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Original Article

Microbial load and diversity in the gastro-intestinal tract of cultured Nile tilapia (*Oreochromis niloticus*) and hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂) in Ilorin Ilorin Metropolis, Nigeria

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Abstract: This study investigated microbial load and diversity of gastro-intestinal tract of the cultured Nile tilapia (*Oreochromis niloticus*) and hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂) in Ilorin metropolis, Nigeria. A set of apparently healthy Nile Tilapia and hybrid catfish were obtained from a fish farm in Ilorin metropolis. After dissecting the fish sample aseptically, the entire alimentary canals of the specimens were divided into foregut, midgut and hindgut. Then bacterial isolates were characterised, following standard operating procedures for gram reaction, morphology, motility, catalase and oxidase reactions, citrate utilization, coagulase production, starch hydrolysis, sugar fermentation, and eventual identification of the resultant colonies. The moulds were examined based on their micro-morphology as well as the colour and micro-morphology of their sporulating structures and conidia. The results of the study revealed that microbes were present in the entire gastro-intestinal tract of cultured hybrid catfish and Nile tilapia with highest microbial load found in the hindgut of the two fish species under study. Also, larger number of bacteria diversity indices were found in the hindgut of cultured Nile tilapia, while the hindgut of cultured clariid catfish had higher fungi diversity indices.

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Introduction

Gut microbes perform a variety of nutritionally important functions. They improve nutritional intake and utilization. It is well-known that dietary fiber cannot be digested in the small intestine of fish as there are no endogenous enzyme which can do so. Dietary fiber and other complex polysaccharides are broken down by gut microbes producing short-chain fatty acid (SCFAs) and lower intestinal pH inhibits the growth of harmful bacteria producing toxins (Scott et al., 2008) and conversely, SCFAs serve as precursors to develop certain beneficial gut bacteria that could offer probiotic effects (Swennen et al., 2006; Ogueke et al., 2010). They enhance the growth of lactic acid bacteria and bifidobacterium (Gibson et al., 2004; Nugent, 2005), which improve a host's health (Gibson and Roberfroid, 1995) by promoting beneficial effects on glucose and lipid metabolism (Clements and Choat, 1995; Gray, 2006; Scott et al., 2008). Fish gut microflora varies with the nature, and complexity of the digestive tract (Cahill, 1990) and they could be autochthonous or allochthonous.

In addition, digestion and utilization of feed are enhanced by presence of gut-associated microbiota (Ghosh et al., 2002; Saha et al., 2006; Nayak, 2010). These microbes are known to play a complementary role of breaking down feed in fishes by producing exogenous enzymes that can degrade starch or cellulose (Bairagi et al., 2002; Saha et al., 2006; Banerjee et al., 2016) and chitin (Banerjee et al., 2015). Fish gut microflora have the capacity of using complex polysaccharides such as mannose, xylose, raffinose, and cellulose; but ordinarily endogenous enzymes lack this capacity (Kar and Ghosh, 2008; Ray et al., 2010). The microbes break down organic matter in complex polysaccharides for their survival. They can also denature anti-metabolite in feed, thereby improving feed utilization (Ghosh and Ray, 2017). In

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the same vein, gut microbes have been reported to have the capacity to degrade phytate; Das and Ghosh (2014) and Khan and Ghosh (2012) reported that phytase is present in yeasts isolated from the gastro-intestinal tracts of four major carps. Similarly, tannase-producing microbiota have been isolated from the gastro-intestinal tracts of some freshwater fish (Mandal and Ghosh, 2013a, b). Mondal et al. (2008) reported that fish gut provides a conducive environment for establishment of microbes owing to the large amount of nutrients therein. Bacteria have been reported to constitute the majority of gut colonizing microbiota (Ray et al., 2012).

