

Estimation of the Nutritional Efficiency of Azolla Plant for Common Carp Fish (*Cyprinus carpio*)

Woroud M. Abdullah, Suaad k. Abd-Al-Wahab

Biology Department, Education for Pure Science, University of Diyala, Iraq

Corresponding Email: Woroudmohammed4@gmail.com

ORCID: <http://0009-0006-5141-1311>

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Abstract:

Background: The experiment was carried out in glass aquaria (100 × 40 × 40 cm) with juveniles of common carp (*Cyprinus carpio* L.) that were obtained from a local fish breeder in Jadidat Al-Shatt, Diyala Governorate, from 25 October 2024 until 5 January 2025. Each aquarium was stocked with 10 fish and three replicates per each treatment. The treatments consisted of commercial feed that contained 35.58% crude protein (control), second one with 80% commercial feed and 20% dried Azolla, third group with 80% commercial feed and 20% fresh Azolla. The fish were fed at the rate of 5% of their weight (total biomass) in 3 meals a day with the feed amount being adjusted every two weeks. Water was changed every two days (replaced 70% of total aquarium water) using clean water.

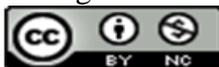
Aims: The planned study was designed to assess the potential for Azolla to serve as a partial protein substitute in carp diets and to check their impacts on growth rate, growth and relative growth rate.

Results: The statistical analysis indicated that a significant difference was found among the treatments as the fresh Azolla treatment significantly resulted in the highest weight gain (3.48 g. fish⁻¹) among all treatments compared to dried Azolla (2.36 g. fish⁻¹) and control treatment (1.94 g. fish⁻¹). Similarly, for growth rate, the dried Azolla treatment had the highest growth rate (67.50 g. fish⁻¹), which was significantly greater than the control treatment (39.00 g. fish⁻¹). The diet based on dried Azolla also resulted in the highest relative growth rate (245.88%) compared to the fresh Azolla treatment (161.10%). In addition, the dried Azolla treatment had the best feed conversion ratio of 2.935 g. fish⁻¹ when compared to control and fresh Azolla treatment (3.250 and 3.343 g. fish⁻¹ respectively).

Conclusions: In conclusion, the study found that feeding common carp diets with Azolla improved growth performance when compared to feeding commercial feed only. It was also found

that replacing 20% of commercial feed with Azolla improved growth performance indicators without any effects on fish health.

Keyword: Feed efficiency, Azolla fern, Relative growth rate, Growth rate



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Introduction:

The common carp (*Cyprinus carpio* L.) belongs to the Cyprinidae family and is among the native fish species of inland waters in Iraq. It is found all over the world (Al-Faisal et al., 2020), and is among the most economically important fish species to Iraq (Mahmood *et al.*, 2023). It is often produced in Iraqi fish farms, with other fish species like silver carp and grass carp. Fish are an important food source and provide highly nutritious food, containing important minerals and nutrients. Fish provide around 16% of total animal protein consumed globally, making them a very important and efficient source of protein for humans (Najem *et al.*, 2024). Aquaculture production has faced many challenges, especially in relation to population increases, as there is pressure regarding food resources and increasing demand for feed ingredients. In recent years, the rising costs of feed production have risen over 50%, which has caused feed manufacturers to innovate formulations suitable for the needs of the aquaculture industry (Akintan et al., 2024). Fishmeal, which is derived from wild fish, is still a major component in aquafeeds and an important source of protein. Nevertheless, rising demand, unsustainable fishing practices, high costs, and unreliable supply chains have created deficits that require an urgent search for cheaper and nutritionally beneficial alternative protein sources. Plant-based feed ingredients are a sustainable, efficient, and economically viable alternative (Hussain *et al.*, 2024; Action, 2020).

Azolla is a small aquatic fern found in lakes, ponds, and wetlands that is commonly utilized as a biofertilizer when growing rice and is best known for its ability to float on water surfaces in temperate and tropical conditions (Padhlary and Behera, 2017). The protein content present in Azolla ranges from 23% to 27%. Azolla is easily cultivated and grows quickly enough for use as supplemental feed in a variety of domestic and cultivated animals, including goats, sheep, fish, buffalo, and cattle (Katole *et al.*, 2017). Studies demonstrate successful application of Azolla microphylla in animal diets due to its excellent nutritional profile (Chatterjee *et al.*, 2013). Additionally, Azolla is known to support healthy growth and boost immune capabilities in carp and other fish. Nonetheless, Azolla is not recommended to be used as an all-inclusive diet as it can impede growth rates of fish (Sahu *et al.*, 2020). Choudhury et al. (2018) suggested that Azolla supplemented into fish feed improved fish growth and enhanced overall health of the fish. The high crude protein content (19-30% of dry weight) in Azolla makes it an ideal feed ingredient for improving feed efficiency and reducing feeding costs. Research suggests that fish fed dried or fresh Azolla diets have good growth performance, with dietary inclusion levels

