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Comparative Study on Bacterial and Fungal Loads from Siphoned and Unsiphoned Culture of African Catfish (Clarias gariepinus) Hatchlings

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Abstract
This study was carried out to isolate, identify and determine the microbial loads that affect the culture of hatchlings (stage whereby hatchlings absorbed their yolk) in siphoned and unsiphoned water in fish culture. The hatchlings were separated into two groups of triplicate sampling bowls and labelled siphoned and unsiphoned. Samples were analysed for presence of bacteria and fungi using standard methods. The microbial analysis of the siphoned and unsiphoned culture of hatchlings was based on Total Viable Count (TVC) and Total Coliform Count (TCC). The bacterial examination was conducted to isolate and identify bacterial isolates. Serial dilution and inoculation were carried out using sterile media on crushed hatchling samples. Water sample was analysed by inoculating on Tryptone Soy Agar (TSA). For fungal identification both crushed hatchling and water were cultured on Sabouraud Dextrose Agar (SDA), Isolates were examined macroscopically by colony shape, size, colour, and growing pattern, observed under microscope and identified with the help of fungal identification key. Bacterial identification was based on colonial, morphological and biochemical characteristics of colonies. The biochemical test were carried out on the bacterial isolates using catalase test, coagulase test, motility test, triple sugar iron test, indole test and motility test. One way anova was the statistical analysis used for all the results obtained in this experiment. The results showed that the bacterial isolates were *Pseudomonas* sp., *Staphylococcus* sp., *Escherichia coli*, *Streptococcus* sp., while the fungi include *Penicillium* sp., *Aspergillus* sp., and *Mucor* sp. The bacterial isolates showed trending pattern with an increase in count from the first day to the seventh day in unsiphoned as against the siphoned water sample. Conversely, the fungal isolates showed an inconsistent load value between siphoned and unsiphoned from the first day to the seventh. This study revealed that bacterial isolates are more associated than the fungal isolates in the hatchlings culture. The fungal load isolated are relatively lower and significantly different ($P<0.05$) than the bacterial load observed.
Keywords: Bacterial, Fungal, African Catfish, Culturing methods, Hatchlings
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1. Introduction

Fishes are a prolific group of animals which consists of all gill bearing aquatic vertebrate animals that lack limbs with digits, fishes are ideal for farming since they require less space, time, money and have a higher feed conversion ratio (Al-Niaeem *et al*., 2013). Fish is an important source of food, income, employment and recreation for people globally. It is a very important source of animal protein for both man and livestock in developed and developing countries. Fisheries and aquaculture are integral parts of agriculture which have the capacity to increase the country's GDP (Gross Domestic Product) and can solve the unemployment problem for our teeming youths if adequately managed (FAO, 2007). In both natural and culture conditions, disease have serious impacts on total fish production. It is universally recognized as one of the most serious threats to the commercial success of aquaculture (CCAC, 2005). In seed production of African catfish (*Clarias gariepinus*), high losses are recorded during egg stage and this is responsible for reduction in total production. The main reduction is caused by fungal infection especially at the spawning stage. They also hinder proper functioning of organs especially in young fishes and can also cause skin irritation which may lead to reduction in commercial value of adult fishes. Almost every freshwater fish is exposed to at least one species of fungus during its life time (Czecuzuga *et al*., 2011). Disease outbreaks have threatened profitable and viable operation throughout the world. However, studies on pathogenic or opportunistic fungi from fish farms and aquacultures are very few, quite limited and in many instances missing. There are few published literature concerning major fungal pathogens affecting African catfish. Hence, this study is to identify the microbial load in siphoned and unsiphoned catfish hatchlings culture in the first week of production at Bells University of Technology, Ota.

2. Materials and Methods

2.1 *Sample collection*

A bowl of newly hatched fry sample of the same brood stock was collected from a fish farm at Ota, Ogun State. The hatchling was cultured for a week in experimental bowls during which the experimental work was carried out.

2.2 *Experimental Procedure*

The hatchlings were divided into 6 experimental bowls, 3 for siphoned and 3 for unsiphoned bowls as rule for replicate treatment. The specimen for analysis was taken once daily during the experimental study.

