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BACTERIA ASSOCIATED WITH *Clarias gariepinus* and *Heteroclarias* (BURCHELL, 1822) FINGERLING FED *Musca domestica* LARVA

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ABSTRACT

In Nigeria, with the present economic depression, cost of fish diets is increasing daily not just because of inflation but because most of the feedstuff used in preparing fish diets face serious competition from man as food. Live food organisms are important for the growth of fish during culture especially at their early stages of development (fry and fingerling stages). Bacteria on the other hand, are responsible for many fish diseases especially those associated with environmental stresses such as poor handling. This study therefore examined the bacteria associated with *Clarias gariepinus* and *Heteroclarias* fingerlings fed with *Musca domestica* larva. Eighty fingerlings of *C. gariepinus* of an average weight of 2.3gm were obtained from a hatchery in Abeokuta Ogun State. Droppings of poultry (100%) were collected in jute bag from University of Agriculture, Abeokuta, Ogun State, Nigeria, poultry farm for maggot production. The harvested larva were then fed to *C. gariepinus* fingerlings. The microbiological analysis was then carried out on the fingerlings fortnightly. The results revealed the presence of micro-organisms in the fish species examined. 41 samples (76%) were affected with bacteria while 13 samples (24%) had no growth. The species of bacteria present in the fish were *Enterobacteria species*, *Escherichia coli and Proteus species.* These microorganisms are either of pathogenic, food poisoning, food spoilage or of epidemiological importance.

Keywords: **Bacteria, *Clarias gariepinus*, *Heteroclarias* fingerlings, *Musca domestica* Larva and Fish Diseases.**

INTRODUCTION

According to Olufemi (1998), bacteria are responsible for many fish diseases especially those associated with environmental stresses such as poor handling.

They are always present in water and they almost always occur in small or large numbers, on skin or inside healthy fish. Representative of 25 bacteria genera have been implicated as pathogens of freshwater and/or marine fish (Austin and Austin, 1985). Olufemi *et al*., (1991) isolated eight different bacterial organisms, each originating from a different case, from the African catfish *Clarias gariepinus*, in tropical fresh water earthen ponds in Ibadan. Ogbondeminu and Okaeme (1986), Fasanya *et al.,* (1988) and Ibiwoye *et al.,* (1989), found that most of the bacterial microflora associated with the skin of Clarias and Tilapia species were Gram negative rods and cocci- usually *Escherichia* and *Citrobacter*. The Gram positive cocci were mainly *Staphylococcus* and *Corynebacterium* and *Bacillus* species. Thus, a considerable number of bacterial diseases of fresh water fish have been recognized in Nigeria.

Solomon *et al.,* (1996) revealed that in Nigeria, with the present economic depression, cost of fish diets is increasing daily not just because of inflation but because most of the feedstuff used in preparing fish diets face serious competition from man as food. Some of such feedstuff includes soyabean, maize, sorghum and groundnut cake. This increasingly high demand for these feedstuff by man and consequently the high prices calls for the attention of fish nutritionist the world over to search deeper into the environment for less competitive feedstuffs that will cost little or nothing for inclusion in fish diets. Since fish farming like any other business is aimed at profit maximization, all efforts aimed at reducing cost of feeding fish will be highly appreciated. In nutrition studies, priority is always given to protein requirement: because protein is the single nutrient that is required in the largest quantity for growth (Lovell, 1981).

According to Ugwumba and Abumoye (1998) live food organisms are important for the growth of fish during culture especially at their early stages of development (fry and fingerling stages). Such live organisms are largely plankton, insects and worms. Their use increase the rate of survival of fish during these critical stages because they contain more protein required for growth, low metabolizable energy which cannot develop into fat and they are easily digested by fish and they reduce the rate of infection.

According to Furman *et al.,* (1959) insects, especially various fly larvae (maggots) and beetles readily feed on fresh manure, converting residual protein

