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Effects of Changing pH on the Nutritional Value and Histological Structure of Apple Snail (*Pila Globosa*)

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ABSTRACT

Pila globosa is found in freshwater ecosystems such as ponds, streams, rivers, lakes, rice fields, irrigation canals, and wetlands with low salinity. In this study, we aimed to analyze the change in the nutritional levels and histological changes of Pila globosa in changing pH levels. To study the pH tolerance, the snails were exposed to different pH (4, 5, 6, 8, 9, 10). At pH 4 and 10, snails showed 100% mortality rate within 24 hours. The snails treated with pH 5, 6, 8, and 9 survived throughout the experiment. These snails were kept in the treated water for about 72 hours (3 days). After that, biochemical and histological studies were performed for nutritional level. The foot and digestive gland were used for histological evaluation. The control group, having pH 7, showed a mean value of 0.133±0.003 gm/gm protein in foot tissue. The snails with pH 5, 6, 8, 9 showed a mean value of 0.122± 0.004, 0.127±0.003, 0.142±0.003 and 0.146±0.003 gm/gm of tissue protein, respectively. The histological analysis of the foot showed the changes in disruption of the epidermis and formation of large vacuoles. And the histological analysis of the digestive gland showed more K corpuscles in pH 5 and pH 9 in comparison with the other two treated groups and the control.

Keywords: *Pila globosa*, digestive gland, foot, K corpuscles

I. INTRODUCTION

The genus Pila includes about 30 species of snails, with *Pila globosa* being one of the most significant. *P. globosa* is mainly found in the Oriental and Ethiopian regions. In the Oriental region, it occurs in India, Sri Lanka, Burma, Thailand, Indonesia, Malaya, Vietnam, and the Philippines, while in the Ethiopian or Afrotropical Region, it is found in Africa, Arabia, and Madagascar. It inhabits freshwater ecosystems such as ponds, streams, rivers, lakes, rice fields, irrigation canals, and wetlands with low salinity. The environment where the organism lives is affected by various abiotic and biotic factors. One important factor for aquatic organisms is pH, which influences enzyme activity. Temperature, light intensity, salinity, and pH are vital for the survival and distribution of organisms [1], and maintaining optimal pH is crucial for key biological processes.

The significance of molluscs has increased significantly, providing substantial socio-economic benefits to local communities. Historically, molluscs have been an important food source, playing a crucial role in diets. Traditional knowledge about their various uses—both as food and medicine—has greatly contributed to their potential exploration in scientific research [2]. Freshwater gastropods, in particular, are in high demand due to their accessibility and ease of collection, which aids the economic well-being of tribal communities [3]. The species *Pila globosa* is edible and has been traditionally consumed by Aboriginal people [4]. Additionally, it has medicinal uses, treating various ailments such as rheumatism, heart disease, high blood pressure, asthma, rickets, calcium metabolism issues, nervousness, and dizziness [4]. Furthermore, it is known to provide essential vitamins and minerals that may be lacking in the diet.

Various tribal communities around the world consume snail meat, and a study noted that 29 groups of tribal people in Bangladesh specifically consume the flesh of *P. globosa* [5]. In some rural areas of Bihar, certain freshwater mollusc species are particularly enjoyed by middle- and working-class families [6]. The raw meat of *Pomacea canaliculata* contains 78.10% water, 15.62% protein, 0.8% fat, 2.60% ash, and 2.88% carbohydrates, while the boiled meat contains 77.40% water, 13.67% protein, 0.4% fat, 4.1% ash, and 4.43% carbohydrates [7]. In *P. globosa*, the lipid and protein content have been found to be 2.30% and 33.81%, respectively [8]. The snail trade is an important component of international trade [9], and globally, the demand for snail meat is growing, especially in developing countries [10]. However, several factors, including climate change, water pollution, and overexploitation of molluscs, have significantly contributed to the decline in snail populations and production [11].

The survival and distribution of organisms are determined by environmental factors [12]. One important factor is pH, and at certain pH values, enzymes work best. Freshwater snails can tolerate a wide range of pH. However, extreme pH environments, particularly acidic conditions, are known to induce ionoregulatory imbalance in freshwater molluscs, as observed in the snail *Elimia flava* when exposed to an acidic pH [13]. In their study, Sullivan et al. [12] observed that when the snail *Biomphalaria glabrata* was exposed to a wide range of pH values, the snails were only found in areas where the pH was tolerable for them. In solutions with pH 2.0 and pH 12.0, they died within one hour. Snails at pH 3.0 and 11.0 did not die immediately, but within 24 hours, all snails at pH 11.0 were dead.

