EFFECT OF ALKALINE pH ON FUNGAL RESISTANCE, TOTAL PROTEIN AND TOTAL LIPIDS OF *Clarias gariepinus* SKIN MUCUS

***NWABUEZE, A. A., G. B. EKO, and J. O. AGBENEWEI**
Department of Fisheries, Delta State University, Asaba Campus, Asaba, Nigeria.
***Corresponding Author**: aanwabueze@gmail.com, +234 806 413 8580

ABSTRACT
The effect of alkaline pH on fungal resistance, total protein, and total lipids on the skin mucus of Clarias gariepinus was investigated. C. gariepinus in renewal static bioassay were subjected to alkaline pH, alkaline + buffer solution, for comparison with fish from control. Antifungal activities of mucus from treatments and control fish were determined using a sensitivity test. Mucus was analyzed for total protein and total lipid contents using the Kjeldahl method and Soxhlet extractor respectively. Zones of inhibition were used to determine the sensitivity of mucus against fungal isolates. Fungi isolated were Aspergillus flavus, Aspergillus niger, Mucor mucedo, Rhizopus stolonifer, and Penicillium species. C. gariepinus mucus from treatments and control exhibited antifungal activity against fungal isolates. Mucus of C. gariepinus from control had higher zones of inhibition as compared with treatments. M. mucedo with 19.4mm zone of inhibition was more sensitive (P>0.05) to the antifungal activity of C. gariepinus. A. niger had significantly (P<0.05) lower zones of inhibition in alkaline pH. Total protein (30.65%) and total lipid (8.23%) were highest in alkaline pH mucus (P>0.05). This study shows that alkaline pH probably reduces the antifungal activity of C. gariepinus mucus and may elicit less resistance to fungal species.
Keywords: Antifungal, pH, protein, lipids, fish

INTRODUCTION

Water is the cultural environment for fish and acts as physical support in which fishes carry out life processes and functions such as feeding, swimming, breeding, digestion, and excretion (Dauus and Ansa, 2010). Access to an adequate, regular, and constant supply of high-quality water is important for fish survival. Danba *et al.* (2015) noted that good growth in the water body is determined by the levels of dissolved oxygen, pH, hardness, turbidity, alkalinity, nutrients, and temperature. Fish skin mucus is considered as the first line of defense against infection through the outer protective layer of the fish skin, protecting against environmental factors like microorganisms, toxins, pollutants, adverse pH, and hydrolytic enzymes (Woof and Mestecky 2005). It plays an important role in maintaining the health of the fish especially under controlled farming where the level of stress and infections could be high (Patel *et al*., 2017). Protein is a major component of crude fish mucus followed by carbohydrate and lipids and acts against pathogen such as bacteria, fungi and other parasites (Manivasagan *et al.*, 2009; Ambuchzhian *et al*., 2011; Estehan, 2012; Al-Arifa *et al.*, 2013; Tyor and Kumari, 2016). The antibacterial and antifungal activities of fish mucus have been demonstrated in several fish species (Pethka, 1995; Hellio *et al.*, 2002; Wei *et al.*,2010; Gobinath and Ravichandran, 2011; Patil *et al.* ,2015; Balasubraaman *et al*., 2016) and this activity seems to vary from one species to the other and can be specific towards certain pathogen (Hiwarale *et al*., 2016). Hydrogen ion concentration, pH, is one of the important factors in fish culture, indicating the balance between acid and base in the culture water (Bhatnagar and Devi, 2009; Hamalatna and Puttiha, 2014).

Catfish culture is popular in Nigeria. The African catfish, *Clarias gariepinus* is one of the important freshwater species with attributes such as disease resistance, ability to survive long drought, and food scarcity among other aquaculture potentials (Dada and Wonah, 2003). The need for sustainable development has necessitated intensive culture of catfishes to enable sufficiency in food fish and cheap protein production. However, poor culture conditions of most intensive fish culture systems associated with high stocking densities, pollution, feed contamination, incorrect feeding, and feeding regime have elicited the presence of pathogenic and opportunistic microorganisms (Nwabueze, 2011; Nwabueze, 2012). Fungi are commonly associated with water derived from fish culture as a result of pollutions from the environment and incorrect feeding practices and are an important economic limiting factor in aquaculture especially in intensive fish production (Chandan, 2012). Poor water quality greatly affects the ability of fish to produce mucus (Al-Arifa *et al.*, 2013). Solomon *et al.* (2013) reported that the determinant and frequency of monitoring water quality depends upon the rearing intensity of the culture medium.