2.3 *Siphoning process*

A hose was inserted into the culture bowl to discharge contaminated water which was replaced with a fresh one, this was done every 24 hours on a daily basis unlike the unsiphoned culture in which dirt are not removed and the water sample is not changed throughout the period of the experimental study. The exchanged water is to allow reduce the microbial contamination, to reduce ammonia, nitrate reduction, temperature adjustments and bowl cleaning. This will allow for fresh clean water.

2.4 *Materials and Media Preparation*

Material needed were hatchlings, 6 Experimental bowls, *Artemia* feed, pH meter, Thermometer, Agar (Nutrient Agar, Tryptone Soy Agar (TSA), Triple Sugar Iron Agar) and Siphoning hose.

2.5 *Media Preparation*

TSA was used for the isolation of bacteria from the fish samples. The media was prepared according to the manufacturers guide; 40g was dissolved in 1L of distilled water and sterilized by autoclaving at 121°C for 15 minutes. The media was allowed to cool and poured into sterile disposable petri dishes and allowed to solidify.

2.6 *Preparation of Samples / Microbiological Analysis*

A 1:10 dilution of the hatchlings was made by adding 1 g of the crushed sample into 9 ml of sterile distilled water and shaken to obtain a homogenous mixture. The stock solution was serially diluted up to 10^{-5} as described by Willey *et al*. (2008).

2.7 *Microbial Analysis*

The microbial analysis of the siphoned and unsiphoned culture of catfish hatchlings were done using standard microbial techniques on the various microbial isolates as noted in the Culture media.to identify individual microbial species.

2.8 *Bacterial Examination*

The examination was conducted to isolate and identify bacterial species. Hatchling sample obtained was crushed to carry out serial dilution and inoculated on sterile media; the water sample used for culturing was analyzed as well. By inoculating on Tryptone Soy Agar (TSA, Oxoid) using pour plate according to the method used by Cheesbrough (2000).

2.9 *Fungal Identification*

For the isolation and identification of fungi, both crushed hatchlings and water sample were collected and immediately cultured on Sabouraud Dextrose Agar (SDA). The inoculated samples were incubated at 25 °C for 3 to 7 days (if no visible fungal growth was observed within this period, no growth was recorded) according to the method of Melaku *et al*. (2017). Isolate was identified with the help of fungal identification key (FAO, 1995). Pure fungal culture was then cultured by picking a small portion of colony with the aid of sterilized loop and inoculating on a freshly prepared SDA plate.

2.10 *Biochemical Test*

The gram staining technique was used to differentiate the gram positive from the gram negative bacterial isolates based on the gram staining technique (Willey *et al*., 2008).

2.11 *Catalase Test*

A catalase test was conducted to differentiate catalase negative *Streptococcus* from catalase positive *Streptococcus* and *Bacillus* (catalase positive) from *Clostridium* (catalase negative) (Willey *et al*., 2008).

2.12 *Coagulase Test*

This test was carried out for the differentiation between *Staphylococcus aureus* from *S. epidermidis* (Willey *et al*., 2008). The slide coagulase method was employed for this test as described by Ogbulie *et al*. (2001).

2.13 *Statistical Analysis*

The bacterial and fungal loads from the siphoned and unsiphoned culture of hatchlings were computed and analyzed statistically using the Statistical Package for the Social Sciences (SPSS) version 15.0. Means were compared by subjecting data to one way analysis of variance (ANOVA) to test the significance ($P<0.05$).

3. Results

The bacterial load obtained from the siphoned and unsiphoned cultures as shown in Table 1. The bacteria load in siphoned culture system varied as the lowest load was observed in day one with a load 1.3 X 10^4 cfu/g while the highest load was observed in day three (3) with a load of 4.0 X 10^4 cfu/g while the count that of unsiphoned ranged from 1.0 X 10^3 cfu/g to too-numerous-to-count colonies, signifying excessively high microbial load.

TABLE 1: Bacterial count obtained from siphoned and unsiphoned cultures.