Certain chemicals (like pesticides) have significant impacts on non-target organisms, particularly in aquatic environments, where they can affect a variety of species, including invertebrates and fish. A noteworthy study by Otludil et al [14] revealed the effects of endosulfan on the snail species *Planorbarius corneus*, highlighting the complex interactions between environmental factors and aquatic life. This research provides valuable insights into the ecological and toxicological implications of pesticide exposure. During their investigation, histopathological analyses revealed several notable changes in the snails, such as infiltration of amoebocytes, dilation of hemolymphatic spaces between tubules, cell degeneration, abnormal lumen formation, and cell necrosis. Additionally, they observed atrophy of connective tissue in the digestive gland, desquamation of epithelial cells, changes in the number of mucocytes and protein gland cells, the appearance of lipid vacuoles, and atrophy of columnar muscle fibers in the foot and mantle tissues. Toxicological studies have suggested that the intracellular symbiotic corpuscles (designated as C and K), which secrete a protease into the digestive tract, play a role in the accumulation of non-essential toxic elements and are directly affected by certain chemicals [15]. Notably, Arrighetti et al [16] reported other histopathological alterations in the digestive gland, including hemocyte infiltration, an increase in the number of K corpuscles, epithelial atrophy, and acinar necrosis.

Building upon these findings, the current study aims to explore the effects of pH variations on the nutritional value and histological characteristics of the apple snail (*P. globosa*). This work is expected to contribute to a deeper understanding of how environmental changes can affect the health and well-being of aquatic organisms.

II. MATERIALS AND METHODS

A. Collection of samples

Healthy freshwater snails, *Pila globosa,* were collected from the ponds in Alagjari village, which is located in Boko circle of Kamrup (R) District, Assam.

B. Experimental Design

After collection (Figure 3), the snails were maintained in earthen pots (Figure 2) and acclimatized for 7 days in our laboratory conditions. The snails were fed with green leafy vegetables. pH levels 4, 5, 6, 8, 9 and 10 were used to carry out the experiment against a control group having pH 7 for 3 consecutive days. Concentrated HCl and NaOH were used throughout the experiment to prepare the required pH. The estimation of physiochemical parameters of water, namely temperature, pH, and dissolved oxygen were analysed by standard methods described in APHA [17]. After 3days of treatment, the animals were sacrificed, and the edible parts were collected for biochemical and histopathological observations. The Bradford method [18] was used to measure the amount of total protein in the meat. A routine eosin-hematoxylin staining procedure was used for the histopathological study of the foot and digestive gland.

III. RESULTS

A. Survey report:

The village of Alagjari is predominantly inhabited by the Rabha and Bodo communities, who regard snails, known as pila (Figure 1), as a delicious and nutritious food source. They prepare a variety of recipes using snails, including a traditional curry that incorporates black grams (Figure 4). The local belief is that consuming snails can enhance eyesight, alleviate gastrointestinal issues, help with asthma, improve night vision, support normal kidney function, and reduce joint pain.

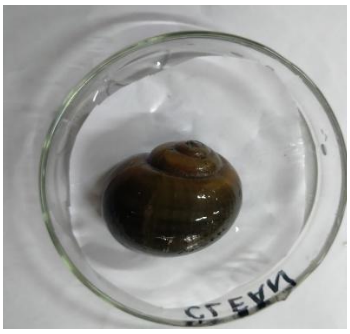

Figure. 1. Pila globosa

Figure. 2. Earthen pots are used to culture experimental animal

Figure.3. Collection of the specimens

Figure.4. Processing of the snails for food

B. Behavioral Observation:

Throughout the treatment period, we closely monitored the snails and noted a striking phenomenon: those exposed to extreme pH levels of 4 and 10 experienced a complete mortality rate (100%), within 24 hours. As they succumbed to the harsh conditions, an excessive amount of mucous was secreted by the snails (Figure 5), serving as a desperate adaptive mechanism to cope with the rapid changes in pH levels. In stark contrast, the snails treated with pH levels of 5, 6, 8, and 9 displayed remarkable resilience, surviving undeterred throughout the entirety of the experiment. Their ability to thrive in these more balanced conditions highlights the critical role that pH plays in their overall well-being.

Figure. 5. Excessive Mucous Secretion

C. Total protein

In the analysis of meat portions, a noticeable decline in total protein content was observed at acidic pH levels of 5 and 6, with values recorded at 0.122±0.001 and 0.127±0.001 gm/gm of tissue, respectively. In stark contrast, at the more alkaline pH levels of 8 and 9, the total protein content significantly increased, measuring 0.142±0.001 and 0.146±0.001 gm/gm of tissue, respectively (Figure 6). These changes were evaluated in comparison to the control, which had a total protein level of 0.133±0.001 gm/gm of foot tissue, underscoring the impact of pH on protein composition in meat.