and other nutrients into biomass which is a high quality animal feed stuff. While, incorporating and concentrating nutrient concentration and bulk of the manure residue thus reducing pollution potential 50-60% or more. Because of its much higher value, this feedstuff can be economically hauled significant distance to relieve local nutrients overload. Also, while occupying the manure, the insects aerate and dry it, reducing odours. Maggots modify the micro flora of manure, potentially reducing harmful bacteria. The high valve insect feedstuff, reduction of the manure mass, moisture content, offensive odour and pollution potential are the rewards for good management of such a system. Insect digestion of manure most of the time relates to maggot production. High reproductive and growth rates makes flies (maggots in the larvae growth stage) the best candidates. Flies that have been used experimentally to produce manure include house flies (*Musca domestica*), face flies (*Musca autumunalies*), Blow flies (*Musca autumunalies*) blow flies (Usually *sarcophaga sp.*) and the black soldier flies (*Hermetia illucens*). Except for the black soldier fly (Furman et al., *Loc. cit.*), all of these are considered pests as adult due to their disease vector potential, behaviour and preferred habitats. House Fly common name for the most familiar species of nonbiting muscoid fly found in the vicinity of human habitations throughout the world; is often a carrier of such diseases as typhoid fever, cholera, dysentery, trachoma, and anthrax. The adult fly transmits disease by contaminating food with disease organisms it has picked up on its hairy legs or has ingested and then regurgitated. The taste-sensitive cells of the common house fly are located on its feet as well as on its mouthparts. The female lays an average of 150 white eggs in a mass about 1 mm (about 0.04 in) long. The eggs are laid in horse manure or other decaying substances. The female lives about two and one-half months and lays between 600 and 1000 eggs during its lifetime. The eggs hatch in about 12 hours into white, legless larvae called maggots, which grow to 12.5 mm (0.5 in) in length. The maggot pupates in five to six days. The new adult emerges in another four to five days if the weather is warm or in a month or later if weather conditions are unfavorable. On the average, 12 generations of house flies are produced in one year. The house fly belongs to the family Muscidae. It is classified as *Musca domestica* (Microsoft® Encarta® Encyclopedia, 2002).

This study therefore examined the bacteria associated with *Clarias gariepinus* fingerling fed with *Musca domestica* larva.

Figure 1: Pictorial Diagram of an House Fly

MATERIALS AND METHODS

Eighty fingerlings of *C. gariepinus* and *Heteroclarias* of an average weight of 2.3g were obtained from a hatchery in Abeokuta Ogun State. These were stocked randomly at the rate of 10 fingerlings per tank which was filled with 20 litres of water with an acclimation period of 3 days. The experimental diets consisted of a compounded artificial diet of 35% crude protein which served as control diet.

Droppings of poultry (100%) were collected in jute bag from University of Agriculture, Abeokuta, Ogun State, Nigeria, poultry farm. This container was placed in a cool dry place which was found to be dominated by house fly (*Musca domestica)*. The container was moistened with water to prevent drying and exposed for 2 days to allow for *Musca domestica* adults to lay eggs on them. They were then covered and left for between 3 days and 5 days to allow larva to be fully grown before harvesting. The harvested larva were then fed to *C. gariepinus* and *Heteroclarias* fingerlings. The fish were fed to satiation twice daily between the hours of 7.00 am – 8.00 am and 4.00 pm - 6.00 pm.

The microbiological analysis was then carried out on the fingerlings fortnightly. Sterile swab sticks were used to collect swabs from the skin, gill and intestine of the fingerlings (for fingerlings fed *Musca domestica* larva and compounded ration separately). These swab sticks were then packed in aluminum foil and taken to Federal Medical Centre, Abeokuta, Pathological Laboratory for bacteriological examination where the following tests were carried out; gram's reaction, cultural morphology, catalase and coagulase production, citrate utilization, indole production and sugar fermentation (Singleton, 1999). The whole procedure was then repeated three times for validation.

RESULTS

The species of bacteria that were isolated from each part (intestine, mouth, skin) of *Clarias gariepinus* is show in Table 1. Table 2 shows the species of bacteria that were isolated from each part (intestine, mouth, skin) of *Heteroclarias*. Table 3 shows the frequency of occurrence and percentage of isolates in fish samples. Table 4 shows the frequency of occurrence of bacteria in both *Clarias gariepinus* and *Heteroclarias.* Table 5 shows frequency of occurrence of bacteria species in *Clarias gariepinus.* Table 6 shows frequency of occurrence of bacteria in *Heteroclarias.* The morphological and biochemical characteristics of bacteria isolated from fish samples fed with live maggots are shown on Table 7.