DAYS	Bacterial count (cfu/g)	
	Siphoned	Unsiphoned
DAY 1	1.3 X 10^4	3.0 X 10^4
DAY 2	3.0 X 10^4	TNTC
DAY 3	4.0 X 10^4	1.0 X 10^3
DAY 4	1.5 X 10^4	3.0 X 10^3
DAY 5	1.3 X 10^4	1.0 X 10^3
DAY 6	3.0 X 10^4	5.9 X 10^4
DAY7	1.0 X 10^4	TNTC
CONTROL (tap water)	0	NA

Note: all readings are mean of triplicate experiments

Key:
NA – Not applicable
TNTC – Too numerous to count

The fungal counts for both culture systems are presented in Table 2. Days 1 and 7 have no fungal growth for the siphoned culture system while day 2 had the highest load of 5.0 X 10^3 cfu/g. In the unsiphoned system, days 1, 5, and 6 recorded no fungal growth. Conversely, in day 7 the highest load of 4.0 X 10^3 cfu/g was recorded.

TABLE 2: Fungal count obtained from siphoned and unsiphoned cultures.

DAYS	Fungal count (cfu/g)	
	Siphoned	unsiphoned
Day 1	0	0
DAY 2	5.0 X 10^3	1.0 X 10^3
DAY 3	1.0 X 10^3	0
DAY 4	1.0 x 10^3	1.0 X 10^3
DAY 5	2.0 X 10^3	0
DAY 6	1.0 x 10^3	0
DAY 7	0	4.0 X 10^3
CONTROL PLATE	0	NA

Note: all readings are average of duplicate experiments

Key:
TNTC – too numerous to count

Table 3 shows the identification of the isolates through Gram staining and biochemical test of the whole pure culture isolated. The suspected bacteria were *Staphylococcus* sp., *Escherichia* sp., *Streptococcus* sp., and *Pseudomonas* sp. The dominant bacteria isolated from the crushed hatchlings were Gram negative bacteria. Similarly, fungal identification is as shown in Table 4. This was done by observing the outcomes from morphological, colonial characterization and staining using lactophenol cotton blue.

TABLE 3: Characteristics of bacterial isolates and biochemical test

Colonial Characteristics	Biochemical Tests									Probable Organism
	GRAM	CAT	COA	MOT	IND	SLP	BUT	H_2S	GAS	
Yellow, round, raised and cocci in cluster	+	+	+	-	+	R	Y	-	-	*Staph.* sp.
Smooth, regular, rods shape.	-	+	-	+	-	Y	Y	-	-	*Esch.* sp.
Creamy, glossy, cocci in cluster, and round.	-	+	+	-	-	R	Y	-	-	*Pseudo.* sp.
Smooth, irregular, rods shape.	-	+	-	+	+	Y	Y	-	+	*Esch.* sp.
Milky, yellow, convex, round and cocci in chain.	+	-	-	-	-	R	Y	+	+	*Strep.* sp.
Creamy, smooth, irregular, rods.	-	+	-	+	+	Y	Y	-	+	*Esch.* sp.
Creamy, glossy, cocci in cluster and round.	-	+	+	-	-	R	Y	+	-	*Pseudo.* sp.
Creamy, irregular, rods and convex.	-	+	-	+	-	Y	Y	-	+	*Pseudo.* sp.
Creamy, convex, irregular and rods.	-	+	-	+	-	R	R	-	+	*Pseudo.* sp.
Yellow, glossy, cocci in Cluster and raised.	+	+	+	-	-	R	Y	+	-	*Staph.* sp.
Creamy, convex, irregular and rods	-	+	-	+	-	R	R	-	-	*Pseudo.* sp.
Creamy, irregular, rods and convex.	-	+	-	+	-	Y	Y	-	+	*Pseudo.* sp.
Yellow, round, raised and cocci in cluster .	+	+	+	-	-	R	Y	+	-	*Staph.* sp.
Smooth, irregular, rods shape.	-	+	-	+	+	Y	Y	-	+	*Esch.* sp.

Key:

GRAM = Gram staining
CAT = catalase
COA = coagulase
MOT = motility
IND = indole
SLP = slope
GAS = gas production

Staph. sp. - *Staphylococcus* sp.
Esch. sp. - *Escherichia* sp.
*Pseudo. s*p. - *Pseudomonas* sp.
Strep. sp. - *Streptococcus* sp.
Y = acid production
H_2S = hydrogen gas
R = alkaline utilization

TABLE 4: Colonial and morphological characteristics of the fungal isolated from siphoned and unsiphoned hatchlings culture.

