotic diet whereas, prebiotic diet were all cocci and symbiotic had rod shape bacteria. In the

gram reaction, most isolates were gram positive except for two isolates which are gram negative and were noted in the control and probiotic diets. Bacteria isolated in prebiotic and probiotic diet were all catalase positive, the control mostly had catalase positive bacteria and few catalase negative, and isolates were catalase negative in the symbiotic diet. Most isolates were coagulase negative across the diets with the exception of few which were coagulase positive.

Table 2: Cell morphology and biochemical characteristics of isolates from formulated feed

Treatment	Cell morphology	Gram reaction	Catalase	Coagulase	Citrate	Oxidase	Indole	Sugar Tests					
								Glucose	Galactose	Lactose	Sucrose	Fructose	
Ctrl A	Cocci	G+	+	+	+	-	-	+	+	+	+	+	AG
Ctrl B	Rod	G+	-	-	-	-	-	+	+	+	+	+	+
Ctrl C	Cocci	G-	+	-	+	-	-	-	-	-	+	+	-
Pro A	Rod	G-	+	-	+	-	-	+	+	+	+	+	+
Pro B	Cocci	G+	+	+	+	-	+	+	+	+	+	+	+
Pro C	Cocci(in chain)	G+	+	-	+	-	+	-	-	+	+	+	-
Pre A	Cocci	G+	+	+	+	-	+	+	+	+	+	+	+
Pre B	Cocci	G+	+	+	+	-	+	+	+	+	+	+	+
Pre C	Cocci(in chain)	G+	+	-	+	-	+	-	+	+	+	+	+
SymA	Rod	G+	-	-	-	+	-	+	+	+	+	+	+
SymB	Rod	G+	-	-	-	+	-	+	+	+	+	+	+
SymC	Rod	G+	-	-	-	+	-	+	+	+	+	+	+

Key-Ctrl: Control; **Pro:** Probiotic; **Pre:** Prebiotic; **Sym:** Symbiotic; **+**: Positive result; **-:** Negative result; **G+:** Gram positive; **G-:** Gram negative; **AG:** Acidic and Gas Production.

Bacteria isolates from control, prebiotic and probiotic diets reacted positive to citrate, but isolates reacted negatively in the symbiotic diet. Isolates of the control, prebiotic and probiotic diets showed no reaction to oxidase, but positive reaction was seen in isolates of the symbiotic diet. Most isolates showed no reaction for the indole test with the exception of few noted in the prebiotic and probiotic diets. On the other hand, glucose was positive in most of the isolates in

the diets with the exception of few noted in the control, prebiotic and probiotic diets. Most isolates were galactose positive in the diets, but few were observed to be galactose negative in the control and probiotic diet. Isolates were all noted to be lactose positive in the diets except in the control diet which lactose negative was observed. The isolates all reacted positively to sucrose in the diets down the table. However, most isolates were fructose positive with the

exception of few which gave acid and gas production in the control and negative in the probiotic.

Table 3: Cell morphology and biochemical characteristics of isolates of Small intestine of African Catfish (*Clarias gariepinus*)

Treatment	Cell morphology	Gram reaction	Catalase	Coagulase	Citrate	Oxidase	Indole	Sugar Tests				
								Glucose	Galactose	Lactose	Sucrose	Fructose
Ctrl A	Cocci	G+	+	+	+	-	-	+	+	+	+	AG
Ctrl B	Rod	G+	-	-	-	+	-	+	+	+	+	+
Ctrl C	Cocci	G-	+	-	+	-	-	-	-	-	+	-
Pro A	Rod	G-	+	-	+	-	-	+	+	+	+	+
Pro B	Cocci	G+	+	+	+	-	+	+	+	+	+	+
Pro C	Cocci(in chain)	G+	+	-	+	-	+	-	-	+	+	-
Pre A	Cocci	G+	+	+	+	-	+	+	+	+	+	+
Pre B	Cocci	G+	+	+	+	-	+	+	+	+	+	+
Pre C	Cocci(in chain)	G+	+	-	+	-	+	-	+	+	+	+
SymA	Rod	G+	-	-	-	+	-	+	+	+	+	+
SymB	Rod	G+	-	-	-	+	-	+	+	+	+	+
SymC	Rod	G+	-	-	-	+	-	+	+	+	+	+

Key-Ctrl: Control; **Pro:** Probiotic; **Pre:** Prebiotic; **Sym:** Symbiotic; **+**: Positive result; **-:** Negative result; **G+:** Gram positive; **G-:** Gram negative; **AG:** Acidic and Gas Production.