of approximately 10%-25% effective for common carp and levels above 50% resulting in reduced fish growth and feed efficiency. While Azolla may not be suitable as a full stomach contents diet, the inclusion of Azolla into fish feeds, as an ingredient, can substantially reduce feed costs (Mosha et al., 2018). Research on Nile Tilapia has noted improved growth when part of the soybean meal was replaced by dried Azolla powder at inclusion levels of 25%-50%, with no negative effects reported (El-Sayed *et al.*, 2023). Also, Hundare *et al.*, (2018) and Mounes et al. (2020) showed that varying levels of Azolla could replace fishmeal or soybean meal major protein sources of commercial diets while improving fish growth parameters, significantly improved immune response, decreased mortality, and improved aquaculture efficiency. Wet Azolla has been indicated to be more practical and more effective than mixing dried Azolla into formulated feeds (Korsa *et al.*, 2024).

This study therefore intends to assess the feasibility of Azolla as a partial substitute for protein in the diets of carps and measure the effects of fresh and dried Azolla on growth rate, absolute growth rate, and relative growth rate.

Materials and Methods:

Sample Collection

The experiment was conducted from 25 October 2024 to 5 January 2025 in glass tanks measuring 100 cm in length, 40 cm in width, and 40 cm in height. The fish were acclimatized to the experimental conditions for 14 days prior to the initiation of the trial. Juvenile common carp (*Cyprinus carpio* L.) were purchased from a local fish breeder in the Jadidat Al-shatt, Diyala Governorate, The initial weight of the fish was 10.36 grams. Ten fish were stocked in each tank, with three replicates per treatment, as shown in Figure (1).

The experimental feeds included 100% commercial feed. The chemical compositions of the commercial feed and the Azolla plant were analyzed using the Kjeldahl method (Van Dijk *et al.*, 2000). The commercial feed and Azolla plant contained 35.58% and 28.9% crude protein, respectively, as shown in Table (1). The last two feeds were diets of 80% commercial feed and 20% dried Azolla and diets of 80% commercial feed and 20% fresh Azolla.

Table (1): Proximate analysis of the nutritional composition of the commercial feed and azolla plant.

Food provided for fish	Protein %	carbohydrate%	lipid%
Commerical feed	35.580	36.150	11.050
Azolla plant	28.900	19.660	3.305



Figure (1): Experiential Pond.

The fish were given feed at a rate of 5% of total biomass, distributed in an average of three meals daily. The amount of feed was modified biweekly by weighing the fish. The fish were weighed every two weeks using an electronic kitchen scale made in China. About 70% of the pond water was changed every day with supplying clean water (referencing Al-Faragi, 2011). To prepare feed, Azolla plants were partially shade-dried, powdered, and made into a diet by mixing 20% dried Azolla and 80% commercial feed. In a similar method, fresh Azolla was offered to the fish as 20% of the diet mixed with 80% commercial feed before it was presented to the fish.

To guarantee sufficient water quality in the experimental ponds, we monitored a number of environmental factors, including, but not limited to, water temperature (using a mercury thermometer), pH (measured from time to time with a pH Meter, pHS-100, China), and dissolved oxygen (measured from time to time with a DO-meter). These environmental measurements can be found in Table (2).

Table (2): Measurements of environmental parameters in the experimental pond water.

Environmental factors	Measured value		
	Beginning of the experiment	Amidst of the experiment	End of the experiment
Temperature	16.3°	14.7°	14.8°
pH	5.8	7.2	7.1
Dissolved oxygen	8.3	8.1	8.5

Weight gain, absolute growth rate, and relative growth rate were calculated according to the following equations. Weight gain (g) was determined as :

Final weight (g) – Initial weight (g) (Al-Hamadany, 2022)

The absolute growth rate (g day^{-1}) was calculated as :

Final weight (g) – Initial weight (g) / Experimental period (days) (Abedalhammed et al., 2017).

The relative growth rate (%) was calculated using the formula :

Weight gain (g) / Initial weight (g) \times 100 (Al-Qudsi et al., 2023).