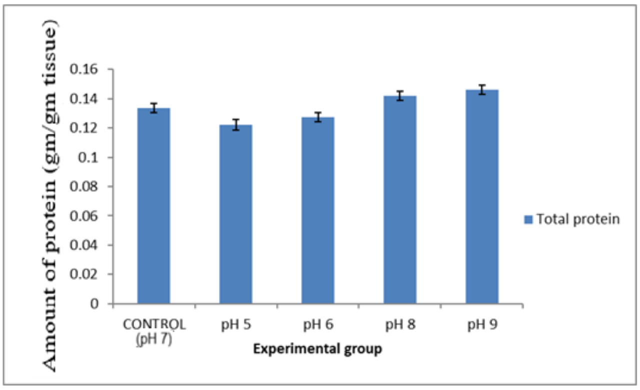

Figure. 6. Showing total protein at different pH levels with their mean ± standard

D. Histological observations

The histological observations of the snail's foot were most pronounced at pH 5 and pH 9. Snails treated with pH 6 and pH 8 showed a small increase in the size of the vacuoles within their muscle fibers. In contrast, those treated with pH 5 and pH 9 exhibited a substantial increase in the size of these vacuoles, along with irregular folding of the epidermis (Figure 7). In comparison, snails in the control group displayed normal histology of the foot. Additionally, the snails in the control group showed normal histology in their digestive glands with K corpuscles. The digestive glands of snails treated with pH 6 and pH 8 demonstrated a slight increase in the number of K corpuscles, whereas the glands of snails treated with pH 5 and pH 9 exhibited a much more significant increase in the number of K corpuscles (Figure 8).

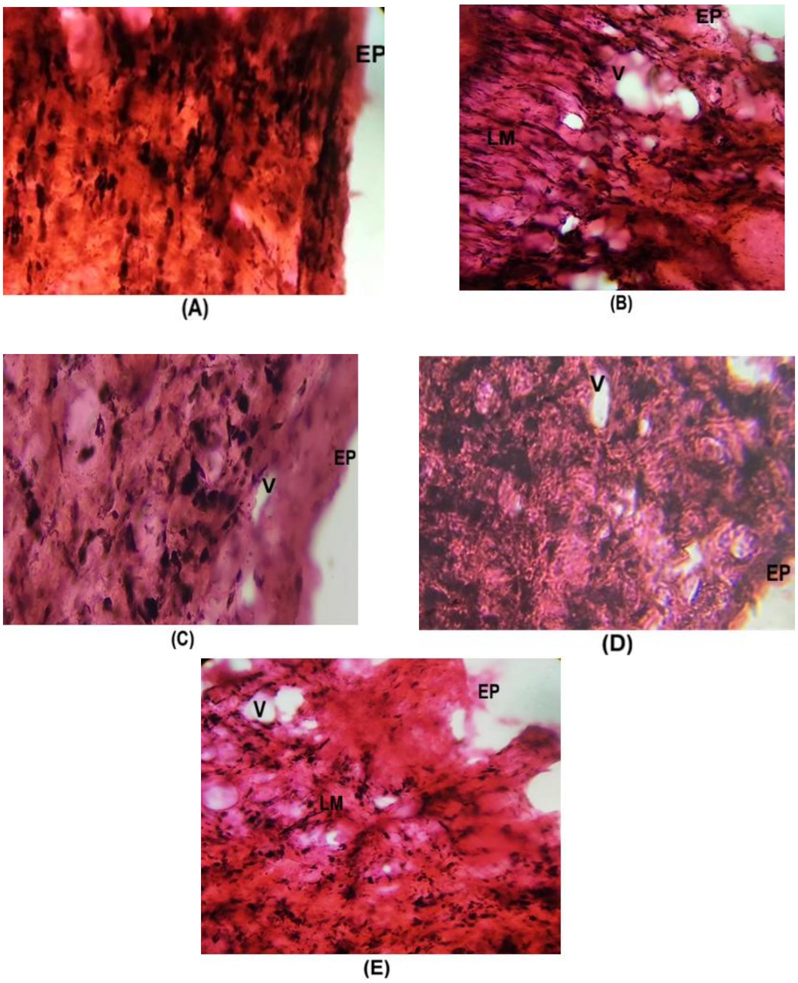

Figure. 7. Histological sections of the foot of *Pila globosa* (A-E) at 40x (A: Control; B, C, D, E treated with Ph 5, 6, 8 and 9, respectively, for 3 days). Here, ES – Vacuoles, LM - Longitudinal muscles, and EP - Epidermis.

