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Article

Vitamin C Dynamics in the Reproductive Tissues of Starved Clarias batrachus: Male vs. Female

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Abstract: This study explores the impact of prolonged starvation on Vitamin C levels in the reproductive tissues of male and female Clarias batrachus. Previous research has primarily focused on mammalian fauna, leaving a gap in understanding the starvation effects on fish in Nepal. Using a 24-dinitrophenylhydrazine method, Vitamin C concentrations in the testes and ovaries were measured after 10, 20, 30, and 40 days of starvation. Findings revealed that under normal conditions, males had higher Vitamin C levels (13.26 mg/100 g) compared to females (8.41 mg/100 g). Prolonged starvation led to a significant decline in Vitamin C levels, with females experiencing a 53% reduction and males a 51% reduction by the end of the 40-day period. These results indicate a differential impact of starvation on male and female reproductive health, underscoring the need for further research into the physiological and biochemical adaptation mechanisms of Clarias batrachus during food scarcity.

Keywords: Catfish, Gonads, Starvation, Vitamin C

1. Introduction

It has been discovered that higher animals can only endure famine for short amounts of time. On the other hand, fish may endure extended periods of starvation in their natural habitats under a variety of stressful circumstances [1].

Numerous bony fish species exhibit the capacity to endure extended periods of famine, which can span from a few months to several years [2]. Due to their easy availability in nearby ponds and rivers, the common freshwater walking catfish, Clarias batrachus, was employed as an experimental animal in this study.

This study aims to learn about the facts, causes, and effects of starvation on animals. Numerous researchers have conducted many comparable studies; however, the majority of their efforts have only focused on mammalian fauna. Research on hunger's impacts on fish has not been conducted much in Nepal. Because fish have a special ability to endure extended hunger through physiological and biochemical changes, it is important to observe and disclose the effects of fasting on fish [3]. Fish health and biochemical makeup are impacted by starvation [4, 5].

Based on the aforementioned information, the current study aims to determine the concentration of Vitamin C in the testes and ovaries of Clarias batrachus throughout a 40-day protracted period of famine by evaluating the level of Vitamin C constituents every 10 days.

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2. Materials and Methods

The methodology of this study involved selecting healthy Clarias batrachus from a nearby pond and transporting them to the laboratory in large clay pots covered with mosquito nets. Upon arrival, the fish were treated with a 0.1% $KMnO_4$ solution for five minutes to eliminate any potential skin infections. Healthy fish with an average length of 18.8 cm and weight of 34.4 g were acclimated in a laboratory setting for twenty days, during which they were fed commercial fish meal twice daily. Feeding was discontinued 24 hours before the experiment began to ensure the alimentary canals were empty. The fish were then divided into four groups (A, B, C, and D), each consisting of ten fish (five males and five females). Group A was subjected to starvation for ten days, while groups B, C, and D were starved for twenty, thirty, and forty days, respectively. Every ten days, fish from each group were dissected to extract and measure Vitamin C levels in the testes and ovaries using the method described by Kanungo and Patnaik (1964) [6], with modifications from Roe (1954) [7]. This involved homogenizing the reproductive tissues in a 6% trichloroacetic acid (TCA) solution, centrifuging the homogenates, and oxidizing the ascorbic acid with bromine water. The resulting solution was filtered, and the excess bromine was removed by passing air through the filtrate. The Vitamin C concentration was then quantified using the 24-dinitrophenylhydrazine method and measured at a wavelength of 520 nm. Standards were prepared to generate a standard curve for calculating the ascorbic acid concentration in each tissue sample, reported as milligrams per 100 grams of wet tissue weight.

3. Results

Under both normal and deprived conditions, males in the current study had comparatively greater levels of Vitamin C than females. The Vitamin C content of the gonads gradually decreased as a result of starvation in the fish. Significant depletion was only seen in the testes and ovaries of the fish after 20 days of fasting. Following 40 days of fasting, the testes had around 51% and the ovaries were approximately 53% depleted of ascorbic acid. The amount of Vitamin C present in the testes and ovaries of Clarias has been shown in Table 1 and Figure 1.

