EFFECT OF ALKALINE PH ON FUNGAL RESISTANCE, TOTAL PROTEIN AND TOTAL LIPIDS OF *CLARIAS GARIEPINUS* SKIN MUCUS

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The effect of alkaline pH on fungal resistance, total protein and total lipids of *Clarias gariepinus* skin mucus was investigated in this study. *C. gariepinus* samples in renewal static bioassay were subjected to alkaline pH, alkaline + buffer solution, and skin mucus collected from the dorsal surface of fish for comparison with mucus from control fish. Antifungal activities of mucus from treatments and control fish were determined using sensitivity test. Total protein and total lipid contents of fish mucus were analyzed using Kjeldahl method and Soxhlet extractor respectively. Zones of inhibition were used to determine the sensitivity of mucus from treatment and control fish against fungal isolates. Fungi isolated from fish culture medium were *Apergillus flavus, Aspergillus niger, Mucor mucedo, Rhizopus stolonifer* and *Penicillium* species. Pooled mucus of *C. gariepinus* from treatments and control exhibited antifungal activity against fungal isolates. Mucus of *C. gariepinus* from control had higher zones of inhibition compared to treatments. *M. mucedo* with 19.4 mm zone of inhibition was more sensitive ($P>0.05$) to antifungal activity of *C. gariepinus*. *A. niger* had significantly ($P<0.05$) lower zones of inhibition in alkaline pH. Total protein and total lipid were highest in alkaline pH mucus. *C. gariepinus* mucus was defensive against fungal pathogens of *C. gariepinus.* This study has shown that alkaline pH probably reduces the antifungal activity of *C. gariepinus* mucus and may elicit less resistance to fungal species. This study is probably novel in reporting the negative impact of alkaline pH on fungal resistance of *C. gariepinus* mucus. Monitoring pH levels especially in intensive aquaculture is important for fish health management.

Key words: Alkaline pH, fungal resistance, total protein, lipids, *Clarias gariepinus*, mucus.

INTRODUCTION

Water is the culture environment for fish and acts as a physical support in which fishes carry out their life processes and functions such as feeding, swimming, breeding, digestion and excretion (Davies and Ansa, 2010). Thus access to adequate regular and constant supply of good quality water is of high importance for fish survival. Danba et al. (2015) noted that good growth in water body is determined by the levels of dissolved oxygen, pH, hardness, turbidity, alkalinity, nutrients and temperature.

Fish skin mucus is considered as a first line of defense against infection through the outer protective layer of the fish skin providing protection against environmental factors like micro organisms, toxin, pollutants, adverse pH and hydrolytic enzymes (Woof and Mestecky, 2005); it plays an important role in maintaining the health of the fish especially in 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; it acts against pathogenic bacteria, fungi and other parasites (Estehan, 2012; Al-Arifa et al., 2013; Tyor and Kumari, 2016). Hydrogen ion concentration, pH being one of the important factors in fish culture, indicates the balance between acid and base of the culture water (Boyd et al., 2011; Bhatnagar and Devi, 2013). Fungi are commonly associated with water derived from fish culture as a result of pollutions from environment and fish feed and is an important economic limiting factor in aquaculture especially in intensive fish production (Mohamed et al., 2017).

The antibacterial and antifungal activities of fish mucus have been demonstrated in several fish species (Hellio et al., 2002; Wei et al., 2010; Gobinath and Ravichandran, 2011; Patil et al., 2015; Balasubraaman et al., 2016; Pethka and Lokhande, 2017) and these activities seem to vary from one species to another and can be specific towards certain pathogens (Hiwarale et al., 2016). Catfish culture is popular in Nigeria. The African catfish, *Clarias gariepinus* is one of the important fresh water 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 feed and feeding regimen have elicited the presence of pathogenic and opportunistic microorganisms (Nwabueze, 2011; Nwabueze, 2012). Poor water quality affects greatly 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 fish environment must be taken into consideration for a successful aquaculture venture (Davies and Ansa, 2010). There is a dearth of literature on the effect of alkaline pH on fungal resistance of fish skin mucus. This study investigates the effect of alkaline pH on fungal resistance, total protein and lipid of *C. gariepinus* skin mucus.