Table 3 reveals the result obtained during the identification and characterization of isolates in the small intestine of African catfish fed supplemented diets. Cell morphology revealed most bacteria isolates were in cocci or rod shape in small intestine of fish fed control and probiotic diet whereas, prebiotic were all cocci and symbiotic were all rod shape.

For the gram reaction, most isolates were gram positive except for two isolates which were gram negative and were noted in the small intestine of fish fed control and probiotic diets. Fish fed prebiotic and probiotic diets were all catalase positive, most were catalase positive in the control but not all whereas, isolates were all catalase negative in the symbiotic.

Most isolates in the small intestine were coagulase negative across the fed diets with the exception of few which were coagulase positive and were noted in the control, prebiotic and probiotic. Isolates were citrate positive in the fish fed control, prebiotic and probiotic diets but isolates were all negative in the small intestine of fish fed symbiotic diet.

Bacteria isolates were observed to be oxidase negative in the small intestine of fish fed control, prebiotic and probiotic diets but were all positive in symbiotic. Most isolates were indole negative with the exception of few noted in the small intestine of fish fed prebiotic and probiotic diets.

On the other hand, glucose was positive in most of the bacteria isolates in the small intestine of fish fed the diets with the exception of few noted in the control, prebiotic and probiotic. Most isolates in the small intestine of fish fed the diets reacted positively to

galactose but few were observed to be galactose negative in the control and probiotic. Isolates were all noted to be lactose positive in small intestine of fish fed the diets except in the control which lactose negative was observed. The isolates were all sucrose positive in the small intestine of fish fed the diets down the table.

However, most isolates were fructose positive with the exception of few which gave acid and gas production in the control and negative in the probiotic. Table 4 reveals the result obtained during the identification and characterization of isolates in the large intestine of African catfish fed supplemented diets. Cell morphology revealed most bacteria isolates were in cocci or rod shape in large intestine of fish fed control, prebiotic and symbiotic diet whereas, probiotic was all rod. The gram reaction were all gram positive in the large intestine of fish fed the diets. However, few isolates were observed to be catalase negative whereas most were positive in the large intestine of fish fed the diets. Isolates of the large intestine were mostly coagulase positive except for few noted in fish fed control, prebiotic and symbiotic diet. Whereas isolates were all citrate positive down the table. Most isolates gave negative reaction to oxidase in the large intestine with the exception of few observed in the control, prebiotic and probiotic. On the other hand, all isolates were indole negative.

However, most isolates gave up acid and gas production in the glucose test but negative reaction were noted in few. Acid and gas production was observed in galactose with few indicating positive and negative reaction observed in control, prebiotic and probiotic.

Table 4: Cell morphology and biochemical characteristics of isolates of Large intestine of African Catfish (*Clarias gariepinus*)

Treatment	Cell morphology	Gram reaction	Catalase	Coagulase	Citrate	Oxidase	Indole	Sugar Tests				
								Glucose	Galactose	Lactose	Sucrose	Fructose
Ctrl A	Cocci	G+	+	-	+	-	-	-	AG	AG	AG	AG
Ctrl B	Rod	G+	-	-	+	-	-	AG	AG	-	-	-
Ctrl C	Rod	G+	+	+	+	+	-	AG	+	AG	-	AG
Pro A	Rod	G+	-	+	+	+	-	AG	AG	AG	-	AG
Pro B	Rod	G+	-	+	+	-	-	AG	AG	AG	AG	AG
Pro C	Rod	G+	+	+	+	+	-	-	+	AG	AG	AG
Pre A	Cocci	G+	+	+	+	-	-	-	-	+	AG	AG
Pre B	Cocci	G+	+	-	+	+	-	AG	AG	AG	+	-
Pre C	Rod	G+	-	+	+	-	-	-	+	-	AG	+
SymA	Cocci	G+	+	+	+	-	-	AG	AG	AG	AG	AG
SymB	Rod	G+	-	-	+	-	-	AG	AG	AG	AG	AG
SymC	Cocci	G+	+	+	+	-	-	AG	AG	AG	AG	AG

Key-Ctrl: Control; Pro: Probiotic; Pre: Prebiotic; Sym: Symbiotic; +: Positive result; -: Negative result; G+ : Gram positive; G-: Gram negative; AG: Acidic and Gas Production.