Colony Characteristics	Morphological Characteristics	Probable Organisms
Black, flat, raised and circular.	Conidia, septate multinucleated hyphae	*Aspergillus* sp.
White with black dots and brittle.	Conidia and septate hyphae.	*Mucor* sp.
Green, rhizoid, circular and carpet like.	Conidia, septate and acoenocytic hyphae	*Penicillium* sp.

4. DISCUSSION

The bacterial load in unsiphoned culture was high in this study. The higher microbial load observed in the unsiphoned system is probably accountable for the high mortality rate at weeks 1 – 2. The high bacterial load observed in the unsiphoned culture may be attributed to the presence of the normal flora and the contaminated environment since microorganisms are ubiquitous. More also, the waste product released by the hatchlings as observed in the culture turbidity. The low bacterial load observed in the siphoned is however attributed to the condition of culture. Hence, the name siphoned indicating that the culture water is changed regularly and replaced with fresh water. The analyzed control water tested okay for domestic purposes. The isolates observed may pose a health concern to the populace according to Adebayo – Tayo *et al*. (2012) while Edun *et al*. (2015) reported that fish can act as an important food vehicle for some zoonotic pathogens such as *Salmonella* and *Vibrio* and the contamination of fish with pathogens is a major public health concern. The results of this study shows that the fungi found were of the genera *Aspergillus, Mucor* and *Penicillium.* Muktar *et al*. (2016) recorded that these isolated genera are the most common fungi infecting fish. Though most fungi were regarded as opportunistic pathogens but few of them are known to cause diseases such as saprolegniasis, Aspergillosis, Scopulariopsis, paecilomycosis and *Penicillium* infections. Saprolegniasis is a localized disease caused by fungi of the order of Saprolegniales (water moulds), Peronosporales and Leptomitales infected fish are lethargic which render it uneatable.

Fadaeifard *et al*., (2011) isolated 8 species of fungi from eggs and brood stock of rainbow trout *Oncorhynchus mykiss*, these isolates were *Penicillium* spp., *Acreomonium* spp., *Alternaria* spp., *Fusarium solani*, *Aspergillus* sp., *Mucor* sp., *Saprolegnia* sp., *Cladosporium* sp. Presence of fungi in culture system causes loss in the production of African Catfish (*Clarias gariepinus*) (Eli and Abowei, 2011) especially at the hatchling stage which accounts for why motility rate was high in the culture system.

Isolation of microbes in the culture system of fish is of great public health significance as the isolates may have health implications on final consumers as reported by Iqbal *et al*. (2012). Growth of fish does not only depend on feeds but also on good environment.

The low bacterial load observed in the siphoned is however attributed to the condition of culture. Hence, the name siphoned indicating that the culture water is changed regularly and replaced with fresh water. Unlike the unsiphoned water culture with higher bacterial load as a result of dirt and other waste products released by hatchlings as waste products.

Water quality management is critical to hatchlings culturing and production, so water quality should be monitored regularly, as this will help in the reduction of the microbial load in the water sample used for culturing. To improve water quality, fresh and clean water must be added and this action requires a reliable source of quality water. The pH of the water used for the experiment ranges between 6.7 and 7.5. The results were analyzed using one way ANOVA, at 95% level confidence interval.

The statistical analysis in this study showed that the mean data obtained for values were significantly different ($P < 0.05$). There is significant difference in the bacterial load in siphoned and unsiphoned hatchlings culture. The highest mean fungal load count for unsiphoned was 14200.00 ± 44803 and in the siphoned culture the fungal load was 1428.57 ± 960.23.

Conclusion

The high bacterial and fungal load caused high mortality and reduction of hatchlings most especially at weeks 1 and- 2. This could endanger the consumers, fish harvested from this pond poses health threat when under cooked. Catfish hatchlings harbour both opportunistic and pathogenic microorganisms.

Recommendation

It is therefore recommended that the environment where the fish ponds are located should be protected from pollutants and weeds which can harbour microorganisms that can find their way into the fish ponds by themselves or by passive process through wind, rainfall, etc. Water supply to the fish culture should be taken and examined in the laboratory for its microbiological quality before stocking. This would give insight to the

possible presence of certain types of microorganisms. Water in the pond should be constantly monitored to eradicate bacteria and fungi. There should also be routine microbial assessment of fish culture during hatching to eliminate their presence and drainage of suspected pond especially at the early stage of fish development.

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