Feed Conversion Ratio (FCR) was calculated as :

Feed intake (g) / Biomass gain (g) (Lapie & Bigueras-Benitez, 1991).

A completely randomized design (CRD) was used in a two-factor factorial arrangement. Statistical analyses were performed using the SPSS software package. Duncan's multiple range test was applied to compare treatment means at the 0.05 probability level (Steel and Torrie, 1980).

Results and Discussion:

Fish weight gain

The data shown in Table 3 reveal highly significant differences between feeding treatment and time period, as well as their interaction. Fresh Azolla produced the greatest average weight gain ($3.486 \text{ g.fish}^{-1}$) while the 60-day rearing period obtained a higher mean value ($3.026 \text{ g.fish}^{-1}$) than the 45-day period ($2.536 \text{ g.fish}^{-1}$). The increased growth observed in fresh Azolla fed fish may be related to the increased activity of digestive enzymes specifically, amylase, lipase, chymotrypsin, and trypsin associated with the feeding treatment with 20% fresh Azolla, that directly translate to weight gain in common carp, and resultantly improved feed conversion rates and growth rate (Rafaey *et al.*, 2023). Both carp and tilapia prefer fresh feed sources, particularly, fresh Azolla, due to its high palatability and consequent feed intake, which ultimately improves growth performance (Hasan & Chakrabarti, 2009). The effective inclusion level of Azolla in fish-feed generally ranges from 10-25% due to nutritional requirements of fish within this range (Mosha, 2018).

Table (3): Increase in fish body weight

Nutrition	Time period		Transaction averages
	After 45 days	After 60 days	
Control	1.928	2.221	1.946 b
Fresh Azolla	3.317	3.785	3.486 a
Dry Azolla	2.364	3.071	2.367 b
Time period averages	2.536 ab	3.026 a	

*Similar letters indicate no statistical differences at a significance level of $P \leq 0.05$.

Growth Rate of Fish

The results in Table 4 indicate significant differences in feeding treatments, experimental periods, and their interaction. The fish fed dry Azolla diet grew out the fastest with an average of (67.50 g. fish⁻¹). The 60-day rearing period recorded the highest mean growth rate (63.89 g. fish⁻¹) as compared to the 45-day period (43.89 g. fish⁻¹).

The growth performance of fish fed on dry Azolla was better than fresh Azolla because dry Azolla contains a higher concentration of proteins, essential amino acids, vitamins, and minerals. This supports greater metabolic activity, increased secretion of digestive enzymes in the intestine, and enhanced metabolic processes in the liver, thereby leading to accelerated growth (Rajan et al., 2021). Like results found in Raju and Benno Pereira (2023), where growth rate and weight gain improved when Azolla was included in the diet as a feed supplement.

Similarly, Korkut *et al.*, (2016) conducted a study in Turkey, where dry Azolla added to the diets of common carp for 60 days had better growth performance compared to fresh Azolla, particularly when comparing diets with shorter feeding periods such as 45 days. This means that the favorable effects of dry Azolla are pronounced over longer rearing periods, potentially due to being more digestible and better stabilizing nutrients.

Feed utilization efficiency is influenced by several biological factors, including species, age, body composition, feed characteristics, and physiological capability for digesting and absorbing nutrients (Ferenczi & Bodnar, 2024). Studies have shown that feeding Azolla with a commercial diet increases survival rates when compared to feeding a commercial diet alone (Wiryat, 2021). This finding is supported by Ahmed et al. (2023), indicating Azolla does not have negative effects on growth performance. Increasing the proportion of fish diets that include Azolla have also shown improved rates of growth likely due to intestinal digestive enzymes optimizing nutrient utilization, digestion efficiency (Shaima *et al.*, 2020).

Table 4. Growth rate of fish (g. fish⁻¹).

Nutrition	Time period		Transaction averages
	After 45 days	After 60 days	
Control	33.67 b	44.33 b	39.00 b
Fresh Azolla	45.33 b	65.00 ab	55.17 ab
Dry Azolla	52.67 ab	82.33 a	67.50 a
Time period averages	43.89 b	63.89 a	

*Similar letters indicate no statistical differences at a significance level of P<0.05.

Relative growth rate of fish

The data in Table 5 indicated significant differences between feeding treatments, experimental periods, and interactions between feeding treatments and experimental periods. Fish given dry Azolla diets had the highest relative growth rate at (245.88 g. fish⁻¹). The experimental period of 60 days also produced a significantly higher mean value (212.48 g. fish⁻¹) in comparison to the 45-day experimental period at (184.80 g. fish⁻¹).