MATERIALS AND METHODS

The experiment which lasted three months (March - May, 2017) was carried out in the Fisheries Laboratory of the Department of Fisheries, Delta State University, Asaba Campus, Asaba. Ninety 4 months old *C. gariepinus* fish (mean weight: 170±10.5 g and standard length: 24.3 ± 1.4 cm) were acclimated in stock tank for 7 days during which fish were fed with commercially available feed at 4% body weight. Ten fish from stock tank 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 alkaline solution (2 ml NaOH) having a pH of 11.5 (0.14 g NaOH/L of borehole water) and kept for 25 min. Fish in Tank B were treated with alkaline solution and buffer (2 N Tri Hydrochloride) according to Al-Arifa et al. (2011, 2013) while fish in Tank C were kept in water with pH of 7.0 for 4 h (Bradford, 1976).

Fungi strains for the study were obtained from homogenized fish culture water which was then serially diluted; 1 ml was measured into petri dish and covered with 15 ml molten potato dextrose agar. It was incubated at 37°C for 72 h. Fungi colonies were subcultured and pure colonies of each isolated fungus were identified according to Gamalat and Galal (2006). Pure colonies were prepared into separate Petri-dishes on potato-dextrose agar.

Epidermal fish mucus was collected from the dorsal surface of fish in treatment and control tanks using sterile spatula. Pooled fish mucus from each experimental group was quantified and centrifuged at 12,000 rpm for 10 min, labeled and stored at -40°C for later analysis (Al-Arifa et al., 2011). Antifungal activities of mucus from treatments and control fish were determined using 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 discs in triplicates, containing the mucus samples were prepared against each fungi isolated (pure cultures) from the water samples of the fish culture medium. The prepared sensitivity discs were then placed on the centre of each Petri dish. The plates were incubated for 48 h. Later, zones of inhibition were used to determine the sensitivity of mucus from treatment and control fish against fungal isolates. The diameters of zones of inhibitions were measured vertically and horizontally with a ruler. The average value of the two measurements was calculated as zone of inhibition of antifungal activities of mucus

against each isolated fungus (Prescott, 1990). A clear zone of inhibition indicates absence of fungal growth while non-visible clear zone around the disc indicates that the fungi are 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 macro-Kjeldahl method (Bradford, 1976; AOAC, 2000). 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 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* treated with different alkaline pH are presented in Table 1.

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

S/N	Fungal Isolates	Treatments		Control
		Tank A (pH 11.5)	Tank B (pH 11.5 + Buffer)	Tank C (pH 7.0)
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 (11.5) + buffer treatment. This is closely followed by alkaline pH (11.5); the highest was 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.

Increasing zones of inhibition for *M. mucedo,*

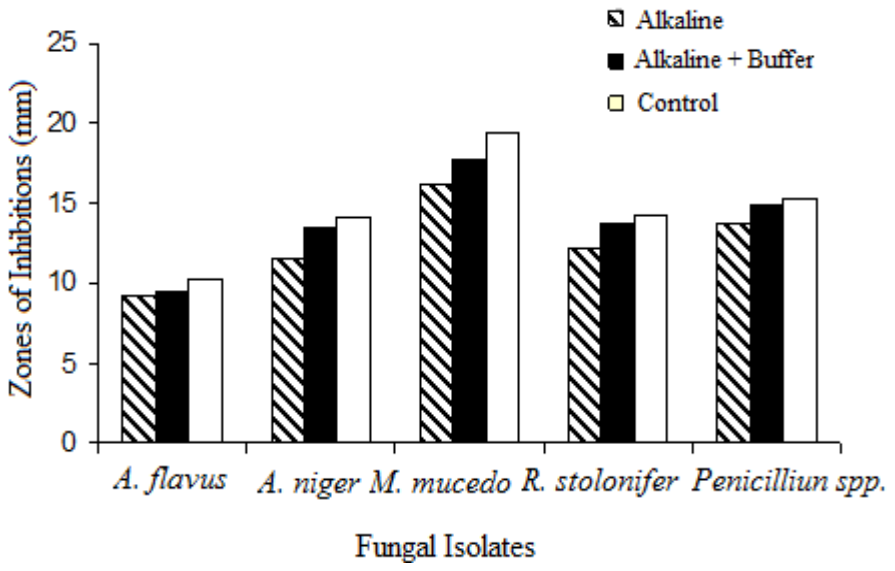

Figure 1. Antifungal activity of *C. gariepinus* skin mucus from treatment and control samples.