Table 1: Bacteria Species Isolated from *Clarias gariepinus*

Sample	Treatments								
	Intestine			Mouth			Skin		
	1	2	3	1	2	3	1	2	3
1	*Entero bacteria sp.*	NG	*Proteus sp.*	*Proteus sp.*	*Entero bacteria sp.*	NG	*Entero bacteria sp*	*Entero bacteria sp*	*Entero bacteria sp.*
2	NG	*E. coli*	*E. coli*	*Proteus sp.*	NG	NG	*Proteus sp.*	*Proteus sp.*	*Proteus sp.*
3	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*Proteus sp.*	NG	*E. coli*	N G	*E. coli*

Key: NG = No growth

Table 2: Bacteria Species Isolated from *Heteroclarias*

Sample	Treatments								
	Intestine			Mouth			Skin		
	1	2	3	1	2	3	1	2	3
1	*Proteus Sp.*	NG	*E. coli*	NG	NG	*E. coli*	*E. coli*	*E. coli*	*E. coli*
2	*E. coli*	*Entero bacteria Sp.*	*E. coli*	*E. coli*	*E. coli*	*E. coli*	*Proteus Sp.*	*Proteus Sp.*	*Proteus Sp.*
3	*Proteus Sp.*	*E. coli*	*Proteus Sp.*	NG	NG	NG	*E. coli*	*Proteus Sp.*	*Proteus Sp.*

Table 3: Frequency of Occurrence and Percentage Microbial Growth in Fish Samples

Samples	*Clarias gariepinus*		*Heteroclarias*	
	Occurrence	%	Occurrence	%
Bacteria growth	20	74	21	78
No growth	7	26	6	22
Total	27	100	27	100

Table 4: Frequency of Occurrence of Bacteria Species in Both *Clarias gariepinus* and *Heteroclarias*

Bacteria Species	Frequency of Occurrence	%
Enterobacteria sp.	6	15
Escherichia coli	19	46
Proteus sp.	16	39
Total	**41**	**100**

Table 5: Frequency of Occurrence of Bacteria in *Clarias gariepinus*

Bacteria Species	Frequency of Occurrence	%
Enterobacteria sp.	5	25
Escherichia coli	7	35
Proteus sp.	8	40
Total	**20**	**100**

Table 6: Frequency of Occurrence of Bacterial Species in *Heteroclarias*

Bacteria Species	Frequency of Occurrence	%
Enterobacteria sp.	8	38
Escherichia coli	12	57
Proteus sp.	1	5
Total	**21**	**100**

Table 7:- Morphological and Biochemical Characteristics of Bacteria Isolated from Fish Samples Fed Live Maggots

Test	**Sample Numbers**								
Morphology characteristics for all samples isolates.	**Intestine**		**Mouth**			**Skin**			
	1	2	3	1	2	3	1	2	3
Nutrient Agar	**Transparent and swarming**	**Smooth and Translucent**	**Not pigmented**	**Transparent and swarming**	**Smooth and Translucent**	**Appeared mucoid**	**Transparent and swarming**	**Appeared mucoid**	**Mucoid**
Mac Conkey Agar	Swarming with irregular	Pinkish colonies	Pink colonies	Swarming pink and irregular	Pink colonies	Pink mucoid	Swarming with irregular	Mucoid and pinkish appearance	Pink mucoid slimy
Gram staining	-	-	-	-	-	-	-	-	-
Motility test	+	-	-	+	+	-	+	-	-
Spore test	-	-	-	-	-	-	-	-	-
Citrate Test	+	-	+	+	-	+	+	-	+
Gelating Hydrolysis	-	-	-	+	-	-	-	-	-
Lactose	-	-	+	-	+	-	-	A	+
Glucose	+	-	+	+	+	+	+	A	+
Manitol	-	-	+	-	+	-	-	A	+
Urea Hydrolysis	+	+	+	-	-	+	+	+	+
Nitrate Reduction Test	+	+	+	-	+	+	+	+	+
MethylRed Test	-	-	-	-	+	-	-	-	-
Optimum Temperature	37^0C	37^0C	37^0C	37^0C	37^0C	37^0C	37^0C	37^0C	37^0C
Coliform Isolated	*Proteus sp*	*E.coli*	Entero bacteria.	*Proteus sp.*	*E.coli*	Entero bacteria	*Proteus sp.*	*E. coli*	Entero bacteria

DISCUSSION

The results revealed the presence of micro-organisms in the fish species examined, the number of samples collected for the experiment was 54 samples out of which 41 samples (76%) were affected with bacteria while 13 samples (24%) had no growth. This high level of microbial growth in the samples indicates the level of microbial proliferation of the environment given their ubiquitous nature (Kerter et al., 1995). The miniature natures of microbes make their existence in an environment unsuspected but nature would have been difficult if it were not for the microbes. Micro-organisms recycle nutrients and elements such as carbon, nitrogen, phosphorus and sulphur in nature (Roberts, 1981).