Moreover, Banerjee and Ghosh (2014) and Das and Ghosh (2014) reported that yeasts are present in fish gut, and yeasts have commonly been isolated from fish gut (Gatesoupe, 2007; Moffitt and Mobin, 2006). Gatesoupe (2007) has reported that the presence of these gut yeasts stimulates the immune system of freshwater fish. Also, yeasts produce several compounds that have enormous organic values such as enzymes and immunostimulants (Chi et al., 2009). Hence, their presence in fish gut has a great value for the host (Romero et al., 2014). Gatesoupe et al. (2005a, b) isolated some fungi from freshwater fish, *Oncorhynchus tschawytscha* and *O. mykiss*, respectively. Genus *Candida* is prominent in the gut of rainbow trout (Gatesoupe et al., 2005a). Similarly, Jimoh et al. (2009a, b) isolated bacteria and fungi, respectively from different gut sections of the cultured and captured *Clarias gariepinus* sampled in Abeokuta, Nigeria. Also, microflora was isolated from different parts of the gastro-intestinal tract of tilapia and *C. gariepinus* obtained from the River Dandaru, Oyo State, Nigeria by Jimoh et al. (2013, 2014), respectively. Paucity of information still exists in exploring gut microbes of different cultured fish species. An attempt is made in this study to investigate the microbial load and diversity in the gut sections of two principally cultured fish species in Ilorin metropolis, Nile tilapia (*Oreochromis niloticus*) and hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂)

Materials and Methods

A set of apparently healthy Nile Tilapia and hybrid catfish were obtained from a fish farm in Ilorin metropolis. The entire alimentary canals of each of the specimens were divided into foregut, midgut and hindgut after the fish sample had been dissected aseptically. Prior to this, the working table was sterilized with absolute alcohol and an oven at 160°C was used to sterilize the glassware for 90 min.

Isolation and characterization of microflora: The different gut sections of each fish in a sterile bottle containing 5 ml sterile distilled water were shaken vigorously to allow the content separate into water. For fungi isolation, the method of Onions et al. (1981) was followed. Using freshly prepared Sabouraud Dextrose Agar medium (SDA), 0.1 ml of each suspension was pour plated, covered and gently swirled to evenly mix up, and it was allowed to gel after which the plates were put in the inoculating chamber for 3 to 4 days. The representative colonies developing from the plates were assembled according to their cultural characteristics, purified by recurrent sub-culturing and maintained on appropriate agar slants as stock culture. The moulds under study were examined on the basis of their micro-morphology as well as the colour and micro-morphology of their sporulating structures and conidia according to Onions et al. (1981). For bacteria isolation, the gastro-intestinal tracts (GITs) of each sample in sterile bottles containing 5 ml sterile distilled water were vigorously shaken to allow the content to detach in water. Using Nutrient Agar, 1 ml of the suspension was taken and serially diluted to 10^{-6}. Using serial dilution and pour plate method, microbial load, isolation and identification of microorganisms were done. After incubation at 37°C for 24 hours, representative colonies emerging from the plates were grouped according to their cultural characteristics, purified by recurrent sub-culturing and maintained on appropriate agar slants as stock cultures. Characterization of bacterial isolates followed standard operating procedures for gram reaction, morphology, motility, catalase and oxidase reactions, citrate utilization, coagulase production, starch hydrolysis and sugar

Table 1. Microbial load (log-CFU/g) of the different sections of gastro-intestinal tract of Nile tilapia (*Oreochromis niloticus*) in Ilorin metropolis.

	Microbial Load	
	Bacteria	Fungi
Foregut	5.89±1.29[b]	3.90±0.19[b]
Midgut	6.06±0.08[b]	4.15±0.22[b]
Hindgut	7.21±0.08[a]	5.00±0.28[a]

Column means with different superscripts were significantly different (P<0.05) from each other

Table 2. Microbial load (log-CFU/g) of different gut sections of the sampled hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis*♂) cultured in Ilorin metropolis.

	Bacteria	Fungi
Foregut	5.28±0.30[a]	3.31±0.15[a]
Midgut	5.46±0.28[a]	3.33±0.17[a]
Hindgut	5.48±0.23[a]	3.38±0.10[a]

Column means with different superscripts were significantly different (P<0.05) from each other

fermentation (Claus, 1992; Harrigan and McCance, 1976; Seeley Jr and VanDemark, 1962). The criteria of Holt et al. (1994) were used in identification of the resultant colonies.

Microbial Count: Counting of the bacteria colonies, which evolved after incubation, was expressed in Colony Forming Unit (CFU)/g. The total fungal counts were expressed as spore/g.

