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Characterization of fish Mucus and Seasonal Impact on their Biochemical Composition

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ABSTRACT

The integumentary layer of fishes is not only the main surface of exchange between them and the external environment, but they also possess other important functions, including the protection of fish from pathogenic attacks. Recently, the integumentary mucus of the fishes has gained importance in the field of biomedical research, because of its ability to tackle infections caused by bacteria, viruses, and fungi, by providing innate immunity to the fishes. It being studied for its potential application in human medicine. It holds valuable antimicrobial bacteria which assist fishes to guard themselves against pathogens in the aquatic ecosystem. In Fish Skin various type of cells present such as mucus cells, club cells and epithelial cells. The fish skin mucus, as an important and effective source of antifungal drugs against several human pathogens and the treatment of their resulting clinical infections. Evolutionary lineage determines the innate immune system of pathogen that are modified through time and environmental factors. In the present study we studied the characteristics of fish mucus in the different ecological zone and also the seasonal impact on their biochemical composition. there are significant differences occurred in concentration of chemical composition of mucus. It was observed that Concentration of protein (982.92µg/ml) and carbohydrate(48.34µg/ml) is high in rainy season while low in summer season. (Protein 91.17µg/ml and carbohydrate16.63 µg/ml).

Key words: *Epidermal mucus, Immunoglobulin's, Biomolecules, harmonize factors, Epithelial cells, pathogen peptide.*

Introduction

Fish mucus layer covers the surface of external body to reduce body friction against water and to protect from abrasion injury. The epidermal fish mucus makes the surfaces smooth and slippery and has mechanical protective nature (Cameron and Endean 1973). However, the composition and rate of mucus secretion has been observed to change in response to microbial exposure or to environmental perturbations such as hyperosmolarity and acidity (Ellis 2001). In fish, the epidermal mucus is the external barrier between the environment and fish which is considered as a key component of innate immunity (Ingram, 1980). The epidermal mucus is produced primarily by epidermal goblet or mucus cells and is composed mainly of water and gel-forming macromolecules, including mucins and other glycoproteins (Shephard, 1993).

The functional properties of mucus depend on its capacity to form a gel on the epithelial surface. The gelforming property is controlled by the amount, size and degree of cross- linking present between the mucus glycoprotein (mucins) (Smith, 1992). Fish mucus also contains numerous immune related components are lysozymes, immunoglobulins components lectins, and antimicrobial peptides (AMPs) (Salinas, 2011). Depending on the fish species, skin mucus varies considerably in viscosity, thickness, and glycoprotein (mucin) content which also represents the major components of mucus (Dash *et al.*, 2018). The major carps under three genera, (Catla

catla), (*Labeo rohita*) and (*Cirrhinus mrigala*). *Catla catla* feeds mainly on Zooplankton at the upper zone of the water body and these are called Surface dweller. *Labeo rohita* mostly feeds on phytoplankton forms found attached to submerged vegetation and these are called mid-dwellar and *Cirhinnus mrigala* feed at bottom biota such as tubifex and other blood worms. Thus, these carps have mutually compatible and complimentary. These are major carp belonging to family Cyprinidae.

Hence, present study the biochemical property of integumentary mucus depending on their ecological habitat of aquatic environment of (Catla *catla*), (*Labeo rohita*) and (*Cirrhinus mrigala*).

Materials and Methods

Fish mucus collection

The integumentary mucus sample was collected form healthy fish(n=10) (*Catla catla*), (Labeo *rohita*) and (*Cirrhinus mrigala*). Fish mucus were collected by modified method of Subramanian *et al.,* fish was starved for one day prior for mucus collection. On the day of mucus collection fish was washed and transferred into sterile polyethylene bag for 10 to 20 minutes and moved front and back to slough off the fish mucus Wei *et al.,* The collected fish mucus thoroughly mixed with equal quantity of physiological saline (0.85% NaCl) solution and centrifuge at 5000 rpm for 10 minutes. The supernatant was stored at 4°C for biochemical studies.