Penicillium species, R. stolonifer, A. niger and *A. flavus* were 16.2, 13.7, 12.2, 11.6 and 9.2 mm for alkaline pH, 17.7, 14.9, 13.8, 13.5 and 9.5 mm for alkaline + buffer pH, 19.4, 15.3, 14.3, 14.2 and 10.3 mm were observed for control respectively. *M. mucedo* with highest zones of inhibition was found to be more sensitive to anti- fungal 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. 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*. Mean total protein and lipid contents of *C. gariepinus* skin mucus are presented in Figure 2. Total protein and lipid in alkaline pH mucus were 30.65 and 8.23% respectively. Total protein (7.36%) and lipid (0.76%) were observed to be lowest in fish mucus from alkaline + buffer treatment.

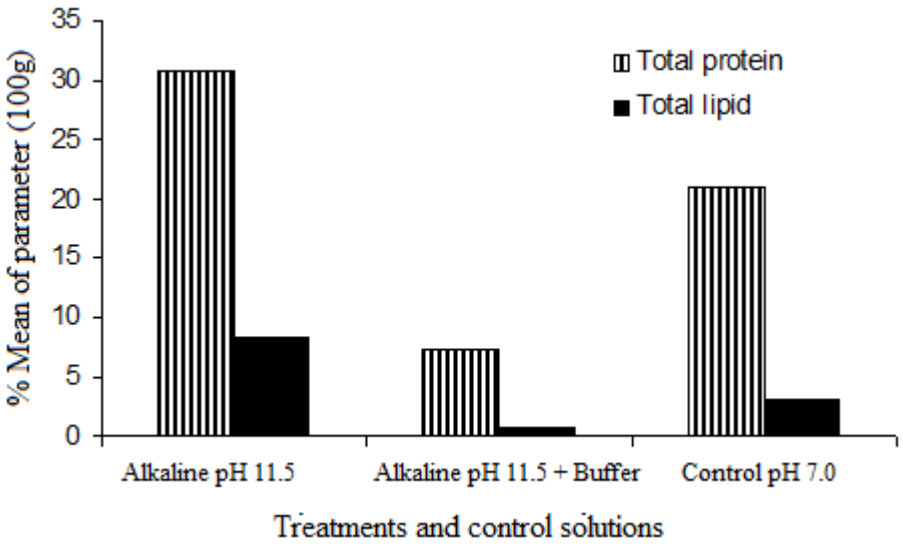

Figure 2. Total protein and lipid contents of *C. gariepinus* skin mucus.

DISCUSSION

Fungi were isolated from *C. gariepinus* culture medium. Fungi are opportunistic microbes existing spontaneously in the air, soil and water of 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 control exhibited antifungal activity against the five fungi isolates from 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 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 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 (CO_2) during the day which causes a rise in pH levels while respiration at night increases CO_2levels and lowers the pH (Tucker, 1984). Adequate compensation by the process of respiration is necessary in order to reduce high pH levels. Wurts and Durborow (1992) noted that in heavily stocked fish ponds, CO_2 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. This infers that total protein is a major component of fish mucus than total lipid (Manvisagan et al., 2009; Tyor and Kumara, 2016). Alkaline conditions irritate the fish. This makes them to 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 mucus of fish in alkaline water compared to the number of 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). This study is novel in reporting the negative impact of alkaline pH on fungal resistance of *C. gariepinus*.

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 negative effect on the defense mechanism of *C. gariepinus* and such conditions should be avoided in fish culture. This finding indicates that high alkaline pH may be a limiting factor in *C. gariepinus* production. This study is probably novel in reporting the negative impact of alkaline pH on fungal resistance of *C. gariepinus* mucus. It is therefore needful and beneficial to monitor fluctuations in pH levels in fish health management in aquacultural practices to prevent stressful conditions for fish.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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