Fish, therefore, live in a challenging environment facing so many problems and are susceptible to attack by pathogens (Al-Arifa *et al.*, 2011). Fish culture water dynamics and management as it relates to the fish environment must be taken into consideration for a successful aquaculture venture (Davies and Ansa, 2010). This study investigates the effect of alkaline pH on fungal resistance, total protein, and lipid of *Clarias gariepinus* epidermal mucus.

MATERIALS AND METHODS

The experiment which lasted three months (March-May, 2017) was carried out in the Fishery Laboratory of the Department of Fisheries, Delta State University, Asaba Campus, Asaba. Ninety 4-months old *Clarias gariepinus* (mean weight: 170 10.5g and standard length 24.3 ± 1.4cm) were acclimated for 7 days in a stock tank during which fish were fed commercial feed at 4% body weight. Ten fish from the stock tanks were randomly distributed in triplicates: Tanks A (A1, A2, A3) and B (B1, B2, B3) as treatments and Tank C (C1, C2, C3) as control. Fish in Tank A were treated with an alkaline solution (2 ml NaOH) having a pH of 11.5 (0.14g NaOH/L) and fish in Tank B was treated with an alkaline solution of pH 11.5 and buffer (pH 7.5, 2 N Tri Hydrochloride) and fish in both tanks kept for 25 minutes according to Al-Arifa *et al.* (2011, 2013). Fish in Tanks A and B were kept for a shorter time to prevent fish kill especially since mucus has to be collected from live fish. While fish in Tank C were kept in water with a pH of 7.0 for 4 hours (Bradford, 1976).

Fungi strains for the study were obtained from homogenized fish culture water which was then serially diluted and 1 ml measured into a petri dish and covered with 15 ml molten potato dextrose agar, incubated at 370C for 72 hours. Fungi colonies were subcultured and pure colonies of each isolated fungus identified according to Gamalat and Galal (2006). Pure colonies were prepared into separate petri-dish on potato-dextrose agar. Epidermal fish mucus was collected from the dorsal surface of fish in treatment and control tanks using a sterile spatula. Pooled fish mucus from each experimental group was quantified and centrifuged at 12,000 rpm for 10 minutes, labeled and stored at -400C for analysis (Al-Arifa *et al*., 2011). Antifungal activities of mucus from the treatments and control fish were determined using a sensitivity test.

Sensitivity discs containing mucus were prepared against each fungal isolate. Sensitivity disc was prepared using filter paper cut into disc-like shapes and then impregnated with 1ml fish mucus collected from the different experimental groups The disc containing the mucus samples were prepared against each fungus isolated (pure cultures) from the water samples of the culture medium. The prepared sensitivity disc was then placed in the center of each petri dish. The plates were incubated for 48 hours.

Zones of inhibition were used to determine the sensitivity of mucus from treatment and control fish against fungal isolates. The diameter of the zone of inhibitions was measured vertically and horizontally with a ruler. The average value of the two measurements was calculated as the zone of inhibition of antifungal activities of mucus against each isolated fungus (Prescott, 1990). A clear zone of inhibition indicates the absence of fungal growth while non-visible clear zone around the disc indicate that the fungal is growing normally. The presence of a fungal lawn means non-sensitivity while the absence means sensitivity or resistance (Nwabueze, 2014).

Fish skin mucus samples were analyzed for total protein and total lipid at the National Institute For Oil Palm Research (NIFOR). Total protein content was estimated by the macro-Kjeldahl method (Bradford, 1976, AOAC, 2000). The total lipid of fish mucus was determined using Soxhlet extractor (Martinez et al., 2009). Data obtained were analyzed using Analysis of Variance ANOVA at a significant level of $P<0.05$.

RESULTS

Fungi isolated from the fish stock tank were *Apergillus flavus, Aspergillus niger, Mucor mucedo, Rhizopus stolonifer,* and *Penicillium* species. Mean counts of fungal isolates from skin mucus of *C. gariepinus* on the treatments are presented in Table 1.