Mucus biochemical characterization: The supernatant was subjected to qualitative and quantitative assays to estimate the biochemical constituents. The supernatant was analysed for biochemical constituents. During the study Protein analysis was done by Lowry assays *et al.,* (1951). Carbohydrate analysis was estimated by Anthrone test.

Lowry assay (Lowry *et al.,* 1951)

This is a biochemical method used to determine total protein in sample.

Procedure

5 ml of Lowry reagent is added to 1.0 ml of diluted protein extract of epidermal mucus which is obtained by proteolytic enzyme assay of epidermal mucus, and then it is allowed to stand for 10 min. at room temperature. Then 0.5 ml of Folin's reagent is added and mixed thoroughly. After 30 min. a blue colour develops. The optical density is measured at 660 nm using spectrophotometer. Distilled water is used as blank. The amount of protein is calculated

Seasonal Effect of Biochemical Composition of Fish Mucus.

Season	Biomolecules	*Catla catla* µg/ml	*Labeo rohita* µg/ml	*Cirhinnus mrigala* µg/ml
Summer Season	Protein	91.171	164.493	188.62
	Carbohydrate	16.663	15.653	16.18
Rainy Season	Protein	982.928	541.536	834.872
	Carbohydrate	48.341	22.929	37.352
Winter Season	Protein	676.531	115.762	116.592
	Carbohydate	30.532	22.346	31.448

Fig. 1. Fish Integumentary Mucus

Fig. 2. Concentration of Protein

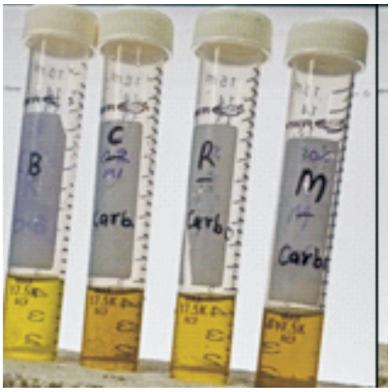
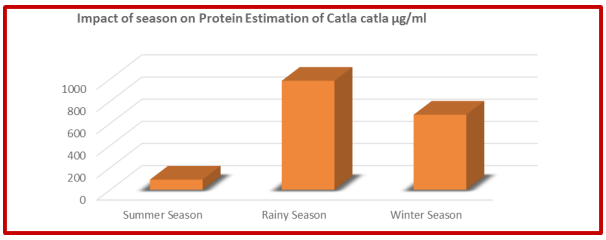
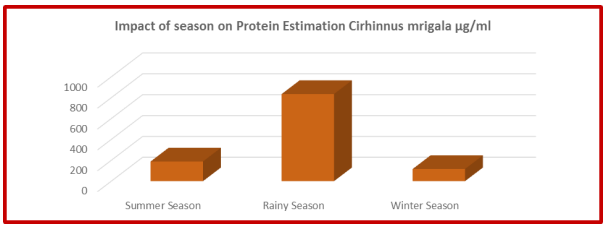
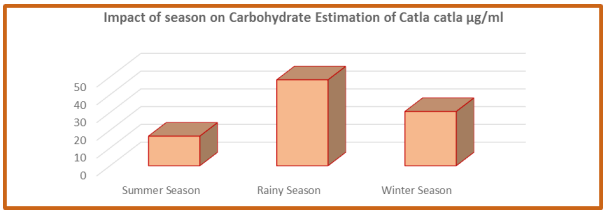
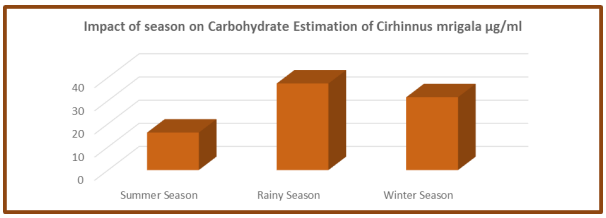