The nature of activities carried out on the land can affect the transmission of pathogenic organisms. This is revealed by the study made to calculate the total number of microbes present in environment where intensive movements were been made (Rahner and Tieraerztliche, 1995). Hence pathogenic intestinal microbes found in dry faeces on the soil can be raised in dust when tread upon by man and their spores setting on clothes of man and animal hairs.

27 samples of *C. gariepinus* were isolated out of which 20 samples (74%) had growth and 7 samples (26%) had no growth. Also in *Heteroclarias*, out of 27 samples isolated 21 samples (78%) had growth while 6 samples (22%) had no growth. The species of bacteria present in the fish were *Enterobacteria species*, *Escherichia coli and Proteus species.*

However, *Escherichia coli* was prevalent than both *Enterobacteria sp and Proteus species* in the intestine of *C. gariepinus.* The mouth of *Clarias gariepinus* haboured more of *Proteus sp.* while, the skin haboured equal level of *Enterobacteria species* and *Proteus sp.*

Nevertheless, the intestine of *Heteroclarias* haboured equal number of *Proteus sp* and *Escherichia coli*, the mouth had only *Escherichia coli* and no other species of bacteria while the skin had more of *Proteus sp* than *Escherichia coli* but no *Enterobacteria species*. According to the results, samples from *Clarias gariepinus* showed a higher presence of *Proteus sp.* (40%) followed by *Escherichia coli* at 35% and least being *Enterobacteria sp.* (25%). But *Escherichia coli* (57%) was prevalent in *Heteroclarias* followed by *Proteus sp.* (38%) and the least being *Enterobacteria sp.* (5%).

In the same vein *Proteus species* presence was higher in *Clarias gariepinus, Escherichia coli* was higher in *Heteroclarias* while *Enterobacteria species* was the least in both *Clarias gariepinus* and *Heteroclarias* put together.

CONCLUSION

From the result of the incidence of microorganisms on *Clarias gariepinus* and *Heteroclarias* fingerlings fed live maggot (the larval stage of housefly) from poultry droppings we can conclude that most of the fish showed the presence of microorganisms.

The presence of these micro-organisms especially *Escherichia coli* indicate that the fish might be feacally contaminated since the maggots feed was from poultry droppings. The presence of *Proteus sp.* might give the fish a poor shelf life when eventually processed for preservation. Most microorganism are either pathogenic, food poisoning, food spoilage or of epidemiological importance. In situations where caught fish were passed through chloroform for bactericidal activity the chloroform action may only be effective on the exposed parts, the skin mainly as most fish are dead and are not capable of swallowing this chemical and so the presence of microorganism in the intestine may remain persistent.

Fish is usually consumed but cases of poisoning through consumption of fish fed live maggots are not common. This may be due probably to antagonistic effect of the microorganisms on one another. *Escherichia coli* is a food poisoning agent causes gastro enteritis (Hobbs and Roberts, 1993). Its presence on fish is of food poisoning implication. *E. coli* is an index of feacal contamination of food and water.

Proteus sp. was also isolated from the fish samples and it is a spoilage importance. *Proteus retigeri* has been reported as a fish pathogen of silver carp (*Hypopthalmichthys molitrix*) in Israel (Roberts, 1978). It is common in soil, vegetation and animal intestines and is of spoilage importance in fish

RECOMMENDATIONS

It is a known fact that fish have to be cooked properly before consumption in Nigeria, therefore the use of live maggot as fish feed is highly recommended based on the fact that:

(1) When using maggots to feed fish, efforts must be made to reduce the level of microbes in the culture medium by the use of good sanitary method.

(2) Fish culturist involved in maggot feeding must be given enough protection against microbial infection to avoid the occurrence of zoonotic diseases.

(3) Eating of uncooked fish should be discouraged as heat helps to eliminate most bacteria.

(4) Trained professionals on fish pathology should be employed so as to ensure low level of microbial presence in cultured fish.

(5) Importation of fingerlings should be under strict surveillance to avoid importation of new strains of infection into the country.

(6) Research on disease of fish, drug and vaccine trails should be encouraged.

(7) There is a need for federal government health workers to educate the public on the risk of unhygienic environment.

(8) Extension officers should be adequately equipped to encourage farmers on low level microbial load technology when using maggots in fish feeding

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