Diversity Study: The following diversity indices were employed for diversity study of the different sections and the entire gastro-intestinal tract of cultured Nile tilapia and hybrid catfish sampled from farms in Ilorin metropolis

$$\text{Shannon Weiner Index (H)} = -\sum_{i=1}^{s} \text{PlnP}$$

$$\text{Simpson Dominance Index } (1 - \text{D}) = 1 - \text{D}$$

Where $\text{D} = \sum_{i=1}^{s} \text{P}^2$.

$$\text{Margalef Richness Index} = {}^{(\text{S}-1)}/_{\ln \text{N}}$$

Where S = total number of species and N = total number of items in the sample.

Statistical Analysis: Data obtained from microbial count were transformed using logarithmic transformation (log CFU), expressed as mean ± SD before subjecting them to one-way analysis of variance using SPSS version 17.0. Duncan Multiple Range Test was used to separate the means where significant difference (P<0.05) existed among the treatment means.

Results

Microbial Load Analysis: Table 1 shows the microbial load in the different sections of the fish sampled. There were significant variations (P<0.05) in the microbial load of the gastro-intestinal tract of Nile tilapia in Ilorin metropolis with hindgut having significantly (P<0.05) higher bacteria and fungi load than other sections of the gastro-intestinal tract. The fungi and bacteria load of foregut and midgut of the sampled Nile tilapia were not significantly different (P>0.05). Table 2 shows the microbial load (log-CFU/g) of different gut sections of sampled hybrid catfish cultured in Ilorin metropolis. The hindgut had the highest bacteria load which was not significantly different (P>0.05) from other sections of the gastro-intestinal tract of hybrid catfish.

Microbial Diversity: Microbial occurrence, distribution, and diversity of the gastro-intestinal tract of Nile tilapia is presented in Table 3. The hindgut of Nile tilapia was colonized by a variety of micro-organisms. It had the highest value of diversity indices, while the midgut had the lowest diversity. Table 4 shows the occurrence, distribution and diversity indices of the different gut sections and the entire gastro-intestinal tract of cultured hybrid catfish. The hindgut harbored a larger number of micro-organisms. The diversity indices increased from the foregut to hindgut

Table 5 reveals the bacteria and fungi diversity indices of different sections and the entire gastro-intestinal tract of Nile tilapia. Comparatively, the bacteria were more diverse than the fungi in the foregut, midgut, and hindgut. Using Margalef's richness as index of assessment, bacteria were more

Table 3. Microbial occurrence, distribution and diversity of the different sections and the entire gastro-intestinal tract of Nile tilapia (*Oreochromis niloticus*) in Ilorin metropolis

Microbial Isolates	Foregut	Mid Gut	Hindgut	GIT
Enterococcus faecalis	1(0.20)	0(0.00)	1(0.11)	2(0.11)
Kleibsiella oxytoca	0(0.00)	0(0.00)	1(0.11)	1(0.06)
Citrobacter freundii	0(0.00)	0(0.00)	1(0.11)	1(0.06)
Escherichia coli	1(0.20)	1(0.25)	1(0.11)	3(0.17)
Shigella flexneri	1(0.20)	1(0.25)	1(0.11)	3(0.17)
Aspergillus flavus	0(0.00)	1(0.25)	1(0.11)	2(0.11)
Aspergillus niger	0(0.00)	1(0.25)	1(0.11)	2(0.11)
Penicillum notatum	1(0.20)	0(0.00)	1(0.11)	2(0.11)
Aspergillus fumigatus	1(0.20)	0(0.00)	1(0.11)	2(0.11)
Diversity indices				
Simpson Dominance Index (1-D)	0.80	0.75	0.89	0.88
Shannon-Weiner Index (H)	1.61	1.39	2.20	2.14
Margalef's richness	1.82	1.37	8.54	7.74

Values in parenthesis are proportion.