Table 1. Vitamin C content (mg/100gm wet tissue) of the gonads in male and female C. batrachus

Organs	Control	Periods of Starvation (in days)			
		10	20	30	40
Testis	13.26 ± 0.17	12.03 ± 0.24	11.49 ± 0.25	8.91** ± 0.25	6.43** ± 0.31
Ovary	8.41 ± 0.16	8.05 ± 0.24	7.59 ± 0.25	5.53** ± 0.25	3.96** ± 0.12

Values are the mean of eight samples of both male and female fish ± SE

** Significant

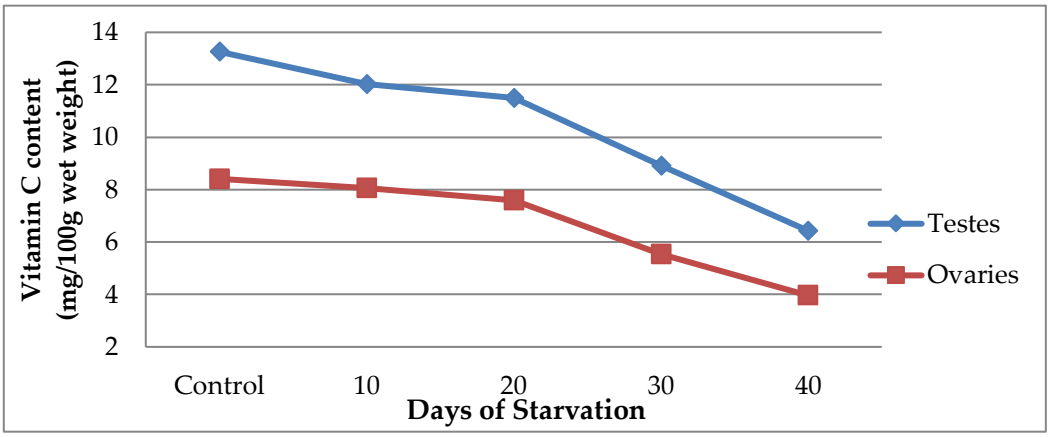

Figure 1. Impact of Starvation on Vitamin C content in the gonads of *C. batrachus*.

4. Discussion

Vitamin C, also referred to as ascorbic acid, is an organic molecule that possesses notable antioxidant characteristics. It occurs naturally in various fruits and vegetables and plays a crucial role in maintaining overall health. Despite a limited understanding of the specific metabolic processes that necessitate ascorbic acid, it is widely believed to function as a catalyst within the oxidation-reduction system, contributing to various biochemical reactions [8]. Additionally, ascorbic acid is recognized for its potent antioxidant capabilities and its ability to mitigate fatigue [9].

The importance of ascorbic acid extends to its essential role in the biosynthesis of connective tissue and the facilitation of wound healing, as highlighted by [10]. Seasonal variations in ascorbic acid levels have been documented, with concentrations tending to rise in the summer months and decline during winter [11]. This fluctuation suggests a potential link between environmental factors and the physiological demand for vitamin C.

Moreover, ascorbic acid is indispensable for the healthy development of precursor cells in various tissues. It plays a vital role in preserving the integrity of intracellular substances, including collagen and mucoprotein, which are crucial components of connective tissues, teeth, bone, and cartilage [12]. The maintenance of these substances is essential for the structural and functional stability of these tissues, underscoring the significance of adequate ascorbic acid intake for overall health and well-being.

Nearly all vertebrates synthesize ascorbic acid from hexose molecules, including glucose. Humans, monkeys, and guinea pigs are the only known species that rely on food sources for ascorbic acid [12]. Like other animals, fish have tissues that contain and manufacture ascorbic acid. The ascorbic acid levels found in the liver, brain and muscles of Clarias batrachus are consistent with findings by [13, 14, 15]. Following ten days of hunger, the ascorbic acid content in the gonads of Clarias batrachus decreases and keeps declining as the number of starving days increases (Table 1).

Starving animals rely on the tissues that make up their bodies to survive [16]. For the synthesis of ascorbic acid, the animal is dependent on the dietary intake of hexoses [17]. When an animal is starving, it consumes carbs quickly, which prevents it from getting enough hexoses. As a result, ascorbic acid synthesis declines during famine, as shown by the body's tissues and organs having lower ascorbic acid concentrations. It is important to note that the trend of decreasing ascorbic acid concentration during famine is similar to that of decreased glycogen concentration. The current observations agree with [15, 18, 19, 20].

5. Conclusion

This study highlights the significant impact of prolonged starvation on Vitamin C levels in the reproductive tissues of Clarias batrachus, revealing a marked depletion in

both male and female gonads, with a 51% reduction in males and a 53% reduction in females after 40 days of food deprivation. These findings underscore the differential physiological responses to starvation between genders, emphasizing the critical role of Vitamin C in maintaining reproductive health under nutritional stress. The results have profound implications for understanding the adaptive biochemical mechanisms in fish, particularly in relation to their resilience to environmental stressors. Further research is warranted to explore the underlying metabolic pathways and potential strategies to mitigate the adverse effects of starvation on aquatic species, contributing to improved management and conservation practices.

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