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**UNIVERSITAS MUHAMMADIYAH SIDOARJO**

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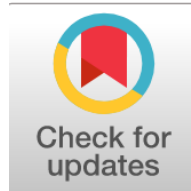
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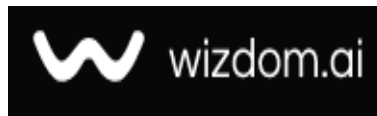
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**Effect of Different Concentrations of Azolla sp. Plant on Protein Concentration in the Gills and Muscles of Grass Carp : Pengaruh Konsentrasi Berbeda Tanaman Azolla sp. terhadap Konsentrasi Protein pada Insang dan Otot Ikan Mas Rumpot**

**Aya Sabah Abdali, aya.abdali@uobasrah.edu.iq (\*)**

*Department of Biology , College of Education Qurna, Basrah University, Iraq*

**Akeil Jameil Mansour, aya.abdali@uobasrah.edu.iq**

*Department of Biology , College of Education Qurna, Basrah University, Iraq*

(\*) Corresponding author

**Abstract**

**General Background** Protein content in fish tissues is closely related to diet composition and nutritional quality in aquaculture systems. **Specific Background** Azolla sp. is a rapidly growing aquatic plant recognized as a low-cost protein source suitable for herbivorous fish such as grass carp. **Knowledge Gap** However, limited information is available regarding tissue-specific protein concentration responses, particularly in gills and different muscle regions, following graded dietary Azolla inclusion. **Aims** This study aimed to evaluate the effects of different Azolla concentrations on protein levels in the gills and muscles of grass carp fingerlings. **Results** One hundred and twenty fish were fed diets containing 0%, 15%, and 30% Azolla, and protein concentrations were determined using the BCA method. The results showed clear variations in protein concentration across tissues, treatments, and experimental periods, with statistically significant differences observed in gill and muscle regions. **Novelty** This study provides region-specific evidence of protein distribution in gills and anterior and posterior muscles in response to Azolla-based diets. **Implications** The findings support the potential use of Azolla as a dietary component in grass carp feeding strategies while highlighting its physiological relevance at the tissue level.

**Keywords:** Azolla Sp., Grass Carp, Gill Protein, Muscle Protein, Plant-Based Feed

**Key Findings Highlights:**

Protein concentrations varied significantly among gills and muscle regions across dietary treatments.

Distinct responses were observed between anterior and posterior muscle tissues.

Graded Azolla inclusion demonstrated tissue-specific nutritional responses.

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## Introduction :

Protein content in fish feed is one of the most important nutrients. Protein levels in the diet significantly affect growth rates, feeding efficiency, and tissue health in fish [1]. Azolla plant is a small, floating aquatic plant that grows rapidly and covers the surface of ponds during the rainy season. It serves as a free food source. A member of the Salviniaceae family, it can be used as a direct fish feed or as a feed ingredient, providing an alternative protein source. It is a low-cost, locally available plant [2] [3]. It is rich in amino acids such as arginine, valine, lysine, and leucine, as well as a rich source of unsaturated fatty acids [4]. The grass carp recently reclassified in the family Xenocypridinae rather than Cyprinidae based on recent evolutionary studies [5], prefers to feed on aquatic plants, including Azolla Pinnata. It has been shown that adding plant protein can improve meat quality by enhancing muscle characteristics, reducing fat accumulation, and boosting collagen production [6] [7]. Studies have also demonstrated that providing optimal protein levels in fish diets increases muscle protein concentration and improves gill function [8]. The current study aims to determine the effect of different concentrations of Azolla in the diet on protein concentrations in the gills and muscles of grass carp fingerlings.

## Materials and Methods

### 2.1 Sample collection

120 samples of grass carp fingerlings (*C. idella*) were collected from the fish farming ponds of the Marine Science Centre/University of Basra between December 2024 and January 2025. The samples were transported using special plastic containers to the Environmental Laboratory of the Department of Life Sciences at the College of Education, Al-Qurna/University of Basra. The fish were acclimatised for ten days. They were then randomly distributed into six glass tanks with dimensions of 40cm x 60cm x 40cm as follows:

1. The two control groups (T1): Each group consisted of 20 fish fed a conventional diet free of Azolla.
2. The two 15% replacement groups (T2): Each group consisted of 20 fish fed a diet containing 15% Azolla.
3. The two replacement groups (T3) (30%): Each group consisted of 20 fish fed a diet containing 30% Azolla.

During the experiment, the fish were fed a laboratory-formulated diet at the studied proportions (Table 1).

T3(30%)	T2(15%)	T1( 0%)	Component
30%	15%	0%	Dried Azolla
20%	35%	50%	Fish meal
25%	25%	25%	Flour