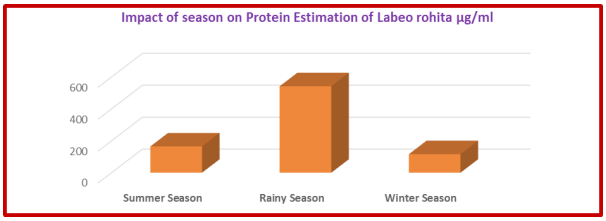
Fig. 3. Concentration of Carbohydrate

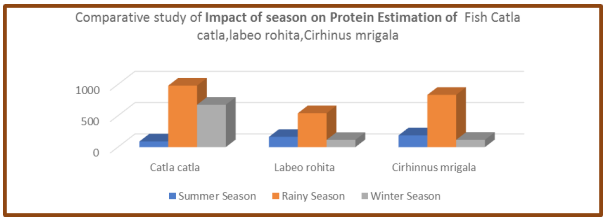


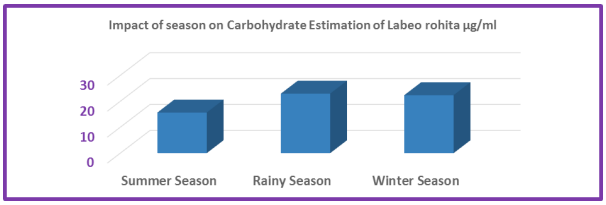


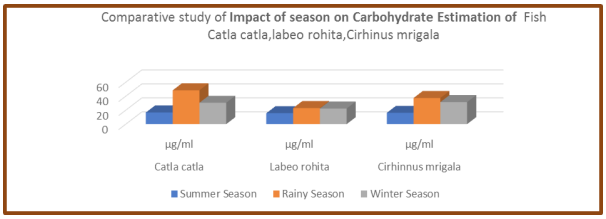












by plotting a standard curve, and Bovine Serum Albumin (BSA) is taken as standard protein.

Carbohydrate estimation-

Anthrone test (Trevelyan *et al.*, 1952)

2 ml of anthrone reagent is poured into clean test tubes. Few drops of fish epidermal mucus is added into the test tubes and mixed. The mixture is allowed to stand for 10 minutes. For standard curve; distilled water is used as blank. The absorbance is taken at 620 nm against blank.

Results and Discussion

In the present study we find out the characteristics of fish mucus find in the different ecological zone and also the seasonal impact on their biochemical composition. there are significant differences that occurred in the concentration of chemical composition of mucus. It was observed that the Concentration of protein (982.92µg/ml) and carbohydrate (48.34µg/ml) is high in the rainy season while low in the summer season. (Protein 91.17µg/ml and carbohydrate 16.63 µg/ml) respectively.

Biochemical nature of the mucus of fish has been investigated by many researchers. According Pickering (1977), mucus layer is a biological interface between fish and their aqueous environment that consist of biochemical diverse secretions from epidermal and epithelial cells. Videler *et al.*, (1999) stated that, mucus, secreted by external epithelial goblet cells possess biochemical antibiotic compounds. Ellis (2001) reported variety of antimicrobial compound such as AMPs, lysozyme, protease and lectins in mucus secreted by epidermal epithelial cells.

In present findings suggest that integumentary mucus of Catla *catla. Labeo rohita* and *Cirrhinus mrigala* is rich protein source and various innate immune components. Which also support our findings that amount of mucus secretion was more in rainy as compared to winters and summer season

and concentration of protein is high in Catla in rainy and winter season. These factors show important role in the susceptibility of fish infection. In this study the seasonal variation of Protein and Carbohydrate concentration in the skin mucus of fresh water fishes which are present in three ecological zone of water.

Conclusion

The fish mucus contains various innate immune component concentrations to formulate new drugs for the therapy of pathogenic microorganisms. These properties of mucus indicate that the fish mucus may be beneficial in aquaculture, human-related problem and the agriculture field.

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Conflict of interest

We declare that we have no conflict of interest.

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