Table 1. Mean (±S.E.M) counts (CFU/mL x 10^3) of fungal isolates from *C. gariepinus* in different pH culture media

S/n	Fungal Isolates	Treatments Tank A (Alkaline)	Tank B (Alkaline + Buffer)	Control Tank C
1	*Aspergillus flavus*	9.70 ± 0.22	9.60 ± 0.42	9.73 ± 0.25
2	*Aspergillus niger*	13.10 ± 0.57	12.73 ± 0.61	13.40 ± 0.24
3	*Mucor mucedo*	17.27 ± 0.71	17.20 ± 0.86	18.60 ± 0.45
4	*Rhizopus stolonifer*	13.47 ± 0.43	13.23 ± 0.71	13.63 ± 0.25
5	*Penicillium* species	14.43 ± 0.52	14.93 ± 0.39	14.47 ± 0.20

A. flavus, A. niger, M. mucedo, and *R. stolonifer* counts were lowest in fish mucus subjected to alkaline pH + buffer treatment, closely followed by alkaline pH and highest in the skin mucus of control sample. Fungal counts of *Penicillium* species were in increasing order in alkaline pH, control and alkaline + buffer media. Figure 1 shows the antifungal activity of *C. gariepinus* skin mucus. Zones of inhibition were generally highest in *C. gariepinus* skin mucus of control.

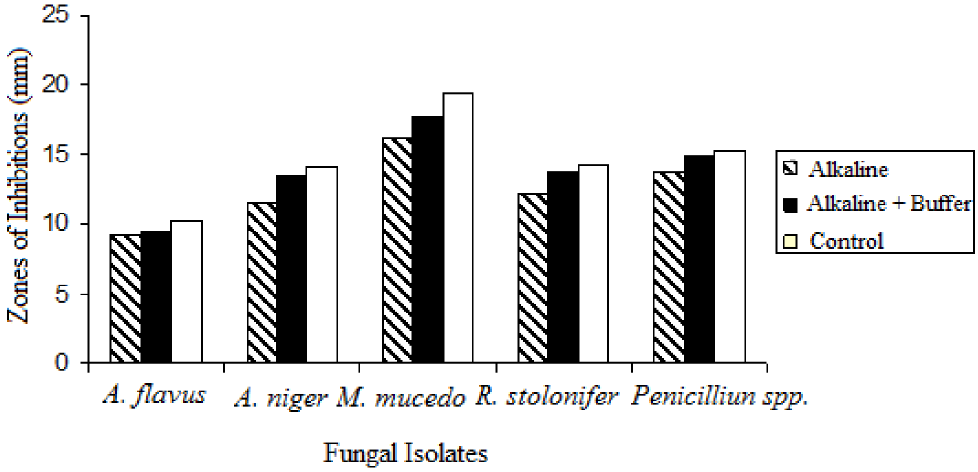

Figure 1. Antifungal activity of *C. gariepinus* skin mucus from treatment and control samples

Increasing zones of inhibition for *M. mucedo, Penicillium species, R. stolonifer, A. niger,* and *A. flavus* were 16.2mm, 13.7mm.12.2mm, 11.6mm, and 9.2mm for alkaline pH, 17.7mm, 14.9mm,13.8mm,13.5mmand 9.5mm for alkaline +buffer pH, 19.4mm, 15.3mm, 14.3mm, 14.2mm, and 10.3mm were observed for control respectively. *M. mucedo* with the highest zones of inhibition was found to be more sensitive to antifungal action of skin mucus of *C. gariepinus*. There was no significance (P> 0.05) difference in mean zones of inhibition for *A. flavus, M. mucedo, R. stolonifer* and *Penicillium* species (Table 2). However, zones of inhibition observed for fish mucus in alkaline + buffer and control were significantly (P <0.05) higher than zones and inhibition observed for *A. niger.* The mean total protein and lipid contents of *C. gariepinus* skin mucus are presented in Table 3.