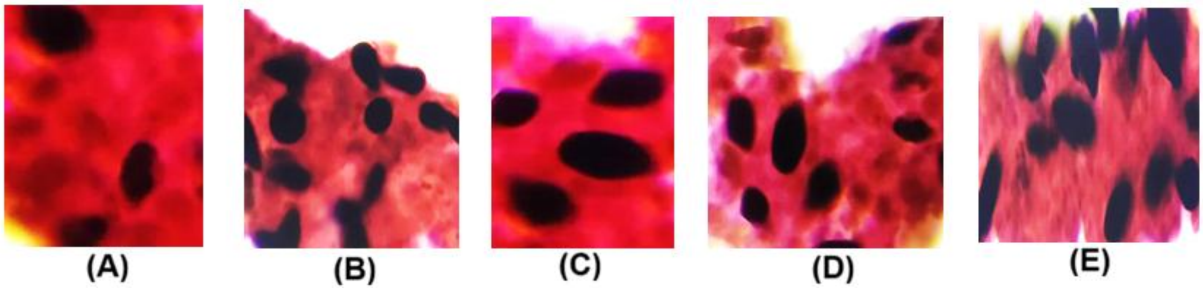

Figure. 8. Histological sections of the digestive gland of *Pila globosa* (A-E) at 40x. (A: Control; B, C, D, E treated with Ph 5, 6, 8 and 9, respectively, for 3 days).

IV. DISCUSSION

In the present study, we observed that an increase or decrease in pH has a profound effect on the physiology of the organisms. Investigating the effects of changing pH on the nutritional value and histological alterations of the snail is essential for understanding its adaptability and health. Apple snails are a significant source of protein, minerals, and nutrients, comparable to other shellfish. Snails, however, also serve as an intermediate host for several zoonotic parasites. A category of parasitic infections, known as snail-transmitted parasitic diseases (STPDs), is harmful to humans and animals and is mainly driven by various trematodes, cestodes, and nematodes [19]. Therefore, proper cooking is essential. Before proper cooking, they must also be cleaned to remove slimy secretions, typically using salt or lime.

Throughout the treatment period, we observed that the snails exposed to extreme pH levels of 4 and 10 experienced 100% mortality rate within just 24 hours. As they succumbed to the harsh conditions, an excessive amount of mucous was secreted by the snails, serving as a desperate adaptive mechanism to cope with the rapid changes in pH levels. In contrast, the snails treated with pH levels of 5, 6, 8, and 9 displayed remarkable resilience, highlighting the critical role that pH plays in their overall well-being. This finding was consistent with the works of Ewald et al. [13], who reported that an extreme pH in environments, especially acidic ones, is known to cause an ionoregulatory imbalance in freshwater molluscs (snail *Elimia flava*) when exposed to pH 4 (acidic).

In the analysis of meat portions (foot tissues), a noticeable decline in total protein content was observed at acidic pH levels of 5 and 6, whereas a significant increase in total protein content was observed at the more alkaline pH levels of 8 and 9, when compared to the control, underscoring the impact of pH on protein composition in meat. In their experiment, Ewald et al. [13] also observed main changes in the metabolites in *Elimia* flava, when exposed to an alkaline pH. They observed an increased level of Glucose (at pH 9) and total protein (at pH 9 and 10).

Histological observations of the snail's foot were most pronounced at pH 5 and pH 9, showing a substantial increase in the size of vacuole within muscle fibers and irregular epidermal folding compared to the snails at pH 6 and pH 8, which had a slight increase. Moreover, snails at pH 6 and pH 8 had a slightly increased number of K corpuscles, while pH 5 and pH 9 showed a significant increase. In contrast, the control group displayed normal histology in both the foot and digestive glands. K corpuscles (brown concretions) are excretory in function [20, 21]. The number of K-corpuscles increases in response to exposure to toxins, suggesting a protective [22], stress-responsive role. Therefore, changes in the histological structure of these cells may influence the overall physiology of the animal.

V. CONCLUSION

Research on the pH requirements of the freshwater snail *Pila globosa* is surprisingly sparse. This is probably because of the complexities associated with studying pH, a critical variable that influences a myriad of metabolic processes. Understanding the specific conditions of the environment in which these snails thrive is essential for predicting how changes in pH might affect their biological functions. Living within an optimal pH range ensures that the vital processes necessary for the snail's survival and overall health are operating efficiently, ultimately contributing to the resilience of this aquatic organism in the face of environmental fluctuations.

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