Table 4. Microbial occurence, distribution and diversity indices of the different sections and the entire GIT of cultured hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂) from Ilorin metropolis

Microbial Isolates	Foregut	Midgut	Hindgut	GIT
Proteus vulgaris	0(0.00)	0(0.00)	1(0.14)	1(0.06)
Bacillus substilis	0(0.00)	0(0.00)	1(0.14)	1(0.06)
Streptococcus faecalis	1(0.25)	1(0.20)	0(0.00)	2(0.13)
Enterobacter aerogenes	1(0.25)	1(0.20)	1(0.14)	3(0.19)
Aspergillus flavus	1(0.25)	1(0.20)	1(0.14)	3(0.19)
Aspergillus niger	0(0.00)	1(0.20)	1(0.14)	2(0.13)
Saccharomyces cerevisae	1(0.25)	1(0.20)	1(0.14)	3(0.19)
Penicillium citrinum	0(0.00)	0(0.00)	1(0.14)	1(0.06)
Diversity Indices				
Simpson Dominance (1-D)	0.75	0.80	0.86	0.85
Shannon-Weiner Index(H)	1.39	1.61	1.95	1.98
Margalef's richness	2.16	2.49	3.08	5.41

Values in parenthesis are proportion.

Table 5. Taxa differential diversity indices of the gastro-intestinal tract of Nile tilapia (*Oreochromis niloticus*) in Ilorin metropolis.

	Foregut		Midgut		Hindgut		GIT	
Diversity Indices	B	F	B	F	B	F	B	F
Simpson Dominance (1-D)	0.67	0.50	0.50	0.50	0.50	0.50	0.50	0.75
Shannon-Weiner Index	1.10	1.39	0.69	0.69	0.69	0.69	0.72	1.39
Margalef's richness	1.24	0.72	1.38	1.28	4.38	2.16	9.38	7.28

GIT: Gastro-intestinal tract B: Bacteria F: Fungi

diverse than fungi in the entire gastro-intestinal tract of Nile tilapia. Bacteria and fungi were equally distributed in the midgut, using Simpson's dominance and Shannon-Weiner (H) as indices of assessment.

Table 6 shows the taxa differential diversity indices of the microbes in different sections and the entire gastro-intestinal tract of cultured hybrid catfish. Comparatively, fungi were more diverse than bacteria in the mid and hind guts, and the entire gastro-intestinal tract of the fish, using Simpson dominance, Shannon-Weiner (H) and Maargalef's richness as indices.

Discussions

The microbial community in the gastro-intestinal tract of fish provides a synergistic role in a host's health, nutrition and development (Romero et al., 2014). The microbial load in cultured Nile tilapia and hybrid catfish in Ilorin metropolis revealed a differential

Table 6. Taxa differential diversity indices of the different sections and the entire gastro-intestinal tract of cultured hybrid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂).

	Foregut		Midgut		Hindgut		GIT	
Diversity Indices	B	F	B	F	B	F	B	F
Simpson Dominance (1-D)	0.50	0.50	0.50	0.67	0.67	0.75	0.69	0.72
Shannon-Weiner Index(H)	0.69	0.69	0.69	1.10	1.10	1.39	1.28	1.31
Margalef's richness	1.44	1.44	1.44	2.09	1.82	2.16	3.08	3.64

GIT: Gastro-intestinal tract B: Bacteria F: Fungi

increment from foregut to hindgut. Comparative assessment of the microbial loads of the two fishes under studies showed that cultured Nile tilapia had higher microbial load than hybrid catfish. Our findings are in tandem with Huber et al. (2004) who observed that bacteria loads vary from fish to fish and across locations. The highest microbial load was found in the hindgut of both fishes in this study, which supports the report of Mountfort et al. (2002) that hindgut harbors larger numbers of microbial community that contribute a great to the energy need of the host fish. Short-chain fatty acid (SCFA) produced by the activities of these microbes have beneficial effect on lipid and glucose metabolism that serve as a source of energy to the host (Gray, 2006; Scott et al., 2008).

The isolated gut microbes of both fish types in this study closely related to what was reported by Jimoh et al. (2009a, b) from captured and cultured *C. gariepinus* sampled in Abeokuta North Local Government, Nigeria. Similarly, Jimoh et al. (2013) isolated *Aspergillus flavus* and *A. niger* from Nile tilapia caught from River Dandaru, Ibadan and some bacteria isolates similar to what is observed in the present study. *Enterobacter* and *Bacillus* spp. observed in this study are in consonance with the report of Jimoh et al. (2014) on microbial flora of the gastro-intestinal tract of *C. gariepinus* caught from River Dandaru Ibadan, Nigeria.