Table 5: Identification of microbial colonies isolated per treatment.

Treatment	Feed	Small intestine	Large intestine
Control	<i>Staphylococcus and Bacillus</i>	<i>Staphylococcus and Bacillus</i>	<i>Staphylococcus and Bacillus</i>
Probiotic	<i>Bacillus, Enterococcus spp and Streptococcus spp</i>	<i>Bacillus, Enterococcus spp and Streptococcus spp</i>	<i>Lactobacillus and bacillus</i>
Prebiotic	<i>Enterococcus spp and Streptococcus spp</i>	<i>Enterococcus spp and Streptococcus spp</i>	<i>Streptococcus spp, Staphylococcus and Lactobacillus</i>
Symbiotic	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus and Streptococcus spp</i>

Lactose, sucrose and fructose all gave acid and gas production with the exception of few which gave a negative and positive reaction all observed in control and prebiotic. Table 6 shows the possible isolates identified in the fed and the intestine of fish fed supplemented diets. The table revealed *Bacillus* was in both the control and probiotic diet, *Enterococcus spp* and *Streptococcus spp* were observed in prebiotic and probiotic diet and *Lactobacillus* was noted in the symbiotic diet. Same was observed in the small intestine of fish fed supplemented diets. On the other hand, *Bacillus spp* was noted in large intestine of fish fed control and probiotic diets, *Staphylococcus* was observed in fish fed control and prebiotic diets. However, *Lactobacillus* was observed only in the large intestine of fish fed supplemented diets. Bacteria proliferation in this trial revealed more population in formulated diets supplemented with prebiotic, probiotic and symbiotic relative to the control. The highest was noted in prebiotic diet and lowest in control diet in terms of numerical values. Fish fed prebiotic and probiotic diets revealed high proliferation relative to the control, however low bacteria proliferation was observed in fish fed symbiotic diet relative to the control, this could be due to the incompatibility of the *Sargassum muticum* and *Parkia biglobosa* present in symbiotic diet. In the large intestine, more bacteria proliferation was recorded in

fish fed probiotic diet relative to the control, fish fed prebiotic and symbiotic diets had similar bacterial proliferation with control. In terms of numerical values, fish fed prebiotic and probiotic diets had better (higher) bacteria proliferation which agrees with the work of Balcázar, *et al.*, (2006). The probiotic bacteria which were identified in this study are *Enterococcus spp*, *Streptococcus spp*, *Lactobacillus* and *Bacillus*. The identified LABs are all proven to be useful to both humans and animals and generally regarded as safe except for *Enterococcus* which are recognized as pathogens for humans and animals (Mayo *et al.*, 2010; Pessione, 2012). Probiotic bacteria (LAB) which are proven to be gram positive non-sporulating, catalase-negative, aerotolerant, rod or coccus shaped organisms in nature in the work of Mokoena (2017). LAB are acid tolerant, devoid of cytochromes and are fastidious organisms which require a complex growth medium (amino acids, vitamins, nucleic acids and minerals components) (Mohankumar and Murugalatha, 2011). LAB produce a variety of antimicrobial substances such as acetic acid, ammonia, lactic acid, ethanol, bacteriocins, diacetyl and reuterin which are able to stop the development of foodborne diseases by inhibiting the growth of food spoilage and pathogenic organisms (Abo-Amer, 2011; Mahrous *et al.*, 2013). LAB have been used as probiotics to reduce intestinal disorders such as lactose intolerance, treatment of

diarrhea, relief from symptoms of constipation and activity against *Helicobacter pylori* responsible for chronic gastritis, peptic ulcers, and gastric cancer (Higashikawa *et al.*, 2010; Yang *et al.*, 2012). They are traditionally used as starter cultures for the fermentation of foods and drinks to improve the storage quality and nutritive value of perishable food such as meat, fish, milk, and vegetables (Halasz, 2009).

Conclusion: The results obtained in the current study shows that supplementation of fish diet with prebiotic, probiotic and symbiotic enhances bacteria proliferation in the gut of *C. gariepinus* and in turn enhances the growth of LABs known to produce a variety of antimicrobial substances which are able to stop the development of foodborne diseases by inhibiting the growth of food spoilage and pathogenic organisms.

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