The increase in relative growth rate could be due to an increase in secretion of digestive enzymes in the intestine, which aids in the digestion and absorption of nutrients, possibly due to the activity of the key digestive enzymes (amylase and protease) (Zou *et al.*, 2019). Furthermore, the polysaccharides contained in dry Azolla, specifically cellulose, act as prebiotics encouraging the

growth of beneficial gut bacteria (for instance *Lactobacillus rhamnosus*), which enhance fish health and the fish's immune response (de la Cruz *et al.*, 2023).

Fish metabolism is significantly affected by feeding frequency as it impacts behavior, foraging activity, and nutrient assimilation. Fish consumed two times daily, displayed less feeding efficiency which led to insufficient digestion and nutrient utilization (Liu *et al.*, 2017). Increasing the number of feeding events increased digestive enzyme activity which subsequently increased nutrient utilization, percent weight gain, and relative growth rate (Hama Amin *et al.*, 2018).

Table (5): Relative growth rate of fish (g. fish⁻¹).

Nutrition	Time period		Transaction averages
	After 45 days	After 60 days	
Control	190.243	187.656	188.950
	ab	ab	ab
Fresh Azolla	150.853	171.350	161.101
	b	b	b
Dry Azolla	213.316	278.446	245.881
	ab	a	a
Time period averages	184.804	212.484	
	b	a	

*Similar letters indicate no statistical differences at a significance level of P<0.05.

Feed Conversion Rate (FCR)

Table 6 shows that there were significant differences due to feeding treatment and experimental periods, as well as their interactions. The diet with fresh Azolla had the top FCR values (3.434 g fish⁻¹), while the mean FCR was greater during the 60-day period (3.580 g fish⁻¹) than the mean FCR of the 45-day period (2.833 g fish⁻¹).

The feed conversion ratio indicates how much of the consumed feed is converted into fish biomass and is among the most important measure of profit for aquaculture businesses (Fadhil *et al.*, 2017). In some cases, dried Azolla outperformed fresh Azolla which may be due to the differences in crude fiber and moisture content. However, it is likely that the crude fiber and moisture content was lower in dried Azolla compared to fresh Azolla, after the processes involved in drying fresh Azolla, which improved the nutrient content. Additionally, this increased the activity of digestive enzymes

located in the gastrointestinal tract leading to better nutrient utilization and faster growth rates (Kumar *et al.*, 2018; Paryanto *et al.*, 2023).

Nevertheless, a high inclusion rate of Azolla in fish diets reduces feed digestibility and nutrient utilization because of its negative effect on the activity of digestive enzymes, leading to lowered growth performance. This reduction is associated with decreased efficiency of digestion as well as reduced feed utilization (Sithara & Kamalaveni, 2008). When Azolla was included at higher levels, there has been a reported increase of ~10% in FCR, possibly due to the reduction or imbalance of essential amino acids, such as tryptophan, and limited threonine availability from Azolla protein. Consequently, fish may consume greater triple feed in compensating for the deficiency when Azolla is included at high levels (Al-Aaraji & Taha, 2020).

In fish production, it has been reported that with Azolla at inclusion rates of up to 40%, profitability improved. However, when the inclusion of Azolla was increased from 10% to 40%, there was a reduction in the level of fish growth and profit margins. Thus, Azolla powder can be used effectively at a 20% inclusion level, for reducing feed costs while keeping growth performance unaffected (Phulia, 2010).

Table (6): Feed conversion rare (g. fish⁻¹).

Nutrition	Time period		Transaction averages
	After 45 days	After 60 days	
Control	2.866 cd	3.633 ab	3.250 ab
Fresh Azolla	3.026 cd	3.842 a	3.343 a
Dry Azolla	2.606 d	3.263 bc	2.935 b
Time period averages	2.833 b	3.580 a	

*Similar letters indicate no statistical differences at a significance level of P<0.05.

Conclusions: The findings of the study reveal that Azolla in diet of fish *Cyprinus carpio* promotes increases in fish body weight compared to conventional commercial feed. The result of substituting 20% of commercial feed with Azolla resulted in improved growth performance in fish without causing adverse health effect.

Recommendations: This study suggests that more thorough investigations should be conducted to evaluate the impacts of various processing methods and formulations of Azolla on quality, sensory characteristics and palatability of fish meat, and also for consumer acceptance and marketability purposes.

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