The results of the present study also reveal that most of the isolates were found in the hindgut sections of GIT as evidenced in the measurement of diversity indices. The foregut and the midgut having lower diversity indices could plausibly be because of pancreatic, bile and acid secretion into the stomach, which could inhibit colonization of these sections by microflora (Guarner and Malagelada, 2003; Jimoh et al, 2014). Anaerobes and facultative anaerobes were found in large number in GIT of fish (Bairagi et al., 2002; Saha et al, 2006). *Bacillus* spp., *Citrobacter* spp. and *Entrobacter* spp. were isolated from the gut sections of three Indian major carp (Ray et al., 2010), which are known to be enzyme-producing bacteria in fish gut. Kar et al. (2008) isolated *B. subtilis* similar to what was observed in this study. Anerobic bacteria such as *Escherichia coli* and *Klebsiella* isolated in the fish gut regions have been reported to contribute a lot to fish nutrition (Clements et al., 2009); they are known to produce amylase (Ray et al., 2007), lipase and glycosidase (Ramirez and Dixon, 2003). The gram negative type among the isolated bacteria, such as *Proteus vulgaris, E. aerogenes, Shigella* and *E. coli*, in this study have enzymes that can digest complex carbohydrates (Ray et al., 2012). They are known to produce short chain fatty acids (SCFAs) mainly acetate, propionate and butyrate (Clements and Choat, 1995; Seeto et al., 1996; Clements, 1997; Stevens and Hume, 2004). Mountfort et al. (2002) reported that an important energy source for fish is the acetate produced by microbial fermentation to fish. *Bacillus subtilis* and *Enterobacter* spp. observed in this study, isolated from GIT of Atlantic salmon and gray mullet respectively, have been reported to produce chitinase (Hamid et al., 1979; Askarian et al., 2012).

Bacteria were more diverse in GIT of Nile tilapia, which is in line with the report of Pond et al. (2006) and Nayak (2010). However, the concentration of bacteria was lower than that of yeast in the GIT of cultured hybrid catfish in this study. This variation might be attributed to the food habit of the two fishes which is known to vary with nature and complexity of gastro-intestinal tract (Cahill, 1990). Higher concentration of the yeast in the gut was observed by Yoshimizu (1980), who found larger number of yeasts in fish intestinal microflora. Andlid et al. (1995)

reported that rainbow trout GIT could contain up to $3x10^6$ yeast cell per gram of intestinal tissue. The values of yeast count recorded in this study was much lower. Andlid et al. (1995) and Sakata et al. (1993) have similar reports to the yeast load found in this study (3.31-5.00 log-CFU/g) for the two fish species under study. A range of 2-4 and 5-7 log-CFU/g was reported by Andlid et al. (1995) and Sakata et al. (1993) as naturally occurring yeast load in the gastro-intestinal tract of freshwater farmed *O. mykiss*. 0-2 log-CFU/g of yeast load was reported by Gatesoupe et al. (2005a) for freshwater farmed *O. mykiss*. Other researchers have reported 0-4 log-CFU/g of yeast load in the same freshwater farmed *O. mykiss* (Gatesoupe et al., 2005b; Waché et al, 2006). Moffitt and Mobin (2006) reported the same value of 0-4 log-CFU/g of yeast load as naturally occurring in freshwater farmed *O. tschawytscha*. Higher yeast concentration observed in this study might be as a result of lower concentration of yeast used as additive in commercial fish feed (Tovar et al., 2002). Tovar-Ramırez et al. (2004) reported that dietary yeast improved gut maturation in sea bass (*Dicentrachus labrax*) larvae. It has immunostimulatory and immunomodulatory effect (Waché et al., 2006; Kutty and Philip, 2008; Song et al., 2010). Tannase activity of *Candida* spp. was reported by Mandal and Ghosh (2013a). *Saccharomyces cerevisae* in fish gut have been identified to stimulate enzyme activities (Waché et al., 2006).

Conclusion

It is evident from the report above that microbes can be found in the entire gastro-intestinal tract of cultured hybrid catfish and Nile tilapia, with the highest microbial load found in the hindgut of the two fish species under study. Larger number of bacteria diversity indices were found in the hindgut of cultured Nile tilapia, while the hindgut of cultured hybrid catfish had higher fungi diversity indices.

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