Table 2. Analysis of Zones of inhibition of *C. gariepinus* skin mucus from treatment and control samples

S/n		Zones of Inhibition		
	Fungal isolates	Alkaline	Alkaline + Buffer	Control
1	*Aspergillus flavus*	9.20a	9.50a	10.33a
2	*Aspergillus niger*	11.57a	13.47b	14.20b
3	*Mucor mucedo*	16.22a	17.73a	19.40a
4	*Rhizopus stolonifer*	12.23a	13.77a	14.33a
5	*Penicillium* species	13.67a	14.90a	15.27a

Table 3. Total protein and lipid contents of *C. gariepinus* skin mucus

	Source of fish mucus		
Parameter	Alkaline	Alkaline + Buffer	Control
Total Protein (%/100g)	30.65	7.36	20.99
Lipid (%/100g)	8.23	0.76	3.10

Total protein and lipid in alkaline pH mucus were 30.65%/ and 8.23%/ respectively. Lowest total protein of7.36%, and lipid, 0.76% were observed in fish mucus from alkaline + buffer treatment.

DISCUSSION

Fungi were isolated from *C. gariepinus* culture water. Fungi are opportunistic microbes existing spontaneously in the air, soil, and water of the fish environment and the ecosystem. Fungal contamination of fish culture water and fish feed has been reported (Hellio *et al.*, 2002; Klinger and Francis-Floyd, 2008; Pethkar and Lokhande, 2017; Mohamed *et al.*, 2017). Skin mucus samples of *C. gariepinus* subjected to alkaline treatments and those on the control exhibited antifungal activity against the five fungi isolates from the fish culture medium. However, skin mucus from control fish with higher zones of inhibition showed more antifungal activity than fish mucus with alkaline pH treatments. This finding indicates that alkaline pH conditions may have altered the defense mechanism of the fish and probably reduced the antifungal activity of *C. gariepinus* skin mucus. Al -Arifa *et al.* (2011) reported a similar finding from the skin mucus of *Labeo rohita* in alkaline pH.

Zones of inhibition were higher in the mucus of *C. gariepinus* in Alkaline + buffer treated culture water than in alkaline pH water without buffer and significantly higher for *A. niger.* These observations point to the fact that the addition of buffer helped to reduce the negative impact of alkaline pH on the fish mucus serving to cushion the stressful effect of high pH on the fish. *A. niger* may have been the worst hit by the effect of alkaline pH treatment. It has been reported that different species and strains of organisms have different levels of tolerance to fluctuations in pH (Guffanti *et al.,* 1980; Sawatari and Yokota, 2007).In practice, buffers greatly affect the final pH of water having a stabilizing impact to effectively neutralize pH ranges when acids or bases are added to water (Boyd *et al.*,2011; Maoxiao *et al.,* 2018). Pond pH varies throughout the day due to photosynthesis and respiratory activities by organisms in water bodies. Photosynthetic phytoplankton use up carbon dioxide (CO2) during the day which causes a rise in pH levels while respiration at night increases CO2levels and lowers the pH (Tucker, 1984). Adequate compensation by the process of respiration is necessary to reduce high pH levels. Wurts and Durborow (1992) noted that in heavily stocked fish ponds, CO2 concentration can become high as a result of respiration which has a lowering effect in pH.

The concentration of total protein in fish mucus was higher than the concentration of total lipid, though not significant, infers that total protein is a major component of fish mucus than total lipid (Manvisagan *et al*., 2009 and Tyor and Kumara, 2016). Alkaline conditions irritate the fish making fish secrete large amounts of mucus but the effectiveness of the alkaline mucus in the defense mechanism of fish is reduced showing that alkaline pH probably has adverse effects on *C. gariepinus* resistance to some fungal species. Al-Arifa *et al.* (2013) reported that alkaline pH enhanced mucus production but also impacted its quality in terms of protein concentration and lectin activity as well as altering its general appearance and odor due to reduced number of proteins in the mucus of fish in alkaline water as compared with several proteins in control fish. Alkaline pH, however, has been observed to have no adverse effect on fish mucosal fatty acid (Al-Arifa *et al.,* 2013).

CONCLUSION

This study has shown that skin mucus of *C. gariepinus* has antifungal properties and may serve as bio-controlling defense apparatus against fungal pathogens in the fish environment. Alkaline conditions have a negative effect on the defense mechanism of *C. gariepinus* and such conditions should be avoided in fish culture. Alkaline pH may be a limiting factor in fish production. It is therefore needful and beneficial to monitor fluctuations in pH levels in fish health management to prevent stressful conditions for fish.

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